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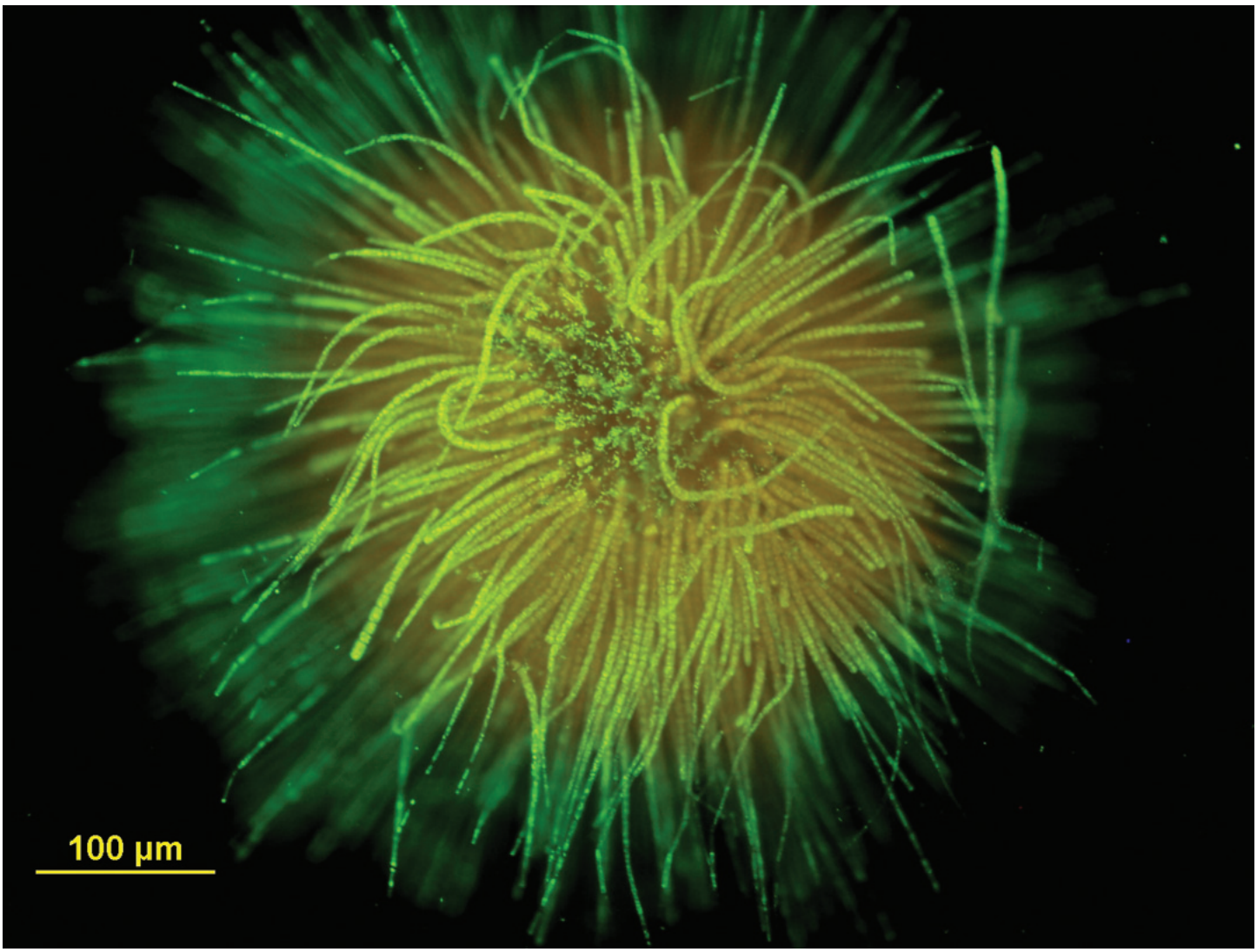
Proceedings of the U.S. Geological Survey Interdisciplinary Microbiology Workshop, Estes Park, Colorado, October 15–17, 2008



Scientific Investigations Report 2010–5146

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

Front cover. *Gloeotrichia* sp. stained with SYTOX® Green; this cyanobacterium was found in Klamath Lake, Oregon. Photomicrograph by Barry H. Rosen (U.S. Geological Survey). Scale in micrometers (μm).

Back cover. Several filaments of *Lyngbya* spp., a cyanotoxin-producing cyanobacterium. These filaments were examined and photographed under epifluorescent microscopy, with excitation between 450 and 80 nanometers (nm) and emission above 515 nm. With this configuration, chlorophyll a appears as a bright red color. These filaments were stained with a nucleic acid stain, SYTOX® Green, which is excluded from healthy cells (leaving them red), while damaged cells appear yellow. This staining thus provides an indication of the health of these cyanobacterial filaments. In this image obtained from a laboratory culture, some of the filaments are exclusively red, some filaments alternate between living cells (red) and those that lost cell membrane integrity (yellow), and some filaments are completely yellow. Photomicrograph by Barry H. Rosen (U.S. Geological Survey).

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Edited by Kay Marano Briggs

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KEN SALAZAR, Secretary

U.S. Geological Survey
Marcia K. McNutt, Director

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Preface

A U.S. Geological Survey Interdisciplinary Microbiology Workshop was held in Estes Park, Colorado, on October 15–17, 2008. Participants came from all USGS regions and disciplines.

This report contains abstracts from 36 presentations and 35 poster sessions and notes from 5 breakout sessions. The seven presentation topics follow:

- Ecology of wildlife and fish disease
- Mechanisms of fish and wildlife disease
- Microbial ecology
- Geographic patterns/visualization
- Public health and water quality
- Geomicrobiology
- Ecosystem function

The six poster session topics follow:

- Wildlife disease
- Disease detection methods
- Water quality
- Microbial ecology
- Metabolic processes
- Tools and techniques

Five working groups met in breakout sessions on October 16, 2008. The highlights for each working group are summarized in this report, and their goals are listed below:

- Working Group I: to plan a Fact Sheet on interdisciplinary microbiology in the USGS
- Working Group II: to plan a USGS interdisciplinary microbiology Web site
- Working Group III: to suggest ways to broadcast and publicize the types of microbiology conducted at the USGS
- Working Group IV: to identify emerging issues in USGS interdisciplinary microbiology research
- Working Group V: to identify potential opportunities for interdisciplinary microbiology work at the USGS

After the workshop, the USGS interdisciplinary microbiology Web site was activated in June 2009 at http://microbiology.usgs.gov/.

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Conversion Factors

Multiply	By	To obtain
	Length	
foot (ft)	0.3048	meter (m)
nautical mile (nmi)	1.852	kilometer (km)
nanometer (nm)	0.00000003937	inch (in.)
micrometer (µm)	0.00003937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
centimeter (cm)	0.3937	inch (in.)
meter (m)	3.281	foot (ft)
megameter (Mm)	621.37	mile (mi)
	Area	
square centimeter (cm^2)	0.1550	square inch (in^2)
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
microliter (µL)	0.00003381	ounce, fluid (fl. oz)
milliliter (mL)	0.03381	ounce, fluid (fl. oz)
liter (L)	1.057	quart (qt)
	Mass	
nanogram (ng)	0.00000000003527	ounce, avoirdupois (oz)
microgram (µg)	0.00000003527	ounce, avoirdupois (oz)
milligram (mg)	0.00003527	ounce, avoirdupois (oz)
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound, avoirdupois (lb)
gigaton (Gt)	1.102×10^9	short ton (2,000 lb)

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

$$°F=(1.8\times°C)+32$$

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

$$°C=(°F-32)/1.8$$

Agenda for U.S. Geological Survey Interdisciplinary Microbiology Workshop, Estes Park, Colorado, October 15–17, 2008

October 15

8:30 a.m.	**Opening:** Welcome, housekeeping announcements, short explanation of how the workshop came to be, and introductions of committee members
8:45 a.m.	**Keynote Speech at Plenary Session:** Ronald S. Oremland: The Ecology of Arsenic: A Lurid Tale of Murder, Mayhem, Microbes, Malodorous Mono Muds, and Mars
9:25 a.m.	**BREAK (15 minutes)**
Section I.	**Ecology of Wildlife and Fish Disease**
9:40 a.m.	David S. Blehert: Bat White-Nose Syndrome: An Emerging Fungal Pathogen?
10:10 a.m.	Gael Kurath: Epidemiology and Evolution of Rhabdoviruses in Fish
10:40 a.m.	Hon S. Ip: The United States Department of the Interior's Avian Influenza Surveillance Program in North America: Laboratory Results
11:10 a.m.	Carter T. Atkinson: Ecology of Vector-Borne Diseases in Pacific Island Birds: Hawaii to New Caledonia
11:40 a.m.	**LUNCH (on site)**
Section II.	**Mechanisms of Fish and Wildlife Disease**
12:30 p.m.	Paul K. Hershberger: Disease Impacts on Populations of Wild Marine Fish
1:00 p.m.	Christopher A. Ottinger: Mycobacteriosis in Chesapeake Bay
1:30 p.m.	Toni E. Rocke: Studies on Plague Pathogenesis in Rodents Using in vivo Imaging
2:00 p.m.	Chad J. Johnson: Role of Wild Rodents in Environmental Transport of Prions
2:30 p.m.	Thierry M. Work: Microbial Diseases in Marine Ecosystems: Challenges and Opportunities
3:00 p.m.	**BREAK (30 minutes)**
Section III.	**Microbial Ecology**
3:30 p.m.	Christina A. Kellogg: Microbiology of Deep-Sea Corals
4:00 p.m.	Andrea L. Foster: Development and Application of Denaturing High-Performance Liquid Chromatography (DHPLC) Methods for the Analysis of Microbial Community Composition
4:30 p.m.	Jayne Belnap: The Role of Biological Soil Crusts in Preventing Desertification of Dryland Ecosystems
5:30 p.m.	**DINNER (on site)**
7:00 p.m.	**Poster Session and Reception**

October 16

Section III.	**Microbial Ecology (continued)**
8:30 a.m.	Russell J. "Rusty" Rodriguez: Endosymbiotic Fungi in Plants: Ecology and Implications for Climate Change
9:00 a.m.	Nicole M. DeCrappeo: Soil Community Dynamics in Sagebrush Steppe and Cheatgrass-Invaded Areas of the Northern Great Basin
Section IV.	**Geographic Patterns/Visualization**
9:30 a.m.	Steve C. Guptill: Microbial Mapping: Stimulating Hypothesis and Informing the Public
10:00 a.m.	Lee De Cola: A Multiscale Approach to the Concept of Regional Health
10:30 a.m.	**BREAK (30 minutes)**
11:00 a.m.	Joseph E. Bunnell: Public Health and Geospatial Distribution
11:30 a.m.	Steve Helterbrand: Critical Habitat Diversity Parameters for Assessing the Geographical Distribution of Plague: A Geographic Information System (GIS)-Based Case-Control Study and Risk Model of Human Plague Cases in the Southwestern United States, 1963–2007
12:00 p.m.	**LUNCH (on site)**
Section V.	**Public Health and Water Quality**
1:00 p.m.	Muruleedhara Byappanahalli: Integrating Quantitative Polymerase Chain Reaction (qPCR) and Predictive Models to Monitor Beach Water Quality
1:30 p.m.	Jennifer L. Graham: Harmful Algal Blooms
2:00 p.m.	Lisa R. Fogarty: Pathogens and Antibiotic-Resistant Bacteria in the Environment
2:30 p.m.	Donald M. Stoeckel: Microbial Source Tracking, Using Quantitative Polymerase Chain Reaction (qPCR), for Enhanced Sanitary Water-Quality Management
3:00 p.m.	**BREAK (30 minutes)**
3:30 p.m.	Ronald W. Harvey: Transport of Microbes in the Subsurface/Physical Interactions of Microbes and Particles
4:00 p.m.	W. Bane Schill: Flow Cytometry in Microbiology
5:00 p.m.	**DINNER (on site)**
7:00 p.m.	**Breakout Working Groups:** There will be several topics the groups will be divided into to address. The notebook you receive upon registration will have your working group assignment.

October 17

Section VI.	**Geomicrobiology**
8:30 a.m.	Ronald S. Oremland: Geomicrobiology/Nanoscience of Toxic Elements Se and Te
9:00 a.m.	JoAnn M. Holloway: Soil Microbial Indices Across Different Geographic Scales
9:30 a.m.	Christopher T. Mills: Using Natural Abundance $\delta^{13}C$ and $\Delta^{14}C$ Values of Phospholipid Fatty Acids to Characterize Bacterial Carbon Cycling Pathways in Deep Aquifers
10:00 a.m.	William H. Orem: Geochemical Controls on Microbial Methylation of Mercury in the Florida Everglades
10:30 a.m.	**BREAK (15 minutes)**
10:45 a.m.	Mark Stanton: An Overview of Bacterial Controls and Influences on Sulfide Mineral Weathering
11:15 a.m.	Mark Marvin-DiPasquale: Defining "Reactive" Inorganic Mercury and its Relationship to Microbial Methylmercury Formation in Aquatic Ecosystem Studies
11:45 a.m.	**LUNCH (on site)**
Section VII.	**Ecosystem Function**
12:45 p.m.	**Special guest** Dr. Erin Muths: A Tale of Disappearing Toads: The Amphibian Chytrid Fungus in the Rocky Mountains: 15-minute presentation followed by a field walk on site highlighting the habitats and life history of the boreal toad and its interactions with the chytrid fungus
2:00 p.m.	**BREAK (30 minutes)**
2:30 p.m.	Richard L. Smith: Natural and Enhanced Bioremediation of Nitrate Contamination in Groundwater
3:00 p.m.	Mark P. Waldrop: Microbial Communities and Carbon Cycling in Response to Global Change
3:30 p.m.	Jill S. Baron: Biogeochemistry of Mountain Watersheds: Through the Microbial Window
4:00 p.m.	Matthew P. Bachmann: Quantifying Microbial Denitrification Rates Using Expressed Functional Gene Abundance
4:30 p.m.	Julie D. Kirshtein: Evaluating Ecosystem Function with Integrated Approaches in Environmental Microbiology
5:00 p.m.	**Closing:** Final comments, next steps

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Presentation Abstracts

Keynote Speech at Plenary Session

The Ecology of Arsenic: A Lurid Tale of Murder, Mayhem, Microbes, Malodorous Mono Muds, and Mars

By Ronald S. Oremland[1]

[1]U.S. Geological Survey, Water Resources Discipline, 345 Middlefield Road, Mail Stop 480, Menlo Park, CA 94205–3561.

Arsenic is a metalloid whose name conjures up images of murder. Nonetheless, certain prokaryotes use arsenic oxyanions for energy generation, either by oxidizing arsenite or by respiring arsenate. These microbes are phylogenetically diverse and occur in a wide range of habitats. Arsenic cycling may take place in the absence of oxygen and can contribute to organic matter oxidation. In aquifers, these microbial reactions may mobilize arsenic from the solid to the aqueous phase, resulting in contaminated drinking water. Future research will build on what is known about arsenic-metabolizing bacteria and their potential impact on speciation and mobilization of arsenic in nature.

Section I: Ecology of Wildlife and Fish Disease

Bat White-Nose Syndrome: An Emerging Fungal Pathogen?

By David S. Blehert[1]

[1]U.S. Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711–6223.

White-nose syndrome (WNS) is a condition associated with an unprecedented bat mortality event in the northeastern United States. Since the winter of 2006–2007, bat declines ranging from 80 to 97 percent have been observed at surveyed hibernacula. Affected hibernating bats often present with visually striking white fungal growths on their muzzles, ears, and (or) wing membranes. Histopathological analyses confirmed that 90 percent (105 of 117) of necropsied bats submitted from WNS-positive sites exhibited an associated cutaneous fungal infection. Direct microscopy and culture analyses demonstrated that the skin of WNS-affected bats is colonized by a psychrophilic (cold-loving) fungus with a unique conidial morphology. The isolates were initially cultured at 3°C and grew optimally between 5°C and 10°C, temperatures consistent with the core temperatures of hibernating cave bat species within the WNS-affected region. There is a growing body of circumstantial evidence supporting an association between WNS and cutaneous fungal infection. Given the hundreds of thousands of hibernating bats found throughout the WNS-affected region, this condition represents an unprecedented threat to bats of the northeastern United States and potentially beyond.

*U.S. Geological Survey, 12201 Sunrise Valley Drive, Mail Stop 301, Reston, VA 20192–0002.

Epidemiology and Evolution of Rhabdoviruses in Fish

By Gael Kurath,[1,2] Evi Emmenegger,[1] Bill Batts,[1] and Jim Winton[1,2]

[1]U.S. Geological Survey, Western Fisheries Research Center, 6505 NE 65th Street, Seattle, WA 98115.

[2]University of Washington, School of Aquatic and Fisheries Sciences, Seattle, WA 98195.

Viral species from the family Rhabdoviridae include some of the most important pathogens that cause epidemic diseases in wild and cultured fish populations. The rhabdovirus species infectious hematopoietic necrosis virus (IHNV) is the most significant viral pathogen of salmon and trout in North America. Genetic typing and phylogenetic analyses of over 650 field isolates of IHNV have revealed that there are three major genogroups of IHNV. These are designated the U, M, and L groups because they occur in the upper, middle, and lower portions of the geographic range of the virus along the Pacific coast. These genogroups vary in genetic diversity, rate of evolution, and virulence in different host species. For example, virus isolates in the U genogroup are homogeneous, with high virulence in sockeye salmon but not in trout. Conversely, M type viruses are genetically diverse, with high virulence in trout but not in sockeye. We suggest that IHNV evolution has occurred from an ancestral sockeye virus by host jumps into trout and Chinook salmon, likely influenced by development of hatchery programs and aquaculture industries. Investigations into the mechanistic basis of U and M host specificity indicate that rapid viral replication early after infection allows the viruses to avoid host immune responses. Epidemiological studies based on genetic typing indicate that a new genotype of IHNV is emerging along the Pacific coast, creating a new threat to steelhead stocks. More recently, another fish rhabdovirus, viral hemorrhagic septicemia virus (VHSV), has emerged as a major pathogen in the Great Lakes region. Since the first outbreak of VHSV in 2005, there have been numerous fish kills, and the virus has been isolated from 25 host species, several of which were not previously known to be susceptible to the VHS virus. Historically VHSV was thought to be a widespread marine fish virus that adapted to cultured rainbow trout in Europe, causing epidemics in European fish farms for several decades. The outbreaks in the Great Lakes are the first time VHSV has caused major disease epidemics in wild freshwater fish. Genetic typing suggests that the Great Lakes VHSV is most similar to marine VHSV isolates from the coasts of North America and that the introduction into the Great Lakes was relatively recent. Current studies of virulence of this new VHSV type are underway to support decisions needed to manage disease caused by VHSV in Great Lakes fisheries.

The United States Department of the Interior's Avian Influenza Surveillance Program in North America: Laboratory Results

By Hon S. Ip,[1] Dirk Derksen,[2] Tina Egstad,[1] Katy Griffin,[1] Alexandria Hauser,[1] Mellisa Houfe,[1] Keynttisha Jefferson,[1] Melissa Kanter,[1] Lucky Karwal,[1] Anson Koehler,[2] Kim Kooiman,[1] Peter Ladell,[1] Renee Long,[1] Kim Miller,[1] Amy Miyamoto,[1] Jessica Montez,[1] Melanie Mossing,[1] Rachel Raddington,[1] Adam Ray,[1] Stephanie Rieger,[1] Hiram Sanchez,[1] Samantha Scott,[1] Evan Sorley,[1] Josh TeSlaa,[1] Jennifer Tuscher,[1] and Thierry Work[3]

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[2]U.S. Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, AK 99508.

[3]U.S. Geological Survey, National Wildlife Health Center, 800 Ala Moana Boulevard, Room 5231, Honolulu, HI 96850.

The global expansion of the distribution of highly pathogenic avian influenza H5N1 virus has raised concern about the role of migratory birds in the long-distance dissemination of the virus. In the United States, the Department of the Interior, in cooperation with many other partners, has developed a risk-based surveillance program for the early detection of the possible introduction of H5N1 by migratory birds. The program identifies target migratory bird species and sampling locations based on their migratory patterns, population size, habitat, potential contact with HPAI-infected birds, and ease of sample collection. Since April 2006, a total of 48,325 birds have been tested. Results show that 1,201 birds may contain an avian influenza virus as identified by matrix real-time reverse-transcription polymerase chain reaction (rRT–PCR) and, of these, 45 samples were positive according to the H5 rRT–PCR test. Twenty-eight H5 viruses were isolated following inoculation in embryonating chicken eggs. The following subtypes were found: 21 H5N2, 5 H5N3, and 2 H5N9 subtypes; however, no H5N1 viruses were found in the birds collected by the Department of the Interior surveillance program. Other avian influenza viruses identified include representatives containing combinations of 14 hemagglutinin and 9 neuraminidase subtypes. Several isolates represent new species records. Genetic sequence analysis of some of the recovered viruses provides support that migratory birds in Alaska are involved in the intercontinental exchange of avian influenza viruses between Asia and North America and hence could be a mechanism for the introduction of H5N1 into North America.

Ecology of Vector-Borne Diseases in Pacific Island Birds: Hawaii to New Caledonia

By Carter T. Atkinson,[1] Dennis A. LaPointe,[1] Michael D. Samuel,[2] Susan I. Jarvi,[3] Ruth C. Utzurrum,[4] and Joshua O. Seamon[4]

[1]U.S. Geological Survey, Pacific Island Ecosystems Research Center, Kilauea Field Station, P.O. Box 44, Hawaii National Park, HI 96718.

[2]U.S. Geological Survey, Wisconsin Cooperative Wildlife Research Unit, University of Wisconsin, Department of Wildlife Ecology, Room 204, Russell Laboratories, Madison, WI 53706.

[3]University of Hawaii, Department of Biology, 200 West Kawili Street, Hilo, HI 96720.

[4]American Samoa Government, Department of Marine and Wildlife Resources, P.O. Box 3730, Pago Pago, American Samoa 96799.

Because of their isolation and often unique evolutionary history, insular species are particularly vulnerable to the introduction of new diseases and disease vectors. The introduction of mosquitoes, avian malaria (*Plasmodium relictum*), and avian pox virus (*Avipoxvirus* spp.) to the Hawaiian Islands has become a classic example of the potential destructive power of introduced diseases and vectors on a vulnerable endemic avifauna. Significant demographic impacts and dramatic changes in the distribution of native Hawaiian forest birds are tied closely to steep altitudinal gradients of disease transmission on the main Hawaiian islands. Transmission of pox and malaria is influenced by a variety of biotic and abiotic factors, including seasonal and altitudinal changes in vector populations that are related to temperature and rainfall, availability of larval habitat for mosquito vectors, presence of suitable disease reservoirs, and potential interactions between pox virus and chronic malarial infections. Keys to maintaining diversity of the endemic Hawaiian avifauna lie at the extremes of altitudinal gradients of disease transmission, both in the lowlands where high transmission rates are fostering natural selection of disease resistance and in remaining high-elevation refugia where restoration efforts are seeking to improve and expand habitat.

In contrast to Hawaii, island archipelagoes of American Samoa, Western Samoa, Fiji, and New Caledonia have a high diversity of indigenous hematozoan parasites, including several distinct lineages of *Plasmodium* and *Haemoproteus, Trypanosoma avium,* and at least two species of filarial worms. Diversity of this parasite fauna increases from east to west across the Pacific as distances to Australasia decrease. Endemic forest birds of eastern Polynesia probably face the greatest risk from introduced vector-borne diseases such as West Nile virus both because of their isolation and because some of these remote island groups have indigenous vectors for transmitting avian pathogens. None of these archipelagos, however, are comparable to Hawaii in terms of remoteness, historical absence of vectors, and diversity of endemic birds.

Section II: Mechanisms of Fish and Wildlife Disease

Disease Impacts on Populations of Wild Marine Fish

By Paul K. Hershberger[1]

[1]U.S. Geological Survey, Marrowstone Marine Field Station, Western Fisheries Research Center, 616 Marrowstone Point Road, Nordland, WA 98358.

