**Blood circulating small non-coding RNAs (c-sncRNAs) as non-lethal biomarkers of reproductive status and gamete quality in pikeperch (Akronym: sAnDeRNA)**

Pikeperch (*Sander lucioperca* L.), belonging to the percid family, has become an important model species in research on reproduction and developmental biology among freshwater fishes cultured in intensive recirculating aquaculture systems (RAS). However, despite significant progress in the intensification of pikeperch aquaculture, the successful expansion of this technology is hindered by the negative impact of RAS on reproductive physiology. The reduced reproductive quality of farmed pikeperch is mostly related to impaired reproductive performance and very low gamete quality in reproductively inexperienced fish (virgin fish, defined as fish attempting spawning for the first time), as well as unpredictable and variable gamete quality in experienced fish (defined as fish attempting spawning for at least the second time). Therefore, there is an urgent need to identify processes affecting the successful induction of the gonadal cycle in virgin fish and variable gamete quality in domesticated pikeperch breeders. For this purpose, innovative and robust research tools, such as circulating small non-coding RNAs (small molecules circulating in blood; sncRNAs). These sncRNAs hold promise as non-invasive biomarkers, safe for fish and easy to measure in the blood, offering precise insights into the reproductive status of fish. While circulating RNAs have been explored as reproductive markers in specific fish species, such as rainbow trout, and in distinguishing sex and monitoring stress in seabass, there is a notable gap in understanding circulating sncRNAs in assessing reproductive success in percids (both females and males). Additionally, there's a lack of research on how circulating sncRNAs change during the onset of puberty or maturation in any fish species.

**Therefore, the main aim of the project is the detailed investigation of changes in circulating sncRNA in the blood plasma of pikeperch throughout two subsequent reproductive cycles (I cycle of virgin fish and II cycle of experienced fish) to explore the connection between circulating sncRNAs and the quality of gametes (eggs and sperm) in fish.** The project will comprise four work packages:

**(1)** establishing a multi-tissues sncRNA and mRNA reference atlas related to blood c-sncRNAs**;**
**(2)** characterizing the kinetics of c-sncRNA repertoire during first induction of reproduction (puberty) in virgin fish in relation to the expression level of blood sncRNA-targets (mRNAs) along reproductive axis (RAxis; which includes hypothalamus, pituitary, gonads, and liver);
**(3)** characterizing the kinetics of blood c-sncRNA repertoire during the reproductive cycle of experienced fish in relation to expression level of sncRNA-targets along RAxis;
**(4)** identifying gamete-quality-linked (i.e., eggs and sperm) and sex-linked specific blood c-sncRNAs in experienced fish during spawning.

For each WP, four independent rearing operations of the spawners (long-term and laborious preparation of the spawners, monitoring of their sexual maturity, pikeperch broodstock management, spawning operation, gamete collection, in vitro fertilization, incubating the eggs, rearing the larvae), followed by intensive molecular analysis, such as sequencing of RNA molecules, and bioinformatic analysis (which will lead to the evaluation and discovery of new forms of small RNAs and to predict their possible functions) will be performed.

The entire project aims to conduct the first comprehensive analysis of small non-coding RNA (sncRNA) molecules in both female and male fish. By integrating the analysis of different RNA molecules, such as sncRNA and mRNA, and exploring potential interactions between them, we aim to unravel the complex mechanisms that lead to reproductive issues in pikeperch. This holistic approach will shed light on the intricate processes affecting the reproductive capabilities of these fish. As part of the project, we will pioneer the exploration of circulating sncRNAs (c-sncRNAs) to identify new, non-invasive biomarkers linked to the quality of gametes. Ultimately, the project's long-term goals are centered around optimizing breeding conditions and refining reproductive techniques. The aim is to enhance overall reproductive efficiency, not only for percids like pikeperch but also for other fish species. This research not only contributes to the advancement of aquaculture practices for pikeperch but also opens new avenues for understanding the reproductive dynamics of various fish species.