

Ph.D. thesis topics 2025/2026 -2nd call

DSP Ochrana vodních ekosystémů / Protection of aquatic ecosystems

ýzkumný ústav rybářský a hydrobiologický / Research Institute of fish Culture and Hydrobiolo odňany	
Supervisor: Assoc. Prof. Martin Bláha, Ph.D.	
Third-generation sequencing: a powerful tool for studying biodiversity in freshwater ecosystems	
Fish migration in a regulated lowland river	
Supervisor: Ganna Fedorova, Ph.D	
Supervisor: Assoc. Prof. Antonín Kouba	
Supervisor: Lukáš Veselý, Ph.D	
Supervisor: Assoc. Prof. Andrea Vojs Staňová	
Supervisor: Prof. Vladimír Žlábek Bioaccumulation dynamics of emerging contaminants in aquatic invertebrates	
Supervisor: Assoc. Prof. Miloš Buřič	



Výzkumný ústav rybářský a hydrobiologický

Research Institute of fish Culture and Hydrobiology

Vodňany





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

Contact

E-mail: <u>blaha@frov.jcu.cz</u> Tel.: +420 389 034 611

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

Sekvenování třetí generace jako nástroj pro studium biodiversity sladkovodních ekosystémů

DSP: Ochrana vodních ekosystémů / Protection of aquatic ecosystems

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 (i) to early detect the spread of non-native aquatic species, (ii) to describe the biodiversity of natural valuable habitats, (iii) develop species-specific protocols for detecting species of interestSuggest possible remedial measures to improve the migratory passability of water barriers for fish.



Possible approaches to reach the aims / to verify the hypotheses

- Performing 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
- Collecting and analysing data about biodiversity and its changes in spots with non-native species presence

References

Dickie, I.A., Boyer, S., Buckley, H.L., Duncan, R.P., Gardner, P.P., Hogg, I.D., ... & Weaver, L., 2018. Towards robust and repeatable sampling methods in eDNA-based studies. Molecular Ecology Resources, 18(5), 940-952, doi.org/10.1111/1755-0998.12907

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., ... & Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews, 81(2), 163-182, doi:10.1017/S1464793105006950

Niedringhaus, T.P., Milanova, D., Kerby, M.B., Snyder, M.P., Barron, A.E., 2011. Landscape of next-generation sequencing technologies. Analytical Chemistry. 83 (12): 4327–41, doi:10.1021/ac2010857

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T., ... & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological reviews, 94(3), 849-873, doi.org/10.1111/brv.12480

Wang, Y., Zhao, Y., Bollas, A., Wang, Y., & Au, K. F., 2021. Nanopore sequencing technology, bioinformatics and applications. Nature biotechnology, 39(11), 1348-1365.

CENAKVA Research program



Supervisor: Bořek Drozd, Ph.D.

Contact

E-mail: drozd@frov.jcu.cz
Tel.: +420 389 034 652

Fish migration in a regulated lowland river

Migrace ryb v regulované nížinné řece

DSP: Ochrana vodních ekosystémů / Protection of aquatic ecosystems

Annotation

European watercourses, including the large, lowland ones, underwent a dramatic change in function with the advent of the Industrial Revolution and the subsequent intensification of land use and urbanisation after World War II. Longitudinal and lateral river connectivity has been lost due to the construction of transverse barriers channelization of streams, branches cutting, drainage, making stream navigable, construction of reservoirs and hydropower plants. This in turn has led to, among many other significant phenomena, the alteration and loss of species diversity of aquatic biota, including fish. Due to the increase in human population, its standard of living and the pressure for the massive exploitation of so-called renewable resources (including the hydropower potential of watercourses), this pressure continues to increase in the 22nd century. This situation also applies to the Slovak-Hungarian part of the Danube, where the construction and operation of the Gabčíkovo hydroelectric dam has destroyed the unique ecosystem of the inland Danube delta, which has left parts of the Danube's branches system on both the Slovak and Hungarian sides, as well as the "Old" Danube (the Danube from Čunovo to Sáp). In both original water systems, changes in the water regime have occurred, manifested by water scarcity, lack of intercommunication, deepening of the channel of the Old Danube, sedimentation, etc., and logically, significant changes in the state of the fish communities.