Pacific herring (*Clupea pallasii*) populations throughout the nearshore and coastal regions of the eastern North Pacific are routinely impacted by infectious and parasitic diseases, including viral hemorrhagic septicemia (VHS), viral erythrocytic necrosis (VEN), and ichthyophoniasis. In an effort to understand, predict, and mitigate the impacts of these diseases on populations of wild marine fishes, we combine a holistic approach consisting of disease surveillances in wild populations with a reductionistic approach consisting of controlled, laboratory-based empirical studies. A major impediment to performing well-controlled laboratory studies was recently overcome with the development of colonies of specific-pathogen-free, immunologically naive Pacific herring. These animals are utilized to understand cause-and-effect relationships involving disease kinetics, acute and chronic impacts of disease, and immunological responses to the pathogens. These relationships can help us to understand the impacts of disease on wild populations in the context of the collapse and failed recovery of adult herring in Prince William Sound, age truncation of adult herring in Puget Sound, and acute and chronic mortalities that occur among juvenile herring throughout the eastern North Pacific.

Mycobacteriosis in Chesapeake Bay

By Christopher A. Ottinger[1]

[1]U.S. Geological Survey, Leetown Science Center, National Fish Health Research Laboratory, 11649 Leetown Road, Kearneysville, WV 25430.

Mycobacteriosis has been present in Chesapeake Bay striped bass since at least the 1980s, as indicated by archived tissue samples. It was not until the summer and fall of 1996–97, however, when large numbers of fish with skin lesions were reported in the Pocomoke River and other tributaries of Chesapeake Bay, that a great deal of public and scientific interest was stimulated about this disease. Mycobacteria-associated infection and disease in Chesapeake Bay striped bass are age dependent. Disease prevalence also appears to have temporal and possibly spatial components, although the latter is difficult to analyze due to the highly migratory nature of this fish. The disease appears to be most severe during the

late summer and fall. Modes of transmission remain poorly understood, although limited data indicate that infections can be transmitted via the water column. Additional data indicate the occurrence of gonadal infection, suggesting the possibility of adults transferring the infection to their progeny. Multiple species of fishes within the Chesapeake Bay have been found to be infected with mycobacteria, suggesting the possibility of trophic transfers of infection. Trophic interactions may also impact striped bass nutrition directly through reductions in prey species numbers. Within the past decade, large numbers of emaciated striped bass have been observed in Chesapeake Bay, and it is thought that this loss in condition may be largely attributed to mycobacteriosis. Laboratory studies indicate that inadequate nutrition can predispose fish, thus enhancing the severity and progression of mycobacteriosis in striped bass. The degree to which poor nutrition contributes to the disease process in the wild striped bass population will likely be difficult to elucidate. Chesapeake Bay population data indicate that the natural mortality rate in the spawning component of the striped bass stock increased beginning in the late 1990s. A cause-effect correlation between increased natural mortality and the mycobacteriosis epizootic is surmised but has yet to be established. The simultaneous occurrences of increased natural mortality along with elevated prevalence of mycobacterial infection have, however, raised concerns about the potential impact of mycobacteriosis on the striped bass population. Studies are currently underway to examine relative return rates of externally diseased and healthy striped bass. This work will provide information on disease-associated mortality in striped bass on a population scale. Additional information on disease-associated mortality in striped bass is being collected through large-scale cross-sectional surveys of mycobacteriosis in mainstem Chesapeake Bay. Modeling of these data with newly developed techniques suggests that mycobacteriosis-associated mortality may be affecting the species at the population level.

Studies on Plague Pathogenesis in Rodents Using in vivo Imaging

By Tonie E. Rocke,[1] Sandra Dawe,[2] and Jorge E. Osorio[3]

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[3]University of Wisconsin-Madison, School of Veterinary Medicine, Department of Pathobiological Sciences, 1655 Linden Drive, Madison, WI 53706.

Plague, caused by the bacterium *Yersinia pestis,* is a flea-transmitted disease of wild rodents that occasionally spills over into humans and other mammals. In the past century, plague caused severe epidemics in many parts of the world, resulting in human deaths and severe economic losses. Fears of a new resurgence of plague have been raised by recent plague outbreaks in regions (India and Madagascar) where the disease had been dormant for decades; these fears were compounded by the isolation of multidrug-resistant strains of *Y. pestis* during the plague epidemic in Madagascar. Furthermore, because of its pathogenicity in humans (particularly the pneumonic form of the disease) and its potential for human-to-human transmission, *Y. pestis* is considered a potential candidate for biowarfare and bioterrorism. These factors have renewed interest in the pathogenesis of *Y. pestis* and host response to infection. In animals, particularly natural rodent hosts like ground squirrels and prairie dogs, the pathogenesis of plague is not well characterized. Traditionally, charting the course of plague entailed sequential euthanasia of infected animals over time, requiring large numbers of animals and the time-consuming tasks associated with determining bacterial load in various tissues and organs ex vivo. As an alternative technique, we are using bioluminescent imaging (BLI) to determine the kinetics of disease progression in vivo in real time, reducing the number of animals required and the animal-to-animal variability. Recombinant, fully virulent, bioluminescent *Y. pestis* C092 was generated using the mini Tn7-transposon to introduce the complete *lux* operon (*luxCDABE*) into the bacterial genome at a unique site. Using a photon-counting, intensified charge-coupled device camera, the dissemination and tissue localization of bioluminescent *Y. pestis* was monitored and quantified in vivo in mice. Differences in the distribution pattern of bacteria were discerned over the course of infection in mice inoculated via different routes of exposure (intradermal, subcutaneous, and intranasal), demonstrating that BLI is capable of distinguishing between different forms of plague disease (bubonic, primary septicemic, and pneumonic). We also monitored plague infection in animals that received different vaccines, demonstrating that BLI can be used quantitatively to measure treatment effects. Bioluminescent imaging offers several advantages for the study of plague pathogenesis, allowing the dissemination of *Y. pestis* to be tracked from the site of exposure through host tissues in real time.

Role of Wild Rodents in Environmental Transport of Prions

By Chad J. Johnson[1] and Dennis M. Heisey[2]

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[2]U.S. Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711–6223.

Prion diseases, or transmissible spongiform encephalopathies (TSE), are a group of progressive fatal neurological diseases. The infectious agent is novel in that it consists of a misfolded form of a normal cellular protein, the prion protein (PrP^C). The disease form of the prion protein (PrP^D)

is highly resistant to inactivation by methods that inactivate other pathogens. Chronic wasting disease (CWD) is the first documented prion disease of a wild mammalian population. CWD agent is maintained in the wild population through lateral transmission. There is growing evidence for animal-to-animal contact and environmental reservoirs contributing to the transmission of the disease agent. The presence of environmental sources of the disease agent indicates a certain background level of exposure of the disease agent to other species in the environment. We are exploring the transmission of CWD to various rodent species, including meadow voles (*Microtus pennsylvanicus*), southern red-backed voles (*Clethrionomys gapperi*), deer mice (*Peromyscus maniculatus*), and white-footed mice (*Peromyscus leucopus*). These rodents scavenge carrion and are an important food source for many predator species. Furthermore, these rodents enter human and domestic livestock food chains by accidental inclusion in grain and forage. All of the rodent species except the white-footed mouse have proven susceptible to interspecies transmission of CWD via the intracerebral route. Meadow voles proved to be most susceptible, with the earliest presentation of disease signs after about 7 months and with 100 percent mortality by 374 days. This high level of penetration indicates a low species barrier in this species to interspecies transmission of CWD. Our main objectives with this study are determining the following: the risk of natural transmission of CWD into rodents, the potential for adaptation and maintenance of a TSE in rodent populations, the properties of any adapted infectious agent, and the effect of prion protein allelic variation on all of the above-listed processes.

Microbial Diseases in Marine Ecosystems: Challenges and Opportunities

By Thierry M. Work[1]

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Diseases in marine ecosystems are becoming increasingly visible, particularly for certain organisms like marine mammals, sea turtles, and corals. In the Caribbean, diseases of corals and echinoderms have led to severe degradation of coral reef ecosystems. Yet our understanding or potential management of many diseases in marine ecosystems is limited by what we don't know. For example, the life cycles of many marine organisms that are hosts to disease are unknown, complicating our understanding of the epizootiology of diseases. The life cycles of many marine parasites are unknown, making their management a problematic issue. Furthermore, many microbial agents present in marine ecosystems cannot be cultured by using conventional techniques, and thus reagents commonly used to identify microorganisms are not suitable or are lacking for marine microorganisms. Genomic techniques provide a partial answer to some of these quandaries; however, detecting the genome of an organism and determining the role this organism plays in actual disease causation are two very different propositions. Some of these quandaries have affected studies of sea turtles, fish, and corals. On the other hand, devising creative solutions to these problems may provide opportunities to form new paradigms about our understanding of wildlife diseases.

Section III: Microbial Ecology

Microbiology of Deep-Sea Corals

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Coral-associated bacteria and Archaea are beginning to be recognized as an important part of the total biology of the shallow-water coral holobiont (the entity composed of the coral animal, zooxanthellae, and mucus/tissue/skeletal-dwelling microbes). Known activities include cycling carbon, producing antibiotics, and fixing nitrogen, while other metabolic roles remain to be discovered. Deep-sea corals have a fundamentally different ecology due to their adaptation to cold, dark, high-pressure environments, and, thus, they have novel microbiota. Submersibles were used to collect samples of both soft corals (gorgonians from waters off the Aleutian Islands) and stony coral (*Lophelia pertusa* from the Gulf of Mexico). The corals were brought to the surface in specially designed containers to prevent contamination from the water column and thermal shock. Additionally, a subset of each sample was preserved at depth, to control for changes in the microbial community that might have resulted from the differences in light, temperature, and pressure experienced as the sample was brought to the surface. Microbes were analyzed by both cultivation and culture-independent methods, making this the first study to include both culture-based and molecular data on deep-sea coral-associated bacterial communities. There are a few similarities between the bacterial symbionts of deep-sea corals and those of shallow-water corals, but both cultured isolates and 16S rDNA clone libraries reveal novel bacteria. Many of these bacteria are similar to prokaryotic symbionts of fish, squid, and methane-seep clams. Others are most similar to psychrophiles from polar waters. The clone libraries also contain a surprising number of mollicute sequences, mainly from the family Mycoplasmataceae. Molecular analysis of bacterial community diversity in *Lophelia pertusa* revealed a marked difference between communities at two study sites that were less than 20 nautical miles apart. This unexpected dissimilarity between the dominant members of these bacterial

communities may be evidence of disease or stress at one site or may indicate biogeographical differences. This work contributes to our understanding of microbial diversity and coral holobiont community structure. Characterizing the microbial communities of deep-sea corals is fundamental to understanding the biology and ecology of deep reefs.

Development and Application of Denaturing High-Performance Liquid Chromatography (DHPLC) Methods for the Analysis of Microbial Community Composition

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Analysis of sequence variation among polymerase chain reaction (PCR) amplicons derived from16S rDNA is currently the standard technique for assessing microbial community composition and diversity in environmental samples. The pooled amplicons are commonly separated for individual sequence analysis by clone library construction or by vertical electrophoresis through a polyacrylamide gel containing an increasing amount of chemical denaturant down the gel (denaturing gradient gel electrophoresis, or "DGGE"). Both techniques have significant drawbacks in terms of expense, labor costs, quantifiability, and ease of downstream processing. Denaturing high-performance liquid chromatography (DHPLC) is a relatively new technique for separating DNA or RNA fragments on the basis of size or sequence variation using reverse phase ion-pairing liquid chromatography. Pooled PCR amplicons are passed through a separation column containing 18C-alkylated polystyrene-divinylbenzene copolymer beads. The beads are electrostatically neutral and hydrophobic. The stationary phase, triethylammonium acetate (TEAA), has both hydrophobic and positively charged ends. It can therefore bind to both the beads and the negatively charged phosphate groups on DNA or RNA fragments, allowing indirect adsorption of the DNA to the separation column beads. Increasing amounts of the mobile phase (25 percent acetonitrile in 0.1 molar (M) TEAA) decreases the hydrophobic interaction between TEAA and the separation column beads, so that the DNA-TEAA ion pair is released to solution. Both ultraviolet (UV) absorption and fluorescence detection of eluted DNA are available, with the latter affording increased sensitivity. DHPLC is more quantitative, reproducible, and automated than gel-based or cloning methods, and it can be coupled to downstream fraction collection, decreasing the preparative procedures required to obtain sequences of PCR amplicons. Case studies have been made of the development and application of DHPLC methods for analysis of 16S rDNA or functional gene fragments for several microbial groups, including all Eubacteria, the sulfate-reducing bacteria, the Archaea, and the Fungi. DHPLC as a method of culture-independent analysis of microbial communities has unique benefits and limitations relative to other methods available or soon to be online at the U.S. Geological Survey (USGS), such as quantitative PCR and methods using phospholipid fatty acids (PLFAs). The technology has potential new applications in the areas of microbial ecology and population biology relevant to the types of investigations the USGS conducts.

The Role of Biological Soil Crusts in Preventing Desertification of Dryland Ecosystems

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Biological soil crusts (BSCs) are a critical component of most dryland ecosystems. The phototropic organisms in these soil surface communities are dominated by cyanobacteria, mosses, and lichens, and, as they often completely cover the large soil interspaces found between plants, they can be the dominant living cover in these ecosystems. Biological soil crusts influence many aspects of dryland function. Cyanobacteria, lichens, and mosses all fix carbon (C) and can be an essential source of C for subsurface soil biota. Most cyanobacteria and cyanolichens that occur in these communities fix nitrogen (N) and can be the dominant source of this often-limiting nutrient for plant and soil communities. The BSC organisms are sticky and help retain wind-deposited nutrients, which can provide up to 75 percent of plant-essential nutrients (for example, N, P, K, Mg, Na, Mn, Cu, and Fe). Crust organisms also contribute to keeping nutrients available to vascular plants by secreting powerful metal chelators. Biological soil crusts can also increase water infiltration and the retention of nutrient-rich dust, seeds, and organic matter. Plant productivity and concentrations of most plant-essential nutrients are often higher in plants when they are growing in soils covered by BSCs relative to adjacent uncrusted soils. Soil food webs are more complex and organisms more abundant under well-developed soil crusts than those under less developed crusts, and thus nutrient cycling is faster when mosses and lichens are present. Biological crusts are also vital in reducing wind and water erosion. Most soil surfaces in drylands lack protection by rocks or plants and depend on BSCs for their stability. Thus, where plant cover is sparse, BSCs reduce erosion from otherwise unprotected soil surfaces. Unfortunately, these communities are highly susceptible to soil surface disturbance, as they are easily crushed and (or) buried when disturbed. Experimental disturbances show that disruptions of soil surfaces result in decreased N and C inputs from soil biota by up to 100 percent. The ability to glue aeolian deposits in place is compromised. While sediment production from BSC-covered surfaces is minimal, production

after disturbance increases by up to 36 times, with soil movement initiated at wind velocities well below commonly occurring wind speeds. Because the disturbance of desert soil surfaces can both reduce fertility inputs and accelerate fertility losses, preservation of these organisms could be a management goal in dryland ecosystems.

Endosymbiotic Fungi in Plants: Ecology and Implications for Climate Change

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Climate change is predicted to negatively impact aquatic and terrestrial ecosystems due to increased frequency and severity of drought, disease, salinization, and biological invasions. This possible increase in droughts will result in difficult decisions concerning water allocations for supporting agriculture, supplying public utilities, generating electricity, and sustaining ecosystems. While significant research effort has been invested in climate predictions and decreasing CO_2, comparatively little effort has gone into mitigating the impacts of climate change. We have developed an *Adaptive Symbiotic* strategy to mitigate impacts of climate change on plants in natural and managed ecosystems. This strategy is based on the fact that fungal endophytes adapt to environmental stress (drought, salinity, disease, temperature) and confer stress tolerance to host plants. Plants appear unable to adequately adapt to stress, thus making fungal symbionts significant adaptive components of plant communities. Symbiotically conferred stress tolerance occurs in a habitat-specific manner, and both plants and fungi switch partners across microhabitats in order to maximize fitness. The mechanism(s) responsible for symbiotically conferred stress tolerance involves diminishing the amount of reactive oxygen generated under stress. In addition, drought tolerance is based on increased water efficiency in symbiotic plants, which require less water for normal growth and development. Therefore, decreased water needs in symbiotic plants can translate into decreased irrigation demands, allowing more water to be allocated for sustaining aquatic ecosystems. I have studied the mechanisms responsible for stress tolerance, the ecological significance of symbiosis, and the use of symbionts to mitigate the impacts of climate change.

Soil Community Dynamics in Sagebrush Steppe and Cheatgrass-Invaded Areas of the Northern Great Basin

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Sagebrush steppe ecosystems in the northern Great Basin have been dramatically altered by the invasion of the exotic annual grass *Bromus tectorum* (cheatgrass). We have extensively studied the effects of this shrub-to-grassland conversion on soil biota in eastern Oregon, southern Idaho, Utah, and Nevada. Surveys of biological soil crust, microbial, and nematode communities using a variety of techniques (line-point intercept, community-level physiological profiling [CLPP], phospholipid fatty acid [PLFA] analysis, terminal restriction fragment length polymorphism [T-RFLP], and sugar-centrifugation and microscopy) have revealed clear differences in biotic community structure and dominance under sagebrush, bunchgrass, cheatgrass, and interspace areas. Preliminary data suggest that sagebrush soils have higher saprophytic fungal diversity and abundance and biological soil crust cover (cyanobacteria, mosses, and lichens), while cheatgrass soils have higher amounts of arbuscular mycorrhizal fungi and little to no crust development. We are in the process of elucidating the consequences of these differences for ecosystem processes by using isotope pool dilution techniques in conjunction with bacterial and fungal antibiotic treatments. Our specific objective is to determine how bacteria and fungi contribute to nitrogen production and consumption in sagebrush and cheatgrass rhizosphere soils. At larger spatial scales, we have discovered soil community patterns related to edaphic factors at cheatgrass-invaded sites across the Great Basin. Initial findings show that sites with high pH values (>8.0) can be characterized as having little to no biological soil crust development and tend to be dominated by bacteria and bacterial-feeding nematodes. Finally, we have quantified how restoration and management treatments, such as prescribed fire and nutrient additions, temporarily increase or decrease certain groups of soil organisms. Ultimately, we hope to use this knowledge to increase native plant restoration success in the sagebrush steppe.

Section IV: Geographic Patterns/ Visualization

Microbial Mapping: Stimulating Hypothesis and Informing the Public

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John Snow's 1855 map of the cholera outbreak in London is well known to epidemiologists, biologists, and cartographers. While it is unlikely that Snow, upon studying the map, had an "ah hah" reaction and rushed to remove the handle of the Broad Street pump, the map remains an iconic example of the power of being able to geographically visualize microbial activity. Microbial activity (from genetic mutations to outbreaks of disease) does not occur on a homogeneous plane, but rather varies by geographic location. By attaching spatial and temporal tags to our observations of microbial occurrences, we can use these data in a wide variety of visualization and analysis methods. These techniques can be used to inform the public by displaying the results of disease surveillance and outbreak investigations. The mapping of arboviral diseases conducted by the U.S. Geological Survey (USGS) under a collaborative agreement with the Centers for Disease Control and Prevention (CDC) is an example (see http://diseasemaps.usgs.gov). The spatial and temporal juxtaposition of disease events in wildlife and human cases can trigger insights into methods that allow for the prediction of human outbreaks. For example, West Nile virus (WNV) cases in wild birds can be used as a predictor of human cases on a county-by-county basis. Along similar lines, environmental conditions that promote the growth of host, vector, or microbe populations can be monitored by using remote sensing and field observations. Areas at greater risk of malaria or Rift Valley fever outbreaks have been identified by using such techniques.

A Multiscale Approach to the Concept of Regional Health

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Health certainly implies the ability of an organism to restore physiological equilibrium when disturbed, but is it meaningful to speak of regional health? A region is a bounded unit of space, and for some purposes, the human body (approximate size 1 square meter) can be considered a small region, whose health is in part determined by processes at the cellular and microbial level of 1 micrometer (10^{-6} meter). Let us reflect on this size range and ask if the concept of health applies at the megameter (10^{6} meter) level. Some islands and lakes are clearly bounded regions of this size, as are some watersheds. The demography, landscape, hydrology, and wildlife of the 160,000-square-kilometer (km^2) Chesapeake Bay watershed (CBW) region are being modeled, and questions of its health arise, but in what sense? We can ask if the region's constituent organisms are healthy, but because the members of this ill-defined set form an impossibly complicated trophic web, the health of the region cannot be any kind of sum of the health ratings of its organisms. More narrowly, we can investigate the health of the set of human inhabitants: compared to a 1:1,000 random sample of global humans, the 15 million inhabitants of the CBW are probably taller, heavier, and likely to have a longer life expectancy. Although we might investigate whether regional environmental conditions threaten public welfare, aggregate human health can only be one among many indicators of regional health. Still at the macro level, CBW scientists are modeling and measuring the status, variation, and trends of such regional systems as atmospheric and water chemistry, land cover and the remotely sensed greenness cycle, and wildlife health and biodiversity; they are finding that many of these indicators are outside of the ranges typical of the region's 10,000-year history. We may build on this notion of regional "physiology" by asking whether regional processes are resilient enough to reestablish some kind of equilibrium within the next few human generations. Although it is quite likely that the chemistry, landscape, and biodiversity of the CBW will attain a new equilibrium, many indices will be very different from preindustrial ranges, and in this sense, the region currently cannot "pass its physical."

To return to the micro scale, the modern theory of infection and immunity suggests a tradeoff between endemicity and virulence: impaired host functioning restricts highly destructive pathogens from infecting large numbers from a population. Because the disequilibrium of the CBW is due to human activities, the region's capacity to host projected numbers of humans will be depressed by negative loops that will limit the prospects for continued regional exploitation, even though some kind of future equilibrium—either managed or "natural"—is likely. The evolving concept of regional health, although difficult to operationalize, can facilitate the development of regional clinical practice allied to the broadening field of public health.

Public Health and Geospatial Distribution

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The value in mapping disease occurrence is to illuminate the underlying *cause* of an outbreak, which may then enable mitigation measures to be taken to prevent further spread of a similar future disease. The earliest example of using a spatio-analytical approach to solving an epidemiological riddle is generally credited to John Snow, who mapped a major outbreak of cholera in 19th century London, during a time before germ theory was well accepted, and hypothesized that there was a causal association between the putative source of the contagion and case locations. He convinced city officials to remove the handle of a pump dispensing contaminated water—an intervention that promptly quelled the outbreak. A more recent example of how understanding geospatial distributions of diseases may lead to improved preventive measures is provided by geospatial analysis of Lyme disease in the Middle Atlantic and northeast regions of the United States. Tick abundance is likely a more reliable measure of the effect of landscape features on Lyme disease risk than human cases. Geographic information system (GIS) techniques were used with statistics incorporating spatial autocorrelation to tick distribution data, and multiple regression analysis was performed. GIS assisted with secondary and tertiary calculations based on raw data, and the investigations revealed significant associations between tick abundance and specific environmental covariates, including, counter-intuitively, soil type. Because a digital subset of soil data at much higher resolution and with a more extensive set of attributes was subsequently made available, a second analysis was conducted. When State Soil Geographic (STATSGO) data (1:250,000, from the Natural Resources Conservation Service [NRCS]) were used, well-drained soils were found to be significantly positively associated with tick abundance, in keeping with previous literature reports. Upon analysis using Soil Survey Geographic (SSURGO) data (1:24,000, from the NRCS), however, it was found that poorly drained soils, too, could be positively associated with tick abundance. This apparent contradiction was resolved by considering precipitation factors and soil water-holding capacity. This example demonstrates the power of a GIS approach in examining environmental influences on factors controlling human disease risk. Observation of previously obscured patterns has enabled the generation of hypotheses now being tested to explain factors responsible for biologically based spatio-analytical trends. If researchers can better understand the effects of environmental parameters on disease vector distribution, then public health officials will likely be able to improve intervention strategies. Other examples where analytical tools have been used to generate testable public health research hypotheses include: fluorosis in China; African trypanosomiasis (sleeping sickness); Hurricane Mitch; cadmium in the Netherlands; and malaria in Mexico and Guatemala.

