Investigators' Annual Reports 2003

Yellowstone National Park

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Yellowstone Center for Resources
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The National Park Service thanks the researchers who have contributed to our knowledge and understanding of Yellowstone. This report was compiled and edited by Alice Wondrak Biel and Christie Hendrix.

Foreword

Since the dawn of scientific wondering, human inquiry has led to the exploration, and often alteration, of almost everything in our world, at every scale—from the landscape of the moon to the human genome. In the national parks, however, through varying definitions and to varying degrees of success, the National Park Service has attempted to "preserve natural conditions" for the past 130 years. Their long-term preservation of natural resources makes parks reservoirs of information of great value to humanity. Today more than ever before, America's national parks are viewed as more than pleasuring grounds and nature preserves. In addition to using science as a means to improve park management, parks are centers for broad scientific research and inquiry.

There are 173 IARs in this year's Investigators' Annual Reports, which for the first time is divided into two parts for ease of reference. For easier reference, projects related to Yellowstone's geothermal environment comprise part I, while part II is devoted to research on the park's terrestrial environment. As has been typical in recent years, the largest sections are Microbiology (32 reports) and Ecology (25 reports). Principal and co-investigators listed in the 2003 index number some 496 people.

This report should not be seen as the body of that knowledge, but rather as its skeleton. Contact information is provided so that readers may learn more about the projects and results described here. Project findings are also available on the NPS website <https://science1.nature.nps.gov/research/ac/ResearchIndex >.

All persons who wish to conduct their own research in Yellowstone are required to apply for a permit. Information on permitting procedures is available from the Research Permitting Office, Yellowstone Center for Resources, P.O. Box 168, Yellowstone National Park, WY 82190, or at <http://www.nps.gov/yell/technical/researchpermits/index.htm>.

Contents

Part I. Geothermal Environment

Contaminants/Hazardous Materials

Project title: Microbial Cleaning of Engine Parts Investigation

Principal investigator: Dr. Leah Matheson
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Report number: 26149
Co-investigators: Timothy McDermott

Purpose: To develop thermophilic microbial culture that is able to digest carbonized, charred engine-oil residue clogging turbine engine parts.

Findings: The research conducted by MSE found that soil and water samples from Yellowstone National Park, incubated with scrapings of the carbonized material from tank engine parts, supported thermophilic microorganisms at 45, 55, and 65°C. Each of these enrichments was inoculated into a series of tubes containing test coupons, manufactured by MSE from stainless steel, that had been baked in BP Turbine Engine Oil 2380 to form occluding deposits in small holes within the parts. After incubation, the parts were flow-tested and then sonicated and flow-tested again. Results indicate that the enrichments did indeed loosen the baked-on oil deposits and significant flow improvement was achieved. However, due to experimental difficulties it is not possible to state conclusively the ultimate cleaning resolution that can be achieved with the thermophilic enrichments. Evidently, the microbes do play a beneficial part in the flow increases observed but more work is needed to determine optimal conditions and process sequence so that an engineered approach to a final engine part cleaning technology can be developed.

Project title: Enhanced DNAPL Degradation by Thermophilic Bioaugmentation of Electrical Resistance Heating

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Report number: 27780

Co-investigators: Timothy McDermott

Purpose: To develop a novel DNAPL bioremediation technology.

Findings: This Small Business Innovation Research Phase I project was funded by the National Science Foundation (NSF) and investigated the feasibility of isolating a consortium of thermophilic microorganisms that degrade chlorocarbons to benign products. Currently we are continuing this research to demonstrate the success of a novel technology using electrical resistance heating (ERH) in combination with these organisms to degrade dense nonaqueous phase liquid (DNAPL) compounds in situ. We have proposed to show that this hybrid thermophile/ERH technology could be used where conventional bioremediation has not typically been used (i.e., in source zone situations). Initial work by Drs. Matheson and McDermott, which was funded by the Interagency DNAPL Consortium, showed that thermophilic bacteria from a contaminated site at Cape Canaveral, Fla., played a role in the degradation of chlorinated hydrocarbons at elevated soil temperatures during an in situ ERH demonstration. The results of these experiments showed that trichloroethylene (TCE) biodegradation occurred due to the presence of indigenous thermophilic microbes at the site. Our present work has shown that moderately thermophilic bacteria were found to degrade TCE to 1,2cis-dichloroetheylene (cis-DCE), and possibly vinyl chloride (VC).

Geochemistry

Project title: Mercury Atmospheric Flux from Yellowstone's Geothermal System

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Report number: 27850
Co-investigators: Mark Engle, Mae Gustin, Nancy Hinman, David Krabbenhoft, Eric Miller, James Schauer, David Susong

Purpose: Quantifying both natural and anthropogenic emission sources of mercury to the atmosphere is critical to our understanding of global environmental mercury cycling and loadings, which have increased significantly in the last 100 years. Natural atmospheric emissions of mercury from geothermal and hydrothermal activity in Yellowstone National Park are a potentially significant source that has not been previously quantified. We propose to measure mercury air concentrations and surface-to-air flux rates in the park for a week in mid-July and the first two weeks in September. The research is a collaborative effort of leading environmental mercury researchers around the U.S.: from the Idaho National Engineering and Environmental Laboratory, the U.S. Geological Survey, the University of Montana, the University of Nevada–Reno, and the University of Wisconsin. The purpose of the July trip is to coordinate the research with the National Park Service staff, explore acceptable sampling sites, develop sampling strategies, and to perform reconnaissance measurements at a few selected locations (Norris, Mud Volcano, Old Faithful, Lake). In September, a more intensive sampling effort will be performed. Air concentrations will be measured in parking lot/service road locations using state-of-the-science Tekran lab analyzers and in selected off-road locations using small battery-powered air pumps and gold traps. An initial estimate of the scale of Yellowstone's mercury emissions will be made based on this data, and a more comprehensive sampling program may be developed for future years to develop a comprehensive total flux estimate. We expect to publish peer-reviewed journal articles on the results.

Findings: Fluxes varied widely across the sites, ranging from an expected low diel response (0 to 3.5 ng/m^2 hr max) at the Swan Lake Flat background site to very high values of 2,400 ng/m^2 hr on the fan below Roaring Mountain and 2,900 ng/m^2 hr (1-hour sample) at the Emerald Spring site in Norris Geyser Basin (Table 1, next page).

Table 1. Mercury flux results.

Sample Site	Area (km^2)	Flux (ng/m^2 hr) Max.	Avg.
Roaring Mountain	2.75	2,400	1,600
Norris Geyser Basin	3.20	2,900	554
Frying Pan Springs	0.007	512	397
Beryl Spring	0.015	212	80
Nymph Lake	0.0085	38	20
Lava Creek Tuff	1,220	19	15
Swan Lake Flat	--	3.5	0
Mammoth	--	Negligible	Negligible

Fluxes measured at the acidic hydrothermally altered sites were very high. At Roaring Mountain, chamber inlet concentrations exceeded 40 ng/m^3, and flux continuously increased from 1,300 to a relatively stable 2,300 to 2400 ng/m^2 h as the flux chamber flow rate was increased (to a max of 26 LPM). The 1 hour portable samples at the six Norris sites varied widely, ranging from 22 ng/m^2 h near Echinus Geyser to 2,900 ng/m^2 h at the Emerald Springs site. The other sites at Norris had flux values of 77 (Steamboat Geyser), 79 (Constant Geyser), 89 (Whale's Mouth), and 203 ng/m^2 h (Bathtub Spring). The remaining acid hydrothermal site, Frying Pan Spring, also produced high fluxes (daytime average of 400 ng/m^2 h and max of 500 $ng/^2$ hr). The fluxes measured at these acid hydrothermal areas are much higher than the average daily fluxes measured in active geothermal areas in Nevada (70.6 ng/m^2 hr) or the Long Valley, Calif., geothermal area (max of 132 ng/m^2 h) but lower than the maximum average daily fluxes found in some Hg mining areas in the western U.S. (e.g., 4,845 ng/m^2 h at Sulphur Bank, Nev.). Although these fluxes are high relative to other known geothermal sources, the measured air concentrations at all sampling locations were well below human health exposure guidelines. Additional flux measurements were made on the fan at Roaring Mountain on October 13 and 14 to confirm the high rates observed there in September. The results (max of 2,100 ng/m^2 h, 20-h average of 934 ng/m^2 h) confirmed the high September measurements, showed a strong diel response with marked nocturnal increases that correlated well with air temperature and wind speed, and showed fairly uniform flux across the different sites. If we assume our fall measurements are representative of average annual meteorological conditions, an order of magnitude estimate of annual Hg emissions may be made by multiplying the average flux obtained at characteristic sampling features (e.g., acid hydrothermally altered area) by the total surface area of those features, obtained from recent geographical information system (GIS) data (Table 1). These calculations indicate that three areas contribute appreciably to the total annual emissions in the Norris–Mammoth area: 1) Roaring Mountain 22 kg, Norris Geyser Basin 3–16 kg, and the Lava Creek Tuff formation 160 kg. The higher Norris value is the average of all six sites, while the lower value excludes the low (–22 ng/m^2 h at Echinus) and high sites (2900 ng/m^2 h at Emerald Spring). Total annual emissions from the Norris–Mammoth area are estimated to be 26–38 kg. The total annual emissions from all of the acid hydrothermally altered areas in Yellowstone (36.4 km^2) are estimated to be from 153 to 200 kg, using the average flux value (628 ng/m^2 h) from the three acid sites (Roaring Mountain, Norris, and Frying Pan Spring). This is significantly higher than the 37 kg/y estimate for hydrothermal alteration around all active geothermal areas in Nevada and the total emissions estimate of 110 kg/y for the Long Valley Caldera, California.

Project title: The Microbiogeochemistry of Sublacustrine Hydrothermal Vents in Yellowstone Lake

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Report number: 26244
Co-investigators: Russell L. Cuhel, Jim Maki, Charles Wimpee

Purpose: Exploration and sampling of underwater hydrothermal springs, plumes, associated biota and geochemical features within Yellowstone Lake. Delineation of the role of these systems in the microbiogeochemistry of the lake, including their relative activity, temporal variation and chemistry, and their influence on microbial communities associated with hydrothermal activity.
Understanding physical, chemical, and biochemical characteristics of organisms that thrive at high temperatures.

Findings: During 2003, based on parameters measured in previous years, we have been grouping the different active areas of the lake into domains, namely: North Basin (which includes Steamboat Point, Mary Bay, Sedge Bay, and several other sites around that area), Stevenson Island (deepest sampling site), and West Thumb. Some of the measurements included: silicate, chloride, total CO_2, methane, hydrogen sulfide, reduced iron, reduced manganese, and dark CO_2 fixation. It is clear that the enrichment of certain elements is not widespread throughout the lake and is not uniform either. These severely impact the microbiology and physiological response of the microbial populations on the different areas. Another relevant component that adds another dimension to the domains picture, and that is temperature. Differences in temperature in the diverse vents vary within the different domains as well as within the domains themselves. Chloride is an element that is enriched in hydrothermal areas, this has been observed consistently throughout the years research, West Thumb has been an area where chloride is more enriched that in other areas of the lake. Methane has been consistently absent from the West Thumb area and it is found in higher concentrations in Mary Bay and Stevenson Island vents.

This year, there were vents with a low concentration. Hydrogen sulfide was present in high concentrations in the North area of the lake, as well as in Stevenson Island, which showed the highest concentrations, but was not found in West Thumb. Chemosynthesis results show the trends we have been observing in the past years, where Mary Bay and Stevenson Island have areas that show a significant chemosynthetic response. Metals such as manganese and iron oxides are an important component of the solid phase material on the bottom of the lake in an oxidized form. Hydrothermal vents in Mary Bay have high concentrations of reduced manganese an iron. The solid phase samples in Mary Bay show the signs of oxidation of manganese and iron, some of the solids have a patina of minerals, whereas other areas have thicker layers of the oxides. Reduced iron is found in very low concentrations in ventwaters in the West Thumb area. The solid material shows an abundant covering of manganese and iron oxides present in the area. The distribution and diversity of methane-oxidizing (methanotrophic) bacteria and methane in both the water column and hydrothermal vents of Mary Bay. Estimations of the number of methanotrophs are being made with a Most Probable Number-Polymerase Chain Reaction (MPN-PCR) technique using specific primers for the gene of an enzyme that is found in all known methanotrophs, particulate methane-monooxygenase. Methanotroph numbers have been and the numbers in the water column were estimated to be between 1.0 x 100 to 5.0 x 104 cells/ml. In the water column of Mary Bay, the highest estimations of cells have been observed with the highest concentrations of methane. However, this does not hold true for hydrothermal vents. In vent water samples methanotroph numbers ranged between 2.5 x 102 and 4.75 x 104 cells/ml. Archaeal diversity in the water column: In samples collected during summer stratification, using primers specific for the 16S renal gene in *Archaea* and the PCR, we were able to amplify genes from samples collected below the thermocline but not in the epilimnion. The data suggest that the thermocline is somewhat of a barrier to mixing of the Archaea from the vents and the hypolimnion into the epilimnion. The archaeal DNA from a couple of the samples has been cloned and the clones examined using Amplified rDNA Restriction Analysis (ARDRA).

Project title: Geochemical Monitoring of Selected Features at the Norris Geyser Basin, Yellowstone National Park: Baseline Chloride and Sulfate Measurements

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Report number: 26853
Co-investigators: Carey Gazis, Virginia Rodriguez, Matt Vitale

Purpose: The extreme variability of thermal activity at Norris Geyser Basin is confirmed by the studies of Fournier et al. and Friedman, who conducted the pH and chloride content of Yellowstone hot spring waters. My investigation builds on those studies. Previous investigations indicate that seasonal disturbances are characterized by variations in the discharge characteristics of many springs in Norris Geyser Basin. The objectives of this study are to (1) conduct chemical and field-monitoring of Norris Geyser Basin that builds on previous studies by Fournier et al. and Friedman; (2) collect data from different temporal (minutes to months) and spatial scales on the chemistry of the geothermal waters in the basin, which will be used to quantify and describe variability of chloride and sulfate flow to map out mixing histories of the waters throughout the basin; and (3) explain the details of the seasonal disturbances in the basin and develop a conceptual model of its plumbing system that explains the mixing characteristics in a spatial and temporal way.

Findings: Last summer I proceeded with these objectives and collected 102 samples (60ml) for trace elements and 102 samples (30ml) for anions to be analyzed at Central Washington University labs. Because of the very active season in the basin this past summer, I had to limit my features to seven and monitor just once a week instead of twice as I did in July. The features show variability for the Cl and SO_4, which were done using the Ion Chromatograph (IC). Additional analysis are currently underway. The trace elements, analyzed by Inductively-Coupled Plasma Mass Spectrometry (ICP-MS), are variable among the features. In particular, Echinus has distinctly different trace elements (As, Mo, Ba, Li, Mn, Cr, Zn, La, Eu, Ho,Yb, etc.) compared to the other sites. Additional trace element data are currently being collected. During winter 2004, the same hydrothermal features will be sampled to facilitate comparison with the samples collected during summer 2003. Changes in the potentiometric surface occurs as water is added to the hydrothermal plumbing system due to melting. Addition of this cooler water may be traced through changes in chemistry of the sampled waters.

Project title: Water Chemistry and Its Relationship to Local Geology: a Yellowstone Case Study

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Report number: 26219
Co-investigators: Clara Cotten

Purpose: This study is an ongoing component of Geology 329 taught from the Indiana University Geologic Field Station, Cardwell, Montana. During the field-based class, undergraduate students involved with several environmentally oriented programs (chemistry, biology, ecology, geology, and environmental science) on campus, are involved in their first intensive field experience. The objectives of the Yellowstone study are twofold. First, the Yellowstone field trip is a unique opportunity to look at an ecosystem that is heavily influenced by hydrothermal activity, which is in stark contrast to the riparian and mountain systems found in study areas of the Tobacco Root Mountains. During the weeks preceding the Yellowstone trip, the students engage in the collection of field measurements of various aquatic systems encountered in their study areas. This data (including oxidation-reduction potential, pH, temperature, and specific conductance) is used as a comparative set against the data collected in the select thermal features of Yellowstone. The range of values encountered in the Yellowstone features gives some extreme values for real world data sets and illustrates how temperature controls many of the other chemical variables and microbial ecosystems. Plotting the data on topographic maps gives some notion of the distribution, and compartmentalization of the thermal features, in contrast to typical watershed/groundwater studies. In addition, the real time data that the students collect and plot is compared to the plots of the field data from the USGS Bulletin 1303, which was collected in the 1960s, and the data from previous classes. The data illustrates the geologically ephemeral nature of the features when compared to time scales of other geologic processes observed and discussed during the course.

