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Mercury in water and biomass of microbial communities in hot springs of Yellowstone National Park, USA

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Abstract

Ultra-clean sampling methods and approaches typically used in pristine environments were applied to quantify concentrations of Hg species in water and microbial biomass from hot springs of Yellowstone National Park, features that are geologically enriched with Hg. Microbial populations of chemically-diverse hot springs were also characterized using modern methods in molecular biology as the initial step toward ongoing work linking Hg speciation with microbial processes. Molecular methods (amplification of environmental DNA using 16S rDNA primers, cloning, denatured gradient gel electrophoresis (DGGE) screening of clone libraries, and sequencing of representative clones) were used to examine the dominant members of microbial communities in hot springs. Total Hg (THg), monomethylated Hg (MeHg), pH, temperature, and other parameters influential to Hg speciation and microbial ecology are reported for hot springs water and associated microbial mats.

Several hot springs indicate the presence of MeHg in microbial mats with concentrations ranging from 1 to 10 ng g^{-1} (dry weight). Concentrations of THg in mats ranged from 4.9 to 120,000 ng g^{-1} (dry weight). Combined data from surveys of geothermal water, lakes, and streams show that aqueous THg concentrations range from 1 to 600 ng L^{-1}. Species and concentrations of THg in mats and water vary significantly between hot springs, as do the microorganisms found at each site.

1. Introduction

Geothermal features (volcanoes, geysers, hot springs, fumaroles) are known as geologic sources of Hg to the environment (Nraigu, 1989; Christenson and Mroczek, 2003; Rytuba, 2005). Releases of Hg from geological sources has been found in both gaseous and aqueous forms, but recent research focuses almost entirely on gaseous Hg emissions (Gustin et al., 1996; Engle and Gustin, 2002; Christenson and Mroczek, 2003). While a few researchers have used ultra-clean sampling techniques to investigate THg concentrations in water

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(McCleskey et al., 2004) and air samples (Hall et al., 2006) associated with geothermal features, most research on Hg in geothermal systems occurred before incorporation of clean sampling protocols for trace metal collections (Patterson and Settle, 1976). This raises concerns for environmental managers regarding the application of the historic datasets in watersheds containing or receiving geothermal waters. Moreover, investigations of organo-Hg compounds in geothermal systems are rarer and focused primarily on gaseous emissions from hot springs (Hirner et al., 1998). As a result, important biogeochemical processes and transport mechanisms of Hg are poorly understood in geothermal systems. Subsequently, policy-makers are unable to make informed decisions in regions containing geothermal environments because geochemical and microbial data do not exist.

Extensive research in non-geothermal aquatic systems has demonstrated the importance of microbial processes in the cycling of Hg. Methylation of Hg(II) to the more toxic, bioaccumulated form, MeHg, is facilitated by microorganisms, primarily SO_4-reducing bacteria (Compeau and Bartha, 1985; King et al., 2000). Bacteria can also degrade MeHg via oxidation or reduction pathways (Oremland et al., 1995; Marvin-DiPasquale and Oremland, 1998). Reduction of Hg(II) to Hg(0) and MeHg to Hg(0) is achieved by microorganisms using the *mer* A and/or *mer* B genes. The reductive pathways are believed to have evolved in geothermal environments where microorganisms needed to find a mechanism to survive and thrive in the presence of high concentrations of toxic trace metals (Nazaret et al., 1994; Barkay et al., 2003; Barkay and Wagner-Dobler, 2005). Once again, little information is currently available to evaluate microbial interactions with Hg in geothermal water.

Recent advances in molecular analytical techniques (RNA- and DNA-based polymerase chain reaction (PCR) coupled with DGGE, cloning, sequencing and phylogenic analysis) have improved our ability to describe and characterize microbial community structure in geothermal and other natural environments that are not amenable to traditional culturing techniques (Ferris et al., 1996; Burr et al., 2006). Likewise, advances in analytical techniques of atomic fluorescence detection and mass spectrometry have improved the ability to detect and quantify low-concentrations of Hg species and reaction pathways in the environment (Olson and DeWild, 1999; DeWild et al., 2002). Here these powerful tools are employed together in a novel setting—geothermal features—with the goals of:

- increasing current knowledge of Hg concentrations and speciation in geothermal water and biomass,
- evaluating important biotic and abiotic controls on Hg biogeochemistry, and
- increasing understanding of Hg–microbe interactions in geothermal environments.

In this paper, the authors relate initial findings of Hg distribution and speciation in chemically diverse hot springs in Yellowstone National Park and describe the molecular methods used to examine microbial communities in a subset of hot springs of representative geochemical diversity.

2. Field sites

The physical and chemical diversity among geothermal features in Yellowstone National Park (YNP) is unsurpassed among the world's geothermal regions. The US Geological Survey (USGS) investigations of water chemistry of YNP hot springs, geysers, and streams (Ball et al., 2002; McCleskey et al., 2004) reveal that water chemistry varies greatly between hot springs in close proximity. A small subset of hot springs were studied that represent the broadest possible range of YNP hot spring geochemistry contained within a single watershed. Reconnaissance work included searching for hot springs that demonstrated obvious microbial activity and evidence of Hg methylation. An underlying assumption of this work is that biotic processes are the primary mechanism of Hg methylation, though this hypothesis is unproven for hot springs.

