



Ph.D. thesis topics 2023/2024

DSP Rybnářství / Fishery

Supervisor: M.Sc. Olga Bondarenko, Ph.D.

- Mechanisms of potassium signaling in fish spermatozoa motility / Signalizace draselnými ionty při motilitě spermií u sladkovodních ryb

Supervisor: M.Sc. Serhii Boryshpolets, Ph.D.

- Effect of viscosity on fish sperm motility / Vliv viskozity na pohyblivost spermií ryb
- Short-term storage of freshwater fish spermatozoa: improvement and application / Krátkodobé uchování spermatu sladkovodních druhů ryb: vývoj a aplikace

Supervisor: doc. MSc. Borys Dzyuba, Ph.D.

- Sperm aging in relation to cryoresistance in fishes / Stárnutí spermií ve vztahu ke kryorezistenci u ryb

Supervisor: prof. Ing. Martin Flajšhans, Dr. rer. agr.

- Mosaicism in sturgeons / Mosaicismus u jeseterů

Supervisor: prof. Ing. Otomar Linhart, DrSc.

- DNA methylation in heterogenous populations of fish spermatozoa after their aging *in vitro* / Methylace DNA v heterogenních populacích rybích spermií po jejich stárnutí *in vitro*

Supervisor: doc. Ing. Jan Mráz, Ph.D.

- Development of new fish products / Vývoj nových rybích výrobků

Supervisor: MVDr. Veronika Piačková, Ph.D.

- Factors affecting fish immunity in relation to infectious diseases / Faktory ovlivňující imunitu ryb v souvislosti s infekčními chorobami

Supervisor: doc. Ing. Martin Pšenička, Ph.D.

- Effect of light on early embryo development in fish/ Vliv světla na raný vývoj embrya u ryb

Supervisor: Ing. Vlastimil Stejskal, Ph.D.

- Using of novel strategies to improve nanovaccine effectiveness in salmonids and percids / Využití inovativních strategií ke zvýšení účinnosti nanovakcín u lososovitých a okounovitých ryb

Supervisor: Mgr. Otakar Strunecký, Ph.D.

- Composition of the microbiome in intensive aquaculture systems; monitoring and assembly of microbial consortium for pre-inoculation based on beneficial microorganisms / Složení mikrobiomu v intenzivní akvakultuře: jeho analýza a příprava konsorcia prospěšných mikroorganismů pro inokulaci

Supervisor: doc. Mgr. Radka Symonová, Ph.D.

- (Cyto)genomics in biodiversity assessment and conservation of fish / Využití (cyto)genomiky pro stanovení a ochranu biodiverzity u ryb



Supervisor: M.Sc. Olga Bondarenko, Ph.D.

Contact

E-mail: obodarenko@frov.jcu.cz

Phone number: +420 387 774 607

Mechanisms of potassium signaling in fish spermatozoa motility / Signalizace draselnými ionty při motilitě spermií u sladkovodních ryb

Annotation

Ion exchange in general, and K⁺ efflux specifically, is involved in regulation of such a crucial part of the fertilization process as spermatozoa motility. In fishes, spermatozoa are immotile in testis and seminal fluid, reaching motility by contact with water during spawning due to abrupt changes in the surrounding ion concentration (K⁺, Na⁺, Ca²⁺, Mg²⁺, Cl⁻), pH and osmolality. In freshwater fishes, spermatozoa activation is species specific: for species like sturgeons and trouts, decrease of environmental K⁺ was shown to be the first trigger of motility initiation, whereas for other species, like some cyprinids, spermatozoa can be activated in presence of high potassium. However, the mechanism of K⁺ exchange and its regulation of sperm motility remains largely uncharted in general in fishes and freshwater fish species. Thus, the main goal of present study is to identify some K⁺ channels in spermatozoa of taxonomically distant fish species (like cyprinid, sturgeons and salmonid) and their role in regulation of motility.

Specifically, the study is aimed to:

- Identify the presence and types of some K⁺ channels expressed in spermatozoa membrane of cyprinids (carp, zebra fish), salmonids (rainbow trout) and sturgeons (sterlet) by PCR and Western blotting methodology,
- Obtain the full-length gene sequence of identified K⁺ channel. Heterologous expression of the detected channel in model cells (HEK, CHO) will be performed. Model cells will be used for scrutinizing of K⁺ transport via target channel by using fluorescent microscopy method.
- Apply obtained knowledge from model to spermatogenic and sperm cells to determine the role of target K⁺ channel in spermatozoa physiology of different species.

