Biogeochemical Transformations at Critical Interfaces in a Mercury Perturbed Watershed

ORNL Mercury Science Focus Area 2020 Annual Report

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

Mercury Program Overview

At a time when demand for water is dramatically increasing because of population growth, industrialization, and expansion of irrigated agriculture, freshwater resources supplied by headwater streams and their surrounding watersheds are being threatened by severe pollution from anthropogenic releases of nutrients and trace metals such as mercury (Hg). More than 9,000 waterbodies in the continental United States are impaired. Mercury is the second leading cause of impaired waters—including locations in the Tennessee River Basin—and is responsible for fish consumption advisories in all 50 states (U.S. EPA 2011, 2013).

The economic and societal importance of headwater streams and their surrounding watersheds is exemplified by the Tennessee River Basin (see Fig. 1). Located in the southeastern United States, this river basin consists of a series of nested watersheds that supports ~4.5 million people by supplying water for power generation, industry, recreation, agriculture, and human consumption (Bohac and Bowen 2012) and represents 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. Preserving these freshwater resources for future use requires developing a deeper understanding of the structure and function of watersheds and the processes that govern pollutant transformations in aquatic ecosystems.

The Biogeochemical Transformations at Critical Interfaces in a Mercury Perturbed Watershed Science Focus Area (SFA) —is providing foundational insight on exchange and feedback processes occurring at critical interfaces that

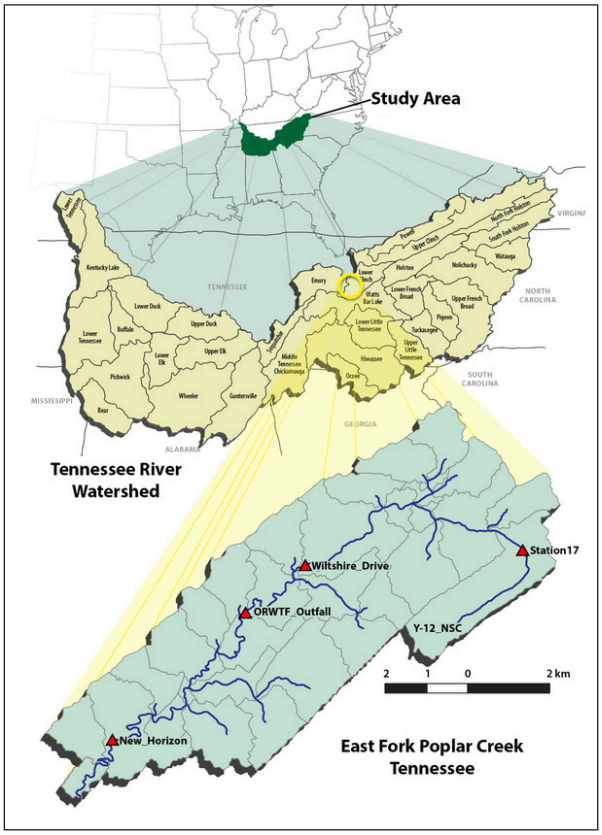

Fig. 1. SFA Study Area. East Fork Poplar Creek (EFPC), which is part of the 40,910-square-mile Tennessee River Basin, is the representative test bed system being studied. This river basin represents the most intensively used freshwater Water Resource Region in the contiguous United States, with estimated withdrawals of >280,000 gallons a day per square mile. The river basin is shown here 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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control Hg fate and transformation. The SFA study area is East Fork Poplar Creek (EFPC), an industrially contaminated freshwater creek in Oak Ridge, Tennessee. Led by Oak Ridge National Laboratory (ORNL), this project is supported by the Subsurface Biogeochemical Research (SBR) program of the Office of Biological and Environmental Research (BER) within the Department of Energy's (DOE) Office of Science.

In FY20, the Critical Interfaces SFA team has (1) added new capabilities to the Advanced Terrestrial Simulator (ATS) modeling software that integrates multiple components of EFPC watershed hydrology, (2) refined our transient availability model by including the impact that sediment type has on net methylmercury (MeHg) production, (3) explored the transcriptional regulation of *hgcA* under different growth parameters, (4) examined complex biogeochemical controls on Hg methylation, and (5) performed assays of the HgcAB complex in an *Escherichia coli* expression host and taken significant steps to evaluate potential cellular metabolites essential for methylation activity. We have also explored the mechanisms of abiotic dimethylmercury formation using density functional theory (DFT) calculations and determined that Hg isotope exchange reactions can alter native Hg isotope compositions, complicating the interpretation of Hg methylation and demethylation assays. We developed a new approach that definitively determined the functional group assignments, electronic structure, and coordination geometry and binding interactions that characterize methanobactin-metal complexes, using this information to help explain the differences in Hg methylation potential caused by different types of methanobactin. Collectively, the aforementioned activities are providing a deeper understanding of Hg transformations in EFPC and allowing us to gain the process knowledge needed to improve predictions of Hg transformations at the scale of individual stream reaches and small watershed catchments.

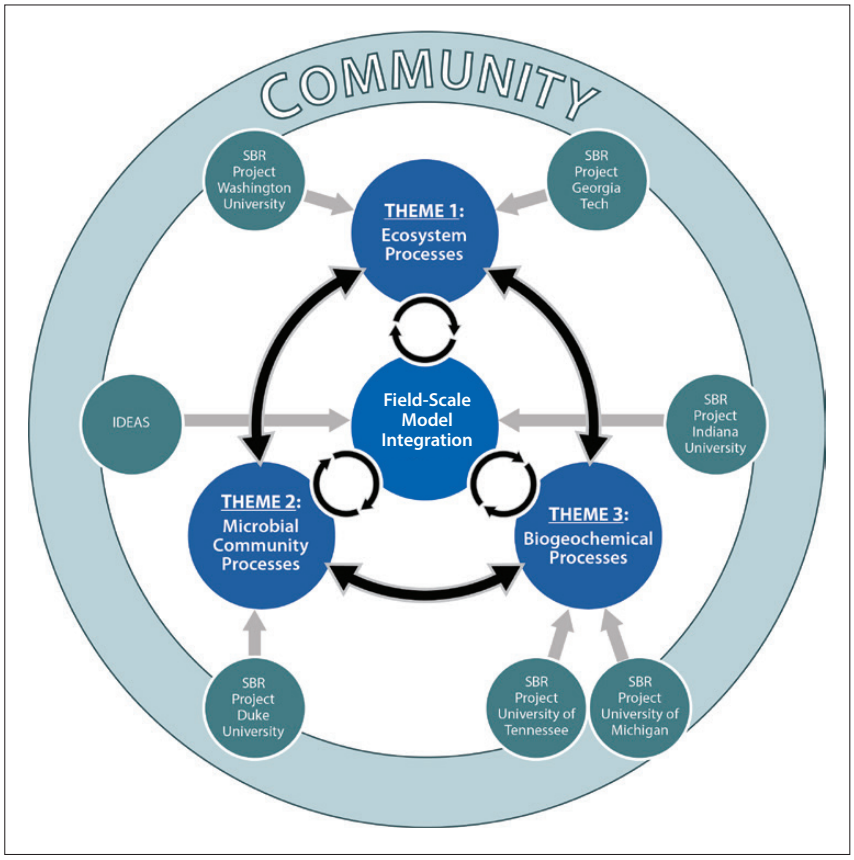

Fig. 2. Integrated, Multiscale SFA Research. Illustration showing the interconnections between themes, modeling activities, Interoperable Design of Extreme-scale Application Software (IDEAS), and various Subsurface Biogeochemical Research (SBR) university projects.

Integrated, Multiscale Research Approach

Critical Interfaces SFA research encompasses three themes—ecosystem processes, microbial community processes, and biogeochemical processes—and a research activity involving field-scale model integration (see 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 observe 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 transformations.
- **Field-Scale Model Integration.** Improves stream reach-to-watershed reactive transport modeling of contaminant and nutrient export. Activities include estimating the volume of transient storage zones (TSZs) and metabolically active TSZs (MATSZs) in EFPC and the mass transfer between TSZs and the creek channel, using nonreactive and reactive tracers to parameterize the field-scale model.

This annual report summarizes Critical Interfaces SFA accomplishments from July 2019 to June 2020, a period representing the second year following the program's triennial peer review in May 2018 and acceptance of the revised plan in September 2018 by BER's SBR program.

Scientific Progress

Theme 1: Ecosystem Features Influencing Mercury Transformation

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.

There are two overarching objectives:

- Identify ecosystem domains and hydro-biogeochemical conditions that govern net MeHg concentrations in EFPC.
- Work iteratively with ongoing modeling activities to inform and support the biogeochemical modeling framework.

These objectives are addressed through a set of hypotheses-driven field and laboratory investigations and the development of a process-rich numerical model to challenge current understanding of watershed processes occurring over broad spatiotemporal scales.

FY19–FY20 Accomplishments

Over the past 12 months, Theme 1 made significant progress toward milestones and published several papers relating to the role of periphyton in Hg cycling and development of 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.

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).

Periphyton biofilms produce a substantial fraction of the overall monomethylmercury (MMHg) flux in EFPC. We examined periphyton MeHg production across seasons, locations, and light conditions, using mercury stable isotopes. A transient availability (*trans av*) kinetic model was used to calculate methylation and demethylation rate potentials ($k_{m,\ trans\ av}$ and $k_{d,\ trans\ av}$, respectively; see Fig. 3). 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. Ambient MeHg and $k_{m,\ trans\ av}$ were significantly correlated. Transient availability rate potentials were 15 times higher for k_m and

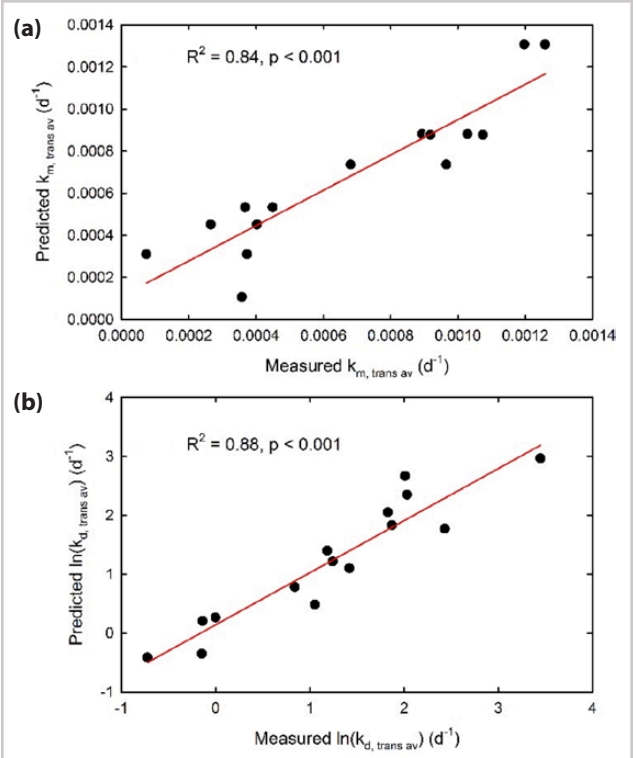

Fig. 3. Modeling Advance. Graphs show significant predictive modeling output for **(a)** methylation and **(b)** demethylation rate potential per day (d^{-1}) determined from the transient availability model (Olsen et al. 2018; Schwartz et al. 2019). No predictive relationship could be developed using traditional modeling approaches.

