Mercury Program Overview

Anthropogenic releases and changing environmental conditions profoundly affect the biogeochemical cycling of trace metals, such as mercury. Mercury can be methylated to form methylmercury (MeHg), a neurotoxin that bioaccumulates in the food web, endangering humans and other biota. While mercury contamination in natural environments results mostly from atmospheric processes (Mason et al. 2006; Mason et al. 2002; Fitzgerald and Lamborg 1998; Lindberg and Stratton 1998), mining and industrial processes can lead to severe local pollution. On the Oak Ridge Reservation (ORR), for example, mercury pollution in the East Fork Poplar Creek (EFPC) watershed is caused by historical mercury use at the Y-12 National Security Complex, where large quantities of mercury were lost to the environment during the 1950s and 1960s.

After making substantial progress over the past 6 years in understanding the processes that govern mercury transformation in contaminated systems, the ORNL SFA program is poised to make new transformational advances in mercury research and, more broadly, subsurface biogeochemistry in the program’s subsequent phase. This next phase seeks to address the following science challenge and goal:

- **Nine-Year Science Challenge:** Determine the coupled hydrobiogeochemical processes that control mercury fate and transformation in low-order freshwater stream systems.
- **Nine-Year Science Goal:** Process-rich predictive capability that integrates field, laboratory, and modeling studies of mercury fate and transformation dynamics across broad spatiotemporal scales in low-order streams.

Low-order freshwater streams, such as EFPC (the project’s representative use case), constitute nearly 90% of the total stream length in the United States and are the most frequently occurring stream type (>85%; Pierson et al. 2008). Furthermore, because of their low hydraulic radius (cross-sectional area and wetted perimeter) and low average water velocity, these stream systems have high water-sediment contact times, which promote in-stream biogeochemical cycling (Haggerty, Wondzell, and Johnson 2002). Numerous studies indicate that low-order
streams play a dominant role in the flow, biogeochemistry, and water quality of downstream higher-order reaches (Alexander et al. 2007; Bernhardt et al. 2003; Waldron et al. 2009; Milliman and Syvitski 1992; Jeong et al. 2012). Additionally, these streams play a prominent role at the terrestrial-aquatic interface because they represent the first aquatic environment encountered by terrestrially derived materials (solutes and particles).

Developing a predictive understanding of mercury and trace metal transport and fate in environmental systems, such as terrestrial surface and subsurface ecosystems, is a formidable challenge that requires deciphering complex processes (i.e., physical, chemical, and biological), deconvoluting how these processes interact with one another, and understanding the factors that control system response over broad spatiotemporal scales.

Exchange and feedback processes at critical interfaces are central for determining fluxes, stocks, and transformation rates of key constituents that control mercury speciation, distribution, and bioavailability, such as oxygen, nutrients, and dissolved organic matter (DOM). Therefore, over the next 3 years [Phase I, fiscal years (FY) 2016–18], the ORNL SFA program will focus on

**Determining the fundamental mechanisms and environmental factors that control mercury biogeochemical transformations at key interfaces in terrestrial and aquatic ecosystems.**

The research outlined in Phase I of the ORNL SFA plan comprises collaborative and complementary research activities that support four research thrusts:

- Ecosystem Features Influencing Mercury Transformation
- Biogeochemical Mechanisms Controlling Mercury Uptake and Methylation
- Microbial Community Functions and Geochemical Influences on Mercury Transformations
- Molecular Structure, Dynamics, and Mechanisms of Mercury Transport and Transformations

This report summarizes progress made during the first quarter of FY16, which represents the initial 4-month period following the program’s triennial peer review (April 2015) and acceptance of a renewal science plan, which occurred in August 2015, by the U.S. Department of Energy’s (DOE) Office of Biological and Environmental Research.