Critical Habitat Diversity Parameters for Assessing the Geographical Distribution of Plague: A Geographic Information System (GIS)-Based Case-Control Study and Risk Model of Human Plague Cases in the Southwestern United States, 1963–2007

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Remotely sensed geographic parameters including land cover, elevation, and slope were used to develop and validate models of peridomestically acquired human plague case sites using a case-control study design for the two largest case foci in the United States. Analysis of 82 cases and 222 control sites from the Four Corners Focus, using 30-meter-resolution data layers from the U.S. Geological Survey's National Land Cover Database and National Elevation Dataset, shows strong correlations of plague incidence with elevation, slope, and vegetative land cover diversity. Higher elevations, above 2,100 meters (m), are strongly correlated with a 2.64 times increased incidence, whereas, lower elevations, below 1,825 m, are correlated with a 3.13 times reduced incidence. Slope greater than 3 percent is strongly associated with a 2.4 times increased incidence, as is a vegetative land cover composed of shrubland, grassland and juniper, within a 500-m buffer zone around sites having a 3.35 times increased incidence. All three parameters together in a simple correlation model produced a 4.81 times increased incidence. A 30-m-resolution risk map (model) was constructed and validated using the 163 North-Central New Mexico Focus case sites and demonstrates a 78 percent accuracy rate. A logistic regression model was separately constructed and yielded a best-fit model, which included elevation, elevation squared, and the same land cover diversity measure. Receiver operating characteristic (ROC) curves were used to evaluate the regression models, with the best model yielding a ROC value of 0.68. Habitat diversity is a critical distribution parameter for human plague risk.

Section V: Public Health and Water Quality

Integrating Quantitative Polymerase Chain Reaction (qPCR) and Predictive Models To Monitor Beach Water Quality

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Fecal indicator bacteria, such as fecal coliforms, *E. coli,* and enterococci, have historically been used to determine the quality of fresh and marine recreational waters. Currently, bacterial culturing requires 18–24 hours to complete, causing a considerable delay in issuing beach advisories. The need for a more rapid test has been emphasized by local and State managers and the U.S. Environmental Protection Agency. Quantitative polymerase chain reaction (qPCR) has been suggested as a potential rapid test for measuring beach water quality. Recent research has shown that enterococci counts measured by qPCR correlate well with swimming-associated illnesses in recreational waters. The objectives of this project were to demonstrate if qPCR is a potential tool for managing recreational beaches for microbiological water quality and to compare qPCR results with results from empirical predictive models. During the summer of 2006, water samples were collected from three southern Lake Michigan beaches and an associated river outfall (Burns Ditch). Culturable enterococci counts (log colony-forming units per 100 milliliters, or log CFU/100 mL) for the beaches were significantly correlated with calibrator cell equivalents (CCE) as determined by qPCR ($R=0.650$, $P<0.0001$, $N=32$). Culturable enterococci densities at the beaches were significantly correlated with Burns Ditch enterococci densities ($R=0.565$, $P=0.001$, $N=32$). Enterococci densities in Burns Ditch were significantly higher than those in beach water ($P<0.0001$). However, there were no correlations or significant differences observed between CCE values at the study sites. Enterococci CCE results for the study beaches correlated well with many independent variables tested in the development of the Swimming Advisory Forecast Estimate (SAFE) predictive model. Regression analysis resulted in a model that incorporated Burns Ditch discharge and an interactive term: log-transformed lake turbidity × wave height. Our results show that analytically, qPCR compares well with the traditional membrane-filtration (MF) method for measuring beach water quality. Thus, qPCR may be a viable alternative approach for determining beach water quality if a standard water-quality criterion for enterococci CCE is developed.

Harmful Algal Blooms

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Harmful algal blooms (HABs) in both marine and freshwater environments cause ecologic, economic, and public health concerns. In freshwater, the majority of HABs are caused by cyanobacteria. Cyanobacteria cause a multitude of water-quality concerns, including potential production of taste-and-odor compounds and toxins. Taste-and-odor compounds cause malodorous or unpalatable drinking water and fish, resulting in increased treatment costs and loss of aquacultural and recreational revenue. Cyanobacteria produce a diverse group of toxins, including hepatotoxins, neurotoxins, and dermatoxins, that have been implicated in human and animal illness and death in at least 35 U.S. States. Acute human toxicoses associated with cyanotoxins have most commonly occurred after exposure through recreational activities; however, many toxins are potent tumor promoters and chronic exposure to low levels in drinking water also may pose health risks. Because of potential human-health risks, several States have recently added cyanobacteria, or the easily measured toxin microcystin, to routine beach monitoring programs, and cyanotoxins currently are on the U.S. Environmental Protection Agency drinking water contaminant candidate list. Despite known human health risks, few studies have assessed the distribution, co-occurrence, and concentration of cyanotoxins in the United States. Federal and State agencies, operators of drinking-water treatment facilities, and resource managers increasingly are faced with decisions about managing cyanobacterial blooms that affect local economies and public awareness, exposure, and health; therefore, representative scientific data are needed to guide management and public health decisions about cyanotoxins and taste-and-odor compounds. During 1999–2007, several studies were conducted to assess occurrence of cyanobacterial toxins and taste-and-odor compounds in U.S. lakes, particularly in the Midwest. Total microcystin was detected in 78 percent of Midwestern lakes ($n=359$) during the summers of 1999–2006 and in 32 percent of lakes nationally ($n=1,150$) during the summer of 2007. Concentrations in these studies exceeded the World Health Organization (WHO) recreational guideline of 20 micrograms per liter (µg/L) in 1 percent of lakes. Cyanobacterial blooms in Midwestern lakes ($n=23$) were sampled in August 2006 to assess occurrence of several classes of toxins, including microcystin, anatoxin, and cylindrospermopsin. Total microcystin was detected in all blooms, with 17 percent of concentrations exceeding

20 µg/L. The taste-and-odor compounds geosmin and 2-methylisoborneol (MIB) also were measured. Geosmin was more common (83 percent of lakes) than MIB (35 percent), but concentrations of both compounds when detected always equaled or exceeded the human detection limit of 10 nanograms per liter (ng/L). These data indicate that toxin and taste-and-odor compounds are widespread in Midwestern lakes and probably nationally, sometimes at levels that cause health concerns.

Pathogens and Antibiotic-Resistant Bacteria in the Environment

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Pathogens, antibiotic-resistant bacteria, and their impacts on human health have been studied in great detail from a clinical perspective. Increases in antibiotic-resistant bacteria and recent waterborne and foodborne outbreaks due to pathogenic bacteria have highlighted the need to better understand the effect the environment has on human health. The U.S. Geological Survey (USGS) Michigan Water Science Center (MI WSC) is currently conducting several studies to better understand the occurrence, distribution, sources, and maintenance of pathogens and antibiotic-resistant bacteria in the environment. Much of the information that exists on the occurrence of pathogens comes only from the clinical setting after the public has already become ill. Nevertheless, several waterborne outbreaks due to pathogens have occurred following exposure to contaminated waters. Shiga toxin-producing *Escherichia coli* (STEC) have been implicated in several waterborne and foodborne outbreaks in the United States. The occurrence of STEC in the environment is evaluated by using multiple lines of evidence that include DNA-based, immunological, and growth-based assays for STEC-related genes. Particular emphasis has been placed on occurrence of these markers with respect to land use, season, and potential fecal sources. Studies have focused on occurrence of the *eaeA* gene that encodes the intimin protein, the *stx1* and *stx2* genes that encode the STEC Shiga toxins 1 and 2, and the rfb_{O157} gene that encodes the O157 somatic antigen in *E. coli.* In addition, studies of enterococci have focused on detection of the human-pathogenic *Enterococcus* surface protein (*esp*) gene. Although the prevalence of antibiotic-resistant bacteria in hospitals has been well documented, the presence of these organisms in the environment has not. Antibiotic-resistant bacteria in the environment may pose a health threat if the organisms are pathogenic or can transfer antibiotic-resistance genes to pathogens. Researchers of the USGS MI WSC and the Toxic Substances Hydrology (Toxics) Program's Emerging Contaminants in the Environment Project are studying the role the environment may play in the development, maintenance, and dissemination of antibiotic-resistant bacteria that pose a potential human health concern. Antibiotic resistance studies use growth assays and molecular methods. The growth assays determine the ability of the organisms to tolerate different environmentally relevant antibiotic concentrations. The molecular methods identify genes or other genetic elements responsible for antibiotic resistance, as well as changes in microbial communities after exposure to antibiotics.

Microbial Source Tracking, Using Quantitative Polymerase Chain Reaction (qPCR), for Enhanced Sanitary Water-Quality Management

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Microbial source tracking (MST) is the science—and art—of distinguishing the origins of intestinal microbes on the basis of host-specific characteristics. MST scientists identify these characteristics by use of host-associated phylogenetic groups (such as human-associated clades of fecal anaerobes in the order Bacteroidales), populations with host-related phenotypes (such as *E. coli* that are resistant to the antibiotics commonly used by humans), or markers relevant to host-microbe interactions (such as the human-associated adhesin gene *esp* in *Enterococcus faecium*). Many MST tools have been proposed and tested, with the most recent family of techniques emerging about 1996. None of the various approaches to MST is universally applicable to water-quality management needs; each has benefits and liabilities in different applications. Applications of MST include traceback analysis to establish epidemiological linkages between contamination sources and receiving waters, screening for prioritized sources of fecal contamination as a basis to select impaired waterways for remediation, and use as a management tool to estimate amounts of fecal-indicator bacteria (*E. coli* or enterococci) coming from different hosts. The need for the last application of MST tools was enhanced with implementation of Total Maximum Daily Load (TMDL) programs across the United States because a TMDL plan requires apportionment of the contaminant (fecal-indicator bacteria, in this case) according to source. Though many MST tools are able to detect the presence of fecal contamination from animal hosts (such as humans, ruminants, dogs, pigs, and others), at this time there is no proven procedure to quantify *E. coli* from various hosts (such as 50 percent from humans, 10 percent from cattle, and 40 percent from wildlife). Despite

the lack of quantitative standard methods for MST, research methods that apply protocols based on quantitative polymerase chain reactions (qPCR) to detect host-associated markers in the environment eventually may allow apportionment of fecal contamination by host, at least in relative terms and perhaps quantitatively. In preliminary research on Fountain Creek, near Colorado Springs, Colo., increases in fecal contamination in areas where human contamination was suspected could be detected by concurrent increases in human-associated MST markers. Other current research addresses the extent to which host-associated MST marker concentrations can be related to fecal-indicator bacteria densities from a host. Fecal indicator-to-MST marker ratios may change over time with various environmental stressors. Despite these limitations, however, quantitative detection of MST markers enhances water-quality management and may allow estimation of the proportion of fecal indicators from various hosts.

Transport of Microbes in the Subsurface/ Physical Interactions of Microbes and Particles

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Starting in the late 1800s, the use of microbial tracers has helped researchers to gain a better understanding of groundwater movement in different types of aquifers. However, the importance of studying the subsurface transport behavior of the microorganisms themselves is now more obvious, largely because of our increasing dependence upon limited and fragile groundwater resources. In particular, widespread contamination of drinking-water aquifers by microbial pathogens and chemical wastes has resulted in an increased interest in factors controlling subsurface microbial transport. The movement of pathogenic bacteria, viruses, and protozoa through aquifers is a major public health concern in the United States, where microbial contamination of water-supply wells contributes significantly to the total number of waterborne disease outbreaks. The intentional injections of nonindigenous bacterial populations into aquifer sediments are being carried out in order to enhance bioremediation of organically contaminated groundwater and to increase oil recovery from less transmissive zones by selective bacterial plugging of more permeable strata. The success of bioaugmentation depends as much upon the ability of the introduced microorganisms to reach the contaminants of interest as their ability to survive in situ. Transport properties of microorganisms introduced into aquifers can have major roles in the transmission of some waterborne diseases, in the success of microbially enhanced oil recovery processes, in the mobility of surface-active or hydrophobic groundwater contaminants, in pore clogging, and in the potential subsurface dissemination of genetically engineered bacteria for biorestoration. However, many of the factors controlling subsurface microbial transport are still poorly understood, including the reversible interactions between microorganisms and solid surfaces. To better study the controls of microbial transport, it has been necessary to improve available methodology for investigating microbial transport behavior in the laboratory and in the field. The multitude of biological and physicochemical factors affecting microbial transport through aquifer materials necessitates a refinement of methodologies, allowing observations under more controlled experimental conditions. Improvements in laboratory and field techniques for studying microbial transport behavior in aquifer materials and new models of porous media are leading to new findings.

Flow Cytometry in Microbiology

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Bacteria are ubiquitous inhabitants of all sorts of environments including freshwaters and saltwaters, thermal vents, arctic snow, acidic mine drainage, petroleum deposits, soils, and sediments. Recent studies demonstrate that bacteria communicate with each other through chemical messaging and even communicate with the cells of plant and animal hosts to modulate physiology, development, and the immune system. Large shifts in microbial populations can be triggered by subtle environmental cues, but once begun, these shifts may themselves cause dramatic ecosystem changes. These changes may result because microbes are responsible for many geochemical processes including metal and sulfur oxidation and reduction, nitrification and denitrification, fermentation, and methane production. Over 2,300 scientific studies (PubMed search) have been published about using small subunit ribosomal RNA to elucidate shifts in microbial assemblages in response to particular environmental stressors. The great majority of those have utilized genetic sequencing of ribosomal RNA to identify the bacteria present and their distribution. While the advent of high throughput automatic sequencing has made these studies feasible, bacteria react quickly to environmental insults, and the application of the sequencing approach to repetitive samples rapidly becomes impractical. The computer programs Primrose and Rose (available at http://www.bioinformatics-toolkit.org/Primrose/index.html) were used to scan bacterial nucleic acid sequences (16S ribosomal DNA) available in public databases and to search for regions that are highly specific to particular phylogenetic groupings. Additionally, several computer programs were written in the Icon Programming Language to further compare these sequences and to modify these sequences from strictly hybridization probes to probes suitable for use in an allele-specific primer extension (ASPE) format. The ASPE format was chosen for further development because

it offers high specificity and relative ease of use. Twenty-six ASPE probes were designed to target a broad range of bacterial phylogenetic groups that are important in numerous and varied environmental settings, as well as in human and animal health. These include the alpha-, beta-, gamma-, delta-, and epsilon Proteobacteria; the Cytophaga-Flavobacterium-Bacteroides Complex; the Cyanobacteria; the Mycobacteria; the Arthrobacteria; the Clostridia; the Streptomyces; the Staphylococci; *Frankia;* the Bacilli; and *Saccharopolyspora.* This suite of ASPE probes was further modified by incorporation of known target sequences (tag) into the 5′ end of the molecule to allow capture on fluorescently bar-coded latex beads that have the matching anti-tag covalently attached to their surface (www.luminexcorp.com). This dedicated flow cytometry system allows the simultaneous detection of up to 100 targets in a sample, and 96 samples can be analyzed in a 96-well microplate format in about 40 minutes. Newer and alternative instrumentation systems are available.

Section VI: Geomicrobiology

Geomicrobiology/Nanoscience of Toxic Elements Se and Te

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Certain anaerobic bacteria respire toxic selenium oxyanions and in doing so produce extracellular accumulations of elemental selenium [Se(0)]. We examined three physiologically and phylogenetically diverse species of selenate- and selenite-respiring bacteria, *Sulfurospirillum barnesii, Bacillus selenitireducens,* and *Selenihalanaerobacter shriftii,* for the occurrence of this phenomenon. When grown with selenium oxyanions as the electron acceptor, all of these organisms formed extracellular granules consisting of stable, uniform nanospheres (diameter, ~300 nanometers, or nm) of Se(0) having monoclinic crystalline structures. Intracellular packets of Se(0) were also noted. The number of intracellular Se(0) packets could be reduced by first growing cells with nitrate as the electron acceptor and then adding selenite ions to washed suspensions of the nitrate-grown cells. This procedure resulted in the formation of primarily extracellular Se nanospheres. After harvesting and cleansing of cellular debris, we observed large differences in the optical properties (ultraviolet (UV) to visible absorption and Raman spectra) of purified extracellular nanospheres produced in this manner by the three different bacterial species. The spectral properties in turn differed substantially from those of amorphous Se(0) formed by chemical oxidation of H_2Se and of black, vitreous Se(0) formed chemically by reduction of selenite with ascorbate. The microbial synthesis of Se(0) nanospheres results in unique, complex, compacted nanostructural arrangements of Se atoms. These arrangements probably reflect a diversity of enzymes involved in the dissimilatory reduction that are subtly different in different microbes. Remarkably, these conditions cannot be achieved by current methods of chemical synthesis.

Certain toxic elements support the metabolism of diverse prokaryotes by serving as respiratory electron acceptors for growth. Here, we demonstrate that two anaerobes previously shown to be capable of respiring oxyanions of selenium also achieve growth by reduction of either tellurate [Te(VI)] or tellurite [Te(IV)] to elemental tellurium [Te(0)]. This reduction achieves a sizable stable-Te-isotopic fractionation (isotopic enrichment factor [epsilon] = –0.4 to –1.0 per milliliter per atomic mass unit) and results in the formation of unique crystalline Te(0) nanoarchitectures as end products. The Te(0) crystals occur internally within but mainly externally from the cells, and each microorganism forms a distinctly different structure. Those formed by *Bacillus selenitireducens* initially are nanorods (~10-nm diameter by 200-nm length), which cluster together, forming larger (~1,000-nm) rosettes composed of numerous individual shards (~100-nm width by 1,000-nm length). In contrast, *Sulfurospirillum barnesii* forms extremely small, irregularly shaped nanospheres (diameter <50 nm) that coalesce into larger composite aggregates. Energy-dispersive X-ray spectroscopy and selected area electron diffraction indicate that both biominerals are composed entirely of Te and are crystalline, while Raman spectroscopy confirms that they are in the elemental state. These Te biominerals have specific spectral signatures (UV to visible light, Raman) that also provide clues to their internal structures. The use of microorganisms to generate Te nanomaterials may be an alternative for bench-scale syntheses. Additionally, they may also generate products with unique properties unattainable by conventional physical and chemical methods.

Soil Microbial Indices Across Different Geographic Scales

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Soil microbial communities participate in mineral weathering through the production of enzymes and organic acids that can accelerate dissolution rates. The intertwining of soil chemistry and microbial ecology was examined through the Geochemical Landscapes pilot study, which addressed variations in soil chemistry on both continental and regional scales. Mineral soils for the continental study were collected along N-S (Manitoba to Texas) and E-W transects (along the 38th parallel) for analysis of major and trace chemical concentrations and indices of microbial composition, including

phospholipid fatty acids (PLFA; *n*=182) and enzyme assays (*n*=251). Continental-scale data revealed distinct patterns of microbial biomass and composition that were largely governed by climatic variations and related variations in organic carbon. PLFA biomass and arylsulfatase activity, which may be related to fungal biomass, were low in regions with low annual precipitation, including the Great Basin, Colorado Plateau, and southwestern Great Plains. A regional-scale study examined the influence of parent lithology on microbial community structure in soils from the California Coast Range and Sierra Nevada Foothills. Upland soils with a bedrock parent (serpentinite, mineralized serpentinite, marine siltstone, volcanic rock) were collected in the Cache Creek watershed in the Coast Range, with alluvial soils collected along a transect following the stream into the Sacramento Valley. Samples were also collected from the Sierra Nevada Foothills to address the influence of the geomorphic setting and climate on serpentine soils. Soils from these parent materials have distinct PLFA biomass and community structure that may be related to Ca/Mg ratios (serpentinite), and toxicity effects of Cr (serpentinite), and Hg (mineralized serpentinite). For instance, PLFA biomarkers (for example, 18:2ω6c, 18:1ω9c) for fungi represent a greater percentage of total PLFA in siltstone parent soils than serpentine soils from the Coast Range. Coast Range serpentine soils tend to have a greater PLFA biomass than siltstone parent soils, possibly due to reduced competition for nutrients from the sparser vegetation associated with serpentine soil. Organic carbon and total PLFA concentrations are greater in serpentine soils from the Sierra Nevada Foothills than in those from the Coast Range; however, the concentration of the PLFA biomarker for fungi is greater in the Coast Range serpentine soils. Mean annual precipitation is somewhat greater at the Coast Range sites (860 millimeters, or mm) than at the Sierra Nevada Foothills sites (680 mm), which should enhance fungal growth. Alternatively, fungal growth in Sierra Nevada Foothills serpentine soils may be inhibited by relatively large concentrations of Cr, which appears to be in a more bioavailable form than in the Coast Range serpentine soils.

Using Natural Abundance $\delta^{13}C$ and $\Delta^{14}C$ Values of Phospholipid Fatty Acids to Characterize Bacterial Carbon Cycling Pathways in Deep Aquifers

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Microorganisms in the deep subsurface compose nearly half of the global biomass and drive the cycling of carbon and other elements. In these nutrient-poor environments, microbes may exploit energy and carbon sources using diverse metabolic strategies: consumption of dissolved and (or) particulate organic matter (OM) by heterotrophic bacteria, conversion of dissolved inorganic carbon (DIC) into biomass by autotrophic microbes, and oxidation of dissolved CH_4 by methanotrophs. Measurements of the natural abundance of stable carbon ($\delta^{13}C$) and radiocarbon ($\Delta^{14}C$) content of subsurface carbon pools can provide insight into microbially mediated carbon transformations. Although challenging in low-biomass subsurface environments, carbon isotope measurements of microbial membrane phospholipid fatty acids (PLFAs) provide important additional information about carbon cycling pathways. Previous pure cultures studies have shown a general trend towards more negative $\delta^{13}C_{PLFA}$ values in the order of heterotrophs, autotrophs, and methanotrophs. This trend is due both to varied $\delta^{13}C$ values of carbon sources and isotope fractionation effects associated with their assimilation. Because $\Delta^{14}C_{PLFA}$ values are inherently corrected for such fractionation effects, they should closely match those of their carbon source. We extracted PLFAs from microorganisms that were filtered from pristine, anoxic groundwaters hosted by both sedimentary and granite rocks in central Japan. The most abundant PLFA structures in both waters were 16:0, 16:1ω7*cis*, *cy*17:0, and 18:1ω7*cis*. A PLFA biomarker for type II methanotrophs, 18:1ω8*cis*, composed 3 and 18 percent of total PLFAs in the sedimentary and granite waters, respectively. The presence of this biomarker was unexpected, given the traditional definition of type II

methanotrophs as obligate aerobes. However, a bacterium that grows aerobically with CH_4 as the sole energy source and produces 56 percent of its total PLFAs as 18:1ω8*cis* was isolated from both waters. The $\Delta^{14}C$ values determined for type II methanotroph PLFAs in the sedimentary (–861 per mil (‰)) and granite (–867‰) waters were very similar to the $\Delta^{14}C$ values of DIC in each water (~–850‰), suggesting that type II methanotrophs ultimately derive all of their carbon from DIC. In contrast, $\delta^{13}C$ values of type II PLFAs in the sedimentary (–93‰) and granite (–60‰) waters indicate that these organisms use different carbon assimilation schemes in each environment. The $\delta^{13}C_{PLFA}$ values (–28‰ to –45‰) of non-methanotrophic bacteria in the sedimentary water indicate that >65 percent of total bacteria are heterotrophs, and dead $\Delta^{14}C$ values (~–1,000‰) of some PLFAs suggest that many heterotrophs utilize ancient OM, perhaps from lignite seams within the sedimentary rocks. The more negative range of $\delta^{13}C_{PLFA}$ values determined for the granite water (–42‰ to –66‰) is likely the result of a microbial ecosystem dominated by chemolithoautotrophy and (or) CH_4 cycling.

