Biogeochemical Transformations at Critical Interfaces

ORNL Mercury Science Focus Area (SFA) 2019 Annual Report

Determining the mechanisms and environmental controls on mercury fate and transformation in streams

Mercury Program Overview

Freshwater resources supplied by streams and their surrounding watersheds are vital to the global economy. Before the 20th century, the water supplied by these systems was sufficient to support a range of services (e.g., energy production and human consumption). However, demand for these resources has grown dramatically because of population growth, industrialization, and expansion of irrigated agriculture. Furthermore, as demand increases, the availability of clean water is diminishing because of severe pollution from anthropogenic releases of nutrients and trace metals such as mercury (Hg).

The economic and societal importance of headwater streams and their surrounding watersheds is further exemplified using the Tennessee River Basin (TN river basin) (Fig. 1). This river basin, located in the southeastern United States, consists of a series of nested watersheds that encompasses portions of seven states (Tennessee, Virginia, North Carolina, Alabama, Georgia, Mississippi, and Kentucky) and supports ~4.5 million people by supplying water for power generation, industry, recreation, agriculture, and human consumption (Bohac and Bowen 2012). The basin provides ~8% of the U.S. total power via thermoelectric and hydroelectric plants (ranking fourth in overall power production). Furthermore, the TN river basin, its associated headwater streams, and their surrounding watersheds represent the most intensively used freshwater Water Resource Region in the contiguous United States, with estimated withdrawals of >280,000 gallons a day per square mile.

Headwater streams and their surrounding watersheds supply a significant portion of the liquid water available for

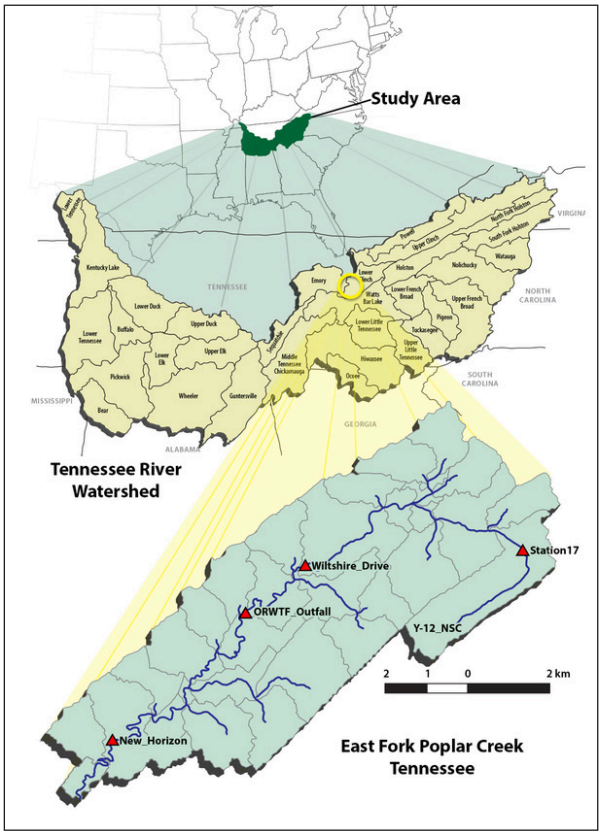

Fig. 1: Study Area of the Critical Interfaces Science Focus Area. East Fork Poplar Creek (EFPC), which is part of the 40,910-square-mile Tennessee River watershed, is the representative test bed system being studied. This watershed and its associated streams represent the most intensively used freshwater Water Resource Region in the contiguous United States, with estimated withdrawals of >280,000 gallons a day per square mile. Shown here is the river basin as 32 segmented, 8-digit hydrologic unit codes (HUC-8). The EFPC watershed is nested in the Lower Clinch HUC-8.

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human consumption. This limited supply is being threatened by severe pollution from anthropogenic releases of nutrients and trace metals, especially in cultivated and urban environments (Vorosmarty et al. 2005). This is especially true for the trace metal Hg, where anthropogenic inputs (2.7 to 27.3 tons per year primarily from coal-fired power plants) are significantly greater than releases from natural sources (0 to 4.9 tons per year; Tercier-Waeber and Taillefert 2008; Pacyna et al. 1995). Mercury is a pervasive global pollutant that can be methylated to form toxic methylmercury (MeHg), which bioaccumulates in aquatic food webs, endangering humans and other biota. With over 9,000 impacted systems in the continental United States, Hg is the second leading cause of impaired waters—including locations in the TN river basin—and is responsible for fish consumption advisories in all 50 states (U.S. EPA 2013, 2011). Developing scientific approaches that enable a deeper understanding of pollutant cycling in streams and their surrounding watersheds is critical for preserving freshwater resources.

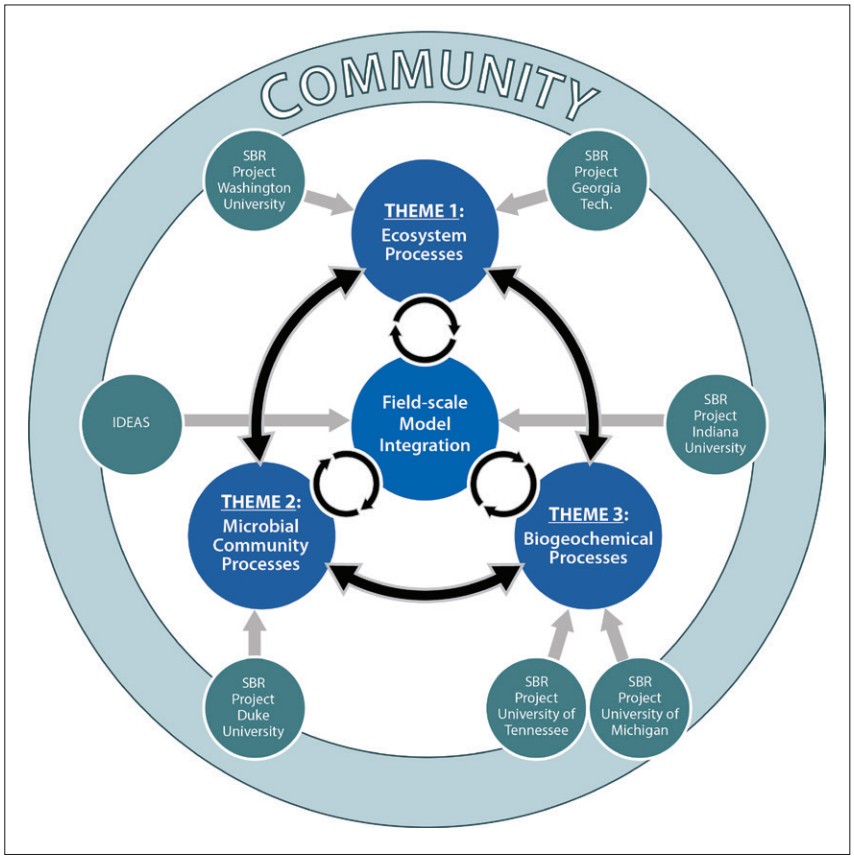


Fig. 2: Integrated, Multiscale SFA Research. Illustration showing the interconnections between themes, modeling activities, Interoperable Design of Extreme-scale Application Software (IDEAS), and various SBR university projects.

To enable a predictive understanding of Hg cycling in stream systems both locally and globally, the Biogeochemical Transformations at Critical Interfaces in a Mercury Perturbed Watershed Scientific Focus Area (Critical Interfaces SFA) is providing foundational insight on exchange and feedback processes occurring at critical interfaces that control mercury fate and transformation (Fig 2). This project, led by Oak Ridge National Laboratory, is supported by the Subsurface Biogeochemical Research (SBR) program within the Department of Energy's (DOE) Office of Biological and Environmental Research (BER).

Systems-level understanding of stream function has evolved from one of a passive conduit draining its watershed to one that hosts hot spots of biogeochemical transformations that exert a controlling influence on water quality and ecosystem health. Deeper understanding is needed, however, of the mechanisms operating in transient storage zones (TSZs) surrounding the stream channel where prolonged contact times between water and sites of high biogeochemical activity occur. TSZs are locations where the downstream movement of water is delayed in comparison to the main channel flow. Metabolically active transient storage zones (MATSZs), a subset of TSZs that are microbially active, are important hot spots where a substantial portion of carbon, nutrient, and trace metal processing occurs, influencing stream biogeochemistry and, ultimately, downstream water quality.

Integrated, Multiscale Research Approach

Developing a predictive understanding of Hg and, more broadly, trace element transport and fate in these environmental systems requires deciphering complex processes (i.e., physical, chemical, and biological), deconvoluting how these processes interact with one another, and understanding the factors that control system response over broad spatiotemporal scales.

Critical Interfaces SFA (CI-SFA) research encompasses three themes—ecosystem processes, microbial community processes, and biogeochemical processes—and a research activity involving field-scale model integration (Fig. 2).

- **Ecosystem Processes.** Through a combination of field- and laboratory-scale studies, research investigates Hg biogeochemical transformations in hyporheic zone sediments and the influence of nutrient additions on net MeHg production and microbial community composition in field-derived periphyton biofilms.
- **Microbial Community Processes.** Research seeks to (1) understand the contributions of known Hg-methylating organisms to observed Hg methylation rates and extents in biofilm lifestyles using synthetic and natural microbial communities; (2) determine the breadth and depth of Hg-methylating species; and (3) determine the biochemical roles of the proteins (HgcA and HgcB) that facilitate MeHg production.
- **Biogeochemical Processes.** Research elucidates key biogeochemical mechanisms controlling Hg bioavailability and microbial transformation of inorganic Hg to MeHg in simplified, but field-relevant, laboratory experiments. Activities include (1) investigating complex biogeochemical processes and their interactions controlling Hg species transformation and availability for cellular uptake and methylation and (2) using molecular-scale computational approaches to elucidate key biogeochemical mechanisms governing Hg speciation and microbial transformation.
- **Field-Scale Model Integration.** Improves stream reach-to-watershed reactive transport modeling of contaminant and nutrient export. Activities include estimating the volume of TSZs and MATSZs in East Fork Poplar Creek (EFPC) and mass transfer between TSZs and the creek channel using nonreactive and reactive tracers to parameterize the field-scale model.

This annual report summarizes the CI-SFA accomplishments from June 2018 to June 2019, a period representing the first year following the program's triennial peer review in May 2018 and acceptance of the revised plan in September 2018 by the SBR program within DOE BER.