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**Fig. 1. Key biogeochemical interfaces.** Interfaces are common boundaries between two or more system compartments or phases where steep gradients develop and govern the fate and transformation of material crossing those gradients.
References Cited in This Section


Scientific Progress and Select Research Highlights

**Task 1: Ecosystem Features Influencing Mercury Transformation**

**Overarching Objectives**

The general objectives of Task 1 are to (1) identify ecosystem compartments and hydrobiogeochemical conditions that govern net methylmercury concentration in EFPC and (2) understand the extent to which groundwater–surface water exchange drives mercury transformations in EFPC. These objectives are addressed through a set of hypotheses-driven field and laboratory investigations. Additionally, the SFA program is developing a process-rich numerical model to integrate past results and challenge current understanding of watershed processes occurring through space and time. The model will be used in an iterative fashion with experiments to help inform the design of experiments and subsequently to refine the model based on experimental results.

**First Quarter FY16 Accomplishments**

For the period October–December 2015, significant progress was made within Task 1 under the new project plan. During this period, much of our effort was devoted to manuscript preparation. One manuscript was accepted for publication, another was submitted for review, and substantial progress was made on three other manuscripts. Two presentations were given at national and international meetings. We refined our experimental protocol for quantifying mercury methylation and methylmercury demethylation rates mediated by periphyton based on results of our initial experiments. Detailed experimental plans for the coming year, which include close collaboration with Task 3 for gene target and microbial community assays, are being finalized. We maintain our water quality monitoring efforts in support of (1) out-year milestones for Task 1; (2) continuous improvement to our conceptual model of watershed processes including, but not limited to, mercury cycling; (3) design and interpretation of future field-scale experiments; and (4) the development of a process-rich numerical model of the lower EFPC system. Finally, we have been meeting with the modeling staff (led by Dr. Scott Painter) to discuss model development and clearly identify goals and milestones for this effort in the current fiscal year.
Task 2: Biogeochemical Mechanisms Controlling Mercury Uptake and Methylation

Overarching Objectives
The overarching goal of Task 2 is to gain a fundamental understanding of the key geochemical and biochemical mechanisms controlling mercury sorption, uptake, and transformation at the microbe-fluid and particulate (mineral) interfaces. We attempt to answer the following specific scientific questions:

- What are the key geochemical and biochemical variables and their interactions affecting mercury-DOM complexation, mercury–cell surface interactions, cellular uptake, and methylation?
- How does photoredox transformation of mercury-DOM influence mercury reactivity and bioavailability?
- What are the specific molecules in DOM and proteins in and out of the cell membrane that complex mercury?
- Under what conditions do suspended particles become a net sink or source of bioavailable mercury in EFPC?

First Quarter FY16 Accomplishments
For the period October–December 2015, we made significant progress toward understanding the mechanisms and geochemical controls on mercury and methylmercury species transformation within the Task 2 project. We published one technical manuscript related to “thiol-facilitated cell export and desorption of methylmercury by anaerobic bacteria” in Environmental Science and Technology (EST) Letters. We also completed and submitted two technical manuscripts for publication—one focused on photochemically driven mercury sulfide formation and decreased methylmercury production in water (submitted to EST Letters), and the other was about the climate warming effects on methylmercury production in Arctic soil resulting from collaborative work between the ORNL Mercury SFA and Next-Generation Ecosystem Experiments–Arctic (NGEE–Arctic) projects (under review, Nature Geoscience).

In the published EST Letters manuscript, we studied the factors affecting methylmercury export and its distribution in cells, on cell surfaces, and in solution by two known mercury methylators, Geobacter sulfurreducens PCA and Desulfovibrio desulfuricans ND132. We found that thiols, such as cysteine, greatly facilitate desorption and export of methylmercury, particularly by PCA cells. In cysteine-free assays (4 h), <10% of the synthesized methylmercury was found in solution; >90% was associated with PCA, of which ~73% was sorbed on the cell surface and 19% remained inside the cells. In comparison, 77% of the methylmercury was in solution, leaving ~13% of the methylmercury sorbed and ~10% inside the ND132 cells. These results are different from previous views that methylmercury, once formed inside the cell, is rapidly excreted from cells. Our results demonstrate that methylmercury export is bacteria specific, time dependent, and influenced by thiols, implicating important roles of ligands, such as natural organic matter (NOM), in methylmercury production and mobilization in the environment.

