Managing Aquatic Mercury Pollution:
Strategies to Quantify Mercury Biomethylation Potential in Sediments

Helen Hsu-Kim, DUKE UNIVERSITY
hsukim@duke.edu

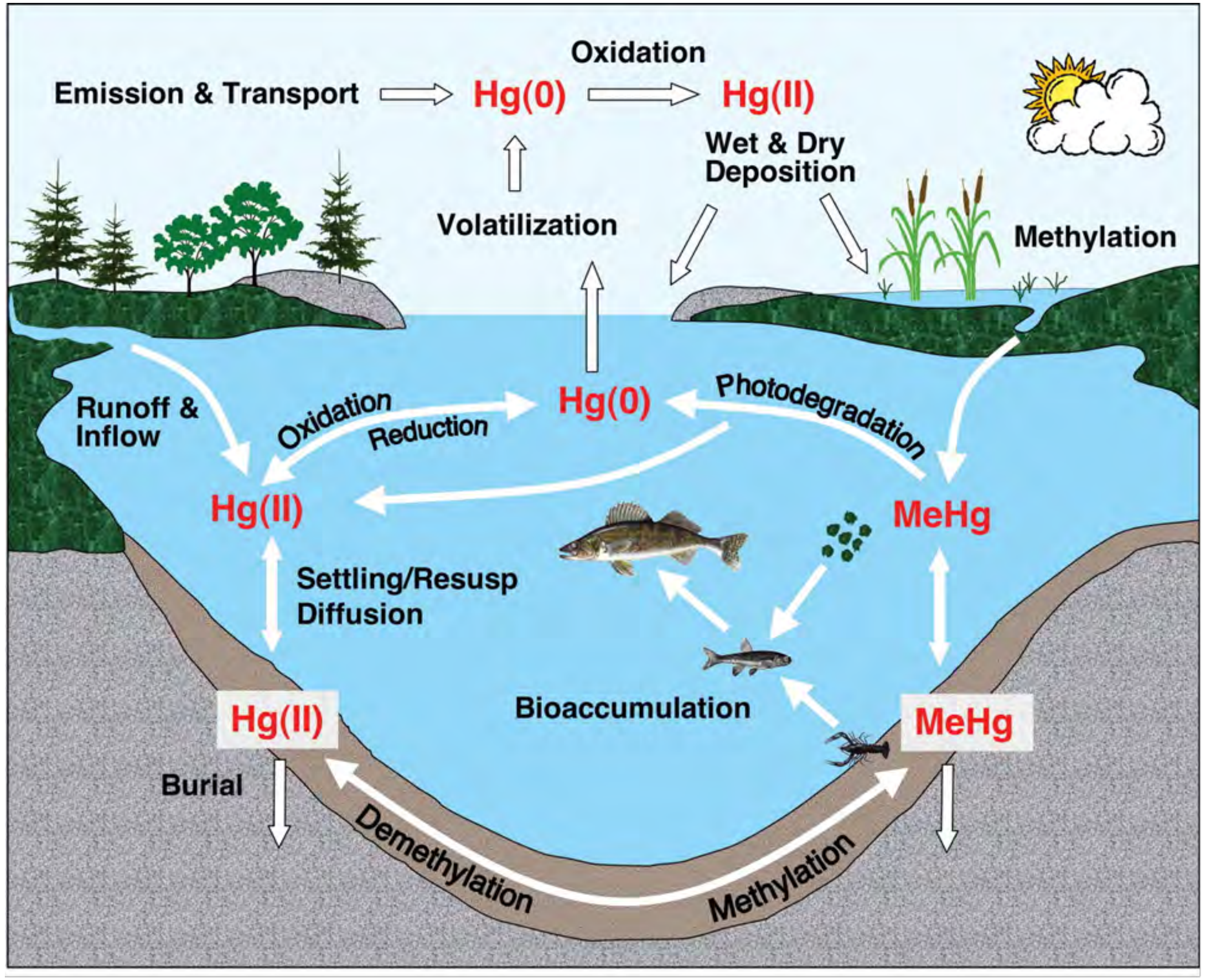
Udonna Ndu, Natalia Neal-Walthall, Marc Deshusses, DUKE UNIVERSITY
Dwayne Elias, Geoff Christensen, Caitlin Gionfriddo, OAK RIDGE NATIONAL LABORATORY

NIH National Institute of Environmental Health Sciences
Superfund Research Program

R01ES024344

DUKE
EDMUND T. PRATT, JR. SCHOOL OF ENGINEERING

Methylmercury: the driver of risk at Hg-contaminated sites



Engstrom, 2007, *PNAS*

- Mercury biomagnifies in aquatic food webs as monomethylmercury (MeHg)
- MeHg is produced by anaerobic microorganisms

Management of Mercury-Contaminated sites

Onondaga Lake (NY)
cleanup estimate: ~$500 million

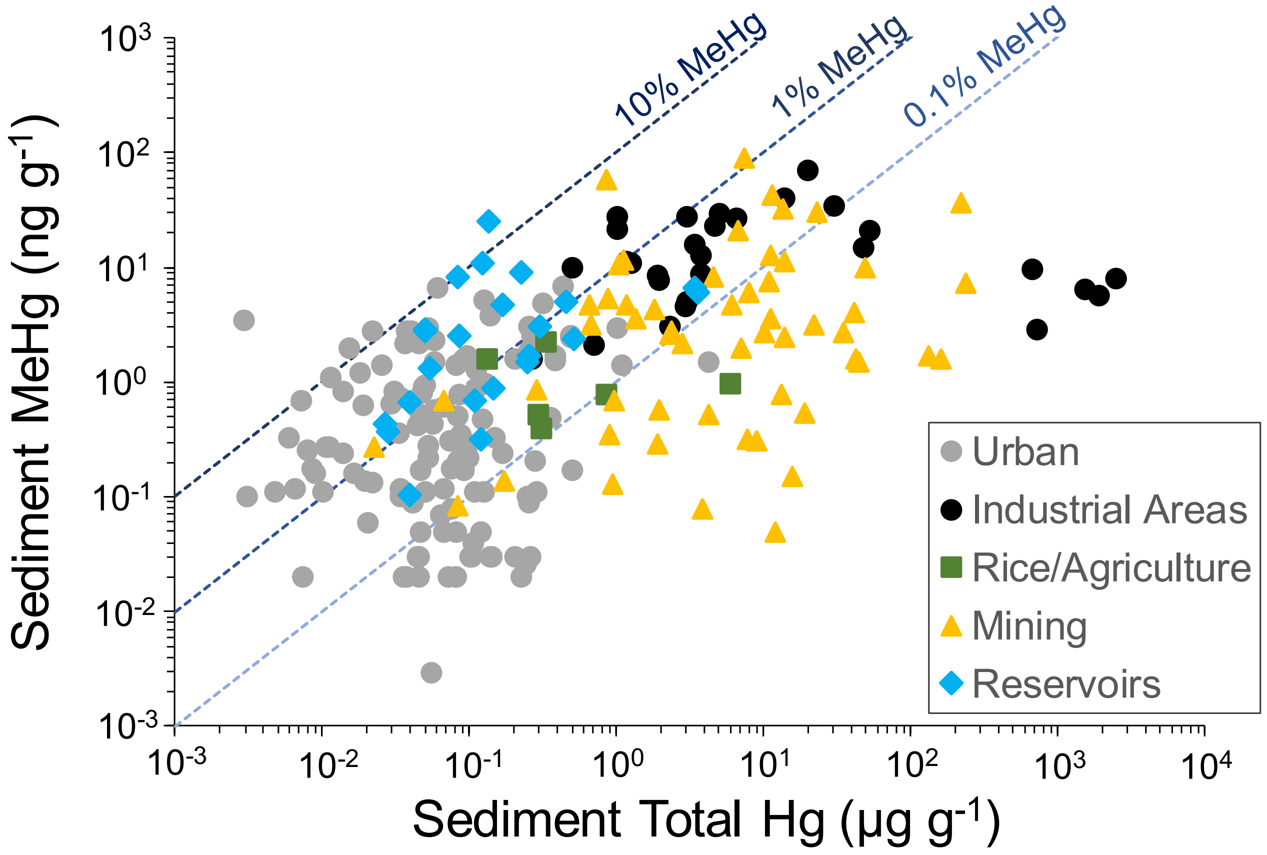
East Fork Poplar Creek (Tennessee)
cleanup estimate : ~$3 billion