This study will result in clarification of fundamental mechanisms of spermatozoa activation and motility. Since taxonomically distant species will be under study, we expect to obtain species specific differences in levels of K⁺ channels expression and their involvement in motility regulation, highlighting aspects of reproductive system evolution in fishes.



Fakulta rybnářství
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: M.Sc. Serhii Boryshpolets, Ph.D.

Contact

E-mail: sboryshpolets@frov.jcu.cz

Phone number: +420 387 774 615

Effect of viscosity on fish sperm motility / Vliv viskozity na pohyblivost spermií ryb

Annotation

Gametes' functioning is essential for reproduction success, especially in species with external fertilization when spermatozoa should reach the egg for a pretty short time. Thus the properties of the environment play a crucial role in this process. The viscosity of the environment is one of these essential environmental factors likely contributing to spermatozoa motility regulation in many species. The main objective of the prospective project is to understand and evaluate the effect of environmental viscosity on the performance of spermatozoa and their progression efficiency. In this project, the effect of different viscosity on spermatozoa behavior will be tested in combination with other external factors as osmolarity, ion composition, temperature, etc. The obtained results will be compared with specific effects of ovarian fluid in selected model freshwater species: carp, trout, sterlet. Particular attention will be devoted to the ancient Elasmobranchii species (sharks; skates; and freshwater rays), spermatozoa of which have relatively large size and specific spiral shape, essential for the interaction with the viscous environment. The study will involve spermatological approaches focused on visualization and analyses of moving spermatozoa and flagellar: phase-contrast, high-speed, fluorescent microscopy; image analysis and processing, CASA; basic programming and statistical approaches; fertilization test, etc.

Short-term storage of freshwater fish spermatozoa: improvement and application / Krátkodobé uchování spermatu sladkovodních druhů ryb: vývoj a aplikace

Annotation

Short-term storage is a conventional procedure used during the artificial reproduction of many domestic animals, including fish. This procedure is quite challenging for freshwater fish because of particular features of spermatozoa, such as activation of motility during contact with water and urine, short duration of motility, sensitivity to environmental factors (pH, ion composition, temperature, etc.). In addition, many species possess their unique properties and reaction to environmental signals, which requires the consideration of species specificity for developing the proper procedures. This study aims to elaborate new methods of short-term sperm storage and test the existing ones in several freshwater model species (carp, trout, sterlet, etc.). Many current methods use different media compositions to keep the cell immotile and prolong the storage time in other species. Nevertheless, the issue is still not solved completely. In particular, it is due to the fact that exact reasons for sperm quality decay are not clear. In this study, we will evaluate sperm quality parameters by different methods and combine them with varying storage conditions to improve storage outcomes and understand the reason for quality loss. We will also test natural antibacterial extracts, which should replace antibiotics during short-term storage and artificial reproduction in fisheries practice. This study will involve various approaches of sperm quality evaluation, including sperm motility, swelling rate, metabolic activity, DNA fragmentation, fertilization tests, etc.



Fakulta rybnářství
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: doc. MSc. Borys Dzyuba, Ph.D.

Contact

E-mail: bdzyuba@frov.jcu.cz

Phone number: +420 389 034 614

Sperm aging in relation to cryoresistance in fishes / Stárnutí spermií ve vztahu ke kryorezistenci u ryb

Annotation

Recent progress in applying fish sperm cryopreservation for aquaculture of salmonids indicates a good potential of this approach in breeding other fishes. The development of fish sperm cryobanking in our Faculty is associated with the historically leading position of RIFCH in the Czech Republic in applying this methodology in the National Programme on Conservation of Farm Animal Genetic Resources. However, the application of sperm cryopreservation in aquaculture is not yet implemented in the Czech Republic. That is because of two main reasons: 1) not proved effectiveness of the application of sperm cryopreservation protocols at the aquaculture level, and 2) the absence of easy, not-expensively realisable whole technological cycle of cryopreserved sperm use, which includes sperm collection, cryopreservation, storage, and application.