Geochemical Controls on Microbial Methylation of Mercury in the Florida Everglades

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Mercury (Hg) is a global contaminant emitted from industrial sources that exhibits very high levels of deposition in south Florida. Mercury deposited on the vast wetland ecosystem of the Everglades can increase in toxicity through conversion of Hg^{2+} deposited in rainfall to methylmercury (MeHg). The conversion of inorganic mercury to MeHg is microbially mediated, primarily by sulfate-reducing bacteria. MeHg is a potent neurotoxin that is also highly bioaccumulative. It poses a potential health threat to wildlife and humans, primarily through fish consumption. The Everglades has some of the highest levels of MeHg in fish on record. Our studies over the past decade have been aimed at understanding the principal controls on microbial MeHg production in the freshwater Everglades. Perhaps the most surprising outcomes of our research have been the documentation of extensive sulfur contamination in the Everglades and the elucidation of the role of sulfur as a major control on microbial MeHg production. The extensive use of sulfur in agriculture to the north of the Everglades and the resulting transport of sulfur (as sulfate) to the Everglades through canals dissecting the ecosystem result in stimulation of microbial sulfate reduction and net increases in the amount of MeHg produced in areas that would otherwise be sulfate limited. The effect of excess sulfate load on MeHg production in the Everglades, however, is complex. Sulfate increases MeHg production through stimulation of microbial sulfate reduction, but buildup of sulfide in soil porewater inhibits MeHg production. The balance between these two effects of sulfur influences both the magnitude and location of MeHg production in the Everglades. The complex relationship between sulfur geochemistry and MeHg production in the Everglades was first deduced from field studies at various sites in the ecosystem. This hypothesis has been confirmed by experimental studies using mesocosms. These mesocosm studies show that increasing sulfate concentrations up to about 15 milligrams per liter (mg/L) increases microbial MeHg production. At sulfate concentrations greater than 15 mg/L, buildup of sulfide from microbial sulfate reduction inhibits MeHg production, probably by inhibiting microbial uptake of Hg^{2+} through changes in the speciation of mercury in soil porewater. Dry/rewet cycles also affect MeHg production in the Everglades. These cycles were shown to temporarily increase surface water sulfate concentrations (due to oxidation of reduced sulfur in soil), stimulating microbial sulfate reduction and MeHg production. A unique combination of conditions in the Everglades, including high mercury deposition, sulfate contamination, and favorable environmental conditions (extensive wetland area, wet/dry cycles, high dissolved organic carbon), results in high levels of MeHg in Everglades biota.

An Overview of Bacterial Controls and Influences on Sulfide Mineral Weathering

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The weathering of sulfide minerals by bacteria is a critical geochemical process in the generation of acid rock drainage and acid mine drainage (ARD/AMD). The dominant bacteria mediating this process are in the genus *Acidithiobacillus* (formerly *Thiobacillus*). These microbes, commonly referred to as thiobacilli, oxidize ferrous iron (Fe^{2+}) and sulfide sulfur (S^{2-}) in sulfide minerals. Thiobacilli prefer low-pH environments (<5.0), and some are able to grow at pH values near 1.0. Generation of ARD or AMD is perhaps the most deleterious consequence of sulfide mineral weathering. The acidic pH values and high dissolved metal concentrations

can negatively affect water quality and impair the survival of aquatic and terrestrial organisms. The role of bacteria in enhancing pyrite (FeS_2) dissolution via iron oxidation has been well studied. During abiologic pyrite decomposition at acid pH (<5), aqueous ferric iron oxidizes pyrite (ferrous) iron:

$$3+ \qquad 2+ \qquad - \qquad .$$

The rate-limiting reaction is formation of aqueous ferric iron. Iron-oxidizing thiobacilli can accelerate the oxidation of solution ferrous iron from 1,500 to 78,000 times faster than the abiologic rate; rate increases up to a millionfold have been noted. Higher numbers of bacteria sorbed to the mineral surface generally correlate with increases in dissolution rates of pyrite and marcasite. The dissolution of other sulfides mediated by bacteria is mostly unknown and poorly understood. More work is needed to determine the specific role of bacteria in the dissolution of monosulfides such as sphalerite (ZnS) and mixed-metal sulfides such as arsenopyrite (FeAsS), which can be major sources of metals in AMD settings. A current U.S. Geological Survey (USGS) laboratory study comparing the abiotic and bacterial dissolution of ZnS indicates little difference in rates in short-term experiments. Previous USGS field and laboratory studies from several ARD/AMD sites were done to quantify the numbers of thiobacilli and their effect on iron oxidation. In one such study in acidic iron fens in the San Juan Mountains of southwestern Colorado, the presence of *Leptothrix,* a filamentous bacterium associated with iron oxidation, indicated active iron oxidation. Yet numbers of thiobacilli were generally low (10^2 to 10^3 cells/milliliter, or cells/mL) compared to numbers in similar environments. This bacterial weathering process is currently being used for the extraction of metals from sulfide ores using heap leach methods. Significant progress has been made in studying this important biogeochemical process.

Defining "Reactive" Inorganic Mercury and Its Relationship to Microbial Methylmercury Formation in Aquatic Ecosystem Studies

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Mercury (Hg) contamination of aquatic ecosystems has long been recognized as a persistent environmental problem that threatens both wildlife and human health. The conversion of inorganic divalent mercury (Hg(II)) to the more toxic methylmercury (MeHg) is a process largely facilitated by sulfate-reducing bacteria in anoxic sediments. Recent advances in the study of Hg biogeochemistry have begun to unravel the most relevant environmental factors that ultimately control the formation of MeHg, both in terms of those that mediate the activity of the Hg(II)-methylating bacteria and those that mediate the availability of Hg(II) to those bacteria. The current evidence suggests that only a small fraction of the total Hg(II) may be available for Hg(II)-methylation; this fraction is termed the "reactive" inorganic mercury (Hg(II)R) fraction. An operationally defined measure of Hg(II)R has been developed and is being used across a wide range of mercury ecosystem studies currently being conducted by the U.S. Geological Survey (USGS). Results from an international study comparing multiple methods used to quantify specific mercury fractions indicate that the Hg(II)R method provides a good proxy for the pool of Hg(II) truly available for Hg(II)-methylation. Deep core profiles from San Francisco Bay demonstrate that Hg(II)R concentrations are highest in surface sediment and decrease with depth. Additional geochemical evidence from multiple ongoing studies suggests that the size of this pool is controlled by sediment redox conditions and the association of Hg(II) with solid-phase reduced-sulfur minerals. Laboratory oxic/anoxic slurry experiments simulating sediment perturbations (such as dredging or slough scouring) demonstrate that the oxidation of deeply buried sediment can increase the Hg(II)R pool size up to sixtyfold. This result is presumably related to the reoxidation of the reduced-sulfur minerals to which nonreactive Hg(II) is strongly bound, and the subsequent liberation of Hg(II) (as Hg(II)R). These findings have significant implications for dredging operations and for the reconstruction of wetland habitat with dredge material. A major conclusion of this body of research is that defining the Hg(II)R pool, and the factors that control it both spatially and temporally, is a critical component of environmental studies that aim to understand microbial MeHg production dynamics within and across ecosystems.

Section VII: Ecosystem Function

Natural and Enhanced Bioremediation of Nitrate Contamination in Groundwater

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Nitrate is a prevalent contaminant in groundwater systems, originating from a variety of agricultural, industrial, and wastewater-disposal practices. Denitrification is a key process that can affect the fate and transport of nitrate in the subsurface and the ultimate delivery of nitrogen to surface waters. A long-term study of the factors that affect the in situ function of subsurface denitrification has been conducted in a sand-and-gravel aquifer on Cape Cod, Mass. The aquifer has been contaminated by the discharge of dilute, treated wastewater, which has resulted in a large contaminant plume

that is characterized by geochemical gradients of fixed carbon and nitrogen that provide a suitable redox environment for denitrification to occur naturally. This study features natural gradient tracer tests (primarily with ^{15}N) as in situ activity assays to quantify rates of denitrification within the geochemical and hydrologic context of the aquifer. These tests can quantify individual steps of the denitrification pathway and the overall effect the process has on nitrogen oxide speciation and nitrogen loads. In zones in which the process was most active, low-level concentrations of nitrite, nitrous oxide, and nitric oxide could all be detected, and it was demonstrated that turnover of these intermediate pools was a sensitive short-term approach for quantifying nitrogen and electron flow via denitrification. Combined tracer test and laboratory studies with aquifer core material suggested that both inorganic (hydrogen, Fe(II), and possibly methane) and organic (acetate and bulk dissolved organic carbon (DOC)) electron donors were fueling denitrification, but that nitrate reduction to ammonium or nitrate/nitrite reduction coupled to ammonium oxidation (anammox) were apparently insignificant nitrate-consuming processes. Another aspect of the study was to determine approaches that could be used to enhance denitrification as a nitrate remediation tool, particularly for situations where the process was electron donor limited. Autotrophic, hydrogen-coupled denitrification was particularly effective at nitrate removal, but it was restricted to a pump-and-treat approach due to limited hydrogen solubility in water. In situ enhancements with formate or Fe(II) were also tested. Both stimulated denitrification. Formate stimulation was relatively slow and produced stoichiometric, persistent concentrations of nitrite. Adding nitrate to an Fe(II) zone very quickly stimulated denitrification, with only transient accumulation of nitrous oxide (no nitrite). Delivery of Fe(II) to the subsurface could be difficult in many cases, but production of insoluble Fe(III) oxides could have additional benefits by removing other concurrent contaminants, such as phosphate, arsenic, or sorbable organic compounds.

Microbial Communities and Carbon Cycling in Response to Global Change

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Global change phenomena such as climate warming, permafrost thaw, wildfires, and drought are affecting terrestrial ecosystem biogeochemistry, particularly in northern latitudes, but also in the continental United States. Soil microbial communities are critical to the carbon biogeochemistry of ecosystems, for they decompose as much carbon as is annually photosynthesized by plants. There is strong evidence that variation in the composition of the below-ground microbial community affects the way in which ecosystems function and can affect regional to global biogeochemistry. Particular functional groups of microorganisms, such as decomposer fungi, have a disproportionate effect on elemental cycles. For example, in northern latitude soils, climate warming is accelerating permafrost thaw and wildfire intensity, altering the abundance of soil decomposers and, thus, directly affecting rates of biogeochemical processes. This and other types of microbial community information can be used by the next generation of mechanistic microbial-based C cycling models. A next step will be to merge bioinformatics with geoinformatics that can be used to build a spatially explicit map of microbial biogeography that is linked to environmental and process data. Such a map has many uses beyond understanding ecological principles that structure community composition and diversity. Such spatially explicit information can potentially be used for assessing how global change will affect microbial communities and the biogeochemical processes (such as C, N, and S cycling) within specific regions. Researchers from the U.S. Geological Survey (USGS), U.S. Department of Agriculture Forest Service (USFS), U.S. Department of Energy (DOE), and the National Science Foundation (NSF) are currently conducting several continental-scale inventories, and these inventories can be linked to a multi-institution effort to study vulnerabilities within the carbon cycle. Microbial communities are central to biogeochemical processes, and linking microbial information with biogeochemical models and biogeographical data is a new frontier for microbiological research within the USGS.

Biogeochemistry of Mountain Watersheds: Through the Microbial Window

By Jill S. Baron,[1] Donald H. Campbell,[2] and David W. Clow[2]

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Nitrogen cycling has been a focus of research for many years in Loch Vale Watershed, a long-term ecological research and monitoring site in Rocky Mountain National Park. Research began in Loch Vale in 1982 and is a joint effort of the Biological Resources Discipline and the Water Resources Discipline Water, Energy, and Biogeochemical Budgets (WEBB) program. Even in this high-elevation catchment, which is 82 percent exposed rock or ice, most nitrogen measured in surface waters bears an isotopic signature indicative of microbial processing. Even the interstitial sediments between rocks above the tree line are microbially active. Our studies of the microbial role in nitrogen cycling have included monitoring, in situ fertilization and laboratory experiments of both soils and lake waters, regional surveys, ecosystem modeling, and most recently, studies of the origin of mass-specific acid, alkane, and chlorin fractions from the

recent past preserved in lake sediments. Our work has helped develop insight into the influences of atmospheric nitrogen deposition, and more recently, into the effects of changing climate on high-elevation terrestrial and aquatic ecosystems.

Quantifying Microbial Denitrification Rates Using Expressed Functional Gene Abundance

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Hydrogeologists interested in certain types of contaminant remediation problems have benefited from several recent advances in molecular techniques for assessing gene expression in subsurface microbial communities. One such technique, reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), has made possible the quantification of functional gene transcripts as a proxy for the rate of processes facilitated by microbial enzymes. RNA and DNA were extracted from the model organism *Paracoccus denitrificans* grown in batch culture under denitrifying conditions; the RNA and DNA were analyzed for gene and transcript abundance to determine how the expression of functional denitrifying genes varies with measured bulk denitrification rates. Measured denitrification rates ranged from 1.6 to 57 milligrams per liter per hour (mg/L/hour), and relative transcript abundances ranged from 15 to 276 copies per milliliter (copies/mL) when normalized to the expression of the constitutive RNA polymerase encoding gene rpoB. The relationship between *nirS* transcript abundance and denitrification rate is linear (R^2=0.97) at rates above 5 mg/L/hour, suggesting that RT-qPCR may be an effective tool for assessing the rate of denitrification by some common denitrifying organisms in nutrient-rich environments. In environments where more traditional methods are limited by low diffusion rates or poor tracer recovery, this new technique can determine a rate from soil or pore-water samples as small as 1 milliliter. The determination of an instantaneous transformation rate in a single sample makes this method well suited to measuring rapidly changing rates and facilitates incorporation of microbiological data into groundwater models of reactive contaminant transport. Future potential applications of this new approach include improved models of subsurface nutrient cycling and the fate and transport of a variety of common groundwater contaminants with known microbially mediated pathways, including arsenic, copper, mercury, and several volatile organic compounds.

Evaluating Ecosystem Function with Integrated Approaches in Environmental Microbiology

By Mary A. Voytek,[1] Julie D. Kirshtein,[1] Michael B. Lowit,[1] and Bess B. Ward[2]

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Microorganisms are involved in the biogeochemical cycling of nutrients in the environment. Biotransformations of nitrogen for example impact the availability and fate of nitrogen in the environment. Microorganisms involved in these biotransformations occur with functional redundancy; that is, there may be different types of organisms performing the same function in a given environment, using either variants of the same enzyme or slightly different enzymes to catalyze the transformations. The relationship between environmental factors and functional gene diversity of ammonia-oxidizing bacteria (AOB) was investigated across a transect from the freshwater portions of the Chesapeake Bay and Choptank River out into the Sargasso Sea. The temporal and spatial variation in ammonia oxidizer (bacterial and archaeal) diversity of the functional gene, *amoA,* was assessed by clone libraries and (or) functional gene microarray analyses, and their total abundance was assessed by quantitative polymerase chain reaction (qPCR) analysis. Library constructs and hybridization patterns along the transect showed clear variations in *amoA* community composition. Cluster analysis of communities along the transect detected three groupings. Members of Cluster 1 were most closely related to upper bay (freshwater) sediment probes, and Cluster 3 was dominated by the marine *Nitrosospira*-like probe signal. Discriminant analysis of the *amoA* groupings indicated that Clusters 1 and 2 were positively correlated with temperature and nitrate concentration, while Cluster 3 was negatively correlated with concentrations of chlorophyll a, dissolved organic carbon, and particulate nitrogen and carbon, suggesting that different *amoA* sequences represent organisms that occupy different ecological niches within the estuarine/marine environment. The lowest diversity in the bacterial AOB was observed in samples from the Sargasso Sea and in samples collected at most stations during the winter. AO-Archaea were most abundant in the Sargasso Sea and in the freshwater sites particularly during periods of high flow. Studies such as this have begun to elucidate the fundamental ecological question of the relationships between the functional complexity and genetic diversity of microbial communities, ecosystem function, and environmental variables, which until now have been virtually unknown.

Poster Session Abstracts

Wildlife Disease

Temporal Dynamics of Mixed Avian Influenza Infections in Waterfowl

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From the National Surveillance Program, up to 20 percent of wild waterfowl sampled that were positive for avian influenza (AI) were infected by more than one isolate. Mixed infections are important in the ecology of AI because they create the opportunity for reassortment and generation of potentially new, more virulent genetic combinations of the virus. In this study, we will infect mallards with two isolates of low pathogenic AI, sequentially and simultaneously, and measure the proportions of each isolate in the infected birds. Isolates generated from birds with mixed infections will be sequenced and examined for evidence of reassortment. Thus, we will be able to determine the reassortment rate in dually infected birds.

Experimental Infection of a North American Raptor with Highly Pathogenic Avian Influenza H5N1

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Highly pathogenic avian influenza (HPAI) is primarily a disease of poultry and waterfowl. However, several species of wild raptors have been found in Eurasia after mortality events with HPAI H5N1. Should HPAI H5N1 reach North America in migratory birds, several species of raptors are at risk, not only from environmental exposure, but also from eating dead or infected birds. In this study, we used American kestrels as a surrogate species of North American raptor to examine the effects of HPAI H5N1 infection in terms of dose response, viral shedding, pathology, and survival in this species and other more endangered/threatened species. These experimental data showed that kestrels are highly susceptible to HPAI regardless of dose. All birds succumbed to infection within an average of 4–5 days and shed significant amounts of virus both orally and cloacally. Examination of tissues revealed that the virus was spread throughout the body, but the highest levels were found in the brain. This finding corresponded to the expression of symptoms, which were predominantly neurological. This is the first experimental study of HPAI in a North American raptor species and highlights the potential risks to other falcon, eagle, and condor species if HPAI comes to North America.

Forecasting the Space/Time Spread of West Nile Virus

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Forecasting depends on autocorrelation; events $Z(X, T)$ at a point X and an instant T tend to occur in runs and clusters—and diseases can occur in similar organisms. I use the 2003 West Nile virus outbreak among humans and birds to illustrate these ideas. The map shows that the epidemic was concentrated in the New York and upper Rocky Mountain region of the United States, but data were reported at the county level for three broad taxonomic classes and most of the human reports came from counties also reporting bird cases. A time series analysis shows that the three kinds of reports all occurred around the same time of year, with avian preceding human preceding veterinary cases. And when the counties are binned by week of first reporting, we see that most of the counties reported avian cases several weeks before human cases. We can use this conjunction to estimate relative risk: the extent to which knowledge of an avian report increases the probability of later human reports. This technique is illustrated for week 29, showing that later occurrences of human reports are more likely in counties reporting avian cases, and the forecasts predict about one-quarter of the variance. Finally, those counties reporting human cases during the year form an induced subgraph of the 3,141 U.S. counties, suggesting that spatial autocorrelation is likely to improve forecasting by using activities in neighboring regions to predict reports.

Viral Fitness Assays Used in vivo To Investigate Virulence Tradeoffs in an Acute Rhabdovirus of Rainbow Trout

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We have developed a system to assess viral fitness in live juvenile rainbow trout (*O. mykiss*) using field isolates of the rhabdoviral pathogen infectious hematopoietic necrosis virus (IHNV). In these assays, juvenile trout are infected with selected strains of IHNV that can be distinguished genetically. Groups of approximately 25 trout are infected by immersion with each strain alone, or with a 1:1 mixture of the two strains. Progeny virus populations are characterized after 3 days of in-host replication. During development of the assay, we showed that IHNV strains of equal virulence that co-circulate stably in an aquaculture setting have equal fitness in these assays. More recently, we have worked with two strains that reproducibly differ in virulence. In assays initiated with a 1:1 ratio of high-virulence (HV) and intermediate-virulence (IV) strains, the progeny virus populations after 3 days of in-host replication had a mean HV:IV ratio of 3:1. Thus, these strains differ in fitness, with the higher virulence strain being more fit. The correlation of higher virulence with higher fitness indicates a virulence tradeoff that may explain the persistence of high-virulence IHNV in the field. We are using modified assays to investigate the contribution of different stages of the virus infection cycle to the difference in viral fitness observed between HV and IV. Comparative assays using injection of virus rather than immersion indicated that ability to enter the host was partly responsible for the higher fitness of HV, but HV also had higher in-host replication than IV. Analysis of virus populations shed into the water indicates that both viral strains are shed at equal rates. In all our results, data from individual fish provide a high-resolution picture of the variability inherent in natural viral infection processes.

Diversity and Pathogenicity of *Avipoxvirus* from Hawaiian Forest Birds

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Reports by early naturalists suggest that pox viruses (*Avipoxvirus* spp.) have been affecting native Hawaiian forest birds for over 100 years. Clinical signs of the cutaneous form of pox in Hawaii include nodular lesions on unfeathered areas of the body, especially the feet. *Avipoxvirus* species are exceptional in that they possess genes that can modify innate host immunity, and therefore may play a significant role, with malaria, in the decline of native forest birds. To evaluate diversity and pathogenicity of *Avipoxvirus,* isolates from lesions collected from seven species of birds from four Hawaiian islands were cultured in Muscovy duck embryonic fibroblasts. We sequenced a fragment of the 4b core protein gene. To evaluate within-individual variation, multiple clones were sequenced from several individuals. Samples included isolates collected from 1993 to 2004. Ancient DNA techniques were used to obtain and confirm 4b sequence from a museum specimen of an infected `Elepaio (*Chasiempis sandwichensis*) collected in 1900 on Hawaii Island. Phylogenetic analyses indicate that at least two variants of pox exist in Hawaii that are approximately 6 percent divergent from each other. Within-individual variation appears minimal, suggesting that individuals are infected by a single pox variant. The pox variants we recovered in chickens appear to be fowlpox variants and are distinct from the canarypox-like variants detected in Hawaiian passerines. Reciprocal experimental infections suggest that one variant appears more virulent, and that neither long-term immunity nor reciprocal immunity was induced. We conclude that the two variants are either antigenically distinct or may induce only short-term immunity.

Disease Detection Methods

Detection of *Batrachochytrium dendrobatidis* (Chytrid Fungus) in Water and Amphibian Swabs Using PCR: Towards a Life History Assessment

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The fungal pathogen *Batrachochytrium dendrobatidis* (Bd) causes chytridiomycosis, a disease implicated in amphibian declines on five continents. A Bd-specific polymerase chain reaction (PCR) assay used to test swabs of amphibians for Bd has demonstrated that Bd is widespread in the United States and globally. Using the existing laboratory assays and primers, we developed a method to test for Bd in water by filtration, followed by extraction of DNA from particulates retained on the filter. Initial tests confirmed that Bd exists in the environment outside its amphibian hosts; however, insufficient data were available to indicate the range or temporal variability of Bd zoospore densities in ponds. Recently, we modified the field method to facilitate sample collection and preservation from remote sites, and we conducted monthly to bimonthly sampling from ponds in Oregon where amphibian populations (primarily *Rana catesbeiana*) have histologically confirmed infections with Bd. Seasonal patterns in zoospore densities in water and on frogs are shown in relation to water temperature and amphibian life cycle. Future testing will help elucidate the most likely exposure mechanisms for amphibians by defining the location and partitioning of Bd in ponds. Together with swabbing of amphibians, these PCR methods are expanding our knowledge of the lifecycle of Bd in the environment and its interaction with amphibians, and this expansion will foster the development of better management strategies for mitigating this global threat to amphibians.

