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Vodňany

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The Dynamic Interplay of Germ and Somatic Cells in Gonadal Tissue Regeneration in Fish

Dynamická souhra zárodečných a somatických buněk při regeneraci tkáně gonád u ryb

Annotation

The gonad is a very complex organ consisting of distinct types of germ cells, including stem cells, differentiating cells capable of meiosis and production of gametes, while being continuously supported by somatic cells facilitating hormonal regulation, creating the germ cell niche and blood-testes barrier. Although a significant advancement in germ cell and somatic gonadal cell biology has been achieved, we still lack knowledge on how gonads can regenerate as a whole organ, particularly in vertebrates. Such a knowledge gap limits current strategies for restoring the function of the defective gonad.

Understanding how injured gonadal tissue can recruit distinct cell types and restore its size and function in a very dynamic fashion is crucial for identifying the key regulatory and developmental mechanisms behind this process. In turn, this can offer a novel view of processes within the testis, their regulation, and the potential development of innovative strategies for infertility treatment through gonadal function restoration.

To open this field, this thesis topic aims to discover the key processes facilitating successful gonadal regeneration using tractable fish models – zebrafish and killifish with particular attention to gonadal somatic cells. This thesis will present a comprehensive study on gonadal regeneration in fish, focusing on the unique regenerative capacity of fish gonads and the interplay between the somatic and germ cell lineages during this process. The proposed research employs a multifaceted approach, combining advanced molecular techniques, including RNA sequencing, detection of molecules, novel genetic models, sophisticated cell ablation techniques, and in vitro experiments to elucidate the mechanisms underlying gonadal regeneration.

The main hypothesis

Published results and our preliminary data suggest that fish gonad is a highly potent tissue in terms of gamete production while retaining an exceptional capacity to regenerate after an injury. We hypothesize that the regeneration process of the fish gonad will be unique as it contains lineages of germ cells and somatic cells, which do not occur together in other body tissues. We presume that somatic cells play a primary and essential role during regeneration.

Aim(s) of the Ph.D. thesis

- Develop a novel platform for studying gonadal regeneration in fish
- Characterize the dynamic of the regeneration process using various techniques such as histology, immunohistochemistry, lineage tracing, and in situ hybridization.
- Take advantage of already available genetic models in our laboratory to dissect key factors for successful regeneration.
- Perform in-depth transcriptomic characterization to gain insights into the regeneration process.

Possible approaches to reach the aims / to verify the hypotheses

Research will be performed on small-bodied fish models - zebrafish (*Danio rerio*) and turquoise killifish (*Nothobrnachius furzeri*). A partial resection of the gonad will be performed by surgery. Operated fish will then be sampled and processed for routine histology, immunolabelling, in situ hybridization chain reaction, and analysis of cell cycling. This will yield a robust dataset characterizing all the major events during the gonad regeneration. Next, we will use already established zebrafish and killifish genetic models in our laboratory to selectively target specific pathways during the regeneration process. Based on the transcriptomic analysis, we expect to generate a rich dataset that will provide us with a novel set of pathways and candidate genes that we will study in depth using gene editing.

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CENAKVA Research program

RP1 Reproductive and genetic procedures for fish biodiversity conservation and aquaculture

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Application of outdoor "Pond raceway system" for diversification of aquaculture in Central Europe

Využití venkovního pontonového chovu ryb v rybnících k diverzifikaci akvakultury ve střední Evropě

Annotation

Current freshwater fish production is only based on the culture of two main species – common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) cultured in Europe mainly in production ponds and water through- and recirculating- aquaculture systems, respectively (Policar and Adámek, 2013).

The future technology and technical development of production systems and their practical application for different species are the key strategy steps for the getting of diversified, profitable and long-term sustainable inland fish aquaculture production. All mentioned production systems (ponds, water through- and recirculating- aquaculture systems) have had a several technological problems: high dependence on water supply, unstable fish production depending on weather and season, loading of nutrients to open waters, demand on energy, staff and precise operations (Bostock et al., 2016; Liu et al., 2023). The development and application of new aquaculture technology like as the Pond raceway system is very important for the getting of new aquaculture production system to combine the advantages of several production systems (such as pond and RAS) with sustainable environmental, economic and production concepts related to climate, culture and social conditions and tradition of Central Europe. The application of newly developed Pond raceway system for several highly valuable fish species (such as pikeperch- *Sander lucioperca*, Eurasian perch – *Perca fluviatilis*, European catfish – *Silurus glanis*, largemouth bass – *Micropterus salmonids*, etc.) can help to diversify aquaculture production and also increase attractiveness and profitability of this developed system (Policar et al., 2019; Verdegem et al., 2023).