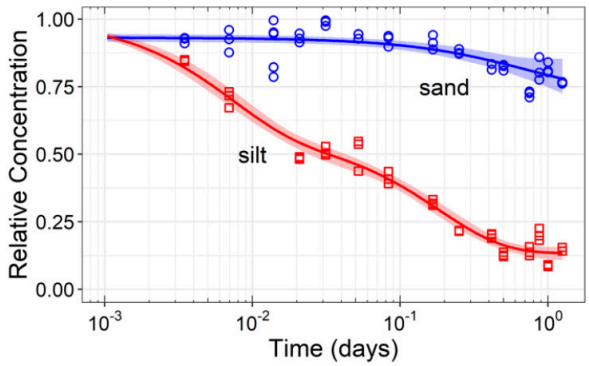

Fig. 4. Methylmercury (MeHg) Sorption onto Sandy (blue) and Silty (red) EFPC Sediments over Time. Symbols represent observations, and lines and ribbons represent multisite kinetic model fit and 95% confidence interval, respectively.

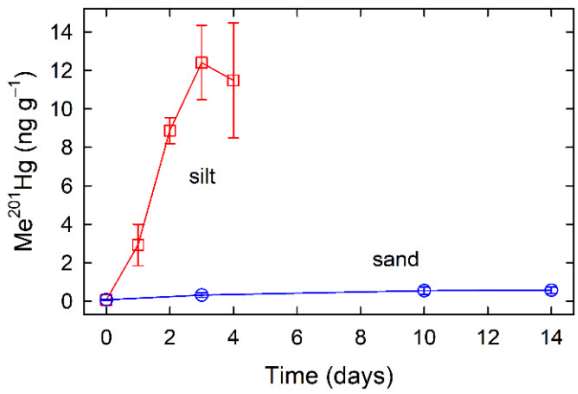

Fig. 5. MeHg Generation in Sandy (blue) and Silty (red) EFPC Sediments. Symbols and error bars represent the average ± standard deviation of triplicate samples.

9 times higher for k_d compared to full availability rate potentials ($k_{m,\ full\ av}$ and $k_{d,\ full\ av}$) calculated at 1 day. No significant model for the prediction of $k_{m,\ full\ av}$ or $k_{d,\ full\ av}$ could be constructed using light, season, and location. In addition, there were no significant differences among treatments for the full availability $k_{m,\ full\ av}$, $k_{d,\ full\ av}$ or net MeHg calculated using the full availability rate potentials. The $k_{m,\ full\ av}$ was not correlated with ambient MeHg concentrations. The current results underscore the importance of applying transient availability kinetics to MeHg production data when estimating MMHg production potential and flux (Schwartz et al. 2019).

We have extended our methylation-demethylation assays and kinetic model to include creek sediments from TSZs. Our work developed a kinetic model for net MeHg production in EFPC sediment that accounts for competing processes that may reduce Hg availability for methylation and MeHg availability for demethylation. This transient availability model combines kinetic expressions for multisite sorption of Hg and MeHg, Hg(II) reduction/Hg(0) oxidation, and methylation/demethylation kinetics. We conducted experiments in two EFPC sediment types: silty and sandy (see Fig. 4). Stable Hg and MeHg isotopes were used to determine Hg and MeHg sorption rates to the sediments and to track methylation and demethylation in sediment slurry microcosms. The silty sediment has a long water residence time and is relatively anoxic, carbon rich, and metabolically active compared to the sandy sediment (see Fig. 5). We found a much higher MeHg production potential in the silty sediment compared to that of the sandy sediment. The high MeHg production potential, coupled with the long water residence time, indicates that the silty sediment would have greater overall MeHg production in EFPC. However, the sandy sediment displayed lower MeHg sorption, indicating that a large proportion of the MeHg produced could be readily delivered to the water column. Our results will be incorporated into a field-scale model of EFPC to predict MeHg fluxes within the watershed (Schwartz et al., in preparation).

Status of FY20 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 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. Additionally, we are preparing a proposal to the DOE Joint Genome Institute's (JGI) Biological and Environmental Research Support Science (BERSS) program to perform metagenomics on periphyton samples.

Milestones 1c and d: *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 Laboratory at ORNL, which are critical to successful completion of these studies, have been completed. Initial trial runs were started then suspended due to the work-from-home posture at ORNL.

FY21 Plans

In FY21, 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

Muller, K. A., C. C. Brandt, T. J. Mathews, and S. C. Brooks. 2019. "Methylmercury sorption onto engineered materials." *Journal of Environmental Management.* **245**:481–88. DOI:10.1016/j.jenvman.2019.05.100 (data DOI:10.17632/jcfwd5sg4w.1).

Schwartz, G. E., T. A. Olsen, K. A. Muller, and S. C. Brooks. 2019. "Ecosystem controls on methylmercury production by periphyton in a contaminated freshwater stream: Implications for predictive modeling." *Environmental Toxicology and Chemistry.* **38**(11):2426–35. DOI:10.1002/etc.4551.

Submitted or In Preparation

Mohamed, R. A. M., S. C. Brooks, C.-H. Tsai, T. Ahmed, D. Rucker, A. Ulery, E. M. Pierce, and K. C. Carroll. "Geostatistical study of streambed hydrologic attributes including censored data and anisotropy." *Journal of Hydrology. In review.*

Murphy, S., G. E. Schwartz, and S. C. Brooks. 2020. "Demethylation or sorption? The fate of methylmercury in the presence of manganese dioxide." *Environmental Engineering Science. In review.*

Pathak, A., R. Jaswal, R. Rathore, M. Agarwal, X. Xu, J. R. White, S. Brooks, and A. Chauhan. "Characterization of environmentally-relevant bacterial and fungal strains from historically contaminated metalliferous soils using metagenomics coupled with diffusion chambers and microbial traps." *Frontiers in Microbiology. In revision.*

Rucker, D. F., C.-H. Tsai, K. C. Carroll, S. C. Brooks, E. M. Pierce, A. Ulery, and C. DeRolph. "Bedrock architecture, soil texture, and hyporheic zone characterization combining electrical resistivity and induced polarization imaging." *Journal of Applied Geophysics. In review.*

Schwartz, G. E., C. M. Gionfriddo, D. S. Jones, A. Soren, J. T. Bell, D. A. Elias, and C. C. Gilmour. "Abundance and diversity of *hgcAB*$^{+}$ microbes in Chesapeake salt marsh soils: Relationships to MeHg and site biogeochemistry." *Journal of Geophysical Research: Biogeosciences. In preparation.*

Tsai, C.-H., S. C. Brooks, D. F. Rucker, A. Ulery, E. M. Pierce, and K. C. Carroll. "Sherwood, Damköhler, and Péclet dimensionless number correlations of hyporheic zone, two-domain, transient-storage exchange." *Journal of Hydrology. In preparation.*

Data Products Released

1. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2019. DOI:10.12769/1569761.
2. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2019. DOI:10.12769/1569762.
3. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2019. DOI:10.12769/1569818.
4. Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 16.2 Water Year 2019. DOI:10.12769/1569821.

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?

FY19–FY20 Accomplishments

Over the past 12 months, Theme 2 has published a number of manuscripts and made significant progress toward our milestones, including (1) determining alternative (native) functions of HgcAB, (2) developing an accurate three-dimensional (3D) structural model of the HgcAB complex, (3) identifying and attempting to isolate novel Hg methylators from EFPC sediments, and (4) developing model microbial communities to reflect the EFPC community.

Determining Alternative HgcAB *Functions*

The native biochemical function of HgcAB remains elusive, but its identification is important to understand what controls Hg methylation and how metabolic states and environmental conditions impact activity. 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 FY20, we have made progress in 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 environmentally (versus mammalian) derived *Desulfobulbus* that does not methylate Hg. We performed an evolutionary analysis of HgcAB using all available genomic and metagenomic data (>4,000 sequences). While we observed the previously recognized groupings that include Deltaproteobacteria, methanogenic Archaea, and Firmicutes, there are numerous other organisms that possess HgcAB, 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) the genes may move within communities at time scales that are shorter than speciation (i.e., recent HGT events).

We have continued work related to physiological experiments for native function and completed numerous batch culture bottle experiments with *Desulfovibrio desulfuricans* ND132 wild-type and mutant strains (Δ*hgcAB*, Δ*metH*, Δ*cobT*, Δ*hgcA*:T101A, Δ*hgcA*:C93A, Δ*hgcA*:N90A, and Δ*hgcA*:N90P) grown in defined media with various substrates (e.g., pyruvate, fumarate, lactate, sulfate, formate, and acetate). We chose mutant strains related to carbon and Hg cycling that exhibited differences in Hg-methylation capability compared to the wild-type (e.g., 0 to 246%). Additionally, we continue to analyze the large omic dataset obtained from the Environmental Molecular Sciences Laboratory (EMSL; user proposal 50174). Preliminary results show differences in substrate consumption, acetate production, and transcription of one-carbon (C1) metabolism genes between mutant strains and wild-type under fermentative and sulfate-reducing conditions.

We hypothesize that the native physiological function of HgcAB may be related to C1 metabolism for acetyl-CoA and methionine biosynthesis, metal resistance, or metalloid methylation. Clues to the native biochemical function of HgcAB may lie in determining the environmental conditions that control expression and translation of *hgcA*. Therefore, we are investigating transcriptional regulation of *hgcA* under different growth parameters using *D. desulfuricans* ND132 as a model organism. We utilized both molecular reverse transcription–quantitative polymerase chain reaction (RT-qPCR) and metaomic (RNA-seq) methods to test whether changes in *hgcA* expression occurred when wild-type ND132 cells were grown in conditions that require the postulated

biochemical functions of HgcAB (e.g., +/– formate, methionine, arsenate, and Hg). Indeed, our results indicate that *hgcA* expression is significantly regulated across the growth stages of *D. desulfuricans* ND132 under some but not all conditions tested. We also explored whether deletion of *hgcAB* in *D. desulfuricans* ND132 hindered growth or produced any major phenotype in environmental test conditions. We measured differences in the proteome, metabolome, and metal speciation in growth media between wild-type and Δ*hgcAB* cultures. Significant differences in substrate consumption, acetate and biomass production, and expression of C1 metabolism proteins were observed between the strains under fermentative and sulfate-reducing conditions.