Findings: On July 4, 2003, Yellowstone geologists Henry Heasler and Cheryl Jaworowski accompanied the group through the Upper Basin at Norris. The group took four sets of field measurements (oxidation-reduction potential, pH, temperature, and specific conductance) at 13 thermal features along the public boardwalk. Over lunch, the data were compiled and plotted on a copy of the map figure from Rowe et al. The data was then compared to the published data from USGS Bulletin 1303 to see which features were new in the last 40 years, which had cooled or were inactive, and where the current hot spots were today. Data were compiled and sent to the Norris Ranger Station to add to the database. Later in the afternoon, the group visited Octopus Spring for a look at the controlling factors in the distribution of microbial communities. Groups again took field measurements along the runoff channel to observe how temperature controls the chemistry and the distribution of the various microbial communities. Students plotted their data along their sketched map of the spring and runoff channels. Eight water samples were collected, and H_2S (HS-), SO_{42}-, and Cl- were trapped with Zn-acetate, BaCl, and $AgNO_3$, respectively, to form precipitates from the pool, and three points of increasing distance from the spring. These precipitates were filtered, weighed, and the concentrations of these constituents calculated by the students two days later at the Field Station. Care was exercised to leave the features as undisturbed as possible, and to avoid reactants' making it into the environment.

Project title: Low-Temperature Release of Magmatic Gases

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Report number: 28134
Co-investigators: Deborah Bergfeld, B. Mack Kennedy, Jacob Lowenstern, Mathijs C. van Soest

Purpose: We will collect samples of water from selected cold springs in the park and analyze them for dissolved magmatic gases, mainly carbon and helium. We will also make some measurements of diffuse CO_2 emissions from soils. The main goal of this investigation is to determine if substantial amounts of magmatic gases are transported by the cold groundwater system and if diffuse emissions of magmatic gas are important in areas away from the main thermal fields. The main area of interest is the NW sector of the Park between Norris Geyser Basin and Hebgen Lake, where seismic tomography suggests a large gas reservoir in the subsurface. No chemicals will be released to the environment and physical disturbance to springs and soil surfaces will be minimal.

Findings: We located and sampled twenty springs within the park and in the area between the park and Hebgen Lake. Sampling sites inside the park include three springs in the Madison Valley, two springs on Pass Creek, one spring on Grayling Creek, and six springs on the Gallatin River. We achieved the desired coverage over the target area. Initial carbon isotope results indicate a variety of carbon sources in these springs, but helium isotope analyses are not yet complete; so at this time we have no definitive evidence for magmatic carbon in any of the springs. Nevertheless, the findings are of sufficient interest to justify another round of sampling in 2004, with more intensive sampling of springs near those springs found most interesting in the 2003 survey.

Project title: Geochemistry of Thermal Springs

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Report number: 28626
Co-investigators: Julie Michelle Kotler, David Krabbenhoft, John Mocko, Aaron Tenesch, Cindy Wilson, William Woessner
Purpose: (1) Identify physicochemical or hydrological factors that control geochemistry of thermal springs. (2) Document temporal and spatial variations in thermal spring chemistry in spring deposits. (3) Investigate mutual effects of microbial populations and geochemistry. (4) Investigate interaction of thermal springs with shallow groundwater environments.

Findings: Work in 2003 focused on the relationship between solution chemistry and the timing and nature of solids formed in hot springs. Although largely focused on higher temperature systems, the interaction between hot surface water and cooler groundwater also produces solids that are unique to thermal areas. <u>Groundwater–Surface Water Interactions.</u> Seasonal changes in water chemistry were observed in groundwater at Rabbit Creek. Iron increased in groundwater near Rabbit Creek during winter, as did sulfide. Such changes indicate that suboxic conditions develop in shallow groundwater during winter. Saturation of soils, effectively isolating shallow groundwater, is the likely explanation for development of suboxic conditions. In previous years, release of reduced elements from groundwater to surface water was observed but could not be confirmed this year. Such events are probably short-lived and if not sampled at the right time, are not observed. Continuous, or at least more frequent, monitoring of water chemistry would be needed to determine the timing and conditions for registering such exchange.

Chemistry of Sinter Deposits and Hot Springs. Chemical changes can affect the pH and redox conditions of thermal springs. Thus, it makes sense to use signatures of elements affected by these factors to determine the chemical history of a thermal spring or area. In this study, we focused on changes in the concentrations and forms of aluminum and silicon in thermal springs of different compositions. An unnamed geyser in Shoshone Geyser Basin and Trinoi Geyser (Krontoskii Reserve, Kamchatka, Russia) are used to represent different spring chemistries that might result from changes over a long time period or may result from original differences in host rock composition. Nuclear magnetic resonance (NMR) spectra acquired at the Wiley Environmental and Molecular Science Laboratory illustrate differences in Al between the unnamed and Trinoi Geyser. Trinoi Geyser shows significant quantities of both tetrahedrally coordinated aluminum and octahedrally coordinated aluminum. The unnamed geyser has no octahedrally coordinated aluminum. Such differences in Al atomic environment may be attributed to effects of solution chemistry on the speciation of dissolved forms of Al. Speciation affects properties of solid deposition and therefore is the likely explanation in the observed differences in NMR spectra. Spring chemistry, therefore, affects the properties of solids formed there and may yield evidence of spring changes in the past. In addition to major elements, minor elements can and do become concentrated in mineral precipitates. The species of elements incorporated into such deposits depends on its chemical form in solution. Important complexants that affect chemical speciation are organic compounds. These components also affect photochemical reactions of redox reactive elements in surface water, which in turn affect the composition of solids formed from such solutions. Studies of organic components of hot springs indicate the presence of extremely polar organic compounds. Additional studies are necessary to further identify the organic compounds. Another chemical marker for hot spring deposits is mercury. Aside from its environmental significance, the presence of mercury in hot spring deposits indicates specific, limited conditions for its incorporation. Mercury is also photochemically active and may provide a good comparison for photochemical reactions involving iron, reactive oxygen species, and sulfur compounds. Mercury was measured in acid-sulfate and alkaline-chloride hot springs. Concentrations did not vary predictably between different areas indicating a complicated relationship between source, chemical evolution of water compositions, and hydrogeology.

Project title: Sulfur Speciation and Redox Processes in Mineral Springs and Their Drainages

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Report number: 28892
Co-investigators: James Ball, JoAnne Holloway, Blaine McCleskey

Purpose: The primary objective is to determine the actual speciation of dissolved sulfur species as they undergo oxidation and volatile losses for H_2S. Intermediate sulfoxy anions such as thiosulfate have been implicated as complexing agents in the formation or ore deposits and as monitors of volcanic activity. We hope to relate sulfur speciation in hot springs and their drainages to rates of oxygen diffusion and solubility.

Findings: We sampled about 47 hot springs and overflow drainages in the Park and determined the H_2S and thiosulfate concentrations at most of these. We continue to find rapid volatilization and oxidation of H^2S and the rapid oxidation to thiosulfate. We have also found a few more springs where thiosulfate is present in higher concentrations than H_2S and we are attempting to relate this unusual occurrence to the degree of mixing of thermal fluid with shallow dilute ground waters.

Project title: Arsenic Geochemistry in Yellowstone National Park: Occurrence, Speciation, and Transformations

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Report number: 28893
Co-investigators: James Ball, JoAnne Holloway, Blaine McCleskey

Purpose: This proposal focuses on the source, transport, and fate of arsenic in the hydrothermal waters and drainages of Yellowstone National Park, especially the effect of speciation on arsenic mobility. Arsenic(III) and arsenic(V) will be determined routinely for a variety of thermal waters from different areas and determinations of arsine, methylated arsenic, and thioarsenites will be attempted on waters from selected locations.

Findings: We have found that arsenic occurs predominantly as the reduced form As(III) in discharging hot springs and it quickly oxidizes to As(V) in the overflows once the reduced sulfur concentrations decrease to low levels. The arsenic oxidation rate is at least seven orders of magnitude faster than inorganic rates (uncorrected for temperature effects). This faster rate can only be achieved by arsenic-oxidizing microbes. Arsenic also behaves both as a conservative and a non-conservative element in the hydrothermal waters of Yellowstone but the main zone of non-conservative behavior is found at or near the ground surface.

Project title: Geochemistry, Microbiology, and Stable Isotope Systematics of Hydrothermal Systems in Yellowstone National Park

Principal investigator: Dr. Wayne (Pat) Shanks
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Report number: 28355
Co-investigators: Jeff Alt, Steve Harlan, Lisa Morgan

Purpose: Many ancient epithermal precious-metal and mercury deposits occur in veins, hydrothermal explosion breccias, and siliceous sinter hosted in lake beds and volcanic rocks related to ancient hot springs. The objectives of this task are to understand mineralizing conditions and geochemical processes in sublacustrine hydrothermal vents in Yellowstone Lake and at subaerial vents parkwide. In particular, we are using minor and trace elements and stable isotope studies (H, C, N, O, and S) to understand processes of hydrothermal mineralization in Yellowstone Lake and to track toxic and nutrient elements from the hydrothermal vents into the micro- and macro-fauna of the lake and into the Greater Yellowstone Ecosystem. In this regard, we have collected multiple samples of sublacustrine hydrothermal vent fluids and associated hydrothermal mineral deposits from Mary Bay, Steamboat Point, Sedge Bay, Stevenson Island, and from several localities in West Thumb. In addition, we collected water samples and estimated stream flow for the 46 largest streams that feed into Yellowstone Lake to provide a comprehensive picture of trace element and water fluxes in the lake. Knowledge of elemental fluxes is critical to understanding the effects of natural hydrothermal vents on water quality in the lake, and the effects of toxic elements on native species such as the Yellowstone cutthroat trout. Cutthroat trout feed in shallow sublacustrine vents ("cutthroat Jacuzzis"), and are critical to the ecosystem because they spawn in streams and are food sources for otters, eagles, and bears. We are also sampling numerous subaerial hydrothermal systems to understand hydrothermal processes of mineralization and relations of these processes to microbial ecology, in collaboration with Tina Vesbach, Anna-Louise Reysenbach, and the NPS in the context of the parkwide microbiological inventory. We are sampling hot springs, mineral deposits, and altered rocks at many sites parkwide.

Findings: In 2003, water and solid samples from Yellowstone Lake hydrothermal vents were collected in West Thumb, and in the northern lake at off Storm Point and near the large crater south of Mary Bay (informally referred to as Elliot's Crater. Subaerial water samples were collected at Nymph Lake (new vent), Highland Glen/Africa Basin, Violet Springs, Mammoth, Norris, Washburn, Mud Volcano, Rainbow, Coffeepots, Joseph's Coat, Hotspring Basin, West Thumb, Mudkettles/mushpots, Wahb, Calcite, Gibbon/Artist Paint Pots, Lower, Midway/Rabbit Creek, Upper, Lone Star, and Crater Hills. All of these samples have been analyzed for complete chemical and stable isotopic compositions and data are currently being interpreted and prepared for publication.

Project title: Student Project to Measure Geochemistry of Thermal Springs

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Report number: 28040
Co-investigators:

Purpose: To instruct students about the geochemistry of thermal springs. The students under my supervision will conduct in situ measurements of temperature, pH, and conductivity.

Findings:

Location	Temp. (deg.C)	pH	Sp. Cond. (microS/cm)
Norris GB			
Echinus Geyser	72	3.75	2,000
Cistern Geyser	81	5	3,400
Pinwheel Geyser	50	4.2	1,500
Whale's Mouth	51	7.5	1,750
Mammoth HS			
Narrow Gage Spg.	60	6.8	2,700

GEOPHYSICS/SEISMOLOGY

Project title: Mapping the Floor of Yellowstone Lake Using High-Resolution Bathymetry, Seismic Reflection, and Submersible Remotely Operated Vehicle

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Report number: 28280
Co-investigators: David Lovalvo, Pat Shanks

Purpose: Our initial goals of obtaining a high-resolution bathymetric map of Yellowstone Lake and unequivocally characterizing many lake bottom bathymetric features such as faults, fissures, slumps, hydrothermal deposits, explosion craters, submerged shorelines, and glacial deposits have been achieved. Subbottom seismic reflection profiles, combined with towed magnetometer data obtained at about 200-m line spacing, have discerned zones of active, present-day geothermal fluid migration through sediments or hydrothermally altered sediments. Our surveys (1999–2002) have identified submerged faults, explosion craters, hydrothermal vents, domal structures, and landslide deposits have improve understanding of the interrelationship of these features, their causes, and influences by deeply circulating hydrothermal fluids. A next phase is to work with NPS fishery biologists in identifying locations of spawning sites for introduced lake trout. The surveys give an accurate picture of the geologic forces forming Yellowstone Lake and how geology affects the aquatic biosphere. In 2003, we plan to focus on specific sites in Yellowstone Lake using the submersible (ROV) remotely operated vehicle with a sub-bottom seismic profiler on specific sites. Such sites in the lake include a very active hydrothermal area interpreted as an inflating domal structure (the Inflated Plain), several young faults, detached landslide blocks, glacial features, and a few other active hydrothermal areas. If time and access to equipment allows, we may survey Duck Lake and Indian Pond. Turbid Lake would also be desirable to survey but its location may present significant logistical access problems that would need to be coordinated with the park.

Findings: In 2003, we collected sub-bottom seismic reflection profiles at selected sites in Yellowstone Lake. That data is currently being processed and analyzed. We are working with YNP/NPS fishery biologists to help characterize the substrate that is favorable for spawning of lake trout, which will contribute to resource management goals. In 2003, we worked with individuals from the University of Minnesota who have developed a new real-time in situ sensor into active hydrothermal vents on the lake bottom. The sensor was able to measure pH, H_2S, H_2, and temperature. We continue to work on publications that have evolved from this work. These products include several maps and research papers.

Project title: Operation and Development of an Earthquake and Volcano Information System at Yellowstone and Ancillary Research on the Geodynamics of the Yellowstone Hotspot

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Report number: 28411
Co-investigators:

Purpose: The Yellowstone seismic and GPS network is the main monitoring system of the Yellowstone Volcano Observatory (YVO) and serves as part of the NSF project, "Geodynamics of the Yellowstone Hotspot." The network is an integrated, real-time system for recording earthquakes and ground motion of Yellowstone National Park and surrounding area focusing on the Yellowstone volcanic field and associated fault zones. Specific objectives include: (1) operation of the 23-station seismic and five-station GPS (Global Positioning System), (2) routine station installation, maintenance and upgrading, (3) data recording, processing, analyses, and archiving and (4) web accessible distribution of the data from these systems. Information from this network are provided both via the web and verbally to the National Park Service and USGS management, NPS public safety and interpretation groups, as well providing online data for scientific research for all users. In this report period we expanded the efforts of the seismic and GPS monitoring efforts in Yellowstone utilizing the University of Utah as the base of operations. The USGS Volcano Hazards Program jointly funds this cooperative project with partial support form the National Park Service for fieldwork. The primary products of the project are earthquake catalogs, ground deformation data, the services of a regional earthquake and precise GPS recording and information center, including timely release of unusual volcanic and earthquake activity reports to the USGS and the NPS. In addition, the National Science Foundation and the University of Utah have provided matching funds for GPS stations and for basic research on the Yellowstone Hotspot project.

Findings: Norris Geyser Basin Disturbance. The University of Utah responded to the summer 2003 Norris geyser basin disturbance by installation in collaboration with the NPS of portable monitoring equipment. This included five high precision GPS stations and seven broadband seismographs. This equipment operated for approximately August–Sept 2003. This disturbance included an increase in ground temperatures of up to 94°C over a 0.1 km sq. area, killing vegetation along a ~200 m long zone of the Back Basin and prompting closure of the area to visitors. In response to these changes, a temporary seismic and geodetic monitoring network was deployed by the University of Utah with the assistance of UNAVCO and IRIS. Data collection began in August 2003, with a dense, 0.75 km^2 array of seven broadband seismographs and five precision GPS stations across the disturbed area. Results from the seismic monitoring allowed us to study the presence and movement of hydrothermal fluids, while the GPS data indicated rather high ephemeral ground motions exceeding cm/yr of uplift and SW motion of the Back Basin. Together with ancillary temperature and fluid flow data by the NPS, the information suggests migration of shallow hydrothermal fluids that drove the deformation. Yellowstone Seismicity. Epicenters of 1,124 earthquakes (M<= 4.4) were located in the Yellowstone region during the reporting period. Earthquakes occurred throughout the caldera with the largest event, a M 4.4 event in August 2003 that occurred just south of the park boundary on the Mt. Sheridan fault zone. Activity continued in the West Yellowstone–Norris zone, the western caldera and in the area east of the caldera in the Absaroka Mtns. GPS Monitoring of Yellowstone Ground Motion. During the report period, principal effort was focused on: (1) operation of the five-station permanent GPS network, and (2) a major GPS field campaign of monitoring of the Yellowstone Plateau. Station positions for 2003 were combined to obtain the station velocities for the network. Results show that the Yellowstone Plateau exhibits continued parkwide crustal deformation of ~4 mm/yr SW extension with a concentrated zone of uplift from 1995–2000 centered at northwest caldera of ~1 cm/yr, a decrease from that we noted in our earlier monitoring of ~2 cm/yr. This is in contrast to the central and eastern caldera that continues to be subsiding. Geodynamics of the Yellowstone Hotspot. The study includes seismic and GPS investigations of the possible plume-plate interaction that is hypothesized for the Yellowstone volcanic field. Regional deformation by GPS reveals dominant NE–SW extension of the entire system. Seismic tomography reveals low-velocity crustal bodies beneath Yellowstone and an upper mantle P- and S-wave low-velocity anomaly that extends to the NW to depths of ~400 km. The mantle body is considered a mantle plume likely produced by return eastward flow of the upper mantle. *Publications*—In total, University of Utah Yellowstone researchers have published and presented papers on their Yellowstone research projects including: (1) submitted five papers to national journals, (2) made nine presentations at national meetings, (3) gave seven invited presentations.