Nymph Lake is part of the watershed drained by the Gibbon River. The watershed lies along a north–south fault stretching from Norris Geyser Basin to Mammoth Hot Springs. Geothermal chemistry is predominantly acidic at the southerly end of the fault and basic at the north end. The sites included both acid-sulfate hot springs and neutral pH-chloride hot springs draining to the eastern shore of Nymph Lake (Fig. 1). These springs have widely varying temperature, pH, and water chemistry (Table 1) that create ideal "field laboratories" for the investigations. There are also several hot springs and fumaroles on the west side of Nymph

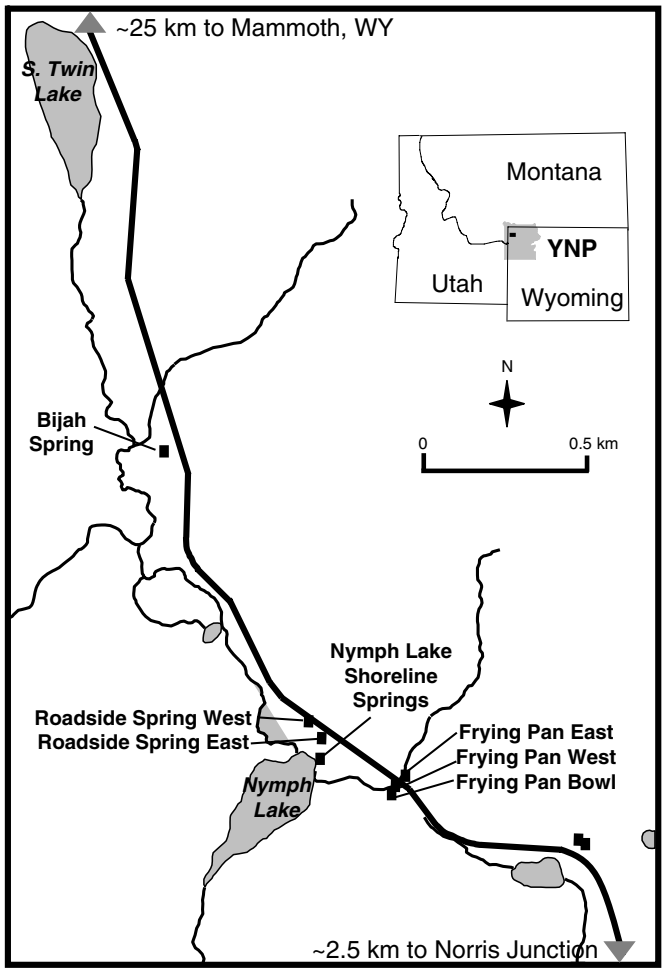

Fig. 1. Map of geothermal features near Nymph Lake, Yellowstone National Park (YNP). Sampling sites are Roadside Springs (East and West), Frying Pan Springs (Bowl, East, West), and Bijah Spring. Inset shows location of YNP in northwestern United States. Dot in YNP is location of the geothermal areas in main map.

Lake, as well as hot springs flowing into Nymph Lake from the lake bottom.

Soil around the hot springs are typically sinter nodules that form as silica-rich source water, which is cool at the surface. Vegetation encroachment upon the hot springs is found at circumneutral (Roadside Spring-West) and basic hot springs (Bijah) more than at acidic hot springs (Roadside-East, Frying Pan Springs).

Outflow channel sediment is a combination of microbial biomass and co-forming minerals that are collectively referred to as microbial mats. Biomass and mineral appearances of the microbial mats differ greatly between sites representing major hot spring types. Microbial mats are present in the outflows of all of the hot springs, although color, thickness, and mat coverage vary with season and hydraulic conditions within individual springs and associated outflow.

2.1. Low pH sites

Frying Pan sites (West, East and Bowl) and Roadside Spring-East site, have elemental S precipitates and tan or pink ''streamers'' near geothermal sources, which are thin filamentous strands of biofilm encrusted with sulfurous precipitates. However, downstream microbial mats differ as water chemistry changes with loss of sulfides and heat and gain of oxygen. As waters cool to 35 °C, Frying Pan Bowl sites develop thick, gelatinous purple-green mats of *Zygogonium* (Weigert and Fraleigh, 1972) that fill outflow channels. These lush mats contrast greatly with sediment at Roadside Spring-East

Table 1
Hot springs aqueous geochemistry

	Bijah Spring	Frying Pan Bowl Spring	Frying Pan Spring-East	Roadside Spring-West	Frying Pan Spring-West	Roadside Spring-East
T (°C)	79.0	36.4	80	65	56	60
pH	8.6	2.3	2.2	6.4	2.6	3.5
Fe(II) (mg L^{-1})	0.24	n/c	n/c	0.01	n/c	0.25
SO_4 (mg L^{-1})	105	n/c	n/c	73.2	n/c	220
H_2S (mg L^{-1})	<0.002	n/c	n/c	0.15	n/c	0.004
Alkalinity-HCO_3 (meq L^{-1})	290	n/c	n/c	170	n/c	b.d.l.
Cl (mg L^{-1})	177	n/c	n/c	454	n/c	167

This paper reports samples as below detection limit (b.d.l.) when the concentration is less than the larger of the daily detection limit or method detection limit.
n/c indicates that a water sample was not collected for the parameter indicated.

