Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

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# Ph.D. thesis topics 2025/2026
# DSP Ochrana vodních ekosystémů / Protection of aquatic ecosystems

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

## Vodňany

# Supervisor: Assoc. Prof. Martin Bláha

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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 Research program**

RP4 Freshwater ecosystems in the era of global change

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## Fishpond ecosystems in an era of global change

**Ekosystém rybníka v éře globálních změn**

**Annotation**

Central European fishponds represent unique, manmade freshwater ecosystems that are important and integral to the landscape (Pechar et al., 2002). Fishponds used mostly for fish production represent the predominant form of standing water bodies in Central and Eastern Europe (from < 1 ha to several hundred ha, depth 1–4 m) (Francová et al., 2019). Long-term excessive nutrient loading in the last century led to both enhanced fish production and significant environmental degradation of fishpond ecosystems (Adámek et al., 2023; Pechar 1995). Such environmental degradation has manifested in impaired fish production, frequent cyanobacterial blooms, and hypoxic events (mostly in deep water layers) with periodic fish kills alongside direct effects on eco-services, such as degraded recreational uses or biodiversity support (Baxa et al., 2021). Recently, climate change has deepened some of these negative processes (Orság et al., 2023). Consequently, the current fishponds have become less predictable regarding production processes and thus also less cost-efficient, more stochastic, and greatly unstable systems.

Additionally, the water quality in fishponds has come under scrutiny and pressure from the public and state authorities. The current unfavorable condition of fishpond ecosystems only reflects intentional eutrophication of fishponds in the last decades. The overall heavy nutrient burden needs complex solutions coupled with adjusted stocking management. With the continued tightening of EU rules regarding open water quality conditions, close collaboration of scientific institutions with the fish producers is needed. The proposed thesis should reveal the functioning of the fishpond ecosystem under the changing climate and find the balance between the profitability of fish production on one side and eco- and social services on the other.

**The main hypothesis**

- Reasonable fish stocking can keep both economic profitability and eco-services in fishponds.
- Climate change shifts fishpond ecosystems to nitrogen limitation, which proliferates in cyanobacterial blooms, anoxic and further unstable conditions.
- With increasing water temperature and/or fish stock, the diversity of phyto- and zooplankton decreases.

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

- Describe the functioning fishpond ecosystem under changing climate
- Find equilibrium between fish culture and non-production services of fishponds
- Suggest usable fishpond management based on size, position and historical data.

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

- Analysis of available long-term data on fishpond environmental parameters and fish production
- Collecting and analysing data about the fishpond ecosystem under different conditions of management
- Carry out specific experiments to reveal the limitations and capabilities of the fishpond ecosystem
- Use modern methods of metabarcoding (from eDNA by nanopore sequencing) to reveal the diversity of particular communities in the fishpond ecosystem

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**CENAKVA Research program**

RP4 Freshwater ecosystems in the era of global change

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## Invasive freshwater crayfish and their influence on other freshwater taxa

### Invazní druhy raků a jejich vliv na ostatní vodní organismy

### Annotation

European freshwaters are facing to high rates of colonization by invasive animal species. These species represent deliberately or accidentally introduced animals into areas outside their natural range, established in new environments, spreading uncontrollably, and causing substantial problems in ecological but also economic costs. The effects of biological invasions are pronounced by anthropogenic changes of habitats, pollution, and climate change effects due usually to high adaptability and resistance of unwanted species. No country is immune to the colonization of animal alien species. The proposed PhD thesis is aimed mainly on the very successful group of alien invasive species in European waters – North American freshwater crayfish. Six of them are included in the list of the invasive species of European concern, and their handling and transport is therefore limited. Unfortunately, it is almost impossible to manage their populations when established. They can spread and affect valuable endangered species such as several bivalve species, native crayfish or fish. Proposed work will employ several experimental and observational approaches in both laboratory and field studies to evaluate the impacts on different aquatic organisms in terms of their behavior, competition and predator – prey interactions with assessment of the influence of different environmental conditions as temperature, habitat complexity, water flow. Results of this PhD thesis will extend the knowledge about basic life processes of invasive organisms and their impacts in novel environment and on native organisms. Last but not least, results will be published in peer-reviewed journals and presented at international conferences.