Geosedimentology

Project title: The Emergence of Scale-Invariant Architecture in Rimmed-Pool Carbonate Terraces: Abiotic Controls in Surface Hot Springs and Subsurface Cold Springs

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Report number: 28406
Co-investigators: Nigel Goldenfeld, Alison Murray

Purpose: In order to more accurately interpret the geological record of microbial life, the search is intensifying for definitive criteria with which to discriminate among biologically controlled, biologically induced, and inorganically precipitated aragonite and calcite crystals. We have observed that rimmed-pool carbonate terraces in terrestrial hot springs at Yellowstone National Park and subterranean cold springs in Illinois Caverns are strikingly similar with respect to their macro-scale morphology, depositional patterns, and crystalline architecture. Yet the two depositional settings are dramatically different with respect to all fundamental environmental parameters, including water temperature, water chemistry, carbonate mineralogy, and local climatic conditions. An integration of techniques in concepts in geology, physics, groundwater geochemistry, and hydrology to identify the fundamental physical and/or biological processes operating in these profoundly different hot spring and cave environments to produce nearly identical crystallization patterns and morphologies. The questions to be addressed include: (1) What physical and chemical environmental conditions are present during the precipitation of rimmed-pool carbonates in surface hot springs and subsurface cold springs? (2) Can quantitative analysis of the emergent scale-invariant patterns of rimmed-pool carbonate terraces from these extremely different environments identify unifying physical controls? (3) Once identified, can these inorganic physical controls on carbonate precipitation be used to discriminate between biotic and abiotic carbonate deposition in these and other settings? We propose to complete a three-phase study. In the first phase, comparative in situ measurements of the natural physical parameters (water temperature, flow rates) and chemical reactions at the margin of travertine and speleothem rimmed pools will be completed. In the second phase, in situ crystallization experiments will be done to track the interplay between fluid dynamics and crystalline architecture to create the rimmed-pool morphologies. In the third phase, theoretical and computer modeling of these data, including the evaluation of physical phenomena such as self-organization, scale invariance, spatial-temporal patterns, chaotic dynamics, and fat-tailed probability distributions, will be completed to identify underlying physical controls on rimmed-pool carbonate terrace architecture.

Findings: The research conducted this year was to understand the carbon cycling pathways in different facies of travertine deposits at Angel Terrace, part of the Mammoth Hot Springs complex. Microbial mats or biofilm-encrusted carbonate rocks were collected for lipid and carbon isotope analyses along a temperature gradient from 71°C in the vent facies to 24°C in the distal-slope facies. Phospholipid fatty acids (PLFA) show distinct patterns in different facies. The vent facies is dominated by 20:1_ (34.1%) followed by 18:0, 18:1_, and 16:0 as major compounds. This pattern is consistent with lipid profiles of known thermophilic *Aquificales*. The pond and proximal slope have similar PLFA patterns with 16:0 being the most abundant fatty acid (56.7 and 54.5%, respectively) followed by 18:1_, 18:0, 18:2_6 and 16:1_ as major compounds. The biomarker 20:1_, however, is negligible (<0.5%), indicating the minor abundance of *Aquificales* in these facies. The distal slope has 16:0 and 18:1_ as the most abundant fatty acids (30 and 24.8%, respectively), but also has appreciable amounts of i15:0, 18:3_3, and 20:0–24:0 in addition to 16:1_ and 18:2_6 that are present in the pond and proximal slope. The presence of i15:0 indicates contribution from bacteria and the presence of polyunsaturated fatty acids and long-chain (>20 carbons) fatty acids suggest contributions from eukaryotes. The 13C values of total organic carbon (TOC) decrease from –16.1 in the vent to –23.5 in the distal slope; however, lower values occur in the pond (–26.0) and proximal slope (–28.0). Total fatty acids (TFA) in the vent are enriched in 13C by 3.7% relative to TOC, supporting the notion that the biosynthetic pathways may be dominated by *Aquificales* using the reversed tricarboxylic acid cycle. TFA in the pond and proximal slope are depleted in 13C by 6.9% and 5.9%, respectively, relative to TOC, which are characteristic of the Calvin cycle performed by cyanobacteria or other chemoautotrophic bacteria. TFA in the distal slope have 13C values (–24.0) close to TOC, suggesting the involvement of other biosynthetic pathways in addition to the Calvin cycle. Results of this study demonstrate that lipid biomarkers can provide valuable information on changing microbial communities in different depositional environments; carbon isotope fractionations on the other hand can provide insight into the operating biosynthetic pathways associated with different organisms in the changing environment. This integrated approach may serve as a powerful tool for identifying functional metabolism within a stratified community as well as community compositional shifts in modern hot spring systems.

Project title: The Structure, Facies, and Deposition of Siliceous Sinter Around Thermal Springs: Implications for the Recognition and Study of Early Life on Earth and Mars

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Report number: 26369
Co-investigators:

Purpose: This is an ongoing project. The purpose is the same as in my previous permit application and the annual reports for 1999, 2000, 2001, and 2002.

Findings: No field work in Yellowstone was done during 2003, although a number of silica deposition rate experiments, placed in hot springs and run-out systems in prior years (see annual reports for 2002 and 2001) were left in place during 2003. Research in 2003 consisted mainly of writing and working with materials already at Stanford. Published paper: Lowe, D.R., and Braunstein, D., 2003. Microstructure of high-temperature (>73°C) siliceous sinter deposited around hot springs and geysers,

Yellowstone National Park: The role of biological and abiological processes in sedimentation: *Canadian Journal of Earth Sciences,* v. 40, pp. 1611–1642.

Geothermal/ Volcanology

Project title: Monitoring Caldera Unrest at the Yellowstone Caldera: A Global Positioning System (GPS) Crustal Deformation Study and Hot Springs Temperature Study by the Eastern Illinois University Geology Field Camp

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Report number: 28400
Co-investigators: Sid Halsor, Robert Jorstad, William Toothill

Purpose: The purpose of our study is to monitor caldera unrest at the Yellowstone caldera by annually collecting GPS data and hot spring temperatures and pH from a network of data collection points. Although the data collected in this study can contribute to more detailed monitoring efforts of the caldera, the primary goal of this project is to provide an ongoing, hands-on field experience for undergraduate geology majors at the Eastern Illinois University Geology Field Camp.

Findings: During our fifth annual GPS survey on June 25–26, 2003, we deployed three Trimble 4000Ssi receivers at pre-determined GPS control stations in the park. These stations are located along a NW–SE traverse across the western part of the Yellowstone caldera. Each year, data is collected continuously for 48 hours from eight control stations. The GPS team sets up each station and trains students in overall instrument monitoring before leaving them with the receivers for four-hour intervals. From 2000–2002, our deformation results showed that the western region of the Yellowstone caldera had subsided, uplifted, and then subsided. Horizontal displacements in 2002 had southern and western components, in contrast to northerly displacement in 2001. There is no apparent relationship between the pattern of uplift/subsidence and horizontal displacement direction and the caldera structure. Results from the 2003 survey were still being processed when this report was submitted. A more detailed report of this study, including the 2003 data and its interpretation is available at: <http://spruce.gis.wilkes.edu/yellowstone/>. A second component of our study is monitoring hot springs temperatures at selected thermal areas within the Yellowstone caldera. Our study area consists of three separate thermal areas in the Lower Geyser Basin located along Rabbit Creek, White Creek, and Sentinel Meadows. Students measure the temperature of each hot spring using thermocouples and determine its location using a GPS unit. Baseline temperature data for our study was collected on June 28–29, 2000, and baseline pH data and digital imagery was collected on June 26–27, 2002. On June 25–26, 2003, we revisited the study areas and collected data on about 150 hot springs. All three study areas showed a general decrease in hot spring temperatures, in contrast to the gradual increases that we noted from 2000–2002. Springs in Sentinel Meadows thermal area are consistently the hottest of all the study areas, with temperatures typically ranging from 85–96°C. From 2000–2002, springs had either remained stable or shown temperature increases of 3–4°C. During the past year however, most changes were towards cooler temperatures, lowering between 5–9°C. The pH in Sentinel Meadows springs was relatively unchanged, ranging from 6.2–8.7, and exhibited a positive correlation with temperature. There is no correlation between pH and location or pool size at Sentinel Meadows. The Rabbit Creek thermal area exhibited the greatest range in temperatures of the three study areas, 40–95°C. Between 2000–2002, about 40% of the springs showed modest temperature increases, typically between 3–7°C. In the past year, however, about 25% of the springs in the Rabbit Creek area have had temperature decreases, mostly between 3–10°C. Rabbit Creek has the widest variance in pH of the three study areas (6.0–9.5), although a majority of the springs have a pH between 7.5–9.0. No significant changes in pH were noted in 2003. There is no correlation between temperature and pH at Rabbit Creek. Larger pools tend to have higher pH values than smaller pools, and there appears to be a geographic pattern of water pH at Rabbit Creek. Thermal features in the White Creek Group range in temperature from 53–95°C. In this group, more springs had shown temperature decreases than increases between 2000–2002, although most had remained unchanged. In 2003, most changes were towards lower temperatures with about 25% of the springs showing temperature decreases between 3–10°C. The White Creek Group has pH values between 6.3–8.9, but most are above 7.5. Similar pH values were recorded in 2003. There appears to be no relationship between pH and temperature, geographic distribution, or pool size. Maps and data tables for this study can be viewed at <http://oldsci.eiu.edu/geology/camp/YNP/ynpres.htm>.

Project title: Eruption Observation of Selected Remote Geysers

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Report number: 27587
Co-investigators: Carlton E. Cross, Tara M. Cross

Purpose: To obtain eruption interval and duration measurements of selected remote geysers at Shoshone Geyser Basin (Double Geyser, geysers in North Group), Gibbon Geyser Basin (Phoenix Geyser, Oblique Geyser), Heart Lake Geyser Basin (Glade Geyser), Lone Star Geyser Basin (Buried Geyser, Unnamed geyser SW of Lone Star–Shoshone trail bridge).

Findings: At Heart Lake Geyser Basin, Glade Geyser erupted every 16.8–22.5 hours, with an average of 20.4 hours (11 intervals) July 9–18, 2003), representing a slightly increased eruption frequency over that observed in 2002. At Shoshone Geyser Basin, Double Geyser erupted every 84–97 minutes with an average of 89 minutes (31 intervals) during July 11–13, 2003. A small but significant new geyser, informally named "Hydra Geyser," or "The Hydra," continued its activity for a second summer, erupting (June 22–August 7) in a series of 1–5 eruptions spaced at intervals of 56–128 minutes with an average of 88 minutes. Series began at irregular intervals every 8–97 hours. At Gibbon Geyser Basin, Phoenix Geyser entered its ninth year of eruptions since reactivating in 1995. Intervals during July 18–August 10 were 3.7–4.8 hours, with an average of 4.2 hours (129 intervals).

Project title: Contemporary Surface Deformation of the Yellowstone Caldera

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Report number: 26426
Co-investigators: Michael Poland

Purpose: We will conduct precise leveling surveys every few years at Yellowstone National Park to assess the character of vertical deformation within the caldera system.

Findings: To prepare for leveling surveys that are planned for late summer 2004, we spent two weeks in 2003 locating and documenting survey benchmarks along park roads. Our reconnaissance over the past two years has yielded the following results: 349 benchmarks confirmed in good condition, 7 benchmarks confirmed destroyed, 34 benchmarks presumed destroyed, 36 benchmarks searched for but not found, and 88 benchmarks not yet been searched for. We anticipate locating the remaining 88 marks during fieldwork in 2004, and will produce a map and database that documents the locations and specifics of each survey marker.

Project title: Hydrocarbon Distribution Among Hot Springs and Fumaroles in Yellowstone National Park

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Report number: 28251
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Purpose: To examine variability in volatile hydrocarbon contents in hot springs and fumaroles. In this study, a total of 26 hot springs and fumaroles were sampled within the Yellowstone caldera, along the caldera rim, and outside the caldera for the determination of hydrocarbon composition. The results of this study will be written up in a Master's thesis.

Findings: Twenty-one different hydrocarbon compounds present in the gas phase were identified. Death Gulch Thermal Area samples had the highest overall concentrations of hydrocarbons (22,000 ppm, not including methane). Of the sites with hydrocarbons heavier than methane, the fumarole adjacent to Beryl Spring had the lowest overall hydrocarbon concentration (84 ppm, not including methane). Methane, the predominant hydrocarbon found in the gases, was detected in all of the Yellowstone samples. After methane, ethane (up to 13,000 ppm) and propane (up to 11,000 ppm) were the most abundant hydrocarbons. Concentrations of the higher hydrocarbons generally decreased with increasing carbon number with the exception of benzene (up to 495 ppm). Three different hydrocarbon distributions, related to location, are evident in the Yellowstone samples. With the exception of Ochre Springs, samples collected within the Yellowstone caldera contained no hydrocarbons higher than methane in the gas phase. Samples collected along the northeastern rim of the caldera and outside of the caldera had high concentrations and diversity of alkanes; no alkenes, with the exception of benzene, were detected in these samples. Finally, some samples have much lower concentrations and diversity of alkanes, but they contain the alkenes propene and i-butene, the aromatics benzene and toluene, and dimethylsulfide. These differences in hydrocarbon composition are likely due to temperature as well as maturity of source material.

Project title: Geologic Dating and Detailed Mapping of Hydrothermal Features—Phase One, Test and Demonstration

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Co-investigators:

Purpose: When visitors, park service personnel, or scientific researchers ask the simple question, "How old is that geyser?" there is only one published date from the 1950s, which was early in the development of carbon-dating techniques, to answer (indirectly) the question. New techniques of 14C dating of bulk samples of sinter and laser mapping provide opportunities that did not exist nearly a half century ago to provide more accurate information about the age and geologic development of hydrothermal systems. This proposal is phase one, the technique-testing phase, of what, if successful, will become a multiphase effort. Phase two will focus the techniques on Old Faithful Geyser to provide new data and maps for resource management, park interpretation, and the planned visitor center. Phase three will expand the research to other areas of Yellowstone Park. This proposed research consists of two parts, both of which, while significant and meaningful in themselves, are designed to address critical issues that will be faced in Phase two, Old Faithful. The first part is to investigate direct and indirect dating of hydrothermal deposits. The second part is to do a laser-based detailed map of a hydrothermal deposit, for analysis of geologic development of that deposit. Hydrothermal systems have killed trees. 14C dating killed trees may give indirect evidence of when a system developed or expanded. Unfortunately, very little is known about silica deposition and carbon preservation in wood that is impacted by thermal deposits. This proposal will investigate carbon preservation and dating potential of "bobby-sox" and thermal-killed trees. A new dating technique will also be tried, which allows 14C dating of siliceous sinter by extracting and dating organic matter that incorporated in the deposits. This proposal requests that samples of silica-impacted trees be collected from the Lower Geyser Basin area and from the trees due east of Grand Geyser. Two samples of surficial sinter, sufficient to test for carbon concentration and to, if possible, date, are requested from the terraces on the southeast side of Castle Geyser. If the

Park Service has already collected a well-documented sinter sample from Castle or another site, and is willing to have it be used in this effort, new collection of sinter for dating would not be required. Interpretation of surficial hydrothermal deposits may allow a detailed relative chronology of the geologic history of a system to be interpreted. New techniques of laser mapping will allow quick, highly detailed morphologic maps of hydrothermal deposits to be developed. Fist-sized samples of sinter from lower terraces on the southeast side of Castle Geyser will be collected, to correlate newly mapped hydrothermal features with established sinter facies concepts. This proposal requests that permission be granted to map Castle Geyser, as its height and variety of sinter deposits, as well as the relatively long and moderately predictable interval between eruptions, will provide an excellent test of the mapping methods.