(pH 3.5, $T = 60$ °C), where a powdery film of yellow-orange to silver-gray precipitates form as water cools.

2.2. Circumneutral to basic pH sites

Roadside Spring-West (pH 6.5, $T = 65$ °C) has dark silver-gray precipitates at the source, but downstream mats take on a rich chocolate brown color as Fe oxides form. Thereafter, peach-colored precipitates blend with the chocolate brown mat.

Bijah Spring is hotter and more alkaline (pH 8.7, $T = 79$ °C) than the other hot springs in this study and sediment near the source appears to be fused sinter nodules. As outflow water cools to 65 °C, a bright blue-green mat develops that transitions into a fluvial fan with a thin, rust-colored microbial mat covering the hard sinter. Macrophytes and light green algal mats were present below the rust-colored community as the water cooled even further. The outflow channels of all hot springs were unfrozen, with adult flies living on the mats during the December 2004 survey of potential research sites.

3. Methods and materials

3.1. Field collections

Datasets combined from McCleskey et al. (2004), Krabbenhoft (unpublished data), and this study show that filtered THg concentrations in water vary greatly among YNP hot springs, geysers, and streams (Fig. 2). To avoid cross-contamination of samples from high Hg to low Hg sites, accepted clean sampling protocols (Patterson and Settle, 1976; Olson and DeWild, 1999) were used throughout sample collection and laboratory analyses, with no reuse of filter holders or sampling lines between springs. Solids and water samples were collected and placed into pre-cleaned Teflon or glass bottles with Hg-free certification. Distance from hot spring source, temperature, and microbial appearance were noted at each water and sediment sampling location.

Hot springs source waters were collected in a 1 L grab sample and filtered the same day through quartz fiber filters ashed at 550 °C for 4 h. Filtrate was collected into acid-washed Teflon bottles or certified Hg-free bottles, preserved with low-Hg HCl, and shipped overnight to the USGS Mercury Laboratory (Middleton, Wisconsin) for THg and MeHg analyses. Methods for temperature, pH, sulfide, SO_4, alkalinity and Cl are described in McCleskey et al. (2004).

Surface sediment and mats were collected from the upper 1 cm depth in hot springs and outflows in locations having distinctive coloration suggesting differing geochemical conditions relative to upstream mats. Samples were placed on dry ice until transported to the Montana State University laboratory, where they were thawed in a laminar flow hood, homogenized, and split for THg, MeHg and DNA analyses, and dry weight and loss on ignition determinations.

3.2. Mercury analyses

Filtered and unfiltered water samples were analyzed for MeHg using distillation and ethylation and chromatographic separation with cold vapor atomic fluorescence spectrometry (CVAFS) detection as described in DeWild et al. (2002). Filtered and unfiltered water samples were analyzed for THg using EPA method 1631 described in Olson and DeWild (1999). A method detection limit of 0.04 ng L^{-1} was determined for THg and MeHg, and daily detection limits were calculated as well.

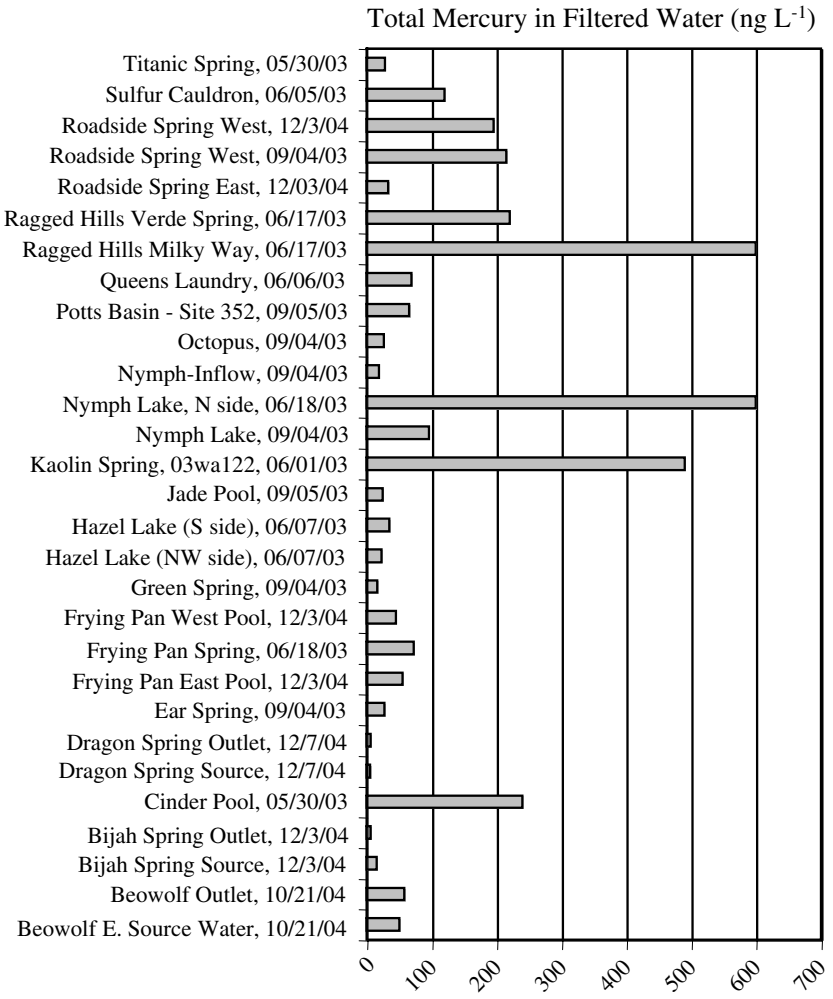

Fig. 2. Total Hg in filtered waters of Yellowstone National Park hot springs as sampled by S.A. King (2004), D.P. Krabbenhoft (September, 2003), and D.K. Nordstrom (June, 2003).