Penobscot River estuary (Maine)
cleanup estimate : >$130 million

Management of Mercury-Contaminated sites

Benchmarks for Site Assessment

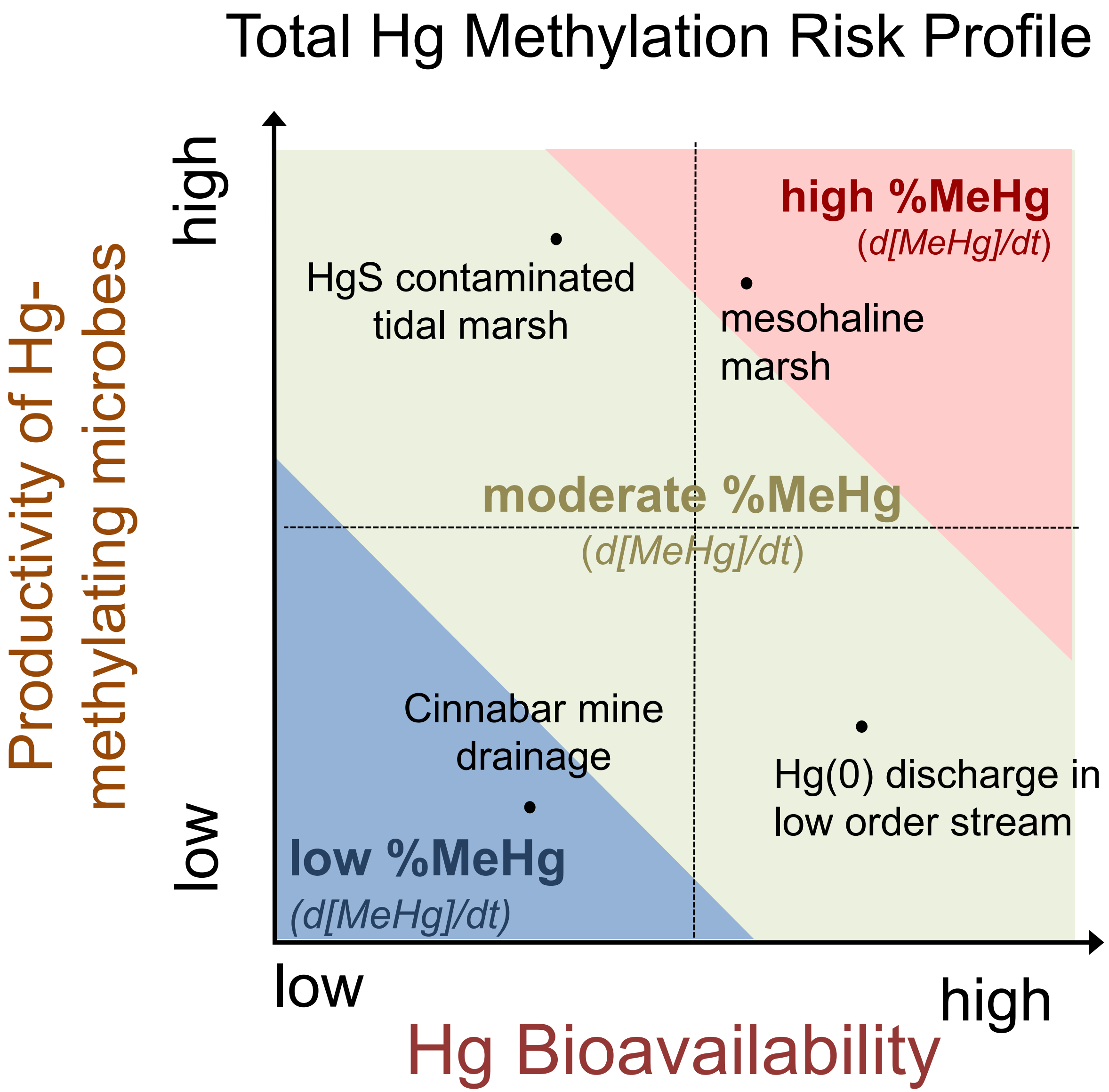
Challenges:
- Total Hg content is a poor predictor of risk
- Current water quality standard: MeHg in fish



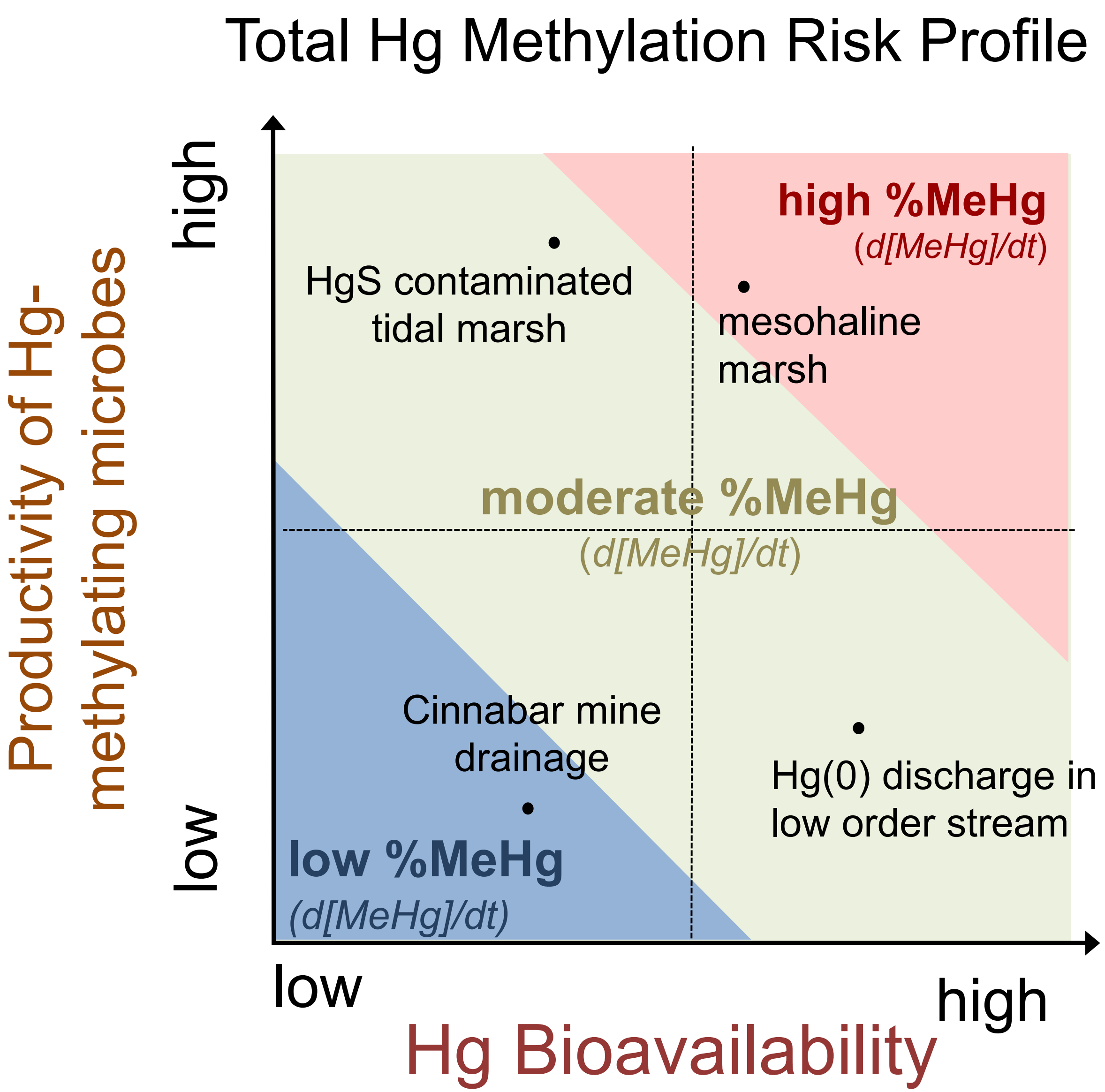
Needs:
- More functional shorter-term target for watershed management & remediation
  (e.g., Biomethylation potential of Hg)

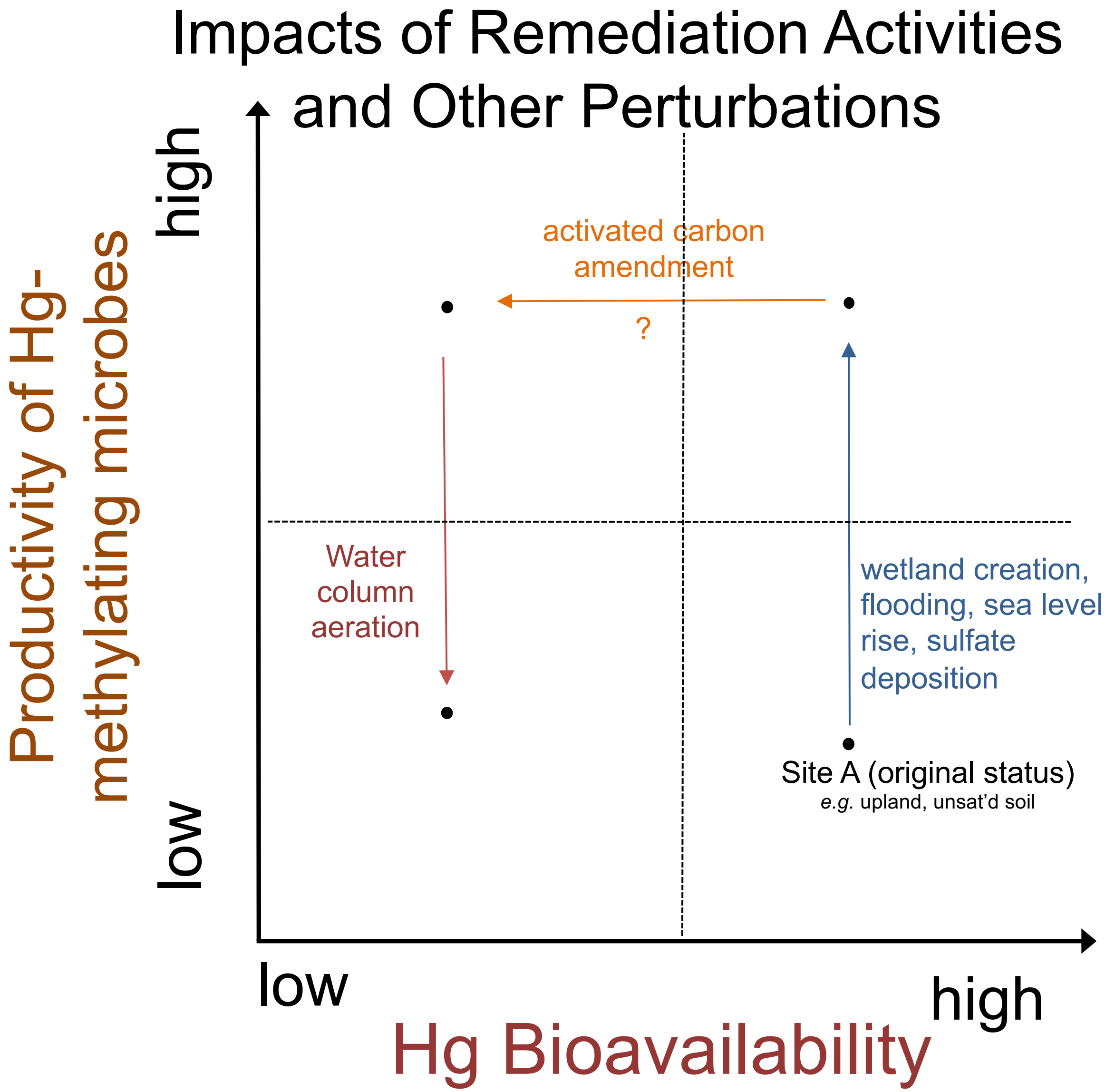
Hsu-Kim et al. (2018) Challenges and Opportunities in Managing Aquatic Mercury Pollution. *Ambio*. 47: 141-169

Why do we need a model to predict Hg methylation potential?



Why do we need a model to predict Hg methylation potential?