In addition, the communication of the Old Danube with the rest of the river system is severely limited at the upper end (below Čunovo), and on the Hungarian side the Old Danube has been dammed by the Dunakiliti dam, so it is generally assumed that the original Danube channel has lost its role as an important European biocorridor. However, how fish, especially rheophilic species, have actually responded to these changes in terms of behaviour, migration and habitat preferences is not yet known. In addition, the newly built Danube Canal, built for shipping and hydropower generation, is dammed by the Gabčíkovo Dam. Thus, the migration of rheophilic species, especially long-distance migrants such as sturgeons, seems to be significantly impaired or completely prevented in the SK-HU part of the Danube. However, sufficient empirical data needed for a credible assessment of the behaviour, fish migration and migratory passability of the Gabčíkovo barrage system for fish is still lacking. Therefore, it is necessary to obtain these data by direct observation of the tagged migratory rheophilic fish species through the target area using telemetry surveys. Data analysis will then provide valuable information on fish movement, migration passability and the use of different parts of the Danube SK-HU by key fish species (with a special emphasis on sterlet as an umbrella fish species of the Danube R.).

The main hypothesis

• The altered conditions of the former Danube river bed (Old Danube) do not represent attractive/preferred habitats for migratory fish species and they do not seek it out.



- The original biocorridor of the Old Danube (Čunovo Sáp) does not allow sufficient or any fish migration due to flow regulation and construction of barriers.
- The newly constructed Danube Canal does not provide sufficient migratory passability of the river due to the construction of the Gabčíkovo dam, which does not allow migration passage for fish.
- The migratory (in)passability of the entire Gabčíkovo barrage system is partial or total and allows only upstream, downstream or no migration at all.
- Migratory fish, due to the changed conditions, have developed a different strategy to overcome the barriers and use the habitats compared to the former natural state.

Aim(s) of the Ph.D. thesis

- Determine the current migratory passability of the Gabčíkovo barrage system for key rheophilic fishes (with a special focus on sturgeons).
- Describe the annual migratory pattern in the use of individual sections of the SK-HU Danube for key rheophilic fishes.
- Estimate the current role of the Old Danube as a biocorridor for migratory rheophilic fish species.
- Suggest possible remedial measures to improve the migratory passability of water barriers for fish.

Possible approaches to reach the aims / to verify the hypotheses

- Catch, individually tagging of key rheophilic fish species.
- Performance of the long-term telemetry surveys (combination of continuous data acquisition and active telemetry from the boat) to track the movement of tagged fish and collect data on water depth and temperature utilized by fish through the target areas.
- Statistical evaluation of data (R software) and drawing conclusions from the data sets.

References

de Leeuw, J. J., Winter, H. V. (2008). Migration of rheophilic fish in the large lowland rivers Meuse and Rhine, the Netherlands. Fisheries Management and Ecology, 15(5–6), 409–415.

Birnie-Gauvin K, Aarestrup K, Riis TMO, Jepsen N, Koed A. (2017). Shining a light on the loss of rheophilic fish habitat in lowland rivers as a forgotten consequence of barriers, and its implications for management. Aquatic Conserv: Mar Freshw Ecosyst., 27: 1345–1349.

Deak, G., Badilita, A.M., Dănălache, T., Tudor, M. (2014). Use of acoustic telemetry for providing an insight into sturgeons' behaviour and migration routes on lower Danube, J. Environ. Prot. Ecol, 15(3), 954-964.

Honţ, S., Paraschiv, M., Iani, M. I., Stefanov, T., Lenhardt, M., & Oprea, L. (2018). Long Distance Migration of Beluga (Huso huso) and Stellate Sturgeon (Acipenser stellatus) in Lower Danube River in Relation with Iron Gate II Dam. Bulletin UASVM Animal Science and Biotechnologies, 75(1).

Kubala M, Farský M, Pekárik L. (2019). Migration patterns of sterlet (Acipenser ruthenus, Linnaeus 1758) in the Middle Danube assessed by 1 year acoustic telemetry study. J Appl Ichthyol., 35: 54–60.