Water Quality

Microbial Quality of Water in Selected Principal Aquifers of the United States

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As part of the National Water-Quality Assessment (NAWQA) program, microbiological data were collected from more than 1,200 wells in 22 NAWQA study units during 1993 through 2004. These wells were used for monitoring purposes, sources of water for domestic and public supplies, and irrigation. The wells constituted 3 major NAWQA sampling networks representing 16 principal aquifers. Samples of untreated groundwater were tested for concentrations of fecal-indicator bacteria and the presence of coliphage viruses. Summaries of these data showed that nationwide, coliform bacteria occur relatively frequently, as nearly 30 percent of all wells tested positive. Coliphage viruses, however, were rare, as fewer than 4 percent of untreated water samples from domestic and public supply wells tested positive. Coliform bacteria generally were more prevalent in samples from domestic wells than from public supply wells, in fractured or porous rock materials than in unconsolidated materials, and in principal aquifers with median well depths ranging from 100 to 200 feet than in aquifers with median depths less than 100 feet or greater than 200 feet. Of the 16 principal aquifers studied, waters most affected by the presence of coliform bacteria were those in the Valley and Ridge, Floridan, and Piedmont and Blue Ridge aquifers, where more than 50 percent of sampled wells tested positive. Waters least affected were those in the Columbia Plateau, High Plains, Stream and River Valley, Coastal Lowlands, Southeastern Coastal Plain, Basin and Range, and Snake River aquifers, where less than 20 percent of wells tested positive. The Valley and Ridge wells had the highest concentrations of total coliform bacteria with a median of 2 colony-forming units per 100 milliliters (CFU/100 mL) and a maximum of 1,600 CFU/100 mL. High concentrations of *Escherichia coli* (greater than 300 CFU/100 mL) were reported for wells completed in the Valley and Ridge aquifer in the eastern United States, as well as the Mississippian-Pennsylvanian aquifer and the Ordovician aquifer in Tennessee. More than 50 percent of wells completed in carbonate or crystalline rock types, such as in the Valley and Ridge and Floridan aquifers, tested positive for coliform bacteria. Conversely, less than 5 percent of wells completed in unconsolidated materials, such as those in the Snake River aquifer, or in basalt with sand and gravel interbeds, such as compose the Basin and Range aquifer, tested positive. Although lithologic composition might be one factor in

the occurrence of fecal-indicator bacteria in the aquifers, others, such as geohydrologic characteristics, proximity of contaminating sources, interactions with surface water, or well-construction features (including age of the well), likely control the presence and transport of these bacteria in the groundwater.

Quality Control of Quantitative Polymerase Chain Reaction (qPCR) for Environmental Water Samples

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Quantitative polymerase chain reaction (qPCR) requires routine quality control (QC): mainly, sample replication, a serially diluted standard curve, and a no-template control. Environmental water samples, however, require additional QC because of their more complex matrix. They may contain nontarget microorganisms whose DNA/RNA could overload the extraction process. Environmental samples also may contain PCR-inhibiting compounds that are coextracted with the target DNA/RNA. These PCR inhibitors may eventually lead to false-negative results or skewed quantitation. Therefore, it is important to incorporate controls into various steps of the process to guard against sample loss and skewed data. Internal controls (IC) are additional QC used to measure both DNA/RNA extraction efficiency and qPCR efficiency. When extracting a surface-water sample for a microbial source tracking (MST) assay, for example, there could be so much interference that only a small portion of the DNA is recovered. This interference-related loss can be estimated by coextracting an organism that is similar to the target but not found naturally in environmental samples. When running an assay for the direct detection of pathogens, larger sample volumes are typically used in order to enhance detection limits, but the larger the sample volume, the more PCR inhibitors are included in the sample. Matrix inhibition can be estimated by adding a known amount of the target into a separate PCR master mix, which can be run in parallel with nonspiked environmental samples. Full detection of the matrix spike indicates that the qPCR was not retarded by inhibitors. The USGS Ohio Water Microbiology Laboratory (OWML) currently uses noncompetitive IC, which means that the target and the IC use different primers and probes and the qPCR reactions are run separately. However, the OWML is currently testing a new competitive IC, which means that the target and the IC will share primers but have different probes and can be run in the same reaction. Use of competitive IC is an exciting new approach that will allow matrix inhibition to be run for each target in the same reaction as the test analysis.

Response of Groundwater Bacteria from Cape Cod, Mass., to Low Concentrations of Antimicrobials

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There is increasing focus on the protection of groundwater resources due to the growing reliance on groundwater for human consumption. Many antimicrobials from anthropogenic sources have been detected in low but significant concentrations in groundwater, which may disrupt groundwater microbial communities and lead to direct and indirect human health consequences. However, it remains unclear if the current detected levels of antimicrobials and the concomitant presence of antimicrobial resistance alter bacterial community structure and, thus, preclude bacteria from serving important ecological roles as decomposers, nutrient transformers, and contaminant removers. Recently, a field-scale test performed in Cape Cod, Mass., assessed the effects of sublethal concentrations of sulfamethoxazole (SMx) on microbial community viability. Microbial viability was compared between groundwater communities with no previous exposure to SMx (pristine) and those with previous exposure to SMx (contaminant-impacted). After 2 weeks of SMx exposure, viability staining with applied stains indicated that the ratio of viable to nonviable cells changed from 8:1 in both communities to 1:24 in the contaminant-impacted community and 1:762 in the pristine community. Thus, the pristine microbial community appeared much more sensitive to SMx exposure than the contaminant-impacted community. On the basis of these field data, we hypothesized that SMx exposure may inhibit some important microbial processes, such as denitrification. The removal of nitrate, a predominant groundwater contaminant, by microbially mediated denitrification was significantly reduced after adding 100 micrograms per liter (100 µg/L, or 0.39 µM) of SMx to a denitrifying groundwater isolate in the laboratory. The ecotoxicological impacts of SMx alone and in concert with trimethoprim (TMP) and lincomycin (LM) at environmentally significant levels on this isolate and on a groundwater-denitrifying community have been tested. In addition, the influence of a *sul* gene, which codes for resistance to SMx, on denitrification inhibition, has been studied.

Antibiotic Resistance in an Urban Watershed with Combined Sewer Overflows

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The U.S. Geological Survey (USGS), in cooperation with the City of Omaha, conducted a combined sewer overflow (CSO) monitoring project to measure the water-quality impacts of CSOs on receiving streams in the Papillion Creek watershed in Omaha, Nebr., between May and September 2007. Constituents analyzed in the samples include organic wastewater compounds, nutrients, metals, *E. coli,* chloride, biochemical oxygen demand, chemical oxygen demand, hardness, and bacterial antibiotic resistance. The monitoring network in the watershed consisted of 2 CSOs, 1 stormwater outfall, and 12 stream sites. Most stream samples analyzed for antibiotic-resistant bacteria were collected during scheduled nonstorm times. The scheduled samples were generally collected on the third Tuesday of every month. Stream samples were also collected during one CSO event. Samples were collected from the CSOs and stormwater outfall during selected storms during the study period. The antibiotic-resistance analysis indicated that up to 95 percent of the *E. coli* from a specific stream site were resistant to cephalosporin antibiotics. The cephalosporin-resistant *E. coli* (CRE) could be correlated with wet or dry weather samples (as indicated by specific conductance). Vancomycin-resistant enterococci were also detected in CSOs and in one stream sample. A cluster analysis of CRE concentrations indicated that the CSO samples and stream samples collected during or immediately after CSO discharges were associated with high CRE concentrations. In general, chemical concentrations from samples in this group contained higher trace metal concentrations and a greater number of wastewater chemical detections than the rest of the samples.

Use of Carboxylated Microbial-Sized Microspheres for Estimating *Cryptosporidium parvum* Oocyst Transport within Russian River (Sonoma County, Calif.) Bank Filtration Sediments

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Bank filtration, as practiced domestically and internationally, involves drawing surface water through riverbank and aquifer sediments to nearby collector wells. Generally, it is used as a cost-effective pretreatment option to reduce many common microbial and chemical contaminants. The efficacy of bank filtration systems to remove oocysts of the protistan pathogen *Cryptosporidium parvum* is particularly important because of their well-documented episodic high abundances in surface waters and high resistance to chemical disinfection. This presentation details small-scale laboratory studies using carboxylated microspheres and oocysts in assessing their transport potential through physically and geochemically heterogeneous sediments from a bank-filtration site along the Russian River in Sonoma County, Calif. Varied methodologies were used to assess how oocyst-sized microspheres are affected by passage through poorly sorted riverbank sediments between river bottom and horizontal collector wells. Initial field experiments suggested that the near-surface sediments, which are high in metal oxide content (~4 percent), have a high capacity for sorptive filtration of oocyst-sized microspheres (2–5 micrometers (µm) in diameter). Subsequent laboratory investigations revealed that metal oxide coatings on sediments were significantly related to the degree of microsphere and oocyst recovery. By contrast, grain size was an inadequate predictive tool for microsphere or oocyst recovery. This result suggests that the primary mode of microsphere and oocyst removal was likely sorptive rather than physical filtration. Pretreatment of sediments with low levels of natural organic material or SDBS (sodium dodecyl benzene sulfonate) anionic surfactant significantly decreased sediment efficiency for removal of oocysts or microspheres. Finally, while metal oxides on sediment surfaces may currently control or limit the transport of oocysts from river water to collector wells, our results suggest that slight changes in dissolved organic carbon content may reduce oocyst removal rates within this operating bank filtration system and make this water system more vulnerable to waterborne pathogen contamination.

Examination of the Watershedwide Distribution of *Escherichia coli* along Southern Lake Michigan: An Integrated Approach

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Recent research has highlighted the occurrence of *Escherichia coli* in natural habitats not directly influenced by sewage inputs. Most studies on *E. coli* in recreational water typically focus on discernable sources (for example, effluent discharge and runoff) and fall short of integrating riparian, nearshore, onshore, and outfall sources. An integrated beachshed approach that links *E. coli* inputs and interactions would be helpful to understand the difference between background loading and sewage pollution; to develop more accurate predictive models; and to understand the differences among potential, net, and apparent culturable *E. coli*. The objective of this study was to examine the interrelatedness of *E. coli* occurrence from various coastal watershed components along southern Lake Michigan. The study shows that once established in forest soil, *E. coli* can persist throughout the year, potentially acting as a continuous nonpoint source of *E. coli* to nearby streams; year-round background stream loading of *E. coli* can influence beach water quality. *E. coli* is present in highly variable counts in beach sand to depths just below the water table and to distances at least 5 meters (m) inland from the shore, providing a large potential area of input to beach water. In summary, *E. coli* in the fluvial-lacustrine system may be stored in forest soils; sediments surrounding springs, bank seeps, stream margins, and pools; foreshore sand; surface water; and groundwater. While rainfall events may increase *E. coli* counts in the foreshore sand and lake water, concentrations quickly decline to prerain concentrations. Onshore winds cause an increase in *E. coli* in shallow nearshore water, likely resulting from resuspension of *E. coli*-laden beach sand. When examining indicator bacteria source, flux, and context, the entire beachshed as a dynamic interacting system should be considered.

Seasonal Stability of *Cladophora*-Associated *Salmonella* in Lake Michigan Watersheds

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The bacterial pathogens *Shigella, Salmonella, Campylobacter,* and shiga toxin-producing *E. coli* (STEC) were recently found to be associated with *Cladophora* growing in southern Lake Michigan. Preliminary results indicated that the *Salmonella* strains associated with *Cladophora* were genetically identical to each other. However, because of the small sample size (n=37 isolates) and a lack of information on spatial-temporal relationships, the nature of the association between *Cladophora* and *Salmonella* remained speculative. In the present study, we investigated the population structure and genetic relatedness of a large number of *Cladophora*-borne *Salmonella* isolates (n=133) from Lake Michigan, as well as those isolated from stream and lake water (n=31), aquatic plants (n=8), and beach sands and sediments (n=8) from adjacent watersheds. *Salmonella* isolates were collected during 2005–2007 between May and August from Lake Michigan beachsheds in Wisconsin, Illinois, and Indiana. The genetic relatedness of *Salmonella* isolates was examined by using the horizontal, fluorophore-enhanced repetitive polymerase chain reaction (rep-PCR) DNA fingerprinting technique called HRERP. While the *Salmonella* isolates associated with *Cladophora* exhibited a high degree of genetic relatedness (≥92 percent similarity), the isolates were not all genetically identical. Spatial and temporal relationships were evident in the populations examined, with tight clustering of the isolates both by year and location. These findings suggest that the relationship between *Salmonella* and *Cladophora* is likely casual and is related to input sources (for example, wastewater, runoff, and birds) and the predominant *Salmonella* genotype surviving in the environment during a given season. Our studies indicate that *Cladophora* is likely an important reservoir for *Salmonella* and other enteric bacterial pathogens in Lake Michigan beachsheds, which in turn may influence nearshore water quality.

Real-Time Estimation of Taste-and-Odor Occurrences in Cheney Reservoir, Kansas

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Cheney Reservoir, Kansas, is one of the City of Wichita's primary drinking-water supplies. Cyanobacteria-related taste-and-odor events in reservoirs are a concern because of aesthetics and water-treatment costs. Since 2000, the U.S. Geological Survey, in cooperation with the City of Wichita, has operated real-time water-quality monitors on the North Fork of the Ninnescah River, the main tributary to Cheney Reservoir, and in Cheney Reservoir. Real-time water-quality variables measured since 2000 include water temperature, specific conductance, pH, dissolved oxygen, turbidity, and chlorophyll fluorescence (an estimate of algal abundance). Data collected during 2001–2003 were used to develop a real-time water-quality model to estimate Cheney Reservoir geosmin (an earthy odor compound) concentrations in real time. Multiple regression analysis was used to develop a relation between geosmin concentrations and the real-time measured sensor variables turbidity and specific conductance. The resulting model is used to provide hourly estimates of geosmin concentration on the World Wide Web at http://nrtwq.usgs.gov/ks/. Evaluation of the model indicates that, within existing model limits (turbidity of <36 formazin nephelometric units (FNU), specific conductance of 790–915 microsiemens per centimeter (µS/cm)), geosmin estimates are conservative (overestimates are more likely than underestimates). However, model probabilities for exceeding the human detection limit of 10 nanograms per liter (ng/L) are fairly robust (probabilities were accurate for 76 percent of measured geosmin values during 2001–2008). Several additional real-time sensors have been installed in Cheney Reservoir since the development of the initial geosmin model including ones to measure the following: wind speed and direction, light, nitrate, and phycocyanin fluorescence (an estimate of cyanobacterial abundance). These new variables may facilitate additional model development and enhance understanding of the factors driving cyanobacterial bloom development and taste-and-odor occurrence. Ongoing studies at Cheney Reservoir will refine the relations between reservoir and inflow conditions and taste-and-odor occurrences and develop similar models for the cyanobacterial toxin microcystin. The City of Wichita currently uses the real-time geosmin estimates, along with other variables measured in real time, to make management decisions that have helped decrease water-treatment costs.

Cyanotoxins in Midwestern Lakes and Reservoirs

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Cyanobacteria (blue-green algae) produce a diverse group of toxins that target fundamental cellular processes and affect a wide range of organisms. Cyanobacterial toxins (cyanotoxins) have been implicated in human and animal illness and death in more than 50 countries worldwide, including at least 33 U.S. States. Human toxicoses associated with cyanotoxins most commonly have occurred after exposure through drinking water or from contact through recreational water activities. The hepatotoxin microcystin is believed to be one of the most common cyanotoxins. Because microcystin is a potential human-health risk, several studies were conducted to determine the occurrence of microcystin in Midwestern water resources. During the summers of 1999–2001 and 2004–2006, 359 lakes in Missouri, Iowa, southern Minnesota, and eastern Kansas were sampled for microcystin. Most lakes were sampled two to four times in one year or all years. Microcystin commonly was detected in the region, and 78 percent of lakes sampled had detectable concentrations (≥0.1 microgram per liter (µg/L) as determined by enzyme-linked immunosorbent assay (ELISA)) at least once. Total microcystin concentrations ranged from 0.1 to 52 µg/L (median=0.2 µg/L), with 21 percent of lakes having concentrations >1 µg/L and 4 percent having concentrations >10 µg/L. Microcystin concentrations >20 µg/L are considered to have a high risk for adverse health effects during recreational exposure. Seasonal studies were conducted on nine lakes during 2001–2004. Peak microcystin concentrations occurred in June to December; 66 percent of the lakes had peaks in September or October. More recent studies have examined the presence of other cyanotoxins in Midwestern lakes. Resource managers, drinking-water treatment-plant operators, lake associations, and local officials increasingly are faced with making decisions about cyanobacterial blooms that affect public awareness, exposure, and health. Although anecdotal reports are common, few studies have documented the occurrence of toxins other than microcystin in cyanobacterial blooms throughout the United States because few analytical methods are available. In addition, although the general factors affecting cyanobacterial bloom formation are well known, specific factors driving individual toxic occurrences are unclear. Understanding the

range of cyanotoxins that are present and identifying the biological, physicochemical, and hydrological factors affecting toxin occurrence are key to effective management of water resources and minimization of human-health risks.

The South San Francisco Bay Baylands Mercury Project: Mercury Status of Pond A8, Alviso Slough, and Alviso Marsh, Based on Sediment

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As part of the South Bay Salt Pond Restoration Project, construction is slated to begin in 2009 on a size-adjustable notch in the levee between Pond A8 and Alviso Slough. In preparation and to establish preconstruction conditions, an assessment of mercury (Hg) concentrations and chemical form was conducted for the top 2 centimeters (cm) of surface sediment of Alviso Slough (main channel and fringing marsh), of Pond A8, and in 17 additional reference marsh sites throughout the South San Francisco Bay. Total mercury (THg) and methylmercury (MeHg) concentrations were highest overall within Pond A8. Vegetated marsh sites along Alviso Slough also had almost twofold higher average THg and inorganic reactive mercury (Hg(II)R) concentrations than the combined group of reference marsh sites, although MeHg concentrations were not significantly different between Alviso and reference marsh groupings. Across all sites, Hg(II)R concentration was related to sediment oxidation-reduction conditions, such that the most chemically reducing sites (for example, Pond A8) had the lowest percentage of Hg(II)R (0.08 ± 0.06 percent of THg) and the most chemically oxidized sites (for example, vegetated marshes) had the highest percentage of Hg(II)R (2.4 ± 1.4 percent of THg). In spite of the lower Hg(II)R concentrations in Pond A8, the higher MeHg concentrations in this habitat (compared to both vegetated marshes and Alviso Slough main channel) appear to be driven by high rates of microbial activity that are stimulated by the high loading of readily degraded organic matter, in the form of phytoplankton. We predict that by facilitating the tidal flushing of Pond A8, and thus decreasing the amount of phytoplankton deposition to the benthos, MeHg concentrations in surface sediment will eventually decrease within Pond A8.

Microbial Ecology

Enumeration of Viruses and Prokaryotes in Deep-Sea Sediments and Cold Seeps of the Gulf of Mexico

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Little is known about the distribution and abundance of viruses in deep-sea cold-seep environments. Like hydrothermal vents, seeps support communities of macrofauna that are sustained by chemosynthetic bacteria. It was hypothesized that sediments close to these communities would be more microbially active and therefore would host higher numbers of viruses than nonseep areas.

Methods.—Push cores were taken at six Gulf of Mexico sites at water depths below 1,000 meters. These sites included reference sediment near coral gardens, brine seeps, microbial mats, an urchin field, and a pogonophoran worm community. Once on deck, cores were immediately transferred to a 10°C cold room where sediment samples (0–2 centimeters (cm)) were collected from the core tops. Replicates were sonicated in sterile, 0.02-micrometer (μm)-filtered deionized water for 30 seconds on ice to extract the viruses and bacteria, followed by a low-speed centrifugation (800 xg) for 1 minute to pelletize the larger particles. Immediately after extraction, aliquots of the supernatant were filtered onto 0.02-μm filters, stained with SYBR Gold, mounted on glass slides, and frozen at –20°C. The slides were counted by epifluorescence microscopy within 48 hours.

Results.—Bacterial counts were an order of magnitude lower in sediments directly in contact with macrofauna (urchins, pogonophorans) than counts for all other samples (10^7 vs. 10^8 cells/gram dry weight) and were highest in areas of elevated salinity (brine seeps). Viral counts were lowest in the reference sediments and pogonophoran cores (10^8 virus-like particles/gram dry weight), high in brine seeps (10^9 VLP/g dry wt), and highest in the microbial mats (10^{10} VLP/g dry wt). Virus-bacteria ratios (VBR) ranged from <5 in the reference sediment to >30 in the microbial mats and >60 in the urchin field.

Summary.—Viral counts and VBR ratios were all significantly greater than those reported from sediments in the deep Mediterranean Sea and in some cases were higher but on par with recent data from cold seeps off Japan. This result indicates that greater microbial activity in or near cold-seep environments results in greater viral production and higher numbers of viruses.

Microbial Decomposer Communities in Alaskan Permafrost Soils and Their Response to Thaw

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Permafrost-protected soil carbon in boreal forest ecosystems represents a significant portion of the approximately 500 gigatons (Gt) of C in the soil organic matter of boreal regions. The magnitude of this thermally protected carbon pool makes it particularly important to the global C cycle within the context of global climatic change. Permafrost has acted as a C sink for thousands of years, yet it currently has been warming at a rate of 1°C per decade, making the C contained within it potentially available for decomposition. Thawing permafrost opens a latch into a globally important C reservoir that could be released to the atmosphere (as CO_2) and rivers (as dissolved organic carbon, DOC), affecting greenhouse warming and aquatic chemistry. A gap in our current knowledge is the extent to which permafrost-protected C is available for microbial metabolism once soils thaw. Current indications are that organic matter contained within permafrost is relatively labile, since it is not protected from decomposition by physical protection or humification mechanisms. However, we have little understanding of the microbiology of permafrost soils, which could significantly affect the rate of decomposition of permafrost C after thaw. Our aim was to use quantitative molecular techniques to examine the abundance of microbial decomposer functional groups in permafrost soils, the enzymes they encode, and their rates of respiration under both aerobic and anaerobic conditions in a simulated summer thaw at 5°C. We compared microbial and chemical characteristics of active layer and permafrost soils from black spruce stands in three distinct geographic regions: Coldfoot, Hess Creek, and Smith Lake, Alaska. We chose these regions because they span a range of permafrost conditions from shallow active layers and mineral-associated permafrost layers to thick active layers and deep organic permafrost soils. Soil carbon and nitrogen concentrations did not differ between active layer and permafrost soils within sites, and neither did the relative abundance of total bacteria and methanogens. In contrast, total fungal abundance and basidiomycete abundance were strongly reduced in permafrost soils. We tested whether the reduction in fungal abundance in permafrost soils could affect the turnover of soil carbon in thawed permafrost. We incubated soils under aerobic and anaerobic conditions at 5°C for 3 months. We are currently examining the changes in microbial respiration and enzyme activities that result from the incubation, as well as microbial population shifts. We are testing the hypothesis that low fungal biomass in permafrost soils will reduce the rate of decomposition of organic matter during summer thaw.

Fungal and Methanogen Functional Groups in Boreal Forests and Wetlands in Interior Alaska: Abundance, Diversity, and Process in the Face of Environmental Change

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Boreal forest ecosystems cover nearly 1.2 billion hectares (ha) of the surface of the Earth, equivalent to 17 percent of the total land surface, and contain approximately 90 gigatons (Gt) of carbon (C) in vegetation and 470 Gt C in soil organic matter and forest floor litter. Such large pools of C within the boreal ecosystem, equivalent to half of the total carbon within all terrestrial ecosystems, make it particularly important to the global carbon cycle and global climatic change. If the carbon balance in boreal ecosystems were significantly altered, then the rate of accumulation of carbon within the atmosphere could accelerate, resulting in a positive feedback to climate change. Moisture is a primary control over plant community distributions and C cycling processes in interior Alaska. Most soil carbon is contained in saturated soils having low decomposition rates. Changes in moisture and temperature have the potential to release large quantities of soil carbon to the atmosphere. Therefore, we have begun a comprehensive assessment of pools and fluxes of carbon across a moisture gradient, fungal and bacterial communities affecting C cycling, and the sensitivities of microorganisms to environmental change.

