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# Ph.D. thesis topics 2025/2026

## 2nd call

### DSP Rybářství / Fishery

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

##### Vodňany..... 2

###### **Supervisor: Olga Bondarenko, Ph.D. .... 3**

Evaluation of Risks from Environmentally Prevalent Antibiotics and Antimycotics Targeting Fish Sperm Ion Channels:  
Impacts on Motility Activation and Fertilization Success..... 3

###### **Supervisor: Serhii Boryshpolets, Ph.D. .... 5**

Study of Sperm Physiology and Motility in Bitterlings in the Context of Reproductive Strategy: Comparison with Zebrafish  
and Carp ..... 5

###### **Supervisor: Assoc. Prof. Borys Dzyuba ..... 7**

Sperm cryopreservation strategies for small-bodied fishes: optimising slow freezing and vitrification for practical  
application ..... 7

###### **Supervisor: Evgenia Gazo, Ph.D. .... 9**

Long-term consequences of early life DNA damage in fish embryo ..... 9

##### Hydrobiologický ústav BC AV ČR / Institute of Hydrobiology CAS České Budějovice..... 11

###### **Supervisor: Prof. Jan Kubečka..... 12**

Population dynamics of fish in reservoirs ..... 12

###### **Supervisor: Assoc. Prof. Radka Symonová..... 14**

Molecular background of the developmental switch from planktivory to piscivory in pikeperch brain ..... 14



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





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### Evaluation of Risks from Environmentally Prevalent Antibiotics and Antimycotics Targeting Fish Sperm Ion Channels: Impacts on Motility Activation and Fertilization Success

Hodnocení rizik environmentálně prevalentních antibiotik a antimykotik zaměřených na iontové kanály rybího spermatu: Dopady na aktivaci pohyblivosti a úspěšnost oplodnění

### DSP: Rybářství / Fishery

#### Annotation

Fish reproduction plays a vital role in maintaining the balance of aquatic ecosystems and supporting the commercial success of aquaculture. A key factor in successful fertilization is the motility of spermatozoa, which depends significantly on the proper functioning of ion channels and pH regulation. For species that reproduce externally, such as many freshwater fish, sperm motility is activated upon water exposure, making it vulnerable to disruption by environmental pollutants, particularly pharmaceuticals. Antibiotics and antimycotics, which are known to inhibit potassium ( $K^+$ ), calcium ( $Ca^{2+}$ ), and hydrogen ( $H^+$ ) channels, pose considerable threats to sperm function (Espinosa et al., 2000; Brenker et al., 2018; Lahnsteiner et al., 2005; Sánchez-Tusie et al., 2014). Specific drugs like neomycin and erythromycin affect potassium channels, oxytetracycline impacts calcium channels, and miconazole interferes with hydrogen channels, all leading to reduced sperm motility and lower fertilization success (Kümmerer, 2009).

While the effects of these pharmaceuticals on mammalian sperm are well-documented, their impact on fish sperm is less understood, presenting a crucial gap in ecological risk assessments and the development of mitigation strategies. These substances are commonly found in aquatic environments, including wastewater and global river systems, indicating potential long-term reproductive risks to fish populations.

This topic provides a comprehensive framework for exploring the intersection of environmental pollution, reproductive biology, and ecological risk, establishing a basis for future research and remediation strategies in aquatic systems.

#### The main hypothesis

- **Pharmaceutical Impact Hypothesis:** Antibiotics and antimycotics prevalent in aquatic environments adversely affect the fish sperm motility.
- **Ion Channel-Targeting Hypothesis:** Different classes of pharmaceuticals selectively inhibit potassium ( $K^+$ ), calcium ( $Ca^{2+}$ ), and hydrogen ( $H^+$ ) channels in fish sperm, resulting in distinct patterns of decreased motility and fertilization success
- **Species-Specific Sensitivity Hypothesis:** Sensitivity to pharmaceuticals varies by species due to differences in ion channel expression and function in various freshwater fishes.

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

- Characterize the effects of these pharmaceuticals on sperm motility and fertilization success.



- Conduct a systematic assessment of how pharmaceuticals interact with ion channels in fish gametes.
- Evaluate the spermatozoa sensitivity of various fish species to these pharmaceuticals to identify those at greatest risk.