At the moment, the time between collection and cryopreservation is essential, and from practice, it is clear that it should be minimised as after several hours of storage spermatozoa decrease their cryoresistance. That complicates experimentation with sperm needed for methodology improvement and, more critically, the implementation of the whole cycle of cryopreserved sperm application at the aquaculture scale. Simply saying, precisely storage time between sperm collection and cryopreservation is the missing point for effective sperm cryobanking. It is impossible to realise easy sample transfer between sites of sperm collection and cryopreservation without solving this point.

The rapid decrease of sperm cryoresistance after its collection is associated with complex changes in sperm physiological and biochemical parameters, generically known as "sperm aging." These changes are described recently regarding physiological and biochemical sperm parameters. In turn, it is unclear if sperm aging can be overcome to preserve sperm cryoresistance.

This PhD study focuses on understanding sperm aging physiological mechanisms responsible for decreasing sperm cryoresistance. The proposed PhD study is oriented on investigating changes in physico-chemical properties of spermatozoon membrane (e.g., ability to maintain cellular volume, lipid content) and bioenergetical parameters occurring during sperm aging (in vitro storage) in relation to their cryoresistance. The study is based on previous studies performed and is associated with currently running projects in the Laboratory of physiology of reproduction of USB.



Fakulta rybnářství
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: prof. Ing. Martin Flajšhans, Dr. rer. agr.

Contact

E-mail: flajsh@frov.jcu.cz

Phone number: +420 389 034 608

Mosaicism in sturgeons / Mosaicismus u jeseterů

Annotation

Mosaicism in fish, i.e. presence of two (or more) cell lines with different karyotypes originating from a single zygote is relatively rarely described in teleosts, namely in hybrid complexes (e.g. in genus *Cobitis*). It is more frequently found in sturgeons originating from artificial reproduction, and it leads to abnormal cleavage of the zygote and to the death of abnormal morula, rather rarely to development of viable mosaic individual. Auto- or allopolyspermic fertilization is reported to be its reason (Igorova et al., 2018a,b), as well as mitotic failure during the early embryonic development (Fopp-Bayat et al., 2021, 2022) leading to $1n/2n$ or $2n/3n$ mosaicism. Polyploidization experiments appeared to be a proper technique to develop mosaicism in sturgeons. Using combination of polyploidization techniques that have been applied to study the effect of whole genome duplication on fitness of sturgeons in last years, we obtained a whole scale of $2n/3n$, $2n/4n$ or $2n/6n$ mosaics (Lebeda et al., 2020) and we managed to keep some of them yet alive. The task of the candidate will be to induce these mosaics again or to study the older mosaic individuals which survived to-date. The goal of the dissertation will be to describe yet not studied/published types of mosaicism, distribution of cell lines in various tissues (fin and blood cells as the most frequent sample types for ploidy analysis which, however in case of mosaics can lead to a wrong conclusion; if possible further in muscle, gills, kidney, liver and gonads), to determine their ploidy levels, to perform flow cytometric cell sorting into lines and to do microsatellite genotyping following selected markers in order to assess the origin of the cell lines. If possible, the candidate should also focus on proteomics of mosaic tissues, gonads and gametes of mosaic individuals and detection of sex-specific markers. The candidate will learn polyploidization techniques, flow cytometric approaches, cell sorting and molecular genetic and proteomic methodologies.



Fakulta rybnářství
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: prof. Ing. Otomar Linhart, DrSc.

Contact

E-mail: linhart@frov.jcu.cz

Phone number: +420 389 034 743

DNA methylation in heterogenous populations of fish spermatozoa after their aging *in vitro* / Methylace DNA v heterogenních populacích rybích spermií po jejich stárnutí *in vitro*

Annotation

This is the first study elaborating the genome variability in different fish sperm populations after *in vitro* aging, which can serve as a foundation for better understanding of sperm aging in vertebrates. Sperm DNA methylation patterns are important for the development of embryos health of offspring. The evaluation will be done at the levels of whole genome, gene transcription analysis, global methylation as well as the phenotype and functional changes in populations of fresh/young sperm compared to the populations of aged sperm. Student will focus on determination of the DNA methylation pattern during sperm aging in heterogeneous population of motile/non motile spermatozoa with and without a damaged cell membrane. The expression level of genes involved in the differential methylation of DNA will also be estimated, as well as transcripts associated with embryonic development in the middle blastula, depending on the quality of the aged sperm populations.