Developing a Model of the HgcAB *Complex*

Our understanding of the Hg methylation pathway is limited in part because we lack complete structural models of HgcA and HgcB, and the identities of other proteins likely involved remain 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 FY20, we generated an accurate model of the HgcAB complex by combining metagenome sequence data, coevolution analysis, and Rosetta calculations. 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.

Identifying and Isolating Novel Hg Methylators

Although we have some idea which me thylators are present in EFPC, none have been isolated or characterized at the genomic level. In FY20, we have taken steps to develop more robust, rapid, and cost-effective approaches to identify and isolate candidate strains from EFPC sediments. 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 sediments 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 have now produced 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.

The distribution and activity of *hgcAB*$^+$ organisms are important components in developing predictive models for Hg methylation from stream reach to watershed scales. Work at the Smithsonian Environmental Research Center (SERC) and at ORNL has mainly focused on this activity, including expression of HgcAB. To understand organismal distribution, we have employed several approaches to measuring *hgcAB* in DNA in nature. In FY20, a critical development was the publication of a new approach, the use of *hgcAB* amplicon specific sequencing (Gionfriddo et al., in review). Combined with the most current library of *hgcAB* sequences, this approach provides very high depth sequencing, allowing a much fuller and more detailed look at the types of microbes that harbor *hgcAB* in various environmental settings. SERC used this approach to study how the *hgcAB*$^+$ distribution in EFPC changes in response to changing electron acceptor conditions. This approach was used in parallel with the ORNL clade-specific qPCR primers to better quantify organisms in each major group of methylators. *Proteobacteria* were the dominant *hgcAB*$^+$ organisms in the creek at the time and place of sampling, followed by Chloroflexi and methanogens. Firmicutes and Nitrospirae made up only small percentages of the *hgcAB*$^+$ organisms. Over a month, the community structure of *hgcAB*$^+$ organisms did not change significantly at the phylum level in response to different electron acceptors. However, community structure did change significantly at the family level, with increases in *Desulfobulbus* in sulfate ($+SO_4$) bottles and increases in *Desulfarculaceae* and *Ruminococcaceae* in iron [+Fe(III)] bottles. Sulfate enhanced *de novo* MeHg production from a ^{201}Hg spike much more than nitrate or Fe(III), although biogenic sulfide inhibited MeHg accumulation. MeHg was strongly correlated with sulfate-reduction rates. Total microbial activity did not predict MeHg production or accumulation. Overall, we observed that sulfate-reducing bacteria, particularly *Desulfobulbus,* disproportionately contributed to MeHg production and accumulation in

this experiment using freshwater sediments (Schwartz et al., in preparation).

SERC has also been evaluating the distribution of *hgcAB*$^+$ organisms in relation to biogeochemistry across a salinity gradient of marsh soils in Chesapeake Bay. This work is a collaboration among Theme 1, Theme 2, and SERC. The study included quantification of *hgcAB*$^+$ organisms by clade using the ORNL qPCR primers and in-depth evaluation of community structure using *hgcA* amplicon specific sequencing, including the first test in natural samples of the new ORNL *hgcAB* universal primers (Gionfriddo et al., in review). Like the Terminal Electron Acceptor Process (TEAP) study, we found a disproportionate contribution to MeHg accumulation from Deltaproteobacteria. Salinity, overall microbial activity, and the relative abundance of *hgcAB*$^+$ Deltaproteobacteria were the main drivers of net MeHg accumulation.

An important next step in understanding and modeling the role of community structure in MeHg production will be to understand the relative levels of expression of *hgcAB* among microorganisms and across activity gradients. To help develop protocols for measuring *hgcAB* expression in nature, SERC is evaluating how expression changes across growth cycles in our model organism ND132. This work involves determining appropriate methods for isolating RNA, creating complementary DNA (cDNA), and quantifying *hgcA* in the cDNA. The critical choice of appropriate housekeeping genes for normalization of expression has been informed by the transcriptomic and proteomic work on native function in ND132.

In addition, we are in the process of renaming our model Hg-methylating SRB, ND132, and getting it into publicly available culture collections. The Gilmour lab wild-type strain was deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ) this year (DSM 110689). Approved in the American Type Culture Collection (ATCC), deposition is pending re-opening of laboratories after the pandemic. A paper formally describing the strain and its renaming to *Pseudodesulfovibrio mercurius* (sp. nov.) is complete and ready to submit to the *International Journal of Systemic and Evolutionary Microbiology* as soon as ATCC receives the culture for deposit.

Developing Model Microbial Communities and Fitness Assay

In FY20, we have made steady progress toward understanding the effect of multispecies interactions on MeHg generation and developing model microbial communities to reflect the EFPC community. Large-volume (1- to 4-L), single-species and dual-species batch cultures have been completed in bottles and bioreactors to characterize growth and chemical profiles for methylating and nonmethylating sulfate-reducing bacteria and methanogens, including *Desulfobulbus propionicus*, *Desulfobulbus oligotrophicus*, *Methanospirillum hungatei*, and *Methanococcus maripaludis*. We measured a suite of analytical parameters throughout the growth curve for each culture, including growth rates based on optical density (OD) and protein content, Hg methylation, MeHg demethylation, *hgcA* expression, qPCR and fluorescence *in situ* hybridization (FISH) determination of relative cell abundance, simple metabolites (anions/cations/organic acids), sulfide concentration, and pH. Methylation/demethylation analyses are ongoing due to the pandemic, but the pairings of methylating and nonmethylating organisms allow us to disentangle the specific effect of syntrophic interactions on MeHg generation in organisms with very different metabolisms (i.e., sulfate reduction versus methanogenesis). Continuing efforts are underway in FY20 to increase the complexity of multispecies cultures to recapitulate the range of metabolisms present in EFPC sediment, including the addition of *Syntrophus aciditrophicus* and *G. sulfurreducens*.

With respect to cell fitness under laboratory conditions, deletion of *hgcAB* does not significantly change the growth rate of *D. desulfuricans* ND132. However, maintenance of these genes across evolutionary time scales suggests that they provide an important fitness benefit in the environment. We hypothesize that the fitness effects of an *hgcAB* deletion are environmentally dependent and that these genes are conditionally dispensable under standard laboratory conditions. In conditions where *hgcAB* are providing their native function, we expect to see a fitness defect in a Δ*hgcAB* strain compared to the wild-type. So far, we have measured competitive fitness between wild-type and Δ*hgcAB* ND132 in more than 20 different media compositions. Although additional experiments are needed, our current results show that the strains grow differently when mixed together compared to when grown separately, despite minimal genetic differences between the two strains. In turn, this result suggests that HgcAB is involved in interactions between cells of ND132. Ongoing work is exploring these interactions in more detail.

Status of FY20 Milestones

Milestone 2a: Identified existing neutral, single-nucleotide mutations for fitness and performed several fitness assays using various metals, both higher and lower as well as absent from growth media.

Milestones 2b and g: Generated new antibodies and accomplished initial cell sorter runs toward isolating EFPC Hg-methylating species.

Milestone 2c: Completed use of comparative genomics for elucidating *hgcAB* pathways.

Milestone 2d: Have not yet begun transformation of hgcAB into naïve hosts to mimic HGT.

Milestones 2e and f: Completed characterization of microbial growth and Hg methylation in monocultures and two co-cultures, and are working toward characterizing a synthetic community.

Milestones 2h and i: Submitted manuscript (under review) on metagenomics-enabled co-evolution and protein-protein interaction studies for HgcA and HgcB. We are also initiating protein-protein interaction studies for HgcAB-associated proteins for biochemical pathway elucidation.

FY21 Plans

In FY21, 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 completion of the multiomics analysis using transcriptomics, proteomics, metabolomics, and lipidomics from several wild-type and mutant cultivations as above.
- Continue to characterize growth rate, metabolism, Hg methylation, and MeHg demethylation in co-cultures before moving onto tri- and perhaps quad-communities.

Manuscripts

Published or In Press

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 of Chemical Research.* **52**(2):379–88. DOI:10.1021/acs.accounts.8b00454.

Bowman K., E. Collins, A. Agather, C. Lamborg, C. Hammerschmidt, D. Kaul, C. L. Dupont, G. A. Christensen, and D. A. Elias. 2019. "Distribution of mercury-cycling genes in the Arctic and equatorial Pacific Oceans and their relationship to mercury speciation." *Journal of Limnology and Oceanography.* **65**(S1):S310–20. DOI:10.1002/lno.11310.

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. "Determining the reliability of measuring mercury cycling gene abundance with correlations with mercury and methylmercury concentrations." *Environmental Science & Technology.* **53**(15):8649–53. DOI:10.1021/acs.est.8b06389.

Cooper, C. J., K. Zheng, K. W. Rush, A. Johs, B. C. Sanders, G. Pavlopoulos, N. C. Kyrpides, M. Podar S. Ovchinnikov, S. W. Ragsdale, and J. M. Parks. 2020. "Structure determination of the HgcAB complex using metagenome sequence data: Insight into the mechanism of mercury methylation." *Communications Biology.* **3**:320. DOI:10.1038/s42003-020-1047-5.

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Hwang, H., A. Hazel, J. C. Smith, J. C. Gumbart, and J. M. Parks. 2020. "A minimal membrane metal transport system: Dynamics and energetics of *mer* proteins." *Journal of Computational Chemistry*. **41**(6):528–37. DOI:10.1002/jcc.26098.

Lian, P., L. Guo, D. Devarajan, J. M. Parks, S. L. Painter, S. C. Brooks, and J. C. Smith. 2020. "The *AQUA-MER* databases and aqueous speciation server: A web resource for multiscale modeling of mercury biogeochemistry." *Journal of Computational Chemistry*. **41**(2):147–55. DOI:10.1002/jcc.26081.

Submitted or In Preparation

Gilmour, C. C., C. M. Gionfriddo, and D. Elias. "An overview of our understanding of Hg methylation." *Frontiers in Microbiology*. *In preparation*.

Gilmour, C. C., A. Soren, C. M. Gionfriddo, M. Podar, J. D. Wall, S. D. Brown, J. K. Michener, M. S. Goñi-Urriza, and D. Elias. "*Pseudodesulfovibrio mercurius* sp. nov., a mercury-methylating bacterium isolated from Chesapeake Bay sediment." *International Journal of Systematic and Evolutionary Microbiology*. *To be submitted*.

Gionfriddo, C. M., A. M. Wymore, D. S. Jones, M. M. Lynes, G. A. Christensen, A. Soren, C. C. Gilmour, J. D. Wall, C. C. Brandt, M. Podar, A. V. Palumbo, and D. A. Elias. "Expanded Hg-methylator diversity in nature, using an improved *hgcAB* primer set and direct high-throughput sequencing." *Frontiers in Microbiology*. *In review*.

