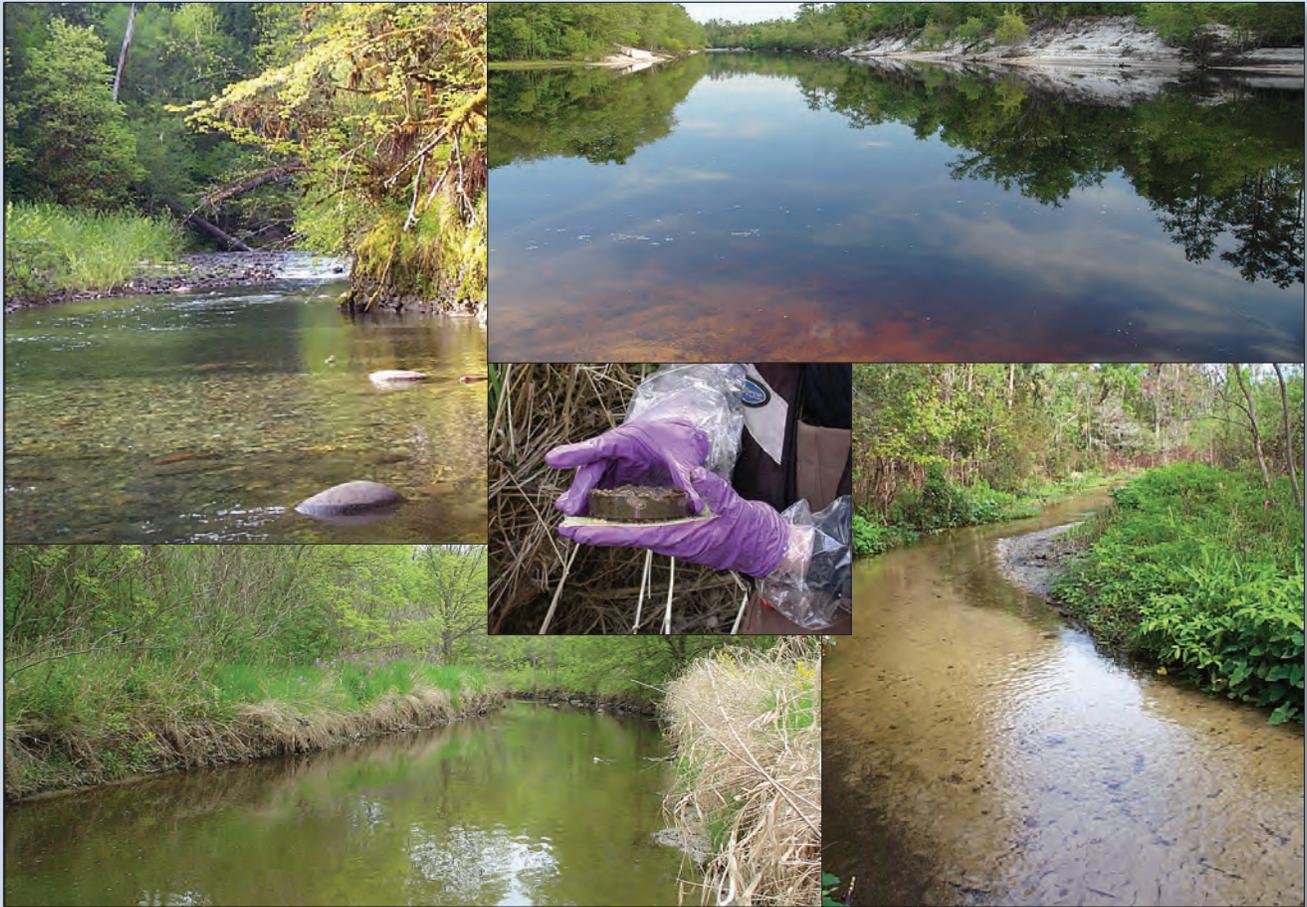**National Water-Quality Assessment Program**

Total Mercury, Methylmercury, Methylmercury Production Potential, and Ancillary Streambed-Sediment and Pore-Water Data for Selected Streams in Oregon, Wisconsin, and Florida, 2003–04

Data Series 375

Total Mercury, Methylmercury, Methylmercury Production Potential, and Ancillary Streambed-Sediment and Pore-Water Data for Selected Streams in Oregon, Wisconsin, and Florida, 2003–04

By Mark C. Marvin-DiPasquale, Michelle A. Lutz, David P. Krabbenhoft, George R. Aiken, William H. Orem, Britt D. Hall, John F. DeWild, and Mark E. Brigham

National Water-Quality Assessment Program

Data Series 375

U.S. Department of the Interior
U.S. Geological Survey

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DIRK KEMPTHORNE, Secretary

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Foreword

The U.S. Geological Survey (USGS) is committed to providing the Nation with credible scientific information that helps to enhance and protect the overall quality of life and that facilitates effective management of water, biological, energy, and mineral resources (http://www.usgs.gov/). Information on the Nation's water resources is critical to ensuring long-term availability of water that is safe for drinking and recreation and is suitable for industry, irrigation, and fish and wildlife. Population growth and increasing demands for water make the availability of that water, now measured in terms of quantity and quality, even more essential to the long-term sustainability of our communities and ecosystems.

The USGS implemented the National Water-Quality Assessment (NAWQA) Program in 1991 to support national, regional, State, and local information needs and decisions related to water-quality management and policy (http://water.usgs.gov/nawqa). The NAWQA Program is designed to answer: What is the condition of our Nation's streams and ground water? How are conditions changing over time? How do natural features and human activities affect the quality of streams and ground water, and where are those effects most pronounced? By combining information on water chemistry, physical characteristics, stream habitat, and aquatic life, the NAWQA Program aims to provide science-based insights for current and emerging water issues and priorities. From 1991–2001, the NAWQA Program completed interdisciplinary assessments and established a baseline understanding of water-quality conditions in 51 of the Nation's river basins and aquifers, referred to as Study Units (http://water.usgs.gov/nawqa/studyu.html).

Multiple national and regional assessments are ongoing in the second decade (2001—2012) of the NAWQA Program as 42 of the 51 Study Units are reassessed. These assessments extend the findings in the Study Units by determining status and trends at sites that have been consistently monitored for more than a decade, and filling critical gaps in characterizing the quality of surface water and ground water. For example, increased emphasis has been placed on assessing the quality of source water and finished water associated with many of the Nation's largest community water systems. In addition, national syntheses of information on pesticides, volatile organic compounds (VOCs), nutrients, selected trace elements, and aquatic ecology are continuing.

The USGS aims to disseminate credible, timely, and relevant science information to address practical and effective water-resource management and strategies that protect and restore water quality. We hope this NAWQA publication will provide you with insights and information to meet your needs, and will foster increased citizen awareness and involvement in the protection and restoration of our Nation's waters.

The USGS recognizes that a national assessment by a single program cannot address all water-resource issues of interest. External coordination at all levels is critical for cost-effective management, regulation, and conservation of our Nation's water resources. The NAWQA Program, therefore, depends on advice and information from other agencies–Federal, State, regional, interstate, Tribal, and local–as well as nongovernmental organizations, industry, academia, and other stakeholder groups. Your assistance and suggestions are greatly appreciated.

Matthew C. Larsen
Acting Associate Director for Water

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Conversion Factors

Multiply	By	To obtain
Length		
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
micrometer	0.000001	meter (m)
nanometer (nm)	0.000000001	meter (m)
Area		
square meter (m^2)	10.76	square foot (ft^2)
Volume		
cubic centimeter (cm^3)	0.06102	cubic inch (in^3)
liter (L)	61.02	cubic inch (in^3)
milliliter (mL)	0.001	liter (L)
microliter (µL)	0.000001	liter (L)
Mass		
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)
milligram (mg)	0.01	gram (g)
nanogram (ng)	0.000000001	gram (g)
Density		
gram per cubic centimeter (g/cm^3)	62.4220	pound per cubic foot (lb/ft^3)
Flow Rate		
milliliters per minute (mL/min)	0.06102	cubic inches per minute (in^3/min)
Radioactivity		
becquerel (Bq)	27.027	picocurie (pCi)
disintegrations per minute (dpm)	0.01667	becquerel (Bq)
nanocurie (nCi)	1,000	picocurie (pCi)
Electrical potential		
millivolt (mV)	0.001	volt (V)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8×°C)+32.$$

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L), micrograms per liter (µg/L), or nanograms per liter (ng/L). Concentrations for some chemicals are given as molarity (M), which is defined as moles per liter, where a mole is $6.022 × 10^{23}$ atoms of a pure compound or element. Multiply concentrations expressed as millimolar (mM) by 10^{-3} to obtain moles per liter, and multiply concentrations expressed as micromolar (µM) by 10^{-6} to obtain moles per liter.

Datum

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Abbreviations and Acronyms

AVS	acid volatile sulfur
CVAFS	cold vapor atomic fluorescence spectrometry
DOC	dissolved organic carbon
h	hour
IC	inorganic carbon
LOI	loss on ignition
MDP	MeHg degradation potential
MPP	MeHg production potential
NAWQA	National Water-Quality Assessment Program
OC	organic carbon
ORP	oxidation-reduction potential
ppb	parts per billion
rpm	rotations per minute
RSD	relative standard deviation
s	second
SAOB	sulfur antioxidant buffer
SR	sulfate reduction
SRB	sulfate-reducing bacteria
SRM	standard reference material
SUVA	specific ultraviolet absorbance
TC	total carbon
TN	total nitrogen
TP	total phosphorus
TRS	total reduced sulfur
TS	total sulfur
μm	micrometer
USGS	U.S. Geological Survey
UV	ultraviolet
w/v	weight (of solute) per volume (of solvent)
WMRL	Wisconsin Mercury Research Laboratory

Total Mercury, Methylmercury, Methylmercury Production Potential, and Ancillary Streambed-Sediment and Pore-Water Data for Selected Streams in Oregon, Wisconsin, and Florida, 2003–04

By Mark C. Marvin-DiPasquale, Michelle A. Lutz, David P. Krabbenhoft, George R. Aiken, William H. Orem, Britt D. Hall, John F. DeWild, and Mark E. Brigham

Abstract

Mercury contamination of aquatic ecosystems is an issue of national concern, affecting both wildlife and human health. Detailed information on mercury cycling and food-web bioaccumulation in stream settings and the factors that control these processes is currently limited. In response, the U.S. Geological Survey (USGS) National Water-Quality Assessment Program (NAWQA) conducted detailed studies from 2002 to 2006 on various media to enhance process-level understanding of mercury contamination, biogeochemical cycling, and trophic transfer. Eight streams were sampled for this study: two streams in Oregon, and three streams each in Wisconsin and Florida. Streambed-sediment and pore-water samples were collected between February 2003 and September 2004. This report summarizes the suite of geochemical and microbial constituents measured, the analytical methods used, and provides the raw data in electronic form for both bed-sediment and pore-water media associated with this study.

Background

Mercury (Hg) is an environmental contaminant, is the most widespread cause of fish-consumption advisories (U.S. Environmental Protection Agency, 2007a), and is the second leading cause of impaired waters in the United States (U.S. Environmental Protection Agency, 2007b). Current Hg levels in the environment are due to a combination of natural and anthropogenic sources. Mercury enters most aquatic environments predominantly through wet and dry atmospheric deposition of primarily inorganic forms from combustion sources (U.S. Environmental Protection Agency, 1997), although some locations have substantial mercury inputs related to mineral deposits, mining, or industrial uses (Wiener and others, 2003). Methylation of inorganic mercury [Hg(II)] produces methylmercury (MeHg), an organic form of mercury that readily biomagnifies in aquatic food webs (Wiener and others, 2003). The methylation process is largely mediated by bacteria in aquatic bed sediment (Gilmour and others, 1992; Fleming and others, 2006). Methylmercury commonly occurs in fish at concentrations sufficient to be a toxicological concern for humans and wildlife that consume those fish.

The USGS National Water Quality Assessment Program (NAWQA) studied mercury in eight stream ecosystems in the United States (fig. 1, table 1) from 2002 to 2006 (Brigham and others, 2003). These studies assessed hydrologic, geochemical, and ecological controls on mercury transport and speciation, Hg(II) methylation, and MeHg bioaccumulation. As part of this study, mercury and related measures were characterized in stream water, bed sediment, pore water, and aquatic biota.

This report summarizes methods and data for the bed-sediment and pore-water sampling component of the study. Sampling was conducted from February 2003, through September 2004. Other reports describe the environmental settings of the studied streams (Bell and Lutz, 2008), and more detailed bed-sediment and pore-water sampling methods (Lutz and others, 2008).