Methods for Quantifying Mercury Biomethylation Potential

The conventional approach: Equilibrium speciation

Particulate

Mineral phase
$HgS_{(s)}$

Sorbent
Organic matter
$FeS_{(s)}$

K_{sp} or K_d

$\rightleftarrows$

Dissolved

weakly complexed
$Hg(OH)_2$, $HgCl_2$, $HgCl_3^-$

$\downarrow\uparrow$

strongly complexed
$Hg(HS)_x$, Hg-DOM

$\longrightarrow$

Bioavailable

Hg-methylating microbes:

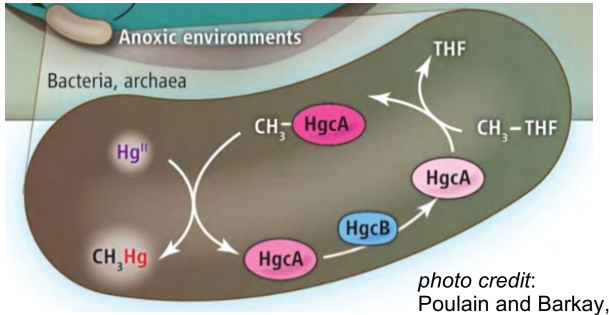


photo credit:
Poulain and Barkay, 2013, *Science*

$\longrightarrow$ **MeHg**

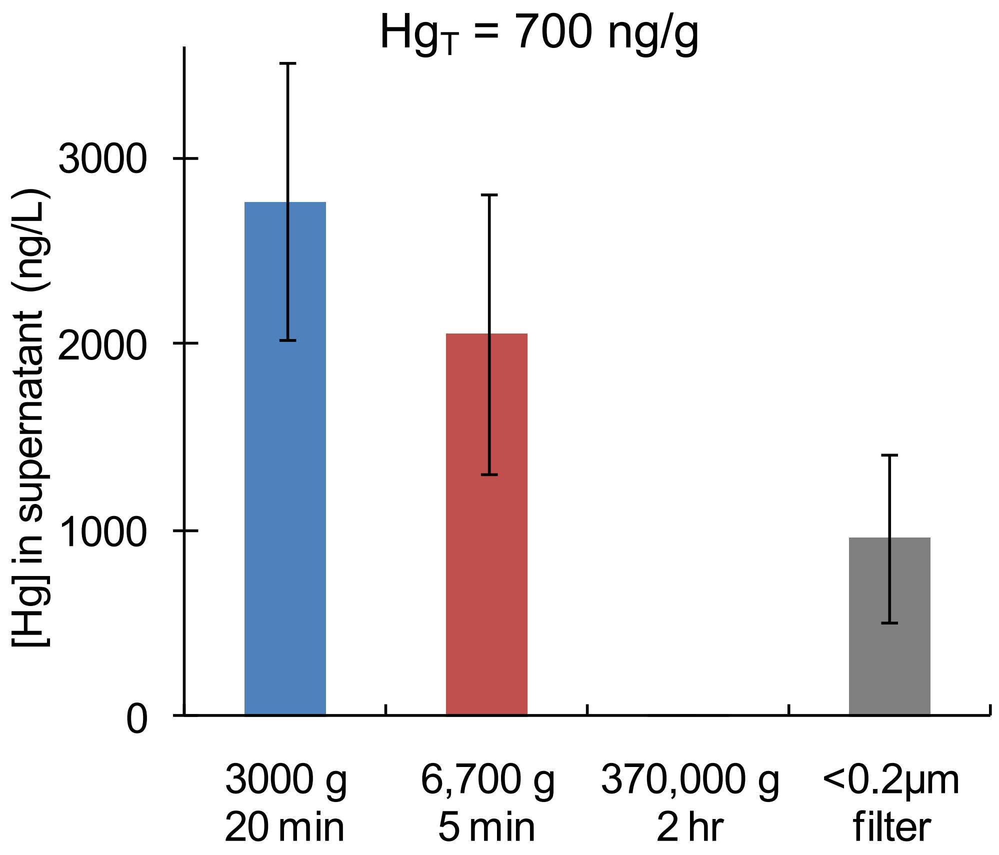
- All have the HgcA and HgcB proteins
- Ubiquitous in anaerobic niches (sulfate reducers, Fe reducers, methanogens)

Parks *et al.* 2013 *Science*
Gilmour *et al.* 2013 *ES&T*

$Hg^{2+} + xHS^- \leftrightarrow Hg(HS)_x^{2-x}$ $\quad K_{Hg(HS)x}$

$Hg^{2+} + DOM \leftrightarrow Hg\text{-}DOM$ $\quad K_{HgDOM}$

Hg speciation in benthic settings

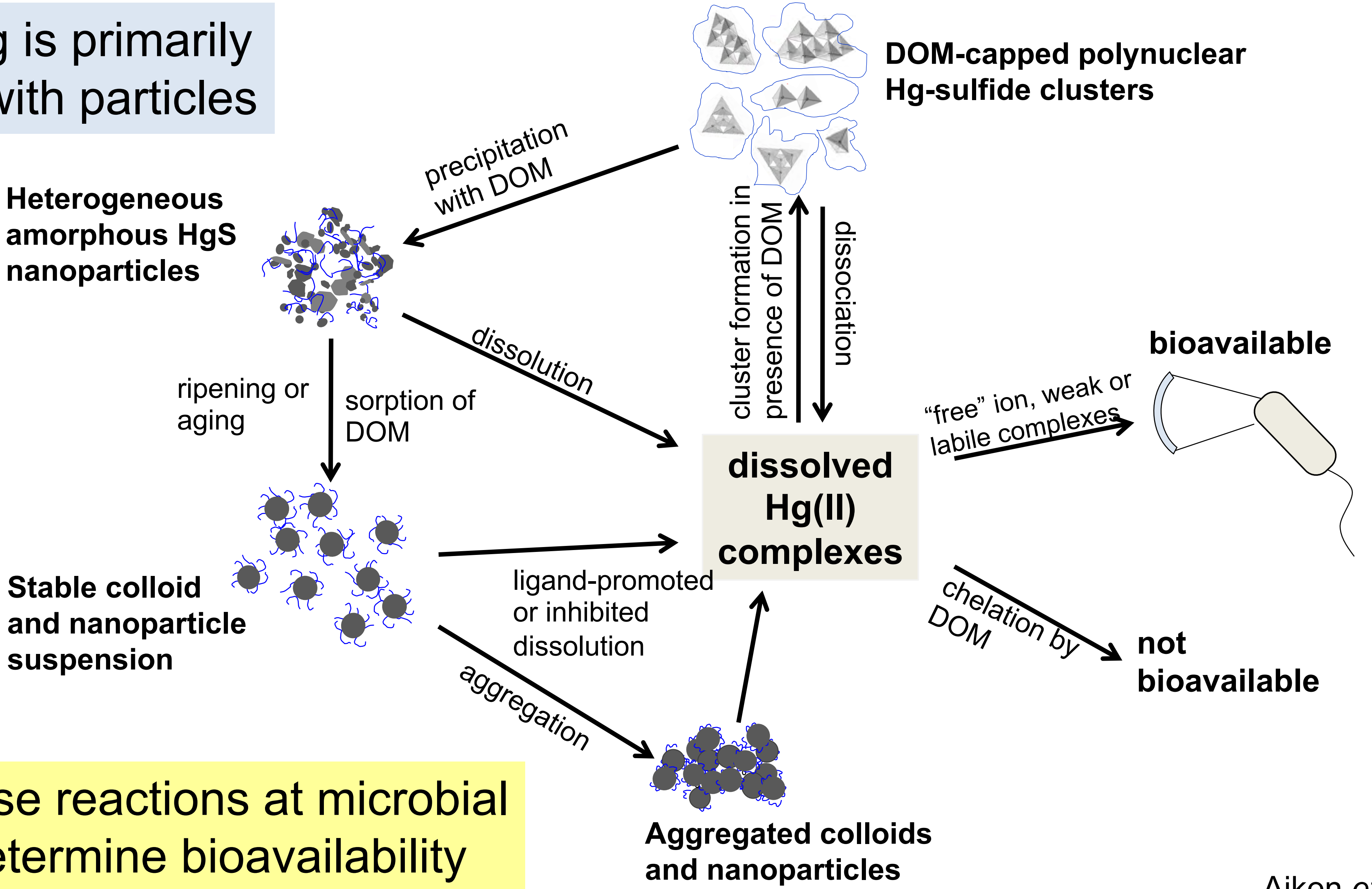


Sediment porewater of a freshwater lake

Most of the mercury in porewater is bound to particles

Bioavailability of Mercury for Methylation: An Alternative Approach

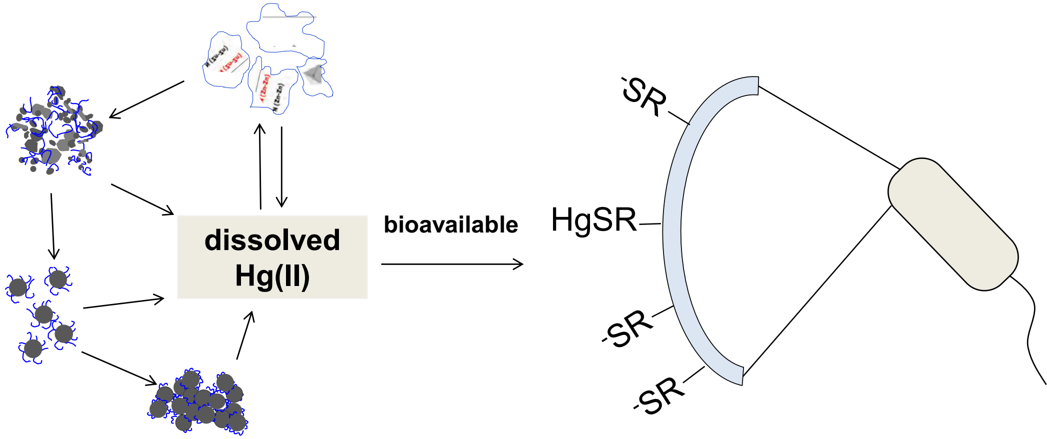
Inorganic Hg is primarily associated with particles