Lian P., Z. Mou, R. C. Johnston, S. C. Brooks, B. Gu, N. Govind, S. Jonsson, and J. M. Parks. "Mechanisms of dimethylmercury formation facilitated by nanoparticles." *Environmental Science & Technology*. *In revision*.

Schwartz, G. E., C. M. Gionfriddo, D. S. Jones, A. Soren, J. T. Bell, D. A. Elias, and C. C. Gilmour. "Abundance and diversity of *hgcAB*$^{+}$ microbes in Chesapeake salt marsh soils: Relationships to MeHg and site biogeochemistry." *Journal of Geophysical Research: Biogeosciences. In preparation*.

Wilpiszeski, R. L., A. M. Wymore, C. M. Gionfriddo, M. Podar, and D. A. Elias. 2020. "Effects of syntrophic interactions on MeHg generation." *PLOS Biology. In preparation*.

Theme 3: Biogeochemical Complexity and Molecular Mechanisms of Hg Transformations

The overarching goal of Theme 3 is to gain a fundamental understanding of complex biogeochemical processes and their interactions [e.g., dissolved organic matter (DOM), microbes, particulate organic matter (POM) and minerals, and water chemistry in EFPC] controlling Hg species transformation and availability for cellular uptake and methylation. Our specific objectives are to address the following scientific questions:

- What are the 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?

FY19–FY20 Accomplishments

Theme 3 made significant progress toward milestones over the past 12 months and has published six papers and submitted nine manuscripts that are currently under review. The studies on which these documents are based are mostly focused on understanding a complex, yet finite set of geochemical and biomolecular processes controlling Hg transformations and net MeHg production in the environment. A robust predictive understanding of Hg biogeochemical transformations requires knowledge about the underlying molecular mechanisms and coupled interactions between microbes, Hg-binding organic and inorganic ligands, and minerals.

Roles of Methanobactin (MB) on Hg Methylation and Structural Characterization of Hg-MB Complexes

Methanotrophic bacteria and the copper [Cu(II)]-chelating chalkophore MB have been implicated in the biogeochemical cycling of Hg. Our recent studies demonstrated that MBs enable some methanotrophs to degrade MeHg (Lu et al. 2017), but they can also influence the bioavailability of inorganic Hg(II) for MeHg production by anaerobic bacteria (Yin et al. 2020). Methanobactins are small, post-translationally modified peptides, which strongly bind Cu(II) ions and other late-transition metals such as Hg(II), and thus are hypothesized to limit Hg(II) uptake and methylation by the methylating bacteria. Contrary to expectations, however, MB produced by the methanotroph *Methylosinus trichosporium* OB3b (OB3b-MB) enhanced the rate and efficiency of Hg(II) methylation more than that observed with thiol compounds (e.g., cysteine) by the Hg-methylating bacteria, *D. desulfuricans* ND132 and *G. sulfurreducens* PCA (Yin et al. 2020). Compared to no-MB controls, OB3b-MB decreased the rates of Hg(II) sorption and internalization, but increased methylation by five- to seven-fold, suggesting that Hg(II) complexation with OB3b-MB facilitated exchange and internal transfer of Hg(II) to the HgcAB proteins required for methylation. Conversely, addition of excess amounts of OB3b-MB or a different form of MB from *Methylocystis* strain SB2 (SB2-MB) inhibited Hg(II) methylation. These observations underscore complex interactions among microbial communities and the roles of exogenous metal-scavenging compounds produced by methanotrophs in controlling net production and bioaccumulation of MeHg in the environment.

To understand the underlying biochemical mechanisms of MB influences on Hg methylation and MeHg degradation, we investigated the complexation of Hg(II) by MB from *Methylocystis* sp. SB2 by ultraviolet-visible absorption spectroscopy and fluorescence spectroscopy, and we compared the experimental data to electronic structure calculations using time-dependent DFT. Our results reveal that binding of Hg(II) to MB results in a characteristic red shift along with changes in the amplitude of electronic excitations originating from two chromophores near the ligand binding site. In addition, a Hg(II) concentration–dependent fluorescence enhancement suggests that binding

of Hg(II) increases local structural rigidity near the metal-binding site. This study represents the first combined spectroscopic and computational characterization of metal binding by MBs, providing insights into MB interactions with Hg(II). Furthermore, we conducted a crystallization screening of Hg(II)-MB complexes aimed at gaining insights into the structure of MB complexed to Hg(II). However, time-resolved studies of Hg(II)-MB complexed and other late-transition metals revealed that Hg(II)-MB complexes undergo a series of time-dependent transformations over periods of minutes to days, which are not observed for Cu(II) or zinc [Zn(II)]-MB complexes.

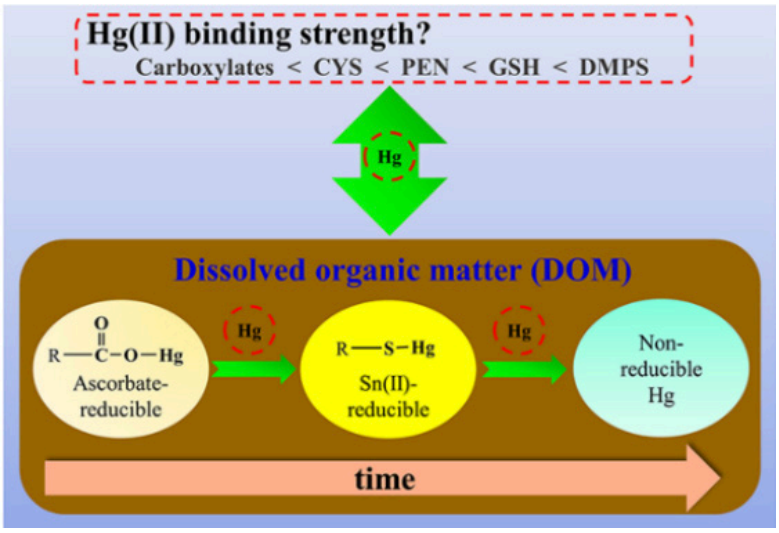


Fig. 6. Kinetics of Mercury (Hg) Binding with Dissolved Organic Matter (DOM). Illustration of competitive ligand exchange reactions for Hg from the weak carboxylate to the strong thiolate ligands, and to the strongest nonreducible fractions within DOM over time. [Reprinted with permission from Liang, X., et al. 2019. "Stepwise reduction approach reveals mercury competitive binding and exchange reactions within neutral organic matter and mixed organic ligands." *Environmental Science & Technology.* **53**(18): 10685–94. DOI: 10.1021/acs.est.9b02586. Copyright 2019 American Chemical Society.]

Environmental Complexity on Hg-Ligand-Cell Interactions and Biochemical Mechanisms

We have developed a novel stepwise reduction approach to enable differentiation of Hg species bound to various organic and inorganic ligands in complex natural systems and thus to tease out competitive ligand-binding reactions with Hg (Liang et al. 2019). The kinetics of Hg binding with heterogeneous natural DOM has been hypothesized to result from competitive interactions among different organic ligands and functional groups of DOM for Hg(II), but experimental protocol is lacking to determine Hg binding with these ligands and their relative binding strengths, as well as dynamic exchange reactions. A stepwise reduction approach using ascorbic acid (AA) and stannous tin [Sn(II)] was devised to differentiate Hg(II) species in the presence of two major functional groups—the carboxylate-bound Hg(II) is reducible by both AA and Sn(II), whereas the thiolate-bound Hg(II) is reducible only by Sn(II). Using this operational approach, the relative binding strength of Hg with selected organic ligands was found, in order: dimercaptopropanesulfonate (DMPS) > glutathione (GSH) > penicillamine (PEN) > cysteine (CYS) > ethylenediamine-tetraacetate (EDTA) > citrate, acetate, and glycine at the ligand-to-Hg molar ratio < 2 (see Fig. 6). Dynamic, competitive ligand exchanges for Hg from weak carboxylate to strong thiolate functional groups were observed among these ligands and within DOM, and the reaction depended on the relative binding strength and abundance of thiols and carboxylates, as well as reaction time. The new approach could thus offer a means to assess relative binding strengths of Hg with a suite of natural organic ligands and their competitive exchange reactions. Our results provide additional insights into dynamic exchange reactions of Hg within heterogeneous DOM in controlling the transformation and bioavailability of Hg in natural aquatic environments.

The new approach also enables us to discover an overlooked isotope exchange phenomenon in tracing Hg biogeochemical transformations with enriched Hg isotopes, which have been widely used as tracers in field and laboratory investigations of Hg fate and transformation, such as methylation and demethylation. Few studies, however, have considered concurrent isotope exchange between newly spiked and native ambient Hg in reactions, which are found to alter Hg isotope redistribution and thus its transformation dynamics (Zhang et al., in review). We investigate isotope exchange reactions of divalent Hg(II) species in various environmental matrices, including Hg-contaminated sediments, low-molecular-weight (LMW) thiols, and DOM. We found that spiked ^{198}Hg readily exchanges with sediment-associated Hg despite concurrent Hg adsorption and immobilization on the particles. Spiked ^{198}Hg also rapidly exchanges with ^{200}Hg bound to LMW thiols and DOM in solution within minutes. The exchange results in no net changes in chemical speciation but redistributions of Hg isotopes bound to

the ligands proportional to their molar fractions. Most importantly, the reaction alters Hg bioavailability and therefore the rates and magnitude of microbial methylation. These findings suggest a potentially wide occurrence of Hg isotope exchange in environmental systems and, if not considered, it could lead to biased risk assessments of new Hg input to the natural ecosystem.

The production of MeHg in anaerobic bacteria and archaea is mediated by the *hgcAB* gene cluster. Proteomics and immunoblot data suggest that the abundance of the proteins HgcA and HgcB in cells of the model Hg-methylating bacterium *D. desulfuricans* ND132 is extremely low. In a collaboration with Steve Ragsdale (University of Michigan), we have co-expressed HgcAB in a pETDuet vector in an *E. coli* BL21 cell line, which also contains the plasmids pBtu and pRKISC to improve cobalamin uptake iron-sulfur cluster assembly, respectively. We conducted Hg methylation assays with *E. coli* cell lysates, demonstrating that the heterologously co-expressed HgcAB complex is active and able to convert added Hg(II) to MeHg. In addition, to facilitate purification of the HgcAB complex for spectroscopic and structural characterization, we expressed a His_6-tagged HgcAB in *E. coli*, which also showed methylation activity in cell lysates, albeit at lower levels compared to untagged HgcAB. Interestingly, the methylation activity of all constructs is substantially enhanced after adding cell lysates from deletion mutants *(ΔhgcAB)* of *D. desulfuricans* ND132 and *G. sulfurreducens* PCA. Thus, we further explored the role of cellular metabolites on Hg methylation activity by screening a series of cellular metabolites potentially involved in Hg methylation. We identified a positive correlation between the levels of exogenously added S-adenosyl methionine and Hg methylation rates. The present results establish the foundation for biochemical, spectroscopic, and structural characterization of the HgcAB complex, which will provide essential information about its role in MeHg methylation and the links to Hg uptake and cellular metabolism. Ultimately, our studies will provide improved understanding of the key geochemical and biochemical controls and interactions affecting Hg species transformation and availability for cellular uptake and methylation in complex environmental systems.