Scientific Progress

Theme 1: Ecosystem Processes

Theme 1 research examines the biogeochemical controls on Hg methylation and demethylation within the context of the flowing creek system and its connection with the surrounding watershed. Emphasis is on field-based investigations with supporting laboratory work to elucidate mechanisms. Our overarching goals are (1) to identify ecosystem domains and hydro-biogeochemical conditions that govern net MeHg concentrations in EFPC and (2) to work iteratively with ongoing field-scale modeling activities to inform and support the biogeochemical modeling framework. The following questions are being addressed:

- What biogeochemical factors or characteristics affect net MeHg production in EFPC transient storage zones?
- What are the distinguishing biogeochemical properties of Hg-methylating transient storage zones in EFPC sediments?
- Among the known Hg-methylating clades (Deltaproteobacteria, Firmicutes, Archaea), is one clade dominant in EFPC sediments?
- How do nutrient levels in EFPC affect periphyton community structure and function with specific emphasis on Hg cycling reactions? How do these characteristics change with changing nutrient levels?

FY18–FY19 Accomplishments

Over the past 12 months, Theme 1 made significant progress toward milestones, publishing several papers relating to the role of periphyton in Hg cycling and developing predictive models of those reactions. Additional papers have been published reporting on the effect of Hg(II) sorption on MeHg production and on improvements to predicting the equilibrium aqueous speciation of Hg. In addition to journal articles, Theme 1 also has released a number of data products publicly.

Role of Periphyton in EFPC Mercury Cycling

Previous work in EFPC led us to hypothesize that key controls on net methylation occur within the stream or on the stream bed and, specifically, that periphyton may play an important role in MeHg production. This hypothesis was tested by measuring the rate of Hg methylation and MeHg demethylation using periphyton samples collected from the field. Between-site differences in net methylation for samples collected from an upstream versus downstream location were driven by differences in the demethylation

rate constant (k_d). In contrast, the within-site seasonal difference in net methylation was driven by changes in the methylation rate constant (k_m). Samples incubated in the dark had lower net methylation due to k_m values that were 60% less than those incubated in the light. Disrupting the biofilm structure decreased k_m by 50% and resulted in net demethylating conditions. Overall, the measured rates resulted in a net excess of MeHg generated and suggest that intact, actively photosynthesizing periphyton biofilms harbor zones of MeHg production, possibly making a substantial net positive contribution to the creek's MeHg budget (Olsen et al. 2016).

Laboratory measurements of Hg methylation often exhibit kinetics that are inconsistent with first-order kinetic models. Using time-resolved measurements of filter-passing Hg and MeHg during methylation/demethylation assays, a multisite kinetic sorption model, and re-analyses of previous assays, we showed that competing kinetic sorption reactions can lead to time-varying Hg and MeHg availability and apparent non-first-order kinetics in Hg methylation and MeHg demethylation. Our new transient availability model employing a multisite kinetic sorption model for Hg and MeHg describes the range of behaviors for time-resolved methylation/demethylation data reported in the literature including those that exhibit non-first-order kinetics. Additionally, we showed that neglecting competing sorption processes can confound analyses of methylation/demethylation assays, resulting in rate-constant estimates that are systematically biased low. Simulations of MeHg production and transport in a hypothetical periphyton biofilm bed illustrated the implications of our new model and demonstrated that methylmercury production may be significantly different than projected by single-rate first-order models (Olsen et al. 2018).

We examined periphyton MeHg production across seasons, locations, and light conditions using mercury stable isotopes. Methylation and demethylation rate potentials were calculated using the transient availability kinetic model ($k_{m,\ trans\ av,}$ and $k_{d,\ trans\ av,}$ respectively). Light exposure and season were significant predictors of $k_{m,\ trans\ av,}$ with greater values in full light exposure and in the summer. Season, light exposure, and location were significant predictors of $k_{d,\ trans\ av,}$ which was highest in dark conditions, in the spring, and at the upstream location. Light exposure was the controlling factor for net MeHg production, with positive production for periphyton grown under full light exposure and net demethylation for periphyton grown in the dark. Transient availability rate potentials were 15 times higher for k_m and 9 times higher for k_d compared to the standard practice of calculating these values using full availability models ($k_{m,\ full\ av}$ and $k_{d,\ full\ av}$) calculated at 1 day. Using the full availability approach there were no significant predictive relationships among rate potentials and environmental factors (Fig. 3). Our results underscore the importance of applying transient availability kinetics to MeHg production data when estimating MeHg production potential and flux (Schwartz et al., in review).

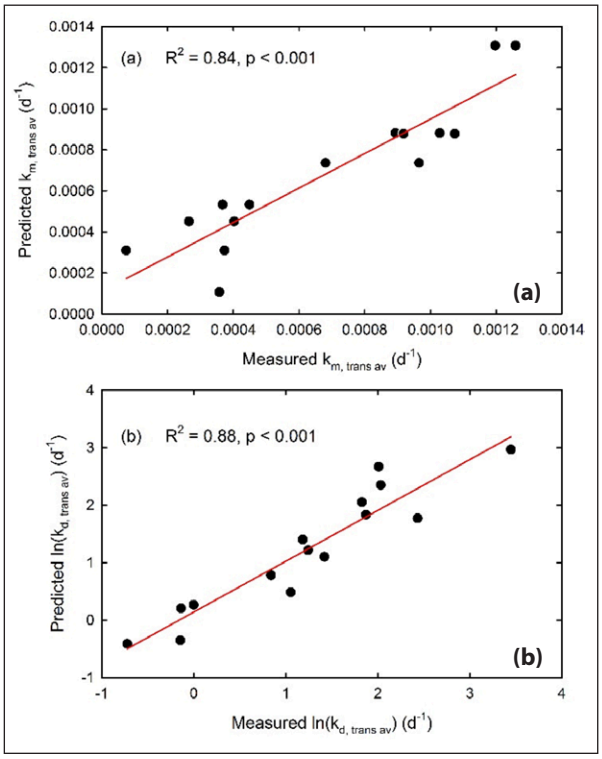


Fig. 3: Modeling Advance. Significant predictive modeling output for **(a)** methylation and **(b)** demethylation rate potential per day (d^{-1}) determined from the transient availability model (Olsen et al. 2018; Schwartz et al., in review). No predictive relationship could be developed using traditional modeling approaches.

Status of FY19 Milestones

Milestone 1a. Conduct studies of Hg methylation and MeHg demethylation potential with stream sediments. We have completed these methylation/demethylation assays as a function of sediment texture. Ancillary experimental data are being collected to refine the structure of and to parameterize our transient availability model as it applies to sediments.

Milestone 1b. Sediment microbial community structure and function; hgcAB phylogeny and abundance. Sediment samples from our methylation/demethylation assays have been provided to Theme 2 for analysis.

Milestones 1c and 1d. *In situ* and *ex situ* translocation experiments and community analyses. We have conducted several trials to refine methods and approaches

for these experiments. Upgrades to the Aquatic Ecology Lab, which are critical to successful completion of these studies, are on schedule to be completed by mid- to late November 2019.

FY20 Plans

In FY20, Theme 1 planned activities include:

- Continue long-term stream gauging and water quality monitoring activity.
- Complete studies to parameterize the transient availability model for sediments and prepare manuscript.
- Continue preliminary experiments in support of the translocation studies.

Manuscripts

Published or In Press

Guo, L., S. L. Painter, S. C. Brooks, J. M. Parks and J. C. Smith. 2019. "A probabilistic perspective on thermodynamic parameter uncertainties: Understanding aqueous speciation of mercury." *Geochimica et Cosmochimica Acta*. *In press*.

McManamay, R. A., F. Linam, T. J. Mathews, S. C. Brooks, and M.J. Peterson. 2019. "Scaling mercury biodynamics from individuals to populations: Implications of an herbivorous fish on mercury cycles in streams." *Freshwater Biology* **64**(5):815–31. DOI: 10.1111/fwb.13265.

Muller, K A., and S. C. Brooks. 2018. "Effectiveness of sorbents to reduce mercury methylation." *Environmental Engineering Science* **36**(3):361–71. DOI: 10.1089/ees.2018.0375.

Dickson, J. O., M. A. Mayes, S. C. Brooks, T. L. Mehlhorn, K. A. Lowe, J. K. Earles, L. Goñez-Rodriguez, D. B. Watson, and M. J. Peterson. 2019. "Source relationships between streambank soils and streambed sediments in a mercury-contaminated stream." *Journal of Soils and Sediments* **19**(4):2007-2019. DOI: 10.1007/s11368-018-2183-0.

Pathak, A., R. Jaswal, P. Stothard, S. Brooks, and A. Chauhan. 2018. "Draft genome sequence of *Pseudomonas* sp. strain B1 isolated from a contaminated sediment." *Genome Announcement* **6**(25):e00518–18. DOI: 10.1128/genomeA.00518-18.

Submitted or In Preparation

Muller, K. A., C. C. Brandt, and S. C. Brooks. "Methylmercury sorption onto engineered materials." *Journal of Environmental Management*. *In revision*.

Schwartz, G. E., T. A. Olsen, K. A. Muller, and S. C. Brooks. "Ecosystem controls on methylmercury production by periphyton in a contaminated freshwater stream: Implications for predictive modeling." *Environmental Toxicology and Chemistry*. *In review.*

Eller, V. A., T. L. Mehlhorn, S. C. Brooks, D. P. Harper, M. A. Mayes, E. M. Pierce, M. J. Peterson, and A. Johs. "Evaluation of sorbent materials for removal of mercury from contaminated freshwater ecosystems." *Science of the Total Environment*. *In review*.

Pathak A., M. Agarwal, R. Jaswal, S. Brooks, X. Xu, C. Jagoe, and A. Chauhan. "Comparative proteogenomics of three mercury resistant strains isolated from two DOE contaminated ecosystems." *Cells*. *In review*.

Pathak A., R. Jaswal, S. Brooks, X. Xu, and C. Jagoe. "Metagenomics-based multi-taxonomic survey of the soil microbiota as a function of variable mercury gradients." *Frontiers in Microbiology*. *In review.*

Data Products Released

1. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2015. doi:10.12769/1490688
2. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2016. doi:10.12769/1490689
3. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2017. doi:10.12769/1490690
4. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2018. doi:10.12769/1490691
5. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 16.2 Water Year 2015. doi:10.12769/1490692
6. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 16.2 Water Year 2016. doi:10.12769/1490694
7. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 16.2 Water Year 2017. doi:10.12769/1490695
8. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 16.2 Water Year 2018. doi:10.12769/1490696
9. Riscassi, Ami L., and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2012. doi:10.12769/1489524
10. Riscassi, Ami L., and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2013. doi:10.12769/1490223
11. Riscassi, Ami L., and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2014. doi:10.12769/1489825
12. Riscassi, Ami L., Kenneth A. Lowe, and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2015. doi:10.12769/1489828
13. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2016. doi:10.12769/1489830
14. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2017. doi:10.12769/1489831

15. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2018. doi:10.12769/1489832

16. Riscassi, Ami L., and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2012. doi:10.12769/1490225

17. Riscassi, Ami L., and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2013. doi:10.12769/1490227

18. Riscassi, Ami L., and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2014. doi:10.12769/1490228

19. Riscassi, Ami L., Kenneth A. Lowe, and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2015. doi:10.12769/1490231

20. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2016. doi:10.12769/1490234

21. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2017. doi:10.12769/1490236

22. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2018. doi:10.12769/1490237

Theme 2: Microbial Community Processes

The overall goals of Theme 2 are to (1) understand the mechanisms of Hg methylation at the molecular scale and the consequences to the cell in planktonic and biofilm lifestyles, whether in isolation, synthetic, or natural microbial communities; (2) determine the breadth and depth of Hg-methylating species; and (3) elucidate the biochemical roles of HgcA and HgcB. Our research is designed to answer the following questions:

- How widespread is the ability to methylate Hg, and what are the relative contributions from different microbial clades to the overall net pool of MeHg generated in different types of environments, specifically in EFPC?
- What genes and metabolic traits are required for function and maintenance of *hgcAB*?
- What environmental conditions alter HgcAB expression?
- What is the biochemical (native) function of HgcA and HgcB in the absence of Hg?
- Can sequence-inferred HgcAB structural models provide a mechanistic framework for testing structure function hypotheses of Hg binding, methylation, and potential involvement of other proteins in the methylation process?
- Do mutations to *hgcAB* affecting Hg methylation also change organismal fitness under certain environmental conditions?
- Does the overall cellular metabolism and MeHg generation change in multispecies cultures versus single organism cultures?