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RP4 Freshwater ecosystems in the era of global change

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## Nature-inspired approaches for wastewater treatment and reuse

**Přírodou inspirované přístupy k čištění a opětovnému použití odpadních vod**

**Annotation**

Every day we are using water and releasing many chemicals to wastewater. With the growing global concern over water scarcity and pollution, there is a critical need for innovative approaches to wastewater treatment that not only effectively remove contaminants but also recover valuable resources from wastewater streams.

Conventional wastewater treatment is not effective enough to remove pollutants from the wastewater, thus wastewater effluents are recognized as the main source of micropollutants in the aquatic environment. Presence of a wide range of emerging contaminants (such as pharmaceuticals, personal care products, perfluorinated compounds) in wastewater treatment plants effluents was reported.

Intensive research is going on to improve elimination of micropollutants from wastewater. So called green approaches, which promote sustainable and environmentally friendly methods for treating wastewater while also reducing the environmental impact of traditional wastewater treatment processes are gaining a lot of attention nowadays. Natural treatment methods include, for example, the use of stabilization ponds for wastewater treatment. This is a cheap and relatively effective way to reduce the content of nutrients and micropollutants in wastewater. Constructed wetlands are also used for wastewater treatment and have the potential to remove a wide range of micropollutants.

Treated wastewater represents a sustainable water source that could be used for fish farming. However, the reuse of treated wastewater poses certain safety risks. Until now, attention has been paid to the risk of microbial contamination or the accumulation of toxic metals in fish produced this way. However, less information is available on the possible contamination of fish with micropollutants.

**The main hypothesis**

- Additional wastewater treatment steps can improve quality of wastewater effluents
- Water plants can contribute to the elimination of micropollutants from wastewater
- Wastewater reuse for the fish production can be beneficial using the proper practise of fish handling

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

- To assess the effectiveness of stabilisation pond and constructed wetlands for the elimination of micropollutants from wastewater.
- To evaluate the bioaccumulation of micropollutants in water plants.
- To analyze the content of micropollutants in fish produced in the pond fed with treated wastewater. In case these substances will be detected, to monitor the duration of their purification after transferring the fish into clean water.

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

- Collection of water, plant and fish samples; development and validation of extraction procedures.
- LC/MS analysis of micropollutants in collected samples.
- Data analysis, risk assessment.

**References**

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**CENAKVA Research program**

RP2 “New” pollutants in the environment and their effect on freshwater ecosystems

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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**

NAZV projects:

- 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 Research program**

RP2 “New” pollutants in the environment and their effect on freshwater ecosystems

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## Effects of thyroid-disrupting chemicals on fish and amphibians, focusing on their immune systems

### Účinky chemických látek narušujících funkci štítné žlázy na ryby a obojživelníky se zaměřením na jejich imunitní systém

**Annotation**

Endocrine disruptors, i.e. substances that interfere with the functioning of the hormonal system of exposed organisms, are among the most threatening environmental pollutants. They deserve special attention because they can cause, for example, reproductive system abnormalities, reduced fertility, thyroid dysfunction, or metabolic disorders (diabetes, obesity). Since immunity is susceptible to reprogramming by environmental chemical and endocrine signals, it is thought that endocrine disrupters may also adversely affect the immune system of exposed animals. However, it is often unclear whether the immunotoxicity of endocrine disruptors is dependent on, or completely independent of, endocrine toxicity. Thyroid-disrupting chemicals are a class of endocrine disruptors with a high potential to interfere with the immune system, as the literature data suggest that different immune pathways are under distinct control by thyroid hormones during early vertebrate development. In addition, numerous thyroid-disrupting chemicals have been documented to disrupt immune endpoints (McGuire and Robert, 2023).

Thyroid-disrupting chemicals have been reported in various types of environments, including the aquatic environment. The results of our recent nationwide screening for anti-thyroid activity in surface waters showed that this activity was detected in more than one-third of the water samples (Šauer et al., 2024). It is, therefore, essential to study the effects of thyroid-disrupting chemicals on aquatic animals, especially fish and amphibians, in more detail, as these lower vertebrates already have a fully developed hypothalamic-pituitary-thyroid axis. Research into thyroid-disrupting chemicals has focused on their adverse effects on early development in fish and amphibians. However, it can be hypothesised that thyroid-disrupting substances could also negatively affect their immune response.

**The main hypothesis**

Some endocrine disruptors reported to be present in aquatic environments interfere with the hypothalamus-pituitary-thyroid axis. As the thyroid controls several immune pathways, thyroid-disrupting chemicals may also affect the immune response of fish and amphibians living in polluted areas. Consequently, animals with an impaired immune response may be more susceptible to pathogens normally found in the aquatic environment.