Findings: During 2003, our study of Castle Geyser and thermally impacted trees continued. For reader information, Castle Geyser is shown in the fourth photograph from the left on the header photos for the NPS Research Permit and Reporting System web sites. During 2003, specific progress included analyses of gathered wood and silica samples, two undergraduate senior capstones, and an abstract and poster session for the Geological Society of America (GSA) annual convention. So far in 2004, an abstract has been prepared for delivery at a GSA section meeting. References to all publications are included below. In brief, we had mixed results. The dating of wood samples largely failed, due to selected trees having contamination from young 14C. One tree, however, from the drainage of Gem Pool in the Upper Geyser Basin, dated about 190 years BP. Dendrochronologic analysis of cores collected from trees were unsuccessful, as the trees were too brittle to allow complete core preservation. Future researchers may wish to try larger diameter cores, in the hopes of getting more complete samples. The study of sinter deposition at Castle Geyser was highly successful, and led to the recognition of pervasive microbial presence in sinter deposits, even in splash zones near the top of the geyser. One sample of sinter was 14C AMS dated at about 930 years BP. This date, which must be considered preliminary until further dating confirm or refute it, is interesting because it is much younger than previously speculation of the age of this geyser had been (in the range of 5,000 to 15,000 years).

3-D laser mapping had mixed results. As noted last year, field problems with GPS links and processing issues with software code failed to link each data point collected with unique latitude-longitude-elevation coordinates. However, the mapping itself allows easy identification of several generations of different types of activity at Castle Geyser. Preliminary interpretation of the maps suggests that periods of hot spring activity preceded pool-type geyser activity, which was followed by development of the present cone geyser. It is possible that further laser mapping will lead to more detailed interpretations of the geologic development of the geyser edifice. Publications: Ricketts, Erin. 2003. Comparison of dating methods and Yellowstone's silicified trees with other specimens of petrified wood: Capstone paper, Pacific Lutheran University Department of Geosciences, 31 pp.; Thompson, Denise. 2003. Textural clues and microbial processes associated with precipitation of siliceous sinter: Castle Geyser, Yellowstone National Park: Capstone paper, Pacific Lutheran University Department of Geosciences, 49 pp.; Thompson, Denise. 2003. Textural clues and microbial processes in association with precipitation of siliceous sinter: Castle Geyser, Yellowstone National Park: Geological Society of America Abstract, Annual meeting, Seattle, Washington; Foley, Duncan. In press (2004). How does your geyser grow? 3-D laser scanning and preliminary 14C dating of Castle Geyser, Upper Geyser Basin, Yellowstone National Park, Wyoming: Geological Society of America Rocky Mountain/Cordilleran Section combined abstracts, Boise, Idaho, May 2004.

Project title: Chloride Flux Monitoring

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Co-investigators: Shaul Hurwitz

Purpose: Because chloride flux is a surrogate for heat flow, long-term measurements of chloride flux will provide indication of changes to the geothermal system feeding Yellowstone. It will also provide a baseline to assess future effects of development of both geothermal and gas and oil extraction adjacent to the park. In addition to collections from the major rivers, monitoring of major thermal areas, e.g., Norris Geyser Basin, Mammoth Hot Springs, will also be carried out.

Findings: Annual chloride flux leaving the park varies and is influenced by water discharge. However, the annual chloride flux, corrected for climatic factors, shows a decline of approximately 10% over a 20-year period. A similar decline has also been documented for Mammoth Hot Springs.

Project title: Monitoring High Frequency Transients in the Hydrothermal System of Yellowstone National Park

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Report number: 26460
Co-investigators: Evelyn Roeloffs

Purpose: By tracking groundwater pressure and temperature changes in Yellowstone's active hydrothermal system, we hope to improve our general understanding of processes linking transients in hydrothermal systems with seismic and volcanic processes. The deployment of a pressure-temperature sensor in Yellowstone presents an outstanding opportunity to examine hypotheses relating the inflation/deflation cycles in the area with fluid pressure changes, or the involvement of fluids in triggering seismicity. The data will enable the evaluation of groundwater changes as an additional tool in the monitoring effort of volcanoes.

Findings: In addition to a temperature-depth profile measured after drilling of the Y-7 hole in the Biscuit Basin parking lot in 1967, the temperature profile in the well was measured four more times between October 1999 and March 2002 by scientists from the University of Colorado. Since we started our research in the Y-7 well in May 2002, we have logged the temperature two more times. Despite the low precision of these temperature logs (manual measurement every 10 ft.), they might indicate some temperature transients in the hydrothermal system. For example, the single log measured during the winter period (December 1999) might indicate some large temperature changes associated with the annual groundwater cycle. On June 20, 2003, we measured the depth to the water table (66.04 cm), and then we installed two thermocouple wires at depths of 72 m (bottom of the hole at a temperature of 141.7°C) and 18 m (an area inferred from the drilling log to be of high permeability), and a thermistor cable at a depth of 4.5 m below the ground surface. All these probes measured water temperature every hour, and measurements were recorded on a data logger. Data from June 2003 to the beginning of February 2004 indicates that (1) the temperature variations in the two thermocouples varied by a maximum of 0.2°C, which is only slightly above the analytical precision, and most probably associated with the large temperature fluctuations within the enclosure containing the data logger, and (2) the shallow thermistor shows both daily fluctuations and seasonal variation with a wavelength longer than 7 months and an amplitude greater than 5°C. This seasonal fluctuation was also measured by the same thermistor between September and December 2002. We have not analyzed the cause for this transient, but with this observation, we plan to modify our thermal monitoring of the well, to obtain more constraint. On June 20, 2003, we also re-deployed a modified pressure transducer at a depth of 30 ft. (after the transducer failed the year before). The transducer measured water pressure in the well every minute, and the data was recorded on a Campbell data logger. The transducer measured pressure until November 17, 2003, when it failed again. The nearly five month data shows that pressure fluctuated at various frequencies, and that there was a gradual pressure increase until failure occurred. We plan to analyze the data in the near future, and design a more robust tool after we diagnose the cause of failure.

Project title: Gas Geochemistry of the Yellowstone Volcanic System

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Report number: 26182
Co-investigators: Deborah Bergfeld, William Evans, Andrew Ouimette

Purpose: We will collect geothermal gases from selected thermal areas to aid in monitoring the Yellowstone volcanic system. Our goals are the following: (1) Provide baseline data for the composition and variability of gases emitted from Yellowstone geothermal areas, (2) Aid current and future studies focused on gas flux, which typically lack detailed chemical analysis, (3) Delineate zones of primary upflow of gases from the magmatic environment. In 2003, we propose to sample ~20 features in the Upper Geyser Basin, Lone Star Geyser, Hillside Springs, and Smokejumper Hot Springs. We also propose limited sampling at Norris Geyser basin to aid in understanding current patterns of ground deformation and genesis of new thermal features. Specific vents for study will be chosen in collaboration with park geologist Henry Heasler, who will serve as an advisor for this project. No chemicals will be released to the environment and physical disturbance of sampled springs and fumaroles will be minimal.

Findings: We collected gases from over twenty fumaroles in the Upper and Lower Geyser Basins, Smokejumper Hot Springs, Beryl Spring, and the Norris Geyser Basin. Gases were sampled via a titanium tube and plastic tubing and introduced into an evacuated gas bottle partly filled with 4N NaOH solution. In addition, we collected condensates of steam from each thermal feature and samples of liquid water from hot springs where gases were also collected. Samples were analyzed by a combination of gas chromatography, wet chemistry and mass spectrometry. We determined that the gases represent mixtures of deep magmatic gas with gases derived from surface waters and rocks. Samples from the Norris Back Basin, site of a hydrothermal disturbance in July 2003 were among those with characteristics most similar to surface waters. This implies that the disturbance at Norris is a phenomenon related to increased boiling of shallow waters.

Project title: Volcano Emissions

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Report number: 26917
Co-investigators: Michael Doukas, Terrence Gerlach

Purpose: Research on various gas emissions from fumaroles, soils, and other sources within Yellowstone National Park for the purpose of defining a background level of emissions to which future measurements of anomalous degassing during volcanic or tectonic unrest could be compared.

Findings: On May 7–8, a two-day airborne mission was conducted by fixed-wing aircraft in order to make volcanic gas measurements of plumes in the air over the park. The plumes from several geyser basins, thermal features, and fumaroles in the western portion of YNP were profiled and measured, primarily for carbon dioxide.

Project title: Crustal Structure and Composition of Yellowstone National Park: Relation of Crustal Structures to Geology, Hydrothermal Alteration, and Seismic Activity

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Report number: 28276
Co-investigators: Steve Harlan, Pat Shanks

Purpose: A high-resolution aeromagnetic survey flown over Yellowstone National Park shows a broad spectrum of contrasting magnetic patterns reflecting variations in rock composition, types and degree of alteration, and crustal structures. This method has identified fractures and areas of alteration that previously have not been mapped, identified the extent of individual geologic units and structures, and estimated the magnitude of hydrothermal alteration. Magnetic gradient trends follow the mapped north–south Basin and Range structural trends. These trends are at small scales such as in the hydrothermal basins and at larger scales such as with fault systems suggesting that the regional stress field localizes much of the present-day hydrothermal activity. We will continue analysis of new high-resolution aeromagnetic survey over most of YNP and compare with magnetic susceptibility and remanence measurements. A recent finding from the integrated study of the aeromagnetic characteristics of hydrothermal areas in tandem with our hydrothermal explosion and Yellowstone Lake studies has identified a NE-trending structure subparallel with the NE-trending fissures on Elephant Back. This structure has not previously been described in the literature and may be active and reflect the inflation and deflation of the Yellowstone caldera. Several young hydrothermal explosion craters and features occur on this structure. We plan to continue studying this feature.

We also plan to continue analysis of the high-resolution aeromagnetic survey over most of YNP and compare with magnetic susceptibility and remanence measurements, focusing on the Quaternary rhyolite lavas and ignimbrites. Samples will be collected and analyzed for magnetic remanence and susceptibilities of fresh and altered volcanic and sedimentary rocks. Manuscripts describing the aeromagnetic data in terms of its usefulness in mapping volcanic flows, faults, and zones of alteration are planned. We plan to compare magnetic susceptibility, zones of alteration, oxygen isotopes, and total magnetic intensity of specific volcanic units. Magnetic fabric analyses and interpretation of possible flow directions in ignimbrites and lava flows in the Yellowstone Plateau volcanic field, based on anisotropy of magnetic susceptibility, will continue and be complimented with granulometric and component analyses. Visual inspection of the aeromagnetic map with superposed geologic features suggests that there may be distinctive magnetic anomaly minima associated with mapped zones of hydrothermal alteration of the source rocks. To investigate this, we want to accomplish three objectives: (1) quantify and verify the relationship between the lows and alteration and use the aeromagnetic data to map the extent of alteration zones beneath covered areas; (2) analyze the aeromagnetic map for trends which might delineate the structural fabric of older geologic structures which controlled the loci of volcanism within the Yellowstone system; and (3) map textural measures of the aeromagnetic anomaly field which might be related to rock lithologies and thus be of use in elucidating the geologic structure. To do this, we will apply new analysis tools in conjunction with Mark Gettings (USGS, Tucson) to the data and field check the data. A paper describing the method and the case study will be published.

Findings: In 2003, selected oriented core samples from various volcanic units in YNP (Lava Creek Tuff, Quaternary rhyolite lava flows) were processed and analyzed for magnetic properties (remanence, magnetic fabric). Analyses of these samples and interpretation of the data is continuing and eventually will be published in a peer-reviewed journal.

Project title: Mapping, Chronology, And Geochemistry of Hydrothermal Explosion Deposits in YNP

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Report number: 28712
Co-investigators: Ken Pierce, Pat Shanks

Purpose: Hydrothermal explosion craters and associated breccia deposits are commonly observed features in Yellowstone National Park. Visitors to popular thermal areas like Upper and Lower Geyser Basin, Norris Geyser Basin, and West Thumb Geyser Basin often see deep conical pools of thermal water and large rock fragments of explosion breccia littering the landscape. Each of these pools is an explosion crater, and they occur throughout the Park at all scales from sub-meter to kilometer diameters. Because hydrothermal explosions can occur at any time, they constitute a potential hazard to visitors in the park. Mary Bay, a 3-km-diameter embayment on the north shore of Yellowstone Lake, is a major hydrothermal explosion crater complex that erupted about 13,000 years BP and deposited an apron of explosion breccia for several kilometers around Mary Bay. Other major explosion craters in the Park include: Indian Pond, Turbid Lake, Duck Lake, Fern Lake, Pocket Basin, Roaring Mountain, and a newly discovered site in the Sulfur Hills. Smaller hydrothermal eruptions, at Biscuit Basin, Porkchop Geyser, Seismic Geyser and other localities have been observed in recent years. Our objective is to use geologic and stratigraphic studies, geochronology (principally 14C analyses of carbon), the new high-resolution aeromagnetic data, seismic data from the monitoring network in the Park, spectral imaging (AVIRIS) and geochemical, mineralogical, fluid inclusion studies to understand better the mechanisms of hydrothermal eruptions, the causes of such events, regional seismicity, and possible relations to recently discovered regional inflation/deflation cycles cycles.

Detailed stratigraphic studies in the Mary Bay explosion breccia deposit have identified unusual sand deposits which may be possible large wave deposits. One sand below the breccia can be traced as far as 5 km north of Yellowstone Lake into the Pelican Valley. We suggest that this sand represents a deposit from an earthquake-generated tsunami-like wave, which in turn triggered the explosion of the 13,000-year-old Mary Bay explosion crater complex. Our studies will continue to evaluate the potential of such event occurring in the near future. Our work will continue to examine and analyze geothermal vent locations, physical characteristics of the deposits and vents and their distribution and chronology, and analyze stable isotopes and fluid inclusions to determine the deposits' temperature of formation and composition of fluid. We will examine details of these hydrothermal systems and their relation to magmatic activity, faulting, and changes in post-glacial lake levels. Geothermal fluid changes that are potentially mineralizing within the 640,000-year-old caldera may reflect contrasting time scales: the inflation/deflation cycles occur on a millennial time scale whereas the hydrothermal explosions occur within minutes. Changes in pressure and flow rate would dramatically change as mineralized geothermal fluids are released and confining pressures drop which may significantly contribute to mineralization. Finally, high-resolution sonar imaging, seismic reflection, and submersible surveys in Yellowstone Lake have identified sublacustrine hydrothermal craters, vents, domal structures, and gas pockets.

Findings: In 2003, field studies of plastically deformed lake sediments along the wave cut terraces of Yellowstone Lake continued. Several radiometric ages of felsic clasts from the Mary Bay explosion breccia were obtained. Two manuscripts on hydrothermal features in YNP are in progress and will be published in 2004.

Project title: Potential Global Impact of an Eruption From Yellowstone Caldera

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Report number: 26521
Co-investigators: Ben Mason

Purpose: This project will investigate the petrology of the three main volcanic eruption deposits in

Yellowstone National Park; the Lava Creek Tuff, the Mesa Falls Tuff, and the Huckleberry Ridge Tuff. Analyses of pumices from these deposits will enable the quantification of sulfur and other volatiles released from each eruption. This in turn will provide an estimate of the potential global impact of the three eruptions. Similar studies have been carried out on huge eruptions from Toba caldera, Indonesia, and La Garita caldera, Colorado. Fieldwork must be carried out to carefully select pumice samples from each of the three pyroclastic deposits in order for analysis. This will be carried out in association with Dr. Jake Lowenstern. The collection of these samples is integral to the project, although it should only take a couple of weeks. These samples will subsequently be analysed in Cambridge University (UK) and Orleans (France), in collaboration with Dr. Bruno Scaillet.

Findings: Samples of pumice were collected from Tuff Cliff inside YNP and at locations outside the park. The samples are now awaiting analysis.