FY18–FY19 Accomplishments

Over the past 12 months, Theme 2 published a number of manuscripts and made significant progress toward milestones, including (1) determining alternative (native) functions of HgcAB, (2) developing an accurate 3D structural model of the HgcAB complex, and (3) identifying and isolating novel Hg methylators from EFPC sediment.

The native biochemical function of HgcAB remains elusive, and its identification is important to understand what controls Hg methylation and how metabolic states and environmental conditions impact activity. The model systems previously used (*Desulfovibrio* ND132 and *Geobacter sulfurreducens*) have relatively elaborate metabolisms, complicating efforts to identify alternate HgcAB functions. We know HgcAB has moved across microbial genomes through independent horizontal gene transfer (HGT) events (Podar et al. 2015). Certain species pairs suggest recent pathway gain or loss, with examples in the genera *Desulfovibrio*, *Desulfobulbus* (Deltaproteobacteria), *Desulfosporosinus*, *Desulfitobacter*, *Clostridium* (Firmicutes), and *Methanocella* (Archaea). If HgcAB is specifically associated with other genes as part of a physiological pathway, such genes would also be lost or gained. In FY19, we made progress elucidating which genes localize with *hgcAB* during HGT, thereby giving the recipient organism the ability to methylate Hg. We also conducted physiological/metabolic experiments with follow-on omics analysis to determine the carbon pathways used by HgcAB. By determining the co-localizing genes and differential expression, we are gaining clues as to the native function of *hgcAB*. We evaluated the methylation activity of *Desulfobulbus oligotrophicus*, which did not produce MeHg. This is the first report of an environmental (versus mammalian microbiome) derived *Desulfobulbus* that does not methylate Hg. We performed an evolutionary analysis of HgcAB using all available genomic and metagenomic data (>4000 sequences). While we observed the previously recognized groupings that include Deltaproteobacteria, methanogenic Archaea, and Firmicutes, there are numerous other organisms that include HgcAB in their genomes, interspersed with clades that follow phylogenetic profiles. This indicates that (1) the diversity of organisms that

potentially methylate is much higher than previously anticipated when we discovered HgcAB and (2) that the genes may move within communities at time scales that are shorter than speciation (i.e., recent HGT events).

With regard to physiological experiments for native function, we have completed 150 mL batch culture bottle experiments with *D. desulfuricans* ND132 wild-type and mutant strains (Δ*hgcAB*, Δ*metH*, Δ*cobT*, Δ*hgcA*:T101A, Δ*hgcA*:C93A, Δ*hgcA*:N90A, Δ*hgcA*:N90P) grown in defined media with various substrates (e.g., pyruvate, fumarate, lactate, sulfate, formate, acetate). We chose mutant strains related to carbon and Hg cycling that exhibited differences in Hg methylation capability compared to wild-type (e.g., 0–246%). These experiments compared growth and metabolite profiles of the various *D. desulfuricans* ND132 gene deletion strains to wild-type to test whether deletion or mutation of *hgcAB* affected cellular metabolism in ND132. Over the growth curve, we monitored OD600, total protein (BCA analysis), major anions, and organic acids (e.g., lactate acetate, pyruvate) to determine if changes in central metabolism coordinated to changes in MeHg generation between wild-type and mutant strains. At the end of exponential growth, cells were preserved for transcriptomic, metabolomic, proteomic, and lipidomic analyses, which were performed this year at the DOE Environmental Molecular Sciences Laboratory (EMSL) (user proposal 50174). We are in the process of analyzing the omics data; preliminary results show significant differences in substrate consumption, acetate production, and transcription of C1 metabolism genes (specifically, certain amino acid synthesis pathways) between mutant strains and wild-type under fermentative and sulfate-reducing conditions.

Our understanding of the Hg methylation pathway is limited in part because we lack complete structural models of HgcA and HgcB, and the identity of other proteins likely involved remains unknown. Thus, obtaining accurate model structures of HgcAB and any partner proteins that interact directly with them will provide key insight into Hg methylation and possibly alternative functions of HgcAB. In FY19, we made significant progress in creating accurate metagenome-based 3D structural models of HgcA and HgcB. This was achieved by combining metagenome sequence data, coevolution analysis, and Rosetta calculations to generate a structural model of the HgcAB complex. Our analysis revealed that there is essentially no interaction between the two domains of HgcA, but HgcB binds to both of these domains in the assembled complex. The conserved pair of Cys residues in HgcB may bind Hg(II) and position it to accept a methyl group. In addition, there is evidence for domain motion in HgcA that likely plays an important role in methyl transfer. These findings provide mechanistic insight into the biochemical mechanism of Hg methylation and may also reveal other reactions catalyzed by HgcAB.

Although we have some idea which methylators are present in EFPC, none have been isolated or characterized at the genomic level. In FY19, we have taken steps to develop more robust, rapid, and cost-effective approaches to identify and isolate candidate strains from EFPC sediment. In particular, we have focused on developing methods for anaerobically cell-sorting and growing methylators on agar plates, resulting in several successful runs with strains of *Geobacter sulfurreducens* and *Desulfobulbus propionicus*. Recently, we characterized the diversity of some prime targets for isolation from EFPC sediment using a combination of 16S rRNA amplicon sequencing, fractionation of cell populations by flow cytometry, and microbial purification on Nycodenz gradients. These results indicate that there are at least a half dozen species of both *Desulfobulbus* and *Geobacter* present in the EFPC sediments. We performed antibody immunolabelling assays comparing reactivity on various culture collection isolates and are in the process of generating new antibodies that will recognize a broader range of target species, which will be used to selectively isolate and grow methylators from EFPC. These efforts not only will allow us to determine if the EFPC system contains novel methylators, but also will provide EFPC system–relevant strains for use in our co-culture experiments. Together, these activities will provide the necessary microbiological data for integrating geochemical and hydrological data into large-scale models.

Status of FY19 Milestones

Milestone 2a: Identified existing neutral single nucleotide mutations for fitness. Initiated protocol development studies to increase fitness assay throughput.

Milestone 2c: Initiated comparative genomics for elucidating *hgcAB* pathways.

Milestones 2e and f: Completed characterizing microbial growth in monoculture and synthetic communities and initiated experiments to validate culture conditions and characterize metabolism and Hg methylation rates.

Milestones 2h and i: Drafted manuscript on metagenomic-enabled co-evolution and protein-protein interaction studies for HgcA and HgcB. Will also initiate protein-protein interaction studies for HgcAB-associated proteins for biochemical pathway elucidation.

Milestone 2g: Initiated the isolation and characterization of new EFPC methylators and potential MeHg demethylators.

FY20 Plans

In FY20, Theme 2 planned activities include:

- Continue sequence analysis efforts to determine diversity of Hg-methylating microbes in EFPC in collaboration with Theme 1.
- Continue to develop protocols for identifying, isolating, and characterizing novel EFPC methylators and demethylators.
- Continue co-evolution and protein-protein interaction studies for HgcAB biochemical pathway elucidation.
- Continue efforts to determine the alternative (native) biochemical function of HgcAB. These efforts include fitness assays and computer-controlled bioreactor experiments to evaluate substrate dependence.
- Continue isolate species cultivation studies followed by mixed species cultivation.
- Continue to characterize growth rate, metabolism, Hg methylation, and MeHg demethylation in synthetic communities.

Manuscripts

Published or In Press

Ndu, U., G. A. Christensen, N. Rivera, C. M. Gionfriddo, M. Deshusses, D. A. Elias, and H. Hsu-Kim. 2018. "Quantification of mercury bioavailability for methylation using diffusive gradient in thin-film samplers." *Environmental Science & Technology*. **52**(15):8521–8529. DOI: 10.1021/acs.est.8b00647.

Gilmour C. C., A. L. Bullock, A. McBurney, M. Podar, and D. A. Elias. 2018. "Robust mercury methylation across diverse methanogenic Archaea." *mBio*. **9**(2):e02403-02417. DOI: 10.1128/mBio.02403-17.

Asaduzzaman, A. M., D. Riccardi, A. T. Afaneh, S. J. Cooper, J. C. Smith, F. Wang, J. M. Parks, and G. Schreckenbach. 2019. "Environmental mercury chemistry – In silico." *Accounts Chemical Research*. **52**, 379–88. DOI: 10.1021/acs.accounts.8b00454.

Devarajan, D., P. Lian, S. C. Brooks, J. M. Parks, and J. C. Smith. 2018. "Quantum chemical approaches for calculating stability constants of mercury complexes." *ACS Earth and Space Chemistry*. **2**, 1168–178. DOI: 10.1021/acsearthspacechem.8b00102.

Submitted or In Preparation

Gionfriddo, C. M., A. M. Wymore, D. S. Jones, M. M. Lynes, G. A. Christensen, R. L. Wilpiszeski, A. Soren, C. C. Gilmour, J. D. Wall, C. C. Brandt, M. Podar, A. V. Palumbo, and D. A. Elias. "Updated technique for PCR amplification of Hg-methylation genes (hgcAB) from environmental samples." *Environmental Science & Technology*. *In review*.

Christensen G. A., A. J. King, J. G. Moberly, C. M. Miller, A. C. Somenahally, S. J. Callister, H. M. Brewer, M. Podar, S. D. Brown, A. V. Palumbo, C. C. Brandt, A. M. Wymore, S. C. Brooks, C. C. Gilmour, C. M. Gionfriddo, M. W. Fields, J. D. Wall, and D. A. Elias. 2019. "How reliable are hgcA abundance measurements and do they correlate with mercury and methylmercury concentrations in the environment?" *ISME Journal*. *In review*.