CENAKVA Research program



Supervisor: Ganna Fedorova, Ph.D.

Contact

E-mail: gfedorova@frov.jcu.cz

Tel.: +420 775 360 674

Nature-inspired approaches for wastewater treatment and reuse

Přírodou inspirované přístupy k čištění a opětovnému použití odpadních vod

DSP: Ochrana vodních ekosystémů / Protection of aquatic ecosystems

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

Fedorova, G., R. Grabic, K. Grabicova, J. Turek, T. Van Nguyen, T. Randak, B. W. Brooks and V. Zlabek (2022). "Water reuse for aquaculture: Comparative removal efficacy and aquatic hazard reduction of pharmaceuticals by a pond treatment system during a one year study." Journal of Hazardous Materials 421.

Mackulak, T., M. Mosný, J. Škubák, R. Grabic and L. Birošová (2015). "Fate of psychoactive compounds in wastewater treatment plant and the possibility of their degradation using aquatic plants." Environ Toxicol Pharmacol 39(2): 969-973.

Zaibel, I., D. Zilberg, L. Groisman and S. Arnon (2016). "Impact of treated wastewater reuse and floods on water quality and fish health within a water reservoir in an arid climate." Science of the Total Environment 559: 268-281.

CENAKVA Research program

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



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

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

Supervisor: Assoc. Prof. Antonín Kouba

Contact

E-mail: <u>akouba@frov.jcu.cz</u> Tel.: +420 607 622 137

Macrobrachium nipponense: a new threat to European freshwater ecosystems

Macrobrachium nipponense: nová hrozba pro evropské sladkovodní ekosystémy

DSP: Ochrana vodních ekosystémů / Protection of aquatic ecosystems

Annotation

Freshwater ecosystems are increasingly affected by biological invasions, resulting in significant ecological and socio-economic consequences. One emerging non-native species spreading across Europe is the Oriental prawn Macrobrachium nipponense. It is therefore essential to monitor its distribution and investigate its potential impacts on native taxa. This thesis aims to monitor sites where this species has recently been detected, as well as other locations considered critical to its potential future spread. Genetic methods will be employed to elucidate the introduction pathways of non-native populations. Potential impacts on native taxa, ranging from competition for resources to predation and disease transmission, will be assessed using a multi-methodological approach that includes laboratory experiments, genetic markers, and stable isotope analysis of the invaded communities. Species distribution models will be used to predict both current and future areas of potential establishment.

The main hypothesis

The Oriental river prawn is an emerging invasive species in European freshwater ecosystems. Its recent spread is likely facilitated by multiple introduction pathways, and its establishment may exert measurable ecological impacts on native biota through trophic interactions, competition, and potential disease transmission. These impacts, when combined with suitable environmental conditions and ongoing climate change, are expected to promote the species' further spread and invasiveness across Central and Western Europe.

This hypothesis is supported by the following observations and considerations:

- Oriental river prawn has established populations in both thermally altered and naturally temperate waters in Central Europe. Its life-history traits, such as high fecundity, the ability to reproduce in freshwater, and thermal tolerance, are consistent with those of other successful invasive species.
- Preliminary evidence suggests introductions may occur via the pet trade, as seen with non-native crayfish, or through unintentional means such as ballast water discharge. Population genetic analyses will be used to determine whether spatially disjunct populations have independent origins.
- As a generalist omnivore, the Oriental river prawn may impact both native and non-native species through predation and resource competition. These potential interactions will be investigated experimentally.
- The species may act as a vector for pathogens such as Aphanomyces astaci, thereby contributing to indirect negative effects on native crayfish populations.



The hypothesis will be tested using a multidisciplinary approach combining field monitoring, laboratory experiments, stable isotope analysis, genetic tools, and species distribution modelling to ensure a comprehensive and robust assessment.

Aim(s) of the Ph.D. thesis

 This project will monitor the spread of a novel non-native species in Europe, assessing its introduction pathway, several potential impacts on other taxa, role in the invaded trophic web, as well as its potential distribution, and invasiveness and impact risks.