Methylmercury in mat material was extracted by additions of KBr, $CuSO_4$, and methylene chloride according to DeWild et al. (2002). Back extraction of MeHg into reagent water and detection using direct ethylation/purge and trap/chromatographic separation/pyrolysis was carried out by CVAFS. This extraction procedure eliminates known interferences from organic matter, recalcitrant solids, and sulfides, as well as greatly reducing potential MeHg artifact generation from high concentrations of inorganic Hg interactions with organic compounds. The method detection limit is 0.08 ng g^{-1}. All concentrations for sediment MeHg are reported as dry weight.

Concentrations of THg in mat material were determined according to published methods (Olson and DeWild, 1999). An aliquot of homogenized mat material was digested and oxidized with aqua regia overnight. The sample was diluted with 5% bromine monochloride, pre-reduced with hydroxylamine hydrochloride to remove free halogens, and Hg(II) was reduced to Hg(0) with stannous chloride. The Hg(0) produced was purged and trapped onto a Au trap, thermally desorbed, and detected using CVAFS. The detection limit was 0.3 ng per digestion bomb using this method. Percent relative standard deviation for field replicate samples ranged from 9% at sites with fine, homogeneous mixtures of biomass and mineral precipitates to 80% at sites with coarse, heterogeneous mixtures of biomass and sinter nodules. All concentrations for THg are reported as dry weight.

3.3. DNA extraction and PCR reaction

Microbial populations were identified using the approach described by Burr et al. (2006) expanding upon the pioneering work of Ferris et al. (1996). DNA was extracted from mat samples using the DNA FastDNA® SPIN Kit for Soil (Q-Biogene) by following the manufacturer's instructions. DNA extracts from environmental samples or purified cultured clones were amplified with a PTC-100 Programmable Thermal Controller from MJ Research using 15–30 ng of DNA. The non-GC clamp primers, 1070F: ATGGCTGTCGTCAGCT (bacterial) and 1392 R:ACGGGCGGTGTGTAC (universal) (Ferris et al., 1996), and the 2X Master Mix (Promega) were mixed with 15–30 ng of DNA in nuclease-free water provided by the Wizard® *Plus* Minipreps DNA Purification System (Promega) or DES solution provided in the FastDNA® SPIN Kit for Soil (Q-Biogene). Primers were synthesized by IDT® Integrated DNA Technologies and used at a concentration of 12 μM. To avoid contamination, all reaction batches were measured and mixed in the laminar flow hood. A negative control, consisting of a sample to which DNA template was not added, was included in all experiments. The temperature and cycling programs used for amplification of the 16S rDNA are described as follows: *Program 1:* (1) 94 °C for 2 min; (2) 94 °C for 45 s; (3) 55 °C for 45 s; (4) 72 °C for 45 s; (5). Repeat 2–4 for 25 cycles; (6) 72 °C for 7 min; (7) 4 °C cooling and hold. DNA from sediment at hot springs was sometimes difficult to amplify with program 1. Therefore, to qualitatively compare microbial diversity between all hot springs, a two-stage program was used to amplify DNA from environmental samples. In this program, 5 μL of the resulting PCR product from the first stage of Program 2 were used as a template for the reactions for the second stage. Program 2 (two-stage PCR): (1) 94 °C for 8 min, (2) 94 °C for 40 s, (3) 55 °C for 30 s, (4) 72 °C for 30 s, (5) Repeat 2–4, 10× (first stage) and (5) Repeat 2–4, 30 cycles (second stage), (6) 72 °C for 5:00 min, (7) 4 °C cooling and hold. The PCR products were visualized on 1.5% agarose gels and run for 30 min at 80 V in 1X Tris-Acetate-EDTA buffer. Gels were stained with ethidium bromide solution and rinsed in water for 10 min. PCR products from environmental samples or purified cultured clones were purified using a QIAquick Gel Extraction Kit (Qiagen Inc.) according to manufacturer's instructions.

3.4. Cloning

Cloning of the environmental DNA was performed with TOPO® TA Cloning® Kit (Invitrogen) and the OneShot® Chemical Transformation kit following the manufacturer's instructions. After a 24 h incubation period at 37 °C, single white colonies were restreaked onto LB agar plates containing 50 μg mL^{-1} ampicillin and 40 μL X-gal. After the next incubation period, single white colonies were grown in liquid medium containing kanamycin to assure presence of the cloned fragment. The Wizard® *Plus* Minipreps DNA Purification System (Promega) was used to isolate and purify the recombinant plasmids.