Theme 3: Biogeochemical Processes

The overall goal of Theme 3 is to gain a fundamental understanding of the complex biogeochemical processes and their interactions that control Hg species transformation and availability for cellular uptake and methylation. These processes and interactions involve, for example, dissolved organic matter (DOM), microbes, particulate organic matter and minerals, and water chemistry in EFPC. Our experiments are designed to address the following specific scientific questions:

- What are the specific (or dominant) Hg-binding organic ligands or molecular compositions (e.g., thiolates in DOM), and how do they competitively interact and control Hg speciation?
- What are the Hg-binding domains on cell membrane and cytosols, and how do cells competitively interact with extracellular organic and inorganic ligands for Hg binding, uptake (either passive or active), and ultimately methylation?
- How does environmental complexity (e.g., DOM, microbes, and minerals) influence Hg species distribution and availability for cell sorption, uptake, and methylation?
- What is the impact of methanotrophs on net MeHg production in TSZs and periphyton? What is the role of methanobactin on Hg speciation and MeHg degradation?

FY2019 Accomplishments

Significant advances have been made in understanding complex biogeochemical processes and interactions that control Hg species transformations and availability for cellular uptake and methylation. Most notably, we recently determined whether Hg(II) can be taken up passively or actively by methylating bacteria, such as *D. desulfuricans* ND132. Contrary to current views of active Hg(II) uptake, we found that active metabolism is not required for cellular Hg(II) uptake, and Hg can get into cells without a specific transporter (An et al. 2019). Our work suggests that intracellular Hg binding and handoff to HgcA, rather than uptake, is likely the driving factor for Hg methylation. Also, for the first time, we investigated Hg stable

isotope fractionation and its mechanisms during abiotic dark oxidation of dissolved elemental Hg(0) by DOM and thiols and observed that DOM- and thiol-induced Hg(0) oxidation results in both mass dependent fractionation (MDF) and mass independent fractionation (MIF). Our work is featured in *Environmental Science & Technology* Editor's Choice (Zheng et al. 2019), as it provides additional experimental constraints on interpreting Hg isotope signatures, with important implications for the use of Hg isotope fractionation as a tracer of the Hg biogeochemical cycle. We also determined under what conditions minerals and particulates may become a sink or a source for Hg(II) methylation and found that the mineral-bound Hg(II) may be directly available for microbial uptake or methylation (Zhang et al. 2019; Zhao et al. 2019). Our work highlights the importance of Hg(II) partitioning at particulate-water interfaces and the role of particulates as a significant source of Hg(II) for methylation in the environment. Lastly, we investigated the enzymatic activity of HgcAB proteins catalyzing Hg methylation in *D. desulfuricans* ND132 cell lysates (Date et al. 2019). Our results show that Hg methylation mediated by HgcAB is oxygen sensitive, irreversible, and follows Michaelis-Menten kinetics. These results provide first-of-a-kind data regarding the kinetics of Hg methylation in sulfate-reducing bacteria.

Hg stable isotope fractionation has been widely used to trace Hg sources and transformations in the environment, but many important fractionation processes remain unknown. We recently observed that DOM- and thiol-induced Hg(0) dark oxidation results in both MDF with enrichment of heavier isotopes in the oxidized Hg(II) and a small negative MIF owing to nuclear volume effects. The measured enrichment factors for MDF and MIF (ε^{202}Hg and E^{199}Hg) ranged from 1.10‰ to 1.56‰ and from -0.16‰ to -0.18‰, respectively. These results agreed well with theoretically predicted values for equilibrium fractionation between Hg(0) and thiol-bound Hg(II). The observed equilibrium fractionation is likely controlled by isotope exchange between Hg(0) and Hg(II) following the production of the Hg(II)-thiol complex. However, significantly attenuated isotope fractionation was observed during the initial stage of Hg(0) oxidation by humic acid and was attributed to the kinetic isotope effect (KIE) due to slower isotope exchange. This is the first study of Hg isotope fractionation during dark abiotic oxidation of Hg(0) by DOM and thiol compounds. The results provide additional constraints on interpreting Hg isotope signatures, with important implications for using Hg isotope fractionation as a tracer of Hg biogeochemical cycling in the environment.

We also know that, in natural freshwater and sediments, Hg(II) is largely associated with particulate minerals and organics, but it remains unclear under what conditions these particles may become a sink or a source for Hg(II) in complex environmental systems and whether the particulate-bound Hg(II) is bioavailable for microbial uptake and methylation. Concurrent interactions can occur between Hg(II) and minerals, DOM, microbes, and various dissolved ligands. We recently investigated Hg(II) sorption-desorption characteristics on three organo-coated hematite particulates and a Hg-contaminated natural sediment and evaluated the potential of particulate-bound Hg(II) for microbial methylation. We found that Hg(II) rapidly sorbed onto particulates, especially the cysteine-coated hematite and sediment, with little desorption observed (0.1–4%). However, the presence of Hg-binding ligands, such as low-molecular-weight thiols and humic acids, resulted in up to 60% of Hg(II) desorption from the Hg-laden hematite particulates, but <6% from the sediment. Importantly, the particulate-bound Hg(II) was bioavailable for uptake and methylation by a sulfate-reducing bacterium (*D. desulfuricans* ND132) under anaerobic incubations, and the methylation rate was 4 to10 times higher than the desorption rate of Hg(II). These observations suggest direct contacts and interactions between bacterial cells and the particulate-bound Hg(II), resulting in rapid exchange or uptake of Hg(II) by the bacteria. Furthermore, we studied the effects of mercury adsorption on minerals on MeHg production by *D. desulfuricans* ND132, as the adsorbed or solid-phase Hg(II) is commonly assumed immobile or less bioavailable for microbial uptake. We observed that the mineral-adsorbed Hg(II) on both hematite and montmorillonite is not only available for cell uptake and methylation, but also results in a two- to threefold increase in methylmercury production compared to the mineral-free incubation. An optimal Hg(II) methylation is observed at a low to moderate mineral-to-solution ratio (1–5 g L^{-1}). The result is explained by decreased cellular immobilization of Hg(II) but enhanced close interactions between Hg(II) and cells both adsorbed or concentrated on mineral surfaces, leading to increased methylation. However, a high mineral-to-solution ratio inhibits Hg(II) methylation due to a low Hg(II) coverage (per surface area) at high mineral loadings, which limit close contacts between Hg(II) and the cells. These results confirm that the

mineral-adsorbed Hg(II) is directly available for microbial uptake or methylation, although whether the adsorption enhances or inhibits Hg(II) methylation may depend on micro-niches where Hg(II), microbes, and minerals coexist in the natural environment. Our results highlight the importance of Hg(II) partitioning at particulate-water interfaces and the role of particulates as a significant source of Hg(II) for methylation in the environment (Zhang et al. 2019; Zhao et al. 2019).

Interestingly, in studies of Hg isotopes in tracing Hg biogeochemical transformations, such as partitioning and concurrent Hg sorption-desorption reactions in environmental matrixes, we also discovered an important utility (as well as a potential artifact) in using Zeeman cold vapor atomic absorption spectrometry (CVAAS) for quantifying Hg isotopes. Although Zeeman CVAAS has been widely used for environmental Hg detection and quantification for decades, little is known about its utility and potential artifacts in analyzing—in both laboratory and field investigations—Hg with varying isotope compositions. We found that different Hg isotopes respond differently by CVAAS analysis, with ^{200}Hg and ^{202}Hg isotopes exhibiting ~10 times greater signal intensities than ^{198}Hg and ^{201}Hg isotopes. However, all Hg isotopes show a linear correlation between Hg concentrations and the signal intensity, validated by both measurements and theoretical simulations. These results demonstrate that Zeeman CVAAS could offer a convenient, inexpensive tool for determining Hg isotopes, particularly in using single- or dual-labeled Hg isotopes for tracing Hg biogeochemical transformations, such as partitioning, ion exchange, sorption-desorption, and methylation-demethylation in environmental matrixes.

While experimental data generated by Date et al. (2019) enables us to glean insights into the kinetics of Hg methylation catalyzed by HgcA and HgcB, the rapidly growing availability of sequencing data offers a perspective on the diversity of the *hgcAB* genes across a broad range of microorganisms and environments. However, accurate identification of *hgcA* and *hgcB* can be a challenging task due to a high degree of sequence diversity and homology to other genes. We are currently preparing a manuscript that aims to characterize *hgcAB* diversity and presents strategies for accurate identification of *hgcA* and *hgcB* in sequencing data in collaboration with Theme 2.

Status of FY19 Milestones

Milestones 3a and b. Hg-binding ligand characterization and interactions: Molecular characterization of Hg-binding ligands and their competitive interactions.

Milestones 3c, d, and e. Cellular protein characterization and Hg-cell interactions: Characterization of cellular proteins, Hg-cell interactions, and controls on Hg uptake and methylation.

Milestones 3f, g, and h. Biogeochemical complexity on Hg uptake and methylation: Biogeochemical complexity influences on microbial Hg uptake and methylation.

The collection of metagenomic datasets from sediment and periphyton samples is underway (see Theme 2). Preliminary analysis results indicate that alpha- and gammaproteobacterial methanotrophs are present in EFPC sediments.

We have obtained highly purified samples of methanobactin from *Methylosinus trichosporium* OB3b and *Methylocystis sp*. SB2 and started to investigate the complexation of these methanonactins with Hg species. We also conducted a comprehensive crystallization screening of Hg(II)-methanobactin complexes with the aim of gaining insights into the interaction between Hg(II) and methanobactin by X-ray crystallography.

FY20 Plans

In FY20, Theme 3 planned activities include:

- Complete and publish results on a stepwise reduction approach to determine Hg competitive binding and exchange reactions within natural organic matter and mixed organic ligands.
- Continue studies of Hg isotopes in tracing Hg biogeochemical transformations, such as partitioning and concurrent Hg sorption, desorption, and methylation in environmental matrixes.
- Determine the roles of methanobactin in methylmercury degradation by non-methanobactin-producing methanotrophs, as well as study the effects of methanobactin on Hg methylation by *D. desulfuricans* ND132 and *G. sulfurreducens* PCA in collaboration with Jeremy Semrau at the University of Michigan.
- Continue collection and analysis of metagenomic datasets to delineate the prevalence of methanotrophs in EFPC sediments and periphyton.
- Characterize Hg(II)-methanobactin complexes by isothermal titration calorimetry.
- Continue collaboration with Jeremy Semrau and determine the impact of methanobactin on Hg speciation, uptake, and demethylation.
- Evaluate the role of critical environmental factors (such as DOM and pH from TSZs in EFPC) on MeHg degradation by methanotrophs.
- Continue collaboration with Stephen W. Ragsdale at the University of Michigan on the structural and functional characterization of HgcAB.