Status of FY20 Milestones

Milestones 3a and b: Molecular characterization of Hg-binding ligands and their competitive interactions. Completed competitive ligand interactions with Hg(II), and the characterization of MB complexes with Hg(II) and other transition metals. Studies to investigate time-dependent changes in Hg(II)-MB complexes are ongoing.

Milestones 3c, d, and e: Characterization of cellular proteins, Hg-cell interactions, and controls on Hg uptake and methylation. Completed studies of Hg-ligand-cell interactions in controlling Hg uptake and methylation (An et al. 2019; Yin et al. 2020). We also demonstrated activity of the heterologously expressed HgcAB complex and identified potential methyl donors for HgcAB. Additional studies are ongoing to investigate cellular binding proteins and the effects of mixed organic ligands on cellular Hg uptake and methylation.

Milestones 3f, g, and h: Biogeochemical complexity influences on microbial Hg uptake and methylation. Completed studies of biogeochemical complexity (e.g., DOM and minerals) on Hg chemical speciation and microbial Hg uptake and methylation (e.g., Zheng et al. 2019; Zhang et al. 2019; Zhao et al. 2019). Additional studies are ongoing to investigate mixed organic ligands on Hg methylation and MeHg degradation by algae and methanotrophs under varying environmental conditions.

FY21 Plans

In FY21, Theme 3 planned activities include:

- Investigate cellular binding domains and the effects of mixed organic ligands on cellular Hg uptake and methylation.
- Continue studies of Hg isotope exchange reactions in tracing Hg biogeochemical transformations in complex environmental matrixes.
- Investigate MeHg degradation by algae and methanotrophs under varying environmental conditions.
- Delineate the prevalence of methanotrophs in EFPC sediments and periphyton in metagenomic datasets.
- Continue collaboration with Steve Ragsdale at the University of Michigan on the structural and functional characterization of HgcAB.

Manuscripts

Published or In Press

Cooper, C. J., K. Zheng, K. W. Rush, A. Johs, B. C. Sanders, G. Pavlopoulos, N. C. Kyrpides, M. Podar S. Ovchinnikov, S. W. Ragsdale, and J. M. Parks. 2020. "Structure determination of the HgcAB complex using metagenome sequence data: Insight into the mechanism of mercury methylation." *Communications Biology.* **3**:320. DOI:10.1038/s42003-020-1047-5.

Johs, A., V. A. Eller, T. L. Mehlhorn, S. C. Brooks, D. P. Harper, M. A. Mayes, E. M. Pierce, and M. J. Peterson. 2019. "Dissolved organic matter reduces the effectiveness of sorbents for mercury removal." *Science of the Total Environment.* **690**:410–16. DOI:10.1016/j.scitotenv.2019.07.001.

Liang, X., X. Lu, J. Zhao, L. Liang, E. Zeng, and B. Gu. 2019. "Stepwise reduction approach reveals mercury competitive binding and exchange reactions within natural organic matter and mixed organic ligands." *Environmental Science & Technology*. **53**(18):10685–94. DOI:10.1021/acs.est.9b02586.

Yin, X., L. Wang, L. Zhang, H. Chen, X. Liang, X. Lu, A. A. DiSpirito, J. D. Semrau, and B. Gu. 2020. "Synergistic effects of a chalkophore, methanobactin, on microbial methylation of mercury." *Applied and Environmental Microbiology*. **86**:e00122-20. DOI:10.1128/AEM.00122-20.

Zaporski, J., M. Jamison, L. Zhang, B. Gu, and Z. Yang. 2020. "Mercury methylation potential in a sand dune on Lake Michigan's eastern shoreline." *Science of the Total Environment*. **729**:10(138879). DOI:10.1016/j.scitotenv.2020.138879.

Zhao, L., Y. Li, L. Zhang, J. Zheng, E. M. Pierce, and B. Gu. 2019. "Mercury adsorption on minerals and its effect on microbial methylation." *ACS Earth and Space Chemistry*. **3**(7):1338–45. DOI:10.1021/acsearthspacechem.9b00039.

Submitted or In Preparation

Date S., K. Zheng, K. Rush, J. M. Parks, S. W. Ragsdale, and A. Johs. "Biochemical factors controlling Hg methylation activity of HgcAB." *In preparation*.

Eckert, P., A. Johs, J. D. Semrau, A. A. DiSpirito, B. Gu, and E. M. Pierce. "Computational and spectroscopic characterization of methanobactin from *Methylocystis* sp. SB2 and its complexation with transition metals." *Journal of Physical Chemistry A. To be submitted*.

Kang, C. S., X. Liang, P. Dershowitz, A. Schepers, A. Flatley, J. Lichtmanneger, H. Zischka, X. Lu, B. Gu, A. A. DiSpirito, and J. D. Semrau. "Cheating to get ahead: Chalkophore theft by methanotrophs." *The ISME Journal. In preparation*.

Koenigsmark, F., C. Weinhouse, A. Berky, E. Ortiz, E. M. Pierce, W. Pan, and H. Hsu-Kim. "Efficacy of hair total mercury content as a biomarker of methylmercury exposure to communities near artisanal and small-scale gold mining in Madre de Dios, Peru." *Environmental Science & Technology. In review*.

Liang, X. J., A. Johs, J. Zhao, X. Yin, L. Wang, L. Zhang, E. Zeng, D. A. Pelletier, E. M. Pierce, and B. Gu. "Light-independent degradation of methylmercury by algae." *Nature Communications. In review*.

Wang, Q., L. Zhang, X. Liang, X. Yin, Y. Zhang, E. M. Pierce, W. Zheng, and B. Gu. "Rates and mechanisms of isotope exchange between dissolved elemental Hg(0) and Hg(II) bound to organic and inorganic ligands." *Environmental Science & Technology*. *Submitted*.

Xin-Quan, Z., H. Yun-Yun, F. Jiao, B. Gu, L. Yu-Rong, and H. Qiaoyun. "Microbial communities associated with methylmercury degradation in paddy soils." *Environmental Science & Technology*. *Submitted*.

Zhang, L., X. Lu, X. Liang, Q. Wang, Y. Zhang, X. Yin, E. M. Pierce, and B. Gu. "Overlooked isotope exchange reactions in tracing mercury (Hg) biogeochemical transformations with enriched Hg isotopes." *Environmental Science & Technology Letters*. *In review*.

Zhang, L., M. Philben, N. Taş, Z. Yang, E. M. Pierce, D. E. Graham, and B. Gu. "Biogeochemical drivers of methylmercury production in Arctic tundra soils." *In preparation*.

Zhou, X.-Q., Y.-Y. Hao, J. Feng, B. Gu, Y.-R. Liu, and Q. Huang. "Microbial communities associated with methylmercury degradation in paddy soils." *Environmental Science & Technology*. *Submitted*.

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 is iteratively developing, evaluating, and refining 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 possible, for the first time, the tractable representation of redox zonation and other fine-scale geochemical phenomena at reach-to-watershed scales without the need for 3D characterization of hyporheic flow zones. This 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. This 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. 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.

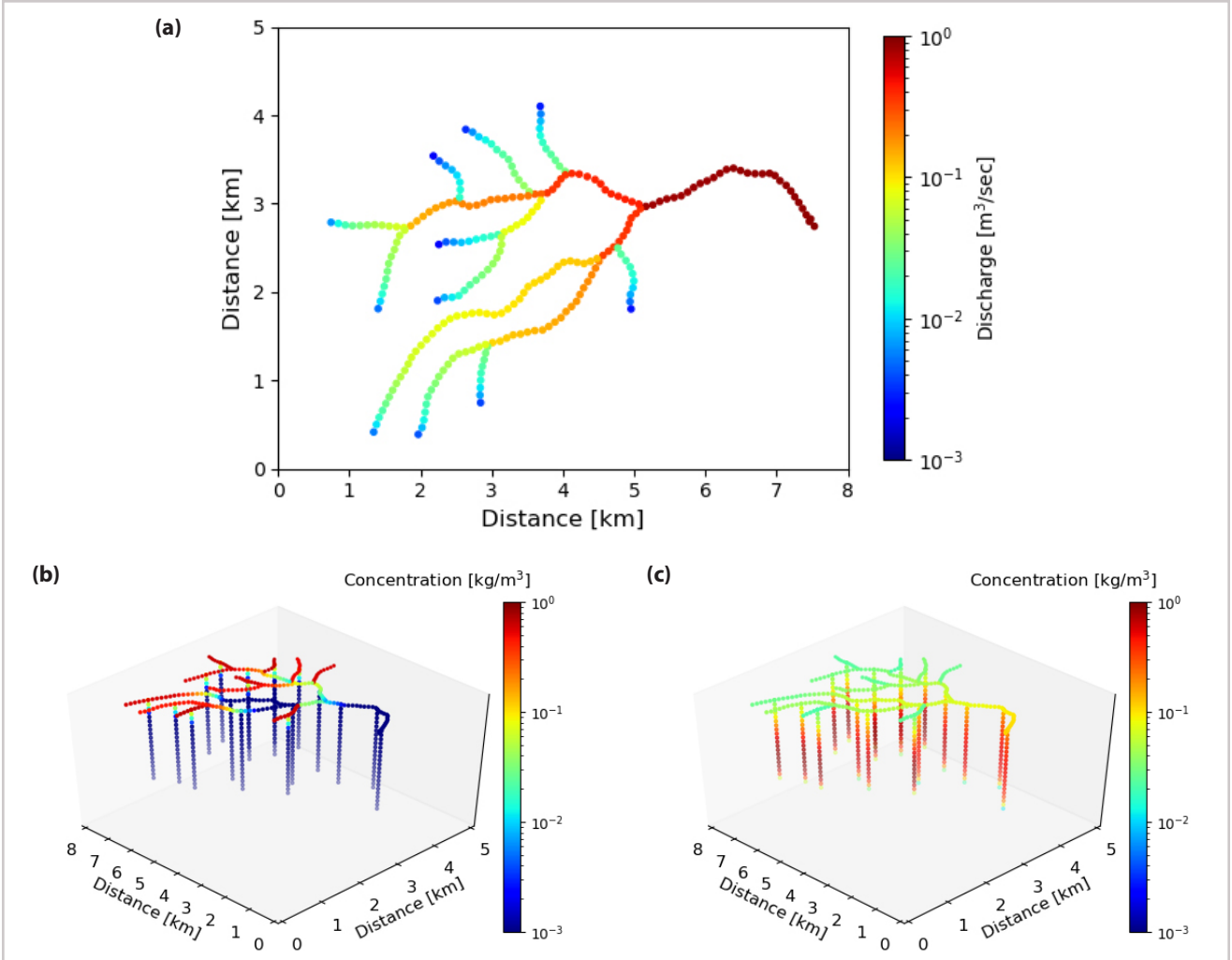


Fig. 7. Multiscale Advanced Terrestrial Simulator (ATS) Simulations. We extended our multiscale model for reactive transport in stream corridors to watershed scales and developed software tools to extract stream network data from online databases to build the necessary model input. Shown in **(a)** is channel discharge for a headwater catchment on the western edge of the Tennessee River Basin. Shown in **(b)** and **(c)** are ATS results for transport of a pulse of conservative tracer through the network. These plots show concentration in both the channel network and in the hyporheic zone subgrid model. For clarity, we show only one hyporheic zone subgrid model below each reach. In **(c)**, the pulse of conservative tracers has ended, but the hyporheic zone still has stored trace that is slowly returning to the stream channel network.