The main hypothesis

- Well-designed Pond raceway system can be effectively used as new aquaculture system in the production ponds with effective fish production and without negative environmental effect.
- Well-designed Pond raceway system can be effectively used for culture of different high valuable fish species (like as pikeperch, Eurasian perch, European catfish, largemouth bass, etc.) in different phases (juvenile and market-able fish).
- Waste nutrients from the Pond raceway system can be effectively used in production ponds for primary production with stable water quality.

Aim(s) of the Ph.D. thesis

- The development and testing of a well-designed the Pond raceway system with an identification of main bottleneck and advantages and describe user manual for aquaculture fish production.
- Effective use of the Pond raceway system for different selected fish species and evaluation of this production performance compared to other production aquaculture system.

- Monitoring of water quality in the Pond raceway system and production pond and describing waste nutrient production, its transfer, and loading between both parts of this system.
- Using waste nutrients in the production pond with the Pond raceway system for effective primary production to control and keep good water quality.

Possible approaches to reach the aims / to verify the hypotheses

- The application of different experiences and data from the designing and operation of the different aquaculture systems.
- Using of the effective principles for aquaculture of selected different fish species included different manuals and handbooks.
- Test of the first prototype of the Pond raceway system for future development and research approaches.
- Analyses of growth and survival rates, body, physiological and fin conditions during culture of different fish species in the Pond raceway system.

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CENAKVA Research program

RP3 Sustainable aquaculture with a responsible water and nutrient management

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The use of histology and immunohistochemistry as a diagnostic tool of health status in animals breed in aquaculture.

Využití histologie a imunohistochemie v diagnostice chorobných stavů organismů chovaných v akvakultuře.

Annotation

Histology is a biological discipline dealing with the microscopic examination of thin stained tissue sections in order to study the structure closely related to function. Histological examination may reveal symptoms of the disease that cannot be detected by conventional macroscopic examination and is, therefore, a suitable diagnostic tool for monitoring the health status of fish. Although the histological methods of processing fish and crustacean tissues are essentially the same as those used in mammals, there are various variations that are important to know for proper histopathological evaluation. Histological examination is often the only method that can accurately determine the structural origin of changes in a tissue or suitably complete and expand the information obtained by other diagnostic procedures. The quality and quantity of information obtained through histopathology varies considerably on the skills of the examiner. Histology as a diagnostic discipline differs considerably from many biochemical methods, where the result can only be obtained by correctly performing a precisely given procedure. The aim of the dissertation is to gain histological skills in the diagnosis of diseases and unhealthy conditions of organisms kept in Aquaculture and to apply these skills to specific diseases practically.

The main hypothesis

- Toxicants and infectious diseases affect tissue structure at the cellular level
- Many changes that are histologically insignificant can be diagnosed by immunohistochemistry in detail

Possible approaches to reach the aims / to verify the hypotheses

- Try immunohistochemical methods for the analysis of histopathologically altered tissues
- Suggest uniform quantification and description of histological changes in selected tissues as the background for the statistic evaluation

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CENAKVA Research program

RP3 Sustainable aquaculture with a responsible water and nutrient management

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Circularity and sustainable aquaculture

Cirkularita a udržitelná akvakultura

Annotation

With the rapid growth of the human population, the need for aquaculture fish production increases. Aquaculture has been the fastest-growing sector of animal production in recent decades. This surge in aquaculture raises questions about its sustainability and environmental impact. Most of the concerns about the sustainability of aquaculture are oriented toward feed and the discharge of waste nutrients (water and sludge) into recipients. In the field of feed, the main problems are the lack of traditional feed ingredients – fishmeal and oil, and the so-called "food feed" conflict between the consumption of the same raw materials for human nutrition and feed purposes. In the field of discharge of waste nutrients into the environment, the main problem is the loading of water with nitrogen and phosphorus, which leads to the eutrophication of water. The European strategy FOOD 2030 aims to introduce as many principles of sustainability and circularity as possible into the food production system by 2030. The aim is to reduce the negative impacts of food production on the environment, reduce dependence on fossil fuels and non-renewable sources of fertilizers, reduce waste production, reduce food feed conflict, and use waste and by-products as much as possible.

The main hypothesis

- Is it possible to reuse wastewater and sludge from various aquaculture systems in a sustainable and practically viable way?
- Is it possible to incorporate various by-products and waste into fish feed without compromising productivity and fish quality and be economically viable?
- Is it possible to reuse by-products from fish processing for product development or feeds without negative impacts on quality?

