Cryopreservation of sperm is a game-changing tool in the world of fish breeding, allowing us to store the genetic material of valuable fish for really long periods. This technology supports hatchery operations, breeding programs, facilitating efforts to conserve fish species, helping manage sustainable fish populations. But its potential goes beyond just preservation - it also opens doors for studying the biological secrets of how fathers influence the development of their offspring. For example, our recent research with Eurasian perch has uncovered genes that are passed down from fathers and control key aspects of early development, like the visual system. These findings suggest that sperm cryopreservation imposes a kind of "selection pressure," where only the strongest sperm survive and are able to fertilize eggs. This phenomenon, known as "cryo-selection," not only affects which sperm succeed but may also influence the traits of the next generation through non-genetic factors like epigenetic changes. Despite the growing recognition of cryo-selection's importance, there is still much we do not know about its full impact. While we already understand it shapes progeny traits, we still lack a deep understanding of how it influences growth, survival, and overall performance, as well as how it affects the larvae at the molecular level. Furthermore, we do not yet know if the male offspring obtained from cryopreserved sperm inherit the ability to withstand cryopreservation, as their fathers do. This is also crucial because the widespread use of sperm cryopreservation could bring risks - like lower breeding efficiency and reduced genetic diversity - if we do not fully understand these processes. This is why studying the broader effects of sperm cryopreservation is essential for ensuring its reliable and sustainable use in aquaculture and conservation.

Therefore, the **CRYO-LARVA project proposes a unique, multi-species, and multi-level approach to better understand the developmental consequences of cryo-selection.** Through this project, we will compare how sperm from fish raised in different environments and exposed to different cryoprotectants perform, and whether these traits are passed down across generations. Over time, the findings will shed light on non-genetic inheritance, revealing new pathways for improving selective breeding strategies.

The CRYO-LARVA project will use five species of fish - rainbow trout, sterlet, pikeperch, burbot, and zebrafish - to explore several important questions: **1.** How does cryopreservation affect the molecular and physical traits of rainbow trout and sterlet larvae, especially when different cryoprotectants are used? **2.** How do different rearing environments (such as recirculating aquaculture systems vs. natural environment) impact the larvae of pikeperch and burbot, when fertilized with cryopreserved sperm? **3.** What are the long-term, transgenerational effects of sperm cryopreservation on zebrafish larvae? **4.** What common and species-specific patterns can we identify in the molecular and developmental impacts of cryopreservation across different species?

For the first time, this project will examine how cryopreservation, combined with environmental conditions and the type of cryoprotectant used, impacts the development and molecular profile of fish larvae. It will also explore how these effects can be passed down through generations, using zebrafish as a model species. Unlike most studies, which focus only on earliest life stages like embryos, CRYO-LARVA will also look at how cryopreservation influences the later stages of life, including larvae and juveniles. This more comprehensive approach will give us a fuller picture of how cryopreservation shapes the fish's wildly understand development, paving the way for innovations in breeding and conservation efforts. By studying how traits related to cryo-sensitivity are inherited, the project will also fill in critical knowledge gaps about the long-term, intergenerational effects of cryopreserved sperm. The ultimate goal of CRYO-LARVA is to provide new insights into how cryopreservation affects fish, particularly through non-genetic inheritance, and how we can use later this knowledge to optimize breeding programs**.** Project will also provide a groundbreaking look at the biological effects of sperm cryopreservation. All this findings will help ensure the sustainability of aquaculture practices, support genetic conservation efforts, and deepen our understanding of how fathers influence the traits of their offspring.