The field-scale modeling activity is a partnership activity with the Interactive Design of Extreme-scale Application Software (IDEAS)–Watersheds project. The jointly funded activity has subtasks related to model development, estimation of parameters from field-scale tests, and initial demonstrations.

FY19–FY20 Accomplishments

We extended our multiscale model implementation in the ATS to watershed scales. The capability is now fully functional for conservative tracers. Tools for extracting stream network characteristics from online databases and building the computational meshes needed for the model were developed. An example watershed simulation built this way is shown in Fig. 7. We also made significant progress merging that capability with PFLOTRAN reactive chemistry through the Alquimia interface and expect to complete that in the third quarter (Q3) of FY20. Software tools to estimate model parameters and their uncertainties from the results of in-stream tracer tests using the Markov Chain Monte Carlo method were also developed. We are currently testing those tools using published tracer test data.

Status of FY20 Milestones

As described in the FY19 annual report, our originally proposed work on modeling EFPC tracer tests has been revised to focus first on published tracer test results from other systems including H. J. Andrews Experimental Forest. Work on modeling nonreacting tracers in streams with unsteady channel discharge is on track for completion in Q4 of FY20.

FRA2b work was split into two parts. The first manuscript, introducing our new multiscale model, was published in FY19. The second part, describing the implementation in ATS and extension to river networks, is on track for completion in Q3 of FY20.

Work on FRA2a, an extension of Alquimia to accommodate the PHREEQC computer program, is delayed because ATS's Alquimia interface needed to be updated to be consistent with the current transport capabilities in ATS. We will work with PFLOTRAN for reactions in the immediate future.

FY21 Plans

In FY21, we will extend our work on tracer test analysis to model reactive tracer tests conducted at Andrews Forest and prepare a journal article on that work. We also will begin modeling planned reactive tracer tests in EFPC to help design and evaluate the planned tests.

Manuscripts

Submitted or In Preparation

Painter, S. L., A. Jan, and E. T. Coon. "Accounting for the biogeochemical effects of hyporheic exchange flows in network-scale models of reactive transport in streams and rivers." *Water Resources Research. In preparation.*

Painter, S. L., A. Jan, and E. T. Coon. "Toward more mechanistic representations of biogeochemical processing in stream and river networks: A multiscale strategy for representing the biogeochemical effects of hyporheic exchange flows." *Frontiers of Water. In preparation*.

Rathore, S., S. L. Painter, E. T. Coon, and A. Jan. "On the modeling of tracer transport in streams with unsteady channel discharge." *Water Resources Research*. *In preparation*.

Select Research Highlights

In FY20, a total of 31 manuscripts have been published or submitted by the Critical Interfaces SFA. Of these publications, 15 are published or in press, bringing the total to 142 for the Critical Interfaces SFA since its inception. Of these 142 publications, 127 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 2 of the 31 published or submitted manuscripts.

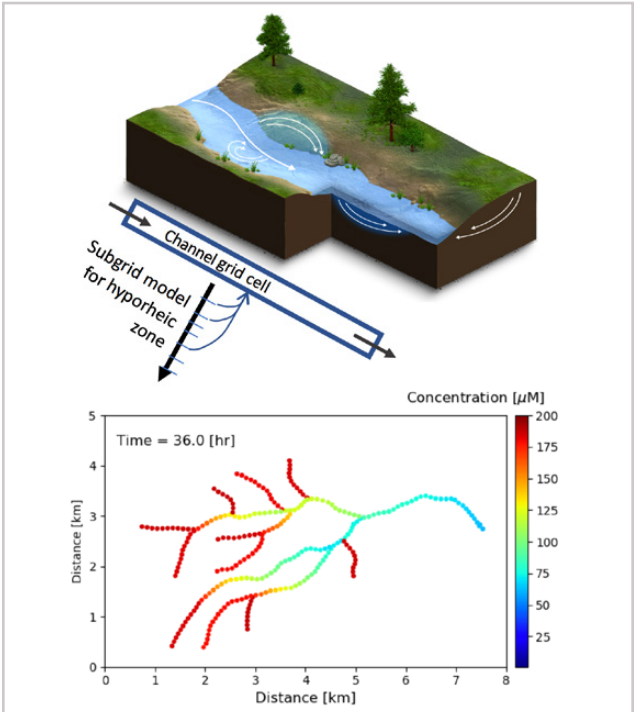
Research Highlight

Representing the effects of biogeochemical hotspots in basin-scale models

A new multiscale simulation approach enables more detailed representation of biogeochemical processing of carbon, nutrients, and metals in basin-scale models

The Science

Highly localized biogeochemical hotspots are widely recognized to play an outsized role in controlling the transformation of nutrients and contaminants in streams and rivers, but they are difficult to represent in field-scale models. In collaboration with the IDEAS–Watersheds project, we developed a new multiscale representation of transport and transformation in stream corridors. The new model, CHAnnel Network with Lagrangian Subgrid (CHANLS), associates a subgrid model for hyporheic zone reactive transport with each channel grid cell. The subgrid models are written in Lagrangian form with hyporheic age replacing travel distance. This novel application of stochastic subsurface hydrology theory makes it possible to efficiently represent an ensemble of flowpaths and account for flowpath diversity through the biogeochemically active hyporheic zone. We implemented the CHANLS conceptualization in the open-source community software ATS using the Alquimia application programming interface to access the biogeochemical reaction modeling capability in the PFLOTRAN simulator. Methods for estimating key model parameters related to hyporheic exchange rates and hyporheic residence times from stream tracer tests were developed and tested.



Top: Depiction of hyporheic exchange in the stream/river corridor with a schematic of our new multiscale model for reactive transport. **Bottom:** Nitrate concentration from a watershed-scale simulation of microbially driven denitrification.

The Impact

For the first time, detailed, fine-scale understanding of nutrient, carbon, and metal biogeochemical processes in the hyporheic zone can be incorporated at the societally relevant scales needed to understand effects on downstream water quality.

Summary

In contrast to traditional methods for modeling reactive transport in streams and rivers, the CHANLS conceptualization allows more detailed biogeochemical process understanding to be represented at the appropriate fine spatial scale of those processes while remaining tractable at field scales. The implementation in ATS provides a platform for next-generation water quality modeling tools.

Publications

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.

Painter, S. L., A. Jan, and E. T. Coon. "Accounting for the biogeochemical effects of hyporheic exchange flows in network-scale models of reactive transport in streams and rivers." *Water Resources Research. In preparation.*

Research Highlight

Surprising effects of a chalkophore, methanobactin, on mercury methylation

Substantially enhanced mercury methylation is observed with some forms of methanobactin produced by some methanotrophs, but not by others

The Science

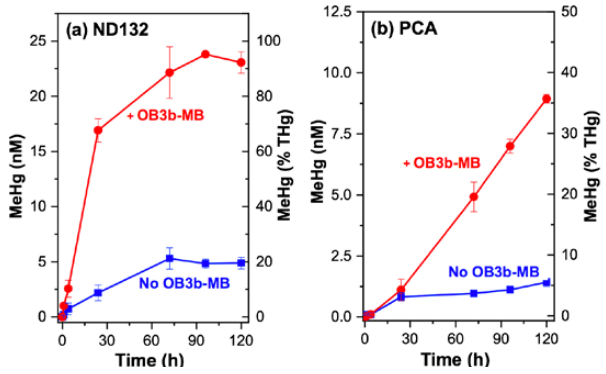
Microbial production of MeHg, a neurotoxin, is a great health and environmental concern as it can bioaccumulate and biomagnify in the food web. A chalkophore or a copper-binding compound, termed methanobactin (MB), has been shown to form strong complexes with Hg(II) and also enables some methanotrophs to degrade MeHg. Unknown, however, is whether Hg(II) binding with MB can also impede Hg(II) methylation by other microbes. Contrary to expectations, MB produced by the methanotroph *Methylosinus trichosporiu*m OB3b (OB3b-MB) enhanced the rate and efficiency of Hg(II) methylation more than that observed with thiol compounds (such as cysteine) by the Hg-methylating bacteria *D. desulfuricans* ND132 and *G. sulfurreducens* PCA. Compared to no-MB controls, OB3b-MB decreased the rates of Hg(II) sorption and internalization, but increased methylation by five to seven fold, suggesting that Hg(II) complexation with OB3b-MB facilitated exchange and internal transfer of Hg(II) to the HgcAB proteins required for methylation. Conversely, addition of excess amounts of OB3b-MB or a different form of MB from *Methylocystis* strain SB2 (SB2-MB) inhibited Hg(II) methylation, likely due to greater binding of Hg(II).

The Impact

Collectively, these results underscore the complex roles of exogenous metal-scavenging compounds produced by methanotrophs in controlling net MeHg production and bioaccumulation in the environment.

Summary

Although the genetic basis of Hg methylation is known, factors that control net MeHg production in the environment are poorly understood. We show that Hg methylation can be substantially enhanced by one form of an exogenous copper-binding compound (MB) produced by some methanotrophs, but not by another. This novel finding illustrates that complex interactions exist between microbes and that these interactions can potentially affect net MeHg production *in situ*.



Methanobactin (MB) from *M. trichosporium* OB3b (OB3b-MB) can substantially increase methylmercury (MeHg) production by anaerobic bacteria, such as **(a)** D. desulfuricans ND132 and **(b)** *G. sulfurreducens* PCA. [Reprinted by permission from Yin, X., et al. 2020. "Synergistic effects of a chalkophore, methanobactin, on microbial methylation of mercury." *Applied & Environmental Microbiology*. **86**(11): e00122–20. DOI:10.1128/AEM.00122-20.]