Base composited from National Atlas of the United States state boundaries of the United States, 1:2,000,000, 2005.
Albers Conical Equal Area Projection, referenced to North American Datum of 1983.

Figure 1. Locations of stream sites sampled for the National Water-Quality Assessment Program's mercury studies in bed sediment and pore water, 2002–04.

Table 1. U.S. Geological Survey stream sites sampled for the National Water-Quality Assessment Program's mercury studies in bed sediment and pore water, 2002–04.

[Location of stream sites are shown in figure 1]

Stream name	Short stream name	Station No.	Site code for tables
Lookout Creek near Blue River, Oregon	Lookout Cr., OR	14161500	OR-LO
Beaverton Creek at Southwest 216th Avenue, near Orenco, Oregon	Beaverton Cr., OR	14206435	OR-BT
Pike River at Amberg, Wisconsin	Pike R., WI	04066500	WI-PR
Evergreen River below Evergreen Falls, near Langlade, Wisconsin	Evergreen R., WI	04075365	WI-EG
Oak Creek at South Milwaukee, Wisconsin	Oak Cr.,WI	04087204	WI-OC
St. Marys River near Macclenny, Florida	St. Marys R., FL	02231000	FL-SM
Santa Fe River near Fort White, Florida	Santa Fe R., FL	02322500	FL-SF
Little Wekiva River near Longwood, Florida	Little Wekiva R., FL	02234998	FL-LW

Field Sampling

Detailed field methods used for bed-sediment and pore-water samples; and methods used for in-field measurements of bed-sediment parameters (oxidation-reduction potential (ORP), pH, and temperature), pore-water parameters (sulfide, ORP, and pH), and piezometric head measurements are provided in a companion report (Lutz and others, 2008). Sampling at each stream site was conducted in three phases, which included initial site characterization (Phase I, 2003), temporal sampling (Phase II, 2003–04), and detailed stream reach characterization (Phase III, 2004). During all three study phases and at all sites, bed-sediment samples were collected from a 0 to 2 cm depth interval, and pore-water samples were pumped from a nominal depth of 2 cm (pumping induces flow, which draws water from shallower and deeper depths). Bed-sediment samples were analyzed for a standard suite of constituents, including mercury speciation, microbial-rate measurements, and ancillary parameters. Pore-water samples were collected for mercury speciation and ancillary parameters.

Spatial Framework for Streambed-Sediment and Pore-Water Sampling

The spatial framework used for streambed-sediment and pore-water sampling and stream-reach characterization are shown graphically in figure 2, summarized here, and detailed in Lutz and others (2008). Key words are boldfaced at first occurrence. All sediment sampling was conducted within a **stream reach**, the length of which was at least 20 times the stream width, and a minimum of 150 (wadeable streams) to 500 m (non-wadeable streams). Within a reach, **zones** of relatively homogeneous sediment occur. These zones could include, for example, fine-grained organic-rich sediment, mixed sand and fines, sandy sediment, and material larger than sand (gravel, cobble, and boulders, all of which were

characterized as larger than sand). The extent of each zone within a reach was determined using a series of **transects** established across stream, along the length of the reach; grain size and loss on ignition (for sand and finer material) were determined at midpoints of each zone, where the zone was intersected by a transect. Detailed geochemical samples also were collected in **sampling areas**; each sampling area is a small (approximately 1–10 m^2) area within a specific zone type.

Phase I: Initial Site Characterization

The first field effort entailed the collection of samples from two to four sampling areas in each stream, aimed at capturing the spatial variability of bed-sediment and pore-water parameters. Sampling areas were distributed across different sediment zone types (based on observable grain size and organic content), to provide a wide range of rates of MeHg production potentials, and to be representative of the bed-sediment types in the stream reach. Multiple bed-sediment and pore-water samples were composited (Lutz and others, 2008) from each sampling area. Sampling areas were approximately 1–10 m^2 in area (depending on the size and spatial heterogeneity of the sediment zone). Although surficial bed sediment (0–2 cm) and pore water (2 cm) were of primary interest for these studies, additional samples were collected during Phase I for depth-profile characterization of select parameters. Long cores (measuring about 20 cm) of bed sediment were retrieved from a single, undisturbed location within each sampling area and frozen; samples from various depth intervals were analyzed for sulfur speciation and general sediment chemistry. Pore-water samples were collected at various depths, with each sample representing a composite drawn from the same depth at a number of locations within the sampling area. In addition, stream-water samples were collected just above the sediment-water interface using the pore-water sampling apparatus. Latitudes and longitudes of sampling areas are given in appendix 1.
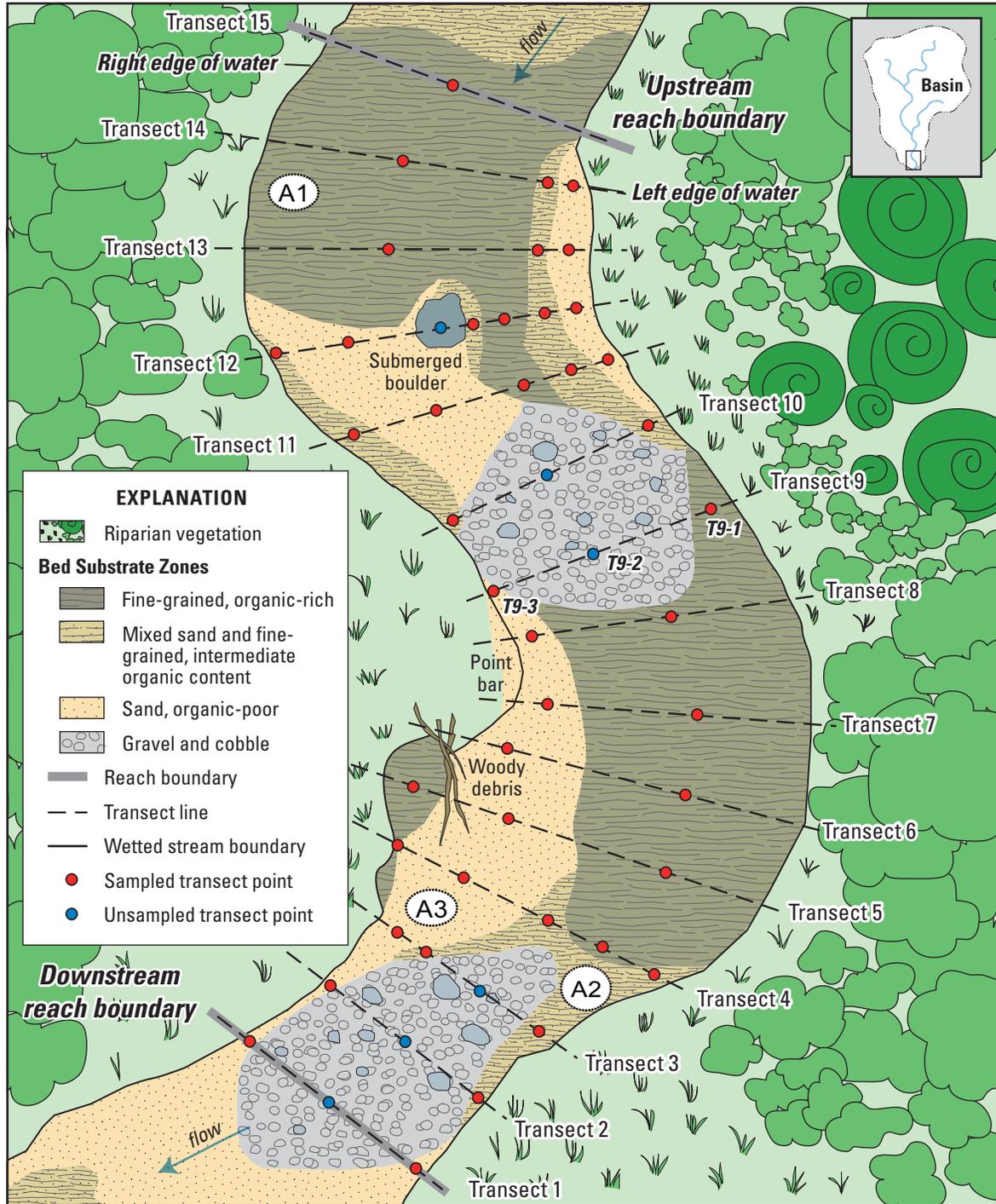
Figure 2. Conceptualized spatial framework for streambed-sediment sampling.
Zones of interest for detailed geochemical sampling were (1) primarily fine-grained organic-rich material, (2) mixed sand and fine-grained material, and (3) primarily sand, and were sampled in **sampling areas** A1, A2, and A3, respectively. Zones composed of material larger than sand (gravel, cobble, boulders) were not sampled, but the extent of these zones was determined by the multiple-transect approach and was used in reach calculations.

Phase II: Temporal Sampling

Temporal sampling areas were selected based on the analytical results of Phase I spatial geochemical sampling: at each stream, the sampling area with the highest measured MeHg production potential was selected for Phase II (temporal) sampling. Temporal trends related to benthic Hg cycling at each site were measured using this single benthic sampling area. Each temporal sampling area was sampled a total of five times (Phase I and III included) over a 13–17 month period (Florida: February 2003 through August 2004; Oregon: May 2003 through September 2004; Wisconsin: June 2003 through July 2004). The time interval between sampling events ranged from 50 to 212 days (about 2–7 months), depending on the region.

Phase III: Detailed Reach Characterization

Given (a) the inherent within-stream biogeochemical spatial heterogeneity observed in the benthic substrates of these streams, and (b) the limited number of sampling areas used to characterize bed-sediment and pore-water biogeochemistry at each stream during Phases I and II, Phase III was designed as a two-tiered approach to more effectively characterize the spatial distribution of Hg-related parameters in each stream. The first tier entailed intensive reach characterization aimed to characterize bed substrate organic content, as measured by loss on ignition (LOI), and grain size, as measured by percent fines (that is, percent smaller than 0.063 mm). The second tier entailed spatial biogeochemical sampling of three to seven sampling areas per stream; at each stream, one of these was the temporal sampling area and the remaining were representative bed-sediment zones selected from within the stream reach (wherever possible). Samples collected from these areas were analyzed for mercury species concentrations, microbial MeHg production potential, and other primary constituents that are more labor-intensive to collect and more expensive to assay, in addition to organic content and grain size. Field sampling and data analysis methods associated with Phase III sampling are detailed in Lutz and others (2008).

Bed-Sediment and Pore-Water Parameters and Associated Methods

Bed sediment and pore water collected in the field were sampled according to protocols detailed by Lutz and others (2008). Five U.S. Geological Survey laboratories were used for the following classes of samples:

1. Field-frozen, whole bed-sediment and acid-preserved, filtered pore-water samples (multiple depths) for mercury speciation and organic content analyzed by the USGS Wisconsin Mercury Research Laboratory (WMRL), Middleton, Wisconsin.

2. Chilled mason jars of sieved (1 mm) bed sediment (composite 0–2 cm surface interval) for microbial rate assays and subsequent subsampling for ancillary bed-sediment and pore-water parameters associated with these microbial rate samples analyzed by the Methylmercury Production and Degradation Potential Rates Laboratory, USGS Branch of Regional Research, Menlo Park, California.

3. Field-frozen or otherwise preserved whole bed-sediment and filtered pore-water samples (multiple depths) for ancillary bed-sediment and pore-water characterization (for example, complete sulfur speciation and elemental analysis) analyzed by the Sulfur Geochemistry Laboratory, USGS, Reston, Virginia.

4. Chilled pore water (multiple depths) for dissolved organic carbon (DOC) and specific UV absorbance (SUVA) analyzed by the Carbon Geochemistry Laboratory, USGS Branch of Regional Research, Boulder, Colorado.

5. Whole bed-sediment samples for grain-size characterization analyzed by the USGS Iowa Water Science Center Sediment Laboratory, Iowa City, Iowa.

Streambed-sediment and pore-water parameters measured are listed in table 2. Subsequent sections of this report detail the methods used for each constituent.

Streambed-Sediment Analyses—Whole Sediment Samples

Whole streambed-sediment samples were analyzed for numerous mercury-related measures. Relative deviations, defined as one-half the absolute value of the difference between replicate measurements, are provided for replicate determination in the data files (appendixes 1-6). Average relative deviations, expressed as a percentage of the mean measurement for a replicate pair, are summarized for selected analytes in section, "Mercury Speciation." For triplicate analyses, variability is reported as percent relative standard deviation (RSD), which is defined as 100 • [standard deviation / mean].