Possible approaches to reach the aims / to verify the hypotheses

 Several complementary and integrated approaches can be employed, aligned with the objectives outlined above. These include field monitoring (using trapping, hand searching, eDNA sampling, and associated genetic analyses), trophic ecology, behavioural and functional response experiments, species distribution modelling, and impact and invasiveness risk assessment.

References

- Bláha, M., Schrottenbaum, A., Weiperth, A., Hammerschmied, U., Graf, W., Csányi, B., ... & Kouba, A. (2025). Macrobrachium nipponense (De Haan, 1849) continues to spread in the Danube: first records in Germany and Hungary. Knowledge & Management of Aquatic Ecosystems, 426: 9.
- Oficialdegui, F. J., Bláha, M., Prati, S., Lipták, B., Weiperth, A., Bányai, Z. M., ... & Kouba, A. (2025). Contrasting patterns of genetic variability in pet-traded red swamp crayfish Procambarus clarkii and its feral populations. Freshwater Biology, 70: e70008.
- Lipták, B., Prati, S., Oficialdegui, F. J., Apfelová, M., Pekárová, S., Kautman, J., ... & Kouba, A. (2024). First populations of invasive red swamp crayfish flourish in Slovakia. BioInvasions Record, 13: 825-841.
- Mojžišová, M., Weiperth, A., Gebauer, R., Laffitte, M., Patoka, J., Grandjean, F., Kouba, A., Petrusek, A. (2024). Diversity and distribution of Aphanomyces astaci in a European hotspot of ornamental crayfish introductions. Journal of Invertebrate Pathology, 202: 108040.
- Kouba, A., Oficialdegui, F. J., Cuthbert, R. N., Kourantidou, M., South, J., Tricarico, E., ... & Haubrock, P. J. (2022). Identifying economic costs and knowledge gaps of invasive aquatic crustaceans. Science of the Total Environment, 813: 152325.
- Haubrock, P. J., Oficialdegui, F. J., Zeng, Y., Patoka, J., Yeo, D. C., & Kouba, A. (2021). The redclaw crayfish: A prominent aquaculture species with invasive potential in tropical and subtropical biodiversity hotspots. Reviews in Aquaculture, 13: 1488-1530.

CENAKVA Research program



Supervisor: Lukáš Veselý, Ph.D.

Contact

E-mail: <u>veselyl@frov.jcu.cz</u> Tel.: +420 728 486 172

Freshwater food webs under biotic and abiotic stressors

Vliv biotických a abiotických stresorů na sladkovodní potravní řetězce

DSP: Ochrana vodních ekosystémů / Protection of aquatic ecosystems

Annotation

Freshwater ecosystems occupy less than 1% of the Earth's surface but support approximately 10% of global biodiversity, including one-third of all vertebrate species (Strayer & Dudgeon, 2010). The structure of freshwater food webs is characterized by numerous interconnected trophic units (Vander Zanden & Rasmussen, 2001), whose interactions drive the fluxes of energy and matter as well as the stability of these ecosystems (Barrios-O'Neill et al., 2015; Sih et al., 1998). However, these energy flows and the overall stability of freshwater ecosystems are susceptible to a range of biotic and abiotic stressors—including elevated temperatures, eutrophication, biological invasions, pollution, habitat modification—and interactions. These stressors can induce irreversible changes in ecosystem structure and function.

Mentioned stressors can significantly influence both the availability and quality of food resources within aquatic systems (Schälicke et al., 2019). Food quantity refers to the dietary energy (i.e., caloric content) accessible to consumers, whereas food quality refers to its nutritional value, determined by biochemical composition—specifically the compounds essential for physiological processes such as somatic growth, survival, and reproduction (Müller-Navarra et al., 2000). When key nutritional resources are absent and cannot be substitute by alternative sources, individual fitness may decline, potentially leading to changes in population dynamics (Martin-Creuzburg et al., 2008; Schälicke et al., 2019).

Accordingly, a central objective in trophic ecology is to elucidate species interactions and to determine how various food sources are assimilated into consumer biomass. It is also essential to identify which food sources are most critical for sustaining organisms across different trophic levels (Ahlgren et al., 1992; Dalsgaard et al., 2003), and how these trophic relationships are modulated by biotic and abiotic environmental stressors.

