# Flow Cytometry as a Tool to Assess the Responses of *Mytilus edulis* Digestive Cells to Contaminants Present in the Arctic

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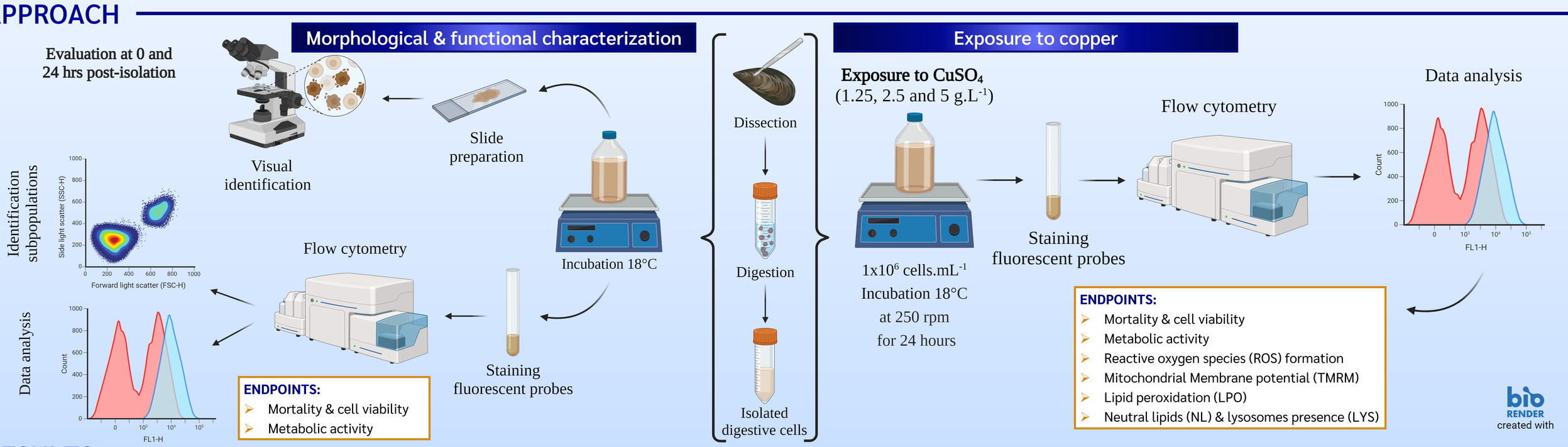
## BACKGROUND

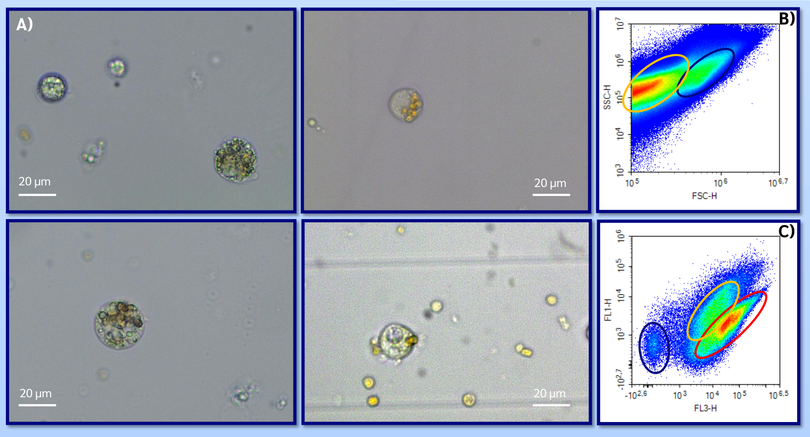
Primary cell cultures from aquatic organisms are being increasingly developed for use in toxicity testing and mechanistic studies in response to xenobiotics. Whereas fish cells are commonly used, less is known about primary cells derived from aquatic invertebrates such as bivalves. Digestive glands play a key role in the metabolism and biotransformation of xenobiotics in mussels *Mytilus sp.,* making digestive cells relevant *in vitro* models for studying pathways and toxic mechanisms of xenobiotics. Flow cytometry (FCM) can be used as a tool to evaluate the toxicological effects of contaminants in individual cells. However, its potential use in integrated effects assessment and next generation risk assessment (NGRA) has not yet been fully explored. Accordingly, the aim of this study was to investigate the sensitivity and applicability of FCM to quickly screen the effects of model chemicals towards digestive cells isolated from *Mytilus edulis*.

## APPROACH




## RESULTS

### Morphological & functional characterization



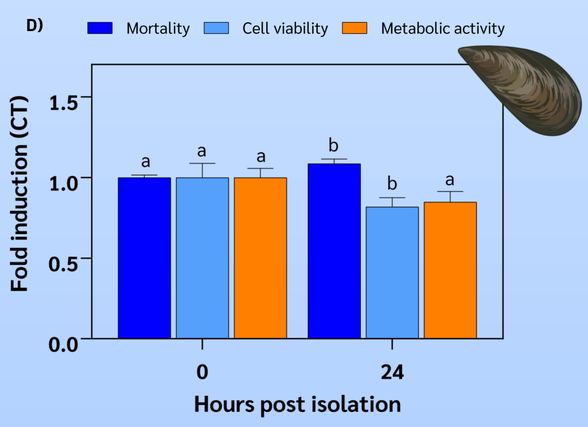




**Figure 1** – A) Representative images of *Mytilus edulis* digestive cells at x40 magnification. B) Size (FSC-H) and forward (SSC-H) density plot and C) fluorescence density plot (FL1 and FL3) of digestive cells with subpopulations highlighted. D) Mortality, viability and metabolic activity of isolated digestive cells over 24 hours, expressed as fold induction from the control (CT) at time 0. Different letters represent statistical differences between time points ($p$<0.05).