Table 2.   Streambed-sediment and pore-water parameters analyzed for the National Water-Quality Assessment Program's detailed mercury studies, 2002–04.

[Details regarding field preservation techniques are detailed in Lutz and others (2008). mm, millimeter]

Parameter	USGS laboratory
Sediment analyses—whole sediment samples	
Total mercury (THg)	Wisconsin Mercury Research Laboratory, Middleton, Wis.
Methylmercury (MeHg)	Wisconsin Mercury Research Laboratory, Middleton, Wis.
Reactive mercury ($Hg(II)_R$)	Wisconsin Mercury Research Laboratory, Middleton, Wis.
Total carbon, nitrogen and sulfur (TC, TN, TS)	Sulfur Geochemistry Laboratory, Reston, Va.
Organic carbon (OC)	Sulfur Geochemistry Laboratory, Reston, Va.
Total phosphorus (TP)	Sulfur Geochemistry Laboratory, Reston, Va.
Grain size (GS)	Iowa Water Science Center Sediment Laboratory, Iowa City, Iowa
Acid volatile sulfur (AVS)	Sulfur Geochemistry Laboratory, Reston, Va.
Disulfides (FeS_2)	Sulfur Geochemistry Laboratory, Reston, Va.
Solid phase sulfate minerals	Sulfur Geochemistry Laboratory, Reston, Va.
Organic sulfur	Sulfur Geochemistry Laboratory, Reston, Va.
Pore-water analyses—field filtered pore water	
Total mercury (HgT)	Wisconsin Mercury Research Laboratory, Middleton, Wis.
Methylmercury (MeHg)	Wisconsin Mercury Research Laboratory, Middleton, Wis.
Dissolved Organic Carbon (DOC)	Carbon Geochemistry Laboratory, Branch of Regional Research, Boulder, Colo.
Specific Ultra Violate Absorbance (SUVA)	Carbon Geochemistry Laboratory, Branch of Regional Research, Boulder, Colo.
Sulfide (S^{2-})	In-field measurement
Sulfate (SO_4^{2-})	Sulfur Geochemistry Laboratory, Reston, Va.
Chloride (Cl^-)	Sulfur Geochemistry Laboratory, Reston, Va.
Fluoride (F^-)	Sulfur Geochemistry Laboratory, Reston, Va.
Bromide (Br^-)	Sulfur Geochemistry Laboratory, Reston, Va.
Nitrate (NO_3^-)	Sulfur Geochemistry Laboratory, Reston, Va.
Ammonium (NH_4^+)	Sulfur Geochemistry Laboratory, Reston, Va.
Phosphate (PO_4^{3-})	Sulfur Geochemistry Laboratory, Reston, Va.
Oxidation-reduction potential (ORP)	In-field measurement
Sediment analyses—1-mm sieved sediment	
Methylmercury Production Potential (MPP) Rate	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Methylmercury Degradation Potential (MDP) Rate	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Microbial Sulfate Reduction (SR)	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Acid Volatile Sulfur (AVS)	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Total Volatile Sulfur (TRS)	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Acid extractable ferrous iron ($Fe(II)_{AE}$)	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Amorphous ferric iron ($Fe(III)_a$)	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Crystalline ferric iron ($Fe(III)_c$)	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Bulk density	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Dry weight	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Organic content	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Oxidation-Reduction Potential (ORP)	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
pH	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Pore-water analyses—pore water isolated from 1-mm sieved sediment samples	
Sulfate (SO_4^{2-})	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Chloride (Cl^-)	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Ferrous iron ($Fe(II)_{pw}$)	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.
Acetate	Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.

Mercury Speciation

Sediment mercury speciation assays included total mercury (HgT), methylmercury (MeHg), and 'reactive' inorganic divalent mercury [$Hg(II)_R$]. Samples collected for mercury speciation were frozen in the field immediately after collection (Lutz and others, 2008), and maintained frozen until analyzed by the USGS WMRL (Middleton, WI).

Total Mercury

Sediment HgT was assayed according to Olund and others (2004). In brief, thawed, homogenized subsamples were digested in Teflon® bombs with strong acid, after which they were treated in sequence with bromine monochloride, hydroxylamine hydrochloride, and stannous chloride ($SnCl_2$) to convert all mercury species to gaseous elemental Hg^0. The gaseous Hg^0 was purged from aqueous solution, captured on a gold trap, thermally desorbed, and then quantified using a cold vapor atomic fluorescence spectrometer (CVAFS; Tekran® Model 2600 Mercury Detector; Toronto, Canada).

This method has an absolute detection limit of 0.3 ng. The standard reference material (SRM) routinely used as a quality-assurance standard was IAEA-405 (estuarine sediment), with a certified value for HgT of 810 ng/g dry wt. Average (± standard deviation) SRM recovery was 102.0 ±10.4 percent (n=35) for all batches of NAWQA sediment samples assayed. Matrix spikes were conducted in duplicate (per batch) by adding a known amount of $HgCl_2$ solution to the sediment digestate. Average matrix spike recoveries were 92.5 ±7.2 percent (n=50). Although most samples were run only once, method analytical precision was tested on about 11 percent of all samples, by assaying select environmental samples in triplicate. The average RSD of these triplicate assays was 14.2 ±14.3 percent (n=31).

Methylmercury

Sediment MeHg was assayed using a standard USGS protocol (DeWild and others, 2004). Field-frozen samples were thawed and homogenized. MeHg was extracted from subsamples using potassium bromide, copper sulfate, and methylene chloride, and then back-extracted into de-ionized (DI) water (Milli-Q®). The extractant was pH adjusted and then ethylated with sodium tetraethyl borate. The ethylated-MeHg species was purged from aqueous solution, trapped, thermally desorbed, separated on a gas chromatographic column, reduced to elemental Hg^0 using a pyrolytic column, and detected using CVAFS.

This method has an absolute detection limit of 0.08 ng/g of wet or dry sediment (as-processed). The standard reference material (SRM) routinely used as a quality

assurance standard was IAEA-405 (Estuarine sediment) with a certified value for MeHg of 5.4 (4.96–6.02) ng/g dry wt (as inorganic Hg). Average (± standard deviation) SRM recovery was 76.5 ±17.4 percent (n=39) for all batches of NAWQA sediment samples assayed. Although most samples were run only once, method analytical precision was tested on selected samples, by assaying select environmental samples in triplicate. The average RSD of these triplicate assays was 22.0 ±21.1 percent (n=39).

Inorganic Reactive Mercury

Sediment "reactive" mercury [$Hg(II)_R$] is methodologically defined as the fraction of HgT in a sediment sample that has NOT been chemically altered (for example, digested, oxidized or chemically preserved) and that is readily reduced to elemental mercury (Hg^0) by an excess of $SnCl_2$ over a short exposure time. This operationally defined parameter was developed as a surrogate measure of the fraction of inorganic Hg(II) that is most likely available to the bacteria responsible for MeHg production, and is used in combination with radiotracer-derived methylation rate constants to calculate potential rates of MeHg production in sediment samples (see section, "Methylmercury Production"). Although there is no standard method for this parameter, the procedure described below is modified from similar approaches previously published (Kieu, 2004; Marvin-DiPasquale and Cox, 2007), and has been used in several studies of mercury methylation (for example, Marvin-DiPasquale and others, 2006).

Recent experimental evidence suggests that the $Hg(II)_R$ assay effectively measures the fraction of Hg(II) that is associated with simple anions (for example, $HgSO_4$, $HgCl_2$) in sediment pore water and/or Hg(II) that is weakly adsorbed to particle surfaces (Marvin-DiPasquale and others, 2006). Both of these fractions are likely available to sediment microbes for Hg(II)-methylation. In a related set of experiments, the concentration of $Hg(II)_R$ measured in a suite of freeze-dried and homogenized environmental samples ranging over four-orders of magnitude in HgT (1–24,000 µg/g), was strongly correlated ($r^2 = 0.97$) with the amount of MeHg produced when these freeze-dried samples were mixed (at a constant HgT amendment amount) with fresh sediment containing active populations of Hg(II)-methylating bacteria (Bloom and others, 2006). These results suggest that the $Hg(II)_R$ fraction provides a reasonable surrogate measure of the fraction of HgT that is potentially available for Hg(II)-methylation.

Inorganic reactive mercury is analyzed as follows: field frozen samples were initially thawed in a refrigerator overnight and subsequently homogenized using a Teflon® spatula. Subsamples (1–5 g) were weighed and transferred

into acid-washed 250 mL glass gas-flushing bottles (bubbler flasks). Reagent water (50 mL of Milli-Q®) and 0.25 mL of 12 M HCl were added to each flask, followed by 0.5 mL of $SnCl_2$ solution (30 g/L in 5 percent HCl). Each flask was immediately capped and placed on a shaker. Samples were gently shaken and purged for 30 min with ultra-high purity N_2 gas (200–300 mL/min), during which time the Hg^0 gas (resulting from the reduction of $Hg(II)_R$ with $SnCl_2$) was passed through a soda lime trap (to remove acid vapors) and subsequently trapped on a gold-coated glass bead trap. The Hg^0 was then thermally desorbed from the gold trap to the analytical system and detected using CVAFS (Tekran® Model 2500 Mercury Analyzer; Toronto Canada). Standard curves generated from HgT water sample assays were used to calibrate the $Hg(II)_R$ sample results.

Each set of 12 bubbler flasks consisted of 7–8 $Hg(II)_R$ samples, 1–2 additional replicates of a randomly picked $Hg(II)_R$ sample, 2 bubbler blanks and 1 standard recovery sample. Bubbler blanks consisted of 50 mL of Milli-Q® water, HCl, and $SnCl_2$ only (no sediment). Standard recovery samples consisted of Milli-Q® water, HCl, $SnCl_2$ and 100 µL of 10 ng/mL inorganic Hg(II) standard. Standard recovery samples were 96.3 ±3.5 percent of the theoretical 1.0 ng absolute value (n=15). The relative deviation of replicate samples (average ± standard error) was 16.8 ±5.3 percent (n=12 sets).

Additional Ancillary Sediment Geochemical Measures

In addition to the ancillary sediment geochemical parameters, which are associated with the sediment composite samples collected for microbial rate assays, additional sediment samples were collected for geochemical characterization of conditions at the time of field collection. These sediment samples were placed in airtight containers (30-mL polypropylene jars), immediately frozen, and shipped frozen to the Sulfur Geochemistry Laboratory, USGS, Reston, VA, where all subsequent analyses were conducted.

Elemental Analysis of Carbon, Nitrogen, Phosphorous, and Sulfur

Field frozen samples were thawed under refrigeration, stirred until homogeneous, subsampled into a Petri dish, and weighed. Sediment water content was determined after drying sample to constant weight at 60° C. The dry sediment was ground to a powder and analyzed for total carbon (TC), organic carbon (OC), total nitrogen (TN), and total sulfur (TS) using a Leco 932 CNS Analyzer (Leco Corporation, St. Joseph, MI, USA). TC, TN, and TS were measured directly. OC was determined after removal of inorganic

carbon (IC) in an HCl acid vapor chamber (Hedges and Stern, 1984; Yamamuro and Kayanne, 1995). All samples were analyzed at least in duplicate. Analytical precision (percent relative standard deviation, or RSD) was about 2 percent for TC, 4 percent for OC and TS, and 3 percent for TN. IC is reported as the calculated difference between TC and OC (%IC = %TC – %OC).

Total phosphorus (TP) was determined by a method slightly modified from that of Aspila and others (1976). Samples were dried at 60°C, cooled to room temperature in a dessicator, weighed, and placed in acid cleaned and combusted ceramic crucibles. Weighed sediment samples (0.4–0.6 g) were baked at 550 °C for 2 h, cooled, and then transferred into acid clean plastic centrifuge cones containing 45 mL of 1 M HCl. The original crucibles were rinsed with 5 mL of 1 M HCl, which was added to the centrifuge cones for a final volume of 50 mL of 1 M HCl. The samples were extracted in the 1 M HCl for 16 h on a shaker. An aliquot of each extract was centrifuge filtered using Millipore ultrafree-CL HVPP low-binding Durapore centrifuge filters (0.45 µm pore size), then neutralized with a 1 M NaOH solution, and transferred to plastic test tubes. The filtered aliquots were analyzed for phosphate using the phospho-molybdate method (Strickland and Parsons, 1972) and a Brinkmann PC900 fiberoptic colorimeter. Percent RSD for the TP analysis was ±3. Elemental ratios (C/N, C/P, N/P, C/S) are reported on a molar basis.