The main hypothesis

- Trophic and non-trophic interaction strength in multiple consumer systems will be shaped by stressors and their interaction. But consumer response will depend on their functional group.
- Availability and quality of utilized food sources by consumers might vary due the effect of biotic and abiotic stressors.
- Energy transfer from low levels of trophic chain to higher trophic levels might be modified by given stressors.
- The primary producers quality and biomass might be affected by biotic and abiotic stressors

Aim(s) of the Ph.D. thesis

 To assess differences in energy transfer through food webs between affected and non-affected ecosystems



• To reveal the relationship between multiple consumers and its prey under different biotic and abiotic stressors

Possible approaches to reach the aims / to verify the hypotheses

- Functional response approach in mesocosm with variate consumers and its prey under specific environmental stressors, and their combinations, followed by modelling of future scenarios
- Collecting the data from affected ecosystem (consumer, prey, environmental data) for stable isotopes/ fatty acid analysis.
- Applications of statistical data analysis (GLM, ANOVA, ANCOVA, Bayesian models, predictive models)

References

- Ahlgren, G., Gustafsson, I.-B., & Boberg, M. (1992). Fatty acid content and chemical composition of freshwater microalgae. Journal of Phycology, 28(1), 37–50. https://doi.org/10.1111/j.0022-3646.1992.00037.
- Barrios-O'Neill, D., Dick, J.T.A., Ricciardi, A., MacIsaac, H.J., & Emmerson, M.C. (2015). Predator-free space, functional responses and biological invasions. Functional Ecology, 29(3), 377–384. https://doi.org/10.1111/1365-2435.12347
- Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., & Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology, 46, 225–340. https://doi.org/10.1016/S0065-2881(03)46005-7
- Martin-Creuzburg, D., Wacker, A., & Basen, T. (2008). Interactions between limiting nutrients: Consequences for somatic and population growth of Daphnia magna. Limnology and Oceanography, 53(2), 436–444. https://doi.org/10.4319/lo.2008.53.2.0436
- Müller-Navarra, D.C., Brett, M.T., Liston, A.M., & Goldman, C.R. (2000). A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature, 403, 74–77. https://doi.org/10.1038/47469
- Schälicke, S., Martin-Creuzburg, D., & Wacker, A. (2019). Influence of dietary polyunsaturated fatty acids on fitness and body elemental composition of Daphnia galeata. Limnology and Oceanography, 64(4), 1516–1526. https://doi.org/10.1002/lno.11133
- Sih, A., Englund, G., & Wooster, D. (1998). Emergent impacts of multiple predators on prey. Trends in Ecology & Evolution, 13(9), 350–355. https://doi.org/10.1016/S0169-5347(98)01437-2
- Strayer, D.L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: recent progress and future challenges. Journal of the North American Benthological Society, 29(1), 344–358. https://doi.org/10.1899/08-171.1
- Vander Zanden, M.J., & Rasmussen, J.B. (2001). Variation in δ 15N and δ 13C trophic fractionation: Implications for aquatic food web studies. Limnology and Oceanography, 46(8), 2061–2066. https://doi.org/10.4319/lo.2001.46.8.2061

CENAKVA Research program



Supervisor: Assoc. Prof. Andrea Vojs Staňová

Contact

E-mail: vojsstanova@frov.jcu.cz

Tel.: +420 387 774 752

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

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

DSP: Ochrana vodních ekosystémů / Protection of aquatic ecosystems

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.

References

Svítková, V., Nemčeková, K., Drdanová, A.P., Imreová, Z., Tulipánová, A., Homola, T., Zažímal, F., Debnárová, S., Stýskalík, A., Ryba, J., Bača, Ľ., Šimunková, M. M., Gál, M., Mackuľak, T. and Vojs Staňová, A. (2024). "Advancing wastewater treatment: The efficacy of carbon-based electrochemical platforms in removal of pharmaceuticals." Chemical Engineering Journal 500: 156946.

