# Development of an *In vitro* Marine Fish Bioassay for Characterizing Environmental Impact of Industry Development in the Arctic

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## Introduction

❖ The ongoing changes in the Arctic, such as rising temperatures & diminishing sea ice, are likely to result in increasing industrial activities, including oil, gas & aquaculture operations.

❖ To address the effects of these anthropogenic activities New Approach Methodologies (NAMs), using *in vitro* methods were implemented to quickly assess the toxicity of compounds of concern.

❖ **The main goal of the study was to develop & implement an *in vitro* testing system originating from the marine fish, Atlantic Halibut** (*Hippoglossus hippoglossus*), by investigating chemical & metal effects on the liver (hepatocyte) cells & evaluate their use in predicting environmental consequences from future anthropogenic activities in the Arctic region.

**This goal will be achieved by multiple tasks:**

1) Develop & optimize primary hepatocyte cell isolation, cell characterization (morphology, physiology & structure), culturing & exposure of Atlantic Halibut to model compounds (e.g. Copper (Cu), β-Estradiol (E2) & 2,3,7,8-Tetrachlorodibenzodioxin (TCDD)).

2) Assessing hepatocytes sensitivity & responsiveness of key indicators of toxicity such as cytotoxicity (Cell membrane integrity, Metabolic activity) detoxification Phase I (Ethoxyresorufin-O-deethylase, EROD; Cytochrome P450 1A, CYP1A), endocrine disruption (Vitellogenin, Vtg), & Reactive oxygen species (ROS).

3) Toxicity assessment of a selection of substances relevant to the Arctic environment.

## Experimental design

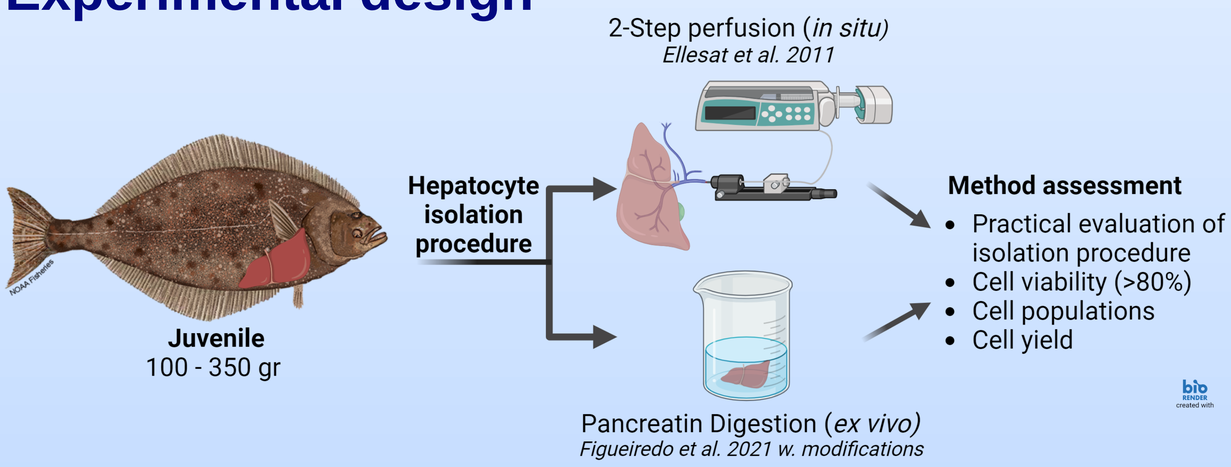


***Figure 1.*** *Assessment of optimal hepatocyte isolation procedure for Atlantic Halibut. Two methods, in situ 2-Step perfusion [1] & ex vivo Pancreatin Digestion w. (no Histopaque) & wo. modifications were evaluated [2]. The optimal method was identified as the ex vivo Pancreatin digestion protocol w. modification.*