Sulfur Speciation

Speciation of sulfur in the sediment involved wet chemical fractionation of the total sulfur into the following forms: (1) acid volatile sulfur (AVS) or monosulfides, (2) disulfides (usually dominated by pyrite or FeS_2 (s), (3) sulfates (solid phase sulfate minerals in the sediment), and (4) organic sulfur. Prior to beginning sulfur speciation analysis, the weight of sediment needed to yield at least 20 mg of Ag_2S or $BaSO_4$ precipitate is estimated from the total sulfur in the sample. This fractionation scheme does not include analysis for elemental sulfur. The overall sulfur speciation scheme used is adapted from Tuttle and others (1986), and details of the procedure used are outlined in Bates and others (1998).

Thawed sediment was transferred to a tared reaction chamber flask and weighed. A volume of 30 mL of 5 percent $AgNO_3$ solution was added to a culture tube, and a Pasteur pipette was attached to the tubing outlet and inserted into the $AgNO_3$ solution. A 50 mL volume of 6 M HCl was added to the reaction chamber through the injection port, and the acidified samples were stirred and heated (just below boiling temperature) in the closed reaction chamber until the silver nitrate solution in the culture tube became clear (about 5 h), indicating all AVS has been collected from the sample. The

precipitated AgS was filtered onto a pre-weighed 0.4 μm Nuclepore® filter, and dried overnight in a desiccator. AVS (monosulfide) content was calculated from the weight of AgS collected, the total sediment wet weight, and the sediment water content. In cases where the water content was not determined, the AVS wet weight was determined.

After collecting the AgS precipitate from the AVS extraction, a new culture tube containing 30 mL of 5 percent $AgNO_3$ was attached to the distillation apparatus. Freshly reduced $CrCl_2$ solution in 1 M HCl (Tuttle and others, 1986) was added (60 mL) to the reaction chamber through an injection port. The reaction vessel contents were stirred and heated (just below boiling temperature), and the H_2S gas generated was trapped as AgS precipitate in the $AgNO_3$ solution. The distillation was allowed to proceed until the $AgNO_3$ solution turned clear (about 3–4 h). The AgS precipitate was collected by filtration, dried and assayed by weight, as described for the AVS separation.

The contents of the reaction vessel remaining after the AVS and disulfide extraction were then filtered through a 0.4 μm Nuclepore® filter. Hot (boiling) Milli-Q® water was rinsed through the material on the filter pad, keeping the total filtrate volume to less than 300 mL. The filter pad with residue was transferred to an open Petri dish, dried overnight in a desiccator, weighed, and saved for analysis of organic sulfur. The filtrate was brought to a boil, 10 mL of saturated Br_2 solution was added, and boiling was continued until the yellow color in the filtrate fades. Then, 10 mL of 10 percent $BaCl_2$ solution is added, and boiling was continued for at least 15 min, during which time the appearance of barium sulfate ($BaSO_4$) precipitate would indicate the presence of any sulfates in the solution. Under conditions of very low amounts of sulfate, solutions were boiled down to a volume of less than 100 mL until $BaSO_4$ appeared. Heating is stopped, the solution is covered, and $BaSO_4$ is allowed to precipitate for at least 3 h (overnight for samples with very low sulfate content). The $BaSO_4$ precipitate is filtered onto a 0.4 μm Nuclepore® filter in a glass filtering apparatus. The filtrate is discarded, and the filter is transferred to an open Petri dish and dried in a desiccator. The weight of the recovered $BaSO_4$ is recorded, and the sulfate mineral fraction is calculated from the original sediment wet weight and the sediment water content.

The weighed residue on the Nuclepore® filter, remaining from the sulfate mineral extraction (described earlier), is transferred to a weighed crucible. A quantity of Eschka fusion mixture at least three times the sample weight is added and the Eschka's mixture and sample is thoroughly mixed. The surface of the mixture is flattened and more Eschka's mixture is added to completely cover the surface. The crucible is reweighed, and the exact weight of the Eschka's mixture is recorded. The crucible is covered with a lid, transferred to a kiln, heated to 800 ºC for 2 h. The contents of the crucible are transferred to a flask and boiled in Milli-Q® water for 30 min. The suspension is filtered through a 9.0 cm ashless filter paper in a clean Buchner funnel, and the filtrate is collected in a clean filter flask. The filtrate is heated and concentrated HCl is added until the pH of the solution is ≤ 4. The solution is brought to a boil and 10 mL of saturated Br_2 is added. The solution is boiled until the yellow color fades, and 10 mL of 10 percent $BaCl_2$ solution is added to precipitate sulfates as $BaSO_4$. The solution is boiled for at least 15 min, and allowed to cool for at least 3 h. The solution is filtered through a tared 0.4 μm Nuclepore® filter in a glass filtering apparatus to recover the precipitated $BaSO_4$. The filter is then placed in an open Petri dish and dried in a desiccator overnight. The weight of the $BaSO_4$ is determined, and organic sulfur is back calculated from the original sediment wet weight and the sediment water content.

Oxidation-Reduction Potential (ORP)

Sediment ORP was measured in the field immediately after sample collection. Measurements were made with an Orion 250A pH / mV meter and a silver-silver chloride, platinum sensor, ORP electrode (Orion 9180BN, Thermo Scientific) by inserting the electrode into the streambed-sediment matrix. Calibration of the probe was assessed either with a two-point redox couple standard, or with the manufacturer's ORP standard (Orion 967961, Thermo Scientific). ORP measurements were converted to Eh (millivolts, relative to the standard hydrogen electrode) using standard procedures (Nordstrom and Wilde, 2005), using standard half-cell potentials for silver:silver chloride in saturated KCl. Eh values for dates when ORP calibration checks differed by 10 mV or more from the accepted values are reported as estimated (E code in appendixes).

Grain Size – Sand/Silt Split

Grain size, greater or less than 62 μm (the sand/silt split), was assayed at the USGS Sediment Laboratory in Iowa City, Iowa, using a standard wet sieve method (Matthes and others, 1992).

Pore-Water Analyses—Field Filtered Pore Water

This section describes analytical methods for filtered pore water. Sampling methods are detailed in a companion report (Lutz and others, in prep). Field-submitted blank sample data collected for quality-control purposes were sampled through the pore-water sampling apparatus (Lutz and others, in prep.), and are presented in table 3. Replicate quality-control data are presented in appendix 5.

Table 3. Field blank-water data.

[Blank samples were collected through pore-water sampling equipment at Oak Creek at South Milwaukee, Wisconsin, on March 12, 2004. •, multiplied by]

Constituent	Concentration
Sample time = 0848, pesticide-grade organic-free water (OmniSolv® EMD)	
Dissolved organic carbon, filtered pore water, milligram per liter	0.3
Ultraviolet absorbance at 254 nanometers (1 centimeter path length), filtered pore water, unit per centimeter	.001
Specific ultraviolet absorbance at 254 nanometers (1 centimeter path length), filtered pore water, liter per (milligram of dissolved organic carbon • meter)	.3
Sample time = 0849, Milli-Q® water from USGS Wisconsin Mercury Laboratory	
Methylmercury, filtered pore water, nanogram per liter	<0.04
Total mercury, filtered pore water, nanogram per liter	.37
Sample time = 0849, Inorganic blank water from USGS Ocala, Florida	
Dissolved organic carbon, filtered pore water, milligram per liter	0.3
Ultraviolet absorbance at 254 nanometers (1 centimeter path length), filtered pore water, unit per centimeter	.001
Specific ultraviolet absorbance at 254 nanometers (1 centimeter path length), filtered pore water, liter per (milligram of dissolved organic carbon • meter)	.3
Sulfide, filtered pore water, microgram per liter	<.1
Sulfate, filtered pore water, milligram per liter	<.1
Chloride, filtered pore water, milligram per liter	<.1
Fluoride, filtered pore water, milligram per liter	<.08
Bromide, filtered pore water, milligram per liter	<.1
Nitrate, filtered pore water, milligram per liter	<.1
Ammonium, filtered pore water, microgram per liter	4.67
Phosphate, filtered pore water, microgram per liter	6.15

Mercury Speciation

Pore-water mercury speciation assays included HgT and MeHg, which were performed at the USGS Mercury Laboratory in Middleton, WI. For both parameters, pore-water samples collected in the field were stored in Teflon® vials, preserved on-site with 1 percent HCl (final concentration), and stored in a dark cool location until their return to the USGS (Lutz and others, 2008), where they were refrigerated until being assayed. The pore-water mercury parameters thus reflect the field conditions at the time the sample was collected.

Total Mercury

Pore-water HgT was assayed according to USEPA method 1631 (U.S. Environmental Protection Agency, 2002). Bromine monochloride (BrCl) was added to the sample container to oxidize all forms of Hg to the Hg(II) oxidation state. After 5 days at 50°C, the BrCl is neutralized by the addition of hydroxylamine hydrochloride (NH$_2$OH*HCl). Following neutralization, stannous chloride (SnCl$_2$) is added to the sample to reduce Hg(II) to volatile elemental Hg^0, which is subsequently purged from solution and trapped on gold-coated glass beads (sample trap). The Hg^0 was then thermally desorbed onto a second gold trap (analytical trap) and from that again thermally desorbed and detected by CVAFS. Some samples high in organic matter were pretreated in an ultra violet (UV) digester to remove the organic color from the sample.

Methylmercury

Pore-water MeHg was assayed by aqueous phase ethylation, followed by gas chromatographic separation with CVAFS detection (DeWild and others, 2002). This method has been used to determine MeHg concentrations in filtered or unfiltered water samples in the range of 0.040–5 ng/L. The upper range was extended to higher concentrations when necessary by distilling smaller sample volumes or ethylating less of the distillate.

Additional Ancillary Pore Water Geochemical Measures

The following pore-water parameter measurements were made either in the field or on samples that were preserved in the field to capture the field conditions at the time the sample was collected.

Dissolved Organic Carbon and Specific UV Absorbtion (SUVA)

Pore-water DOC samples collected and filtered in the field were stored and shipped on ice, and subsequently refrigerated until further analysis. Measurements were made in duplicate using the platinum-catalyzed persulfate wet oxidation method on an O.I. Analytical Model 700 TOC Analyzer (Aiken, 1992). The average standard deviation for replicate DOC measurements was determined to be ±0.2 mg/L. The method detection limit is approximately 0.2 mg/L as carbon.

Ultraviolet (UV_{254}) absorbance measurements were made on filtered subsamples collected for DOC analysis. Analyses were conducted at room temperature using a Hewlett-Packard Model 8453 Photo-diode array spectrophotometer at 254 nm wavelength with distilled water as the blank and utilizing a 1 cm path length quartz cell. Results are reported in dimensionless absorbance units. The 254 nm wavelength is commonly associated with the aromatic moieties of DOC (Chin and others, 1994). The quartz cell was rinsed with a small volume of sample before adding sample for analysis. The cell was then rinsed with distilled water before analyzing the next sample. Standard deviation for a UV_{254}-absorbance measurement at 254 nm is ±0.002 absorbance units.

$SUVA_{254}$, was then calculated as the UV_{254} absorbance of a sample divided by the DOC concentration. $SUVA_{254}$ indicates the nature or "quality" of DOC in a given sample and has been used as a surrogate measurement of DOC aromaticity (Chin and others, 1994; Weishaar and others, 2003). SUVA values are reported in units of L/(mg C * m) and have a standard deviation of ±0.1 L/(mg C * m).

Sulfide

Pore water collected for sulfide analysis (3 mL) was preserved in the field by the addition of 3 mL of sulfur antioxidant buffer (SAOB) (Brouwer and Murphy, 1994) to the sample in a small plastic container. Analysis of sulfide was carried out within 6–8 h of collection using a sulfide ion-specific electrode, which was calibrated just prior to each field trip. The average RSD of replicate measurements of a single sample was ±7 percent for sulfide. The method detection limits was approximately 0.1 μg/L (ppb) sulfide.

pH

Pore-water pH was measured in the field within 6–8 h of sample collection using a semi-micro electrode and two-point buffer calibration. The average percent RSD of replicate measurements of a single sample was ±10.

Conductivity and Total Dissolved Solids

Conductivity and total dissolved-solids measurements were carried out by electrochemical measurements within 6–8 h of sample collection using an Orion Conductivity/ Salinity/Total Dissolved Solids electrode (U.S. Environmental Protection Agency Method 120.1). The electrode was calibrated using an Orion standard solution, as recommended by the manufacturer. The average percent RSD of replicate measurements of a single sample was ±2. The method detection limits were approximately 0.1 μS/cm and 0.1 mg/L.