We also made a novel finding that photo-irradiation of mercury-DOM complexes in water by either solar light or ultraviolet results in the precipitation of mercury sulfide (or metacinnabar beta-HgS), with concurrent loss of mercury reactivity and up to 80% decreased methylmercury production by a methylating bacterium, Geobacter sulfurreducens PCA. Our results not only identify a new pathway of abiotic photochemical formation of HgS mineral, but also provide a mechanism that explains why newly deposited Hg(II) is readily methylated but, over time, progressively becomes unavailable for microbial uptake and methylation.

Additionally, in collaboration with the NGEE–Arctic project, we studied climate warming effects on methylmercury production in Arctic soils and found that methylmercury production increased >10 fold in both organic- and mineral-rich soil layers at warmer (8°C) compared to subzero (~2°C) temperatures. The presence and type of labile soil organic carbon, such as reducing sugars and ethanol, were particularly important in fueling the rapid initial biosynthesis of methylmercury. Methylation was positively correlated with ferrous ion and methane production, and freshly amended mercury was more readily methylated than preexisting mercury in the soil. Our results indicate that climate warming and permafrost thaw could enhance methylmercury production by an order of magnitude, impacting Arctic terrestrial and aquatic ecosystems by increased levels of mercury exposure through bioaccumulation and biomagnification in the food web.

Task 3: Microbial Community Functions and Geochemical Influences on Mercury Transformations

Overarching Objectives
The overarching objectives of Task 3 are to (1) characterize and understand the diversity and abundance of the newly discovered 2-gene cluster (hgcAB) that is responsible for Hg(II)-methylation and (2) elucidate the complete biochemical pathway for mercury methylation. To this end, the project is pursuing these activities:
First Quarter FY16 Accomplishments

The genes (hgcAB) and subsequently expressed proteins (HgcAB) are required for bacterial mercury methylation to occur, and we have focused efforts of the microbiology team on developing tools to characterize these genes and proteins across the range of scales in the ORNL Mercury SFA. We have nearly completed development of qualitative and quantitative DNA and mRNA primers for hgcAB.

These primers include a universal set for determining the organisms that are present and possess hgcAB and quantitative primers for each methylating clade: Deltaproteobacteria, Firmicutes, and methanogenic Archaea. The reasoning is that each clade methylates at different rates and to different extents in pure cultures, so delineating this difference in methylation potential is essential. The first publication, now in author review, will detail the development and initial testing of the primers. Further, for this paper we also repeated the hgcA primer protocols of the three publications in the literature and compared our results to theirs. Our second paper will utilize these primers on eight different sites along with metagenomes and 16S rRNA sequencing, as well as site geochemistry to determine the efficacy of our primers in the environment, their sensitivity and robustness, and their quantitative accuracy as compared to other methods. Most of the effort expended in quarter 1 of FY16 has been devoted to this work.

We are also characterizing hgcAB and HgcAB to determine the native function of these genes and proteins; that is, what biochemical function do they perform in the absence of mercury. To this end, we have employed several tactics including deletion mutagenesis of ~14 genes that we suspect are involved in the methyl group transfer to mercury as well as the two electrons required for reduction of the cobalamin cofactor in the active site of HgcA. All these mutants have been cultivated under several different regimes of carbon and electron sources and electron acceptors to determine the effects on cell metabolism as well as mercury methylation. The results are still being processed.