Fire and Ice: Examining Fungal Mechanisms of Recalcitrant Carbon Decomposition in Response to Fire in the Boreal Forest Landscape

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Heterotrophic microbial communities within boreal forest ecosystems control the fate of large pools of soil organic carbon (C) through decomposition and mineralization, yet the factors that control microbial communities are not well understood. Boreal forest ecosystems contain approximately 470 gigatons (Gt) of C in soil organic matter and forest floor litter and thus have a significant effect on the global C cycle. Here we examine the landscape-level variation in microbial community composition in order to understand (1) the primary factors that control microbial communities in boreal forest ecosystems and (2) the effects of variations in community composition on the cycling of C. Soil drainage and fire are two of the most important factors affecting boreal forest structure and function. We hypothesized that the biomass and diversity of soil fungi would increase with successional stage (5 and 60–120 years after a fire) and with decreasing soil moisture. We further hypothesized that increased fungal biomass and diversity would increase the capacity of the soil microbial community to decompose recalcitrant carbon. Fungal community composition and diversity were analyzed by denaturing gradient gel electrophoresis (DGGE) and denaturing high performance liquid chromatography (DHPLC) of the fungal internal transcribed spacer (ITS) region of community DNA. Fungal biomass was determined by quantitative polymerase chain reaction (qPCR) analysis of the ITS region. Functional analysis of the microbial community was performed by using oxidative enzyme activities (required to degrade recalcitrant C) and qPCR of phenol oxidase functional genes. Fire and higher soil moisture reduced the diversity and size of the soil fungal community by 50 to 75 percent, correlating to reduced (50 to 75 percent) phenol oxidase enzyme activities, primarily in the fibric (F) horizon. Lower phenol oxidase enzyme activities may, in turn, reduce the decomposition of recalcitrant carbon, or the “slow” pool of carbon used in modeling studies. We also discovered fungal mats appearing in a small number of F horizon samples from unburned soils that had tenfold higher phenol oxidase enzyme activities than the surrounding soil community. The organism composing the fungal mat, when present, dominates the soil fungal community. As such, it may be an example of a single microbial population that strongly affects the turnover of soil C at small scales within boreal ecosystems, yet what controls its distribution or activity is unknown.

In situ Measurement of Microbially Catalyzed Nitrification and Denitrification Rates in Stream Sediments Receiving Coalbed-Methane Discharge, Powder River Basin, Wyoming

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Dissolved methane collected from deep subsurface coal beds is a significant source of domestic energy production. Water from coalbed-methane (CBM) wells typically contains several reduced chemical species including ammonium (1–8 milligrams nitrogen per liter (mg N/L)) that can affect the quality of surface water and groundwater. The transport and fate of ammonium are influenced by microbial activities occurring in sediments of streams receiving water discharged after CBM recovery. In the presence of oxygen, nitrifying bacteria oxidize ammonium, producing nitrite and nitrate, both of which can be subsequently reduced to nitrogen gas by denitrifying microorganisms. Thus, microbial activity can potentially influence the nitrogen status of CBM discharge waters and serves as the only mechanism for permanent nitrogen removal in situ. We examined microbially catalyzed nitrification and denitrification in CBM discharge streams using in situ sampling chambers designed to capture a parcel of water and sediment and observed short-term temporal changes in stream chemistry. Rates of dissolved inorganic nitrogen (DIN) removal were highest (3.5 milligrams nitrogen per square centimeter per hour (mg N/cm^2*hr)) in light incubations when oxygen levels were temporally high at locations where ammonium levels were elevated (>4.9 mg N/L). This high rate of DIN removal resulted in accumulation of nitrite and nitrate. However, these endproducts accounted for <15 percent of the ammonium consumed, suggesting that an additional fate for ammonium exists at this site. At the same location, the rate of DIN removal in the dark (0.3 mg N/cm^2*hr) was <10 percent of the value observed in the light. Although microbial nitrate and nitrite consumption was observed under both light and dark incubations, the rate was faster under the latter condition when oxygen levels were lowest. Nitrogen cycling in CBM discharge water exhibits a diel pattern that is likely related to the flux of oxygen in stream sediments. In situ sampling chambers can be used to estimate microbial activity rates in CBM discharge streams and gage the impact of those processes on stream geochemistry.

Microbial Methanogenesis in Laboratory Incubations of Coal: Implications for a Sustainable Energy Resource in Subsurface Coal Beds

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Methane desorbed from subsurface coal seams contributes about 8 percent of the total natural gas produced in the United States. This value is expected to increase over the next several years as a growing proportion of energy demands is supplied from unconventional reservoirs. Isotopic analyses of gas samples from several geographically separate coal beds indicate that a substantial proportion of the sorbed methane is biogenic in origin. Furthermore, previous studies have shown the ability of microbial consortia to degrade coal in aerobic laboratory incubations. These findings suggest that stimulation of microbial methane production in subsurface coals may provide a sustainable source of domestic energy. To address this prospect, we assessed the ability of indigenous microbial populations to produce methane in coal maintained under anaerobic conditions in the laboratory and investigated factors that influenced the rate and extent of the process. Several freshly collected coals of different rank were examined for their ability to support methanogenesis in mineral medium alone or amended with different nutrients such as hydrogen (4 kilopascals (kPa)), formate (20 millimolar (mM)), or acetate (25 mM). Microbial methane production was distinguished from abiotic desorption by subtracting methane generated in replicate incubations that contained bromoethanesulfonic acid (5 mM), an inhibitor of methanogenesis. The extent and rate of methane production varied among the different coals. A relatively shallow (400 meters (m)), immature coal exhibited a rate of 700 nanomoles of methane per day per gram of coal (nmole CH_4*day^{-1}*g $coal^{-1}$), a value comparable to previous observations of contaminated sediments. Methane production was negligible in a deeper (650 m), relatively mature coal obtained from the same borehole, although the same material exhibited a rate of about 80 nmole CH_4*day^{-1}*g $coal^{-1}$ after a formate amendment. In contrast, hydrogen proved to be ineffective as a methanogenic substrate, although this electron donor was rapidly consumed in coal incubations. A filter-sterilized warm water extract of spent coal renewed methanogenesis in incubations no longer generating methane, suggesting the cessation of methane production was not due to moribund cells or the accumulation of an inhibitory compound, but rather the lack of suitable electron donor. Viable methanogenic consortia were present in most of the coal samples examined in this study, and their activity could be enhanced by electron donor amendment, which presumably supports microbial growth. Furthermore, the observation of rapid hydrogen consumption uncoupled from methanogenesis suggests that competition exists for this compound. The success of efforts to stimulate methanogenesis in subsurface coal beds will likely be influenced by the nature of the electron donor.

Chemical Character and Lability of Carbon Across a Chronosequence of Drained Sediments of a Drying Arctic Lake, Interior Alaska

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Drying of lakes over large regions in the arctic and subarctic has been occurring during the past several decades, exposing lake sediments to aerobic conditions. This drying likely leads to changes in sediment carbon (C) chemistry and lability due to microbial degradation and encroachment of terrestrial vegetation. Characterization of C chemistry and lability in these drained sediments is critical to understanding its role as a biotic substrate and as a source of greenhouse gases. We collected sediments from a drying lake in interior Alaska, including flooded sediments (T0) and areas continuously or intermittently drained for approximately 5 years (T1), 15 yrs (T2), and 30 yrs (T3). We characterized sediment C chemistry (elemental and pyrolysis-gas chromatography/mass spectrometry analyses) and lability of solid and leachable C. Organic C content of surface sediments decreased from 16 to 10 grams of carbon per gram of sediment (g C/g sediment) from T0 to T3, while dissolved organic carbon (DOC) yield and aromaticity were greatest from T3 sediments. Incubation results (3.5 months, 10°C) indicate that C in T3 sediments is least labile. This finding suggests that C is quickly oxidized from drained sediments, leaving recalcitrant C behind.

Indicators of Ecosystem Health and the Impact of Mineralized Terrane

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Studies of soil quality and ecosystem health employ a wide variety of approaches designed to monitor ecosystem functioning within an array of natural and anthropogenic disturbances. Definitions of soil health vary, but they typically include the soil's role in mediating nutrient, water, and energy cycles in support of associated flora and fauna. The limited mobility and inherent sensitivity of soil microbial communities to external stressors make them potentially well suited for the study of stressors on ecosystem health. Additional measures of plant community characteristics can provide a more complete picture of the impact of a natural or anthropogenic disturbance on an ecosystem due in part to the roles of vegetation in phytoremediation, the hydrologic cycle, and nutritional value to fauna. This study is focused on metal-microbe-vegetation interactions in mineralized terrane of terrestrial ecosystems and impacts on ecosystem functioning. Relationships among microbial and plant activity and composition and abiotic soil parameters will be used to develop ecosystem health indices that could be used in support of geoenvironmental assessments and reclamation monitoring. An extensive reconnaissance study within several Western U.S. mining districts of contrasting mineralogy and climate is underway, examining the differences in metal-biogeochemical interaction between mineralized and adjoining nonmineralized areas in various ecosystems. Study areas include a sage-grassland site impacted by copper/molybdenum, an oak-pine-grass site impacted by nickel/chromium, and two sage-pinyon-juniper sites impacted by acid sulfate mineralization. Preliminary data will include (1) soil biota: microbial biomass (phospholipid fatty acids analysis, PFLA), enzyme activity (fluorescein diacetate (FDA) hydrolysis), functional diversity (Biolog Ecoplate), and C/N mineralization potential (lab incubation); (2) vegetation: density, cover, and biomass; and (3) soil abiotic measures: total and bioavailable metals, pH, texture, electrical conductivity, and bulk density.

Climate-Induced Changes in Nitrogen Dynamics in Loch Vale Watershed, Rocky Mountain National Park

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Mountain terrestrial and aquatic ecosystems are responsive to external drivers of change, especially climate change and atmospheric deposition of nitrogen (N). We explored the consequences of an overlay of climate change on an alpine and subalpine watershed in the Colorado Front Range that has long been the recipient of elevated atmospheric N deposition. Mean annual nitrate concentrations increased by 33 percent, and mean annual nitrogen export has increased by 28 percent from the Loch Vale watershed since 2000. Measured inorganic nitrogen values since 2000 are the highest observed since monitoring began in 1982. The substantial increase in nitrogen dynamics comes as a surprise, because atmospheric N deposition has not increased during this period. Coincident with the increase in watershed nitrogen loss and stream nitrogen concentrations, there has been a period of below-normal precipitation and an increase in temperatures, especially mean annual temperature, which increased from a mean of 1.3°C for the years 1985–1999 to a mean of 1.7°C for 2000–2006. The temperature increase is driven by a strong increase in July mean and minimum temperatures. Nitrate concentrations, as well as the weathering products calcium and sulfate, were higher for the period 2000–2006 in rock glacier meltwater at the top of the watershed, suggesting minimal influence of alpine and subalpine vegetation and soils. We conclude that the observed N increases in Loch Vale are climatically induced, caused by melting ice in glaciers and rock glaciers that have exposed microbially active sediments. The phenomenon observed in Loch Vale may be indicative of nitrogen release from ice features worldwide as mountain glaciers retreat. In regions that are chronically ultra-oligotrophic, additional nitrate may stimulate algal productivity and affect species assemblages, such as we have already observed.

Metabolic Processes

Laboratory Studies of Biogenic Methane Production from Coal by Microbial Consortia: Identifying Organisms, Reactions, and Intermediate Products

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Many isotopic studies have indicated that some coal beds produce biogenic methane. Little is known, however, about the organisms, reactions, and intermediate products involved in the process of biogenic methane formation from geopolymers like coal. Laboratory microcosms of coal and nutrients, some with an added microbial consortium (WBC–2) enriched from a modern wetland, were used to study the production of methane from coal. Subbituminous coal samples from the Wilcox Group (Zavala County, Tex.) and the Fort Union Formation (Campbell County, Wyo.), both known to produce biogenic methane in the field, released 56 and 16 standard cubic feet per ton (scf/ton) of methane, respectively, in laboratory experiments with WBC–2 added. Generation of methane from coal involves a consortium of microorganisms that ferment complex organic compounds to methanogenic substrates, which are then converted to methane. Microbial biomass in the coals was low and methane generation was limited by methanogen growth, which did not always occur. When WBC–2 was added, organic compounds released from the coal, including aromatics and long-chain hydrocarbons (fatty acids and alkanes), were degraded over about 70 days. Acetate accumulated initially, then decreased as methane was formed. WBC–2 also produced methane in coal-free treatments with organic compounds such as octadecanoic, hexadecanoic, benzoic, and vanillic acids, confirming that coal intermediate products can be fermented to methane precursors. There was a shift in the WBC–2 microbial population grown in coal microcosms, indicating growth of some new dominant members specific to coal fermentation. Phylogenetic identification of community members in the coal incubations will be useful for understanding and manipulating in situ coal-bed populations.

Microbially Induced Temperature Changes in a Petroleum Hydrocarbon Plume

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The degradation reactions of organic contaminants are often exothermic. Given this, the degradation of organic contaminants in an aquifer should produce measurable temperature increases if the heat is generated faster than it is dissipated. The groundwater contaminant plume at a crude oil spill site near Bemidji, Minn., has been undergoing aerobic and anaerobic biodegradation for 28 years. At this site, the theoretical degradation of 100 milligrams per liter (mg/L) of phenol, a representative compound, under aerobic conditions could generate a 2°C increase in groundwater temperature with no heat loss and an aquifer heat capacity of 2,494 joules per liter per degree Celsius (J/L-°C). The temperature in the aquifer was measured with an accurate thermistor (≤±0.01°C) that was lowered to multiple depths in 13 monitoring wells along a groundwater flowpath. The measurements were taken from 0.15 to 12.62 meters (m) below the water table. Temperatures ranged from 6.35°C in the background aquifer to 9.19°C just under the crude oil source. These data revealed a thermal plume co-located with a previously observed area of benzene, toluene, ethylbenzene, and xylene (BTEX) biodegradation under iron-reducing and methanogenic conditions. The results indicate that evidence of exothermal microbial reactions within contaminant plumes can be detected by using sensitive and detailed temperature measurements in wells.

Denitrification-Coupled Iron Oxidation in Cultures from Aquifer Sediments from Cape Cod, Massachusetts

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The ability of microorganisms to use Fe(II) as an electron donor for the reduction of nitrate via the denitrification pathway was examined within a groundwater contaminant plume on Cape Cod, Mass. The plume originated from the disposal of treated wastewater and contained high concentrations of nitrate. Field studies had previously shown nitrate reduction occurring in an anoxic Fe(II) zone along with a decrease in the concentration of Fe(II). Core material

was collected from this reduced-iron zone within the aquifer and used to create anaerobic enrichment cultures containing reduced iron, organic carbon, and nitrate. The enrichments exhibited anoxic iron oxidation in conjunction with nitrate reduction to nitrogen over a period of several weeks. Growth experiments showed that nitrate (2 millimolar (mM)) and acetate (1 mM) concentrations were depleted within 2 weeks while Fe(II) decreased at a much slower rate with about half of the initial concentration oxidized to Fe(III) after 12 weeks. The enrichments were also capable of autotrophic denitrification when grown with Fe(II) and hydrogen as electron donors but were not capable of autotrophic growth solely on Fe(II). Preliminary studies indicate that these enrichments are able to reduce perchlorate, a contaminant found in some drinking water and groundwater sources, when substituted for nitrate. Several bacterial strains were isolated from the enrichments and subsequently identified using 16S rRNA gene sequencing. Most of the isolates belonged to the phyla *Proteobacteria* (mostly Burkholdera and Xanthomonads) and *Actinobacteria* (Cellulomonads) and were distinct from known strains of iron-oxidizing denitrifiers. Growth experiments with the isolates indicated differences in their ability to reduce various nitrogen oxides in the presence of iron. The results of these studies provide details of a potentially important redox reaction occurring in nitrate-contaminated aquifers because nitrate intrusion into iron-reducing zones may be an increasingly common situation in many subsurface environments.

Cycling of Methylmercury in the Sediments of the Penobscot River Estuary, Maine

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Due to the presence of high concentrations of organic matter and sulfate, estuarine sediments may produce a great deal of methylmercury (MeHg). Using equilibrium dialysis samplers and molecular analysis of the resident microbial population in contaminated sediments of the Penobscot River estuary (Maine), we observed a correspondence between the density of the sediment sulfate-reducing bacteria and methylation in pore waters. We also observed rapid MeHg demethylation close to the sediment-water interface (SWI) that may be due to the presence of iron-reducing bacteria that are dominant close to the SWI, or to the presence of *mer* genes that may be expressed in contaminated sediments, such as the ones studied here. We studied Hg methylation in intact sediment cores by manipulating the location of the redoxcline in the laboratory. Induced shoaling of the redoxcline was correlated with a shoaling of the net MeHg production zone and an increase in the net MeHg production rate. This study indicates that environments such as saltpans, where the shallow redoxcline leads to the shoaling of the MeHg front, are likely to be Hg methylation and release hotspots in coastal environments.

Role of Microbes in Attenuation and Mobilization of Arsenic at the Lava Cap Mine Superfund Site, Nevada County, California

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Iron-oxidizing, iron-reducing, and arsenic-reducing microbial communities at the Lava Cap Mine Superfund site (Nevada City, Calif.) were investigated using culturing, chemical, and spectroscopic techniques to understand the interplay between processes that attenuate and mobilize arsenic (As) in surface waters. Sheaths formed by bacteria of the genus *Leptothrix* physically support a community of ferrous iron-oxidizing bacteria suspended in the water column. This community induces the precipitation of ferric (hydr)oxide, which has a high sorptive capacity for As (1,000 to >10,000 milligrams per kilogram (mg/kg) As, dry weight). X-ray absorption spectroscopy indicates that arsenic is sorbed to iron (hydr)oxide. This process removes arsenic from water, which is desirable, but it is also potentially reversible. Since iron- and (or) arsenic-reducing bacteria could enhance the desorption of arsenic from iron (hydr)oxide, their numbers were estimated using the most-probable-number technique. The results indicate that iron- and arsenic-reducing bacteria are present in the iron-oxidizing microbial community dominated by *Leptothrix,* and their reducing activity may be responsible for the high iron and arsenic concentrations observed in soil pore waters. Denaturing gradient high-performance liquid chromatography indicates changes in the microbial community in space and time; identification of community members is ongoing. These lines of evidence suggest that a cycle of arsenic mobilization and immobilization exists and is associated with blooms of iron-oxidizing bacteria. Additional studies are needed to determine the factors controlling the flux of arsenic within each compartment of this cycle and the ways that the microbial community responds to and (or) influences this cycling.

Cr(III) Oxidation by Soluble Mn(III) Chelates: A Potential Biogeochemical Pathway for the Enhanced Mobilization of Cr from Spinels

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Soils derived from ultramafic (UM) rocks are enriched in Cr(III), mainly due to the presence of chromite ($FeCr_2O_4$) and other Cr(III)-bearing spinels. Weathering of UM rocks in the Coast Range and Sierra Nevada mountains of California has impacted the geochemistry of alluvial soils in the Sacramento Valley, which are enriched in Cr (30–1,420 micrograms per gram ($\mu g\ g^{-1}$)) relative to the U.S. geometric mean (37 $\mu g\ g^{-1}$). Although much of the Cr in these soils appears to be bound in refractory spinels, some mobilization of Cr is apparent in the coincidence of enriched soils with elevated Cr(VI) in groundwater (up to 50 micrograms per liter ($\mu g\ L^{-1}$)). It was recently reported that dissolution of chromite can be driven by the oxidation of the released aqueous Cr(III) on soil Mn oxides (Oze and others, 2007). We are studying the oxidative dissolution of chromite by soluble Mn(III) as a an additional Cr mobilization pathway. Free aqueous Mn(III) is a strong oxidant but rapidly disproportionates to Mn(II) and Mn(IV). Various chelates such as oxalate and pyrophosphate can stabilize Mn(III) in solution. Mn(III)-oxalate is produced by extracellular enzymes of white-rot fungi and acts as a diffusible oxidant in lignin degradation. Pyrophosphate (PP) is a hydrolysis product of adenosine triphosphate (ATP) and is also a component of some fertilizers. Its reaction with soil Mn oxides can result in soluble Mn(III)-PP. We measured the reduction potential of a 1-millimolar (mM) solution of Mn(III)-PP as a function of pH. The reduction potential decreased from ~1.1 volts (versus standard H_2 electrode) at a pH of 2 to ~0.6 V at a pH of 7. Consistent with these measurements, Mn(III)-PP oxidized a greater percentage of aqueous $CrCl_3$ at lower pH levels. After 2 weeks reaction time, 94, 63, 28, 3, and <1 percent of Cr(III) was oxidized to Cr(VI) at pH levels of 3, 4, 5, 6, and 7, respectively. Forty-five times as much Cr(VI) was produced from chromite grains (63–150 micrometers (μm) in diameter) incubated for 8 days in a Mn(III)-PP solution at a pH of 3 than from grains incubated with solid manganese oxides at the same pH. As expected, the chromite and Mn(III)-PP incubation produced one tenth as much Cr(VI) at pH 5 as at pH 3. Our initial studies of Mn(III)-oxalate suggest its ability to oxidize aqueous $CrCl_3$, but only in a narrow, circumneutral pH range.

Reference Cited

Oze, Christopher, Bird, D.K., and Fendorf, Scott, 2007, Genesis of hexavalent chromium from natural sources in soil and groundwater: Proceedings of the National Academy of Sciences, PNAS, v. 104, no. 16, p. 6544–6549, doi:10.1073/pnas.0701085104, available at http://www.pnas.org/content/104/16.toc.

A Proteomic Profile of a Novel Arsenite-Oxidizing Bacterium *Alkalilimnicola ehrlichii* strain MLHE-1T

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Arsenic speciation, mobility, and toxicity can be greatly influenced by microbial activity (for example, arsenate reduction, arsenite oxidation, methylation, and demethylation). The arsenite oxidizer *Alkalilimnicola ehrlichii* strain MLHE-1T is a haloalkaliphilic gammaproteobacterium isolated from Mono Lake, Calif. It has the unique ability to grow both aerobically as a heterotroph and anaerobically as a chemolithoautotroph by coupling arsenite oxidation to nitrate reduction. The latter ability has implications for arsenic and nitrogen cycling, as well as for carbon fixation in anoxic environments. The genome of MLHE-1T has been sequenced, and the operons for carbon fixation, CO oxidation, nitrate reduction, and arsenic resistance have been identified. Surprisingly, no homologue for arsenite oxidase was found, but two arsenate reductases were discovered. In addition to the dissimilatory nitrate reductase, homologues for nitric oxide reductase and nitrous oxide reductase were found. MLHE-1T lacks a homologue for nitrite reductase, and nitrite is the end product of nitrate respiration. A proteomic approach was employed to understand the complex physiology of this novel bacterium. Two-dimensional polyacrylamide gel electrophoresis (PAGE) was used to separate proteins present in the soluble (for example, cytosolic) and particulate (for example, membrane) cell fractions of the bacteria grown under both oxic and anoxic conditions. Blue native gels were also used to separate individual proteins of membrane-bound complexes. Trypsin-digested samples were analyzed with matrix-assisted laser desorption/ionization time-of flight (MALDI-TOF) mass spectrometry. Proteins involved in aerobic respiration were identified in aerobically grown cells (for example, superoxide dismutase, catalase/

peroxidase I). Most of the Calvin cycle proteins (including RuBisCo) and nitrate reduction (for example, NarH) were identified in the anaerobic fraction. Interestingly, nitrous-oxide reductase (for example, NosZ) was a prominent protein in the anaerobic fractions. In addition, the catalytic subunit, ArrA (designated Mlg_0216 in the annotated genome), of one of the two putative arsenate reductases (ArrDCBAB) was found. Activity assays were subsequently performed to locate potential arsenite oxidases. Blue native and sodium dodecyl sulfate-PAGE (SDS-PAGE) gels revealed that the same Arr homologue exhibited oxidase activity using either oxidized DCIP or methyl viologen, as well as arsenate reductase activity using reduced methyl viologen. Our results suggest that this "respiratory arsenate reductase" functions in reverse as the arsenite oxidase.