Anions: Sulfate, Chloride, Fluoride, and Bromide

Pore-water samples collected for determination of sulfate, chloride, fluoride, and bromide anions ($SO4^{2-}$, Cl^-, F^- and Br^-) were shipped on ice, and refrigerated until analysis using suppressed anion chromatography with conductivity detection. Identification and quantification of individual anions was accomplished using external calibration standards and peak area calculation using Waters Associates Millennium chromatography software. The average percent RSD of replicate measurements of a single sample was ±4, and the method detection limit was approximately 0.05 mg/L, for each anion (excluding major interferences).

Nutrients: Nitrate, Ammonium, and Phosphate

Nitrate was determined by suppressed anion chromatography as described above for anions, using combined conductivity and UV/VIS absorbance detectors. The average percent RSD of replicate measurements of a single sample was ±4, and the method detection limit was approximately 0.05 mg/L (excluding major interferences).

Samples for ammonium and phosphate analysis were frozen on dry ice within 6–8 h of collection, and transported to the Sulfur Geochemistry Laboratory (USGS, Reston, VA) for analysis using standard colorimetric methods (Strickland and Parsons, 1972). The average percent RSD of replicate measurements of a single sample was ±5, and the method detection limit was 0.5 μg/L, for both constituents.

Oxidation-Reduction Potential (ORP)

Pore-water ORP was measured in the field immediately after sample collection. Measurements were made with an Orion 250A pH / mV meter and a silver-silver chloride (platinum sensor) ORP microelectrode (MI-800/710, Microelectrodes, Bedford, NH). Calibration of the probe was assessed either with a two-point redox couple standard, or with the manufacturer's ORP standard (Orion 967961, Thermo Scientific). ORP measurements were converted to Eh, (mV, relative to the standard hydrogen electrode), using standard procedures (Nordstrom and Wilde, 2005).

Streambed-Sediment Analyses—1-mm Sieved Sediment

Splits of whole bed-sediment samples were sieved through plastic sieves with 1-mm mesh to remove large debris (such as sticks, leaves, and pebbles) prior to analyses described in this section.

Microbial Rate Assays

Three microbial process rates were measured in composite surface sediment (0–2 cm) samples: (1) MeHg Production Potential (MPP), (2) MeHg Degradation Potential (MDP), and (3) Sulfate Reduction (SR). The first two processes have obvious direct impacts on sediment MeHg concentrations. The third was measured because sulfate-reducing bacteria (SRB) generally are believed to be the primary microbial group responsible for MeHg production (Compeau and Bartha, 1985; Gilmour and others, 1992).

Sediment samples were collected in the field, transferred to acid-cleaned mason jars, and shipped chilled (on wet ice) to the USGS Methylmercury Production and Degradation Potential Rates Laboratory in Menlo Park, Calif, where they were refrigerated until further processing within 2–10 days (average of 6 ± 2 days; n=94) of the original collection date. All preliminary sample processing was conducted under anaerobic conditions in a nitrogen (N_2) gas flushed glove bag. Sediment was removed from the mason jars and homogenized in plastic bags. Subsamples for each microbial rate assay and ancillary sediment parameter were weighed and transferred into appropriate containers. Microbial rate assays were initiated the same day, while samples for ancillary parameters were preserved and assayed at a later date (see section "Ancillary Sediment Measures Associated with Composite Samples Collected for Microbial Rate Assays").

Methylmercury Production

The mercury radioisotope ^{203}Hg (half-life = 46.5 days) has been used since 1980 to assess potential rates of Hg(II)-methylation in a wide range of environments (Furutani and Rudd, 1980; Gilmour and Riedel, 1995; Guimaraes and others, 1995; Stordal and Gill, 1995; Gilmour and others, 1998; Guimaraes and others, 2000; Marvin-DiPasquale and Agee, 2003; Marvin-DiPasquale and others, 2003). Typically, a constant amount of inorganic ^{203}Hg(II) isotope is added to environmental samples, which are then incubated for a fixed amount of time (hours to days). The fraction of the ^{203}Hg(II) that is converted to radiolabeled methylmercury (Me^{203}Hg) is then normalized by the incubation time to derive an Hg(II)-methylation reaction rate constant (k_{meth}). The resulting differences in k_{meth} values among a suite of samples provides a relative measure of the propensity of the native microbial

communities to convert readily available 'reactive' Hg(II) into MeHg. In most studies, the pool size of *in situ* Hg(II) that is actually available for Hg(II)-methylation is unknown, and the calculated Hg(II)-methylation potential is based only on the amount of ^{203}Hg(II) added to the sample, or on the site specific HgT concentration (Gilmour and others, 1998). Since the development of the reactive mercury (Hg(II)$_R$) assay (as described above), and its application across a range of ecosystems and sediment types, it has become clear that only a small percentage (typically 0.1–5 percent) of HgT occurs as Hg(II)$_R$ (Marvin-DiPasquale and others, 2006; Marvin-DiPasquale and Cox, 2007). Thus, MeHg production rates calculated from ^{203}Hg(II) derived k_{meth} values in conjunction with *in situ* HgT concentrations very likely overestimate actual rates. In the current study, as in other recent investigations (Kieu, 2004; Marvin-DiPasquale and others, 2006; Marvin-DiPasquale and Cox, 2007) ^{203}Hg(II) derived k_{meth} values were used in conjunction with independently measured Hg(II)$_R$ concentrations to calculate *in situ* rates of MeHg production. This approach is advantageous because it accounts for both the activity of the native Hg(II)-methylating microbial population and the site-specific pool size of Hg(II) that is most likely available for methylation.

The general ^{203}Hg(II) incubation procedure, and the associated k_{meth} calculations, have been previously published (Marvin-DiPasquale and Agee, 2003; Marvin-DiPasquale and others, 2003), but the specific conditions used for this study are detailed here. All samples consisted of 3.0 g wet sediment in a 13 cm^3 crimp-sealed serum vial with a Teflon® lined stopper, and a N_2 gas flushed headspace. During Phase I and Phase II, a sample set from a single site consisted of three such vials, two of which were incubated (live samples) plus one killed-control. During Phase III, only one incubated and one killed-control sample was assayed per sediment composite; the duplicate incubated sample was omitted due to the large number of samples collected during Phase III. A working solution of radiolabeled mercuric chloride (^{203}HgCl$_2$; total activity of 15 µCi/mL, in anoxic 59 mM KH$_2$PO$_4$ phosphate buffer, final pH=7.0) was prepared from a concentrated ^{203}HgCl$_2$ stock solution preserved in 1.2 M HCl. The working solution specific activity was always fixed at 1.0 µCi/µg Hg(II), by adding non-radioactive HgCl$_2$ if necessary, so that all samples in the study received the same total Hg(II) amendment and radioactivity. Each sample vial was injected with 100 µL of the ^{203}HgCl$_2$ radiotracer (equivalent to 500 ng Hg(II) per gram of sediment) and homogenized on a vortex unit for 30 s. The injection time was recorded and the killed-control sample was immediately flash frozen in a bath of dry ice plus ethanol. The remaining duplicate live samples were incubated at room temperature (19–21 °C) for approximately 20–24 h, after which they also were flash frozen and the exact time of incubation termination was recorded. All samples were subsequently stored frozen until further analysis.

The Me^{203}Hg produced from ^{203}Hg(II) was extracted into toluene. Thawed sediment was first washed from the serum bottles, into 50-cm^3 fluoropolymer centrifuge tubes, with the following succession of reagents: 4 M urea (4 mL), 0.5 M CuSO4 (2 mL), 6 M HCl (8 mL), and toluene (10 mL). The centrifuge tube was vortexed for 1 min then placed on a rotating shaker for 15 min. Tubes were then centrifuged (3,000 rpm for 5 min) to separate organic and aqueous phases. The toluene phase was decanted to a second fluoropolymer tube, and another 10 mL addition of toluene was added to the first tube. The vortex, rotation, centrifugation, and decanting steps were then repeated, resulting in a 20-mL combined toluene phase containing the Me^{203}Hg. Anhydrous sodium sulfate (Na$_2$SO$_4$) was added (ca. 0.5 g) to the toluene fraction to adsorb trace amounts of water, which may have contained inorganic ^{203}Hg(II). The toluene was subsampled (15 mL) and counted for gamma radioactivity using an EG&G Wallace Gamma Counter (Model 1480) operated in the counts per minute (cpm) mode. The duplicate incubated sample cpm results were corrected by subtracting any resulting radioactivity measured in the killed-control for that sample set, to account for any abiotic ^{203}Hg(II)-methylation or any inorganic ^{203}Hg(II) that was carried over into the toluene phase. Pseudo first-order rate constants for Hg(II)-methylation (k_{meth}, units = 1/d) were calculated as:

$$k_{meth} = \ln(1-f_m)/t ,$$

where
ln is the natural logarithm function,
f_m equals the fraction ^{203}Hg(II) converted to Me^{203}Hg (cpm in the total toluene phase of kill-corrected incubated samples divided by the cpm originally injected), and
t equals the incubation time in days (d). Daily MPP rates (units = ng/g dry sediment/d) are then calculated as:

$$MPP = Hg(II)_R - Hg(II)_R \cdot EXP(-k_{meth} \cdot t) ,$$

where t = 1.0 day and independently measured *in situ* Hg(II)$_R$ concentration values (ng/g dry weight) are used (see section, "Inorganic Reactive Mercury").

The detection limit for this method is based on a greater than 50 cpm difference between incubated samples and the killed-control for that sample set, which corresponds to a nominal k_{meth} value of 3×10^{-5} per day. The actual detection limit varied as a function of actual incubation time, the specific amount of radiotracer amendment (precisely determined at the beginning of the experiment, and the holding time between incubation and the subsequent Me^{203}Hg extraction. The relative deviation for all incubation pairs greater than the detection limit was (average ± standard error) 15.8 ±3.3 percent (n=37 pairs).

Methylmercury Degradation

Methylmercury labeled with carbon-14 radioisotope (half-life = 5,730 years) (^{14}CH$_3$Hg$^+$) has been used to assess benthic MeHg Degradation Potential (MDP) in numerous environmental studies (Ramlal and others, 1986; Oremland and others, 1991; Oremland and others, 1995; Marvin-DiPasquale and others, 2000; Marvin-DiPasquale and Agee, 2003; Marvin-DiPasquale and others, 2003). In the current study, ^{14}CH$_3$Hg$^+$ degradation incubations were conducted in parallel with the ^{203}Hg(II)-methylation incubations, using the same sample preparation, level of replication, killed-control approach, and incubation conditions as described above for MPP. However, MDP samples were injected with 100 μL of a ^{14}CH$_3$Hg$^+$ solution (total activity ranging from 41 to 102 nCi/mL, specific activity = 60 nCi/ng (as Hg)) prepared in anoxic phosphate buffer (final pH=7.0). This resulted in final MeHg amendment concentrations ranging from 4.5–11.4 ng (as Hg) per gram of sediment. Incubations were terminated, and killed-control samples prepared, with the addition of 1 mL of 1 M sodium hydroxide (NaOH) solution to each vial, followed by vortex homogenization and flash freezing. Samples were maintained frozen until further processing.

Pseudo first-order rate constants for MeHg degradation (k_{deg}, units = 1/d) were calculated as:

$$k_{deg} = \ln(1-f_d)/t ,$$

where
f_d equals the fraction of ^{14}CH$_3$Hg converted to (^{14}CH$_4$ + ^{14}CO$_2$) (that is, the total kill-corrected dpm of the ^{14}C gaseous end-products form incubated samples divided by the dpm originally injected), and
t equals the incubation time in days. Daily MDP rates (units = ng/g dry sediment/d) are then calculated as:

$$MDP = MeHg_{sed} - MeHg_{sed} \cdot EXP(-k_{deg} \cdot t) ,$$

where t = 1.0 day and independently measured *in situ* sediment MeHg concentration values (ng/g dry weight) are used (see section, "Methylmercury").

Alternatively, MDP rates are similarly calculated, but with the assumption that the pool of MeHg available for microbial degradation is limited to pore-water MeHg only, such that:

$$MDP = MeHg_{pw} - MeHg_{pw} \cdot EXP(-k_{deg} \cdot t) .$$

where t = 1.0 day and MeHg$_{pw}$ is the MeHg from pore water only, calculated and expressed in terms of whole sediment dry weight content (ng/g dry weight).