Imreová, Z., Vojs Staňová, A., Zažímal, F., Debnárová, S., Vrána, L., Petrovičová, N., Tulipánová, A., Lukáč, T., Végh, D., Stýskalík, A., Mackuľak, T. and Homola, T. (2024). "Low-cost carbon-based sorbents for the removal of pharmaceuticals from wastewaters." Journal of Water Process Engineering 61: 105181.

Kuchtová, G., Hojová, L., Vojs Staňová, A., Marton, M., Vrška, M., Behúl, M., Michniak, P., Vojs and M., Dušek, L. (2023). "The influence of micro-/macro-structure of a boron-doped diamond electrode on the degradation of azo dye Direct Red 80." Electrochimica Acta 464: 142924.

Mordačíková, E., Vojs, M., Grabicová, K., Marton, M., Michniak, P., Řeháček, V., Bořík, A., Grabic, R., Bruncko, J., Mackuľak, T. and Vojs Staňová, A. (2020) " Influence of boron doped diamond electrodes properties on the elimination of selected pharmaceuticals from wastewater." Journal of Electroanalytical Chemistry 862: 114007.

CENAKVA Research program

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



Supervisor: Prof. Vladimír Žlábek

Contact

E-mail: <u>vzlabek@frov.jcu.cz</u> Tel.: +420 777 698 427

Bioaccumulation dynamics of emerging contaminants in aquatic invertebrates

Bioakumulační dynamika emergentních kontaminantů ve vodních bezobratlých organismech

DSP: Ochrana vodních ekosystémů / Protection of aquatic ecosystems

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.

References

Van Nguyen, T., Bořík, A., Sims, J.L., Kouba, A., Žlábek, V., Koubová, A., 2023. Toxicological effects of diclofenac on signal crayfish (Pacifastacus leniusculus) as related to weakly acidic and basic water pH. Aquatic Toxicology, 265: 106777.

Van Nguyen, T., Bořík, A., Velíšek, J., Kouba, A., Žlábek, V., Koubová, A., 2022. Integrated biomarker response in signal crayfish Pacifastacus leniusculus exposed to diphenhydramine. Chemosphere 308, 136382

Koubová, A., Kouba, A., Sims, J., Bořík, A., Brooks, B.W., Žlábek, V., 2024. Bioaccumulation dynamics of ionizable pharmaceuticals in freshwater crayfish. SETAC Europe 34th Annual Meeting, Seville, Spain, May 5-9, 2024.

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 Research program

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



Supervisor: Assoc. Prof. Miloš Buřič

Contact

E-mail: buric@frov.jcu.cz
Tel.: +420 389 034 769

Crayfish behaviour and mother-offspring relationships in crayfish

Chování v souvislosti s mateřskou péčí u raků

DSP: Ochrana vodních ekosystémů / Protection of aquatic ecosystems

Annotation

Crustaceans exhibit extensive parental care, including egg grooming, pleopod fanning, living in burrows with their young and/or moving them to safety. Crayfish exhibit the most complex maternal behaviour among crustaceans. After egg release, females exhibit diverse behaviours, including reduced activity, feeding inhibition, increased aggression and displaying specific postures, as well as using brood pheromones to attract and accept juveniles foraging in the immediate surroundings when danger arises. As with other taxa (e.g. mammals and birds), maternal care is affected by environmental conditions and interactions with conspecifics or individuals of other species, which has detrimental effects on the development of crayfish progeny, resulting in reduced growth and altered behaviour.

Brood parental care is common and necessary among crayfish species, with some variations having been studied in all four crayfish families. Species usually differ in the attachment and sheltering phases. In the families Astacidae and Cambaroididae, the attachment period covers the first postembryonic stage, while in the families Cambaridae and Parastacidae it also covers the second postembryonic stage. The sheltering phase includes the second-stage juveniles in the families Astacidae and Cambaroididae, and the third-stage juveniles in the families Cambaridae and Parastacidae. During this phase, juveniles occasionally leave the female to forage and explore the environment. In unfavourable conditions, this phase can be prolonged. For instance, during dry periods, the young of the red swamp crayfish (*Procambarus clarkii*) may remain with the mother for up to three months, reaching stage 7 in the burrow. Similarly, the development of marbled crayfish (*Procambarus virginalis*) juveniles slows until drought conditions or other stressors occur, such as food deprivation or competition. The mother can also prolong the sheltering phase several times and accept juveniles even after her post-reproductive moult.