Project title: Genesis of Extracaldera Rhyolites and Basalts at the Yellowstone Plateau Volcanic Field During the Past 600 ka

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Report number: 28363
Co-investigators: Eugene I. Smith

Purpose: The purpose of this study is to examine the contemporaneous eruption of <600 ka rhyolites and basalts north of Yellowstone caldera in order to assess the future direction of volcanism at Yellowstone. These extracaldera lavas occur within the Norris–Mammoth corridor (NMC), and represent the most recent volcanism within the plateau. However, little is known about their eruptive ages, geochemistry, or relationship to one another. Information regarding petrogenesis of the rhyolites will determine whether they are derived from a long-lived evolving magma reservoir as opposed to small independent magma batches. This will be accomplished by documenting the eruptive chronology of the extracaldera rhyolites through 40Ar/39Ar dating, determining their geochemical relationships as a function of time, and establishing magma residence timescales using U/Th dating of zircons. During reconnaissance of basalt outcrops a new vent was discovered and mapped. Major and trace element geochemistry and several 40Ar/39Ar dates have been completed on basalts within the NMC, as well as on some basalts outcrops to the southeast and north. The objectives were to locate vents for flows, re-evaluate the volcanic stratigraphy, and determine chemical variation at the (1) regional level, (2) within the NMC, and (3) within individual centers (where those could be identified). Major and trace element geochemical data, as well as isotopic ratios, will be used to put into a temporal framework by dating the lavas.

Findings: Mapping and sampling was completed during June 15–July 12, 2003, and the sampling strategy was based on regional stratigraphy developed by Christiansen. Sample locations for the rhyolitic and basaltic units are situated within the NMC as well as several locations along the Madison River. Rhyolite sampling consisted of collecting samples from each mapped extracaldera dome and flow form the Obsidian Creek and Roaring Mountain Members (OC and RM). Basalt sampling focused on units stratigraphically associated with the Yellowstone (i.e., Undine Falls, Madison River, Swan Lake Flat, and Osprey basalts) and Island Park (i.e., Gerrit, Falls River, and Warm River basalts) caldera cycles. The OC and RM members have previously been characterized as porphyritic and aphyric, respectively, however detailed field and petrographic evidence from this study indicate overlap in phenocryst abundance between the members. Additionally, two new mingled mafic-silicic lavas have been identified (two were previously identified in the OC member), showing that both members record evidence of mafic recharge. Mafic enclaves from the mingled lavas are basaltic-andesite to andesite and represent physically mixed magma.

Previous work suggests extracaldera rhyolites are the product of small, independent magma batches, and are not related to the sub-caldera magma system. However, new geochemical and isotopic data show that the extracaldera rhyolites in the NMC may be related to a single evolving silicic magma system. 40Ar/ 39Ar dating will test the hypothesis that these rhyolites exhibit a progressive chemical evolution with decreasing age. Additionally, the extracaldera rhyolites cluster within a range significantly lower than the caldera-related rhyolites. These data suggest the extracaldera rhyolites erupted from a single evolving source, independent of the main caldera system. Their location may indicate a migration of the current subcaldera magma system, or development of a new magma system of substantial size and longevity north of Yellowstone Caldera; this has implications for the beginnings of a fourth caldera-forming cycle. While sampling Swan Lake Flat basalt, a previously undescribed volcanic vent located 1.8 km southwest of Swan Lake was discovered. Typically basaltic eruptions within the Yellowstone Plateau were Hawaiian in nature, producing low-volume flows that create small shields. These tholeiites vary little in chemical composition and with Nd values of –0.18 to 0.20, are some of the most primitive sampled within the park. The Strombolian eruption style of the Panther Creek volcano adds to the list of potential volcanic hazards in the NMC.

We have compiled a geochemical and geochronological database for basaltic rocks in Yellowstone that contains over 150 analyses and 20 dates. Our database includes samples from modern studies (1963–2002) in addition to 39 new chemical analyses and nine 40Ar/39Ar dates. The database's principal purpose is to determine if the most recent basaltic volcanism within the NMC represents the initiation of a new caldera cycle or the dying phases of the last cycle. Isotopic ratios of the <0.6 Ma basalts suggest a relationship of eNd and 87Sr/86Sr to periods of caldera formation. 87Sr/86Sr changed from 0.707 to 0.703 and eNd from 7 to 0.51 at the inception of the first caldera cycle (2.1 Ma) suggesting injection of asthenospheric basalt into the crust. This injection may have triggered partial melting of the crust that ultimately led to generation of a rhyolitic magma chamber and caldera formation. The isotopic signatures of the <0.6 Ma basalts show both high and low values of 87Sr/86Sr and eNd; however, ages of the basalts are not precise enough to determine a similar temporal trend. Therefore high precision 40Ar/39Ar dates must be determined for the basalts. This work is currently underway.

Project title: Study and Monitoring of Selected Geyser Activity

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Report number: 26620
Co-investigators: Jens Day, James B. Grigg

Purpose: n/a

Findings: As of the end of 2003, data from more than 35 thermal features has been collected and analyzed. Over the winters of 2001–2002 and 2002–2003, about 30 data loggers have been deployed, allowing nearly continuous records of activity. Analysis of the thermal records through the end of 2003 is nearly complete as of February 2004. Some long-term trends are evident in the eruptive activity of several geysers; for example Old Faithful, Daisy, and Castle have all exhibited a gradual increase in interval between eruptions. The Alaska earthquake of November 3, 2002 was followed by large changes in the activity of Daisy Geyser (which decreased its intervals by about an hour) and Castle Geyser, which increased its intervals by about an hour. Changes were observed in the activity of Plume and Depression geysers also. Some geysers returned to pre-quake activity levels (e.g., Daisy Geyser) while others (e.g., Lone Pine and Castle Geysers) have continued in the altered patterns observed post-quake. All of the temperature records and spreadsheets with graphs of activity and statistical analysis of most of the monitored geysers are on file at the Yellowstone Center for Resources and with the author. About 30 data loggers are deployed for the winter, including both the author's loggers and loggers owned by the NPS, and current plans are to continue monitoring of approximately 40 thermal features during the summer of 2004.

Project title: Absolute Gravity and Crustal Deformation in the Yellowstone Caldera

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Report number: 27088
Co-investigators: David C. McAdoo, Mark S. Schenewerk

Purpose: The purpose of our proposed work is to further our understanding of fluid movement and the role of magmatic and hydrothermal activity below the Yellowstone caldera, and in particular, below geyser basins. Our data from 2000–2002 show large oscillatory vertical motions (up to 10 cm) over very short periods (several hours to tens of hours) in the southern end of the Upper Geyser Basin which is indicative of deep pressure fluctuations driven by fluid flow. We have three specific goals for our field work in 2003. (1) We wish to test the pervasiveness and identify the source of the deep pressure fluctuations we have detected below the Upper Geyser Basin in the Yellowstone caldera from gravity and global positioning system (GPS) data. (2) We wish to test whether the pressure fluctuations are unique to the Upper Geyser Basin or are a more common feature of geyser basins. (3) We wish to test how pressure fluctuations in geyser basins are related to background hydrothermal-magmatic behavior and their persistence over long periods of time. In 2003, we wish to conduct GPS campaigns in the Upper, Lower, West Thumb, and Norris geyser basins, and gravity campaigns in Upper and Norris geyser basins, similar to the campaigns we have conducted in 2000–2002. In addition, we would like to explore the possibility of installing tiltmeters and establishing new long-term GPS stations with the National Park Service. We would also like to use a pressure transducer, ultrasonic level monitor, or a simple water level gauge to monitor the water level and discharge of the Chinaman Spring during our gravity observations in the Upper Geyser Basin, and possibly other springs in the geyser basins. This work will be carried out in coordination with the National Geodetic Survey and UNAVCO and is pending funding from the National Science Foundation.

Findings: We acquired several days of short baseline global positioning system (GPS) data in the Upper Geyser Basin area in June 2003. Preliminary processing and analysis of these data indicate that there may be some differential vertical movement between sites separated by several kilometers. This is important for furthering our understanding of geyser basin deformation, and therefore fluid flow in geyser basins, on the timescales of days. We will be pursuing NSF funding for 2005 to follow up on these initial findings, and have funds to collect more data in 2004. In 2003 the results of work from 2000–2002 were presented at the International Union for Geology and Geophysics (IUGG) meeting in Sapporo, Japan (June–July 2003), and the Fall meeting of the American Geophysical Union (December 2003). A research paper was submitted to the online journal G-cubed on the results from 2000–2001, and is currently being revised for resubmission. A research paper on the 2002 results is being prepared for a special issue of the *Journal of Volcanology and Geothermal Research.*

Project title: Preservation of Organisms in Sinter, and Study of Sinter Textures

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Report number: 28563
Co-investigators: Lyall Anderson, Steve Fayers, Ruth Kelman

Purpose: Compare modern processes and preservation of biota at Yellowstone with the early Devonian Rhynie chert of Scotland.

Findings: Conducted fieldwork to continue observations, and augment existing data. Continued monitoring of Liberty/Frog pools at Old Faithful. Investigated Tangled Creek area with Hank Heasler. Checked observations on fossil vents at cliff by Yellowstone Lake. Now preparing paper with Nancy Hinman.

Project title: Physical Volcanology of the Huckleberry Ridge Tuff

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Report number: 27736
Co-investigators:

Purpose: Purpose of study is a detailed investigation of the physical characteristics of the 2.06 Ma Huckleberry Ridge Tuff, in collaboration with Dr. R.L. Christiansen (USGS, Menlo Park, Calif.). Field and laboratory studies on outcrops both within and outside Yellowstone National Park will be used to characterise the distribution and internal "anatomy" of the tuff, and to infer the styles and timings of the parental eruption.

Findings: A total of 16 field days were spent working in Yellowstone National Park, accessing (1) a broad area between Cache Lake, Gardners Hole, Mt. Everts, the Blacktail Deer Plateau, and the Yellowstone River, (2) the Yellowstone River gorge downstream from Canyon Village, and (3) areas east of U.S. Highway 191 in the Fan Creek and Black Butte areas. My work in Yellowstone National Park is part of a much wider-scale study on the Huckleberry Ridge Tuff (HRT), and much of the work in the park is driven by understanding gained from sections outside the park. I report my findings below, numbered according to the areas listed above that were visited.

(1) A major disappointment was to find that several areas mapped by R.L. Christiansen (2001: USGS Professional Paper 729G) as the HRT turned out on close inspection to be the younger Lava Creek Tuff. These areas in particular were the outcrops marked as occurring along each side of Reese Creek, east of Cache Lake, and on the north side of the Yellowstone River between Cottonwood and Crevice creeks. These and other revisions to the designations on the maps presented by R.L. Christiansen will be drawn up as part of my work. On Mount Everts and the Blacktail Deer Plateau, further work was spent characterising the internal structures in, and contacts between, HRT members A and B. The contact between these two members is locally a thin vitric fall deposit (at UTM grid reference 0528914 m E, 4978959 m N), but otherwise is revealed only by textural and jointing changes in this area. Internal vertical variations within members A and B are being documented by counts of lithic and crystal abundances. Previous observations by R.L. Christiansen of a succession of crystal poorer-richer-poorer material in each member are being quantified using the crystal counts in order to decide if these variants can be mapped as separate sub-members and their distributions used to infer particular source areas. Around the area of Golden Gate, I extended the local outcrop area of HRT member C, finding a poorly defined scarp of exceedingly fresh vitric crystal-poor tuff (e.g. at 0521195 mE, 4976006 m N) that forms a mappable area. (2) I visited the small exposure of HRT member A marked on R.L. Christiansen's (2001) map on the true left wall of the Grand Canyon of the Yellowstone River, just down-gorge from Seven Mile Hole. My observations were consistent with this being HRT member A, and the absence of member B suggests that the latter was poorly developed in the gorge. (3) I worked for three days on outcrops in the Fan Creek drainage, and for two days on Black Butte. At the former, exposure was generally poor, but a good member A:B contact was mapped in cliffs at 0501439 m E 49777199 m N, with a conspicuous, 20-cm-thick, cross-bedded deposit along the contact. Such a bed is appearing at enough localities elsewhere in the tuff such that it represents a consistent marker. Evidence gathered to date is, however, not yet adequate to infer whether this bed is a primary (surge) deposit, or represents an interval of aeolian re-working. At Black Butte, an excellent series of exposures record the thinning and onlap of member A on to Tertiary sediments, and vitric fresh material could be worked on that elsewhere is intensely welded and devitrified. A series of inter-leaved beds of cross-bedded and massive material occur along the A:B contact, and individual flow units or packages can be seen thickening outboard towards the line of the modern Gallatin River. These sections are valuable for showing the morphology of the ignimbrite valley fills, but more sections are required along the valley axis. My thanks again to the NPS for the opportunity to work in the park.

Invertebrates

Project title: Respiratory Physiology and Thermal Preference in Thermophilic Aquatic Insects

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Report number: 27925
Co-investigators: Lori Ybarrondo

Purpose: To investigate the respiratory physiology and thermal preference of water scavenger beetles (*Coleoptera: Hydrophilidae*). To investigate territoriality and thermal preference in adult dragonflies, and development in dragonfly niads.

Findings: No activity was conducted this report year.

Microbiology

Project title: Production and Consumption of Trace Gases by Yellowstone National Park Microbial Communities

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Report number: 28824
Co-investigators: Steven Carpenter, Mary Hogan, Scott Robert Miller, Victoria Orphan

Purpose: The spectroscopic detection of potentially biogenic gases using Earth- and space-based spectrometers and interferometers is an important search strategy for the detection of life on extrasolar planets. It is therefore important to be able to evaluate the biogenicity of these gases. Microbial communities on Earth currently control the cycling of trace gases in our atmosphere. Microbial mat communities are our best modern analogs for the communities of organisms that have dominated life on Earth for nearly 80% of the entire history of life. There are extremely complicated interactions (both between the members of microbial communities, and between the entire microbial community and the environment) that control the rates and timing of trace gas production and consumption in these microbial communities. It is through measurements of modern day microbial communities that we can best understand trace gas cycling in these communities over geologic time. We therefore propose to make measurements of trace gas production and consumption by Yellowstone National Park hot spring microbial communities in order to be able to better characterize a biological "signal" in the composition of these trace gases. Yellowstone National Park offers a range of locations in which the rates of production, as well as the contribution of biogenic versus non-biogenic components to the overall rates of trace gas production are expected to vary. This information will be used to help develop our search strategies for life, through the detection of atmospheric biomarkers in the atmospheres of extrasolar planets.

Findings: No activity was conducted this report year.

Project title: Survey of Yellowstone Hot Springs for Green Sulfur Bacteria

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Report number: 28101
Co-investigators: Mary Bateson, Ulrich Nubel, Greta VanSlyke Jerzak, David M. Ward

Purpose: To follow up on our recent discovery of green sulfur bacteria in Yellowstone hot springs and to search for additional occurrences.

Findings: No activity was conducted in YNP this report year.

Project title: Bacteria Living at Low pH and High Temperature

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Report number: 27705
Co-investigators: Scott T. Kelley, Jayanti Mathur

Purpose: Discovery, isolation, and characterization, including phylogenetic (16S rDNA) characterization, of unknown genera of microorganisms living at low pH (<pH 3) and high temperature (>70°C).

Findings: We examined samples microscopically from acid hot springs at several sites including Amphitheater Springs, Roaring Mountain, Sylvan Springs, and Mud Volcano. We focused our study on Evening Primrose Spring (Sylvan Springs). We isolated several thermoacidophiles at pH 3; 70°C and pH 5; 65°C, examined the pH and temperature optima, and temperature tolerance of enrichments and isolates. We completed 16S rDNA sequencing of isolates. BLAST (phylogenetic) analysis of sequences revealed known genera. In a separate study, we developed methods for attachment and fixation of extremophiles for scanning electron microscopy and X-ray microanalysis. We used several isolates from Amphitheater Springs for this study. We completed our characterization of a new genus (based on 16S rDNA sequence) isolated from Amphitheater Springs. We compared Yellowstone 16S rDNA sequences (cultured and non-cultured) to those obtained from similar low pH high temperature springs in New Zealand. Finally, two new flagellar phenotypes that would appear to reduce turbulence in very hot waters were discovered.

Project title: Transition Between Lithouautotrophy and Chemoheterotrophy in Sulfolobus Species

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Report number: 27879
Co-investigators: Ellie Redfield, James Schelert
Purpose: To understand the metabolism of thermoacidophilic *Archaea* in situ.

Findings: No research was conducted in the park year, however samples obtained in previous years were analyzed.

Project title: Research Experience for Undergraduates: Yellowstone National Park Field Trip

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Report number: 26121
Co-investigators: Melissa Cahoon, Darla M. Goeres, John Neuman

Purpose: The Research Experience for Undergraduates Program recruits talented undergraduate students in the area of math, science and engineering for a ten-week intensive research experience in the area of biofilms. The students travel from across the U.S. to participate in the program. The program is sponsored by NSF and housed at the Center for Biofilm Engineering on the Montana State University campus. A critical piece of the research experience is spending the day in Yellowstone National Park, where the students see wild type biofilms and discuss the ethics associated with conducting research in our national parks.