The detection limit for this method is based on a greater than 50 dpm difference between incubated samples and the killed-control for that sample set, which corresponds to k_{deg} values ranging between 2×10^{-3} and 6×10^{-3} per day, depending on the initial $^{14}CH_3Hg^+$ injection solution activity. The relative deviation for all incubation pairs was (average ± standard error) 8.8 ±1.4 percent (n=50 pairs).

Sulfate Reduction

Microbial sulfate reduction (SR) rates were assayed via the $^{35}SO_4^{2-}$ amendment technique (Jørgensen, 1978). Subsamples for SR consisted of 1.5 g of sediment per vial and were collected under anoxic conditions and incubated in parallel with MPP and MDP samples. Replication consisted of duplicate live (incubated) and one killed control sample per site. Samples for SR were amended with approximately 1.0 μCi of carrier-free $^{35}SO_4^{2-}$ (0.05 mL of a 20 μCi/mL working stock of $Na_2^{35}SO_4$). After 20–24 h, incubations were arrested by the addition of 1 mL of 10 percent (w/v) zinc-acetate and subsequent freezing in an ethanol/dry ice bath. Upon thawing, total reduced sulfur (TRS) was extracted via distillation with an acidic chromium solution and the subsequent trapping of volatilized $H_2^{35}S$ in a 10 mL solution of 10 percent (w/v) zinc-acetate, containing 1 drop of antifoam agent (JT Baker antifoam B silicone Emulsion) (Fossing and Jørgensen, 1989). A 1.0 mL aliquot of this solution (containing $Zn^{35}S$ precipitate) was subsampled into a 20 mL scintillation vial containing 8.0 mL Universol liquid scintillation cocktail (MP Biomedicals Inc., Montréal, Canada). Distilled water was added (2.0 mL) and the mixture was shaken vigorously to gel. Radioactivity for the TRS fraction was measured using a Beckman 6000 beta counter. Rate constants for SR were calculated as the fraction of ^{35}S-TRS produced, relative the amount of $^{35}SO_4^{2-}$ added, normalized by the incubation time. Rates of SR were then calculated from the site-specific rate constants and the in situ whole sediment SO_4^{2-} concentration (Marvin-DiPasquale and Capone, 1998). The relative deviation for all kill-corrected samples assayed in duplicate and greater than the assay detection limit was (average ± standard error) 28.3 ±4.1 percent (n=40 sample pairs).

Ancillary Sediment Measures Associated with Composite Samples Collected for Microbial Rate Assays

Ancillary sediment geochemical parameters were sampled under anoxic conditions (in an N_2 flushed glove bag), at the same time and from the same batch of homogenized sediment that was used for microbial rate assays. All these assays were conducted at the USGS Methylmercury Production and Degradation Potential Rates Laboratory in

Menlo Park, Calif. These parameters provide a measure of the geochemical conditions at the time the microbial rate measurements were conducted, rather than conditions in the field. Every attempt was made to minimize changes in redox sensitive geochemistry between field collection and preservation or analysis, including (a) minimal holding times prior to subsampling, (b) completely filling glass mason jars with sediment and parafilm wrapping of the jar lid to exclude oxygen, and (c) cold storage (on wet ice or refrigerated) during the holding period. Even with these precautions, some changes in redox chemistry can occur during the holding period.

Sulfur Speciation

Acid Volatile Sulfur (AVS)

Whole sediment acid volatile sulfur (AVS) was quantification using a modified acid distillation approach (Zhabina and Volkov, 1978). Upon subsampling, 1.0–1.5 g of homogenized whole sediment was accurately weighed (±0.01 g) and transferred into a 10 mL serum vial, under anoxic conditions. Subsamples were preserved with the addition of 5.0 mL of a 10 percent (w/v) zinc-acetate (anoxic) solution. Subsample vials were then crimp sealed with an anoxic N_2 gas phase, homogenized, and stored frozen (-20 ºC) until further analysis. Our laboratory (USGS, Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.) has determined that sample holding times of up to 6 months, under the above chemically preserved, anoxic and frozen conditions, yields no discernible sample deterioration that would affect the analytical results of this reduced sulfur speciation assay.

Upon partial thawing the AVS subsample, the contents of the serum vial were fully transferred to a 3-neck distillation flask while continuously purging the flask with N_2 gas. An acidic solution of titanium chloride (0.35 M $TiCl_2$/ 8.4 M HCl) was then added (20 mL) to the flask through an injection port. The titanium was used to retard the oxidation of sulfide during the distillation process (Albert, 1984). The acid-sediment slurry was then purged for 45 min with N_2 gas (flow rate 135 mL/min), while stirring with a magnetic stir bar, and without heating. The liberated H_2S gas was trapped as ZnS precipitate in a 10 mL solution of 10 percent (w/v) zinc acetate containing 1 drop of antifoam agent (JT Baker antifoam B silicone Emulsion). The ZnS precipitate solution was subsequently vortexed to break up any large particulates, subsampled in duplicate (0.01 – 1.0 mL), and quantified by colorimetric analysis (Cline, 1969).

A concentrated ZnS standard solution was prepared from combining a known weight of solid Na_2S crystal in anoxic 10 percent zinc acetate. A serial dilution of this standard ZnS primary stock was used to prepare a calibration curve

for the S^{2-} colorimetric assay. The AVS concentration in the original sediment sample was back-calculated based on the determination of total S^{2-} in the ZnS subsample and the original wet weight of the acid distilled sediment. Quality assurance consisted of running method blanks, duplicate colorimetric analyses from each ZnS trap, and occasional matrix spike recovery tests (ZnS standard solution added to the distillation flask). No certified reference material is commercially available for the AVS assay. The average daily detection limit for this assay was approximately 1 nanomoles per mL at the level of the colorimetric analysis. For a standard ZnS trapping solution subsample amount of 1.0 mL and a wet-sediment weight of 1.5 g, this resulted in a method detection limit of approximately 0.05 µg/g wet sediment. However, this detection limit can be lowered by using slightly larger sediment weights or Zn-acetate trap subsampling amounts. The relative deviation for all samples assayed in duplicate was (average ± standard error) 14.3 ±1.8 percent (n=93 sample pairs).

Total Reduced Sulfur (TRS)

Whole-sediment total-reduced sulfur (TRS) was assayed from the same samples distilled by heated chromium for the SR assay (above). A subsample (0.01–1.0 mL) of this zinc-acetate trapping solution (containing the $Zn^{25}S$ precipitate) was quantified colorimetrically (Cline, 1969), as per the above AVS section. In the case of the TRS analysis, however, each sample set consisted of three samples (the two incubated $^{35}SO_4^{2-}$ amended samples, plus the killed-control). The assay detection limit is similar to that for AVS. The RSD for all samples sets assayed was (average ± standard error) 18.5 ±2.2 percent (n=93 sample pairs).

Iron Speciation

Acid Extractable Ferrous Iron

Acid extractable ferrous iron (Fe(II)AE) was quantified by weak acid extraction followed by spectrophotometric determination using Ferrozine (Lovley and Phillips, 1986). Sediment was initially subsampled under anoxic conditions (as above), transferred to a glass serum vial that was crimp sealed, and stored frozen with an N2 gas headspace until further processing. Upon thawing and under anaerobic conditions, 1.00 ±0.05 g of sediment was sampled (in duplicate) and transferred into a 15 mL plastic centrifuge tube, to which 10 mL of 0.5 M HCl was immediately added. The centrifuge tubes were vortexed until a homogeneous slurry was achieved, then placed on a shaker table (200 rpm) in the dark for 1 h. After centrifugation, 0.1 mL of the resulting supernatant was subsampled into a test tube containing 5.0 mL of 1.0 g/L

Ferrozine reagent (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4-disulfonic acid, disodium salt hydrate; prepared in 50 mM HEPES buffer and adjust to pH=4). Full color development was achieved after 30 s and the absorbance was measured on a spectrophotometer at a wavelength setting of 562 nm.

A $FeSO_4$ standard solution series was prepared in 0.25 M hydroxylamine hydrochloride and used to prepare a calibration curve for the Ferrozine colorimetric assay. The $Fe(II)_{AE}$ concentration in the original sediment sample was back-calculated based on the determination of total Fe(II) in the subsample and the original wet weight of the acid extracted sediment. Quality assurance consisted of analyzing method blanks, and duplicate analyses of each sediment sample. No certified reference material is commercially available for $Fe(II)_{AE}$. The average daily detection limit for this assay was approximately 0.02 µg/mL at the level of the spectrophotometric analysis. Assuming a 1.0 g sediment sample and a 0.1 mL post-extraction subsample of the supernatent, the resulting in method detection limit is 0.01 mg/g wet sediment. However, this detection limit can be lowered by using slightly larger sediment weights or supernatant subsampling amounts. The relative deviation for all samples assayed in duplicate, and that had values greater than the method detection limit, was (average ± standard error) 7.6 ±0.8 percent (n=86 sample pairs).

Amorphous Ferric Iron

Whole sediment amorphous (poorly crystalline) ferric iron ($Fe(III)_a$) has been shown to be the form of Fe(III) that is most readily available to Fe(III) reducing bacteria (Lovley and Phillips, 1987). After the spectrophotometric determination of Fe(II) in the test tube containing the 0.1 mL $Fe(II)_{AE}$ subsample plus the 5.0 mL of Ferrozine, 0.25 mL of hydroxylamine-HCl was added to the test tube, which was then vortexed. Hydroxylamine-HCl reduces any acid extractable Fe(III) to Fe(II). After 20 min of reaction time, the absorbance at 562 nm was again measured. This represents the total acid extractable iron fraction (Fe_{TAE}). The Fe_{TAE} concentration in the original sediment sample was back-calculated based on the final Fe(II) concentration in the aqueous subsample and the original wet weight of the acid extracted sediment. $Fe(III)_a$ was then calculated by difference, according to: $Fe(III)_a = Fe_{TAE} - Fe(II)_{AE}$.

The $FeSO_4$ standard calibration curve and approaches to quality assurance were the same as those noted for the $Fe(II)_{AE}$ assay (see section, "Acid Extractable Ferrous Iron"). Matrix spike recovery tests were conducted with a $Fe(III)_a$ solution prepared by neutralizing a 0.4 M solution of $FeCl_3$ to a pH of 7 with NaOH (Lovley and Phillips, 1986). Average matrix spike recoveries were 95 ±26 percent (n=15). No certified reference material is commercially available for the

Fe(III)$_a$ assay. The method detection limit is similar to that for the Fe(II)$_{AE}$ assay. The relative deviation for all samples assayed in duplicate, and that had values greater than the method detection limit, was (average ± standard error) 20.9 ±2.7 percent (n=76 sample pairs).

Crystalline Ferric Iron

Whole sediment crystalline ferric iron (Fe(III)$_c$) was determined by extraction with dithionite-citrate and the spectrophotometric quantification of the resulting Fe(II) with Ferrozine (Roden and Zachara, 1996). The initial sediment preservation (freezing under anoxic conditions), and subsequent sub-sampling (1.0 g into a 15 mL centrifuge tube), is the same as described above for Fe(II)$_{AE}$, and was carried out at the same time as the Fe(II)$_{AE}$ and Fe(III)$_a$ determination. Ten mL of a citrate-acetic acid solution (0.2 M sodium citrate/0.35 M glacial acetic acid, pH adjusted to 4.0 with 6 M HCl) was initially added to the centrifuge tubes, which were then vortexed to achieve a homogeneous sediment slurry. Crystalline sodium dithionite was then added (0.5 g) to each centrifuge tube, which were again vortexed. Sodium dithionite reduces amorphous (poorly crystalline) Fe(III)-silicates and crystalline Fe-oxide minerals [including goethite (αFeOOH), hematite (Fe$_2$O$_3$), Ferrihydrite (Fe(OH)$_3$), lepidocrocite (γFeOOH), and magnetite (Fe$_3$O$_4$)] to Fe(II), but only reduces a fraction of the crystalline Fe-silicate minerals (E. Roden, University of Wisconsin, oral commun., 2004). The centrifuge tubes were placed on a shaker table (200 rpm) in the dark for 1 h. After centrifugation, 0.01 mL of the resulting supernatant was subsampled into a test tube containing 5.0 mL of 1.0 g/L Ferrozine reagent. Full color development was achieved after 30 s and the absorbance was measured on a spectrophotometer at a wavelength setting of 562 nm. This represents the total reactive iron fraction (Fe$_{TR}$). The Fe$_{TR}$ concentration in the original sediment sample was back-calculated based upon the final Fe(II) concentration in the aqueous subsample and the original wet weight of the acid extracted sediment. Fe(III)$_c$ was then calculated by difference, according to: Fe(III)$_c$ = Fe$_{TR}$ - Fe$_{TAE}$.