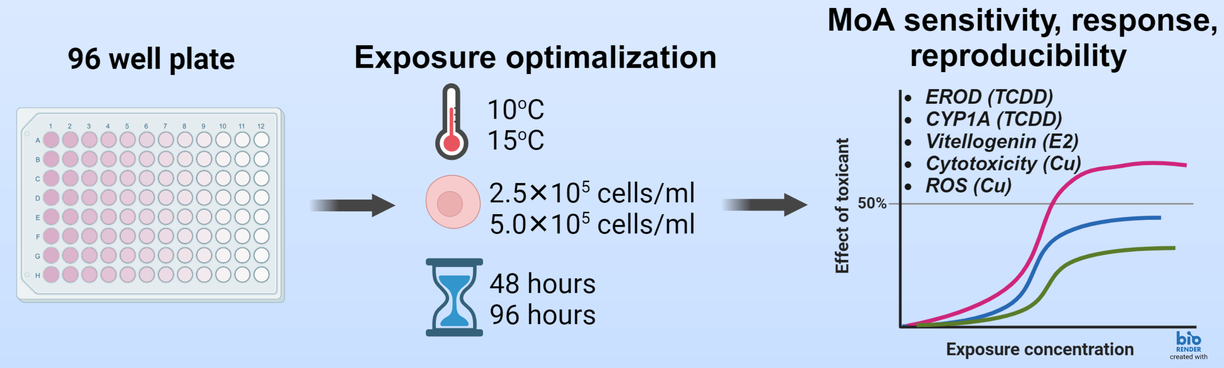


***Figure 2.*** *Isolated primary cells were plated in a 96 well plate in different combinations of cell density, incubation temperature & exposure duration (48 & 96h) to assess the optimal exposure conditions. The hepatocytes were exposed to Cu, E2 & TCDD. Endpoints assessed at each experimental conditions: Vtg (E2), EROD & CYP1A (TCDD), Cytotoxicity: Cell membrane integrity & Metabolic activity (Cu) & Cytosolic ROS (Cu) as previously described [3].*

## Results

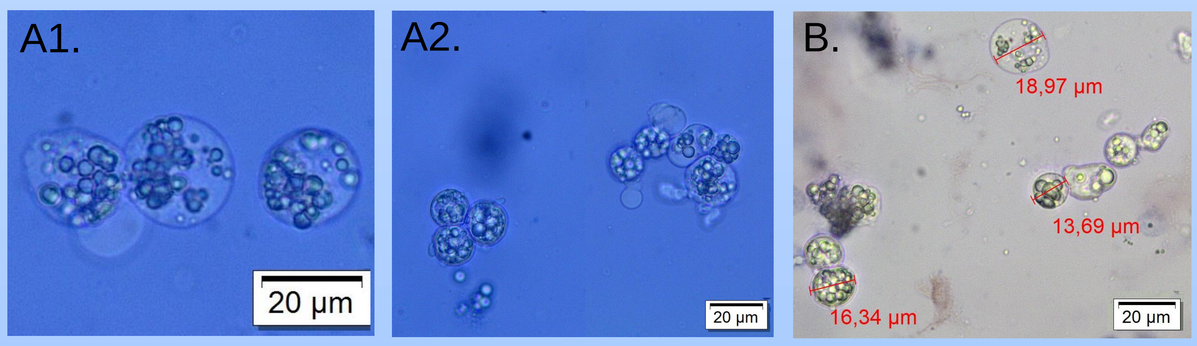


***Figure 3.*** *Isolated primary hepatocytes from juvenile Atlantic Halibut using the ex vivo Pancreatin digestion procedure with modifications [2]. A light microscope was utlized to assess cell viability using Trypan blue (A) & cell diameter (B). Cell diameter was Ø 15.1 ± 2.4 µm (mean ± SD). Visual inspection of the cells implies minimum two different populations (lipid, complexity). Cell viability was 96.6 ± 5.1 % (mean ± SD).*