Tools and Techniques

Flow Cytometry: An Essential Tool in Biomarker Research

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Gains in microbiological knowledge are led by technological advances. Flow cytometry (FCM) technology continues to expand through adaptations of new reagents and protocols. Multiple parameters are measured on cells and nuclei passing singly through a 488-nanometer (nm) laser beam. Data on cell size, light scatter, and specific stain emissions are collected and digitized for computer analyses. Typically, 10,000 to 100,000 cells are analyzed per sample (with replication) within 1.5 minutes. At the USGS National Wetlands Research Center, FCM is primarily applied to environmental studies with aquatic animals and amphibians to study sublethal stress manifested first at the suborganismal level (nuclei, cytoplasm, membranes, and extracellular fluids). Various biomarkers developed to quantify sublethal stress at these different levels of biological organization can be diagnostic and contribute to assessments of animal and ecosystem health.

Specific studies include those described below:

Immune System

- Research on surface glycoconjugates on the parasite (*Perkinsus marinus*) of the eastern oyster (*Crassostrea virginica*) to further understand pathogenic mechanisms. Life cycle changes were noted, similar to those in the malarial parasite.
- Investigation of hemocyte responses of the crayfish (*Procambarus clarkii*) as a nontarget species impacted by fipronil-coated rice. Noted reductions in intraneuronal chloride ions (excitability regulator) were similar to pathogen- or wound-induced responses.

Reproductive Success

- Assays for sperm quality (viability, mitochondrial function, cell counts, spermatogenic stage, DNA integrity, and acrosome reactivity) from impacted watersheds included western mosquitofish (*Gambusia affinis*) from Sonny Bono Salton Sea National Wildlife Refuge and the Imperial Valley in southern California; smallmouth bass (*Micropterus dolomieu*) and yellow perch (*Perca flavescens*) from Chesapeake Bay; razorback suckers (*Xyrauchen texanus*) and common carp (*Cyprinus carpio*) from Lake Mead National Recreational Area and the lower Colorado River; and green swordtails (*Xiphophorus helleri*) from Oahu, Hawaii. Microscopic image analysis and computer-assisted sperm motion analysis (CASA) are two complementary tools used for sperm quality assessment.

Genetic Integrity

- Examination of possible links of DNA impacts with frog abnormalities. Cytogenetic traits of wood frogs (*Lithobates sylvaticus*) from Kenai National Wildlife Refuge, Alaska, were studied by measuring blood cell DNA integrity, caspase 3 enzyme (apoptosis indicator), and DNA repair kinase.
- Exploration of heritable genetic changes and embryonic abnormalities resulting from DNA fragmentation in germ cells; these changes have been studied in many species.

Assessing change is central to understanding animal and aquatic ecosystem health and is germane to the management of their associated resources. The FCM technology has applications in a number of fields.

USGS Interdisciplinary Microbiology Web Site: Marketing USGS Microbiology Expertise and Capabilities

By Bernadette LeMasters[1]

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For anticipating and meeting research needs, a Web presence for USGS microbiology can provide microbiologists with a globally accessible marketing tool for potential collaborators, resource management decisionmakers, and the general public. Web presence in a consolidated Web site could not only offer a comprehensive, "big picture" view of the species and science topics USGS microbiology covers, but also supply information distributed across the Web about the diversity of USGS scientists, research activities, science centers, publications, data, and available tools. This new Web presence could facilitate the interconnection of scientists with peers, leaders, and the public for greater collaboration opportunities and an improved understanding of the role of USGS microbiology in supporting the sound management of the Nation's natural resources.

Application of Phospholipid Fatty Acids in the Evaluation of Post-Katrina Wetland Soils

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The combined effects of Hurricanes Katrina (landfall Aug. 29, 2005) and Rita (landfall Sept. 24, 2005) resulted in a catastrophic loss of wetlands, with an estimated decrease of 562 square kilometers (km^2) of land area (Barras, 2006) along the Louisiana coast of the Gulf of Mexico. A study was initiated following the 2005 hurricane season to characterize storm impacts on coastal marsh soils by measuring soil organic carbon, biogeochemistry of soil pore waters, and soil microbial communities using phospholipid fatty acids (PLFA). Areas selected for study include Caernarvon, which had the greatest land loss through Katrina, and the Barataria Preserve, a unit within the Jean Lafitte National Historical Park. Marshes ranged from freshwater to saline. PLFA concentrations were generally greater in surface soils (28–144 nanomoles of PLFA per gram (nmol PLFA/g) dry soil at 0–5 centimeters (cm)) than in deeper soil (27–77 nmol/g at 10–15 cm; 18–20 nmol/g at 35–45 cm) for soils collected in March 2006. There was a notable exception to this trend. The concentration of PLFAs was greater at 15 cm (51 nmol/g) than at 5 cm (28 nmol/g) in a remnant salt marsh soil from Caernarvon. The ratio of 17:0cy/17:0, a stress indicator, was greater at depths of 5 and 15 cm for this soil (6.4 and 7.3, respectively) than in other soils collected in March 2006 (1.9–6.4 at 5 cm; 1.2–5.4 at 15 cm). The inverted PLFA biomass and elevated 17:0cy/17:0 ratio at this location may reflect disturbance from Katrina 6 months after the storm. Differences in microbial community structure were noted between freshwater marshes and salt marshes, with a general decrease in PLFA concentrations with increasing salinity. A resampling of surface soil in September 2007 showed an increase in PLFA concentrations (64–148 nmol/g) and a decrease in 17:0cy/17:0 ratios (1.5–3.8). In addition, there were shifts in surface microbial communities, including an increase in a16:0 in freshwater marsh soils and an overall increase in 18:1ω9c, a biomarker for eukaryotic microorganisms, including algae and fungi. These shifts may reflect recovery in soil microbial communities 2 years after the landfall of Hurricane Katrina.

Reference Cited

Barras, J.A., 2006, Land area changes in coastal Louisiana after the 2005 hurricanes—A series of three maps: U.S. Geological Survey Open-File Report 2006–1274, 3 oversize sheets, available at http://pubs.usgs.gov/of/2006/1274/.

Breakout Session Outcomes, October 16, 2008

Five working groups met in breakout sessions on October 16, 2008, with goals as listed below:

- Working Group I: to plan a Fact Sheet on interdisciplinary microbiology in the USGS
- Working Group II: to plan a USGS interdisciplinary microbiology Web site
- Working Group III: to suggest ways to broadcast and publicize the types of microbiology conducted at the USGS
- Working Group IV: to identify emerging issues in USGS interdisciplinary microbiology research
- Working Group V: to identify potential opportunities for interdisciplinary microbiology work at the USGS

The highlights for each working group are summarized in table 1.

Working Group I: To Plan a Fact Sheet on Interdisciplinary Microbiology in the USGS

The seven topical sections in the agenda highlight the different types of microbiology conducted within the USGS and showcase the multidisciplinary nature of our research. The task for Working Group I was to consider the topics within each section and develop an outline for a Fact Sheet that would showcase each of these sections. The goal was not to show each and every person's research but to show general research areas that can be highlighted with a few specific USGS examples. A guide would be Fact Sheet 2006–3108, "Conservation Genetics in the USGS" (Jacobs and others, 2006). The purpose of an interdisciplinary microbiology Fact Sheet would be to provide an overview of our microbiological activities for those groups that would use it (customers, collaborators, and the public). It is important to list single discipline contacts at the end of the Fact Sheet and their email addresses and to note that these are the people who could provide more information.

Working Group I members.—Jay Hestbeck, Andrea Foster, Ean Warren, Don Stoeckel, Chris Ottinger, Richard Smith, Jennifer Underwood, Deborah Repert, JoAnn Holloway, David Blehert, Lee De Cola

Microbiology in the USGS, theme of Fact Sheet.—USGS microbiologists conduct prominent, interdisciplinary microbiology research encompassing viruses, bacteria, protozoa, and fungi relevant to central issues facing society today. Leading scientists conduct research spanning disciplines including wildlife health and disease, microbial ecology, public health and water quality, geomicrobiology, and ecosystem function.

Wildlife and Fish Disease

(Wording excerpted from the conservation genetics Fact Sheet:) Infectious disease is a natural component of all ecosystems, affecting the health and abundance of terrestrial, aquatic, and marine animals. Understanding the role of microbes (pathogens) causing these diseases enables managers to adopt strategies to mitigate their influence when necessary. Disease control can be particularly urgent in situations where pathogens alter wildlife population structures or functions within an ecosystem. In general, we strive to understand disease processes, develop diagnostic methods, describe incidence and distribution of significant pathogens, and develop strategies for prevention and control.

Examples.—Bat white-nose syndrome; disease impacts on wild marine fish

Other examples.—Epidemiology and evolution of rhabdoviruses in fish; North American avian flu surveillance; ecology and pathogenicity of *Avipoxvirus* and malaria in Pacific Island birds; mycobacteriosis in Chesapeake Bay; studies on monkeypox pathogenesis in rodents using in vivo imaging; role of wild rodents in environmental transport of prions; microbiology in marine wildlife disease

Wildlife and fish disease contact on Fact Sheet.—David Blehert, dblehert@usgs.gov

Microbial Ecology

Microbial ecology addresses the interaction of microorganisms with the environment. Microbes are ubiquitous in the environment, thriving in aquatic, atmospheric, and terrestrial settings. Microorganisms perform essential functions in these ecosystems, including microbes nutrient (carbon and nitrogen) cycling, organic matter decomposition, and contaminant degradation. Ecosystem health depends on symbiosis between microorganisms and the plant community.

Examples.—Endosymbiotic fungi in plants and their ecology and implications for climate change; soil crusts and their importance to prevention of desertification, ecosystem services, carbon sequestration, and water-quality improvements

Other examples.—Microbiology of deep-sea corals; chromatography for microbial analysis; soil community dynamics in sagebrush steppe and cheatgrass-invaded areas of the northern Great Basin

Microbial ecology contact on Fact Sheet.—Nicole DeCrappeo, ndecrappeo@usgs.gov

Table 1. Summary of breakout session outcomes from Working Groups I–V.

[Detailed notes for each group are in the text, where acronyms are defined]

Working group number and mission	Suggested products	Topics	Status
I, To plan a Fact Sheet on interdisciplinary microbiology in the USGS	Fact Sheet	Fact Sheet should contain examples of USGS microbiology in the following areas: • Wildlife and fish disease • Microbial ecology • Geographic patterns/visualization • Public health and water quality • Geomicrobiology • Ecosystem function	Implement as funds come available
II, To plan a USGS interdisciplinary microbiology Web site	Interdisciplinary Web page	A survey taken by workshop participants to determine Web page emphasis indicated the following four highest ranking areas: • Educate public about USGS microbiology and how it applies to national priorities such as health and resource management • Provide information about the diverse expertise of USGS microbiologists and include contact information • Provide links to USGS centers with microbiology expertise and (or) laboratories • Display USGS publications about USGS microbiology	Highly successful interdisciplinary Web page went live in June 2009. See http://microbiology.usgs.gov/
III, To suggest ways to broadcast and publicize the types of microbiology conducted at the USGS	List of suggested broadcast avenues	• Broadcast: Web site; Fact Sheet; posters • Notify of capabilities: other DOI Bureaus, NOAA, State Department, USAID, EPA, USDA, DOD, State and local governments, NGOs • In all media, include appropriate USGS microbiologist's contact information • Consider: development of environmental microbiology course; conducting USGS science meeting; accepting graduate students	• Web site in place • Posters presented at national meetings • Web site announcement sent to Federal agencies and NGOs
IV, To identify emerging issues in USGS interdisciplinary microbiology research	Identification of emerging issues in microbiology and ways to remain relevant and cutting edge	Priorities: • Technology and tools • Wildlife and ecosystem health • Basic and applied research • Environmental impacts of energy and resource development • Environmental impacts on human and ecological health • Skill set needs including new scientists and highly skilled and trained biological technicians	• Partnerships with other Federal agencies and universities • Where funding is available, include research at the genome level • Conducting some carbon sequestration research
V, To identify potential opportunities for interdisciplinary microbiology work at the USGS	Identification of challenges, opportunities, and impediments to interdisciplinary research	• Identify opportunities for interdisciplinary work • Consider impediments including cost differences between disciplines (salaries and overhead) • Address funding issues and recommend dedicated funding, nontargeted money for integrated science efforts • Develop advisory group to address impediments and develop strategy to encourage interdisciplinary approach	• Researchers are discussing possible collaborative opportunities across disciplines

Geographic Patterns/Visualization

Geographic science contributes to microbiology in four ways. (1) All scientific data should have a spatial and temporal component so that they can be organized and integrated. Geography offers key tools for this integration. (2) The production of useful visualizations of scientific data allows USGS scientists to understand spatial and temporal patterns. These visualizations can benefit from well-developed cartographic and information architecture. (3) Spatial analysis uses fundamental concepts of geographic science to test hypotheses about spatial patterns, interactions among processes, and forecasts of future states. (4) Finally, through the exploration of scale, geography is helping biologists develop new ways of understanding regional health not only as the states of organisms but as the application of clinical principles (homeostasis, resilience, diversity) to the welfare of regions such as islands, watersheds, and even ecoregions.

Example.—Public health and geospatial distribution (mapping vector-borne human diseases such as Lyme disease, plague, West Nile virus, H5N1 avian influenza)

Other examples.—Microbial mapping: stimulating hypothesis and informing the public; medical geography: multiscale insights on the health of regions; identification of human plague risk: the importance of habitat diversity measures

Geographic patterns/visualization contact on Fact Sheet.—Lee De Cola, ldecola@usgs.gov

Public Health and Water Quality

Our population relies upon clean water for drinking and recreation. Microbiological risks to water quality include (un)sanitary waste, fecal contamination from domesticated animals and wildlife, and proliferation of pathogens and toxin-producing cyanobacteria in the environment. The USGS addresses these issues through monitoring fecal-indicator bacteria, pathogens, and cyanobacteria. USGS scientists also work to improve tools such as real-time modeling of *E. coli* concentrations at beaches, to develop and validate rapid methods for beach monitoring, to improve our understanding of the fate and transport of pathogens, and to apply new tools such as fecal source tracking to management of water quality for the protection of human health.

Examples.—Rapid methods for beach health models; harmful algal blooms

Other examples.—Pathogens and antibiotic-resistant bacteria in the environment; bacterial source tracking; transport of microbes in the subsurface/physical interactions of microbes and particles; flow cytometry in microbiology

Public health and water-quality contact on Fact Sheet.—Richard L. Smith, rlsmith@usgs.gov

Geomicrobiology

Geomicrobiology is the study of interaction of microbes with physical processes in soils, surface water and ground water, ice, and the deep subsurface (for example, caves and underground mines). Microbes are Earth's earliest living organisms. The earliest microbes in all likelihood utilized inorganic compounds exclusively for all life functions. Microbes have since evolved to fill all ecological niches, but these earliest life strategies are still important in the modern Earth. USGS scientists are studying the mechanisms by which microbes are involved in the cycling and transformation of potentially toxic trace elements such as arsenic, chromium, mercury, selenium, and tellurium; in the breakdown of silicate and sulfide minerals in soils and mining wastes; in the utilization of inorganic carbon compounds such as methane; and in the stabilization of desert soils. Research sites include the deep subsurface, extreme saline lakes, mining-impacted sites, wetlands, and arid lands.

Examples.—Geomicrobiology/nanoscience of toxic elements As, Se, and Te; microbial methylation of mercury

Other examples.—Geomicrobiology of weathering; using natural abundance $\delta^{13}C$ and $\Delta^{14}C$ values of phospholipid fatty acids to characterize bacterial carbon cycling pathways in deep aquifers; geochemical controls on microbial methylation of mercury in the Florida Everglades; microbial influence on sulfide and weathering

Geomicrobiology contact on Fact Sheet.— Mark C. Marvin-DiPasquale, mmarvin@usgs.gov

Ecosystem Function

Microbes and microbially mediated processes impact nutrient and material flux and transport, energetics, and diversity of ecosystems and ecosystem dynamics. These processes include human-derived changes at global and local levels, microbial responses to these changes, and ecosystem-ecosystem interactions such as transport of microbially derived products from terrestrial to oceanic or freshwater environments. Ecosystem scale can range from soil and subsurface pores to ecotone, oceanic, and continental perspectives.

Examples.—Biogeochemistry of mountain watersheds, evaluating ecosystem function with integrated approaches in environmental microbiology, ecosystem service markets, and carbon sequestration

Other examples.—Tale of disappearing toads: The amphibian chytrid fungus in the Rocky Mountains; natural and enhanced bioremediation of nitrate contamination in groundwater; microbial communities and carbon cycling in response to global change; microbial genetics in groundwater models

Ecosystem function contact on Fact Sheet.—JoAnn Holloway, jholloway@usgs.gov

Reference Cited

Jacobs, Ruth, Haig, Susan, Talbot, Sandy, Winton, James, King, Tim, and Kendall, Kate, 2006, Conservation genetics in the USGS: U.S. Geological Survey Fact Sheet 2006–3108, 4 p., available at http://pubs.usgs.gov/fs/2006/3108/.

Working Group II: To Plan a USGS Interdisciplinary Microbiology Web Site

Ms. Bernadette LeMasters, USGS Bioinformatics, led the breakout session for Working Group II (fig. 1). The purpose of this group was to develop ideas on the creation of an interdisciplinary microbiology Web site, if funding were to become available. Ms. LeMasters had a prototype available as a strawman to give the group a starting point from which to work. The group was asked to address the following during the discussions:

- Define target audiences
- List known customers
- List potential customers
- Discuss titles of the different general microbiology categories and the types of research that might fall under each
- Discuss sample projects that could be highlighted

Figure 1. Moderator and members of Working Group II. **Moderator:** Bernadette LeMasters, National Biological Information Infrastructure (standing, top right). **Group members,** counterclockwise from top right: Joseph E. Bunnell, Geology Discipline, Eastern Energy Resources Team, Reston; Margo D. Corum, Geology Discipline, Eastern Energy Resources Team; Patricia Dick, Biological Resources Discipline, Headquarters; Gael Kurath, Biological Resources Discipline, Western Fisheries Research Center; Christina Kellogg, Geology Discipline, Florida Integrated Science Center; Sandra S. Embrey, Water Resources Discipline, Washington Water Science Center; Christopher Mills, Geology Discipline, Denver Federal Center. Photograph by Cheryl W. Caldwell, USGS.

Moderator's Notes on Focus Group To Discover Attitudes, Beliefs, and Desires for an Interdisciplinary Microbiology Web Site and Reactions to a Prototype

Introduction

Six USGS scientists with experience or expertise in microbiology were assigned to develop ideas for a potential interdisciplinary microbiology Web site. With assistance from moderator Bernadette LeMasters, the group members discussed their information needs and their reactions to a mockup Web site, version 1.

Following is a compilation of the notes the moderator took during the focus group discussion. Despite the organization of the notes into topics, the discussion itself was loosely structured and moved fluidly from topic to topic. Without prompts from the moderator to focus on particular issues, the loose structure often revealed the depth of knowledge that respondents had about Web sites. Without prompting, respondents identified their information needs, explained keyword search terms that would indicate Web site success, identified the costs and benefits of the mockup interface for retrieval of information, and referred to other USGS Web sites as models. The notes from these discussions are compiled for the benefit of future Web site developers within categories of relevance to creating a USGS interdisciplinary microbiology Web site.

Outline of the Discussion

1. Welcome
2. Moderator explained who she is and her purpose as a moderator
3. Assigned timekeeper
4. Asked respondents how they would like to proceed: take a survey, view past National Biological Information Infrastructure (NBII) projects, view related microbiology Web sites, or view the mockup? Participants decided on first viewing the mockup (figs. 2, 3, 4).
5. Passed out hard copies of the mockup pages
6. Took notes as participants give their views

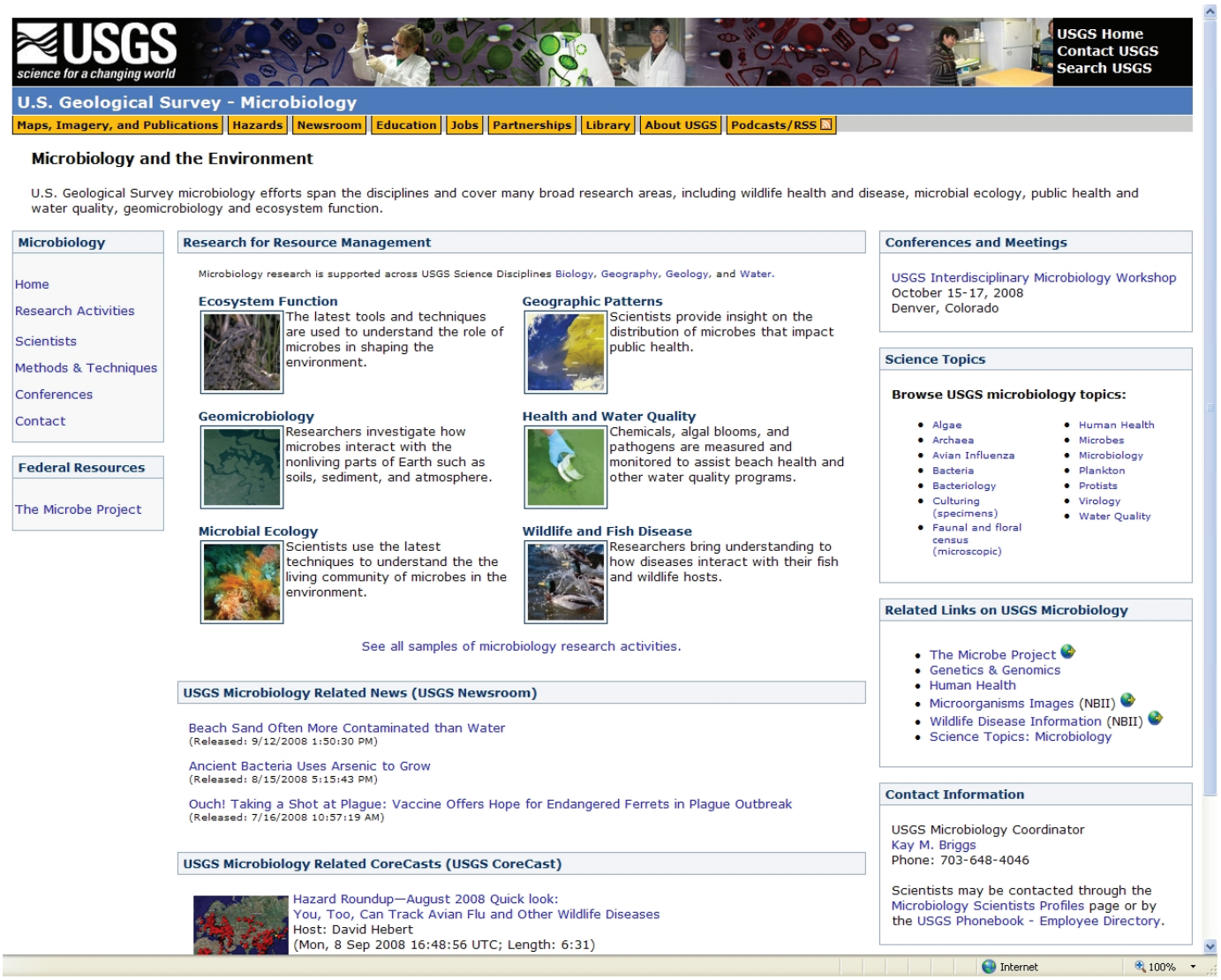

Figure 2. Mockup (version 1) of USGS interdisciplinary microbiology Web site—Home page. After the workshop, the draft Web site was updated and activated at http://microbiology.usgs.gov/.