Lastly, we published a study at the beginning of the quarter in Science Advances (Podar et al. 2015), whereby we interrogated all existing metagenomes for the presence and abundance of hgcA. We found that hgcA is present in low abundance in the open ocean and mammalian micro-organisms but in high abundance in wastewater treatment plants and the thawing Arctic permafrost.

Task 4: Molecular Structure, Dynamics, and Mechanisms of Mercury Transport and Transformations

Overarching Objectives

The goal of Task 4 is to investigate structures, reactions, energetics, and dynamics to understand at the molecular scale how mercury is transformed and transported by biological macromolecules and abiotic species encountered in natural and contaminated environments.

First Quarter FY16 Accomplishments

The proteins HgcA and HgcB are essential for methyl-mercury production by anaerobic bacteria. Our current focus is on identifying biologically important states of HgcA to delineate its functional role in mercury methylation. To obtain sufficient material for spectroscopic and structural studies, we expressed several constructs of HgcA heterologously in Escherichia coli. The constructs include a detergent-solubilized full-length HgcA (fHgcA), the cobalamin-binding domain (CBD), and a maltose-binding protein fusion (MBP-CBD) to increase yield and solubility for spectroscopic studies. Upon purification, all HgcA constructs must be reconstituted with cobalamin. We provided samples of the purified and reconstituted HgcA samples to our collaborators at the University of Michigan for electron paramagnetic resonance spectroscopy. The spectra indicate axial Co-N coordination, which is not consistent with the anticipated axial Co-S coordination predicted for HgcA. This finding may be a result of incomplete reconstitution of the apoprotein with cobalamin, a low affinity of the cobalamin cofactor for the apoprotein, or an unexpected cofactor configuration. To validate our current results, we are collaborating with Task 3 to isolate sufficient amounts of native HgcA protein from Desulfovibrio desulfuricans ND132 by sequential affinity chromatography for spectroscopic characterization.

We are also characterizing the redox and thermodynamic properties of the cobalamin-containing protein HgcA. Redox processes in complex transition metal-containing species often are intimately connected with changes in ligand protonation states and metal coordination number. A major challenge, therefore, is to develop consistent computational approaches for computing pH-dependent redox and ligand dissociation properties of these species.
Extending our previously developed approaches for computing mercury–ligand binding free energies (Riccardi et al. 2013a; Riccardi et al. 2013b) and methyl transfer from cobalamin to Hg(II), we have used density functional theory (DFT) calculations to compute accurate electrochemical and thermodynamic quantities of aquacobalamin as a representative model system. Specifically, we obtained root mean square errors (compared to experimentally determined values) of 90 mV for seven reduction potentials, 1.0 log unit for four pK\textsubscript{a}s, and 1.0 log unit for two equilibrium ligand-binding (K\textsubscript{on/off}) values for the aquacobalamin system. These findings demonstrate the effectiveness of our approach for computing electrochemical and thermodynamic properties of complex, redox-active species relevant to mercury transformation. We plan to apply similar methods to more complex mercury-NOM and HgcA models in future work.

In collaboration with researchers at the University of South Carolina and the Alkek Center for Metagenomics and Microbiome Research at the Baylor College of Medicine, we investigated potential correlations among the appearance of mercury methylation genes, mercury resistance genes, and fetal methylmercury exposure by analyzing whole-genome shotgun sequence datasets of gut microbiome samples (Rothenberg et al. 2015).


Top Cited Publications (as of December 31, 2015)

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<tr>
<th>Publication</th>
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<th>Citations</th>
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<tr>
<td>The Genetic Basis for Bacterial Mercury Methylation</td>
<td>2013</td>
<td>93</td>
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<td>Mercury Reduction and Complexation by Natural Organic Matter in Anoxic Environments</td>
<td>2011</td>
<td>61</td>
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<tr>
<td>Active Transport, Substrate Specificity, and Methylation of Hg(II) in Anaerobic Bacteria</td>
<td>2011</td>
<td>59</td>
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<tr>
<td>Mercury Methylation by Novel Microorganisms from New Environments</td>
<td>2013</td>
<td>47</td>
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<tr>
<td>Kinetic Controls on the Complexation Between Mercury and Dissolved Organic Matter in a Contaminated Environment</td>
<td>2009</td>
<td>41</td>
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ORNL Mercury SFA Research Press Release