Publication

Yin, X., L. Wang, L. Zhang, H. Chen, X. Liang, X. Lu, A. A. DiSpirito, J. D. Semrau, and B. Gu. 2020. "Synergistic effects of a chalkophore, methanobactin, on microbial methylation of mercury." *Applied & Environmental Microbiology*. **86**(11):e00122-20. DOI:10.1128/AEM.00122-20.

COVID-19 Pandemic Impacts

During the week of March 16, 2020, ORNL with DOE's concurrence transitioned ~75% of staff from on-campus to work-from-home status to minimize the potential for community spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the novel coronavirus that causes COVID-19. This action has extended beyond the original 6-week timeline and continued through mid-May 2020. As of June 26, 2020, ~65% of ORNL staff continue to work from home. For the SFA, we have continued to work by revising our planned laboratory- and field-scale research activities and are focusing on completing existing publications. During this period, the SFA team members have published 15 manuscripts with an additional 12 currently under review.

The transition to work from home has interrupted or suspended a number of short- and long-term Critical Interfaces SFA field and laboratory activities, and as of June 26, 2020, it is unclear when ORNL will return to normal operations. As a result, a number of key project milestones are being deferred to FY21. For example, our plan to perform multiple conservative and reactive tracer experiments this summer and fall will be deferred to FY21 because of (1) travel restrictions for key collaborators and (2) a combination of the number of people required to perform the activity and the need to physically distance during execution. The deferral of this activity will ultimately impact our ability to parameterize ATS and fulfill several project objectives for Themes 1, 2, and 3. In mid-May a limited number of laboratory- and field-scale activities were initiated under the Phase I return to campus. These activities require more time to perform safely, especially for tasks that are typically executed more efficiently with additional staff members. The current situation will continue to impact our progress and planning for the triennial renewal, which would be occurring this summer and fall. A select number of these impacted milestones that are critical for achieving project goals include:

- Co-culture experiments for understanding how microbial community composition influences Hg methylation.
- Mesoscale flume experiments for model parameterizations to determine the influence of nutrient dynamics on Hg methylation at reach scale.
- Establishment of new monitoring sites for comparing and contrasting watershed structure and function on solute (e.g., nutrient and Hg) delivery and transformation.
- Studies on the influence of algae on Hg transformations to improve understanding and model parameter development on the role of periphyton biofilm on net MeHg formation.

Postgraduate Spotlight

A key goal of the Critical Interfaces 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 11 years ago. As part of this year's report, we highlight one of these postgraduate researchers—Peter Eckert—who, along with others, has contributed significantly to the overall SFA goals and objectives. See website for a complete list of postgraduate researchers (www.esd.ornl.gov/programs/rsfa/alumni.shtml).

Peter Eckert

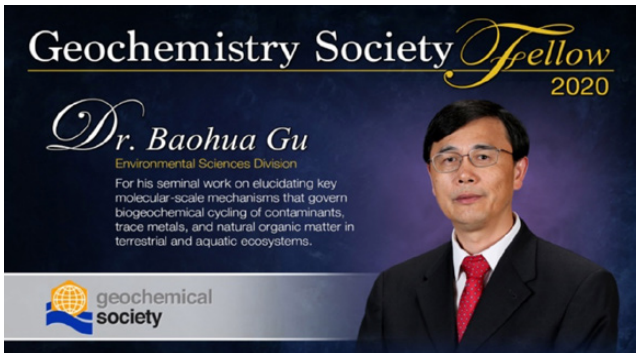
Peter Eckert received his bachelor's degrees in Chemistry and Mathematics from Trinity International University in Deerfield, Illinois. During his undergraduate education, Peter interned for two summers at EMSL at Pacific Northwest National Laboratory. Under the mentorship of Dr. Alexander Laskin, Peter used high-resolution mass spectrometry to study the chemical composition of crude petroleum and atmospheric aerosols. He performed his graduate school research with Dr. Kevin Kubarych at the University of Michigan in Ann Arbor, where he used 2D infrared spectroscopy to study ultrafast vibrational dynamics in small-molecule mimics of the bacterial hydrogenase enzyme's active site. His dissertation, which he defended in May 2018, combined computational and experimental techniques to characterize the molecular dynamics of conformational flexibility, dendritic encapsulation, and vibrational coherence transfer. After completing his graduate research, Dr. Eckert began a postdoctoral appointment at ORNL, where he is working with Dr. Eric Pierce to characterize the complexation of mercury by bacterial exudates, with a focus on Hg complexation by methanobactin, a peptide excreted by methanotrophic bacteria for extracellular copper acquisition. Peter's hobbies include dancing salsa and East Coast swing and reading historical and nonfiction books.

National and International Impact

ORNL Critical Interfaces 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

2019 to June 2020, SFA scientists delivered or published 34 presentations, abstracts, or posters (see Appendix C, p. 23, for details). Described below are team members' contributions to the virtual Goldschmidt 2020 conference, 2020 Environmental System Science Principal Investigators (PI) Meeting, 2019 American Geophysical Union (AGU) Fall Meeting, and the 14th International Conference on Mercury as a Global Pollutant.

Goldschmidt 2020: Several Critical Interfaces SFA team members attended the Goldschmidt 2020 virtual conference held on June 21–26, 2020. Baohua Gu, Eric Pierce, and Dwayne Elias hosted four sessions, and SFA team members also gave a number of presentations at the meeting. During the meeting, Baohua Gu was recognized for being named a Fellow of the Geochemical Society and the European Association of Geochemistry "for his seminal work on elucidating key molecular-scale mechanisms that govern biogeochemical cycling of contaminants, trace metals, and natural organic matter in terrestrial and aquatic ecosystems."



BER's Environmental System Science PI Meeting: SFA team members attended the virtual PI meeting on May 19–20, 2020. Additionally, SFA team members participated in and actively contributed to the May 18, 2020, Environmental System Science Cyberinfrastructure Annual Meeting.

American Geophysical Union Fall Meeting: Several Critical Interfaces SFA team members attended the AGU Fall Meeting in San Francisco, California, on December 9–13, 2019. SFA collaborator Marie Kurz hosted a session titled "Groundwater-Surface Water Interactions: Identifying and Integrating Physical, Biological, Geomorphic, and Chemical Patterns and Processes Across Systems and Scales." SFA team members also gave a number of oral and poster presentations at the meeting.

14th International Conference on Mercury as a Global Pollutant (ICMGP 2019): Several Critical Interfaces SFA team members attended the ICMGP 2019 meeting in Krakow, Poland, on September 8–13, 2019. Baohua Gu gave an invited talk titled "Methanotrophic and Photochemical Demethylation in the Environment." Additionally, several other SFA team members gave oral and poster presentations at the meeting.

Ongoing Collaborative Research Activities

The ORNL Critical Interfaces SFA continues to engage a number of key collaborators in the project (Fig. 8). In FY20, we continued to collaborate with EMSL staff to identify the proteomic and metabolomic signatures that will enable identification of the HgcAB alternative (native) biochemical function. External collaborators, including the South River Science Team, Cynthia Gilmour (Smithsonian Environmental Research Center),

Fig. 8. Key ORNL Critical Interfaces SFA partners.

Adam Ward (Indiana University), Marie Kurz (Drexel University), Helen Hsu-Kim (Duke University), Jeremy Smith (University of Tennessee), Jeremy Semrau (University of Michigan), and K. C. Carroll (New Mexico State University), continue to contribute to SFA milestones.

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 DOE's 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 and Brian Henry), EM headquarter staff (Rod Rimando and Kurt Gerdes), and the Oak Ridge site-specific advisory board.

Organizational Leadership

The scientific objectives of the Critical Interfaces 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 the point of contact with DOE SBR program managers. He speaks to Paul Bayer biweekly on the SFA progress and potential issues.

The three theme leaders are Scott Brooks, Dwayne Elias, and Baohua Gu and the field-scale modeling activity lead is Scott Painter. These leaders along with the broader team meet tri-weekly to provide an update for current research directions, future plans, and changes in staffing. See website for a complete SFA organization chart (www.esd.ornl.gov/programs/rsfa/contacts.shtml).

National Laboratory Investments

ORNL is committed institutionally to the success of the Critical Interfaces SFA program. In FY20, ORNL funded a strategic-hire Laboratory Directed Research and Development project under its Science and Technology Initiative titled "Understanding Complexity in Biological and Environmental Systems." Additional investments were made to modernize the biogeochemistry laboratories in building 1505 of ORNL's Environmental Sciences Division. An estimated value of $11 million in equipment investments were made in FY20 and include the purchase of a (1) Thermo Fisher Scientific Krios G4 cryo-transmission electron microscope, (2) Perkin Elmer NexION 2000B inductively-coupled plasma mass spectrometer, (3) Shimadzu greenhouse gas analyzer, (4) Shimadzu total organic carbon analyzer, and (5) Labconco freeze dryer.

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Gionfriddo, C., A. Wymore, D. Jones, M. Lynes, G. Christensen, A. Soren, C. Gilmour, J. Wall, C. Brandt, M. Podar, A. V. Palumbo, and D. Elias. "Expanded Hg-methylator diversity in nature, using an improved *hgc*AB primer set and direct high-throughput sequencing." *Frontiers in Microbiology. In review*.

Liang, X., X. Lu, J. Zhao, L. Liang, E. Y. Zeng, and B. Gu. 2019. "Stepwise reduction approach reveals mercury competitive binding and exchange reactions within natural organic matter and mixed organic ligands." *Environmental Science & Technology*. **53**(18):10685–694. DOI:10.1021/acs.est.9b02586.

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Schwartz, G., C. Gionfriddo, D. Jones, A. Soren, J. Bell, D. Elias, and C. Gilmour. "Abundance and diversity of *hgcAB*$^{+}$ microbes in Chesapeake salt marsh soils: Relationships to MeHg and site biogeochemistry." *Journal of Geophysical Research: Biogeosciences. In preparation.*

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Zhang, L., X. Lu, X. Liang, Q. Wang, Y. Zhang, X. Yin, E. Pierce, and B. Gu. "Overlooked isotope exchange reactions in tracing mercury (Hg) biogeochemical transformations with enriched Hg isotopes." *Environmental Science & Technology Letters. In review.*

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*. **3**(7): 1338–45. 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

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

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Lian, P., L. Guo, D. Devarajan, J. Parks, S. Painter, S. Brooks, and J. Smith. 2020. "The AQUA-MER databases and aqueous speciation server: A web resource for multiscale modeling of mercury biogeochemistry." *Journal of Computational Chemistry*. **41**:147–55. DOI:10.1002/jcc.26081.