3.5. Denaturing gradient gel electrophoresis

DGGE gel was prepared with a denaturing gradient of urea and formamide with the Bio-Rad DCode™ Universal Mutation Detection System (BioRad Laboratories) as recommended in the user manual. Electrophoresis was run at 60 V for 16–18 h. The PCR for DGGE was performed with a GC clamp on either the primer 1070F or 1392R (Burr et al., 2006). Gels were stained with Sybr®Gold (Molecularprobes) for about 20 min (10 μL Sybr®Gold, 200 μL EDTA, 1000 μL in 100 mL with nanopure water). Subsequently, the gel was scanned with a FluorChem™ 8800 fluorescence imager (AlphaInnotec) and analyzed with AlphaEase Software (AlphaInnotech) by means of 1D lane densitometry.

The identity of environmental DNA fragments was confirmed by DGGE analysis of randomly selected clones side by side with the original PCR products of the of environmental DNA reaction. The 16S rRNA gene inserts from plasmids representing the diversity of the sampled community were then sequenced.

3.6. Sequencing

Plasmids were sequenced by Laragen, Inc. (10755 Venice Blvd., Los Angeles, CA 90034) or Nevada Genomics Center (NGC) (University of Nevada, Reno, NV). Sequences were provided either in .seq or .ab1 file format and processed with the Sequencher™ Software. BLAST searches against the database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/) were performed to identify the organisms most closely related to the cloned environmental 16S

rRNA gene fragments, which represent the dominant organisms in the sampled YNP springs.

4. Results and discussion

The impetus for this study was that preliminary collections of hot spring mats, aquatic vegetation and insects at the east shore of Nymph Lake (Striegl, unpublished) showed evidence of methylation and MeHg bioaccumulation (Table 2). Concentrations of THg were found to be elevated in water samples from two hot springs on the east shoreline of Nymph Lake. Moreover, MeHg in filamentous microbial biomass in the same two hot springs accounted for 5–10% of the THg, whereas less than 0.1% of Hg was as MeHg in the hot spring water. The bioconcentration factors were 1.2 and 12 for microbial communities in these two hot springs.

The preliminary survey in December 2004 of water in several Yellowstone hot springs and outflows showed wide ranges of THg. Concentrations of THg in hot springs source water varied by one order of magnitude between sites (Table 2). Aqueous MeHg concentrations were generally below the detection limit. Regression analyses indicated poor correlation between pH, temperature and Hg species concentrations in water. The highest water concentrations of THg were observed at a site with a pH of 6.4 (Roadside Springs-West), whereas the lowest concentration was found at a site with a pH of 8.6 (Bijah Spring).

Based on chemical modeling (MINEQL+) of cinnabar solubility at increasing temperatures across a range of pH, higher concentrations of aqueous Hg are predicted in geothermal water of lowest pH and highest temperature. However, the results presented here do not support this hypothesis. Filtered water samples of Roadside West had higher concentrations of THg than Frying Pan Springs water (Table 2).

A comparison of microbial mat THg concentrations between Roadside West and Frying Pan Springs also suggests that aqueous phase Hg complexation maybe important at Roadside West, but precipitation is a controlling process at all springs (Table 2). The data suggest that THg is rapidly removed from Frying Pan Springs water by amorphous S precipitates. The churning water of Frying

Table 2
Mercury concentrations in filtered water and microbial mats from hot springs

Site name and sampling location	Water MeHg (ng L^{-1})	Water THg (ng L^{-1})	Mat MeHg (ng g^{-1})	Mat THg (ng g^{-1})
Nymph L. Shoreline Spring #1, at source	0.026[a]	170	0.31[b]	6.3
Nymph L. Shoreline Spring #2, at source	0.43	520	0.53	4.9
Bijah Spring, at source	b.d.l.	15	n/c	n/c
Bijah Spring, 47 m from source	n/c	n/c	0.86	3100
Bijah Spring, 61.5 m from source	n/c	n/c	1.3	160
Bijah Spring, 92 m from source	n/c	n/c	0.16	380
Frying Pan Bowl Spring, purple-green mat	n/c	n/c	9.2	12,000
Frying Pan East Spring, at source	b.d.l.	56	0.12	14,000
Frying Pan East Spring, dark green mat	n/c	n/c	3.0	17,000
Frying Pan East Spring, yellow green mat	n/c	n/c	2.5	120,000
Frying Pan West Spring, at source	b.d.l.	46	0.36	22,000
Frying Pan West Spring, 10 m from source	n/c	n/c	0.08	2800
Frying Pan West Spring, 25 m from source	n/c	n/c	0.17	12,000
Roadside East Spring, at source	b.d.l.	33	0.12	1600
Roadside East Spring, 7 m from source	n/c	n/c	0.11	1200
Roadside East Spring, 13 m from source	n/c	n/c	1.5	9000
Roadside East Spring, 20 m from source	n/c	n/c	1.0	13,000
Roadside West Spring, at source	0.08	200	1.2	46,000
Roadside West Spring, 1.5 m from source	n/c	n/c	0.75	37,000
Roadside West Spring, 4.0 m from source	n/c	n/c	0.22	16,000
Roadside West Spring, 5.5 m from source	n/c	n/c	1.9	52,000
Roadside West Spring, 7 m from source	n/c	n/c	2.4	4100

b.d.l. indicates below detection limit. n/c indicates that a sample was not collected.
Percent relative standard deviations for MeHg analyses of replicate samples range from 10.2% to 15.6% among a wide range of sample matrices. Percent relative standard deviations for THg analyses of replicate samples are required to be less than 5% or data is flagged. None of the samples reported here were flagged.