**Methylmercury: A Dangerous Environmental Toxin**

Researchers from ORNL and the Smithsonian Environmental Research Center published a paper in *Science Advances* that capped a 2+-year-long study seeking to discover how and where methylmercury—a bioaccumulative neurotoxin that poses risks to both people and ecosystems—forms naturally.

ORNL issued a press release about the paper (www.eurekalert.org/pub_releases/2015-10/drnl-fwf100915.php), and an article was published in *Laboratory Equipment* on December 18, 2015 (www.laboratoryequipment.com/articles/2015/12/methylmercury-dangerous-environmental-toxin).

Fig. 2. Molecular structure of methylmercury bound to cysteine. Methylmercury is a bioaccumulative environmental toxin that is formed microbially via a process called methylation.

**Key Collaborators**

Smithsonian Environmental Research Center

![Smithsonian Environmental Research Center](image)

![South River Science Team](image)

![University of Maryland](image)

![University of Michigan](image)

Fig. 3. Key ORNL Mercury SFA collaborators.

**Meetings and Events, Community Involvement**

**American Geophysical Union**

Drs. Elias and Gilmour convened a session at the 2015 AGU Fall Meeting—B13I: Mercury Biogeochemistry, Genomics, and Environmental Change II—on December 14, 2015, in San Francisco, California. Co-organizers included Michael Banks from the University of Massachusetts and James Shanley from the U.S. Geological Survey (USGS).

**FY16 SFA Scientific Advisory Committee (SAC)**

The FY16 SAC meeting is being planned. The tentative meeting dates are early April or May 2016 at ORNL. This year we have added two new SAC members to our distinguished committee—Carl Lamborg from the University of California, Santa Cruz, and Steve Yabusaki from Pacific Northwest National Laboratory. Members currently include Dave Krabbenhoft, USGS; Alex MacKerell, University of Maryland; Richard Sparling, University of Manitoba; and Elizabeth Phillips, DOE Oak Ridge Office of Environmental Management. All currently funded SFA participants will attend to highlight recent progress toward objectives and goals.

**Environmental System Science Principal Investigators (PI) Meeting**

Subsurface Biogeochemical Research program staff plan to attend the PI meeting scheduled for April 26–27, 2016, at the Bolger Center in Potomac, Maryland.

**Goldschmidt 2016**

Mercury SFA team members will host a session titled “Microbiological and Geochemical Controls on Trace Metal Speciation, Transformation, and Transport” at the 26th Goldschmidt Conference in Yokohama, Japan, on June 26–July 1, 2016. The Goldschmidt Conference is the premier international conference on geochemistry. Session

![Goldschmidt Yokohama 2016](image)
organizers include Eric Pierce and Baohua Gu from ORNL along with a number of co-organizers from around the world. They include Christian Mikutta, Swiss Federal Institute of Technology in Zürich, Switzerland; Adrien Mestrot, University of Bern in Bern, Switzerland; Marco Keiluweit, University of Massachusetts, Amherst; and Samantha Ying, University of California, Riverside. The session description can be found at http://goldschmidt.info/2016/program/programViewThemes/.