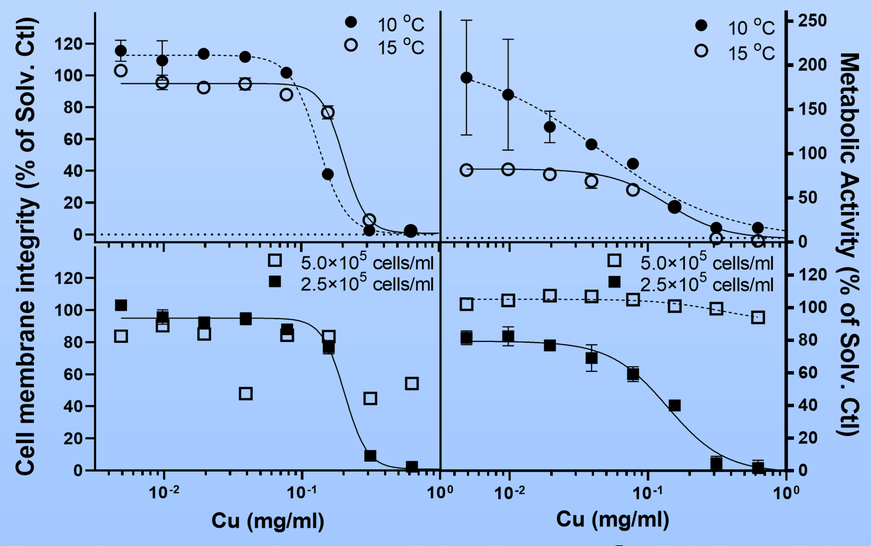


***Figure 4.*** *Effects of temperature (top row, cell density 2.5×10⁵ cells/ml) & cell density (bottom row, exposure temperature 15°C) on membrane integrity & metabolic activity of Atlantic Halibut hepatocytes exposed to Cu for 48 hours. The data (mean ± SEM) were normalized to cell media control (100% viability) & positive control 2.5 mg/ml Cu (0% viability).*

## Conclusion

❖ Visual inspection revealed a minimum of two different cell populations.

❖ Optimal isolation procedure (*ex vivo* pancreatin digestion with modifications) & exposure conditions (15°C, 2.5×10⁵ cells/ml) were established.

❖ Cytotoxicity displayed to be highly sensitive & responsive both in cell membrane integrity & metabolic activity during exposure to Cu at both 48 & 96 hours.

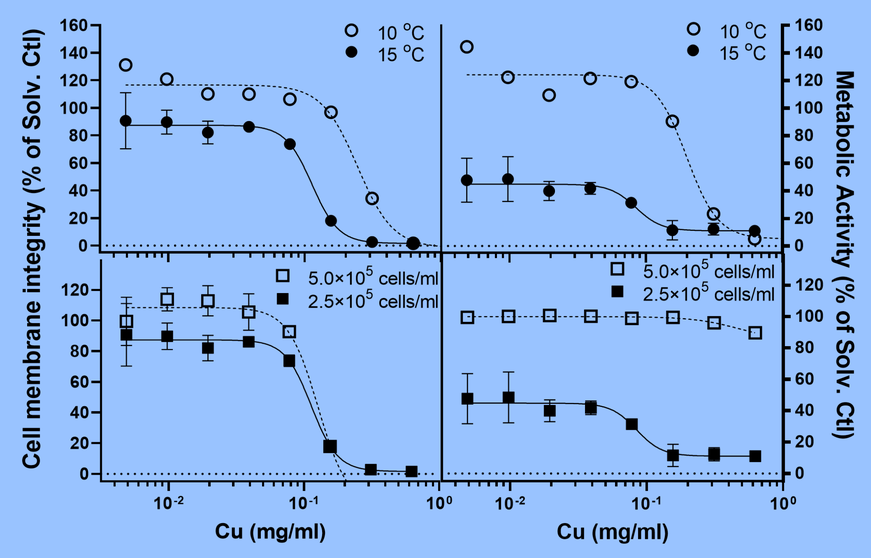


***Figure 5.*** *Effects of temperature (top row, cell density 2.5×10⁵ cells/ml) & cell density (bottom row, exposure temperature 15°C) on membrane integrity & metabolic activity of Atlantic Halibut hepatocytes exposed to Cu for 96 hours. The data (mean ± SEM) were normalized to cell media control (100% viability) & positive control 2.5 mg/ml Cu (0% viability).*

## Future work

❖ Ongoing endpoint analysis of EROD, CYP1A, Vtg, ROS.
❖ Characterize the cell population(s) using fluorescent flow cytometry.
❖ Expose primary halibut cells to a selection of pollutants relevant to the Arctic environment.

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**References:** [1] Ellesat KS *et al*. Mar Environ Res. 2011 Oct;72(4):216-24; [2] Figueiredo *et al*. Int J of Env Res and Pub Heal. 2021; 18(4), p.1380; [3] Petersen *et al*. Aquat tox. 2017; 187, 141-152.

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