[a] Detection limit for water, MeHg = 0.013 ng L^{-1}, for THg = 0.2 ng L^{-1}.

[b] Detection limit for biomass, MeHg = 0.08 ng g^{-1}, for THg = 0.3 ng g^{-1}.

Pan West pool are murky with yellow-tan precipitates and the Frying Pan outflow channel mats show evidence of the same precipitates deposited over sinter nodules.

Concentrations of Hg in microbial mats varied by 5 orders of magnitude for THg and 3 orders of magnitude for MeHg (Fig. 2; Table 2). Concentrations of MeHg in microbial mats were generally comparable to other ecosystems without point sources of inorganic Hg (Krabbenhoft et al., 1995, 2000; Gilmour et al., 1998; King, 2000). The highest concentrations of MeHg were observed in the thick purple-green Frying Pan Bowl mats that fill the channel flowing into the Frying Pan West pool. These mats are noticeably higher in organic matter than other hot springs mats, which may lead to conditions favoring microbial Hg methylation or greater accumulation of MeHg, or both processes. Continuing investigations of geochemical conditions and prokaryotic and eukaryotic organisms will identify microbial populations that may influence the speciation and seasonal distribution of Hg in these hot springs.

The DGGE images of DNA from hot springs microbial communities indicate great diversity between sites of differing pH and temperature (Fig. 3). Sites of lowest pH (Frying Pan Springs sites; Lanes B, C and E) showed fewer gel bands in their DGGE images, indicating a lower diversity relative to sites with neutral pH (Bijah, Lane A, Roadside Springs, Lanes D and F, Fig. 5). Further work at these sites will continue to investigate this diversity by sequencing of individual clone libraries formed from extracted microbial mat DNA. Bijah Springs showed many distinct bands, but far fewer than typical soil and sediment of pH 8.6. This difference is attributed to the high temperatures of the Bijah Springs source water that likely limited colonization to thermophilic microorganisms. Additionally, DGGE profiles for Roadside West and Bijah Springs communities are more similar to each other than to profiles of Frying Pan Springs, which is possibly attributed to a greater similarity in geochemical conditions at the two former sites (Table 1).

DGGE bands representing unique clones for the Roadside West and Bijah communities are numbered and their correspondence to the community profile are presented in Figs. 4 and 5, respectively. Sequencing results verify that microbial communities nearest the Roadside West and Bijah hot spring

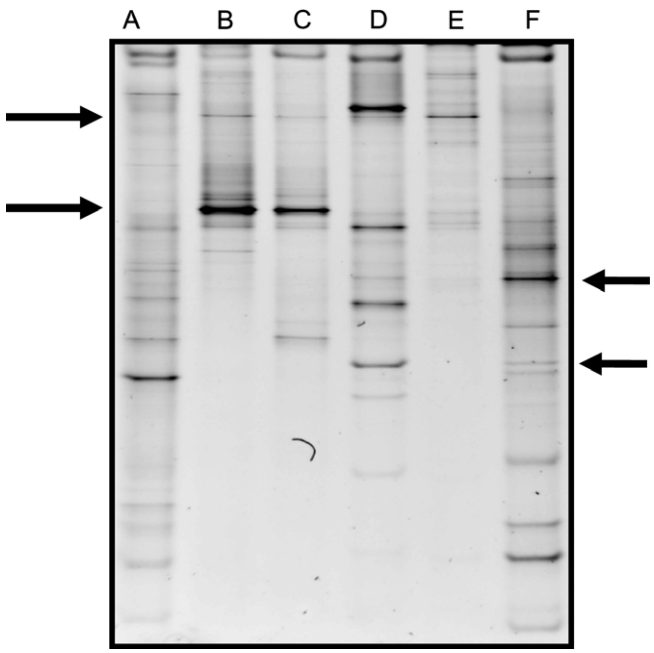

Fig. 3. DGGE image of Roadside Spring-West sites microbial community DNA and corresponding clone isolates DNA. Each lane is the result of two-stage PCR amplification of DNA extracted from hot springs mat/sediments. The number of dark bands on the DGGE image indicates the relative diversity of microbial communities in hot spring sediments. The arrows on the left point to similar bands at Frying Pan Springs sites compared with other sites. Arrows on the right point to similar bands at Roadside Springs compared with other sites. The order of lanes corresponds to the following sites: A—Bijah Springs, B—Frying Pan Bowl, C—Frying Pan Spring-East, D—Roadside Spring-East, E—Frying Pan Spring-West Pool, F—Roadside Spring-West.