Liang, X., X. Lu, J. Zhao, L. Liang, E. Zeng, and B. Gu. 2019. "Stepwise reduction approach reveals mercury competitive binding and exchange reactions within natural organic matter and mixed organic ligands." *Environmental Science & Technology.* **53**:10685–694. DOI:10.1021/acs.est.9b02586.

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Data Products Released

Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2019. DOI:10.12769/1569761.

Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2019. DOI:10.12769/1569762.

Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2019. DOI:10.12769/1569818.

Brooks, Scott C., and Kenneth A. Lowe. 2019. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 16.2 Water Year 2019. DOI:10.12769/1569821.

Appendix C. Presentations and Conferences

Brooks, S. "A top-down perspective towards understanding controls on watershed function." Vanderbilt University, November 8, 2019. Nashville, TN. *Invited.*

Brooks, S., G. Schwartz, T. Olsen, and K. Muller. "Ecosystem controls on methylmercury production by periphyton biofilms in a contaminated stream: Implications for predictive modeling." Goldschmidt Virtual Conference, June 21–26, 2020. Honolulu, HI.

Catalano, J., J. Yan, E. Flynn, N. Sharma, D. Giammar, G. Schwartz, S. Brooks, P. Weisenhorn, K. Kemner, E. O'Laughlin, and D. Kaplan. "Consistent controls on trace metal micronutrient speciation in wetland soils and stream sediments." Goldschmidt Virtual Conference, June 21–26, 2020. Honolulu, HI.

Catalano, J., D. Giammar, J. Yan, N. Sharma, E. Flynn, G. Schwartz, S. Brooks, P. Weisenhorn, K. Kemner, E. O'Loughlin, and D. Kaplan. "Trace metal dynamics and limitations on biogeochemical cycling in wetland soils and hyporheic zones." DOE Environmental System Science Principal Investigator (PI) Virtual Meeting. May 19–20, 2020.

Date, S., and A. Johs. "Delineating the mercury methylation pathway in anaerobic bacteria." Oak Ridge Postdoctoral Association 7th Annual Research Symposium. August 2019. Oak Ridge, TN.

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Eitel, E., A. Moran, H. Shin, N. Patin, A. Bertagnolli, K. Kemner, S. Brooks, C. Pennacchio, D. Kaplan, F. Stewart, T. Dichristina, and M. Taillefert. "Combining geochemical measurements and omics to investigate competitive anaerobic redox dynamics in sediments." Goldschmidt Conference. August 18–23, 2019. Barcelona, Spain.

Flynn, E., G. Schwartz, S. Brooks, D. Giammar, and J. Catalano. "Impact of cobalt on mercury methylation in East Fork Poplar Creek, Oak Ridge, Tennessee." American Chemical Society Spring 2020 National Meeting & Exposition. March 22–26, 2020. Philadelphia, PA.

Gilmour, C., C. Gionfriddo, R. Wilpiszeski, G. Schwartz, S. Brooks, S. Washburn, A. Soren, J. Bell, and D. Elias. "A sediment microcosm study to assess how the community structure of Hg-methylating microbes impacts MeHg accumulation." 14th International Conference on Mercury as a Global Pollutant (ICMGP). September 8–13, 2019. Krakow, Poland.

Gu, B., L. Zhang, X. Yin, L. Wang, X. Liang, A. DiSpirito, and J. Semrau. "Synergistic effects of a chalkophore, methanobactin, on microbial methylation of mercury." DOE Environmental System Science Principal Investigator (PI) Virtual Meeting. May 19–20, 2020.

Gu, B., Q. Wang, L. Zhang, X. Liang, X. Yin, W. Zheng, and E. Pierce. "Isotope exchange between dissolved elemental Hg(0), inorganic Hg(II), and Hg(II)-bound to organic ligands and environmental implications." Goldschmidt Virtual Conference. June 21–26, 2020. Honolulu, HI.

Gu, B., X. Yin, L. Wang, L. Zhang, H. Chen, X. Liang, X. Lu, A. DiSpirito, and J. Semrau. "Microbial methylation of mercury bound to the methanotrophic chalkophore, methanobactin." American Geophysical Union Fall Meeting. December 9–13, 2019. San Francisco, CA.

Gu, B. "Methanotrophic and photochemical demethylation in the environment." 14th International Conference on Mercury as a Global Pollutant (ICMGP). September 8–13, 2019. Krakow, Poland. *Invited.*

Johs, A. "SAXS and SANS enable insights into domain interactions relevant for activity of the mercuric reductase MerA." Oak Ridge National Laboratory Neutron Scattering Workshop. August 28, 2019. Oak Ridge, TN.

Liang, X., K. Zheng, S. Date, J. Parks, S. Ragsdale, B. Gu, and A. Johs. "Biomolecular processes contributing to Hg transformations at critical interfaces." DOE Environmental System Science Community Principal Investigators (PI) Virtual Meeting. May 19–20, 2020.

Mohamed, R., K. Carroll, T. Ahmed, C. Tsai, and S. Brooks. "Use of censored data for improving the geostatistical interpolation of streambed attributes." WM2020. March 8–12, 2020. Phoenix, AZ.

Muller, K., and S. Brooks. "Effect of sorbents on mercury methylation and methylmercury removal from water." 14th International Conference on Mercury as a Global Pollutant (ICMGP). September 8–13, 2019. Krakow, Poland.

Painter, S., E. Coon, and A. Jan. "Multiscale representation of stream hyporheic-zone biogeochemical processes at catchment scales." American Geophysical Union Fall Meeting. December 9–13, 2019. San Francisco, CA.

Pierce, E. M., B. Gu, S. C. Brooks, S. Painter, A. Johs, D. Elias, M. Podar, and J. Parks. "Biogeochemical transformations at critical interfaces in a mercury perturbed watershed scientific focus area." DOE Environmental System Science Principal Investigator (PI) Virtual Meeting. May 19–20, 2020.

Pierce, E. M. "Influence of watershed structure and function on mercury biogeochemical transformations in a freshwater low-order stream." Department of Civil and Environmental Engineering, Vanderbilt University. October 4, 2019. Nashville, TN. *Invited.*

Pierce, E. M. "Influence of watershed structure and function on mercury biogeochemical transformations in a freshwater low-order stream." Department of Earth & Planetary Sciences, University of Tennessee. October 10, 2019. Knoxville, TN. *Invited.*

Schwartz, G., T. Olsen, K. Muller, and S. Brooks. "Kinetics of methylmercury production in periphyton and sediments from a contaminated freshwater stream." 14th International Conference on Mercury as a Global Pollutant (ICMGP). September 8–13, 2019. Krakow, Poland.

Schwartz, Grace, S. Painter, K. Muller, and S. Brooks. "Using transient availability kinetics to scale methylmercury production from microcosms to watersheds." Goldschmidt Virtual Conference. June 21–26, 2020. Honolulu, HI.

Schwartz, G., R. Wilpiszeski, K. Muller, S. Painter, D. Elias, and S. Brooks. "Predicting methylmercury production kinetics in sediment with a transient availability model." DOE Environmental System Science Principal Investigator (PI) Virtual Meeting. May 19–20, 2020.

Semrau, J., C. Kang, X. Liang, P. Dershwitz, A. Schepers, A. Flatley, J. Lichtmanneger, H. Zischka, X. Lu, A. DiSpirito, and B. Gu. "Methylmercury degradation by methanotrophs and its environmental implications." AGU Fall Meeting, December 9–13, 2019, San Francisco, CA.

Stegen, J., E. Arntzen, S. Brooks, X. Chen, V. Garayburu-Caruso, A. Goldman, J. Gomez-Velez, E. Graham, B. Hall, D. Kaplan, M. Kaufman, H. Song, K. Williams, and K. Wrighton. "Pacific Northwest National Laboratory Science Focus Area: Hydrobiogeochemical features and function across basins." DOE Environmental System Science Principal Investigator (PI) Virtual Meeting. May 19–20, 2020.

Stromberg, M., X. Liang, B. Gu, J. Semrau, and A. Johs. "Photosensitivity of methanobactin and its role in the biogeochemical cycling of mercury." Science Undergraduate Laboratory Internship Poster Session. August 2019. Oak Ridge, TN.

Tsai, C., S. Brooks, and K. Carroll. "Modeling the impacts of hyporheic zone heterogeneity on mass exchange and solute transport in East Fork Poplar Creek, Tennessee, USA." American Geophysical Union Fall Meeting. December 9–13, 2019. San Francisco, CA.

Zaporski, J., M. Jamison, B. Gu, and Z. Yang. "Mercury methylation in Lake Michigan sand dunes." American Geophysical Union Fall Meeting. December 9–13, 2019. San Francisco, CA.

Zhang, L., M. Philben, Z. Yang, E. M. Pierce, D. Graham, and B. Gu. "Biogeochemical controls on mercury methylation in Arctic tundra soils." Goldschmidt Virtual Conference. June 21–26, 2020. Honolulu, HI.

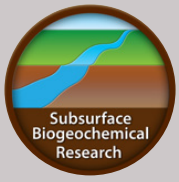
Zhang, L., X. Lu, X. Liang, E. M. Pierce, and B. Gu. "Overlooked mercury isotope exchange reactions in environmental systems." DOE Environmental System Science Principal Investigator (PI) Virtual Meeting. May 19–20, 2020.

Zhang, L., X. Lu, X. Liang, Q. Wang, X. Yin, E. M. Pierce, and B. Gu. "Mercury isotope exchange kinetics and implications for stable isotope tracer studies." American Geophysical Union Fall Meeting. December 9–13, 2019. San Francisco, CA.

Zhang, L., J. An, X. Lu, E. M. Pierce, A. Johs, J. Parks, and B. Gu, "Mercury uptake by *Desulfovibrio desulfuricans* ND132: Passive or active?" 14th International Conference on Mercury as a Global Pollutant (ICMGP). September 8–13, 2019. Krakow, Poland.

Acronyms and Abbreviations

1D, 2D, 3D	one dimensional, two dimensional, three dimensional
ATS	Advanced Terrestrial Simulator
BER	DOE Office of Biological and Environmental Research
DFT	density functional theory
DOE	U.S. Department of Energy
DOM	dissolved organic matter
EFPC	East Fork Poplar Creek
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
LMW	low molecular weight
MATSZ	metabolically active transient storage zone
MB	methanobactin
MeHg	methylmercury
MMHg	monomethylmercury
OD	optical density
ORNL	Oak Ridge National Laboratory
PCR	polymerase chain reaction
PI	principal investigator
POM	particulate organic matter
RT-qPCR	reverse transcription–quantitative PCR
SBR	DOE BER Subsurface Biogeochemical Research program
SFA	Science Focus Area
TSZ	transient storage zone



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