Post Graduate Spotlight

**Bryan Crable**

Bryan Crable received his master’s degree in Biology from Duquesne University and Ph.D. in Microbiology from the University of Oklahoma. Dr. Crable specializes in microbial physiology because he recognized the essential role bacteria play in solving the world’s toughest environmental challenges. His doctoral dissertation was titled “Enzyme Systems Involved in Interspecies Hydrogen and Formate Transfer Between Syntrophic Fatty and Aromatic Acid Degraders and *Methanospirillum hungatei*.” This work demonstrated the role of a novel iron-sulfur (FeS) oxidoreductase complex in catalyzing reverse electron transfer. He was appointed in 2014 as an ORNL postdoctoral research associate under the mentorship of Dwayne Elias to study the native physiological function of HgcA. Dr. Crable is first author on a comprehensive invited review on mercury methylation for the journal *Trends in Microbiology*, first author on a paper published in *Enzyme Research*, and coauthor on seven papers including a publication in *Science Advances*. In FY15, Dr. Crable gave an invited talk at the 2015 American Society for Microbiology General Meeting and participated in the Applied and Environmental Microbiology Gordon Research Conference. Most recently, Dr. Crable was awarded a $130,000 FY16 grant from the DOE-funded Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) program to study the impact of phages on subsurface microbial communities. Outside the laboratory, Dr. Crable is an internationally competitive highland bagpiper and has won awards in six countries.

National Laboratory Investments

ORNL is committed institutionally to the success of the Mercury SFA. In FY15, ORNL invested in new equipment, specifically the Malvern PEAQ-ITC, to support the SFA in making fundamental thermochemical measurements of mercury-ligand interactions. The Malvern PEAQ-ITC was installed in early October and will be used to experimentally determine enthalpies of ligand binding, affinities, and stoichiometry.

Outreach

**Eric Pierce**

- Organized and presented at DOE’s Office of Environmental Management (EM) meeting focused on mercury contamination at EM sites, which was held in September 2015. This meeting was a forum to provide an overview of SFA accomplishments and findings to date.
- Gave an invited oral presentation to the U.S. Army Corps of Engineers Engineer Research and Development Center (ERDC) in Vicksburg, Mississippi, in November 2015.

**Jerry Parks**

- Served on the Scientific Advisory Committee for the Biosciences Division of SLAC National Accelerator Laboratory in October 2015.
- Gave two invited oral presentations in September 2015 on the discovery and characterization of HgcA. One was presented at the University of Oklahoma (Department of Chemistry and Biochemistry), and the other was presented at the University of Tennessee, Knoxville (Department of Biochemistry and Cellular and Molecular Biology).

**Scott Brooks**

- Serves as a co-lead on the Remedial Options Team, Lab and Small Scale Field Testing for the DuPont-led South River Science Team (SRST). In this capacity, he participates in regular conference calls, reviews research plans and results, and consults with SRST staff.
- Attended the SRST annual meeting in Harrisonburg, Virginia, on October 21–22, 2015, and met with SRST staff to discuss and compare our approaches to studying mercury cycling in these two similar watersheds.
## Acronyms and Abbreviations

<table>
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<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AGU</td>
<td>American Geophysical Union</td>
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<tr>
<td>CBD</td>
<td>cobalamin-binding domain</td>
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<td>DFT</td>
<td>density functional theory</td>
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<tr>
<td>DOE</td>
<td>U.S. Department of Energy</td>
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<td>DOM</td>
<td>dissolved organic matter</td>
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<td>EFPC</td>
<td>East Fork Poplar Creek</td>
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<td>EM</td>
<td>DOE Office of Environmental Management</td>
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<tr>
<td>ENIGMA</td>
<td>Ecosystems and Networks Integrated with Genes and Molecular Assemblies</td>
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<td>ERDC</td>
<td>U.S. Army Corps of Engineers Engineer Research and Development Center</td>
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<td>FeS</td>
<td>iron sulfur</td>
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<td>fHgcA</td>
<td>full-length HgcA</td>
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<td>FY</td>
<td>fiscal year</td>
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<td>GSA</td>
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<td>Hg</td>
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<td>NGEE–Arctic</td>
<td>Next-Generation Ecosystem Experiments–Arctic</td>
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<td>NOM</td>
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<td>South River Science Team</td>
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<td>U.S. Geological Survey</td>
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