Manuscripts

Published or In Press

An, J., L. Zhang, X. Lu, E. M. Pierce, A. Johs, J. M. Parks, and B. Gu. 2019. "Mercury uptake by *Desulfovibrio desulfuricans* ND132: Passive or Active?" *Environmental Science & Technology*. **53**(11): 6264–272. DOI: 10.1021/acs.est.9b00047.

Zheng, W., J. D. Demers, X. Lu, B. A. Bergquist, A. D. Anbar, J. D. Blum, and B. Gu. 2019. "Mercury stable isotope fractionation during abiotic dark oxidation in the presence of thiols and natural organic matter." *Environmental Science & Technology*. **53**(4): 1853–862. DOI: 10.1021/acs.est.8b05047.

Date, S., J.M. Parks, K.W. Rush, J. D. Wall, S.W. Ragsdale, and A. Johs. 2019. "Kinetics of mercury methylation mediated by HgcAB." *Appled and Environmental Microbiology*. DOI: 10.1128/AEM.00438-19. *In press*.

Zhang, L., S. Wu, L. Zhao, X. Lu, E. M. Pierce, and B. Gu. 2019. "Mercury sorption and desorption on organo-mineral particulates as a source for microbial methylation." *Environmental Science & Technology.* **53**:2426–433. DOI: 10.1021/acs.est.8b06020.

Lu, X., J. Zhao, X. Liang, L. Zhang, Y. Liu, X. Yin, X. Li, and B. Gu. 2019. "The application and potential artifacts of Zeeman cold vapor atomic absorption spectrometry in mercury stable isotope analysis." *Environmental Science & Technology Letters.* **6**(3):165–70. DOI: 10.1021/acs.estlett.9b00067.

Tang, W., H. Hintelmann, B. Gu, X. Feng, Y. Liu, Y. Gao, J. Zhao, H. Zhu, P. Lei, and H. Zhong. 2019. "Increased methylmercury accumulation in rice after straw amendment." *Environmental Science & Technology*. **53**(11):6144–153. DOI: 10.1021/acs.est.8b07145.

Liu, Y., A. Johs, L. Bi, X. Lu, H. W. Hu, D. Sun, J. Z. He, and B. Gu. 2018. "Unraveling microbial communities associated with methylmercury production in paddy soils." *Environmental Science & Technology*. **52**(22):13110–118. DOI: 10.1021/acs.est.8b03052.

Gu, B., X. Lu, A. Johs, and E. M. Pierce. 2018. "Mercury in water." In *Encyclopedia of Water: Science, Technology, and Society*. P. Maurice (Ed), Wiley. ISBN: 9781119300755.

Chen, Q., A. Johs, X. Lu, H. Chen, J. An, D.A. Elias, E.M. Pierce, J.M. Parks, R.L. Hettich, and B. Gu. 2018. "Quantitative proteomic analysis of biological processes and responses of the bacterium *Desulfovibrio desulfuricans* ND132 upon deletion of its mercury methylation genes." *Proteomics.* **18**(17):1700479. DOI: 10.1002/pmic.201700479.

Lu, X., A. Johs, L. Zhao, E. M. Pierce, and B. Gu. 2018. "Unexpected, copper-enhanced mercury methylation by *Desulfovibrio desulfuricans* ND132." *Environmental Science & Technology Letters.* **5**(6):372–76.

Zhao, L., Y. Li, L. Zhang, J. Zheng, E. M. Pierce, and B. Gu. 2019. "Mercury adsorption on minerals influences microbial methylation by *Desulfovibrio desulfuricans* ND132." *ACS Earth and Space Chemistry*. DOI:10.1021/acsearthspacechem.9b00039.

Submitted or In Preparation

Liang, X., B. Gu, et al. 2019. "A stepwise reduction approach reveals mercury competitive binding and exchange reactions within natural organic matter and mixed organic ligands." *Environmental Science & Technology*. *In review*.

Yin, X., B. Gu, et al., 2019. "Effects of methanobactin on mercury methylation by *D. desulfuricans* ND132 and *G. sulfurreducens* PCA." *Environmental Science & Technology*. *In preparation*.

Field-scale Modeling Activity

The objective of the integrated activity on model development and parameterization is to advance the state of the art in process-based modeling of reactive transport in stream systems using Hg and EFPC as representative use cases. The activity will iteratively develop, evaluate, and refine multiscale modeling approaches, multidisciplinary parameterization strategies, and software frameworks that allow increasingly detailed understanding of the fine-scale biogeochemical processes to be used at their native scales in reach-to-watershed–scale stream models. Central to our strategy is our new multiscale modeling methodology that makes it possible, for the first time, to tractably represent redox zonation and other fine-scale geochemical phenomena at reach-to-watershed scales without the need for 3D characterization of hyporheic flow zones. The approach is based on a recent extension (Painter 2018) of the highly successful residence-time frameworks to accommodate nonlinear multicomponent reactions. The key idea is to solve a 1D reactive transport subgrid system associated with each stream channel grid cell. The auxiliary subgrid system is written in a Lagrangian (travel-time) framework and represents an ensemble of hyporheic pathways that leave and return to the stream channel.

By moving the biogeochemical process representation to the subgrid models, those processes may be represented in great detail at their native spatial scales without averaging over the fine-scale variability in redox states that occurs within sediments and periphyton biofilms. That ability to represent processes at their native scale is a significant advantage over existing field-scale models that require ad hoc "upscaling" of the process representation. That process flexibility is achieved without the large computational demands associated with a fully 3D model. Moreover, the need for detailed characterization of the hyporheic zone is dramatically reduced compared with a fully 3D model. The relevant physical hydrology inputs for the hyporheic zone are the hyporheic exchange flux and hyporheic residence-time distributions.

The field-scale modeling activity is a partnership activity with the Interactive Design of Extreme-scale Application Software – Watersheds (IDEAS–Watersheds) project.

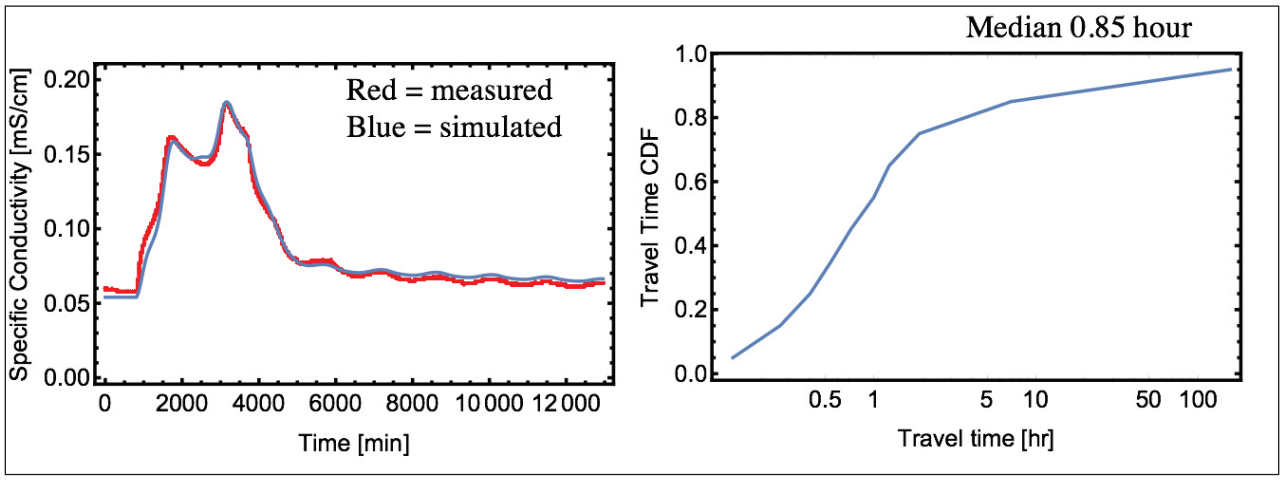


Fig. 4: Application of the new multiscale modeling approach to tracer tests (Ward et al. 2016) performed at the H.J. Andrews Experimental Forest. Shown on the left is a comparison between observed and simulated breakthrough. Shown on the right is the hyporheic travel time distribution estimated from the test.

The jointly funded activity has subtasks related to model development, estimation of parameters from field-scale tests, and initial demonstrations.

FY19 Accomplishments

In FY18 and early FY19, we undertook a complete demonstration of the multiscale framework, using prototype software and focusing on redox zonation and its effect on denitrification in the hyporheic zone (Painter 2018). We also completed an initial implementation of nonreacting transport using the approach in the Advanced Terrestrial Simulator (ATS) software. In addition, we extended the approach to include unsteady flow in the channel. In collaboration with researchers at Indiana University, we successfully modeled a nonreacting tracer test conducted at the H.J. Andrews Experimental Forest, which was affected by strong transients in the channel discharge (Fig. 4). We also investigated the propagation of parameter uncertainty in equilibrium speciation modeling of Hg in aqueous systems (Guo et al. 2019).

Status of FY19 Milestones

After consultation with SBR program managers and in collaboration with the IDEAS project, we have revised some of our early milestones to focus on modeling previously published stream tracer tests. In particular, we are delaying the modeling of tracer tests and biogeochemistry in EFPC and focusing initially on modeling tracer tests conducted in the H.J. Andrews Experimental Forest and other well-characterized systems. That modeling work replaces Milestone FRA2j and is currently on schedule.

Milestone FRA2a, PHREEQC extension of the Alquimia interface, has not been initiated because of a one-year delay in renewing the IDEAS-Watersheds project and a corresponding delay in hiring jointly funded postdoctoral staff. We anticipate this to be a FY20 Q1 activity. Milestone FRA2b, manuscript on ATS multiscale model, was split into two manuscripts, one focusing on the approach and prototypes (completed, Painter 2018) and one describing extension to transient flow and implementation in the ATS software (now anticipated for FY19 Q4).

FY20 Plans

In FY20, planned activities in Field-scale Modeling include:

- Extension of the Alquimia Interface to call PHREEQC biogeochemistry from ATS.
- Continue modeling tracer tests from H.J. Andrews Experimental Forest and similar well-characterized sites.
- Develop tools to perform non-parametric estimation of the hyporheic travel-time distribution.
- Develop manuscript describing modeling and interpretation of published tracer tests.

Manuscripts

Painter, S. L. 2018. "Multiscale framework for modeling multicomponent reactive transport in stream corridors." *Water Resources Research*. **54**(10):7216–230. DOI:10.1029/2018WR022831.

Guo, L., S. L. Painter, S. C. Brooks, J. M. Parks, and J. C. Smith. 2019. "A probabilistic perspective on thermodynamic parameter uncertainties: Understanding aqueous speciation of mercury." *Geochimica et Cosmochimica Acta*. *In press*.