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

The study will be conducted on model animals such as zebrafish and aquaculture-valuable species, such as carp, trout, sturgeon, burbot, perch, and pike perch, depending on their availability. Antibiotics and antimycotics will be chosen based on their documented effects on ion channels (K<sup>+</sup>, Ca<sup>2+</sup> and H<sup>+</sup>) and their frequent detection in wastewater and surface waters, ensuring the study's environmental relevance and scientific significance. The methodological approach is proposed, but not limited by the following steps:

- **Characterization of Pharmaceutical Effects:** To assess the effects of the studied pharmaceuticals on fish spermatozoa performance, Computer-Assisted Sperm Analysis (CASA) will be employed. Additionally, fertilization assays will be conducted in the presence of chosen pharmaceuticals to determine their effect on reproductive success, focusing on changes in fertilization rates.
- **Ion Channel-Targeting Hypothesis:** The effect of selected antibiotics and antimycotics on potassium (K<sup>+</sup>), calcium (Ca<sup>2+</sup>), and hydrogen (H<sup>+</sup>) ion channels in male gametes of different freshwater fish species will be studied using the patch clamp technique. This method will allow precise measurement of ionic currents and provide an understanding of how these pharmaceuticals selectively inhibit ion channels.
- **Species-Specific Sensitivity:** To evaluate species-specific sensitivity to different pharmaceuticals, data obtained from the previous steps will be compared. This comparison will help predict whether other groups of fish with similar spermatozoa activation mechanisms may be similarly affected by the studied antibiotics and antimycotics.

This comprehensive methodological approach will enable the investigation of pharmaceutical effects on ion channels, sperm motility, and reproductive success across multiple fish species, providing insights into species-specific vulnerabilities and ecological risks.

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### **CENAKVA Research program**

RP1 Reproductive and genetic procedures for fish biodiversity conservation and aquaculture





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### Study of Sperm Physiology and Motility in Bitterlings in the Context of Reproductive Strategy: Comparison with Zebrafish and Carp

Studium fyziologie a motility spermií u hořavek v kontextu reprodukční strategie: srovnání s daniem pruhovaným a kaprem

**DSP: Rybářství / Fishery**

### Annotation

Fertilization is the process by which male and female gametes fuse to form an embryo. To reach the egg, spermatozoa must navigate the fertilization environment, guided in some cases by chemotactic cues derived from female reproductive fluids or eggs. The spermatozoon function, including activation, motility, longevity and navigation, is thus linked to the properties of the surrounding environment (Kholodnyy, 2019).

Upon release into the water, spermatozoa are activated by changes in osmotic pressure and ion composition. Osmotic stress regulation is essential for sperm survival in harsh freshwater conditions, and it is mediated by the sperm cell membrane (Herrera et al., 2021). In most species, osmotic differences between the testicular and external environment are responsible for both spermatozoa activation and their damage, restricting the duration of motility. Specific ions may also influence the activation and motility parameters (Alavi & Cosson, 2006).

Sperm production and maintenance are energetically costly (Macartney et al., 2019), and sperm depletion is a common feature of many mating systems, therefore males must allocate sperm strategically to maximize their reproductive success (Turnell et al., 2018). Sperm quantity and quality are directly linked to individual conditions and reproductive strategies.

Thus, reproduction conditions and strategies of small-bodied fishes, such as cyprinid Bitterlings and Zebra fish, may significantly differ from large fishes of the same family as carp. In particular, bitterlings have a unique reproduction when females use a long ovipositor to deposit eggs into the mussel gills through the exhalant siphon, while males ejaculate over the mussel inhalant siphon; the water current carries sperm through the gill cavity, where it must traverse the gill membrane to reach and fertilize the eggs. This highly specialized fertilization environment imposes unique functional challenges on sperm morphology, motility, and endurance, which remain poorly understood and warrant detailed investigation.

### The main hypothesis

- We hypothesize that the unique fertilization environment has led to specific adaptations in sperm physiology in Bitterlings, including activation mechanisms, motility regulation, and motility duration. These adaptations are likely to differ between small-bodied and large-bodied cyprinid species.



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

- To characterize the motility patterns and activation mechanisms of bitterling spermatozoa under varying osmotic and ionic conditions simulating their natural fertilization environment.
- To compare sperm motility parameters (e.g., velocity, motility duration, activation threshold) between bitterlings and other cyprinid species, such as zebrafish or carp.
- To investigate structural and functional adaptations in bitterling sperm morphology (e.g., head and flagellum structure) that facilitate navigation through the mussel's gill cavity.
- To assess how reproductive strategies, body size, and sperm physiology affect fertilization success in different cyprinid fishes.