7. When timer called 30 minutes, passed out the survey about the top goals for the site
8. Reviewed points on chart paper
9. After another 30 minutes, closing questions: Would making a mockup site be worthwhile?
10. Statement of thanks for participating

Materials

- Survey
  - This survey asked participants to complete the statement "A USGS interdisciplinary microbiology Web site should do the following:" by checking "Strongly Agree," "Agree," "Disagree," "Strongly Disagree," or "No opinion" next to a list of predefined goals. Respondents were instructed to complete the survey individually and silently and were encouraged to add their own comments or notes.
- Hard copies of Web site mockup version 1 [After the workshop, the draft Web site was updated and activated at http://microbiology.usgs.gov/.]

Part I: General Goals for a USGS Microbiology Web Site

Introduction

Defining the purpose of a Web site is a critical first step to creating a site that is successful and useful. The

Figure 3. Mockup (version 1) of USGS interdisciplinary microbiology Web site—Research activities page.

following are the ideas, suggestions, and responses of focus group participants related to the goals and needs for a USGS microbiology Web site. Combined are comments made during more loosely structured parts of the discussion, as well as responses to a survey each participant was asked to complete individually.

Information Needs

- Knowing what others in USGS are doing in microbiology [noted by one respondent as the greatest benefit of the workshop itself]
- Knowing who is in my discipline related to microbiology—a list of everyone [One respondent explained he or she was once asked for this information and used Google to search for names.]
- Knowing the capabilities of different USGS laboratories. When a certain expertise is needed, say for a special technique, it is cost-effective to work with a laboratory that already has the expertise and equipment. However, there is no easy way to know which USGS facilities have a needed expertise. [One respondent further explained that he or she used journal article searches to find the best person/lab available.]

Expectations, Requirements, and Preferences

- Top search engines find the Web site within the first page using the key terms "Federal microbiology research." [One respondent said: "No one will search 'USGS microbiology.'"]

Figure 4. Mockup (version 1) of USGS interdisciplinary microbiology Web site—Page of microbiology scientist profiles.

- Web sites to model: Ohio Water Science Center and how it shows the center's capabilities; Florida Integrated Science Center Web site and how it organizes each discipline by tabs

Survey Identifying Web Site Goals

Respondents were asked to finish the statement "A USGS interdisciplinary microbiology Web site should do the following:" and check whether they strongly agreed, agreed, disagreed, or strongly disagreed with, or had no opinion about, a list of predefined goals (table 2). The following four goals received "Strongly agreed" or "Agreed" from every participant.

1. Educate the public about USGS microbiology and how it applies to national priorities such as health and resource management

   Comments: (1) Make sure Web page is designed with USGS science strategy in mind; (2) Yes, but mostly by linking to other sites like ASM's [American Society for Microbiology's] Microbe World; (3) Two most important goals [Respondent drew a line between "Educate the public" goal and "Provide information about the diverse expertise" goal]

2. Provide information about the diverse expertise of USGS microbiologists (e.g., name, title, contact information, and links to project pages)

   Comments: (1) Two most important goals [Respondent drew a line between "Educate the public" goal and "Provide information about the diverse expertise" goal]

3. Provide links to USGS centers with microbiology expertise and (or) laboratories

Table 2. Results of a survey about the proposed USGS interdisciplinary microbiology Web site.

[At the workshop held in October 2008, a survey was conducted of the members of Working Group II; members chose the options below in completing the statement: A USGS interdisciplinary microbiology Web site should do the following: ...]

No.	Goal	Strongly agree	Agree	Disagree	Strongly disagree	No opinion
1.	Educate the public about USGS microbiology and how it applies to national priorities such as health and resource management	5/6	1/6	--	--	--
2.	Educate the public about basic microbiology concepts	1/6	3/6	1/6	1/6	--
3.	Show potential partnering scientists the relatedness of other science topics, such as ecosystem function, to USGS microbiology	3/6	2/6	1/6	--	--
4.	Provide information about the diverse expertise of USGS microbiologists (e.g., name, title, contact information, and links to project pages)	5/6	1/6	--	--	--
5.	Display USGS news releases and broadcasts about USGS microbiology	3/6	2/6	--	--	1/6
6.	Display links to USGS microbiology data sources such as the National Water-Quality Assessment (NAWQA) Program and Wildlife Disease Information Node (WDIN)	2/6	3/6	1/6	--	--
7.	Display USGS publications about USGS microbiology	5/5	--	--	--	--
8.	Display future and past meetings, discussion groups, and conferences of interest to USGS microbiologists	--	3/6	1/6	--	2/6
9.	Display for other scientists how-to materials about microbiology techniques such as rapid detection methods	2/6	1/6	2/6	--	1/6
10.	Provide links to USGS centers with microbiology expertise and (or) laboratories	5/6	1/6	--	--	--
11.	Provide links to related Web sites about microbiology from U.S. agencies	--	4/6	2/6	--	--

4. Display USGS publications about USGS microbiology

   Comments: (1) [5/5 Strongly agreed, but one participant did not respond: "Not sure what you mean by display—links to pdf files? Journal articles"]; (2) For the most part provide links to pubs, methods, etc. only if published. If the material is not published contact info should be provided; (3) Searchable by scientist; (4) Don't duplicate—don't want to update ten pages on the Web about my publications

The following goals also are from the survey but received mixed responses. The goals are ordered highest to lowest after responses were weighted.

1. Display USGS news releases and broadcasts about USGS microbiology
2. Show potential partnering scientists the relatedness of other science topics, such as ecosystem function, to USGS microbiology

   Comments: (1) Show potential partnering scientists (job seekers) what USGS has to offer in microbiology; (2) Yes, but indirectly, by showcasing the research rather than spelling it out
3. Display links to USGS microbiology data sources such as the National Water-Quality Assessment (NAWQA) Program and Wildlife Disease Information Node (WDIN)
4. Display for other scientists how-to materials about microbiology techniques such as rapid detection methods

   Comment: (1) Yes, methods section and an equipment/technique list so you know where to go

5. Educate the public about basic microbiology concepts • Display future and past meetings, discussion groups, and conferences of interest to USGS microbiologists • Provide links to related Web sites about microbiology from U.S. agencies [Three goals having similar weights.]

   Comments: (1) Ok, but of minor importance [in reference to "Display future and past meetings" goal]; (2) Yes, but again, minor component [in reference to "Provide links to related" goal]

Part II: Responses to the Mockup Version 1

Offering a prototype is often one of the best ways to generate feedback and check whether Web designers are on the right track. The following are responses from focus group participants to hardcopies of the USGS interdisciplinary microbiology Web site mockup version 1. The prototype also was made available online; after the workshop, the draft Web site was updated and activated at http://microbiology.usgs.gov/.

Home Page (fig. 2)

- Keep research activities as the main focus of the home page
- Thumbnail image menu should stay; makes scanning easy
- Change research topics to:
  - Climate Change
  - Ecosystem Function
  - Geographic Patterns
  - Geomicrobiology
  - Human Health (simply "Health" is too general—say "Human")
  - Microbial Ecology
  - Water Quality (to include Monitoring Programs, Recreational Health, and Drinking Water)
  - Wildlife Disease ("Wildlife" naturally is understood to include foxes, deer, etc. To include fish and coral, have the photo thumbnail depict fish over coral. After clicking on the thumbnail, have subcategories for terrestrial, invertebrates, aquatic)

Navigation

- "Scientists" in the left-hand navigation: you assume that you will reach a list of people from USGS microbiology. Would be helpful to someone who knows whom they are looking for.
- "Methods and Techniques" may or may not belong separate from research

Research Activities (fig. 3)

- Lists are difficult to navigate; instead, have general information about research activities, then underneath a thumbnail menu that shows research topics
- Looks like a site map; listings such as this should be in a separate "Site map" page
- Be sure to list projects under all possible categories so as not to miss a project because of poor sorting

Microbiology Scientist Profiles (fig. 4)

- List the following for each scientist:
  - Name
  - Research
  - Facility
  - Discipline. List this last, if at all, for the following reasons:
    - A poor reflection of expertise (a person in "Geology" is not necessarily a geologist)
    - Further divides the scientists when the site should be "interdisciplinary"
    - If included, have this deemphasized, perhaps with abbreviations

Working Group III: To Suggest Ways To Broadcast and Publicize the Types of Microbiology Conducted at the USGS

Working Group III was charged to consider several things, including how to broadcast and publicize more broadly the different types of microbiology conducted at the USGS. It would be useful to understand and have a good idea of how people should be notified and who should be notified of USGS work and techniques, methodologies, and products we can provide. This group was charged with coming up with the names of organizations, Federal and State agencies, and other groups that would have needs for USGS skills and services and that would be in a position to spread the word about our capabilities. It was also recommended that the group provide actual contact information of individuals in these suggested groups if they had contact information.

Microbiology Workshop
Breakout Session Recommendations
Group III Chart Notes

Questions:

- How should we broadcast/publicize the different types of microbiology conducted at the USGS?
- How should people be notified and who should be notified of our work/products, etc.?
- What are names of organizations (State, Federal, NGO [nongovernmental organization], etc.) that would be in a position to spread the word?
- What is the contact information for these organizations?

How should we broadcast/publicize?

- Web site
- Fact Sheet/posters/newsletter
- DOI (Department of the Interior) Highlight
- ASM/ASV (American Society for Microbiology, American Society for Virology)—become player within professional societies
- ISSM (International Society for Subsurface Microbiology)

Who should be notified of our work/products?

- NPS (National Park Service)
- FWS (U.S. Fish and Wildlife Service)
- USDA (U.S. Department of Agriculture)
- EPA (U.S. Environmental Protection Agency)
- NAS (National Academy of Science)
- DOI (U.S. Department of the Interior)
- DOD (U.S. Department of Defense)
- BLM (U.S. Bureau of Land Management)
- NOAA (National Oceanic and Atmospheric Administration)
- CDC (Centers for Disease Control)
- State Department/USAID (U.S. Agency for International Development)
- USGS (U.S. Geological Survey)

What are names of organizations that would be in a position to spread the word?

- States/environment/health
- Local government
- NGO (nongovernmental organizations)
- TNC (The Nature Conservancy)
- TWS (The Wildlife Society)
- Audubon
- AFWA (Association of Fish and Wildlife Agencies)
- Conservation International
- SI (Smithsonian Institution)
- CGIAR (Consultative Group on International Agricultural Research)
- Gates Foundation
- Rockefeller Foundation
- UNESCO (United Nations Educational, Scientific and Cultural Organization)
- UNEP (United Nations Environment Programme)
- Environmental journals
- NEON (National Ecological Observatory Network)

How should we acquire contact information?

- Ask for specific names in email distribution to ensure information is received by appropriate contact
- In-person presentations
- Micro-day/conference
- Science tube
- Pod cast
- Educational materials
- National research project
- Environmental journalist

Additional suggestions:

- Environmental micro course
  - Graduate students
  - Open access
  - DVDs
- Develop identity/logo
- USGS science meeting/Need to know what everyone else is doing

Working Group IV: To Identify Emerging Issues in USGS Interdisciplinary Microbiology Research

Working Group IV was to consider what emerging microbiology issues and technologies will be facing USGS microbiologists in the near future. Where should we be headed to remain relevant and cutting edge? How can these suggestions help define our next steps to take after this workshop ends?

Members: Carter Atkinson, Matthew Bachmann, Muruleedhara Byappanahalli, Brian Cole, Nicole DeCrappeo, Jeffrey Hall, Bane Schill, Mark Stanton, Erin Stelzer, Bruce Taggart, Mark Waldrop.

Priority 1 – Technology and Tools

- Remote sensing
- Geographic information systems
- Spatial-temporal models
- Deployable sampling analysis
- Bio-indicators to identify and analyze environmental change
- Microbial biodiversity in soils
- Next generation of microbiology including analytical technology
- Early detection of biological agents for homeland security

Where should we be heading to remain relevant and cutting edge?

- Leverage existing resources and partner up with other agencies and private companies to develop and ground truth these technologies

Resource needs:

- The introduction of new scientists (engineers, biochemists) with appropriate skills and experience on a continual basis
- Highly skilled and trained biological technicians
- Commitment to ongoing professional training and development
- Up-to-date analytical equipment, supplies, and facilities

Priority 2 – Wildlife and Ecosystem Health

- Emerging zoonotic diseases
- How to manage invasive species and pathogens

Where should we be heading to remain relevant and cutting edge?

- Wildlife disease surveillance is a key to early detection of problems in ecosystems. This activity needs to be supported.
- USGS must partner up with other agencies and organizations to exploit its niche and complement partner agency missions that will enhance our ability to detect and manage disease. This includes, but is not limited to, development of new diagnostic technologies and assessment of invasive issues in terrestrial and aquatic (freshwater and marine) systems.
- We need to not only identify new threats, but also determine how they will be addressed. USGS must partner with other agencies and NGOs to develop creative approaches to managing and controlling adverse effects of invasive organisms.
- In order to address invasive issues on a timely basis, USGS needs to be more nimble and be able to shift priorities and resources as needed.

Resource needs:

- Stable funding for wildlife disease surveillance. While not necessarily sexy, this is the best way to enhance our ability to detect problems early before they become difficult to address or out of control.
- The introduction of new scientists with appropriate skills and experience on a continual basis
- Highly skilled and trained biological technicians
- Commitment to ongoing professional training and development
- Up-to-date analytical equipment, supplies, and facilities

Priority 3 – Basic and Applied Research

- Functional genomics
- Metagenetics
- Proteomics
- Metabolomics
- Drug discovery
- Extremophiles

Where should we be heading to remain relevant and cutting edge?

- Integrate genomics, metabolomics, proteomics, and microbiome to understand small- and large-scale

processes in biogeochemistry, disease, and ecosystem health on a nano to tera scale

- Apply our capacity for microbiology in novel uses such as biochips, drug discovery, or energy generation. This application would require USGS partnering with other governmental organizations and industry.

Resource needs:

- Commitment to ongoing professional training and development
- Biochemists
- Highly skilled and trained biological technicians
- Soil microbiologists
- Computational biologists (with associated computing power)
- Distributed computing capability
- Sufficient dedicated laboratory space

Priority 4 – Environmental Impacts of Energy and Resource Development

- Microbiological applications to address energy development and mineral extraction impacts
- Carbon capture
- Linked microbiological, geochemical, and hydrologic impacts of injecting supercritical CO_2 for geologic carbon sequestration

Where should we be heading to remain relevant and cutting edge?

- Study the capture or release of CO_2 through microbial responses to climate change at Arctic latitudes
- Conduct investigations to evaluate effects on microbes if CO_2 is injected or ejected
- Determine and evaluate methods to extract carbon out of waste streams
- Contribute to other governmental agency efforts
- Study effects of carbon sequestration on microbiological communities in subsurface, wetland, and forested ecosystems and how those community changes may in turn affect sequestration
- Evaluate environmental impact of mineral extraction technologies on microbial communities (tar sands, gas extraction, metals, etc.)

Resource needs:

- The introduction of new scientists with appropriate skills and experience on a continual basis
- Highly skilled and trained biological technicians
- Commitment to ongoing professional training and development
- Up-to-date analytical equipment, supplies, and facilities
- Improvements to existing USGS software to accommodate the unique physical and microbiological aspects of CO_2 sequestration; for example, linking PHREEQC to SEAWAT

Priority 5 – Environmental Impacts on Human and Ecological Health

- Emerging contaminants
- Impacts of nanotechnology on the environment

Where should we be heading to remain relevant and cutting edge?

Continuing research on emerging contaminants:

- Pharmaceuticals
- Hormones
- Personal care products
- Antimicrobial agents
- Industrial effluents
- Landfill capacity
- Metals
- Pesticides and fertilizers

Continuing research on impacts of nanotechnology on the environment:

- Nanotech impacts on biological systems

Continuing research on both:

- Biotechnology cooperative research with industry
- Unintended consequences

Resource needs:

- The introduction of new scientists with appropriate skills and experience on a continual basis
- Highly skilled and trained biological technicians
- Up-to-date analytical equipment, supplies, and facilities

Final Thoughts

Knowledge information needs to effectively promote emerging issues

Recommended scientist actions:

- Educate ourselves about USGS Communications Office resources, capabilities, and contacts in order to communicate scientific results to our partners and the general public
- Work together with managers in presenting USGS science to Congressional Members on the Hill in order to most effectively increase their awareness and appreciation of the relevance of USGS science
- Identify and more effectively leverage USGS microbiological scientific capabilities, expertise, and resources in collaboration with one another and with outside partners
- Increase peer-to-peer communications to reduce duplication and cost and maximize research effort. In other words, leverage existing USGS microbiological resources more effectively.

Recommended organizational actions:

- Develop one funding business model that benefits all scientists and disciplines and facilitates broad interdisciplinary collaboration throughout the USGS
- Have managers require less administrative work by scientists, thus allowing a greater focus on research by scientists
- Find ways to streamline and minimize overhead costs at all levels of the organization to maximize available science funding in the USGS
- Work with scientists in developing greater high-throughput analytical capacity
- Recognize the need to solve problems/issues of our partners to remain relevant
- Managers need to listen to and trust our scientists in identifying and developing future microbiology science needs and issues. Through peer-to-peer interactions, our scientists understand microbiology science needs and issues.
- Develop better top down/bottom up communication between scientists and managers. For example, managers (headquarters, regions, areas, and programs) attend many internal and external meetings throughout the year; very seldom do they report to scientists on the issues and opportunities they hear about, nor do scientists see any tangible benefits (such as funding) from these efforts.
- Encourage microbiological researchers and managers to work together in presenting USGS science to Congressional Members on the Hill in order to most effectively increase their awareness and appreciation of the relevance of USGS science
- Identify and more effectively leverage USGS microbiological scientific capabilities, expertise, and resources that other governmental agencies don't possess

Working Group V: To Identify Potential Opportunities for Interdisciplinary Microbiology Work at the USGS

The task of Working Group V was to identify potential opportunities for interdisciplinary work. The ideas could include general topics to specifics. The group was to identify impediments and enhancements for interdisciplinary microbiology to work at the USGS. How can we encourage interdisciplinary microbiological research? Are interdisciplinary microbiology projects useful?

Questions:

- What are potential opportunities for interdisciplinary work?
- What are the impediments and enhancements?
- How can we encourage interdisciplinary research?
- Are interdisciplinary projects useful? **Yes**

Impediments:

- Funding challenges; for example, the Water Resources Discipline (WRD) is difficult to work with because of cost (salaries, overhead)
- Leadership is unaware of what is happening in the field
- Want leadership to recognize "good" interdisciplinary work
- Need more face time with each other
- Need dedicated funding
- It's about effective communication, collaboration, and funding

Recommendations:
How can we encourage interdisciplinary research?

Clarify term "interdisciplinary":

- Identify what defines interdisciplinary
- Foster awareness of others

Meetings/information sharing/networking:

- Host scientific meetings routinely or biannually (include field trips)
- Regional meetings can occur more frequently
- Biannual national meetings

Create a virtual community to link other USGS groups together to include:

- Wiki sites
- Web sites
- Chat room
- Sponsor webinars
- Roster/listing of who's who and what technology is available
- Incorporate Face Book and (or) Community of Practice technology (from Cheryl)
- Video conferencing: we need an effective way to find out what others are doing
- Incorporate technology that will allow folks to have access 24 hours
- Ensure leadership/decisionmakers are connected to this technology as well

Funding:

- Finance people's efforts (especially in Water Science Centers)
- Need dedicated funding
- Have nontargeted money for integrated science efforts

Highlight:

- Identify what interdisciplinary microbiology projects have worked
- Use their success as a model
- Encourage interdisciplinary meetings with other disciplines outside of microbiology where this information can be shared.
- Challenge: Beware of meeting burnout

Web site:

- Set up Web site
- Survey groups who are working in microbiology and build a resource list
- Include who has what technology
- Create a wiki site

Advisory group:

- Recommend creating an advisory group to address the impediments and develop strategy to encourage interdisciplinary research
- Recognize some impediments need to be addressed top-down, collaboration bottom-up
- Establish a email list and have conference calls on science-related areas

Leadership awareness/involvement:

- Important for leaders to have access and utilize these "virtual networks"
- Need for them to know internal capabilities
- Need leadership to be involved in communication loop
- Need a liaison to find a way to get information to the top and make aware of the impediments
- Communication needs to go through center to region to headquarters

Internal/external funding and opportunities:

- Remember collaboration not just internal
- Look for internal and external opportunities to help microbiology to get funding
- Hire staff whose specific function is to find external funding opportunities

USGS

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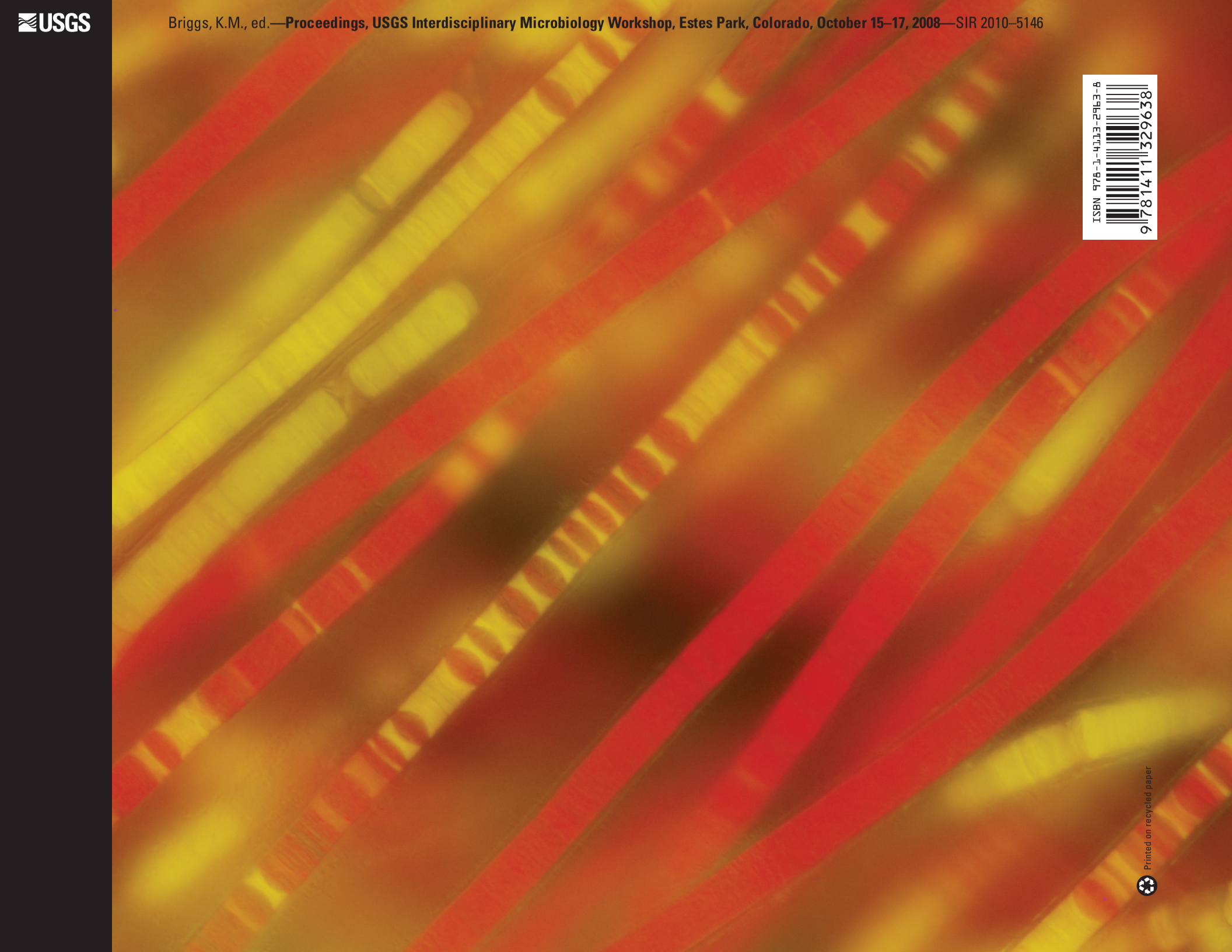

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