Rates of these reactions at microbial interfaces determine bioavailability

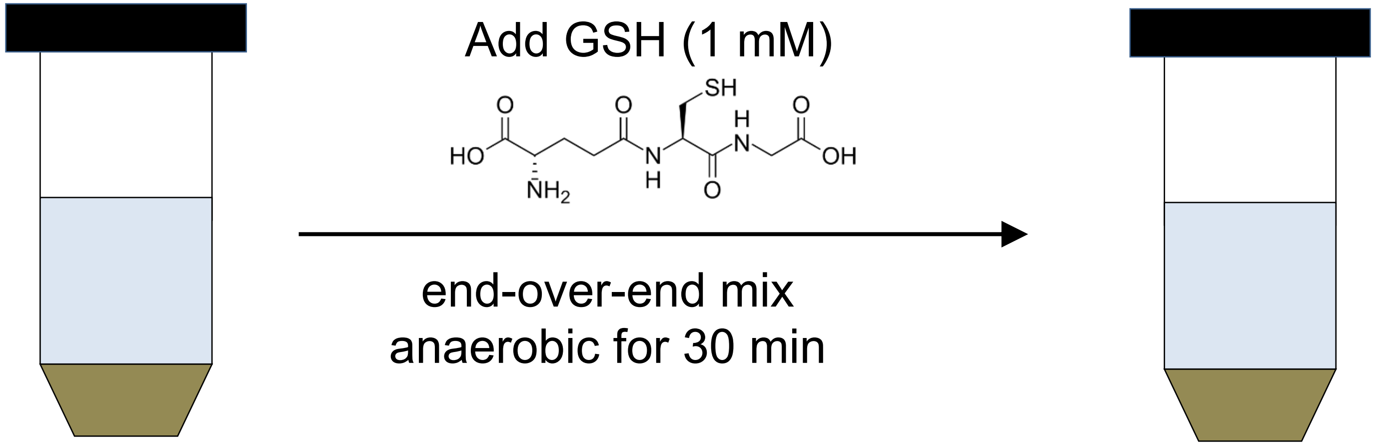
Aiken *et al.*, ES&T 2011

Methods to Quantify Hg Bioavailability



Thiol-based selective extraction

Glutathione (GSH) Extraction



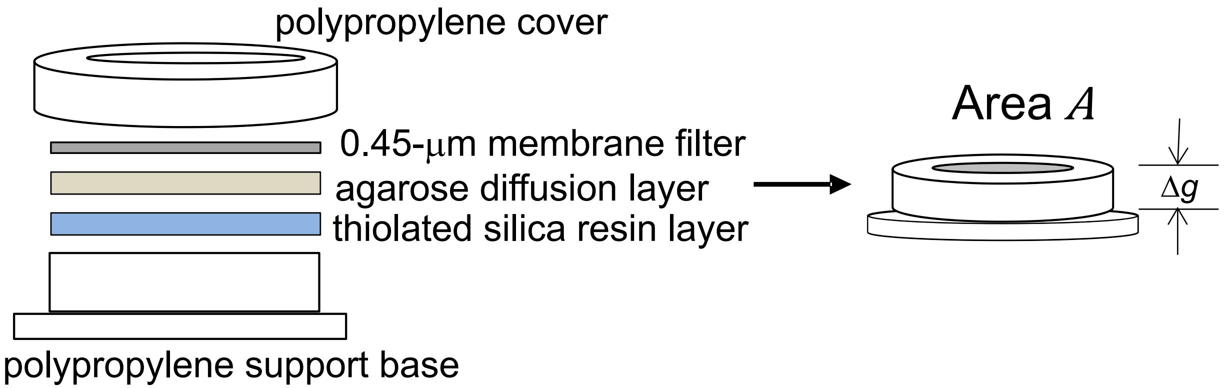
Quantify Hg in
<0.2 μm fraction

Slurry sample

Ticknor *et al., Env Engr Sci* (2015)

Methods to Quantify Hg Bioavailability

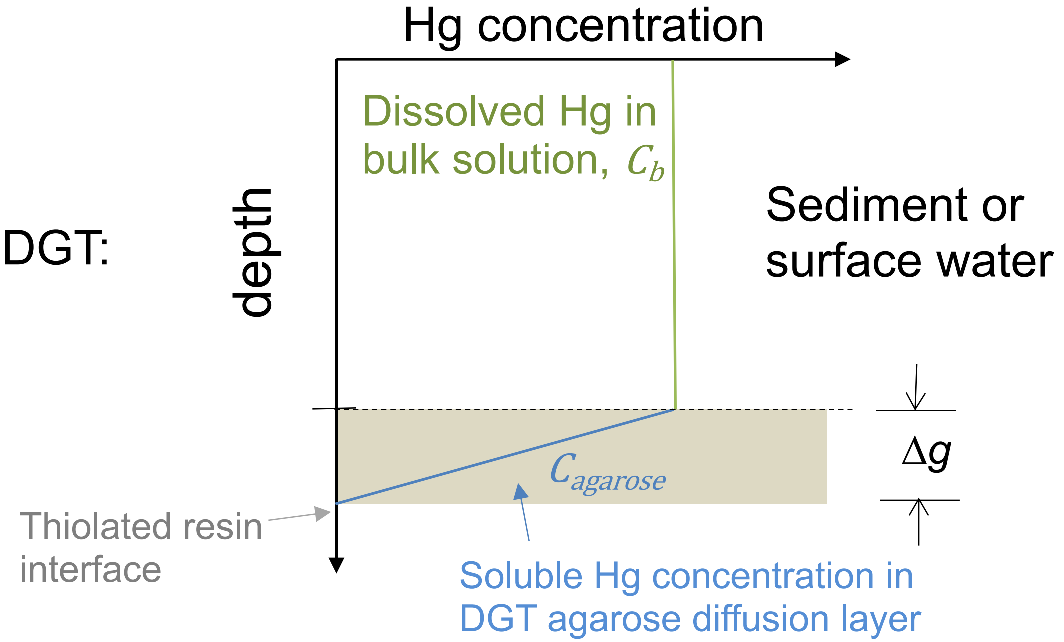
Diffusive Gradient in Thin-film (DGT) samplers



Conventional approach: derive a 'truly dissolved' concentration

$$\frac{m}{C_b} = \frac{D \times A}{\Delta g} \times t$$

Hg uptake into DGT:

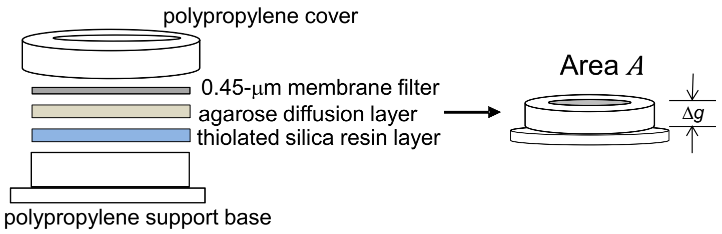


Our approach:

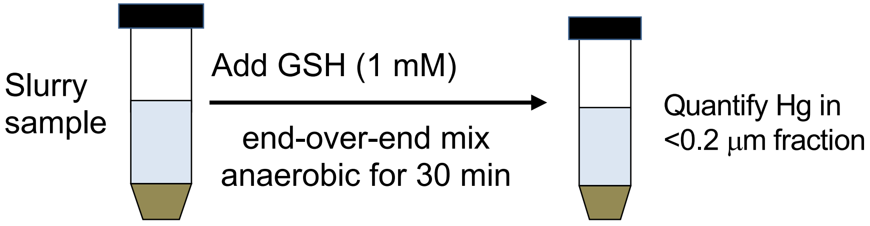
Mass of Hg uptake m $\propto$ reactive Hg fraction

Testing Methods of Quantifying Hg Methylation Potential

Diffusive Gradient in Thin-Films (DGT) passive samplers



Glutathione (GSH) Selective Extraction



Method testing: sediment microcosms

Added Hg: (100-200 ng g^{-1} dw per species)

dissolved ^{204}Hg-nitrate

dissolved ^{196}Hg-humic

^{199}Hg adsorbed to FeS

humic-coated nano-^{200}HgS

sediment slurry with DGT

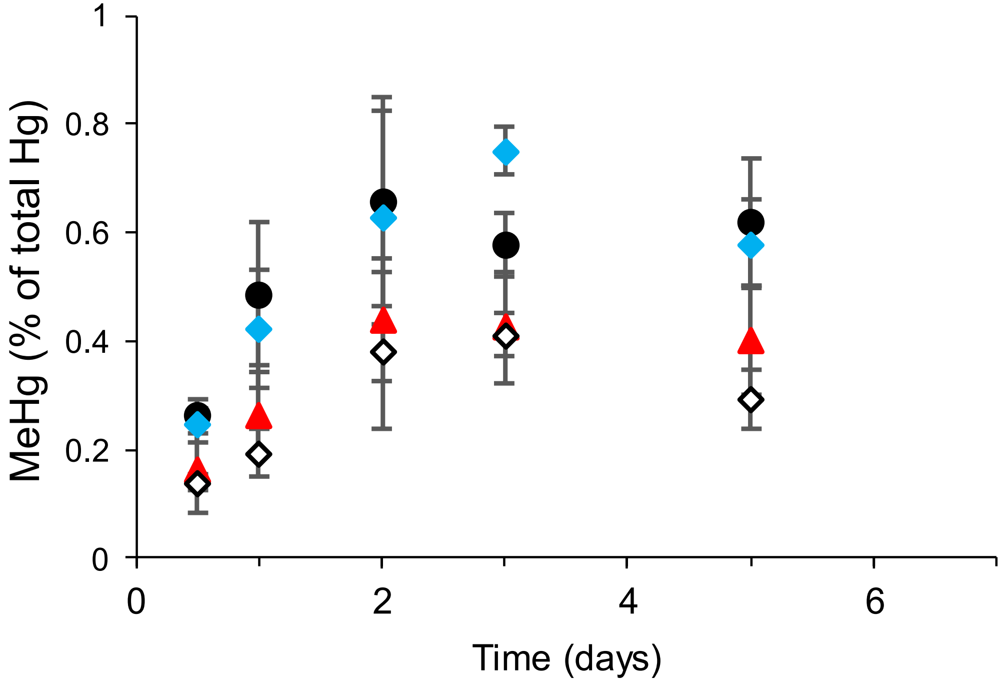
(*sample origin: tidal marsh, freshwater lake*)

Quantify over time:

- MeHg from each isotopic endmember
- Hg on DGTs
- GSH-extractable Hg fraction
- *hgcA* gene copy number and microbial community composition