Aim of the study

The present study proposal will examine the phenotype and DNA changes associated with fish sperm aging with focus on DNA methylation variability in different sperm populations of common carp and zebrafish. The evaluation will be done at the levels of the whole genome, gene transcription analysis, global methylation as well as the phenotypic and functional changes in populations of fresh/young sperm compared to the populations of aged sperm.

Specific objectives

- Analysis of sperm phenotypic and functional changes associated with the heterogeneous populations of fish spermatozoa.
- Determination of the DNA methylation pattern during sperm aging in heterogeneous population depending on the proportion of motile spermatozoa.
- Determination of the level of DNA methylation in aged sperm without a damaged cell membrane.
- DNA methylation will be defined in fish embryos in which the most active old spermatozoa will be used for fertilization compared to the entire sperm population.



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

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

- The expression level of genes involved in the differential methylation of DNA will be estimated, as well as transcripts in embryonic middle blastula development, depending on the quality of the aged sperm populations.

Interational collaboration

Whole-genome bisulfite sequencing and data analysis will be performed in collaboration with INRAE, France (C. Labbe, J.Bob), their bioinformatics laboratory (A. Brionne) and Freie Universität Berlin, Germany (F. Schumacher) for genome evaluation.

Projects of Otomar Linhart

National Agency for Agriculture Research 2021-2025 (QK21010141), GAČR project submitted, 2023-2025 (23-6426S)



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: doc. Ing. Jan Mráz, Ph.D.

Contact

E-mail: jmraz@frov.jcu.cz

Phone number: +420 389 034 643

Development of new fish products / Vývoj nových rybích výrobků

Annotation

Fish is rich in proteins, vitamins, minerals, omega-3 fatty acids, and above all EPA and DHA, which are important for the healthy development of the brain and eyes. Unfortunately, the consumption of fish in the Czech Republic is only about 5 kg/year per inhabitant (ČSÚ, 2019). In the "Nutritional recommendations for the population of the Czech Republic" it is defined that each person should consume at least 400 g of fish per week, which corresponds to 20.8 kg/year. It is clear from the above that the inhabitants of the Czech Republic consume only a quarter of the recommended intake of fish.

The population in the Czech Republic has dietary preferences for consuming a large amount of fat, meat, sausages, fried foods and salt, and has a low intake of vegetables, fruit and fish. These eating habits lead to overweight, obesity and other health conditions. Despite public education about healthy eating habits, these stereotypes fail to change. A person creates eating habits in childhood, carries them into adulthood, and it is difficult to get rid of the wrong ones. Eating habits can be easily shaped in childhood because the child is open to exploring and accepting new habits. However, there are many factors that influence children's preferences, such as family preferences, neophobia, the appearance of food or prejudices about food. Knowing the preferences of preschool children and their eating habits is therefore essential when developing fish products for them. However, factors related to children's fish consumption preferences are not fully understood.

Currently, there are few studies on preschool children's preferences in relation to fish products. In our previous pilot project "Development of new fish products for preschool children", we successfully developed several fish products together with Tilapia s.r.o., which were very positively received by preschool children. These products are already on the market and the company supplies them to more than 200 schools and preschools. During the pilot project, we noticed that the children were very affected by neophobia (fear of new foods). Products that resembled some familiar meat products that children often eat at home (such as sausages, ham, hamburger, meatballs) were well accepted by children because they did not have a neophobic attitude towards them. We also noticed that children were greatly influenced by the way fish products were served and the attitude of teachers, etc. However, these factors need to be further clarified. The aim of this proposal is: To find out which factors influence whether children like to



accept fish products and to what extent. Based on this, develop and optimize fish products for children.