Aim(s) of the Ph.D. thesis

- The aim of the dissertation is to explore the possibilities of using wastewater and sludge from various aquaculture systems will be investigated. Furthermore, the possibilities of using various by-products and waste for fish nutrition will be investigated. Finally, the possibilities of using waste and by-products from fish processing to produce fish products and feed will be examined.

Possible approaches to reach the aims / to verify the hypotheses

- Wastewater reuse - Testing various aspects of aquaponic, biofloc, and irrigation systems. Sludge - testing various aspects of composting, vermicomposting, and mineralization systems. Outcomes will be studied using the LCA method, economic analyses, productivity, and product quality.
- Digestibility trials, retention studies, and growth studies in the RAS Guelph-type system.
- Development of products and testing their quality by chemical, physical, and sensory methods + shelf life studies.

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- OP JAK ITI Aquaculture for Future

CENAKVA Research program

RP3 Sustainable aquaculture with a responsible water and nutrient management

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Unlocking the potential of novel dietary ingredients in shrimp aquaculture

Využití potenciálu nových krmných surovin v akvakultuře krevet

Annotation

Shrimp have been the most-traded aquaculture product for decades, accounting for 15% of total production. However, shrimp farming and aquaculture heavily rely on protein-rich feed ingredients like fishmeal and soybean meal, which are either environmentally taxing or approaching ecological limits (Fearnside, 2001). Additionally, shrimp are among the largest consumers of fishmeal globally, further exacerbating these sustainability challenges (Naylor et al., 2021). To ensure the sustainable growth of the aquaculture industry, it is essential to identify alternative protein sources that reduce reliance on traditional ingredients (Tran et al., 2022). Alternative feed resources must provide sufficient nutritional value to support fish growth and health, be available in ample supply and palatable, remain cost-effective, have minimal environmental impact, and avoid exacerbating feed-food competition. Such ingredients can enhance the environmental sustainability of fish feed, reduce nutrient waste, promote health and stress robustness, and contribute to a circular bioeconomy (Harikrishnan et al., 2023; Abdollahzadeh et al., 2025; Sivaramasamy et al., 2023). Shrimp farming in Europe using RAS or biofloc systems is gaining popularity and is increasingly oriented toward sustainability, technical advancements, and consumer demand for eco-friendly products (Ankamah-Yeboah et al., 2017). These systems demonstrate the potential to enhance production efficiency while minimizing environmental impact.

A comprehensive multi-method approach is essential to address the sustainability challenges of shrimp aquaculture and evaluate the potential of alternative protein sources. Shrimp farming operates within complex systems where biotic and abiotic factors interact intricately. For shrimp farmers, maximizing production efficiency, such as yield per cubic meter, is a critical metric. However, this must be achieved sustainably, carefully considering water quality, shrimp welfare, and stress resilience (Bagheri et al., 2023; Yong et al., 2020). While adopting innovative farming systems like RAS and biofloc demonstrates promising advancements in production efficiency, there is limited knowledge about the nutritional and environmental impacts of novel feed ingredients that could replace traditional protein or lipid sources in aquafeeds.

The main hypothesis

- Alternative ingredients in shrimp aquaculture diets can replace traditional fishmeal and soybean meal.
- Alternative ingredients will provide equivalent or superior nutritional benefits while reducing environmental impact.
- Alternative ingredients will enhance shrimp growth, stress resilience, and immune function.
- Alternative materials may alter the palatability of feed compared to conventional ones

Aim(s) of the Ph.D. thesis

- To identify and evaluate the nutritional value, environmental impact, and economic feasibility of novel feed ingredients for shrimp aquaculture.

- To develop and test feed formulations incorporating alternative ingredients to ensure palatability, digestibility, and nutritional adequacy for shrimp species.
- To analyze the effects of alternative feed ingredients on shrimp growth, survival, stress tolerance, and immune function under varying environmental and climatic stress conditions.
- To quantify the ecological benefits of using alternative feeds, including reduced reliance on fishmeal and soybean meal, environmental impacts, and their compatibility with circular bioeconomy principles.
- To utilize meta-analyses, statistical modeling, and experimental approaches to validate the findings and provide robust scientific recommendations for sustainable shrimp aquaculture.

