

In 2022, and to a lesser extent in the two subsequent years, the Odra River experienced large-scale fish die-offs and the death of other aquatic organisms. These events were linked to blooms of the mixotrophic haptophyte *Prymnesium parvum*, resulting in severe ecological and socio-economic consequences. Studies from various regions of the world indicate that waterbodies affected by *P. parvum* often suffer from recurring blooms and their harmful impacts. Despite the growing global significance of this issue, major gaps remain in our understanding of *P. parvum* diversity, physiology, and toxicity. For example, the genes responsible for the biosynthesis of ichthyotoxic prymnesins (PRMs) were only identified in 2024. However, the environmental and biological factors that regulate their expression are still poorly understood. Furthermore, the relationship between PRM concentrations in bloom samples and the observed fish mortality is not clearly defined.

To address these knowledge gaps, this project proposes a comprehensive and interdisciplinary investigation into the mechanisms, triggers, and consequences of *P. parvum* blooms, with a particular focus on their occurrence in Polish waters. The primary objective is to identify key biotic and abiotic factors that drive the production of toxic metabolites and to elucidate how these compounds affect exposed organisms.

Based on the published data and our preliminary findings, we propose the following hypotheses to be verified in the project: [1] In Polish waters, *P. parvum* blooms consist of metabolically diverse subpopulations, each with distinct toxic potential and different impacts on fish and other aquatic organisms; [2] Environmental conditions significantly influence the growth of these subpopulations, modulate toxin production, and affect overall toxicity; [3] Certain *P. parvum* metabolites, including PRMs, may pose health risks to humans through skin contact or inhalation; [4] Bloom formation and toxicity are linked to complex physiological responses, including changes in the metagenome, transcriptome, proteome, and metabolome of *P. parvum*.

The project will be carried out through six interconnected work packages (WPs): **WP1** - Characterization of metabolic diversity, isolation of key metabolites, and toxicity assessment using human cell lines and animal models; **WP2** - Evaluation of the effects of *P. parvum* cells, lysates, and selected metabolites on fish gill and skin tissue models; **WP3** - Ecotoxicological assessment of *P. parvum* impacts on aquatic invertebrates (zooplankton); **WP4** - Physiological studies on *P. parvum* to determine factors affecting its toxic potential; **WP5** - Analysis of *P. parvum* distribution, environmental conditions, and interactions with the microbiome and microeukaryotes; **WP6** - Data integration and synthesis of project outcomes.

All research tasks will employ sophisticated and modern analytical tools. The project will be carried out by a multidisciplinary team of experts in ecotoxicology, hydrobiology, ichthyology, toxicology, physiology, cell biology, natural product chemistry, molecular biology, and bioinformatics. The outcomes of this project will improve our ability to assess and predict the ecological and health risks associated with *P. parvum* blooms. Moreover, the insights gained may support the development of more effective bloom management strategies and early warning systems.