A specific feature of the Czech fish market is its seasonality, when most carp production is sold during the Christmas period. Despite the tradition of selling live fish, it is necessary to look for ways to supply customers with not only live or processed fish, but also fish products throughout the year. In 2017, processed fish in live weight accounted for only 11% of produced market fish in the Czech Republic. Currently, the majority of products from fish processing plants are delivered to the market in the form of carcasses, halves, fillets or portions. Basically, the only product of fish processing plants intended for direct consumption is smoked fish. One of the possible ways is to focus on the development of fish products that will be boneless, easy to prepare, without a distinct fishy smell and will use a secondary raw material from the processing of fillets - skeletons. Then there are products that imitate popular meat products that customers are used to. As part of a previous project, we managed to develop very interesting fish products for preschool children using African catfish separates. As part of this project, we want to develop a range of fish products for the general population (here the requirements are very different from the already developed children's range) using to a large extent by-products from the processing of African catfish, boneless and imitating popular meat products, such as hamburgers, balls, hot dogs, sausages, meatloaf, salami, etc.

Financial support

CZ.10.2.101/2.1/0.0/21_019/0001364 Development of new fish products for preschool children based on research of their preferences; CZ.10.2.101/2.1/0.0/20_018/0001219 Development of new fish products from African catfish



Fakulta rybnářství
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: MVDr. Veronika Piačková, Ph.D

Contact

E-mail: piackova@frov.jcu.cz

Phone number: +420 387 774 621

Factors affecting fish immunity in relation to infectious diseases / Faktory ovlivňující imunitu ryb v souvislosti s infekčními chorobami

Annotation

The topic is focused on the search for relations between outbreaks of infectious diseases and factors affecting the activity of the immune system of fish. The effects of the external environment (temperature, water saturation with oxygen, pH, organic pollution, etc.) and zootechnical procedures (overcrowding, unsparing handling, qualitatively or quantitatively insufficient feed, or, conversely, the addition of substances with a potentially immunostimulating or medicinal effect, etc.) will be researched.

Attention will be focused primarily on the most important infectious pathogens of common carp, namely carp edema virus (the causative agent of carp edema disease, also called koi sleeping disease, KSD), koi herpesvirus (the causative agent of koi herpesvirosis), carp sprivirus (the causative agent of spring viremia of carp), possibly the bacterium *Aeromonas hydrophila* (one of the causative agents of carp erythrodermatitis and secondary bacterial infections with viral diseases and parasitosis).

During the study, it will be necessary for the student to learn to use molecular-biological diagnostic methods (conventional PCR, qPCR, RT-PCR), hematological and immunological methods (ELISA, virus neutralization test, phagocyte activity, etc.). Student will also be involved in activities related to the rearing of pathogen-free fish, the handling of infection experiments, and sampling in the field.



Fakulta rybnářství
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: doc. Ing. Martin Pšenička, Ph.D.

Contact

E-mail: psenicka@frov.jcu.cz

Phone number: +420 389 034 784

Effect of light on early embryo development in fish/ Vliv světla na raný vývoj embrya u ryb

Annotation

The embryos and larvae of some fish species, such as trout or catfish, are specifically sensitive to light. The aim of this research will be to assess the influence of light, on the early development of different fish species.

The research will be divided to two parts.

In the first part, we will test sensitivity of early embryos of different fish species on different light spectra. Our hypothesis is a species-specific sensitivity of mitochondria to the blue visible light spectrum. We hypothesize differences in reactive oxygen species production and species-specific mitochondrial gene expression in susceptible fish species.

The second aim of this work will be to study the sensitivity of different fish larvae to different light spectra. Some species of fish are photophobic (e.g. catfish), which makes them difficult to rear. The basis for this research will be to test different fish species for their sensitivity to different light spectra and to compare the expression of opsins that detect different light spectra, or the different sensitivity of mitochondria in the retina of the eye.

In the conclusion of both works, we will propose measures to reduce the negative effect of light on early development in fish.



Fakulta rybnářství
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: doc. Ing. Vlastimil Stejskal, Ph.D.

Contact

E-mail: stejskal@frov.jcu.cz

Phone number: +420 737 221 930

Using of novel strategies to improve nanovaccine effectiveness in salmonids and percids / Využití inovativních strategií ke zvýšení účinnosti nanovakcín u lososovitých a okounovitých ryb

Annotation

The measuring of metabolic rates is becoming increasingly important due to the role metabolism plays in enhancing our understanding knowledge of physiology, behavior, evolution, and retaliations to environmental change and intensive aquaculture. The difference between the maximum metabolic rate (MMR) and the standard metabolic rate (SMR), which is the metabolic rate of resting animals, is the aerobic scope (AS), and it indicates fish's ability to sustain aerobic metabolic activity over and above the SMR.