Findings: The trip to Yellowstone Park increased the students' appreciation for field research. Viewing biofilm in a natural environment demonstrated the complex ecology associated with a living biofilm better than any bench-top laboratory system. The students left Yellowstone with a better understanding of the issues surrounding research in a national park.

Project title: A Global Microbial Biodiversity Study of High Temperature Mud Pots: U.S. Component

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Report number: 27232
Co-investigators: Jay Wheeler

Purpose: Biodiversity studies of high temperature environments have centered largely on terrestrial hot springs and deep-sea hydrothermal vents. One globally distributed terrestrial system that has remained largely uncharacterized, and yet a possible rich source of novel extremophiles, is the high temperature Solfatara mud pot. Mud pots are pools of hot, acidic, bubbling clays formed by the dissolution of rock as steam rises from groundwater chambers deep below Earth's surface. The homogeneously mixed viscous clay matrix shows dramatic variability between pots in temperature, pH, and metal speciation, creating a unique environment to challenge the organisms that thrive under these conditions. In light of recent theories suggesting the importance of early clay environments in the abiotic synthesis of simple organic molecules, these mud pots are possibly the best analog to the primordial conditions of early earth under which life may have begun. The extreme physicochemical variability of mud pot systems may support diverse and unique bacterial assemblages rivaling terrestrial hot springs. However, previous studies of mud pots have resulted in the isolation and cultivation of only a few extremophiles. This may be attributed to a lack of understanding of the geochemical conditions under which they thrive. The application of cultivation-independent molecular surveys based on rRNA genes has revealed previously unrecognized microbial diversity, and novel phylogenetic lineages that represent major components of global microbial assemblages. These gene surveys indicate that the dominant proportion of resident bacteria is not represented in culture collections. This approach has been used in other high temperature systems, with dramatic results that have implications pivotal to our understanding of evolution of all the domains of life (*Archaea, Bacteria,* and *Eukarya*) and to the origins of life itself.

Coupling these sophisticated molecular techniques with new micro-geochemical analytical capabilities offers a powerful approach to access and characterize the microbial diversity of these unique high temperature systems. This project is currently supported through a Grant for Research from the National Geographic Society. The project began two years ago with an extensive survey of the mud pots on the North Island of New Zealand. There we optimized and perfected our methodologies with material from easily accessible sites. The results from this initial survey was presented at the International Extremophile meeting in Naples, Italy (Sept., 2002). We then began the global component of the survey with a sampling trip to the mud pots of Yellowstone and Lassen national parks, Costa Rica, and Kamchatka, Russia. Our proposal is to continue the effort in North America by sampling the extensive mud pot systems in Yellowstone and Lassen national parks using some new methodologies that have been successful in the other sites. Our global survey has shown that the microbial biomass in the mud pools is exceptional low. This coupled with the mineral base of the mud makes DNA extraction very difficult. To enhance our DNA recovery we designed and deployed settling tubes that provide access to clean surfaces for bacterial colonization. Briefly, sterile glass tubes containing glass wool are left in the pool for seven days. The glass wool allows pore water to seep into the tube yet excludes the mud itself. The glass wool becomes colonized by a selection of the resident microflora which can be easily extracted when free of the mineral mud. We recognize that this certainly selects for a subset of the flora but have found it to produce a broader image of the community than from DNA extracted directly from the mud. This method is also more reproducible and consistent at producing results.

Findings: During the five-day sampling at Yellowstone this year we successfully visited and sampled six mud pool systems. Collectively, this represented 17 mud pools and five clear pools with temperatures ranging from and 40°C to 108°C and pH from 1.7 to 9.3. The clear pools that were sampled were only those in close association with a mud pool. This was done for later comparative purposes. On average, only 200 mls of mud/water was collected from each pool for molecular and geochemical analysis. The samples were immediately centrifuged and the pore water preserved for later geochemical analysis. DNA was extracted in our hotel room for later analysis. The samples were added to those collected from Costa Rica, New Zealand, and Kamchatka, and are currently being processed. This is a time intensive and costly process and as projected in the proposal should take 1–2 years to complete. Our initial studies have shown that the DNA is very difficult to extract from the mud, probably due to the mineral content being primarily silica. In addition, from some preliminary microscopy it is obvious that the biomass in these samples is very low. Initial surveys of the samples however have demonstrated that these pools do contain a unique microflora when compared to clear pools. The flora are clearly dominated by *Crenarcheaota,* but do seem to contain some *Bacteria*. These preliminary analyses are continuing and should be completed by summer 2004. We would like to return to Yellowstone to complete the survey (pools that were missed) and attempt a series of enrichment experiments. While in New Zealand we encountered the same issues associated with low biomass and poor extraction efficiency. We developed a simple yet effective method to enrich for mud pool microbes. Briefly, a sterile glass tube filled containing glass wool is filled with pore water extracted from the mud. This assembly is then submerged into the pool with a wire leader. The tube is allowed to incubate for one week and then retrieved. The central area of the tube remains clear of mud but heavily colonized by bacteria. Preliminary analysis indicates that the enriched community comprises most if not all of those detected using the direct extraction approach. DNA yields are 100× more using this method.

Project title: Effects of UV Radiation, Desiccation, and Heavy Metals on the Photosynthetic Microorganisms of Hot Springs and Associated Sediments and Hot Spring Travertine Deposits

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Report number: 26952
Co-investigators: Julie Fox, Erich Fleming, Emily Knowles, Tracy B. Norris, Udomluk Sompong, Julie Toplin

Purpose: (A) For the field season 2003 in YNP, one of the objectives was to continue to collect and bring into culture as many cyanobacteria and microalgae from terrestrial mats or crusts as possible, particularly endolithic forms in travertine. The principal goals that frame this research are to ascertain the community composition in these periodically desiccated and frozen habitats by molecular means and to determine the desiccation and freezing tolerance of these microorganisms after they have been brought into culture. (B) The other research project concerns *Cyanidium* and relatives (unicellular red algae that live in thermal, acidic environments). The project is to: (1) Characterize culture isolates in order to collect physiologic and phylogenetic data to establish the genera and species that make up the *Cyanidium* complex and to distinguish them from other thermo-acidophilic eukaryotic algae. (2) Determine if environmental features (e.g., heavy metals, pH, temperature, solar irradiance, competition, desiccation) are correlated with specific *Cyanidium* ecotypes within the various thermo-acidic habitats.

Findings: (A) The work with endolithic cyanobacteria is progressing well with a manuscript in preparation; most of the work currently involves experiments with culture isolates with respect to their genetic identity, desiccation, freezing and temperature tolerances (all work at University of Oregon). (B) The work with *Cyanidium* and related eukaryotic algae involved the collection of live specimens from numerous thermo-acidic habitats over much of Yellowstone National Park. Many strains (>60) were isolated, and the ribosomal 18S DNA has been sequenced for many of these. Preliminary studies have shown that heterotrophic growth of most of the strains is negligible; some are unable to grow with nitrate as the nitrogen source; and growth on soil and water taken from various native sources containing heavy metals was slow to nil, but differed among strains (work done at Montana State U. and U. of Oregon). The tolerance of a few *Cyanidium* strains to various aluminum concentrations has been tested with differences between strains evident.

Project title: Population and Genetic Diversity of Microorganisms Associated with Hydrocarbon Deposits in Thermal Areas

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Report number: 28619
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Purpose: We will investigate the microbiology and geochemistry of naturally occurring hydrocarbon deposits in Yellowstone National Park. The discovery of these hydrocarbon sources in northwestern Wyoming and what is now Yellowstone National Park dates back to the early 1800s. We are primarily interested in the genetic diversity of microorganisms associated with these deposits as the hydrocarbons may have been present for centuries. In contrast, microbiologists have been studying microorganisms from industrially contaminated areas that have been exposed to petroleum hydrocarbons for several decades. The possibility exists that the organisms encountered in the soils surrounding the natural seeps will be novel and/or contain undiscovered metabolic pathways for degrading the hydrocarbons. We will use DNA-based techniques to identify microorganisms that are found within soil samples adjacent to the seeps containing hydrocarbons. In addition to identifying the organisms we will also determine whether genes known to be involved in hydrocarbon degradation are also found in these environments. To complement our molecular work we will also collect soil samples for chemical analyses to determine the types and concentrations of hydrocarbons present. Based on the results of the chemical analyses we will attempt to grow and isolate some of the microorganisms present in the soil samples using predominant hydrocarbons as a growth substrate. We will use our results to characterize the microbial populations associated with these hydrocarbon seeps and to examine the evolutionary relationships between the hydrocarbon-degrading genes detected in these environments with well-characterized genes from extant organisms.

Findings: Two non-thermal hydrocarbon soils sampled near Rainbow Springs in August 2003 (50 grams each site). Samples have been used for molecular and geochemical analysis and have been expended. The samples were used to conduct preliminary analysis of the distribution of 16S rDNA sequences yielding several dominant populations, characteristic of acid-sulfate environments (*Acidosphaera*-like and *Acidithiobacillus*-like). The samples were characterized using GC-MS suggesting a fairly weathered hydrocarbon mixture of linear and branched alkanes ranging in chain lengths from c14 to c30. The samples were assayed for alk genes based on primers developed from known alkane hydroxylase nucleotide sequences. These primers were successful in amplifying several DNA fragments that show reasonable homology to known *Rhodococcus* and *Pseudomonas*-like alkane hydroxylase genes. We plan to continue this effort focused on alkane hydroxylases given that these functions have not been discussed in acidophiles, especially as hydrocarbon substrates may couple with Fe and or S cycling.

Project title: Enhanced Practical Mitigation of Carbon Dioxide

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Purpose: To isolate and characterize thermotolerant cyanobacteria from sites in YNP. These will be used in high temperature carbon dioxide remediating bioreactors that can be retrofitted to existing coal-fired power plants. In year two of this study we have successfully sampled areas at Black Sand Pools, Rabbit Creek and Mammoth Terraces. Isolates have been sent to Ohio University to be evaluated in a fixed film bioreactor.

Findings: The goal of our part of the project, Enhanced Practical Photosynthetic CO_2 Mitigation, was to supply the Ohio University team with thermophilic cyanobacteria and to find optimal conditions for the growth of thermophilic cyanobacteria on artificial substrata. From December 2002 to September 2003, we isolated 36 primary samples from different thermal springs inside and outside of Yellowstone National Park. Coupons of different polymers were used to enhance the efficacy of the sampling of cyanobacteria from environment. These samples were the subjects of further purification. By the end of the project we had 24 frozen unialgal cultures. Six of them have been genotyped. It was found that one isolate belongs to undescribed cyanobacterial genus. Tap water was found to inhibit partially the growth of thermophilic cyanobacteria. Because of this all experiments were carried out in distilled water. Cyanobacterial isolates grew well in standard growth media. However, the cultivation of these organisms with CO_2 aeration required buffering of growth media. Calcium was found to stimulate the generation of cyanobacterial biofilm on artificial substrata. Special calcium-based medium to cultivate cyanobacterial isolates was developed to increase CO_2 trapping and to decrease the contamination of environment with sodium. The environmental alkalinity was found to be a signal for the morphological changes of highly thermophilic strain *Chlorogloeopsis sp.* A method for the selection of acid resistant strains of cyanobacteria was also elaborated. Recommendations for further sampling of cyanobacteria in YNP were made. Two papers are in progress. One describes a new genus of cyanobacteria and the other is about morphological changes promoted by alkaline conditions.

Project title: Analysis of a Eukaryotic Microbial Mat Community Across Environmental Gradients in a Thermal, Acidic Stream

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Purpose: Objective: Characterization of the Nymph Creek microbial mat community using molecular biological techniques and traditional culturing methods in the thermal (T=55°C), acidic (pH=2.7) stream.

Findings: K. Sheehan completed sequencing of several eukaryotic organisms from pure culture isolates. These included *Trichoderma viridae, Chlorophyta sp., Euglena mutabilis,* and an isolate that closely matches an uncultured eukaryote from the River of Fire in Spain. Dr. Jon Martin, Department of Genetics, the University of Melbourne, Australia, collaborated with us to identify an insect present at temperatures up to 45°C in Nymph Creek. This acidic, low-oxygen (during night when there is no photosynthesis by the predominant algal community), hot environment is considered extreme for most insects. Based on cytological analysis, the species was identified as a blood worm, *Chironomus acerbiphilus.* This is the first record for this species in North America. In our previous studies, we identified two sequence-types of *Naegleria* that could represent host amoebae for *Legionella* in situ (Sheehan et al. 2003. Detection of *Naegleria sp.* in a thermal, acidic stream in Yellowstone National Park, *J. Euk. Microbiol.* 50:263–265). Given the presence of potential host organisms for *Legionella* in the stream, the extensive, nutrient-rich biofilm, and stream temperatures favorable for growth, we hypothesized that molecular analytical techniques would reveal *Legionella* sequences in the community. Our pure culture study and clone library analysis revealed sequences that matched with greater than 98% similarity with at least five different species of *Legionella* that are potential human pathogens.

We are currently analyzing the sequence data from this study for a manuscript to be submitted in 2004. Rob Harvey (graduate student) examined the influence of substrates on the distribution of eukaryotic microflora in two acidic streams, Nymph and Alluvium Creeks using cultivation-independent analyses of 18S rRNA gene sequences. The streams differ in that the temperature of Nymph Creek is influenced by active geothermally heated source springs while Alluvium Creek receives no such geothermal input. Samples were collected from Alluvium Creek from three substrates, a lodgepole pine log, a stone surface and a silt sediment. Three test substrates were installed in Nymph Creek, a glass slide, a flat stone, and a small section of lodgepole pine, until covered with green biofilms (one week). DNA was extracted and 18S rRNA gene sequences are being obtained. Rob has sequenced approximately 200 clones and has found a variety of sequences related to fungi and stramenophiles in both creeks.

A list of organisms that are at least 97% similar to the ones detected in our survey includes the following taxa (below). Rob is in the process of examining distributions with respect to substrate in the two acidic streams. Algae: *Chlamydomonas sp., Chlorella sp., Galdieria sp., Lobosphareia sp.*; Fungi: *Capnobotryella sp., Coccodinium sp., Dothidea sp., Oidiodendron sp., Sacricomyses sp., Sclereeroconid sp., Stylodothis sp., Symbiodinium sp., Tricladium sp.*; Stramenophiles: *Coccodinium sp., Chrysopharea sp.* We completed the editing of a book on the microbes in Yellowstone National Park that will be published by Globe Pequot Press: Sheehan, K.B, D.J. Patterson, B.L. Dicks, and J.M. Henson. 2004. *Seen and unseen: the microbes of Yellowstone.* Other publications include Ferris, M.J. Ferris, T.S. Magnuson, J.A. Fagg, R. Thar, K.B. Sheehan, and J.M. Henson. 2003. Microbially mediated sulphide production in a thermal, acidic algal mat community in Yellowstone National Park. *Environmental Microbiology* 5:954–960; Sheehan, K.B., J.A. Fagg, M.J. Ferris, and J.M. Henson. 2003. PCR detection and analysis of the free-living amoeba *Naegleria* in hot springs in Yellowstone and Grand Teton national parks. *Appl. Environ. Microbiol.* 69:5914–5918; Sheehan, K.B., M.J. Ferris, and J. M. Henson. 2003. Detection of *Naegleria* in a thermal acidic spring, *J. Euk. Microbiology* 50:263–265.

Project title: Energy Availability for Photosynthesis-Independent Microbial Ecosystems in Yellowstone Nation Park

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Report number: 27010
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Purpose: Our proposed research will seek evidence for the existence of photosynthesis-independent subsurface communities in YNP springs. We will characterize the availability of energy for anaerobic microbial metabolism in a broad cross-section of springs, with a primary focus on H_2-based processes. Because many of the organisms capable of growing on H_2 can also be supported by small organic acids (e.g., formate, acetate), we will carefully characterize the concentrations and flux of these compounds to determine whether they provide an alternative energy source. We will correlate aquatic chemistry and energetic data with molecular and microscopic assessments of microbial community composition within the anaerobic portions of the springs. Our overall goals are to determine whether YNP plays host to photosynthesis-independent communities, and to establish general criteria (e.g., rock type, fluid flow requirements, etc.) that would aid in better constraining the possible magnitude and distribution of the subsurface biosphere.

