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MASTER THESIS PROJECT

Fish cell culture as a tool to assess the cellular and molecular changes induced by environmental contaminants

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ABSTRACT

The aquaculture industry is one of the most significant food production sectors and it is actively seeking new *in vitro* tools to enhance the welfare of farmed fish. Although the evaluation of immunotoxicity in fish as a tool for environmental risk assessment is very promising, it is still very little explored. Fish health relies heavily on the function of cells from hematopoietic organs, but also on immune cells colonizing the intestine. The wide knowledge gap of the fish immune system and the lack of cell lines to perform *in vitro* studies is a barrier to study fish immunity. The present study aims to investigate the hypothesis that leukocytes isolated from European seabass (*Dicentrarchus labrax*) can be reliable and consistent *in vitro* tool for toxicological studies of environmental pollutants. To address this research question, a protocol for isolating immune cells from the head kidney (HK) and posterior intestine (PI) of European seabass was optimised, coupled with the characterisation of the isolated cell populations. Then, the effects of the exposure of leukocytes to metal contaminants were analysed in the viability and phagocytic activity of the cells. The optimised protocol allows to recover a sufficient yield of cells from the HK. In contrast, the protocol for the isolation of leukocytes from the PI needs further optimisation. Flow cytometry cell sorting was used to separate leukocyte subpopulations based on selective binding to the lectins Wheat Germ Agglutinin (WGA) and *Lycopersicon esculentum* lectin (LEL). Different lectin-binding patterns could be observed, but sorted subpopulations were morphologically homogeneous. The exposure of HK cells to cadmium, copper, and lead for 2 hours did not have a significant ($p \leq 0.05$) impact on cell viability or phagocytic capacity. However, it is important to consider the potential sub-lethal effects on gene expression changes. Therefore, thirteen genes were selected for analysis in HK. So far, it is necessary to optimise the RT-PCR assay to produce reliable and reproducible data for assessment at the molecular level. Overall, the present study provides valuable insights for the development of *in vitro* tools to study the immune system of the European seabass. However, further studies are required for the development of accurate and effective in vitro tool.