Possible approaches to reach the aims / to verify the hypotheses

- A meta-analysis approach to assess alternatives against established dietary ingredients and stress challenges for shrimp species.
- Performing separate feeding trials with different promising alternative ingredients and control groups.
- Feed preference to optimize formulations for palatability and acceptance (choice experiments, behavioural observation, test additives to improve palatability) - use video recording and tracking software to monitor feeding behaviours and preferences (avoidance).
- Digestibility and specific dynamic action (digestibility trials, enzymatic profiling, indirect calorimetry) – analysis of oxygen consumption and carbon dioxide production post-feeding.
- Stress challenges in light of climatic changes after different dietary interventions - exposure to acute or chronic stressors (e.g., heat shock, ammonia stress, salinity shifts) and monitoring physiological and behavioural responses and stress biomarkers.
- Immune function analysis and assessing parameters such as lysozyme activity, complement activity, or oxidative stress markers.
- Analysing the data about performance and production parameters.
- Applications of statistical data analysis (GLM, ANOVA, PCA, Pearson correlations).

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Funding

- Collaboration with Swiss farms, HAFL Bern and laboratory sources

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Harnessing novel microbial ingredients for percid nutrition

Využití nových mikrobiálních složek pro výživu okounovitých ryb

Annotation

Aquaculture is the fastest-growing food production sector, supplying most global seafood, with fed species production surpassing that of non-fed ones (FAO, 2022). This trend has increased the demand for sustainable, cost-effective, and nutritionally adequate feed ingredients that do not compete with human food resources. Promising alternatives include microbial proteins—yeasts, fungi, and bacteria—that convert low-value substrates into high-quality proteins (Bjordal et al., 2022; Hooft et al., 2025; Ruiz t al., 2023; Van Doan t al., 2017.

Wan t al., 2021). Fermentation is another method to enhance plant-based feed ingredients by reducing anti-nutritional factors (ANFs) and improving digestibility (Catalán et al., 2018). These alternative ingredients may also confer health-promoting effects, such as enhanced immune responses and improved gut health in farmed fish (Hoseinifar et al., 2017). This anaerobic process uses microorganisms to break down indigestible polymers into more straightforward, absorbable compounds. Solid-state and submerged fermentation are the main techniques used.

Percid aquaculture, especially involving Eurasian perch (Perca fluviatilis) and pikeperch (Sander lucioperca), is expanding in Europe, mainly in recirculating aquaculture systems (RAS). These systems support sustainability, innovation, and consumer demand for eco-friendly products while enhancing production efficiency and reducing environmental impact. However, several challenges must be addressed, particularly in feed development (Overton et al., 2015). Feed accounts for 70–80% of production costs in carnivorous fish RAS systems (Hoerterer et al., 2022). Despite the availability of species-specific feeds, many farms still use salmonid or sturgeon diets (Król et al., 2021).

Research on percid nutrition remains limited. Advancing this field is essential for optimizing feed, improving sustainability, and supporting the sector's growth. A systems approach is required to balance productivity with water quality, welfare, and stress resilience (Bagheri et al., 2023).

The main hypothesis

- Microbial ingredients in percids aquafeeds can partially replace traditional fishmeal and soybean meal.
- Microbial ingredients will provide equivalent or superior nutritional benefits while reducing environmental impact.
- Microbial ingredients enhance percids, stress resilience, and immune function while not limiting percids growth.
- Microbial sources may alter the palatability of feed compared to conventional ones

Aim(s) of the Ph.D. thesis

- To identify and evaluate the nutritional value and environmental impact microbial feed ingredients for percids aquaculture.

- To develop and test feed formulations incorporating microbial ingredients to ensure palatability, digestibility, and nutritional adequacy for percids species.
- To analyze the effects of microbial feed ingredients on percids growth, gut microbiome, survival, stress tolerance, and immune function under varying environmental stress conditions.

Possible approaches to reach the aims / to verify the hypotheses

- Performing separate feeding trials with different promising microbial ingredients and control groups.
- Feed preference to optimize formulations for palatability and acceptance (choice experiments, behavioural observation, test additives to improve palatability) - use video recording and tracking software to monitor feeding behaviours and preferences (avoidance).
- Digestibility and specific dynamic action (digestibility trials, enzymatic profiling, indirect calorimetry) – analysis of oxygen consumption and carbon dioxide production post-feeding.
- Stress challenges in light of climatic changes after different dietary interventions - exposure to acute or chronic stressors (e.g., heat shock, ammonia stress, salinity shifts) and monitoring physiological and behavioural responses and stress biomarkers.
- Immune function analysis and assessing parameters such as cells composition, lysozyme activity, complement activity, or oxidative stress markers.
- Analysing the data about performance and production parameters
- Applications of statistical data analysis (GLM, ANOVA, PCA, Pearson correlations)

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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

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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