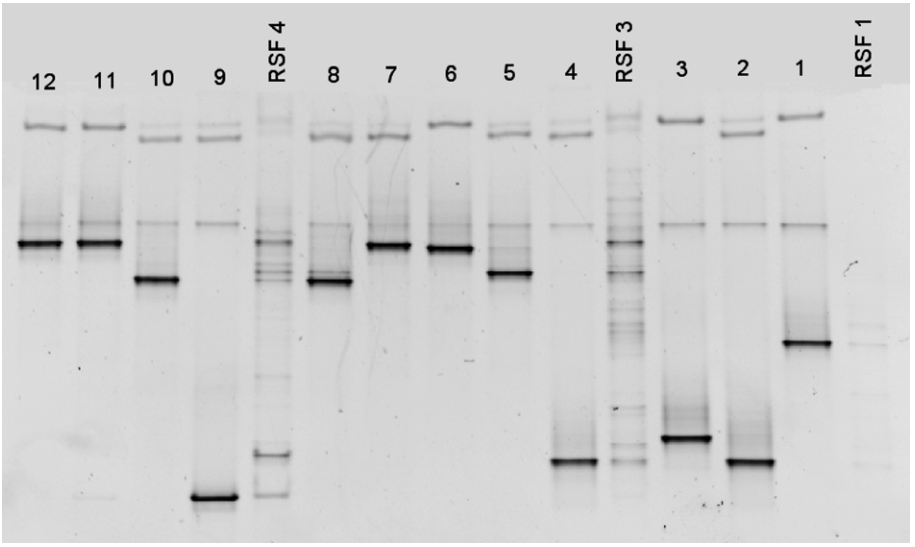

Fig. 4. DGGE image of Roadside Spring-West sites microbial community DNA and corresponding clone isolates DNA. Most closely related microorganism of each clone is listed in Table 3. The image shows single clones randomly picked from the clone library and the corresponding communities in environmental DNA from the RSF sampling site (RSF 1 = 1.5 m from source, RSF 3 = 5.5 m from source, RSF 4 = 7 m from source).

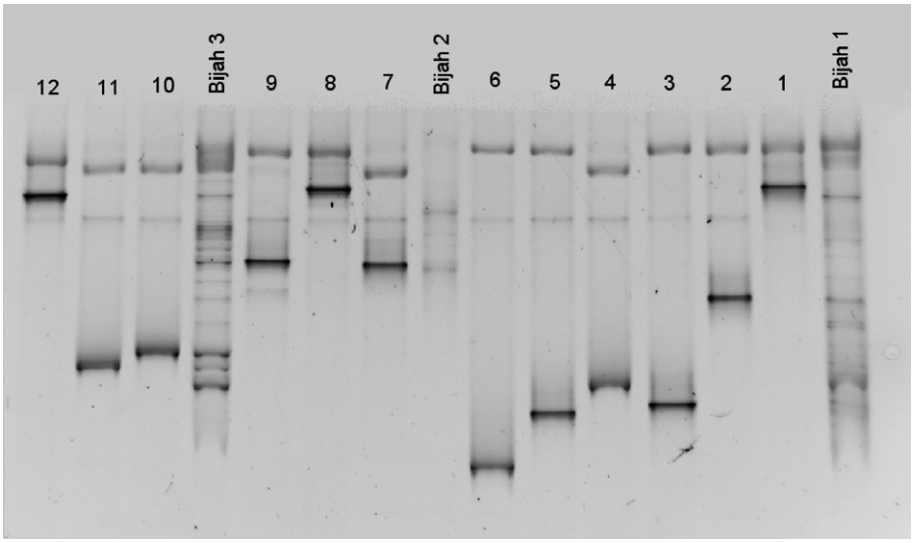

Fig. 5. DGGE image of Bijah Spring sites microbial community DNA and corresponding clone isolates DNA. Most closely related microorganism of each clone is listed in Table 4. The image shows single clones randomly picked from the clone library and the corresponding bands in environmental DNA from the Bijah sampling sites (Bijah 1 = 47 m from source, Bijah = 61.5 m from source, Bijah 3 = 92 m from source).

sources differed, yet *Chloroflexus* was found to be a common organism in each (Tables 3 and 4). The sulfidic hot spring bacterium NPE, a *Chloroflexus* reported by Nubel et al. (2001) and found by D.M. Ward (personal communication) in other YNP hot springs, is a filamentous anoxygenic photoheterotrophic bacterium utilizing organic molecules that are produced by other microbes, although some strains can use hydrogen or sulfide as electron donors, and thus, are chemolithotrophic.

The Frying Pan Bowl site (Table 5) has no organisms common to either of the higher pH hot springs. The sequences from clone libraries had best matches with *Mycobacterium* and other actinobacteria, organisms commonly found in soil. Literature searches did not find any previous reports of

Table 3
Microbial communities at Roadside Spring-West and outflow as determined by 16S rDNA amplification and sequencing of clone libraries developed from microbial mat DNA samples collected 12/03/04

DGGE Band ID	Most similar organism	Similarity (%)	Accession #
RSF1	Environmental sample DNA		
1	Uncultured *Geothermobacterium*	99	AY882756.1
2	Sulfidic hot spring bacterium	100	AJ308502.1
3	Unidentified thermus OPB32	100	AF027021
RSF3	Environmental sample DNA		
4	Sulfidic hot spring bacterium	100	AJ308502.1
5	Uncultured bacterium	98	X84474
6	Uncultured bacterium	99	AB240225.1
7	Uncultured chloroflexi bacterium	96	AY222298.1
8	Uncultured bacterium	100	AF407718.1
RSF4	Environmental sample DNA		
9	*Oscillochloris* sp.	97	AF146831.2
10	Uncultured bacterium clone B25	96	AF407718
11	Uncultured soil bacterium clone 349	98	AY493984.1
12	Uncultured soil bacterium clone 349	97	AY493984.1

According to method of Burr et al. (2006) using DGGE screening of clones.