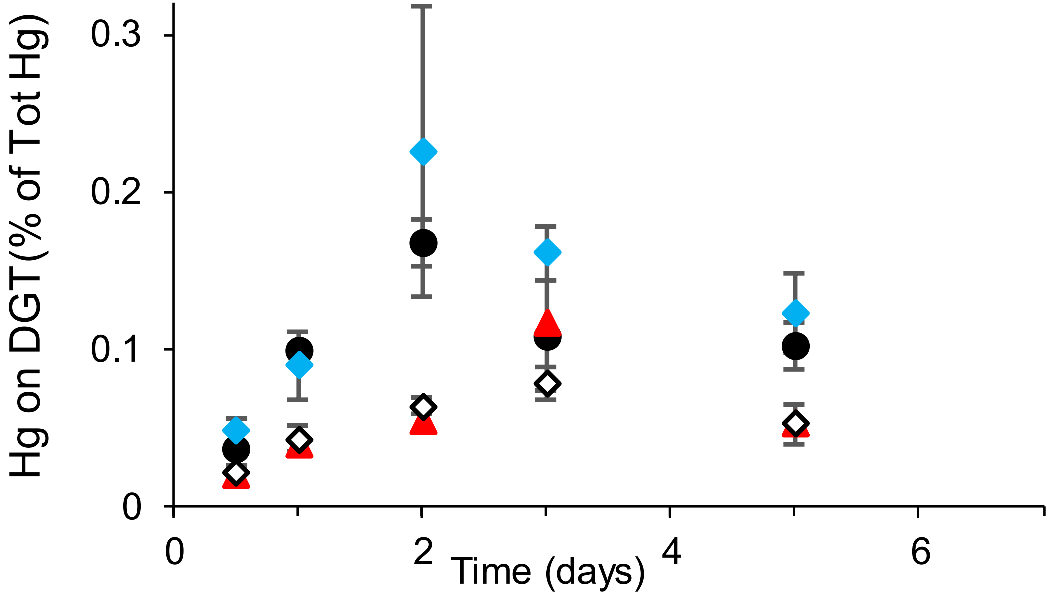
Testing Methods of Quantifying Hg Methylation Potential

Methylation of Hg added to slurries



Tidal marsh (mesohaline) sediment slurry

Uptake of total Hg in DGTs

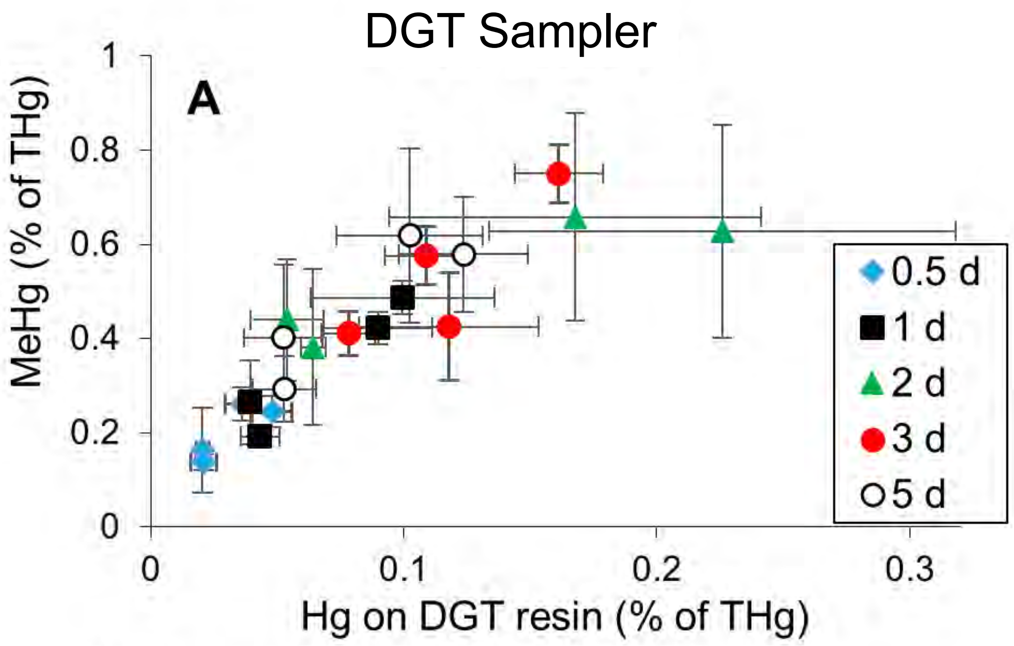


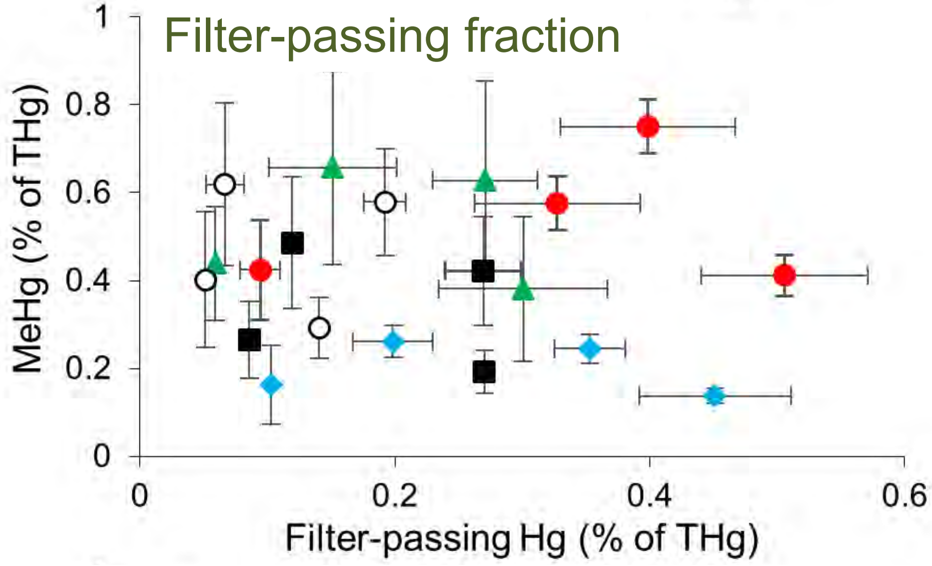
Type of Hg added:

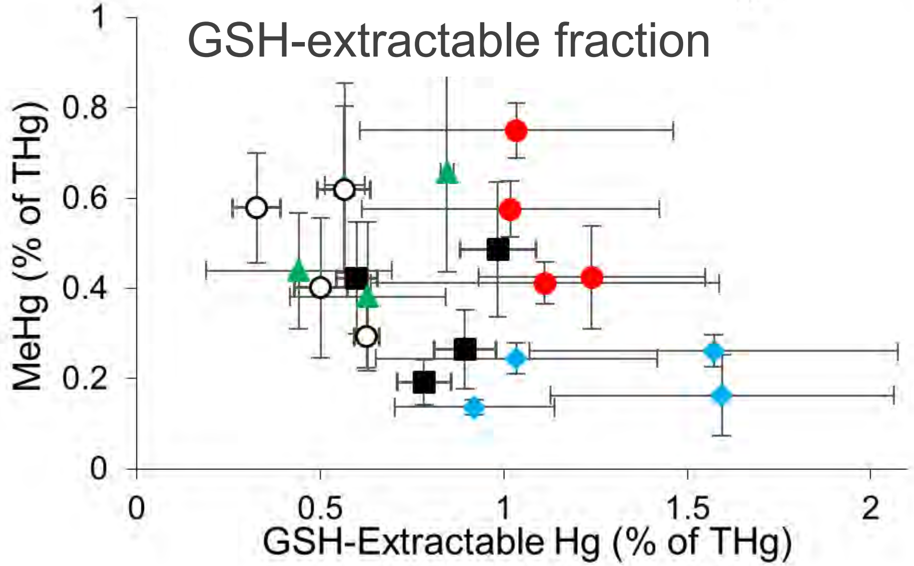
- $^{204}Hg^{2+}$
- ^{199}Hg-FeS
- ^{196}Hg-humic
- nano-^{200}HgS

Ndu *et al.*, *ES&T*, 2018.

Hg uptake in DGTs correlates with MeHg production







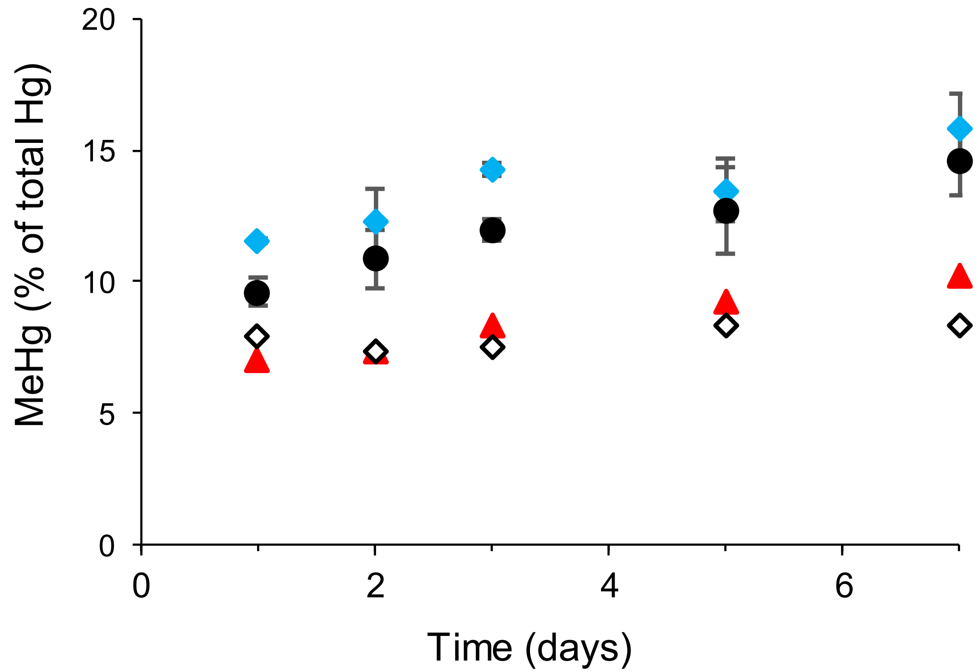
Net MeHg production:
- correlated with uptake on the DGT sampler
- did not correlate with the <0.45 μm or the GSH-extractable fraction

Ndu *et al.*, *ES&T*, 2018.