Environmental hypoxia is a common abiotic stressor in aquaculture systems. Unsurprisingly, many fishes have developed the ability to withstand periods of hypoxia, despite the fact that there is significant interspecific variation and possibly intraspecific variation in the degree and length of hypoxia that may be tolerated. Extensive usage of the critical oxygen tension (Pcrit) as a metric of hypoxia tolerance is defined as the oxygen tension (PO₂) where fish can no longer maintain standard metabolic rate. The relationship between Pcrit and hypoxia tolerance and metabolic responses, however, remains incompletely understood.

In fish, the energy required for feed intake, digestion, absorption, and assimilation is accounted for by a specific dynamic action (SDA). The parameters like magnitude, peak level, peak time, and duration of SDA will be used. These parameters are related to the species, feeding rate, feeding frequency, temperature, feed composition, and quality. For aquaculture, it is important to fully understand the energetics of SDA in order to enhance feed conversion efficiency and energy retention.

Computerized intermittent flow respirometry (Loligo systems; www.loligosystems.com) will be used to measure MO₂, MMR, SMR, Pcrit, and SDA in resting chambers and rearing tanks. The applicant will be involved in SDA measurements in several experiments involving growth studies as well as in studies focused on the characterization of respirometric profiles of different fish populations under intensive culture conditions.



Supervisor: Mgr. Otakar Strunecký, Ph.D

Contact

E-mail: ostrunecky@frov.jcu.cz

Composition of the microbiome in intensive aquaculture systems; monitoring and assembly of microbial consortium for pre-inoculation based on beneficial microorganisms / Složení mikrobiomu v intenzivní akvakultuře: jeho analýza a příprava konsorcia prospěšných mikroorganismů pro inokulaci

Annotation

The work's goal is to characterize the microbiome from intensive aquaculture systems. It simultaneously aims to identify harmful or potentially pathogenic microorganisms. After their identification, these unwanted organisms will be substituted on a competitive exclusion principle by neutral or beneficial strains.

The intensification of fish farming negatively influences fish immunity coupled with a higher incidence of infectious diseases. It causes economic losses due to increased mortality, slower growth, and additional expenses connected with therapeutic treatment. Antibiotics and other chemical compounds are used to prevent and control harmful bacterial infections. Their use often leads to the evolution of more resistant pathogens as well as negatively influences the function of biofilters. The monitoring of fish production in recirculation aquaculture systems (RAS) showed that RAS must provide optimal conditions both for farmed fish and for microorganisms living in its environment, including biofilters.

This work consists of two tasks:

a) RAS microbiome monitoring

The first one is the research of microbiome composition in RAS during the fish production cycles. Microbiome will be sampled from water, tubings, and biofilters. The microbiome composition will be resolved using next-generation sequencing, particularly amplicon sequencing via Oxford Nanopore MinION. Bioinformatic analyses will provide the microbial composition of genera in particular components of RAS. These results will be correlated with the growth figures of fish during their production cycles. Using literature and other sources as GenBank of UniProt will suggest the metabolic features of particular organisms in the documented microbial consortium. Particular attention will be given to i/ critical situations and other issues connected with low fish production to identify the harmful microorganisms. Similar emphasis will be applied to microorganisms in ii/ farming cycles with above-average fish production.

b) Production of microbial premixes enhancing fish production

The microorganisms from ii/ will be cultivated, and subsequently, pure strains will be isolated by standard microbiological techniques. These organisms will be identified using Sanger sequencing.



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

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

Optionally genomic sequencing might be included to document or confirm particular metabolic features of suspected beneficial species. Cultivated organisms that will not be pathogenic and confirm favorable metabolic features, e.g., high denitrification rate, will be combined into microbial premixes. These premixes will be inoculated and tested during fish cultivations in RAS. Premixed microorganisms inoculated in high numbers should competitively exclude potentially pathogenic ones in these artificial environments and simultaneously enhance fish production.

The outcomes of this PhD. thesis will bring more information about the microbial ecology of closed aquatic ecosystems. The topic simultaneously targets improving the current practices in RAS fish farming.