Findings: Our group conducted fieldwork in YNP for five days during September 2003. During this period, we sampled a total of 11 springs in Mammoth Terraces and Midway, Black Sand, and Potts Basins. The following parameters have been or will be analyzed for the samples collected: temperature; pH; gas bubble concentrations of methane, carbon dioxide, and hydrogen; aqueous concentrations of total inorganic carbon (TIC), methane, hydrogen sulfide, sulfate, hydrogen, oxygen, and C1–C5 organic acids; isotopic compositions of TIC, methane, hydrogen sulfide, and sulfate. Analyses for organic acid concentrations and isotopes of methane, hydrogen sulfide, and sulfate are still in progress; all other analyses are complete. Highest levels of hydrogen were observed in a Black Sand Basin site, indicating the greatest abundance of energy for chemotrophic microbes there. Methane levels were highest in Potts Basin samples, followed by some Midway Geyser basin samples. Methane isotope measurements will aid in determining whether biologic or abiologic mechanisms are responsible for these concentrations. Organic acid concentrations are low among samples that have been analyzed thus far, indicating that would-be microbial energy sources must be principally inorganic (e.g., hydrogen). Completion of isotopic analyses will aid in identifying springs where subsurface microbial processes may be occurring. This information will be correlated with calculations of free energy availability for several microbial processes.

Project title: Arsenic Biogeochemistry in Yellowstone National Park

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Purpose: Our work will focus on geochemical and microbiological processes that influence the speciation and behavior of arsenic, sulfur and iron in acidic thermal environments. Given the toxicity and potential negative impacts that As may have on biota in non-park environments, geothermal springs represent an informative model system for understanding how microorganisms metabolize or detoxify As.

Findings: Our work during 2003 focused on several additional acid-sulfate springs found in the Hundred Springs Plain of Norris Geyser Basin, similar in composition and appearance to our original sampling site at Spring No. NHSP106, thermal inventory of YNP (44 43'54.8"N 110 42'39.9"W). At the point of discharge, these springs have a pH of ~3.2, temperatures ranging from 65–85°C and contain approximately 1 mM SO_4, 60 uM H_2S, 60 uM Fe(II), 30–60 uM As(III) and an ionic strength of 20 mM (primarily Na and Cl). Arsenite (As(III)) is often the predominant valence state at the point of discharge, but is rapidly oxidized to arsenate (As(V)) during transport in shallow surface water. These springs exhibit a distinct sequence of well-separated microbial mats covering the spring floor in both longitudinal and lateral directions. During 2003, our efforts were focused on thorough analytical characterization of geothermal waters as a function of distance from geothermal source, and corresponding molecular analysis of bacteria and archaea inhabiting different geochemical zones. We began a detailed effort to characterize dissolved gas concentrations including H_2, CO_2, H_2S, and CH_4 in these springs to ascertain the importance of these species in defining As(III)-oxidizing microbial communities. During 2003, we also initiated efforts to characterize several acidic springs in the Joseph's Coat, Hot Springs Basin and Rainbow Spring geothermal complexes. Aqueous and solid phase samples collected in August 2003 from several geothermal springs have been analyzed for chemical composition at Montana State University. Concentrations of arsenic in the springs sampled vary from 0.2 to 12 mg/L across a wide range of spring types and source water temperatures. Solid phase samples are currently being analyzed for 16S rDNA sequences. The role of specific bacterial and archaeal populations in the oxidation of S, Fe and As is the subject of our continuing effort to understand linkages among microbial populations and geochemical processes in acidic geothermal springs. We are planning on another productive field season in 2004 focused on identifying specific geochemical attributes associated with the diversity and distribution of specific microbial populations in Norris Basin and several similar acid-sulfate springs in other locations of YNP.

Project title: Functional and Molecular Ecology of Hot Spring Microbial Mats

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Purpose: A study of the microenvironmental controls on function and diversity in hot spring microbial mats.

Findings: We have mainly analysed date from previous years field work and have published two scientific papers. Another manuscript is ready for submission. No field activity was conducted this report year.

Project title: Bacterial Diversity of Thermophilic Anoxygenic Phototrophs

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Co-investigators:

Purpose: To isolate and characterize thermophilic (heat-loving) species of anoxygenic phototrophic bacteria. These are photosynthetic bacteria that, unlike green plants, do not evolve molecular oxygen. Several species of these organisms have served as model systems for study of the biochemistry, biophysics, and genetics of photosynthesis. Thermophilic species have evolved heat-stable versions of photosynthetic reaction centers and other photocomplexes, and the nature of these are studied in collaboration with other scientists. My project involves the detection, isolation, and characterization of thermophilic anoxyphototrophs for use in basic science studies of photosynthesis. I am not, nor will be associated with any commercial establishment. All of the pure cultures obtained in my work have been deposited in freely accessible culture collections (American Type Culture Collection and the German Collection of Microorganisms).

Findings: Work was conducted this year on cultures of a new filamentous anoxygenic phototroph related to *Chloroflexus*. This organism, designated strain RSO_1, is a *Roseiflexus* species, and was isolated from Octopus Spring (Lower Geyser Basin). Molecular microbial ecology studies have shown that strain RSO_1-like organisms play a major structural and ecological role in the thick microbial mats of alkaline hot springs that are widespread in Yellowstone thermal areas. Although initially rather difficult to culture, we are now growing strain RSO_1 routinely in the laboratory. The major differentiating features between strain RSO_1 and *Chloroflexus* is its unique phylogeny and its lack of chlorosomes or the bacteriochlorophylls of the *Chloroflexus* group. Further studies will be performed in 2004 to better define the physiological capacities of strain RSO_1. In particular, knowledge of its ability to grow autotrophically and carbon and nitrogen nutrition, as well as temperature requirements and tolerances, will yield a more complete picture of its physiological diversity.

Project title: Biomolecular Diversity in Yellowstone National Park

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Purpose: Diversa is interested in exploring the microbial diversity at Yellowstone. The current focus is mainly on microorganisms that reside in high and low pH environments.

Findings: Diversa did not engage in any research activities in Yellowstone in 2003. We are hoping to engage in research activities later this year (2004). We still do have specimens from previous field sampling trips from Yellowstone, however, we are not doing any work on them until the Servicewide Benefits-Sharing EIS is completed.

Project title: Characterization of the Microbial Rhizosphere Population of Acid and Thermotolerant Grasses Associated with Hot Springs and Microbial Diversity in Thermal Soils in Yellowstone National Park

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Purpose: The objectives of this continuing work has shifted our emphasis from rhizosphere microbial populations to those found in non-rhizosphere environments in thermal soils in various locations in the park. We continue to be particularly interested in assessing the genetic and metabolic diversity of the microbial communities that occur in these understudied environments. Our work includes cultivation and molecular characterizations. The latter is primarily directed at the 16S rRNA gene.

Findings: We have finished the characterizations of two novel thermophiles: (1) a novel genus we have named *Thermobaculum terrenum;* (2) a novel species of *Geobacillus,* proposed name *Geobacillus tepidamans.*

Project title: Linking Phylogeny and Biogeochemistry for the Discovery of Novel Chemolithotrophs Inhabiting Geothermal Gradients in Yellowstone National Park

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Purpose: Norris Geyser Basin in Yellowstone National Park represents one of the most diverse geochemical environments on Earth, and the perfect venue for a comprehensive research thrust aimed at discovering novel chemolithotrophic microorganisms. In terms of described general physiological groups, chemolithotrophs are tremendously underrepresented, particularly given the predominance of inorganic energy sources in our biosphere. The proposed research focuses on Hundred Springs Plain, a dynamic geothermal complex in Yellowstone that is comprised of literally hundreds of thermal acid-sulfate-chloride springs that vary significantly with respect to inorganic constituents and temperature. Both are significant environmental selectors that occur in numerous gradients at this site. These springs contain high concentrations of Fe, S, H_2, and As which represent the predominant potential electron donors driving nonphotosynthetic primary production. Our proposed research plan will utilize novel cultivation and molecular techniques to describe, characterize, and isolate the microbial populations present along geochemical and temperature gradients. This detailed description of the microbiology will be accompanied by a complete analysis of the aqueous and solid phase geochemistry associated with these communities and that no doubt select for, and define, the populations inhabiting these inorganic environments. Our specific objectives are: (1) Characterize aqueous and surface chemical processes associated with S, Fe and As cycling in acid-sulfate-chloride thermal springs, emphasizing the nature of chemical gradients and microenvironmental conditions that define the ecological context of native microbial populations. (2) Employ culture-independent techniques, including a novel PCR approach, to describe native microbial populations, examine community dynamics, and to correlate community composition with the geochemical perspectives derived from Objective 1. (3) Apply more realistic cultivation approaches that account for key environmental factors and that utilize surfaces and flow-through systems to isolate previously uncultured microorganisms or whole communities involved in chemolithotrophic metabolism. In addition to offering significant promise for discovery of previously unknown microorganisms, our proposed studies will contribute significantly to the general development of microbial ecology principles. Our comprehensive, multidisciplinary approach links molecular description with the more difficult task of understanding the relevance of specific microbial populations in their natural environments and the patterns in microbial ecology that reflect the environmental context of specific phylogeny.

Findings: We initiated cultivation efforts aimed at obtaining pure cultures of the different *Hydrogenobaculum* populations that our previous molecular studies have identified. We established a sampling transect that corresponds to temperature and chemical gradients, and have focused all sampling efforts at specific locations along this transect. Thus far, we have enrichments that are supporting at least four different *hydrogenobacula*. Efforts to separate them into pure cultures have not been successful and thus laser tweezer techniques are planned to attempt to isolate single cells that will be used to initiate new enrichment cultures. Thus far, electron donor enrichments that have promoted *hydrogenobacula* growth include i) hydrogen + elemental sulfur, and ii) arsenite. Hydrogen alone has not been successful; hydrogen sulfide enrichments have been inconclusive, although we found that previous *Hydrogenobaculum* isolates taken from the same spring have will grow on hydrogen sulfide. Other enrichments have yielded active Fe_2+ oxidizers at 75°C; 16S rDNA sequence analysis suggests the organisms involved are novel, uncharacterized archaea. In situ enzyme assays suggests hydrogenase is being expressed within the first one meter of the spring, but can not be measured further from the spring source, suggesting that any hydrogen present in the source water gases off rapidly (collaborator W.P. Inskeep has used in-field gas chromatography to document H_2 concentrations in source water to be about 100 nM). We also initiated work with the *cyanidia* in this same spring, examining the contribution of these evolutionarily ancient eukaryotic phototrophs to arsenite oxidation and/or arsenate reduction. We are also interested in their tolerance to sulfide. We have been taking monthly samples measuring UV irradiance at spring side, a complete suite of aqueous chemical analyses, some gases, pH, and temperature. We will attempt to correlate these various environmental parameters with population studies that utilize cultivation and molecular tools (e.g., 18S rDNA, ITS, and rubisco) designed to track population dynamics.

Project title: An Analysis of Soil Microbial Community Structure in an Evolving Thermal Soil Environment

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Purpose: We are taking advantage of the opportunity to characterize soil microbial populations across a temperature gradient established by a geothermal expansion event that occurred in July 1999. We are studying how naturally occurring soil microbial populations respond to a catastrophic perturbation. The site as originally identified as being thermally impacted based on the sudden death of lodgepole pine trees resulting from increased soil temperature. We continue to track changes over time.

Findings: One sample was taken and frozen as part of a long term monitoring project for assessing soil microbial community changes.

Project title: Molecular Diversity and Structure of Photoendolithic Ecosystems

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Purpose: The "endolithic" habitat, pore-space within rocks, is an ubiquitous extreme environment on Earth and may contain a significant contribution to global biomass. Although of large significance to our understanding of biogeochemical processes and microbial diversity, relatively little is known about the identities and properties of the microbial constituents of endolithic ecosystems. We propose here a modest three-year project to survey, identify and study the microbial organisms that occur in selected photosynthesis-driven endolithic communities. Molecular sequence-based methods will be used that do not require pure-cultivation in order to identify organisms and to gain information on them. This consideration is crucial for such environmental analyses because most (>99%) microorganisms in environmental samples are not cultured using standard techniques. The overall, general goal is to contribute to our currently incomplete understanding of the biological makeup of the photoendolithic habitat.

Findings: We have completed sampling endolithic microbial communities and have almost finished analyzing those samples in the laboratory. A peer reviewed scientific paper describing our findings is currently being prepared for submission. In summary, we have found unique microbial communities that inhabit the pore space of chalcedmonic sinter deposits in Norris Geyser Basin. Using molecular phylogenetic methods that do no require the cultivation of microorganisms to identify them, we have determined that these communities are dominated by unique *Cyanidium*-like organisms, as well new species of *Mycobacteria*.

Project title: Phylogenetic Analysis of High-Temperature Ecosystems

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Co-investigators: Alicia Berger, John Spear, Scott Dawson, J. Kirk Harris, Jeff Walker

Purpose: Ongoing research continues to focus on the survey of microorganisms in Yellowstone microbial ecosystems with varying solution chemistries. A molecular approach based on cloning and sequence analysis of the small sub-unit (SSU, 16S RNA (rRNA)) ribosomal gene is used to determine the microbial composition of these ecosystems. Ongoing studies include analyses of sub-aqueous and sub-aerial systems for bacterial, archaeal, and eukaryal life.

Findings: Work from 1999 and 2000 on Well Y-7 in Biscuit Basin, was published in *Yellowstone Science* (Fall 2002 issue, 10(4), pp. 15–21). We found that the sub-surface of Biscuit Basin has a varying temperature of its hot waters over the course of a year. We also found that the Well is rather devoid of life along its 250 foot length and that it has a thermal gradient of 50°C at the surface to 135°C at the bottom. We have an ongoing collaboration with Shaul Hurwitz of the USGS, who has initiated a long-term data-logging study of Well Y-7. In 2001–2002, we measured the bulk aqueous phase hydrogen concentration at a number of hot springs in the park. We found high nM concentrations of H_2 at a number of locations, indicating that hydrogen, rather than sulfur, probably drives primary productivity in this geothermal ecosystem. This is supported by the molecular microbial studies done within the park, where the overwhelming number of organisms utilize H_2 as the basis for their metabolisms. More sites await analysis and we plan to do more field work in 2004. A manuscript on this work is in preparation to PNAS by summer of 2004. John Spear attended the Thermal Biology Institute research meeting at the Old Faithful Snow Lodge in October, 2003. A proceedings from that meeting is being assembled by the TBI at Montana State. We will have two chapters in that: one on hydrogen in Yellowstone and one on Minimum Impact Research. We presented a poster at that meeting on Minimum Impact that applies the principles of "Leave No Trace" to research. In several years of conducting research in the park, we have noticed a trend of research related impacts. The impacts are small compared to that of the general public, still, things should not be left behind, and multiple trails do not need to form. We have submitted a copy of the poster to the Yellowstone Center for Resources.

Project title: Ecology and Geochemistry of Phototrophs in Extreme Environments—Thermal and High Iron

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Purpose: To understand the interactions of phototrophs with each other, other microbes, and the physical/chemical environment in microbial mats. To better understand the origin and evolution of photosynthesis and its impact in the Precambrian. We are seeking to understand the effects of microorganisms and their photosynthetic activity on iron oxidation and mineralization processes in hot spring microbial mats. We are also seeking to understand the effects of iron on the microorganisms. In this complex association, the role of other chemical species will also be studied. An overarching goal is to understand potential biosignatures that may be preserved in the iron geological record on Earth such as in banded iron formations. Biosignatures associated with iron deposits could also be useful in the search for evidence of past life on Mars. The specific objectives for research in 2003 were to: (1) Determine the role of cyanobacterial photosynthesis in oxidizing ferrous iron in situ in the springs; (2) Determine the effects of iron, other reductants, and light intensity on photosynthesis in mat microorganisms; (3) Characterize the range of iron isotope compositions and heterogeneity in solid deposits; (4) Characterize the iron isotope composition and concentration in Chocolate Pots waters in daytime vs. nighttime; (5) Identify lipid biomarkers in mats and sediment cores; and (6) Characterize the primary precipitates in Chocolate Pots iron sediments.

Findings: During the summer field season of 2003, we found that the oxidation of ferrous iron within the mats was directly dependent on cyanobacterial oxygen production within the mats. This was determined using microelectrodes embedded in the mat and shifting quickly from dark to light and back to dark. Furthermore we found that the oxygen production increased with increasing light intensity up to 950 W/m^2 while subsequent iron oxidation was dependent on light intensity up to 270 W/m^2 at which point all the iron was oxidized. Carbon dioxide fixation also increased with increasing light intensity up to the maximum rate which was reached between 200 and 400 W/m^2. While iron stimulated photosynthetic rates, other reductants did not. The methods we developed for collection of water samples under anoxic conditions were very successful, and no aqueous Fe(II) was lost during the filtering and sampling of the anoxic Chocolate Pots waters. These representative fluids collected during the day and nights are actively being analyzed utilizing our newly upgraded instrument at University of Wisconsin. The Fe isotope analysis of the collected fluids and ferrihydrite samples will be completed in the spring thus allowing us to determine the necessary samples that we will need to collect for the 2004 field season.