Hg uptake in DGTs correlates with MeHg production

Freshwater Lake Sediment Slurry
with 1 mM pyruvate

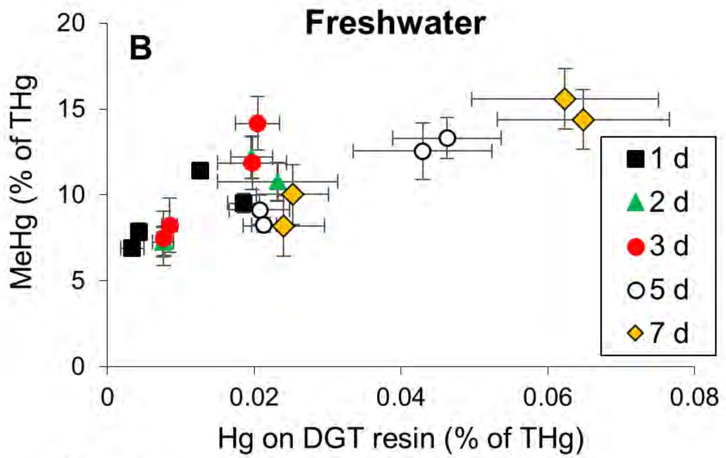
Type of Hg added:
- $^{204}Hg^{2+}$
- ^{199}Hg-FeS
- ^{196}Hg-humic
- nano-^{200}HgS

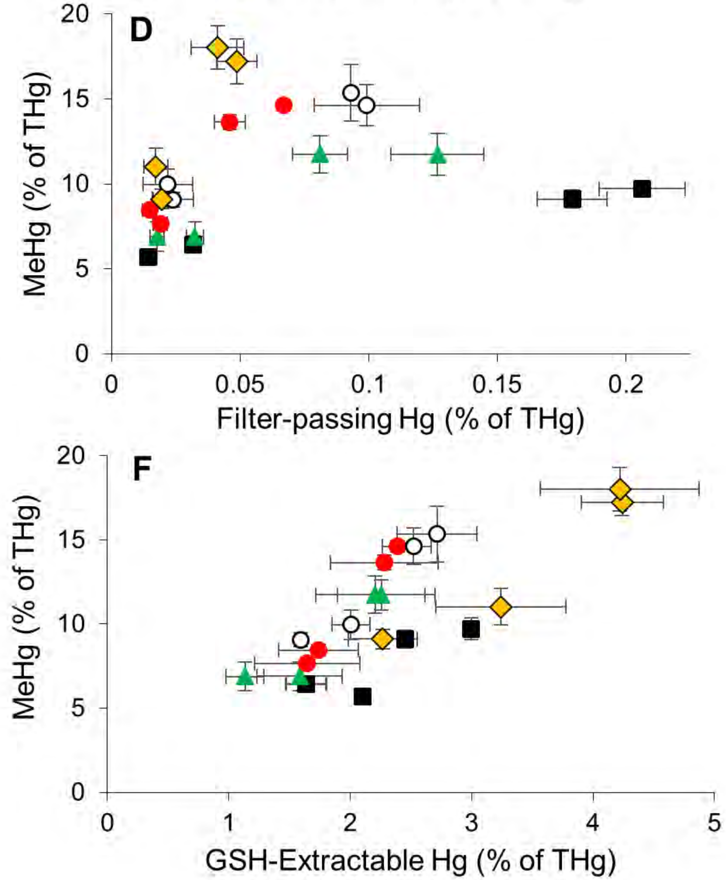


DGT Sampler

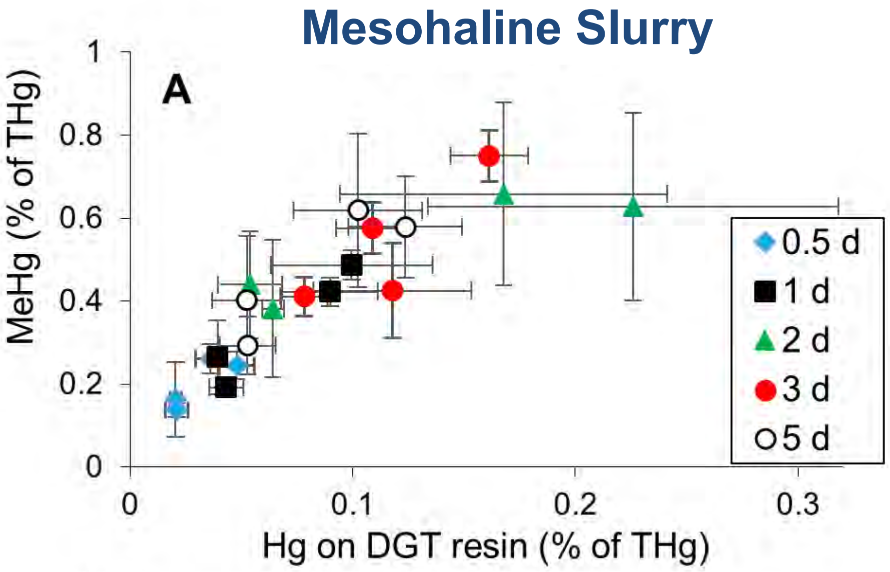
Filter-passing fraction

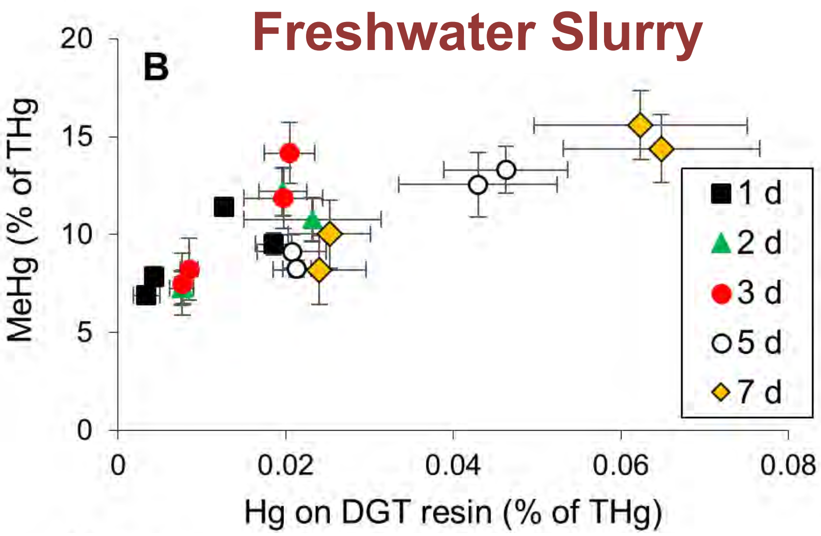
GSH-extractable fraction





Comparing the Hg-Methylating Microbial Communities

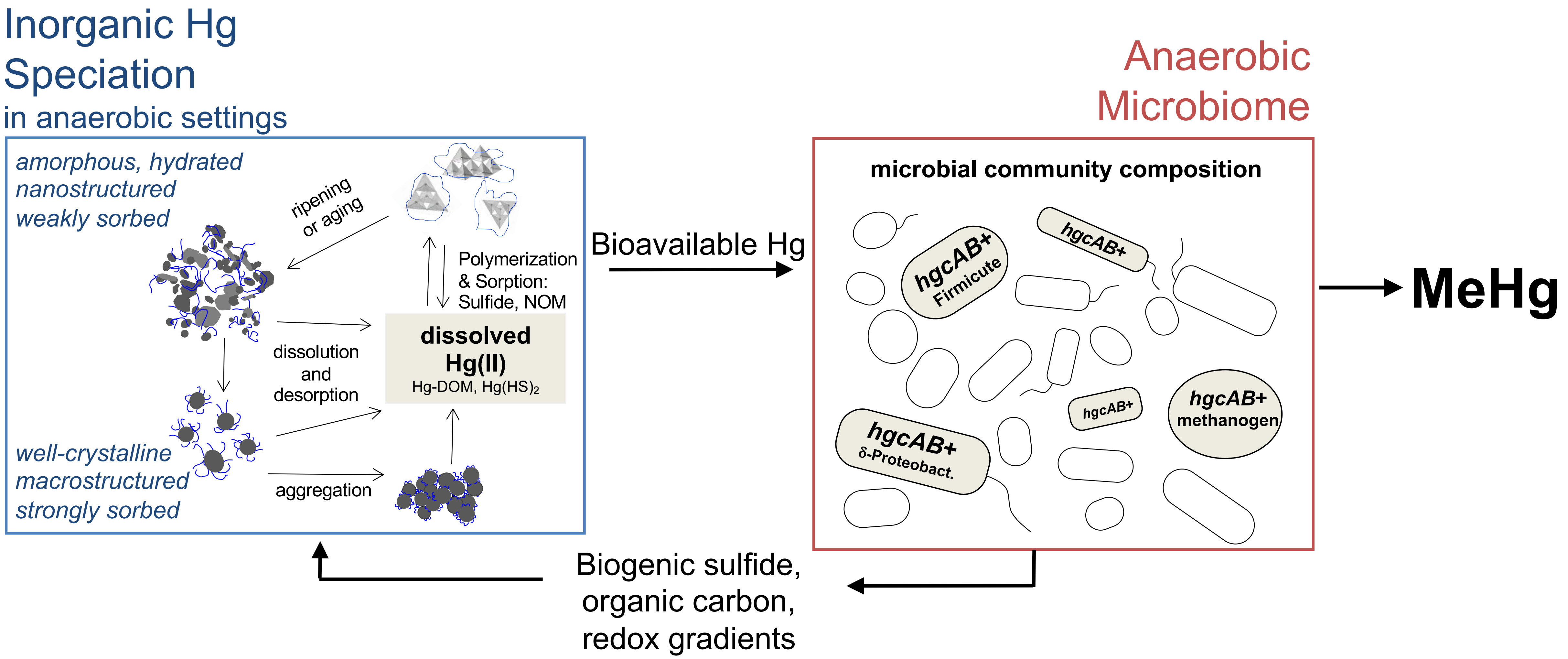




Difference because of abundance of *hgcAB*+ microbes?

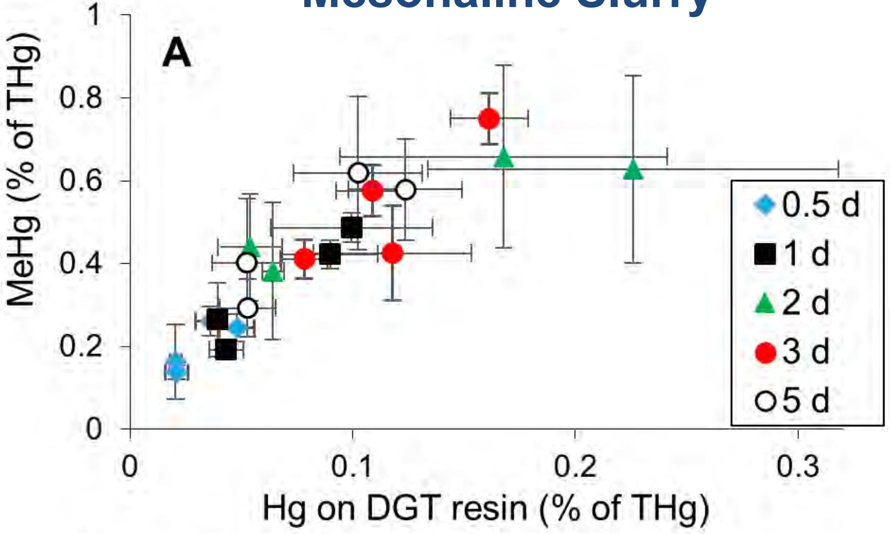
Ndu *et al.*, *ES&T*, 2018.