Supervisor: doc. Mgr. Radka Symonová, Ph.D.

Contact

E-mail: radka.symonova@hbu.cas.cz

Phone number: +420 387 775 893

(Cyto)genomics in biodiversity assessment and conservation of fish / Využití (cyto)genomiky pro stanovení a ochranu biodiverzity u ryb

Annotation

Nuclear ribosomal RNA (rRNA) genes represent the oldest repetitive fraction, the rDNA, universal to all eukaryotes. Their deeply anchored omnipresence reflects in their multiple crucial roles and functions and reaches far beyond ribosomal synthesis and the protein synthesis. Merely the copy number of non-transcribed rDNA is involved in mechanisms governing e.g., maintenance of genome integrity, response to environmental cues, and cellular stress sensing (Symonová & Howell, 2018; Symonová, 2019).

There are several levels and possibilities how to apply analysis of rDNA structure in biodiversity genetics and assessment in combination with genomics and potentially also with molecular cytogenetics. Basically, rDNA structure analysis can be applied to:

1. reveal and understand the hidden biodiversity that cannot be resolved by morphology (eg salmonids);
2. to assess any potential genome infiltration of invasive species (eg *Carassius* through gynogenesis);
3. to examine "genomic condition" and "evolutionary health" in response to environmental and ecological changes based on the rDNA copy number variation (eg cyprinids, salmonids and many more).

The exact types of rDNA analyses to be applied are:

I. Primary sequence and molecular biology of 45S and 5S rDNA including copy number variation (CNV). Partly exploratory and continuation in the current research, partly to be applied as markers of impaired environmental conditions and stress (sensu Araújo et al., 2019; Morton et al. 2020).

II. Secondary structure of ITS2 and 5S rRNA. ITS2 will be used to delimit species and assess their potential to cross/outbreed based on the compensatory base change (reviewed by Symonová, 2019, many orig. papers particularly from algae and plants + insects, hence, currently being transferred to fish, etc. and particularly interesting in asexual invertebrates).

III. Chromosomal localization of 5S and 45S rDNA with FISH as an important factor in all stages of speciation as well as for diagnosis of pure species (many papers, eg Sember et al., 2020; Symonová et al., 2013).



References

- Araújo da Silva F, Feldberg E, Moura Carvalho ND, Hernández Rangel SM, Schneider CH, Carvalho-Zilse GA, Fonsêca da Silva V, Gross MC. 2019. Effects of environmental pollution on the rDNAomics of Amazonian fish. *Environ Pollut.* 252(Pt A):180-187. doi: 10.1016/j.envpol.2019.05.112
- Morton EA, Hall AN, Kwan E, Mok C, Queitsch K, Nandakumar V, Stamatoyannopoulos J, Brewer BJ, Waterston R, Queitsch C. 2020. Challenges and Approaches to Genotyping Repetitive DNA. *G3 (Bethesda)*. 10(1):417-430. doi: 10.1534/g3.119.400771
- Sember A, Bohlen J, Šlechtová V, Altmanová M, Symonová R, Ráb P. 2015. Karyotype differentiation in 19 species of river loach fishes (Nemacheilidae, Teleostei): Extensive variability associated with rDNA and heterochromatin distribution and its phylogenetic and ecologic interpretation. *BMC Evol Biology* 15:251 10.1186/s12862-015-0532-9
- Symonová R, Majtánová Z, Sember A, Staaks GBO, Bohlen J, Freyhof J, Rábová M, Ráb P. 2013. Genome differentiation in a species pair of coregonine fishes: an extremely rapid speciation driven by stress-activated retrotransposons mediating extensive ribosomal DNA multiplications. *BMC Evolutionary Biology*, 13: 42 10.1186/1471-2148-13-42
- Symonová R. 2019. Integrative rDNAomics—Importance of the Oldest Repetitive Fraction of the Eukaryote Genome, *Genes*, 10(5), 345 10.3390/genes10050345.
- Symonová R & WM Howell. 2018. Vertebrate Genome Evolution in the Light of Fish Cytogenomics and rDNAomics. *Genes, Spec. Issue "Chromosomal Evolution"* 9(2), 96 10.3390/genes9020096