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

The aim of this study will be to evaluate the effect of pollutants with anti-thyroid activity on the immune response of exposed fish and amphibians and to assess whether they are more susceptible to pathogens.

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

Model fish and amphibian species will be exposed to substances with anti-thyroid activity during early development when their thyroid and immune systems are forming. These exposed animals and respective controls will then be challenged with pathogens. Model host-pathogen systems will be selected, such as common carp vs. koi herpes virus and African clawed frog vs. rana virus. The immune response and susceptibility of the animals to these pathogens will be studied.

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**Funding**

- In 2025, a project on the same topic will be submitted to the Czech Science Foundation.
- Additionally, other financial sources from the laboratory will be used.

**CENAKVA Research program**

RP2 “New” pollutants in the environment and their effect on freshwater ecosystems

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## Crayfish in a Warming World: Decoding Epigenetic and Metabolic Strategies for Climate Adaptation

**Raci v oteplujícím se světě: Dekódování epigenetických a metabolických strategií pro adaptaci na změny klimatu**

**Annotation**

Aquatic ecosystems are increasingly at risk from the way that global warming is altering essential functions like metabolism, reproduction, and development in ectothermic species like crayfish. Ectotherms are particularly sensitive since their body temperature and physiological processes are influenced by their environment (Beitinger, Bennett, & McCauley, 2000). As keystone species in freshwater environments, crayfish are essential to energy transfer, detritus breakdown, and nutrient cycling, but their existence is becoming more endangered. Temperature profile changes are accelerated by global warming, which leads to metabolic disturbances, decreased reproductive success, and higher death rates (Holdich et al., 2009). Understanding how crayfish respond to heat stress is crucial for biodiversity protection and ecosystem management in the face of climate change. One important environmental factor that affects the metabolic activities of ectothermic organisms is temperature. Warmer temperatures cause metabolic rates to rise, which results in increased energy expenditures for maintenance as opposed to growth or reproduction (Angilletta, 2009).

Particularly during crucial life stages like molting and embryonic development, metabolic stress under high temperatures can impair physiological processes (Stillman, 2003). Long-term exposure to heat stress in crayfish can worsen population reductions by reducing reproductive output through impaired egg production and fertilization success. Additionally, there are frequently trade-offs associated with metabolic adaptations to higher temperatures, such as weakened immune systems or slowed recovery from environmental stress. Histone modifications and DNA methylation are two examples of epigenetic changes that have become important mechanisms enabling organisms to adjust to environmental stimuli. These changes allow rapid phenotypic adaptations to shifting environmental conditions by regulating gene expression without modifying the underlying DNA sequence (Eirin-Lopez & Putnam, 2019). The expression of genes related to stress resistance, energy metabolism, and heat tolerance in crayfish may be mediated by epigenetic responses. For instance, research has shown that higher temperatures can alter DNA methylation patterns, which enhance cellular defense and metabolic efficiency (López-Maury et al., 2008). Similarly, histone modifications that alter chromatin structure can increase the expression of heat-shock proteins, which are essential for cellular stress responses (Tomanek, 2010). These molecular mechanisms enable crayfish to survive and reproduce under suboptimal conditions, improving their resilience to global warming.

To adapt to higher temperatures, crayfish display metabolic changes in addition to epigenetic responses. Because more ATP is needed to sustain physiological functions at higher temperatures, metabolic rate rises (Pörtner & Farrell, 2008). It is difficult for crayfish to balance their energy needs for growth, reproduction, and stress reactions while they are under heat stress. Ectotherms, such as crayfish, can optimize their energy consumption by adjusting their activity patterns and metabolic rates, according to

research using intermittent-flow respirometry techniques (Killen, Marras, & Domenici, 2013). During times of extreme heat stress, for instance, crayfish may decrease locomotor activity to conserve energy, rerouting resources toward cellular survival and repair.

Metabolic plasticity is limited, though. The effectiveness of these adaptations decreases and physiological collapse results if temperatures rise above species-specific thresholds (Stillman, 2003). These metabolic modifications may help non-native crayfish species expand their range into previously inhospitable habitats, as they frequently exhibit higher temperature tolerance than native species (Capinha, Leung, & Anastácio, 2013).