### Exposure to copper

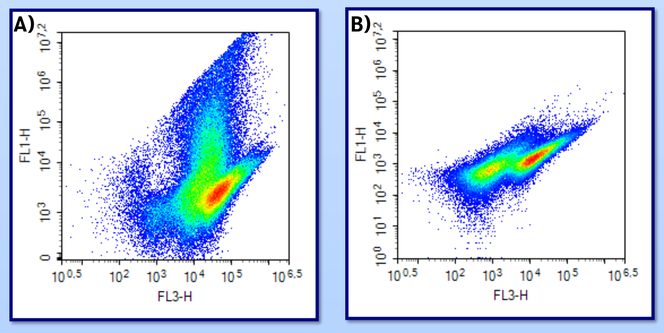



**Figure 2** – Representative fluorescence density plots of digestive cells of *Mytilus edulis* from the control (A) and exposed to 5 g.L$^{-1}$ $CuSO_4$ (B) for 24 hours.

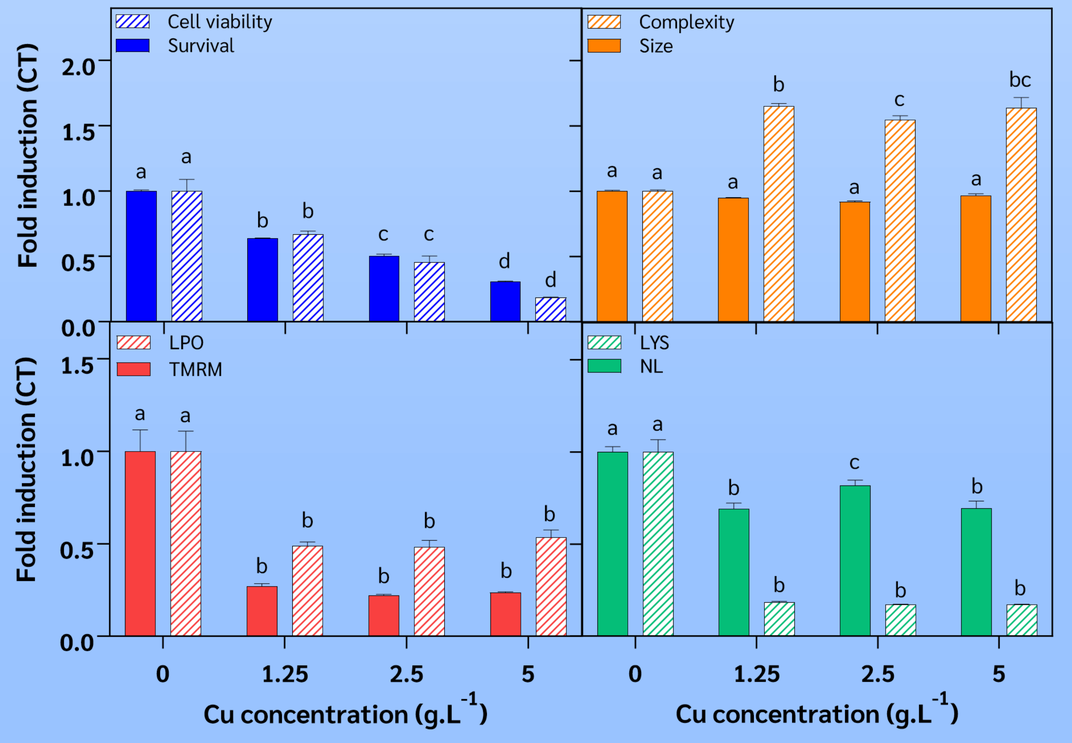



**Figure 3** – Responses of *Mytilus edulis* digestive cells after exposure to $CuSO_4$ for 24 hours, expressed as fold induction from the control (CT). For simplification, cell subpopulations were grouped in one population. LPO – lipid peroxidation, TMRM – mitochondrial membrane potential, LYS – lysosome content; NL – neutral lipid content. Different letters represent statistical differences between concentrations ($p$<0.05).

## MAIN FINDINGS

- **Morphological & functional characterization:**
  - Several cell subpopulations identified ➔ larger cells filled with lysosomes and smaller cells containing few to no lysosomes or lipids. Size ranges ➔ 3.5 to 22.7 µm.
  - Cell subpopulations with ≠ sizes and similar complexity also identified by FCM.
  - Small mortality increase and viability decrease 24 hours post-isolation ➔ cells maintain integrity and biochemical functions over time.
- **Exposure to copper:**
  - Concentration dependent decrease in cell survival and viability ➔ lethal Cu toxicity.
  - Increased cell complexity and decrease in NL and LYS content ➔ reflect ultrastructural changes in cells ➔ presence of detoxification mechanisms.
  - Decreased mitochondrial membrane potential ➔ depolarization of mitochondrial membrane ➔ impact on oxidative phosphorylation and/or activation of apoptosis and cell death.
  - Decreased LPO ➔ point to an efficient activation of the antioxidant defense system.

## FUTURE PERSPECTIVES

- **Ongoing work** ➔ In-depth characterization of digestive cells combining FCM and fluorescent microscopy ➔ identify and understand subpopulations shifts in FCM.
- **Future studies** ➔ Experiments with sub-lethal Cu concentrations and other model compounds (e.g. benzo(a)pyrene) to expand use of digestive cells as *in vitro* tools.
- **FCM** seems to be a **fast**, **accurate** and **reproducible** cost-effective method to quickly **assess** cellular **functions** in mussel **digestive cells** ➔ integrated into assessment of contaminants present in the Artic.

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**References:** Balbi et al. (2017). Comparative Biochemistry and Physiology, Part C: 191; Faucet et al. (2003). Methods in Cell Science: 25.

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