Geochemical vs. Microbiome Controls on Mercury Methylation

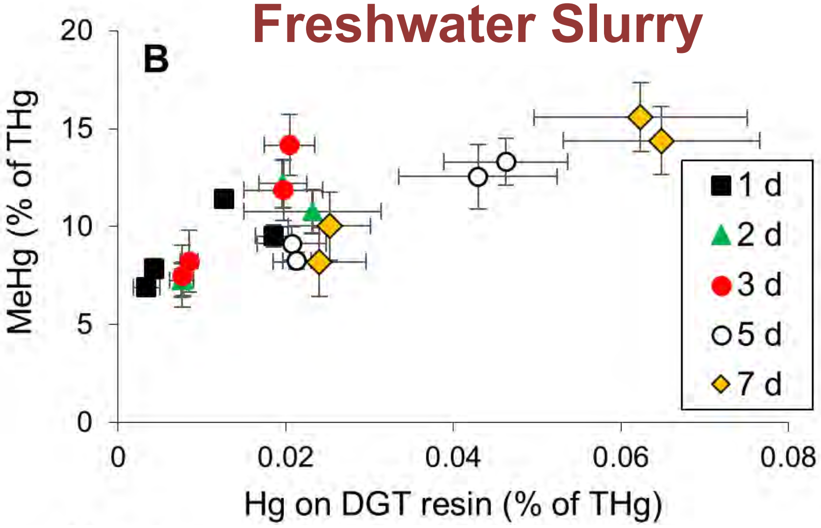


Comparing the Hg-Methylating Microbial Communities

Mesohaline Slurry

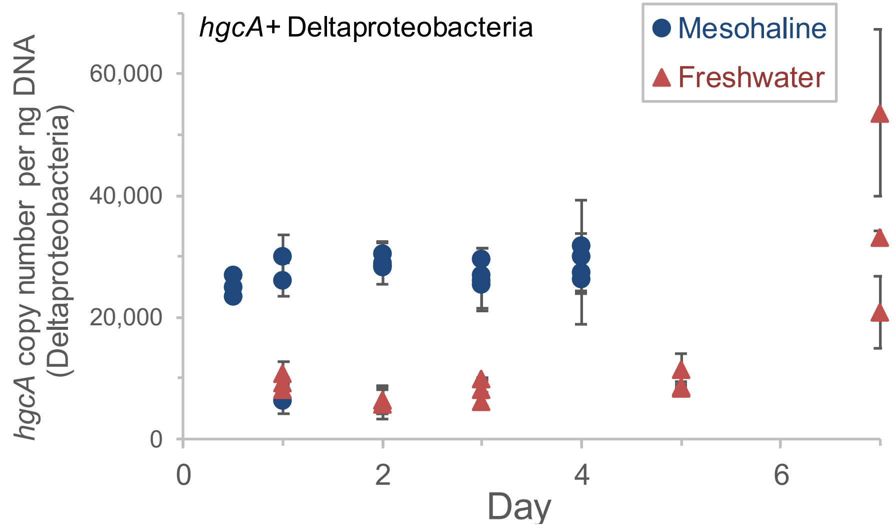


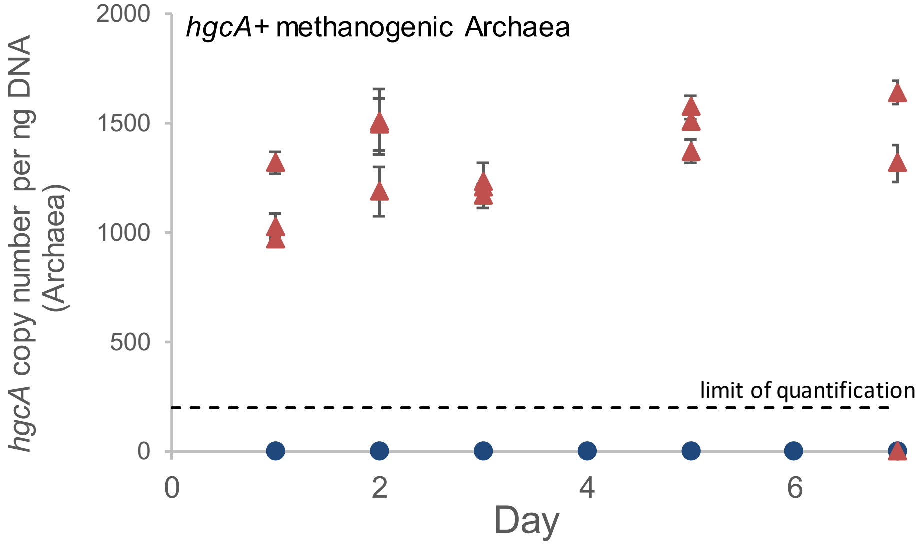
Freshwater Slurry



qPCR *hgcA genes*

Diversity and abundance of methylators from DNA-based approaches





Ndu *et al.*, *ES&T*, 2018.

Next Steps

Can DGTs work in the real world?

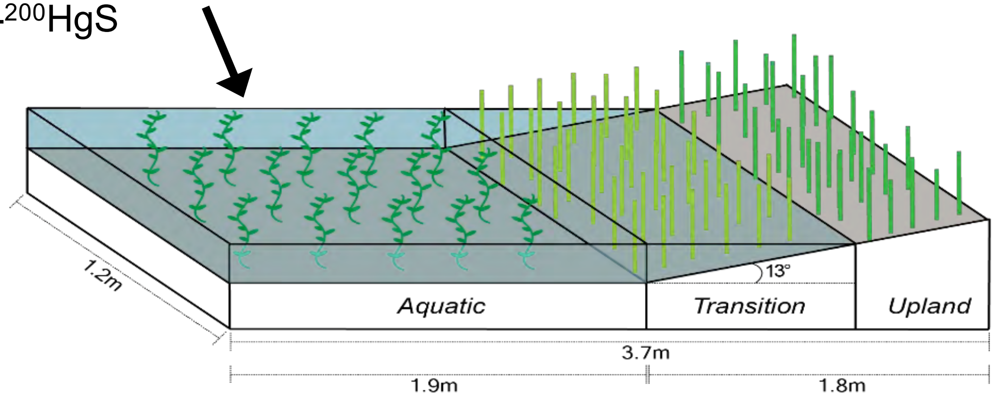
Next Steps

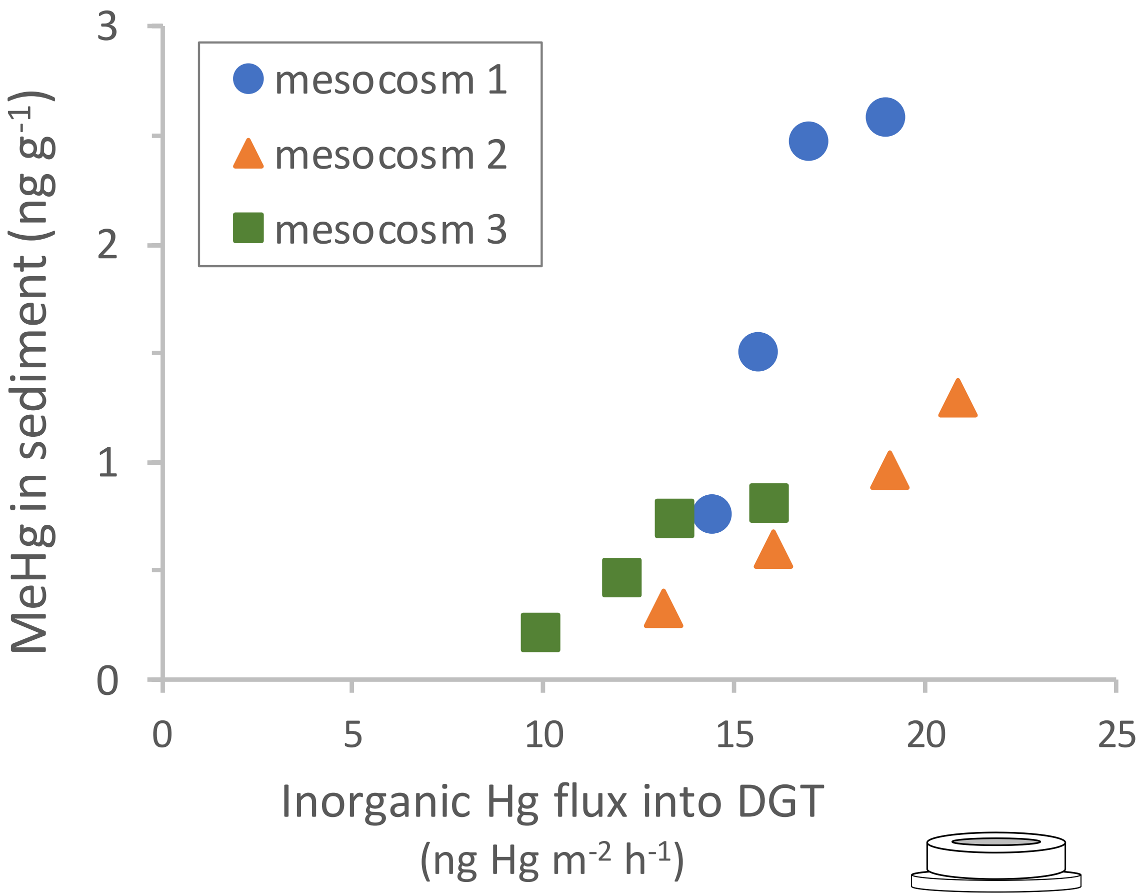
Can DGTs work in the real world?