Select Research Highlights

In FY2019, a total of 26 manuscripts have been published or submitted by the CI-SFA. Of these publications, 18 are published or in press, bringing the total to 113 for the CI-SFA since its inception. Of these 113 publications, 98 are the result of new mercury research, and 15 represent DOE Environmental Remediation Sciences Program projects that were completed with partial SFA funding. In this section, we highlight 4 of the 26 published or submitted manuscripts.

Research Highlight

Ecosystem controls on methylmercury production by periphyton biofilms in a contaminated freshwater stream: Implications for predictive modeling

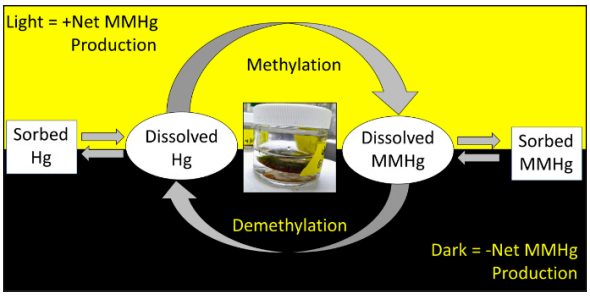
Methylmercury production rates can be predicted from environmental variables

The Science

Periphyton biofilms produce a substantial fraction of the overall monomethylmercury (MMHg) flux in East Fork Poplar Creek, an industrially contaminated creek in Oak Ridge, Tennessee. We examined periphyton MMHg production across seasons, locations, and light conditions using mercury stable isotopes. Methylation and demethylation rate potentials were calculated using a transient availability kinetic model ($k_{m,\ trans\ av,}$ and $k_{d,\ trans\ av,}$ respectively). Light exposure and season were significant predictors of $k_{m,\ trans\ av,}$ with greater values in full light exposure and in the summer. Season, light exposure, and location were significant predictors of $k_{d,\ trans\ av,}$ which was highest in dark conditions, in the spring, and at the upstream location. Light exposure was the controlling factor for net MMHg production, with positive production for periphyton grown under full light exposure and net demethylation for periphyton grown in the dark. Ambient MMHg and $k_{m,\ trans\ av}$ were significantly correlated. Transient availability rate potentials were 15 times higher for k_m and 9 times higher for k_d compared to full availability rate potentials ($k_{m,\ full\ av}$ and $k_{d,\ full\ av}$) calculated at 1d. There were no significant differences among treatments for the full availability $k_{m,\ full\ av,}$ $k_{d,\ full\ av,}$ or net MMHg calculated using the full availability rate potentials. $k_{m,\ full\ av}$ was not correlated with ambient MMHg concentrations. Our results underscore the importance of applying transient availability kinetics to MMHg production data when estimating MMHg production potential and flux.

The Impact

This study presents predictive relationships for the prediction of methylmercury production. These relationships can be incorporated into watershed models of stream function for improved understanding and prediction of Hg cycling in the environment and its impacts on human and environmental health.



Periphyton biofilms grown in the full light during summer produce significantly more net methylmercury than biofilms grown in low light and in the winter. Using environmental variables and a novel transient availability model, significant predictive relationships were developed for mercury methylation and methylmercury demethylation. Traditional analysis methods failed to generate significant predictive relationships.

Summary

Periphyton biofilms are hot spots for Hg cycling in freshwater streams. Advancing our understanding of the rates of methylmercury production will produce improved predictions of stream function and the impacts on human and environmental health.

Publication

Schwartz, G. E, T. A. Olsen, K. A. Muller, and S. C. Brooks. "Ecosystem controls on methylmercury production by periphyton biofilms in a contaminated freshwater stream: Implications for predictive modeling." *Environmental Toxicology and Chemistry. In review*.

Research Highlight

Is particulate-bound mercury available for microbial uptake and methylation?

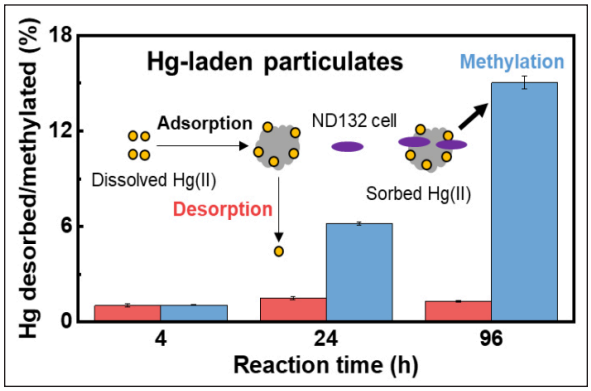
Study reveals important roles of mineral-adsorbed or particulate-bound mercury (Hg) as a significant source of Hg for microbial methylation in the environment

The Science

In natural freshwater and sediments, mercury (Hg) is largely associated with particulate minerals and organics, but it remains unclear under what conditions particulates may become a sink or a source for Hg and whether particulate-bound Hg is bioavailable for microbial uptake and methylation. We investigate Hg sorption-desorption characteristics on minerals and a Hg-contaminated sediment and evaluate the potential of particulate-bound Hg for microbial methylation. Mercury rapidly adsorbs onto particulates, especially the cysteine-coated hematite and sediment, with little desorption observed (0.1–4%). However, the presence of Hg-binding ligands, such as low-molecular-weight thiols and humic acids, results in up to 60% of Hg(II) desorption from the Hg-laden hematite particulates, but <6% from the sediment. Importantly, the particulate-bound Hg(II) is bioavailable for uptake and methylation by a sulfate-reducing bacterium *Desulfovibrio desulfuricans* ND132 under anaerobic incubations, and the methylation rate is 4 to 10 times higher than the desorption rate of Hg(II). These observations suggest direct contacts and interactions between bacterial cells and the particulate-bound Hg(II), resulting in rapid exchange or uptake of Hg(II) by the bacteria. Our results highlight the importance of Hg(II) partitioning at particulate-water interfaces and the role of particulates as a significant source of Hg(II) for methylation in the environment.

The Impact

Our study indicates an alternative pathway in which microbes take up Hg that is more complicated than previously thought: Particulate-bound Hg does not have to be desorbed or dissolved to make it available for microbial uptake and methylation.



The Hg methylation rate is 4 to 10 times higher than the desorption rate of Hg, suggesting direct contacts and interactions between bacterial cells and the particulate-bound Hg for rapid exchange or uptake.

Summary

Mineral- or particulate-bound Hg is often considered unavailable for microbial uptake and methylation. Our study reveals that particulate-bound Hg(II) is readily available for uptake and methylation by a sulfate-reducing bacterium, *D. desulfuricans* ND132, under anaerobic incubations and should be considered in predicting methylmercury production in the natural aquatic environment.

Publication

Zhang, L., S. Wu, L. Zhao, X. Lu, E. M. Pierce, and B. Gu. 2019. "Mercury sorption and desorption on organo-mineral particulates as a source for microbial methylation." *Environmental Science & Technology*. **53**(5):2426–433. DOI: 10.1021/acs.est.8b06020.

Research Highlight

Do microbial cells take up mercury (Hg) passively or actively?

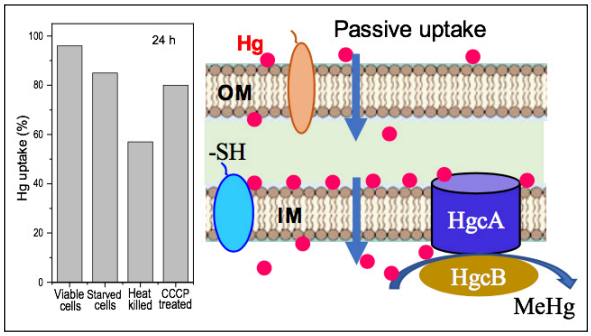
Study reveals that, contrary to current views of active Hg uptake, microbial cells can take up Hg passively without a specific transporter

The Science

Recent studies have identified HgcAB proteins as being responsible for mercury [Hg(II)] methylation by certain anaerobic microorganisms. However, it remains controversial whether microbes take up Hg(II) passively or actively. Here we examine the dynamics of concurrent Hg(II) adsorption, uptake, and methylation by both viable and inactivated cells (heat-killed or starved) or spheroplasts of the sulfate-reducing bacterium *Desulfovibrio desulfuricans* ND132 in laboratory incubations. We show that, without addition of thiols, >60% of the added Hg(II) was taken up passively in 48 hours by live and inactivated cells and also by cells treated with the proton gradient uncoupler, carbonylcyanide-3-chlorophenylhydrazone (CCCP). Inactivation abolished Hg(II) methylation, but the cells continued taking up Hg(II), likely through competitive binding or ligand exchange of Hg(II) by intracellular proteins or thiol-containing cellular components. Similarly, treatment with CCCP impaired the ability of spheroplasts to methylate Hg(II) but did not stop Hg(II) uptake. Spheroplasts showed a greater capacity to adsorb Hg(II) than whole cells, and the level of cytoplasmic membrane-bound Hg(II) correlated well with methylmercury (MeHg) production, as Hg(II) methylation is associated with cytoplasmic HgcAB. Our results indicate that active metabolism is not required for cellular Hg(II) uptake, thereby providing improved understanding of Hg(II) bioavailability for methylation.

The Impact

Contrary to current views of active Hg(II) uptake, we found that active metabolism is not required for cellular Hg(II) uptake, and Hg can get into cells without a specific transporter.



Approximately 60% or more of the added Hg(II) is taken up passively by both live and inactivated cells **(left)**. A schematic representation of passive uptake of Hg and its handoff to HgcAB leading to methylmercury (MeHg) production **(right)**. OM = outer membrane; IM = inner membrane.

Summary

Although recent studies have identified HgcAB proteins as being responsible for Hg(II) methylation by certain anaerobic microorganisms, it remains controversial whether microbes take up Hg(II) passively or actively, and what factors control Hg(II) uptake by methylating organisms. Study suggests that intracellular Hg binding and handoff to HgcA, rather than uptake, is likely the driving factor for Hg methylation.

Publication

An, J., L. Zhang, X. Lu, E. M. Pierce, A. Johs, J. M. Parks, and B. Gu. 2019. "Mercury uptake by *Desulfovibrio desulfuricans* ND132: Passive or active?" *Environmental Science & Technology*. **53**(11): 6264–272. DOI: 10.1021/acs.est.9b00047.