**The main hypothesis**

- Temperature-induced epigenetic modifications (e.g., hypermethylation, chromatin restructuring) and metabolic rate adjustments enable crayfish to survive and grow under thermal stress.
- Thermal adaptation mechanisms may exhibit parallels across related ectothermic species.

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

- Analyze temperature-induced epigenetic modifications, focusing on DNA methylation and histone changes.
- Assess crayfish metabolic rate adjustments under thermal stress using intermittent-flow respirometry.
- Elucidate the role of these mechanisms in supporting crayfish growth and resilience.
- Offer insights for biodiversity conservation and management strategies under climate change scenarios.

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

- Conduct thermal stress experiments at +3°C and +6°C.
- Use genome-wide epigenetic profiling to detect DNA methylation and histone modifications.
- Employ intermittent-flow respirometry to measure metabolic changes during critical development and inactive phases.
- Compare findings with similar ectothermic species for broader ecological insights.

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**CENAKVA Research program**

RP4 Freshwater ecosystems in the era of global change

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## Advanced oxidation processes for effective, ecologic, and safe wastewater treatment

### Pokročilé oxidační procesy pro efektivní, ekologické a bezpečné čištění odpadních vod

### Annotation

One of the most significant environmental challenges is global water pollution, primarily attributed to the increasing presence of synthetic chemical contaminants. Specifically, the discharge of pharmaceuticals has become an alarming issue, mainly due to the growth of the pharmaceutical market and increased consumption. To prevent the discharge of micropollutants, it is important to: (i) change legislation and prioritize hazardous substances and (ii) develop and apply advanced methods for their effective and safe removal. In 2022, under the European Green Deal, the new legislative recast of the Urban Wastewater Treatment Directive should include an additional high-risk substance into the list of controlled micropollutants for quaternary water treatment.

Water treatment is one of the most important technological challenges today, the management of which will determine the living conditions for future generations. The actual technologies using WWTPs cannot effectively eliminate new types of organic micropollutants such as pharmaceuticals, drugs, perfluorinated compounds, personal care products, and many others. Thus, most treatment plants continuously release these types of compounds into the aquatic ecosystem, from where they gradually penetrate other components of the environment and groundwater.

It is evident that pursuing advanced wastewater treatment technologies and developing ways of degrading micropollutants in WWTP effluent is highly desirable. This gap in efficacy highlights the critical demand for innovative, mainly quaternary treatment methods capable of tackling such pollutants with higher precision and efficiency. Membrane technologies have proven well-suited to advanced wastewater processing and can facilitate recycle and reuse of treated effluent. Improve of MBR process performance can be done by combination with other processes. Most perspective processes include various advanced oxidation processes (AOPs) based on boron-doped diamond electrodes, active iron in form Fe(VI), and many others. The principle of electrochemical AOPs consists of the formation of strong oxidants, especially the singlet oxygen, hydrogen peroxide, and hydroxyl radical, which oxidize organic compounds in water and, in most cases, achieve their complete degradation.

Treated wastewater offers a sustainable water source suitable for various applications, such as irrigation and fish farming. However, its reuse comes with potential safety concerns. The identification of degradation and transformation products should be systematically linked to the assessment of their toxicity. However, this process faces several challenges: i) the need to fully understand degradation mechanisms and the identification of all components present in the mixture and ii) the limited availability of analytical standards necessary for studying these substances and characterizing complex mixtures in toxicological studies. Toxicological evaluation should include not only the assessment of individual substances but also an analysis of their potential additive and synergistic effects.

**The main hypothesis**

- Advanced oxidation processes for wastewater treatment can improve the quality of wastewater effluents.
- During the AOPs wastewater treatment processes several set of reactions occurs and there is a demand to study possible pathways for their degradation and transformation.

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

- To assess the effectiveness of different AOP techniques for the elimination of micropollutants in the model and different types of wastewater based on LC-MS/MS analysis.
- To study of creation of degradation and transformation products in model and different types of wastewater samples by HPLC-HRMS analysis.

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

- Collection of different water samples; development and validation of extraction procedures and LC-MS/MS methods.
- LC/MS analysis of selected micropollutants in model and wastewater samples before and after treatment using different AOPs.
- HPLC-HRMS analysis for identification of emerging degradation and transformation products of AOPS
- Data analysis, and risk assessment.

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**CENAKVA Research program**

RP2 “New” pollutants in the environment and their effect on freshwater ecosystems

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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 tree 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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