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

- Evaluate the effects of environmental parameters (e.g., osmolality, pH, ion concentration, ovarian fluid, and water flow) on sperm physiology and motility in bitterlings (zebrafish or carp, if needed).
- Analyze sperm morphology, motility patterns, and flagellar wave propagation in bitterlings using electron microscopy, light microscopy, and advanced image analysis techniques.
- Compare these findings to corresponding data from closely related externally fertilizing cyprinids (e.g., zebrafish or carp).

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### **CENAKVA Research program**

RP1 Reproductive and genetic procedures for fish biodiversity conservation and aquaculture



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### Sperm cryopreservation strategies for small-bodied fishes: optimising slow freezing and vitrification for practical application

**Strategie kryokonzervace spermií u malých ryb: optimalizace pomalého zmrazování a vitrifikace pro praktické využití**

### DSP: Rybářství / Fishery

#### Annotation

This project focuses on developing effective sperm cryopreservation techniques tailored to small-bodied fish species that produce very low volumes of sperm, a group often underrepresented in reproductive biotechnology research. These species include many freshwater ornamental fishes and rare or endangered taxa, for which gamete availability is limited and precise handling is essential.

A central challenge lies in optimising cryopreservation protocols that work with minimal sample volumes while maintaining post-thaw viability and fertilisation capacity. This project, therefore, explores two key strategies:

- Low-volume slow freezing, which allows controlled cryoprotectant exposure and gradual cooling of microliter-scale samples—suitable for species yielding only a few microliters of milt per male.
- Whole-sample vitrification, an ultra-rapid method that avoids ice crystal formation, minimising structural damage—especially relevant for sensitive or low-sperm-output species.

By evaluating the biological outcomes of these methods (motility, membrane integrity, fertility, etc.) across model and non-model species, the project aims to identify scalable, species-adaptable cryopreservation protocols.

This work will contribute to the development of practical cryopreservation platforms that are scientifically robust and feasible for use in the field or in small-scale hatcheries, potentially benefiting biodiversity conservation and the sustainable ornamental fish trade.

The study will be conducted on taxonomically diverse aquarium species and selected small-bodied fish of the Czech fauna.

#### The main hypothesis

- Small volumes of sperm from small-bodied fish species can be effectively cryopreserved using either low-volume slow freezing or whole-sample vitrification; however, the efficiency of these methods is taxon-specific, necessitating the development of species-adapted protocols to ensure practical applicability in biodiversity conservation and ornamental aquaculture.

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

- To develop and optimise cryopreservation techniques suitable for microliter-scale sperm samples from small-bodied fish species by comparing low-volume slow freezing and whole-sample vitrification in terms of feasibility, technical requirements, and biological outcomes.



- To evaluate the post-thaw quality of sperm cryopreserved by different methods by assessing key sperm parameters such as motility, membrane integrity, mitochondrial activity, DNA integrity, and fertilisation success.
- Ensure protocols are low-cost, user-friendly, and compatible with conservation and ornamental aquaculture practices.

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

- Performing small volume sperm slow freezing and whole sample vitrification by different approaches.
- Assess key sperm parameters such as motility, cytoplasmic membrane integrity, mitochondrial status, DNA integrity, and fertilisation success.
- Applications of statistical data analysis (GLM, ANOVA, ANCOVA, PCA, Pearson correlations).

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#### **CENAKVA Research program**

RP1 Reproductive and genetic procedures for fish biodiversity conservation and aquaculture





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### Long-term consequences of early life DNA damage in fish embryo

Dlouhodobé důsledky poškození DNA v raných stádiích vývoje rybního embrya

### DSP: Rybnářství / Fishery

#### Annotation

Proper embryo development is a base for survival and successful reproduction at later stages. However, numerous factors, such as chemical pollution or UV irradiation, may influence embryos of externally fertilizing species, such as most of the fish (Yi et al., 2024). DNA damage or genotoxicity is a mode-of-action of many environmental pollutants which can negatively affect organogenesis (Dey et al., 2023). The negative effects of genotoxic exposure at early developmental stage may impact individuals' survival and reproduction capacity in future.