Added Hg:

- dissolved $^{202}Hg^{2+}$
- dissolved ^{201}Hg-humic
- ^{199}Hg adsorbed to FeS
- nano-^{200}HgS

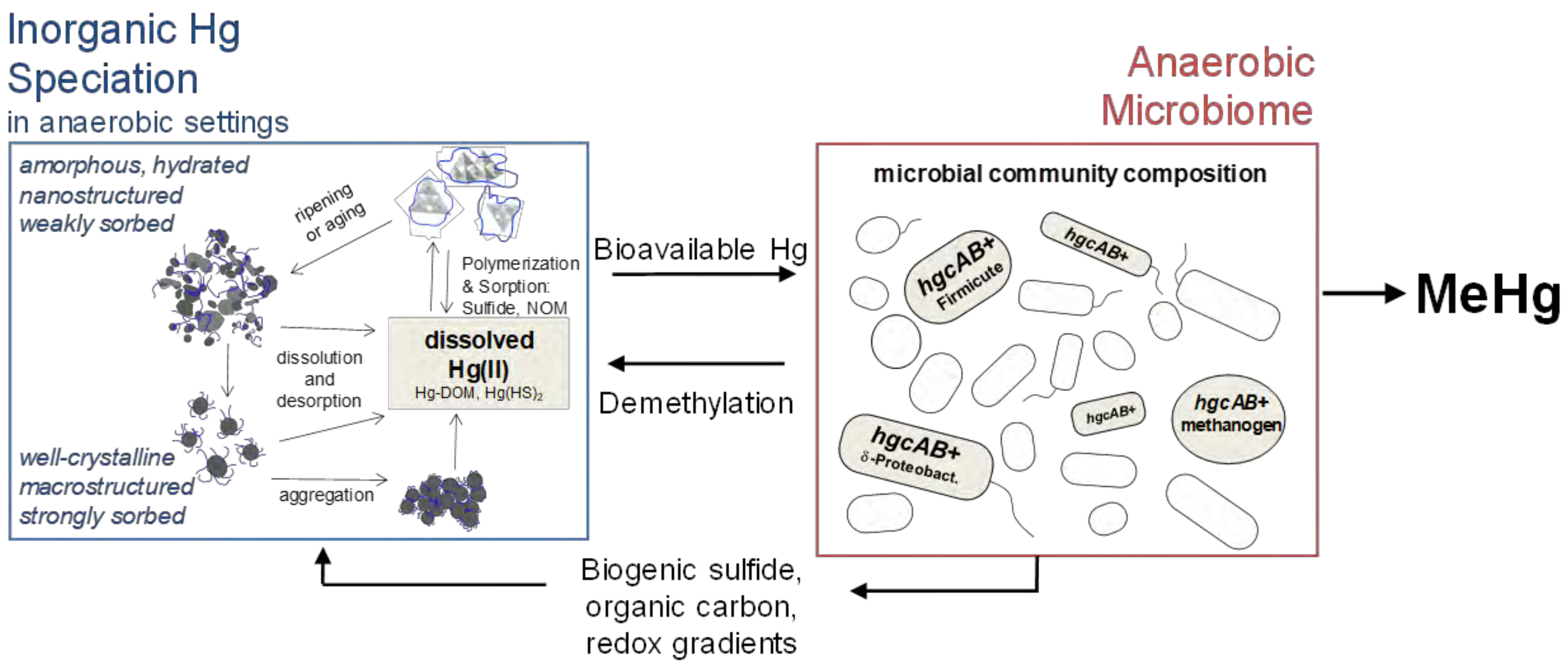
Outdoor freshwater wetland mesocosms.



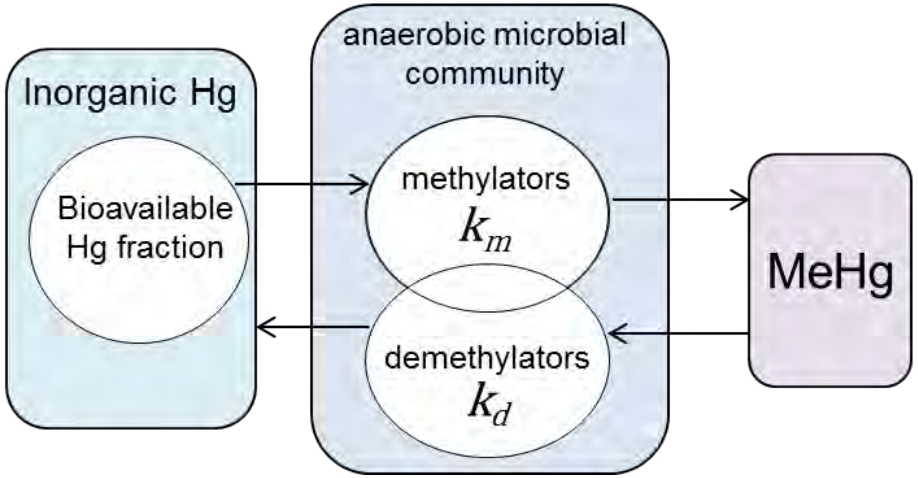


Next Steps

Model for Hg Methylation Potential



A possible simplification…..



Semi-Mechanistic Model

$$\frac{d[MeHg]}{dt} = k_m[bioavailable\ Hg\ flux] - k_d[MeHg]$$

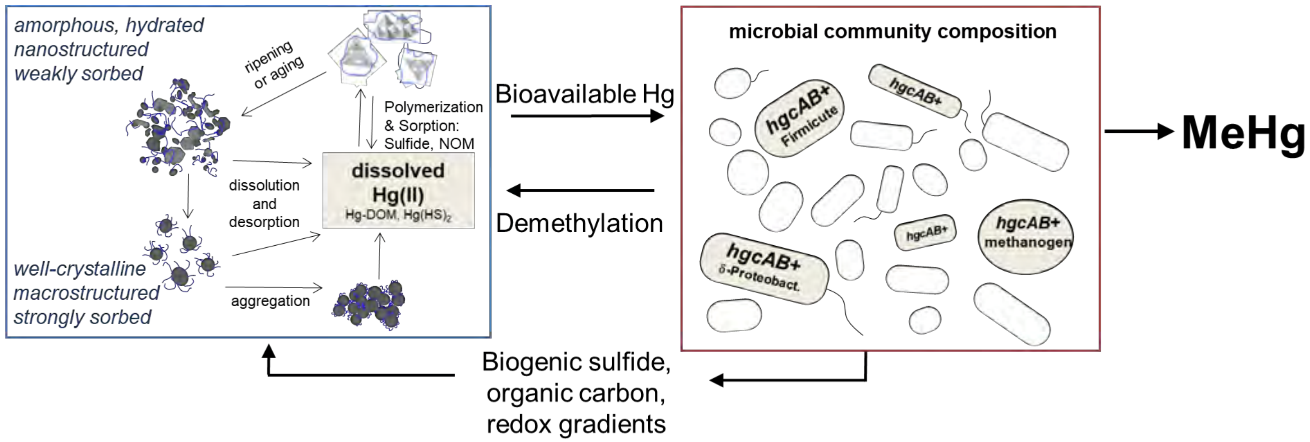
$$k_m = f\left(hgcA_{\delta-proteobact}, hgcA_{archaea}, hgcA_{firmicutes}\right)$$

$$[bioavailable\ Hg] = f(Hg\ uptake\ rate\ in\ DGT)$$

Summary

Mercury: Strategies to Quantify Methylation Potential in the Environment

- Needs for site management & remediation:
  functional measures of MeHg production potential



- Hg bioavailability for methylation: Controlled by reactivity of Hg-S-NOM phases at microbial interfaces

- Quantifying MeHg potential in ecosystems:
  1. Hg bioavailability (Hg uptake rate in DGTs)
  2. Productivity of the methylating microbiome (*hgcA* gene expression?)

Additional questions are welcome!
Helen Hsu-Kim
hsukim@duke.edu

NIH National Institute of Environmental Health Sciences
Superfund Research Program
R01ES024344

References

Aiken, G.R.; Hsu-Kim, H.; Ryan, J.N. (2011). Influence of dissolved organic matter for the environmental fate of metals, nanoparticles, and colloids. *Environ. Sci. & Technol*. *45*, 3196–3201.DOI: 10.1021/es103992s.

Engstrom, D.R., 2007. Fish respond when the mercury rises. *Proceedings of the National Academy of Sciences*, *104*(42), pp.16394-16395.

Gilmour, C.C., Podar, M., Bullock, A.L., Graham, A.M., Brown, S.D., Somenahally, A.C., Johs, A., Hurt Jr, R.A., Bailey, K.L. and Elias, D.A., 2013. Mercury methylation by novel microorganisms from new environments. *Environmental science & technology*, *47*(20), pp.11810-11820.

Hsu-Kim, H.; Eckley, C.S.; Achá, D.; Feng, X; Gilmour, C.C.; Jonsson, S.; Mitchell, C.P.J. (2018). Challenges and Opportunities for Managing Aquatic Mercury Pollution in Altered Landscapes. *Ambio*. 47(2). 141-169. DOI: 10.1007/s13280-017-1006-7

Hsu-Kim, H.; Kucharzyk, K.H.; Zhang, T.; Deshusses, M.A. (2013). Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: A critical review. *Environ. Sci. & Technol*. 47(6), 2441-2456. DOI: 10.1021/es304370g.

Ndu, U.; Christensen, G.A.; Rivera, N.A.; Gionfriddo, C.M.; Deshusses, M.A.; Elias, D.A.; Hsu-Kim, H. (2018). Quantification of Mercury Bioavailability for Methylation Using Diffusive Gradient in Thin-Film Samplers. *Environ. Sci. & Technol*. 52, 8521-8529. DOI: 10.1021/acs.est.8b00647

Parks, J.M., Johs, A., Podar, M., Bridou, R., Hurt, R.A., Smith, S.D., Tomanicek, S.J., Qian, Y., Brown, S.D., Brandt, C.C. and Palumbo, A.V., 2013. The genetic basis for bacterial mercury methylation. *Science*, *339*(6125), pp.1332-1335.

Poulain, A.J. and Barkay, T., 2013. Cracking the mercury methylation code. *Science*, *339*(6125), pp.1280-1281.

Ticknor, J.L; Kucharzyk, K.H.; Porter, K.A.; Deshusses, M.A.; Hsu-Kim, H. (2015). Thiol-based selective extraction assay to comparatively assess bioavailable mercury in sediments. *Environ. Engr. Sci.* 32(7), 564-573. DOI: 10.1089/ees.2014.0526

Zhang, T.; Kim, B.; Levard, C.; Reinsch, B.C.; Lowry, G.V.; Deshusses, M.A.; Hsu-Kim, H. (2012). Methylation of mercury by bacteria exposed to dissolved, nanoparticulate, and microparticulate mercuric sulfides. *Environ. Sci. & Technol*. 46(13), 6950-6958. DOI: 10.1021/es203181m