Research Highlight

Kinetics of enzymatic mercury methylation at nanomolar concentrations catalyzed by HgcAB

Study reveals insights into the biochemistry of mercury methylation by anaerobic bacteria expressing the gene pair *hgcAB*

The Science

Methylmercury (MeHg) is a potent bioaccumulative neurotoxin, which is produced by certain anaerobic bacteria and archaea. Mercury (Hg) methylation has been linked to the gene pair *hgcAB* encoding a membrane-associated corrinoid protein and a ferredoxin. Although microbial Hg methylation has been characterized *in vivo*, the cellular biochemistry and the specific roles of the gene products HgcA and HgcB in Hg methylation are not well understood. Here we report the kinetics of Hg methylation in cell lysates of *Desulfovibrio desulfuricans* ND132 at nanomolar Hg concentrations. The enzymatic Hg methylation mediated by HgcAB is highly oxygen-sensitive, irreversible, and follows Michaelis-Menten kinetics with an apparent K_M of 3.2 nM and V_{max} of 19.7 fmol·min^{-1}·mg^{-1} total protein for the substrate Hg(II). Although the abundance of HgcAB in the cell lysates is extremely low, Hg(II) was quantitatively converted to MeHg at sub-nanomolar substrate concentrations. Increasing thiol/Hg(II) ratios did not impact Hg methylation rates, which suggests that HgcAB-mediated Hg methylation effectively competes with cellular thiols for Hg(II) consistent with the low apparent K_M. Supplementation of 5-methyltetrahydrofolate or pyruvate did not enhance MeHg production, while both ATP and a non-hydrolyzable ATP analog decreased Hg methylation.

The Impact

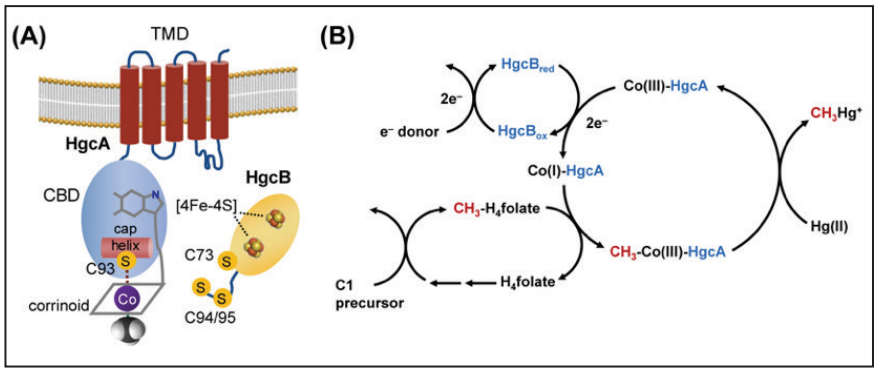
Exposure to neurotoxic MeHg through the consumption of fish represents a significant risk to human health. Anaerobic microbial communities in sediments and periphyton biofilms have been identified as sources of MeHg in aquatic systems, but the underlying biomolecular mechanisms are not fully understood. This study provides insights into the biochemistry of Hg methylation in sulfate-reducing bacteria.

Summary

Anaerobic bacteria that carry *hgcAB* generate MeHg in aquatic environments. Gaining insights into the biochemistry of Hg methylation is important for understanding factors driving Hg methylation. HgcA and HgcB catalyze the formation of MeHg at extremely low Hg(II) concentrations and effectively compete with cellular thiols. Advancing our understanding of microbial MeHg production may inform strategies to curtail the formation of neurotoxic MeHg in the environment.

Publication

Date, S. S., J. M. Parks, K. W. Rush, J. D. Wall, S. W. Ragsdale, and A. Johs. 2019. "Kinetics of enzymatic mercury methylation at nanomolar concentrations catalyzed by HgcAB." *Applied Environmental Microbiology*. DOI: 10.1128/AEM.00438-19.



Structural features of HgcA and HgcB and proposed mechanism of HgcAB-mediated Hg methylation. **(A)** Schematic representation of HgcA and HgcB. TMD = transmembrane domain, CBD = corrinoid-binding domain, [4Fe-4S] = iron-sulfur cluster, C = cysteine. **(B)** Proposed roles of the proteins HgcA and HgcB in Hg methylation. C1 = one-carbon precursor, Co(I)/(III) = cobalt center of corrinoid cofactor and its oxidation state, H_4folate = tetrahydrofolate. CH_3H_4folate = 5-methyltetrahydrofolate, Hg(II) = mercuric mercury, CH_3Hg^+ = methylmercury.

Postgraduate Spotlight

A key goal of the CI-SFA and ORNL is to train the next generation of scientists and engineers. To this end, the SFA has maintained a number of outstanding graduate and postgraduate researchers since its inception 7 years ago. As part of this year's report, we highlight three outstanding postgraduate researchers—Caitlin Gionfriddo, Grace Schwartz, and Lijie Zhang—who have contributed significantly to the overall SFA goals and objectives. See website for complete list of postgraduates (www.esd.ornl.gov/programs/rsfa/alumni.shtml).

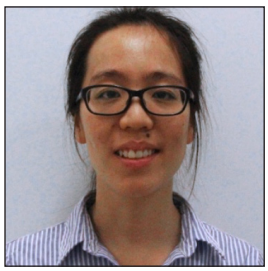
Caitlin Gionfriddo

Caitlin Gionfriddo received her B.S. in Chemistry from the University of South Carolina and completed her masters and Ph.D. in Earth Sciences from the University of Melbourne in Australia. In her postgraduate work she used environmental metagenomic techniques to elucidate biogeochemical controls on mercury cycling in geothermal springs and Antarctic sea ice. She joined ORNL's Biosciences Division as a postdoctoral research associate in late 2017 and is currently working in Dr. Dwayne Elias' lab as part of the Mercury SFA and ENIGMA projects. Her current research focuses on understanding microbial community function and geochemical influences on mercury transformations from the cellular to community level. At ORNL, Dr. Gionfriddo is applying her expertise in community-scale genomics to answer the "who, how, why" of microbial mercury methylation. In FY18 and FY19 she has coauthored five papers, including first authorship on an improved method for identifying mercury methylation genes in the environment. In FY18 she gave a keynote presentation at the 2018 Goldschmidt Conference on her work exploring the "native function" of mercury methylation proteins. She presented her work at BER's 2018 and 2019 Environmental System Science PI meetings and will be presenting at the upcoming International Conference on Mercury as a Global Pollutant in Poland. Recently, Dr. Gionfriddo was awarded a $60,802 FY19 EMSL user proposal to apply multi-omics techniques to explore the physiological role of the mercury methylation proteins, HgcAB, in cellular metabolism. Outside the lab, she applies her chemistry and microbiology knowledge to making beer, kombucha, and sourdough. She also has a keen interest in hiking, botany, and mycology and enjoys the many outdoor activities around East Tennessee.

Grace Schwartz

Grace Schwartz received her Ph.D. in Environmental Engineering from Duke University. She specializes in trace element biogeochemistry, contaminant remediation, and environmental analytical chemistry. Her dissertation work explored the environmental impacts of coal combustion with a specific focus on the biogeochemical transformations and leaching potential of trace element contaminants from coal ash under different ash disposal and spill scenarios. Dr. Schwartz is the first author on three papers examining different aspects of trace element leaching from ash materials, including "Ranking coal ash materials for their potential to leach arsenic and selenium: The relative importance of ash chemistry and site biogeochemistry," which won the 2018 AEESP/Mary Ann Liebert Award for Publication Excellence. After graduating from Duke, she worked as a postdoctoral fellow at the Smithsonian Environmental Research Center, where she developed *in situ* remediation technology for mercury-contaminated wetland sediment. She recently published part of this work in *Environmental Science: Processes and Impacts*. Dr. Schwartz became a postdoctoral research associate at ORNL in Fall 2017. Under the mentorship of Scott Brooks, she is exploring the ecosystem controls governing mercury methylation. Field and laboratory experiments are underway to develop a new kinetic model for mercury methylation in East Fork Poplar Creek sediments and to determine how nutrient concentrations impact mercury methylation by periphyton biofilms. When she isn't in the lab or tromping in the creek, Dr. Schwartz enjoys marathon open-water swimming. Last year she completed her first 10K race and plans to compete in many races in 2019 ranging from 4.4 miles to 10 miles.

Lijie Zhang



Lijie Zhang received her B.S. from Tsinghua University and Ph.D. from Washington University in St. Louis, both in Environmental Engineering. Her doctoral dissertation was titled "Coupling of Geochemical Reactions and Geophysical Properties of Clay Minerals in Energy-related Subsurface Engineered Systems." This work identified the relationship between geochemical reactions of clay minerals and their geophysical property changes

under subsurface-relevant conditions. To broaden her interests in biogeochemistry, Dr. Zhang started her position as a postdoctoral research associate at ORNL in 2018, under the mentorship of Dr. Baohua Gu, to study the molecular mechanisms involved in biogeochemical transformations of Hg under complex environmental conditions. After joining ORNL, she has published a first-author paper in *Environmental Science & Technology* about the role of particulates in mercury sorption, desorption, and methylation. Dr. Zhang also coauthored two papers in *Environmental Science & Technology* and *Environmental Science & Technology Letters*. Outside the laboratory, she serves as a reviewer for more than 10 journals.

National and International Impact

ORNL CI-SFA team members attend strategic conferences in the United States and abroad to gain insights into the state of the science, share project findings and strategies with the broader mercury research community, and identify collaborative opportunities. From July 2018 to June 2019, SFA scientists delivered or published 25 presentations, abstracts, or posters (see Appendix C, page 22, for details). Described below are team members' contributions to BER's Environmental System Science Principal Investigators Meeting and the American Geophysical Union (AGU) Fall Meeting.

BER's Environmental System Science Principal Investigators (PI) Meeting: CI-SFA researchers attended the PI meeting held May 30 to June 1, 2019, at the Bolger Center in Potomac, Md., and gave eight poster presentations. Additionally, members of the SFA team participated and actively contributed to the Open Watersheds town hall and the Open-Science Design Dash sessions.

American Geophysical Union Fall Meeting: Several members of the CI-SFA attended the AGU Fall Meeting in Washington, D.C., from December 10–14, 2018. Scott Brooks along with CI-SFA collaborators K.C. Carroll, Marie Kurz, and Adam Ward hosted a session titled "Coupled Dynamics of Physical, Biological, Geomorphic, Hydrologic, and Chemical Processes in the Hyporheic Zone over a Range of Spatial and Temporal Scales." SFA team members also gave a number of oral and poster presentations at the meeting.

Fig. 5. Key ORNL CI-SFA partners.

Ongoing Collaborative Research Activities

The ORNL CI-SFA continues to engage a number of key collaborators in the project (Fig. 5). In FY19, Themes 2 and 3 collaborated with EMSL staff to identify the proteomic and metabolomic signatures that will enable identification of the HgcAB alternative (native) biochemical function (proposal #50174) and to identify organic molecules responsible for mercury complexation in dissolved organic matter isolated from EFPC using fourier transform ion cyclotron resonance spectroscopy (proposal #48386), respectively. External collaborators Cynthia Gilmour (Smithsonian Environmental Research Center), Adam Ward (Indiana University), and Marie Kurz (Drexel University) continue to contribute to SFA milestones in Theme 2: Microbial Community Processes and the Field-Scale Modeling Activity.