The PhD study will test a hypothesis that DNA damage at an early developmental stage will be reflected in abnormal neuro- and gonadal development at later stages. Thus, water pollution with genotoxic compounds will manifest in reduced performance and reproduction success of fish even at doses which do not induce mortality. This study will combine omics and toxicological approach to explore the negative effects of DNA damage on fish embryo development. Several water pollutants commonly found in European rivers will be tested, such as bisphenols (BPA, BPS, BPF), terbutylazine, chlorpyrifos, and lindane.

Previous studies on gene knock-down and knock-out showed that multiple DNA damage response (DDR) genes are involved in fish embryo development (Dey et al., 2023; Shin et al., 2021). This study will help us to understand how neurodevelopment and gametogenesis could be affected by early-life exposure to environmental contaminants mimicking the situation typically occurring in polluted rivers throughout the world.

We will use two fish species to test the different aspects of DNA damage response in embryo. Specifically, zebrafish (*Danio rerio*) will be used as a model species for standardized embryo toxicity test. Further, the results of our studies will be tested on sterlet (*Acipenser ruthenus*) as endangered species.

#### The main hypothesis

- DNA damage at an early developmental stage will be reflected in abnormal neuro- and gonadal development at later stages
- Early-life stress can sensitize fish embryos to different stressors later in life
- Water pollutants at sublethal concentrations can affect developing fish embryos leading to population decline

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

- To investigate how exposure to genotoxic pollutants at an early developmental stage may affect fish embryo development and whether the phenotype is really related to DNA damage response



- To investigate how exposure to genotoxic pollutants at an early developmental stage may affect fish embryo development and whether the phenotype is really related to DNA damage response

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

- We will investigate the link between genotoxicity and abnormal development in embryos of two fish species. We will use two model genotoxicants and several water pollutants commonly found in European rivers.
- First, we will expose zebrafish embryo at an early stage to genotoxicants. Following the exposures, we will run the comet assay on the embryos to find out doses that induce DNA damage and what type of damage they cause. Further, we will analyze gene expression with RT-qPCR to find genes involved in DNA damage response (Dey et al., 2024).
- In the following tests, we will expose embryos to selected concentrations and let them develop normally up to larval stage. We will perform histological analyses, analyze gene expression and protein profile.
- We will knockdown or overexpress DNA repair genes involved in stress response to test their role in development.
- Finally, we will expose sterlet embryos at early stages of development to the same chemicals. Doses of chemicals will be adjusted for sturgeons as they are more sensitive to water pollution. Comet assay and RT-qPCR will be performed on exposed embryos.
- We will analyze gene expression and protein profiles of hatched larvae. By comparing them to zebrafish we should be able to find universal biomarkers of early-life stress.

### **References**

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### Population dynamics of fish in reservoirs

#### Populační dynamika ryb v nádržích

#### DSP: Rybnářství / Fishery

#### Annotation

Population dynamics of fish in lakes and reservoirs has been studied for decades but is still poorly understood. Recent advances in quantitative sampling promise the improvement thanks to extensive standardized sampling programmes throughout the Europe. Within long-termed monitoring of Czech reservoirs, several decades of quantitative fish data containing the year class strength, survival, growth, and biomass of fish had been collected. New information will come in connection with forthcoming surveys. Using these long-termed sampling campaigns data, the candidate is assumed to create and run mathematical models of the population dynamics of the most important species in model reservoirs. In further steps, the outputs of the models will be confronted to extensive biotic and abiotic background datasets in order to track the main drivers of population changes in fish populations. The topic requires advanced mathematical and modelling skills and deep interest in fish biology and dynamics. Bioenergetic modelling and thinking connected with population dynamics (like the estimation of food consumption of different species) would be also desirable.

#### The main hypothesis

- Quantitative matrix of fish survival and growth increments can be achieved from the combination of Young-Of-the-Year fish sampling methods (fry seining and trawling) and older fish sampling (multimesh gillnets).
- The size structure of the catches of European standard multimesh gillnets reflects well the year class strength of fish.
- The first winter of the life is an important bottleneck deciding the success of individual fish species.
- Finite number of biotic and abiotic predictors determine the natural recruitment success of important fish species.