Table 4
Microbial communities at Bijah Spring and outflow as determined by 16S rDNA amplification and sequencing of clone libraries developed from microbial mat DNA samples collected 12/03/04

DGGE Band ID	Most similar organism	Similarity (%)	Accession #
Bijah 1	Environmental sample DNA		
1	Uncultured bacterium clone O1aB8	99	AY193291
2	Uncultured organism clone MB1003c8	99	AY897755
3	Uncultured firmicute clone SM2H09	99	AF445745
4	*Roseiflexus castenholzii*	96	AB041226.1
5	Uncultured bacterium clone 01aA90	99	AY193179.1
6	*Thermotoga petrophila*	98	AJ872269.1
Bijah 2	Environmental sample DNA		
7	Uncultured bacterium clone 1700a2-40	99	AY917299.1
8	Uncultured planctomycete	97	BX294760
9	*Synechococcus* sp. C1	97	AF132772
Bijah 3	Environmental sample DNA		
10	Uncultured bacterium clone SM1C09	98	AF445666
11	Uncultured *Chloroflexaceae* bacterium clone Hs2_48	99	AF421750.2
12	Uncultured bacterium clone JC2701W_8		

According to method of Burr et al. (2006) using DGGE screening of clones.

thermophilic organisms that related to these best matches, rather they are usually mesophilic microbes often associated with human diseases or soil samples. The Frying Pan Bowl samples were collected from mats ranging from 18 to 60 °C, which suggests a wide range of temperature tolerance for this indigenous community. The results are in agreement with Walker et al. (2005) who have recently shown that 47% of the microbial species found in acidic surface soil of Norris Geyser Basin, YNP, were members of the bacterial division of *Actinobacteria*, and previously unidentified *Mycobacterium* spp. were the most abundant species.

The data show that generally less than 1% of inorganic Hg was present as MeHg in mats (Table 2). However, whether microbial mats are methylating the abundant inorganic Hg is unclear. By sequencing the 16S rDNA of purified cultured clones of the microbial mat from Roadside West Spring, an organism was identified that was closely related to *Geothermobacterium* sp. (99% similarity), which belongs to an early lineage of hyperthermo-

Table 5
Microbial Communities at Frying Pan Bowl as determined by PCR and sequencing of clone libraries developed from microbial mat samples collected 12/03/04

"*Zygogonium*" mat characterization: Most similar organism	Similarity (%)	Accession #
Mycobacterium nebraskense	98	AY368456
Uncultured bacterium	99	AY725260
Mycobacterium sp. JS623	99	AY162028
Uncultured actinobacterium	97	AY743693
Chloroplast *Haslea nipkowii*	98	AF514850

According to method of Ferris et al. (1996), without using DGGE screening.

philic SO_4-reducing bacteria that also contains *Thermodesulfobacterium*. This finding indicates the potential for Hg methylation via SO_4 reduction, as *Thermodesulfobacterium* is a chemolithotrophic SO_4-reducing bacterium. *Thermodesulfobacterium* has also been reported in Joseph's Coat Spring, YNP by Inskeep et al. (2005), and this genus is typically found in aquatic systems associated with volcanic hot springs, deep-sea hydrothermal sulfides, and other marine environments.

There is some evidence that microorganisms other than SO_4 reducers may methylate Hg when utilizing terminal electron acceptors other that SO_4 (Warner et al., 2003). Therefore, it is possible that other microbial populations may influence MeHg concentrations in these hot springs through methylation and demethylation processes or by impacts on the S cycle. The interactions between SO_4-reducing and S-oxidizing microorganisms, as well as SO_4 concentrations (Gilmour and Henry, 1991), may be important influences on Hg biogeochemistry in these hot springs. Additionally, the strong binding of Hg(II) by S precipitates (S^0, HgS, FeS, S_x) and aqueous ligands (sulfides, thiosulfates, polysulfides) may influence the bioavailability of Hg(II) to methylating populations (Benoit et al., 1999; Jay et al., 2000).

5. Conclusions

This study reports some of the first concentrations for THg and MeHg in microbial mats of hot springs and the initial characterization of the corresponding microbial populations that colonize these springs. Inorganic Hg concentrations are high relative to non-geothermal streams and rivers, but MeHg concentrations are comparable to other aquatic systems. Work in progress is focusing on characterizing the Hg speciation in water and gas phases, quantifying transport rates, and identifying microbial populations in hot springs and outflow channels.

Precipitation processes appear to dominate the accumulation of inorganic Hg in hot springs and their outflows. Low pH, high temperatures, and high concentrations of sulfides, thiosulfates and polysulfides may keep aqueous concentrations of THg elevated in geothermal sources, but cooling leads to precipitation and/or adsorption and accumulation of THg in sediment of the outflow channels. Concentrations of MeHg in water are low, but MeHg accumulation is associated with microbial mat material.

Microbial communities differ between hot springs of varying temperature and geochemistry. Sites of similar chemistry share similar DGGE bands and sequencing results verify that *Chloroflexus* was present at two hot springs of slightly different temperature and water chemistry. Microbial diversity appears to be lower at sites with lower pH and S^0 precipitates. Additionally, the dominant microbial populations of these hot springs differ from hot springs with higher pH. Development of clone libraries from amplified 16S rDNA will form the basis for future 16S rDNA sequencing and identification of organisms within the mat communities.

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