Flow Cytometry: A novel, rapid, screening and research tool for methylmercury production activity in aquatic ecosystems?

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Mercury
A contaminant of global consequence

- Responsible for 80% of all fish consumption advisories

- Global burden of disease estimate 1.2-2.4 million years living with disability
  - Higher than hepatitis or Parkinson’s

- Only element with its own international treaty

Global emissions

National deposition

Consumption advisories
Variability of mercury in ecosystems

N=4262 locations

THg (ppm ww)
Pathways of inorganic Hg to bioaccumulation

Inorganic Hg → Methyl Hg → Inorganic Hg → Methyl Hg → Inorganic Hg → Methyl Hg → Fish
Ecological Pathways Conceptual Model

Resource Management

Ecological Pathways and Processes

Environmental Occurrence

Exposure

Adverse Outcomes

Physiology
Methylmercury production – a microbial process

Environmental conditions

Inorganic Hg → Hg-methylating bacteria → Methyl Hg
Hg in the Great Basin and Snake River Drainage

Eagles-Smith et al. 2016
Hells Canyon - 188 thousand ac/ft

Brownlee - 1.4 million ac/ft

Oxbow - 58 thousand ac/ft
Organics and nutrients 

Mercury bioaccumulation through a three-reservoir complex

Slide courtesy of Reed Harris

[Diagram of mercury bioaccumulation through a three-reservoir complex]
Fish mercury concentrations increase through the complex
Aqueous MeHg concentrations through the complex

Variation greater within site (by depth) than across sites
Mercury cycling model for the complex
Primary productivity as a driver of MeHg production
Flow Cytometry

- Instrumented method for quantifying size and type of cells in a fluid
- Originally developed for medical uses (blood cells, etc.)
- Adopted by oceanographers and limnologists for algal cells
- Employed by microbiologists
Flow Cytometry

- Forward scatter estimates cell size
- Side scatter and fluorescence indicative of cell structure and granularity
- Measures light absorption and excitation
• Biplots of scatter and fluorescence allow for differentiation of different cell types
Bacteria determined by size and cell stains

Coarse differentiation

Nucleic acid stains differentiate two primary types

HNA = High nucleic acid content (active cells)

LNA = Low nucleic acid content (inactive cells)
MeHg concentrations and microbial activity through the water column

Spring Fall

Filtered MeHg (ng/L)  Total bacteria  HNA:LNA Ratio
HNA:LNA ratio & Dissolved MeHg (ng/L)
HNA:LNA ratio correlated with aqueous MeHg

All Sites: $R^2 = 0.48$

Stratified Sites: $R^2 = 0.70$

Unstratified Sites: $R^2 = 0.07$
Methylmercury production – a microbial process

- Environmental conditions
- Genetics
- Inorganic Hg
- Hg-methylating bacteria
- Methyl Hg
Discovery of hgcAB gene cluster (2013)

- Insights into molecular mechanisms
- Probing of existing cultures and genetic databases
- Environmental marker

Gilmour et al, 2013
Potential applications of flow cytometry in MeHg monitoring and research

- Sample Fixation
- Hybridization
- Washing
- Cell Count
- Fluorescence
Incorporation of cytometric tools

Water Sampling

Methylmercury concentration

Water samples

Water Depth

Sediment

Cytometric Tools

(developed with Innovation Funding)

Side Scatter

Nucleic Acid Stain

Active cells

Inactive cells

Fluorescence

Call Count

Hg methylation gene probes

Microbial activity markers

Taxonomy of methylating microbes

Environmental processes influencing microbial MeHg production

Management actions

Microbial Community Processes

Methylmercury concentration

Water Depth

Sediment
Summary

- Flow cytometry provides a rapid, low-cost estimate of microbial community composition and activity.

- Ratio of “active” to “inactive” bacteria profile closely matched aqueous MeHg profile.

- Application to other systems?

- Development of molecular/genetic markers?
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