The FeSO$_4$ standard calibration curve and approaches to quality assurance were the same as those noted above for the Fe(II)$_{AE}$ assay. Matrix spike recovery tests were conducted with crystalline forms of either goethite (prepared by and obtained from C. Fuller, USGS Menlo Park, Calif.) or magnetite (commercially obtained). Average matrix spike recoveries were 89 ±24 percent (n=21). No certified reference material is commercially available for the Fe(III)$_c$ assay. The method detection limit is approximately 0.10 mg/g wet sediment, as processed above. The relative deviation for all samples assayed in duplicate, and that had values greater than the method detection limit, was (average ± standard error) 30.4 ±4.6 percent (n=57 sample pairs).

Dry Weight, Porosity, Bulk Density and Organic Content

The sediment parameters bulk density, dry weight, porosity, and organic content were from a single sediment subsample. Duplicate subsamples were taken for these four parameters. A subsample of 3.0 cm^3 of homogenized wet sediment was obtained with a 3.0 cm^3 plastic syringe that has the needle end cut off the syringe barrel. This subsample was transferred into a crucible and weighed. Sediment bulk density (g/cm^3) was then calculated as the weight:volume ratio.

Sediment dry weight and porosity are measured using standard drying techniques (American Public Health Association, 1981a). The crucible was then placed in an oven overnight at 105 °C, placed in a dessicator to cool, then reweighed. The sediment percent dry weight was then calculated as [(dry sediment weight)/(wet sediment weight) × 100]. Sediment porosity (mL porewater per cm^3 of wet sediment) was calculated as the volume of water lost upon drying divided by the original sediment wet volume.

Organic content was calculated via the Loss on Ignition (LOI) assay (American Public Health Association, 1981b). The crucible was then placed in a combustion oven at 500 °C for 4 h, which burns off organic constituents, leaving only mineral material. After cooling and reweighing, the weight loss was calculated, which is a measure of total organic content.

Quality assurance was achieved by assaying all samples in duplicate. The relative deviation for duplicate analysis (average ± standard error) was as follows: (a) bulk density, 2.2 ±0.2 percent (n=93 sample pairs); (b) dry weight, 1.9 ±0.3 percent (n=93 sample pairs); (c) porosity, 3.1 ±0.4 percent (n=92 sample pairs); and (d) organic content, 5.4 ±0.9 percent (n=91 sample pairs).

Oxidation-Reduction Potential (ORP)

In addition to the sediment ORP measurements taken in the field to assess environmental/in situ conditions, sediment ORP also was measured in the methylmercury production and degradation potential rates laboratory to assess changes in ORP between field and laboratory processing of samples. Measurements were made with a platinum band silver - silver chloride ORP electrode (model EW05990-55, Cole Parmer®, Vernon Hills, IL) used in conjunction with a hand held pH/mV multi-meter (Model 59002-00, Cole Parmer®, Vernon Hills, IL). The electrodes accuracy was tested daily with freshly made buffer solutions (pH=7 and pH=4) saturated with quinhydrone, as per the manufacturer's instructions (Cole-Parmer document #P1937). The ORP potential for each solution is measured and the difference between them is calculated. If this value fell within the range of 173 ±4 mV, the probe was determined to be functioning properly. After cleaning thoroughly with reagent water and drying, the probe

was fully inserted into the homogenized sediment, until displaced sediment reached the top of the vial, which was then wrapped in parafilm to minimize any oxygen transfer into the sample. The probe was gently swirled (to exclude any air bubbles). The ORP meter was allowed to equilibrate for a minimum of 10 min, until a stable reading is achieved, prior to recording the millivolt (mV) value. All measurements were taken while sediment was at room temperature (19–22 °C). The ORP meter values were converted to E_h values (the potential, in mV, of the standard hydrogen electrode) using the following conversion provided by the manufacturer:

$$E_h = ORP \text{ (meter value)} + E_R,$$

where $E_R = (-0.718 \times T) + 219.97$ mV, where E_R = is the standard potential for a normal hydrogen reference electrode, in mV; T = temperature (°C).

pH

Sediment pH was measured immediately after homogenization and subsampling approximately 10 cm^3 of sediment into a 20 mL screw top serum vial. Measurements were made with a pH electrode used in conjunction with a handheld pH/mV multi-meter (Model 59002-00, Cole Parmer®, Vernon Hills, IL). The electrode was calibrated daily with fresh commercial pH=7 phosphate buffer and then rinsed clean with reagent water. The probe was then fully inserted into the homogenized sediment, gently swirled to ensure the exclusion of any air pockets, and allowed to stabilized (approximately 45–60 s) until the meter indicated a stable reading.

Pore-Water Analyses—Pore Water Isolated from 1-mm Sieved Streambed-Sediment Samples

Ancillary pore-water parameters were subsampled in the laboratory (USGS, Methylmercury Production and Degradation Potential Rates Laboratory, Menlo Park, Calif.) under anoxic conditions (in an N$_2$ flushed glove bag) at the same time and from the same batch of homogenized streambed sediment that was used for microbial rate assays. These pore-water parameters provide a measure of the geochemical conditions at the time the microbial rate measurements were conducted (that is, 6 ±2 days after field collection). Plastic centrifuge tubes (50 cm^3) were filled to the top with sediment to exclude any significant gaseous headspace. The screw-top threads at the top of the centrifuge tubes also were wrapped in Teflon® tape to ensure a tight leak-free seal upon capping.

The tubes were then centrifuged for 20 min at 3,500 rpm, and subsequently were returned to the N$_2$ flushed glove bag prior to removing the caps for further sample processing. The pore-water supernatant was decanted into the back end of a 10 cm^3 plastic syringe (with the plunger initially removed) that was fitted with a 0.45 um nylon filter (Whatman 25 mm GD/X syringe filter). After replacing the plunger, the pore water was pushed through the filter and into the various containers that had been pre-labeled and prepared for the collection of the various pore-water constituents (that is, sulfate and chloride, ferrous iron, acetate). Occasionally, when the sediment porosity and pore-water volume yielded from the centrifugation step was too low to collect all subsamples required, approximately 20 mL of anoxic (previously boiled and N$_2$ purged) Milli-Q® (ultraclean) water was added to approximately 30 cm^3 of sediment, filling the centrifuge tube completely to the top. The exact weights of the Milli-Q® and sediment fractions were recorded, and the exact pore-water dilution was subsequently calculated (based on the original sediment porosity and bulk density). After capping the tube, these two phases were well mixed (hand shaken or vortexed) until a uniform sediment slurry was achieved. The sample was then centrifuged as described above. This pore-water dilution was subsequently taken into account when calculating the original concentrations of the specific pore-water analytes associated with that diluted sample. Every attempt was made to minimize changes in redox sensitive geochemistry between the time of field collection and subsequent subsampling and analyte specific preservation, as described above for the whole sediment geochemical parameters associated with composite sediment samples collected for microbial rate assays.

Sulfate and Chloride

Sulfate and chloride analyses were conducted on filtered samples of sediment pore water that were collected under anaerobic conditions, transferred to 13 cm^3 crimp sealed serum vials, and stored frozen until analysis. Sulfate and chloride were measured on an ion chromatograph (Dionex Model DX-300, Sunnyvale, CA) equipped with an auto-suppressor, an IONPAC AG4A-SC guard column, AS4A-SC analytical column and mobile phase consisting of 1.8 m*M* Na$_2$CO$_3$ and 1.7 m*M* NaHCO$_3$. Quality assurance included calibration standards prepared from crystalline sodium acetate, laboratory reagent blanks, filter blanks, and analytical duplicates. The relative deviation for all samples assayed in duplicate, and that had values greater than the method detection limit, was (average ± standard error) 11.3 ±1.9 percent (n=82 sample pairs) and 8.2 ±1.5 percent (n=85 sample pairs) for sulfate and chloride, respectively.

Ferrous Iron

The assay for pore-water Fe(II) was adapted from previously published methods (Gibbs, 1979; Faulkner and others, 1999). A known amount (for example, 10–500 µL) of acid-preserved pore water (adjusted pH < 2 with HCl) was added to a test tube containing 5.0 mL FerroZine® reagent. The tube was then vortexed to mix the contents. After full color development (approximately 1 min), a quartz cell cuvette was rinsed three times with 0.5 mL of the solution from the test tube, and then filled. The absorbance at the 562 nm wavelength was measured on a Shimadzu Model UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). Quality assurance measures include calibration standards, laboratory reagent blank, and duplicate analyses. The detection limit for this assay was approximately 0.02 µg/mL, at the level of the spectrophotometric analysis. Assuming a maximum pore-water subsample volume of 0.5 mL, the detection limit for the original pore-water sample was 0.2 mg/L. The relative deviation for all samples assayed in duplicate, and that had values greater than the method detection limit, was (average ± standard error) 2.8 ±1.4 percent (n=7 sample pairs).

Acetate

Acetate analysis was conducted on filtered samples of sediment pore water that were collected under anaerobic conditions, transferred to crimp-sealed serum vials, and stored frozen until analysis. Acetate was measured on an ion chromatograph (Dionex Model DX-300, Sunnyvale, CA) equipped with an auto-suppressor, an IONPAC AG4A-SC guard column, AS4A-SC analytical column and mobile phase consisting of 5 mM $Na_2B_4O_7$. Quality assurance included calibration standards prepared from concentrated commercial stock solutions, laboratory reagent blanks, filter blanks, and analytical duplicates. The method detection limit was 1 µM. The relative deviation for all samples assayed in duplicate, and that had values greater than the method detection limit, was (average ± standard error) 9.8 ±3.9 percent (n=34 sample pairs).

Summary

Mercury contamination of aquatic ecosystems is an issue of national concern, affecting both wildlife and human health. Detailed information on mercury cycling and food-web bioaccumulation in stream settings and the factors that control these processes is currently limited. In response, the U.S. Geological Survey (USGS) National Water-Quality Assessment Program (NAWQA) conducted detailed studies from 2002 to 2006 on various media to enhance process-level understanding of mercury contamination, biogeochemical cycling, and trophic transfer. Eight streams were sampled for this study: two streams in Oregon, and three streams each in Wisconsin and Florida.

Streambed-sediment and pore-water samples were collected between February 2003 and September 2004. Whole streambed sediment samples were analyzed for total mercury, methylmercury, tin-reducible reactive mercury, various forms of sulfur, and selected other geochemical constituents. Filtered pore-water samples were analyzed for total mercury, methylmercury, and additional ancillary geochemical measures. In addition, methylmercury production potential, sulfate reduction rates, and additional ancillary geochemical measures were performed on sieved (1 millimeter) homogenized sediment samples.

This report summarizes the suite of geochemical and microbial constituents measured, the analytical methods used, and provides the raw data in electronic form for both bed-sediment and pore-water media associated with this study.

References Cited

Aiken, G.R., 1992, Chloride interference in the analysis of dissolved organic carbon by the wet oxidation method: Environmental Science and Technology, v. 26, no. 12, p. 2435-2439.

Albert, D., 1984, Improved techniques for measurement of sulfate reduction and pyrite formation rates in sediment: Transactions of the American Geophysical Union, v. 45, no. 906.

American Public Health Association, 1981a, Section 209 C: Total nonfiltrable residue dried at 103-105 °C, in Franson, M.A.H., ed., Standard Methods for the Examination of Water and Wastewater, 15th Edition: Washington, D.C., American Public Health Association, American Water Works Association, Water Pollution Control Federation, p. 94-95.

American Public Health Association, 1981b, Section 209 G: Volatile and fixed matter in nonfilterable residue and in solid and semisolid samples, in Franson, M.A.H., ed., Standard Methods for the Examination of Water and Wastewater, 15th Edition: Washington, D.C., American Public Health Association, American Water Works Association, Water Pollution Control Federation, p. 97-99.

Aspila, K.I., Agemian, H., and Chau, A.S.Y., 1976, A semi-automated method for the determination of inorganic, organic and total phosphate in sediments: The Analyst, v. 101, p. 187-197.

Bates, A.L., Spiker, E.C., and Holmes, C.W., 1998, Speciation and isotopic composition of sedimentary sulfur in the Everglades, Florida, USA: Chemical Geology, v. 146, no. 3-4, p. 155-170.

Bell A.H., and Lutz, M.A., in press, Environmental settings of selected streams sampled for mercury in Oregon, Wisconsin, and Florida, 2002–2006: U.S. Geological Survey Open-File Report 2008–1277.