Although the SFA's primary objective is fundamental science, it is important that project personnel have the opportunity to translate scientific discovery into information relevant to the DOE Office of Environmental Management (EM) and the broader DOE complex. We continue to

fulfill this need through active engagement with local Oak Ridge EM staff (Elizabeth Phillips, Laura Wilkerson, and Brian Henry), EM headquarter staff (Rod Rimando and Kurt Gerdes), and the site-specific advisory panels.

Organization and Leadership

The scientific objectives of the CI-SFA are aligned to the three integrated research themes and one research activity. These themes are managed across the SFA as an integrated team effort. Eric Pierce is the Laboratory Research Manager (LRM) and point of contact with DOE Subsurface Biogeochemical Research program managers. He speaks to Paul Bayer biweekly on SFA progress and potential issues. The three theme leaders are Scott Brooks, Dwayne Elias, and Baohua Gu, and Scott Painter is the Field-scale Modeling Activity lead. These leaders and the broader team meet tri-weekly to provide an update on current research directions, future plans, and changes in staffing. See website for a complete organization chart (www.esd.ornl.gov/programs/rsfa/contacts.shtml).

National Laboratory Investments

ORNL is committed institutionally to the success of the CI-SFA. In FY19, ORNL funded several Laboratory Directed Research and Development projects under its "Integrated Studies of Complex Biological and Environmental Systems" initiative. Projects sponsored under this initiative are developing new tools and techniques and performing complementary science that will eventually benefit this CI-SFA. For example, one project is combining genomic analysis, 3D structure prediction and computational docking, and cell-free protein expression to characterize putative biosynthetic gene products. This project has direct relevance to our ability to construct 3D structures of HgcA and HgcB to computationally probe their biochemical function and to support the design of model-informed experiments. Additionally, renovations have been completed to modernize the biogeochemistry labs in ORNL's Environmental Sciences Division and Biosciences Division. Equipment investments include the purchases of (1) a Zeiss Versa 520 3D X-ray tomography system and (2) a cryo-enabled JEOL-NEOARM (new atomic-resolution analytical electron microscope). Each of these tools will be available to SFA researchers.

Appendix A. References Cited

An, J., L. Zhang, X. Lu, E. M. Pierce, A. Johs, J. Parks, B. Gu. 2019. "Mercury uptake by *Desulfovibrio desulfuricans* ND132: Passive or active?" *Environmental Science & Technology*. **53**(11):6264–72. DOI: 10.1021/acs.est.9b00047.

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Guo, L., S. L. Painter, S. C. Brooks, J. M. Parks and J. C. Smith. 2019. "A Probabilistic Perspective on Thermodynamic Parameter Uncertainties: Understanding Aqueous Speciation of Mercury." *Geochimica et Cosmochimica Acta, In press*.

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Zhao, L., Y. Li, L. Zhang, J. Zheng, E. M. Pierce, and B. Gu. 2019. "Mercury adsorption on minerals influences microbial methylation by *Desulfovibrio desulfuricans* ND132." *ACS Earth and Space Chemistry*. DOI: 10.1021/acsearthspacechem.9b00039.

Zheng, W., J. D. Demers, X. Lu, B. A. Bergquist, A. D. Anbar, J. D. Blum, and B. Gu. 2019. "Mercury stable isotope fractionation during abiotic dark oxidation in the presence of thiols and natural organic matter." *Environmental Science & Technology*. **53**(4):1853–62. DOI: 10.1021/acs.est.8b05047.

Appendix B. SFA Publications

See website for complete list (www.esd.ornl.gov/programs/rsfa/).

Manuscripts

An, J., L. Zhang, X. Lu, E. M. Pierce, A. Johs, J. Parks, and B. Gu. 2019. "Mercury uptake by *Desulfovibrio desulfuricans* ND132: Passive or active?" *Environmental Science & Technology*. **53**(11):6264–72. DOI: 10.1021/acs.est.9b00047.

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Chen, Q., A. Johs, X. Lu, H. Chen, J. An, D. A. Elias, E. M. Pierce, J. M. Parks, R. L. Hettich, and B. Gu. 2018. "Quantitative proteomic analysis of biological processes and responses of the bacterium *Desulfovibrio desulfuricans* ND132 upon deletion of its mercury methylation genes." *Proteomics*. **8**(17):1700479. DOI: 10.1002/pmic.201700479.

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Lu, X., A. Johs, L. Zhao, E. M. Pierce, and B. Gu. 2018. "Unexpected, copper-enhanced mercury methylation by *Desulfovibrio desulfuricans* ND132." *Environmental Science & Technology Letters*. **5**(6):372–76. DOI: 10.1021/acs.estlett.8b00232.

Lu, X., J. Zhao, X. Liang, L. Zhang, Y. Liu, X. Yin, X. Li, and B. Gu. 2019. "The application and potential artifacts of Zeeman cold vapor atomic absorption spectrometry in mercury stable isotope analysis." *Environmental Science & Technology Letters*. **6**(3):165–70. DOI: 10.1021/acs.estlett.9b00067.

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Zheng, W., J. D. Demers, X. Lu, B. A. Bergquist, A. D. Anbar, J. D. Blum, and B. Gu. 2019. "Mercury stable isotope fractionation during abiotic dark oxidation in the presence of thiols and natural organic matter." *Environmental Science & Technology*. **53**(4):1853–62. DOI: 10.1021/acs.est.8b05047.

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Christensen, G.A., C. M. Gionfriddo, A. J. King, J. G. Moberly, C. L. Miller, A. C. Somenahally, S. J. Callister, H. M. Brewer, M. Podar, S. D. Brown, A. V. Palumbo, C. C. Brandt, A. Wymore, S. C. Brooks, C. Hwang, M. W. Fields, J. D. Wall, C. C. Gilmour, and D. A. Elias. "How reliable are *hgcA* measurements and do they correlate with mercury and methyl-Hg concentrations in the Environment?" *Environmental Science & Technology*. *In review*.

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Liang, X., Gu, B., et al. 2019. "A stepwise reduction approach reveals mercury competitive binding and exchange reactions within natural organic matter and mixed organic ligands." *Environmental Science & Technology*. *In review*.

Muller, K. A., C. C. Brandt, and S.C. Brooks. "Methylmercury sorption onto engineered materials." *Journal of Environmental Management*. *In revision*.

Pathak A., M. Agarwal, R. Jaswal, S. Brooks, X. Xu, Charles Jagoe, and A. Chauhan. "Comparative proteogenomics of three mercury resistant strains isolated from two DOE contaminated ecosystems." *Cells*. *In review*.

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Yin, X., B. Gu, et al. 2019. "Effects of methanobactin on mercury methylation by *D. desulfuricans* ND132 and *G. sulfurreducens* PCA." *Environmental Science & Technology*. *In preparation*.

Data Products Released

1. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2015. doi:10.12769/1490688
2. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2016. doi:10.12769/1490689
3. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2017. doi:10.12769/1490690
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5. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 16.2 Water Year 2015. doi:10.12769/1490692
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11. Riscassi, Ami L., and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2014. doi:10.12769/1489825
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13. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2016. doi:10.12769/1489830
14. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2017. doi:10.12769/1489831
15. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2018. doi:10.12769/1489832
16. Riscassi, Ami L., and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2012. doi:10.12769/1490225
17. Riscassi, Ami L., and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2013. doi:10.12769/1490227
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19. Riscassi, Ami L., Kenneth A. Lowe, and Scott C. Brooks. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2015. doi:10.12769/1490231
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Appendix C. Presentations and Conferences

Ahmed, T., S. C. Brooks, R. Mohamed, C.-H. Tsai, and K. C. Carroll. 2018. "Statistical variability of streambed geochemical and hydrologic properties in the hyporheic zone of the East Fork Poplar Creek, Tennessee." 63rd Annual New Mexico Water Conference, October 17–18, 2018. Las Cruces , N.M.

Ahmed, T., S. C. Brooks, R. Mohamed, C.-H. Tsai, and K. C. Carroll. "Statistical variability of streambed geochemical and hydrologic properties in the hyporheic zone of the East Fork Poplar Creek, Tennessee." WM2019, March 3–7, 2019. Phoenix, Ariz.

Angell, E., G. Schwartz, and S. C. Brooks. "Relating soil geochemistry to microbial activity and methylmercury content in creek sediments." Spring 2019 ACS National Meeting, March 31–April 4, 2019. Orlando, Fla.

Cooper, S. J., S. Ovchinnikov, G. A. Pavlopoulos, N. C. Kyrpides, S. W. Ragsdale, A. Johs, M. Podar, and J. M. Parks. "Structure determination of the HgcAB complex using metagenome sequence data." Environmental System Science Principal Investigator Meeting, May 30–June 1, 2019. Potomac, Md.

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Gu, B. "Unraveling the production and degradation of methylmercury toxin in the environment." The 15th International Conference on the Biogeochemistry of Trace Elements (ICOBTE), May 5–9, 2019. http://icobte2019.csp.escience.cn/dct/page/65581 (Invited plenary talk).

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Johs, A. "Biomolecular mechanisms of mercury transformations in the environment." Integrated Diffraction Analysis (IDAT) BER site visit, Advanced Light Source, Lawrence Berkeley National Laboratory. November 6, 2018. Berkeley, Calif.

Johs, A. "Biomolecular transformations of mercury in the environment." Large Scale Structures Seminar Series, Neutron Scattering Division, SNS, ORNL. April 25, 2019. Oak Ridge, Tenn.

Johs, A. "Mercury biogeochemistry in East Fork Poplar Creek." Oak Ridge Institute for Continued Learning, Roane State Community College. June 21, 2019. Oak Ridge, Tenn.

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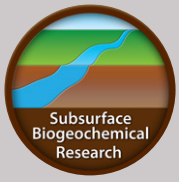
Acronyms and Abbreviations

1D, 3D	one dimensional, three dimensional
AGU	American Geophysical Union
ATS	Advanced Terrestrial Simulator
BER	DOE Office of Biological and Environmental Research
CCCP	carbonylcyanide-3-chlorophenylhydrazone
CI-SFA	Critical Interfaces Science Focus Area
CVAAS	cold vapor atomic absorption spectrometry
DOE	U.S. Department of Energy
DOM	dissolved organic matter
EFPC	East Fork Poplar Creek
EM	DOE Office of Environmental Management
EMSL	DOE Environmental Molecular Sciences Laboratory
Hg	mercury
hgcAB	Hg-methylation gene pair
HgcAB	protein
HGT	horizontal gene transfer
IDEAS	Interactive Design of Extreme-scale Application Software
KIE	kinetic isotope effect
LRM	Laboratory Research Manager
MATSZ	metabolically active transient storage zone
MeHg	methylmercury
MIF	mass independent fractionation
MMHg	monomethylmercury
MOF	mass dependent fractionation
ORNL	Oak Ridge National Laboratory
PI	principal investigator
SBR	DOE BER Subsurface Biogeochemical Research program
SFA	Scientific Focus Area
TSZ	transient storage zone



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