These scenarios are possible due to mother-offspring recognition and the use of specific pheromones, among other factors. However, the effectiveness of their use is also limited by various factors, e.g. hierarchy, pollution or a simple decrease in pH, which can limit the perception of chemical information.

The main hypothesis

- The interactions between mothers and their offspring are affected by chemical, physical and ecological conditions during the sheltering phase.
- The acceptance of juveniles and further parental care can be disrupted by different stress stimuli, even inter-specifically.



Temperature, chemistry, water level, water flow and intra- and interspecific interactions can affect communication among individuals, including mother—offspring relationships.

Aim(s) of the Ph.D. thesis

- To explore the specifics of mother-offspring interactions in different circumstances.
- To identify the consequences of certain types of disturbances to parental care.
- To study crayfish behaviour in experimental and field conditions.

Possible approaches to reach the aims / to verify the hypotheses

- Preparing experimental setups to answer the major research questions and test the hypotheses.
- Exploring crayfish behaviour using video recording under different conditions and comparing with observations from field conditions.
- Application of ethological software to evaluate data.
- Application of statistical data analysis methods.

References

Ameyaw-Akumfi CE. Some aspects of breeding biology of crayfish. University of Michigan; 1976.

Figler MH, Twum M, Finkelstein JE, Peeke HVS, Twum M. Maternal Aggression in Red Swamp Crayfish (Procambarus Clarkii, Girard): The Relation Between Reproductive Status and Outcome of Aggressive Encounters with Male and Female Conspecifics. Behaviour. 1995;132(1–2):107–25.

Figler MH, Blank GS, Peeke HVS. Shelter competition between resident male red swamp crayfish Procambarus clarkii (Girard) and conspecific intruders varying by sex and reproductive status. Mar Freshw Behav Physiol. 2005;38(4):237–48.

Gherardi F. Behaviour. In: Biology of freshwater crayfish. Oxford blacwell science; 2002. p. 258–90.

Guo W, Kubec J, Veselý L, Hossain MS, Buřič M, McClain R, et al. High air humidity is sufficient for successful egg incubation and early post-embryonic development in the marbled crayfish (Procambarus virginalis). Freshw Biol. 2019;64(9):1603–12.

Kaur D., Das K., Kubec J., Buřič, M. Stress conditions extend maternal care and delay juvenile development in crayfish. Curr Zool. 2025; 71(1): 25–32.

Kawai T, Kouba A. Postembryonic development in freshwater crayfish (Decapoda: Astacidea) in an evolutionary context. Vol. 30, Nauplius. 2022. 1–46 p.

Klug H, Bonsall MB. What are the benefits of parental care? The importance of parental effects on developmental rate. Ecol Evol. 2014;4(12):2330–51.

Kubec J, Kouba A, Buřič M. Communication, behaviour, and decision making in crayfish: A review. Zool Anz. 2019;278:28–37.

Little EE. Chemical communication in maternal behaviour of crayfish. Nature. 1975;255(5507):400-1.

Mathews LM. Mother-offspring recognition and kin-preferential behaviour in the crayfish Orconectes limosus. Behaviour. 2011;148(1):71–87.

Pandian TJ. Reproductive Biology of Invertebrates: Asexual Propagation and Reproductive Strategies. John Wiley and Sons Inc, Chechester; 1994. 39–166 p.

Patoka J, Kalous L, Bartoš L. Early ontogeny social deprivation modifies future agonistic behaviour in



crayfish. Sci Rep. 2019;9(1):1-5.

Vogt G. Abbreviation of larval development and extension of brood care as key features of the evolution of freshwater Decapoda. Biol Rev. 2013;88(1):81-116.

Vogt G, Tolley L, Scholtz G. Life stages and reproductive components of the marmorkrebs (marbled crayfish), the first parthenogenetic decapod Crustacean. J Morphol. 2004;261(3):286–311.

Funding

Týmové GAJU, Interreg REWMA.