Bloom, N., Bollen, A., Briscoe, M., Hall, G., Horvat, M., Kim, C., Lasorsa, B., Marvin-Dipasquale, M., and Parker, J., 2006, International Solid Phase Mercury Speciation Exercise (ISPMSE): Introduction and Preliminary Results, *in* Mercury 2006 Abstracts Book: Eighth International Conference on Mercury as a Global Pollutant, Madison, Wisconsin, August 6-11, 2006.

Brigham, M.E., Krabbenhoft, D.P., and Hamilton, P.A., 2003, Mercury in stream ecosystems—New studies initiated by the U.S. Geological Survey: U.S. Geological Survey Fact Sheet 016-03, 4 p.

Brouwer, H., and Murphy, T.P., 1994, Diffusion Method for the Determination of Acid-Volatile Sulfides (AVS) in Sediment: Environmental Toxicology and Chemistry, v. 13, p. 1273-1275.

Chin, Y., Aiken, G., and O'Loughlin, E., 1994, On the molecular weight, polydispersity and spectroscopic properties of aquatic humic substances: Environmental Science and Technology, v. 28, p. 1853-1858.

Cline, J.D., 1969, Spectrophotometric determination of hydrogen sulfide in natural waters: Limnology and Oceanography, v. 14, p. 454-458.

Compeau, G.C., and Bartha, R., 1985, Sulfate-reducing bacteria: Principal methylators of mercury in anoxic estuarine sediment: Applied and Environmental Microbiology, v. 50, no. 2, p. 498-502.

DeWild, J.F., Olson, M.L., and Olund, S.D., 2002, Determination of methyl mercury by aqueous phase ethylation, followed by gas chromatographic separation with cold vapor atomic fluorescence detection: U.S. Geological Survey Open-File Report 01–445, 14 p.

DeWild, J.F., Olund, S.D., Olson, M.L., and Tate, M.T., 2004, Methods for the preparation and analysis of solids and suspended solids for methylmercury: U.S. Geological Survey Techniques and Methods 5-A7, 21 p.

Faulkner, S.P., Hintz, P.E., and Ashby, S.L., 1999, Evaluation of colorimetric methods for measuring reduced (ferrous) iron: Water Quality Technical Notes Collection (WQTN PD-02): U.S. Army Engineer Research and Development Center, Vicksburg, MS, accessed August 15, 2008, at http://el.erdc.usace.army.mil/publications.cfm?Topic=technote&Code=watqual

Fleming, E.J., Mack, E.E., Green, P.G., and Nelson, D.C., 2006, Mercury methylation from unexpected sources: Molybdate-inhibited freshwater sediments and an iron-reducing bacterium: Applied and Environmental Microbiology, v. 72, no. 1, p. 457-464.

Fossing, H., and Jørgensen, B., 1989, Measurement of bacterial sulfate reduction in sediments: Evaluation of a single step chromium reduction method: Biogeochemistry, v. 8, p. 205-222.

Furutani, A., and Rudd, J.W.M., 1980, Measurement of mercury methylation in lake water and sediment samples: Applied and Environmental Microbiology, v. 40, no. 4, p. 770-776.

Gibbs, M.M., 1979, A simple method for the rapid determination of iron in natural waters: Water Research, v. 13, p. 295-298.

Gilmour, C.C., Henry, E.A., and Mitchell, R., 1992, Sulfate stimulation of mercury methylation in freshwater sediments: Environmental Science and Technology, v. 26, p. 2281-2287.

Gilmour, C.C., and Riedel, G.S., 1995, Measurement of Hg methylation in sediments using high specific-activity Hg-203 and ambient incubation: Water Air and Soil Pollution, v. 80, no. 1-4, p. 747-756.

Gilmour, C.C., Riedel, G.S., Ederington, M.C., Bell, J.T., Benoit, J.M., Gill, G.A., and Stordal, M.C., 1998, Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades: Biogeochemistry, v. 40, no. 2-3, p. 327-345.

Guimaraes, J.R.D., Ikingura, J., and Akagi, H., 2000, Methyl mercury production and distribution in river water-sediment systems investigated through radiochemical techniques: Water Air and Soil Pollution, v. 124, no. 1-2, p. 113-124.

Guimaraes, J.R.D., Malm, O., and Pfeiffer, W.C., 1995, A simplified radiochemical technique for measurements of net mercury methylation rates in aquatic systems near goldmining areas, Amazon, Brazil: Science of the Total Environment, v. 175, p. 151-162.

Hedges, J.I., and Stern, J.H., 1984, Carbon and nitrogen determinations of carbonate-containing solids: Limnology and Oceanography, v. 29, no. 3, p. 657-663.

Jørgensen, B.B., 1978, A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. 1. Measurement with radiotracer techniques: Geomicrobiology Journal, v. 1, p. 11-27.

Kieu, L.H., 2004, The seasonal influence of saltmarsh plants (*Salicornia virginica* and *Scirpus robustus*) on methylmercury production and degradation over small spatial scales in South San Francisco Bay: San Francisco, San Francisco State University, Master's Thesis, 72 p.

Lovley, D.R., and Phillips, E.J.P., 1986, Organic matter mineralization with reduction of ferric iron in anaerobic sediments: Applied and Environmental Microbiology, v. 51, no. 4, p. 683-689.

Lovley, D.R., and Phillips, E.J.P., 1987, Rapid assay for microbially reducible ferric iron in aquatic sediments: Applied and Environmental Microbiology, v. 53, p. 1536-1540.

Lutz, M.A., Brigham, M.E., and Marvin-DiPasquale, M., 2008, Procedures for collecting and processing streambed sediment and pore water for analysis of mercury as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 2008-1279.

Marvin-DiPasquale, M.C., Agee, J., Bouse, R., and Jaffe, B., 2003, Microbial cycling of mercury in contaminated pelagic and wetland sediments of San Pablo Bay, California: Environmental Geology, v. 43, no. 3, p. 260-267.

Marvin-DiPasquale, M.C., Agee, J., McGowan, C., Oremland, R.S., Thomas, M., Krabbenhoft, D., and Gilmour, C., 2000, Methyl-mercury degradation pathways: A comparison among three mercury-impacted ecosystems: Environmental Science and Technology, v. 34, no. 23, p. 4908-4916.

Marvin-DiPasquale, M.C., and Agee, J.L., 2003, Microbial mercury cycling in sediments of the San Francisco Bay-Delta: Estuaries, v. 26, no. 6, p. 1517-1528.

Marvin-DiPasquale, M.C., and Cox, M.H., 2007, Legacy mercury in Alviso Slough, South San Francisco Bay, California: Concentration, Speciation and Mobility: U.S. Geological Survey Open-File Report 2007-1240, 98 p. accessed August 15, 2008, at http://pubs.usgs.gov/of/2007/1240/

Marvin-DiPasquale, M.C., Hall, B.D., Flanders, J.R., Ladizinski, N., Agee, J.L., Kieu, L.H., and Windham-Myer, L., 2006, Ecosystem investigations of benthic methylmercury production: A tin-reduction approach for assessing the inorganic mercury pool available for methylation, *in* Mercury 2006 Abstracts Book: Eighth International Conference on Mercury as a Global Pollutant, Madison, Wisconsin, August 6-11, 2006.

Marvin-DiPasquale, M.C., and Capone, D.G., 1998, Benthic sulfate reduction along the Chesapeake Bay central channel. I. Spatial trends and controls: Marine Ecology Progress Series, v. 168, p. 213-228.

Matthes, W.J.J., Sholar, C.J., and George, J.R., 1992, Quality-assurance plan for the analysis of fluvial sediment by laboratories of the U.S. Geological Survey: U.S. Geological Survey Open-File Report 91-467, 37 p.

Nordstrom, D.K., and Wilde, F.D., 2005, Reduction-oxidation potential - Electrode Method (version 1.2), section 6.5 *in* Wilde, F.D., and Radtke, D.B., (eds.), Field measurements: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6, 22 p.

Olund, S.D., DeWild, J.F., Olson, M.L., and Tate, M.T., 2004, Methods for the preparation and analysis of solids and suspended solids for total mercury: U.S. Geological Survey Techniques and Methods 5-A8, 23 p.

Oremland, R.S., Culbertson, C.W., and Winfrey, M.R., 1991, Methylmercury decomposition in sediments and bacterial cultures: Involvement of methanogens and sulfate reducers in oxidative demethylation: Applied and Environmental Microbiology, v. 57, no. 1, p. 130-137.

Oremland, R.S., Miller, L.G., Dowdle, P., Connell, T., and Barkey, T., 1995, Methylmercury oxidative degradation potentials in contaminated and pristine sediments of the Carson River, Nevada: Applied and Environmental Microbiology, v. 61, p. 2745-2753.

Ramlal, P.S., Rudd, J.W., and Hecky, R.E., 1986, Methods for measuring specific rates of mercury methylation and degradation and their use in determining factors controlling net rates of mercury methylation: Applied and Environmental Microbiology, v. 51, p. 110-114.

Roden, E.E., and Zachara, J.M., 1996, Microbial reduction of crystalline iron(III) oxides: Influence of oxide surface area and potential for cell growth: Environmental Science and Technology, v. 30, no. 5, p. 1618-1628.

Stordal, M.C., and Gill, G.A., 1995, Determination of mercury methylation rates using a 203Hg radiotracer technique: Water Air and Soil Pollution, v. 80, p. 725-734.

Strickland, J.D.H., and Parsons, T.R., 1972, A practical handbook of seawater analysis (2nd ed.): Ottawa, Canada, Fisheries Research Board of Canada Bulletin 167, 310 p.

Tuttle, M.L., Goldhaber, M.B., and Williamson, D.L., 1986, An analytical scheme for determining forms of sulfur in oil shales and associated rocks: Talanta, v. 33, p. 953–961.

U.S. Environmental Protection Agency, 2002, Method 1631: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry: Environmental Protection Agency - Office of Water, EPA 821-R-96-012, 38 p., accessed August 28, 2008, at http://www.epa.gov/waterscience/methods/method/mercury/1631.pdf

U.S. Environmental Protection Agency, 1997, Mercury study report to Congress volume III—fate and transport of mercury in the environment: U.S. Environmental Protection Agency, EPA-452/R-97-005, 358 p.

U.S. Environmental Protection Agency, 2007a, 2005/2006 National Listing of Fish Advisories: U.S. Environmental Protection Agency, Office of Water, Fact Sheet; EPA-823-F-07-003, 7 p., accessed August 15, 2008, at http://www.epa.gov/waterscience/fish/advisories/2006/tech.html#website

U.S. Environmental Protection Agency, 2007b National Section 303(d) List Fact Sheet, accessed August 15, 2008, at http://oaspub.epa.gov/waters/national_rept.control

Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., and Fujii, R., 2003, Evaluation of specific ultra-violet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon: Environmental Science and Technology, v. 37, p. 4702-4708.

Wiener, J.G., Krabbenhoft, D.P., Heinz, G.H., and Scheuhammer, A.M., 2003, Ecotoxicology of mercury, Chapter 16, in Hoffman, D.J., Rattner, B.A., Burton, G.A., Jr., and Cairns, J., Jr., eds., Handbook of Ecotoxicology, 2nd edition: Boca Raton, CRC Press, p. 409-463.

Yamamuro, M., and Kayanne, H., 1995, Rapid direct determination of organic carbon and nitrogen in carbonate-bearing sediments with a Yanaco MT-5 CHN analyzer: Limnology and Oceanography, v. 40, p. 1001-1005.

Zhabina, N., and Volkov, I., 1978, A method of determination of various sulfur compounds in sea sediment and rocks, in Krumbein, W.E., ed., Environmental Biogeochemistry and Geomicrobiology, Vol. 3: Methods, Metals, and Assessment: Ann Arbor, Ann Arbor Science Publishers, p. 735-746.

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Appendixes—The following appendixes are data files stored in Microsoft Excel files, and are available for download at http://pubs.usgs.gov/ds/375/

Appendix 1. Sampling Dates (Month/Year) and Locational Information for Streambed-Sediment and Pore-Water Sampling Areas at Each Stream Site.

Appendix 2. Data for Whole (Unsieved) Streambed-Sediment and Pore-Water Samples.

Appendix 3. Data for Sieved Streambed-Sediment and Derived Pore-Water Samples.

Appendix 4. Data for Stream Reach Characterizations.

Appendix 5. Quality-Control Data for Whole (Unsieved) Streambed-Sediment and Pore-Water Samples.

Appendix 6. Quality-Control Data for Stream Reach Characterizations.

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