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

- To create fish abundance/survival/length matrix of cyprinid/percid fish using age readings from otoliths/scales, catch-per-unit-of-effort (CPUE) and length-frequency distribution of multimesh gillnets.
- To find the connection between year class strength determined by Young-Of-the-Year fish sampling methods and by the above analysis of multimesh gillnets catch.
- Using this relationship to determine the first winter survival of cyprinid/percid fish.
- To establish the relationships between Young-Of-the-Year fish abundance and biotic and abiotic predictors during spawning and early ontogeny.



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

- Most fish data for the thesis are already collected and ready to use in databases. However, the information for the current years of the running thesis will be collected in new surveys (Kubečka et al. 2022) and the candidate will take active part in new data collection.
- Analysis of abundance/survival/length matrix will require multiple year analysis of fish length frequency distributions and identification of individual year classes using age-length keys. Both length frequency and age-length keys are available from IHB BC CAS database. It should be possible to track every year class through its lifetime. See the examples at: [https://lipnosimulation.shinyapps.io/fish\\_dynamics\\_simulation/](https://lipnosimulation.shinyapps.io/fish_dynamics_simulation/)
- Young-Of-the-Year fish community abundance is being determined by direct sampling methods (fry seining and trawling) while multimesh gillnets provide only relative abundance and biomass (CPUE/BPUE). The approach of Říha et al., 2023 can be used for putting the two datasets together. This would facilitate tracking the survival over the first winter and throughout the further ontogeny.
- Quantitative data of Young-Of-the-Year fish community available from IHB BC CAS database should be correlated with biotic and abiotic predictors collected for Římov and Lipno reservoirs using common multiple factors regression approaches (GLM, GAMM, Bayesian approaches etc. Tesfaye et al. 2024).

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### CENAKVA Research program

RP4 Freshwater ecosystems in the era of global change





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### Molecular background of the developmental switch from planktivory to piscivory in pikeperch brain

Molekulární mechanismy přechodu k dravému způsobu života v mozku mladých candátů

### DSP: Rybářství / Fishery

#### Annotation

The switch from planktivorous foraging to piscivory is one of the most crucial phases in the pikeperch ontogeny since it determines its chances on surviving the first winter. It is a dynamic phase involving already well-known eco-morphologies like shifting pikeperch niche and acceleration of its growth and of the overall development. While ecological aspects of this switch have been investigated for decades the molecular backgrounds remain almost unknown.

There are several possibilities how to utilize already available transcriptomic (RNA-seq) data of two pikeperch generations from the water reservoir Lipno (Czechia). Although the first two RNA-seq batches were performed with the standard poly-A enrichment approach selecting particularly mRNA transcripts, several potentially important non-coding RNA (ncRNA) species have been identified. Hence, further RNA-seq foreseen will be performed via ribodepletion to catch more ncRNAs and active transposable elements beside mRNA transcripts. Moreover, a special attention will be paid to other ncRNA species, including microRNAs, by further modifications of the currently used protocol.

#### The main hypothesis

- Is the switch of planktivores towards piscivory driven by brain transcription of orexigenic neuropeptides?
- Are non-coding RNA species and transposable elements involved in the regulation of the switch towards piscivory?
- Is there any genetic background of the accelerated switch towards piscivory?
- Are the early piscivores of the same age as the late ones?

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

- Characterization of molecular traits accompanying and following the switch to piscivory
- Reconstruction of regulatory networks of epigenetic mechanisms governing the gene transcription (i.e. interplay between gene transcription, alternative splicing, and activity of ncRNAs and transposons)
- Genetic and ecological exploration the co-existing distinct size classes in the young-of-the-year pikeperch
- Establishing machine learning approach in search for patterns in alternative splicing data (optional)

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

- Field work on the Lipno reservoir – sampling pikeperch brains for RNA isolation and RNA-FISH
- Eco-morphological study further exploring the developmental plasticity of young pikeperch



- Laboratory work – RNA isolation, ribodepletion, sequencing library preparation
- Brain histology and RNA-FISH to brain tissue
- Bioinformatics – transcriptomic data processing, gene annotation, functional annotation of gene networks, differential gene transcription analysis, alternative splicing analysis, assessment of transcriptional activity of transposable elements, identification of molecular sex marker for juvenile pikeperch utilizing BioProject PRJNA561467 data (de los Ríos-Pérez et al., 2020), integration of all results.

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