It does appear that there are small differences in the Fe isotope composition of vent fluids collected at night as compared to the same samples collected during the day. We tentatively suggest that this may reflect photochemical oxidation but because these differences in the Fe isotope composition are small, additional day night samples need to be collected. We have also completed an initial investigation into the mechanism of Fe isotope exchange between aqueous Fe(II) and ferrihydrite. It appears that isotopic exchange is controlled by ferrihydrite surface area and hence isotopic exchange in a natural setting is likely to be small given the relatively large grain size of natural ferrihydrite (~10nm) as compared to the ferrihydrite (3nm) used in our exchange experiments. Therefore, it is unlikely that diagenesis and ageing of ferrihydrite will produce changes in the Fe isotope composition of the ferrihydrite. Our next experimental investigation will focus on determining the Fe isotope fractionation factor between aqueous Fe(III) and ferrihydrite under different rates of ferrihydrite precipitation. Organic biomarkers typical of the green non-sulfur bacteria (wax esters) and several biomarkers typical of cyanobacteria were found within the mats. The lipid biomarkers were also found in the sediments beneath the mats in similar proportions indicating that the community biosignature was not altered in the earliest stages of diagenesis in the iron oxide sediments. X-ray diffraction, energy dispersive spectroscopy, and electron microprobe analyses were used to characterize the primary precipitates and indicated the presence of amorphous two-line ferrihydrite in all the environments. We are currently analyzing further samples and studying the early diagenesis of the iron precipitates to determine what pathway accommodates the transformation of two-line ferrihydrite to more ordered crystalline phases.

Project title: Isolation and Characterization of Thermophilic Microorganisms

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Report number: 27810
Co-investigators: Nathan Varley

Purpose: Isolation and characterization of thermophilic microorganisms, and the isolation and characterization of some of their enzymes.

Findings: We had isolated an apparently previously undescribed gram-negative bacterium from Yellowstone hot springs (tentatively given the name *Cyclobacterium ruber*) during the 2002 field studies in Yellowstone. This isolate is a member of the *Cytophaga-Flavobacterium-Bacteroides* group. Its 16S rRNA sequence most closely resembles (but is not identical) to a red pigmented bacterium *Akait c9* isolated by Stougaard et al. from a cold alkaline spring in Greenland and a recent isolate *(Bella Baltic)* obtained by Brettar et al. from the surface water of the central Baltic Sea. However the Yellowstone isolates (obtained from West Thumb Geyser Basin, Twin Butte Overlook Hot Spring and a small hot spring pool located just upstream from the Five Sisters Hot Spring in the White Creek area), also showed some 16S rRNA sequence similarity to an uncultured *Cytophaga-Flavobacterium-Bacteroides* group 16S rRNA sequence (AF445684) obtained and reported by Fouke et al. from samples taken from Angel Terrace (Mammoth Hot Springs). Thus, during the 2003 field studies in Yellowstone, we sought to see if additional isolates of *Cyclobacterium ruber* could also be cultured from the Angel Terrace area. These samples were collected with the help of Christie Hendrix and Liz Cleveland of the Research Permit Office of the Yellowstone Center for Resources, since the Angel Terrace area is highly visible to the general public. The 2003 samples were taken in the same general area where the Fouke AF445684 sample had been obtained. (Dr. Fouke kindly provided his notes indicating the sample locations.) Although there had been considerable changes in the mineral deposition, hot spring flow, etc., of the Angel Terrace area since Fouke obtained his samples, our 2003 samples were taken in apparently equivalent areas on

Angel Terrace (temperature, etc.). Additional isolates of *Cyclobacterium ruber* were indeed obtained from the 2003 field samples. It is likely that hot springs such as those in the Mammoth Hot Springs area containing high carbon dioxide levels especially favor the growth of *Cytophaga-Flavobacterium-Bacteroides* type bacteria such as the present *Cyclobacterium ruber* isolate.

During the 2003 field season, we also collaborated with Nathan Varley to conduct a field survey and collection of a previously unsampled area (Geyser Creek) to examine the feasibility of being able to collect hot spring samples and then rapidly transport them at ambient temperatures (U.S. Postal Express Mail from the Mammoth Hot Springs post office) to the laboratory at the University of Nebraska Medical Center. Previous studies had shown that collection of hot spring samples permitted the isolation of the largest number of thermophilic microorganisms if sterile glycerol was added to the sample at a (final concentration of 20–30% v/v) and the samples then transported on dry ice. (Long-term storage could then be made at –70°C.) Previous studies had shown that storage and transport of samples at ice temperature resulted in a rapid loss of thermophilic isolates and extended storage of sample at ambient temperatures results in overgrowth of the hot spring samples by mesophilic bacteria. Using the rapid sampling and shipping method it was possible to obtain samples from hot spring to laboratory within 48 hours and the thermophilic isolate recovery was equivalent to that obtained with transport on dry ice. After arrival at the laboratory, the sample from the ambient transport could be stored on 20–30% (v/v) glycerol for future study. During the 2003 field season, samples were also taken for the isolation of various *Meiothermus ruber* type isolates from Yellowstone hot springs to examine the properties of the red vs. the more orange pigmented isolates and these results suggesting that both types are extremely close (by 16S rRNA sequence analysis) and these results will be reported at the 2004 Annual Meeting of the America Society for Microbiology.

Project title: Geochemical Constraints on the Ecology of the Deep Lineages within Bacteria and Archaea

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Report number: 27494
Co-investigators: Amy Banta, Jim Daly

Purpose: (1) To determine the microbial diversity and geochemistry associated with high temperature thermal springs in YNP. (2) Study the ecology of microbial communities inhabiting YNP thermal springs.

Findings: We continued our analysis of samples collected also as part of the Yellowstone Microbial Inventory project (see Dr. Takacs-Vesbach, this report). Over 70 different samples were characterized for bacterial and archaeal diversity providing a framework for future temporal studies. We used *Aquificales*-specific primers to detect members of this group through the park hot springs. Furthermore, we have purified a novel isolate of the *Aquificales* that appears to be a new species of the genus, *Sulfurihydrogenibium*.

Project title: Analysis of Metal Resistance in Yellowstone Bacteria

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Report number: 28662
Co-investigators: Mark E. Delwiche, David Barrie Johnson, Deborah T. Newby

Purpose: Isolation and characterization of thermophilic, thermoacidophilic and other bacteria from locations throughout Yellowstone to identify these microorganisms on the basis of 16S rDNA sequences and determine mechanisms of metal resistance/tolerance at the genetic level in these microbes. Isolates may also be used in other INEEL research (see V. Thompson, W. Apel, this report) screening for novel enzymes.

Findings: A publication entitled "Novel thermo-acidophilic bacteria isolated from geothermal sites in Yellowstone National Park: physiological and phylogenetic characteristics" was published in the journal *Archives of Microbiology* (vol. 180, pp60–68). This publication constituted part of the doctoral degree requirements for Dr. Naoko Okibe, a student at the University of Wales, Bangor.

Project title: Characterizing DNA Methylase and Restriction Enzyme Genes in Environmental DNA

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Report number: 28466
Co-investigators: Richard D. Morgan

Purpose: To discover novel restriction enzyme and methylase genes by directly screening environmental DNA samples.

Findings: One visit to Yellowstone was made in October 2003. Samples of cyanobacterial mat, pool sediment and prokaryote filaments in run off channels were collected in the White Creek, Rabbit Creek and Nymph Creek drainage area. Most samples ranged from 55°C to 90°C and from pH 2 to pH 9.5. We are currently preparing DNA from the samples by a variety of methods, including bead-beating and chemical lysis. Construction of DNA libraries from the sample DNA is underway. The libraries will then be used for identifying restriction-modification systems. We will also characterize a number of16s rRNA sequences from the DNAs. During 2003, work was performed on a DNA library made from a sample collected in the White Creek area. This sample consisted of a mixture of pink, orange, tan, and green filaments growing in the effluent channel of a hot spring at approximately 70°C, pH 8.5. DNA was purified from a 1.5ml sample of these prokaryotic filaments and a library containing approximately 1,000,000 individuals was generated. The methylase selection method was employed to see if several commonly occurring restriction systems might be represented in this library. The endonucleases HaeIII (recognizing 5′-GGCC-3′), MseI (recognizing 5′-TTAA-3′), HinfI (recognizing 5-GANTC-3) and TaqI (recognizing 5′-TCGA-3′) were used. DNAs conferring resistance to each of these endonucleases were identified. Four of these individual DNA fragments were sequenced (one for HaeIII, one for HinfI and two for TaqI resistant DNAs). The sequence of these clones has been deposited in the public database Genbank (for example, accession number AY588130.) These results confirm that restriction modification systems are present in the bacteria and/or archaea inhabiting this thermal environment of Yellowstone NP. The enzymes identified in this initial screen will be further characterized and compared to previously known examples. We plan to form new DNA libraries and to search them for novel restriction endonucleases and methyltransferases.

Project title: Isolation, Identification, and Characterization of Microorganisms Living in Extreme Environments

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Report number: 26734
Co-investigators:

Purpose: The purpose of this study is to teach undergraduate students about techniques of environmentally sampling and analyzing hot spring sources of thermophilic microorganisms, most notably the hot spring environments of Yellowstone National Park. On-site inoculation of media to propagate organisms and analysis of water samples to determine salt levels, heavy metal concentrations, pH levels and temperature variations is performed with the intent of trying to develop new medias for the propagation of extremophilic microbes. In addition, we gain some knowledge about the diversity of the hot spring microhabitats these organisms call home.

Findings: This past year, I had five students come to the park over a two week period for a backcountry trek and sampling of the Heart Lake thermal springs. This has become a popular summer course with the students at my college and I have another group of students eagerly waiting to participate this upcoming summer. I have necessarily limited my course size to five students to limit environmental impact and because it is logistically a manageable number. The most notable finding from this years trip was how variable the arsenic concentrations were in hot springs that were all within 50 yards of each other. One spring had an arsenic concentration of 0.00 ppm and a pH of 4.5, while a large neighboring spring had a pH of 7.5 and arsenic levels of 1.5 ppm. This clearly showed the students just how different the spring environments can be even though they are in the same thermal area.

Project title: Microbial Life in Thermal Environments

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Report number: 27141
Co-investigators: David Mead

Purpose: n/a

Findings: For the past three years, we have had a project to isolate viruses and microbes from thermal springs in YNP and construct libraries of genomic DNA, both from consortia and from single, uncultivated cells. These libraries are sequenced to study the microbiology of the springs. Our field methods involve removal of about two liters of water with minimal impact to the environment. Initial sequence analysis of these libraries is providing a glimpse at the molecular diversity in the hot springs and their associated water columns. We have recently begun a collaboration with the Department of Energy, Joint Genome Institute to sequence a total of 100 megabases of DNA isolated from one hot spring. This will result in one of the most comprehensive molecular surveys of the life in any natural environment. Preliminary analysis of this data indicates that diversity is very high and the community is distinct from the previously described species represented in public databases. When complete, findings relating to the abundance, diversity, biochemistry and morphologies will be published. The sequence data will be deposited in a public database and made freely available to the scientific community.

Project title: Diversity and Habitat Range of Sulfate-Reducing Microorganisms

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Report number: 28402
Co-investigators: Jose R. de la Torre, Jesse G. Dillon, Martin Koenneke, Nicolas Pinel, Linda M. Sauter, Christopher B. Walker

Purpose: Our research at Yellowstone National Park has focused on better defining the diversity of sulfate-reducing bacteria along environmental gradients of pH and temperature. Organisms having the capacity to respire sulfate drive a key step in the global cycling of sulfur and are likely an important biological presence in many of the sulfur-rich geothermal areas within Yellowstone National Park. A long-term objective is to better define the environmental limits of dissimilatory sulfate reduction. Our primary method of assessing population diversity has been comparative sequence analysis of the highly conserved dissimilatory sulfite reductase (DSR) gene. This gene can be selectively amplified from DNA recovered from site material using PCR, as reported by our research group. Comparative sequencing of cloned DSR genes avoids the usual biases associated with culture-based methods of characterization. We complement this molecular characterization with on-site activity measurements and also use more traditional culture-based methods to evaluate cultivable sulfate-reducing bacteria.

Findings: Sulfate respiration by anaerobic microorganisms is a significant biogeochemical process in many of Yellowstone National Park's geothermal features. Previous work by our laboratory and others demonstrated the existence throughout the park of deeply-diverging lineages of sulfate respiring microbes, as defined by the diversity of DSR gene sequences recovered from environmental DNA. To better understand the physiology of these sulfate-utilizing microbes, we continued our studies from 2000 and 2001 by measuring sulfate reduction rates (SRR) in a variety of thermal springs. Although some of these thermal features have been studied previously by our group, we also explored thermal features in the Shoshone Geyser Basin in an effort to better define the range of environmental conditions under which sulfate reduction can be observed. In Mushroom Spring, where sulfate reduction takes place within the context of a photosynthetic microbial mat, we pursued our previous results indicating that SRR varied at different times of the day by measuring SRR at dawn, midday, dusk and midnight. In addition, we used microelectrodes to measure dissolved oxygen and sulfide concentrations along vertical profiles within the mat in conjunction with the SRR measurements. Previous field work by our group indicated that hydrogen might stimulate SRR in sediment from at least one thermal feature. In 2003, we elaborated on these experiments and investigated whether a broad range of potential electron donors (including short chain fatty acids, amino acids, ethanol, hydrogen and methane) could stimulate endogenous SRR. For these studies we focused on two hot spring systems where we had previously measured significant SRR: Obsidian Pool in the Mud Volcano area and Black Sediment Pool in the Nymph Creek area. We are currently analyzing the results of these experiments. Finally, to identify novel thermophilic sulfidogenic microorganisms, we combined enrichment cultivation with sequencing of 16S rRNA and DSR genes. Samples from the three hot springs named above were used to inoculate enrichment cultures containing a variety of electron donors and sulfate as the electron acceptor, at 60°C or 80°C. Sulfide production was observed at 60°C with various organic electron donors or hydrogen. In contrast, at 80°C, sulfide was only produced in cultures using hydrogen as the electron donor, either autotrophically or with acetate as an alternative carbon source. Sulfide was also produced in enrichments containing hydrogen in combination with sulfur, sulfite or thiosulfate as the electron acceptors, at both 60°C and 80°C. In agreement with the observed lower metabolic diversity at higher temperatures, microscopic analysis of these enrichments revealed a lower number of morphotypes at 80°C than at 60°C. Cloning and sequencing of DSR and 16S rRNA genes is now in progress to identify and characterize the potentially lithotrophic thermophilic SRP in our enrichment cultures. The observation that only hydrogen, amongst the various electron donors tested, supported the growth of SRP at 80°C in our cultures argues in favor of the proposed importance of lithotrophic metabolism in these hydrothermal environments.

Project title: Isolation of New Hyperthermophiles and Investigations of Hyperthermophilic Biotopes

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Report number: 27756
Co-investigators: Robert Huber

Purpose: n/a

Findings: From the anaerobic continuous flow fermentor which had been inoculated with samples taken from Obsidian Pool, experiments had been carried out in order to cultivate the novel

Nanoarchaeota species existing therein. The cultivation attempts look already very promising. As final target, we want to describe a new family within the new kingdom of *Nanoarchaeota* existing from Yellowstone National Park. Furthermore, we are in the progress of cultivation and description of the first member of *Korarchaeota* existing in Yellowstone National Park. In a lecture held on the occasion of the First Biannual Workshop on Geothermal Biology and Geochemistry in Yellowstone National Park (October 9–11, 2003), I gave an overview about the ultimate scientific progress. A paper has been submitted for the Proceedings: Stetter, K.O., M.J. Hohn, H. Huber, R. Rachel, E. Mathur, B. Hedlund, and U. Jahn. A novel kingdom of parasitic *Archaea*. Proceedings of the First Biannual Workshop on Geothermal Biology and Geochemistry in Yellowstone National Park (Oct. 9–11, 2003). Submitted 2/3/2004.

Project title: A Microbial Inventory of the Greater Yellowstone Ecosystem Thermal Features

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Report number: 28665
Co-investigators: James Ball, Mike Bobb, Sara Caldwell, JoAnne Holloway, Olan Jackson-Weaver, Blaine McCleskey, Kendra Mitchell, D. Kirk Nordstrom, Anna-Louise Reysenbach, Ann Rodman, Jeremy Vesbach

Purpose: The overall objective of the project is to survey the microbial phylogenetic (16S rRNA of *Bacteria* and *Archaea*) diversity, and describe the geochemical characteristics of 100 thermal sites in the GYE. The survey will be conducted in conjunction with a spatial inventory currently being done by the Yellowstone Center for Resources. The project will result in a microbial inventory housed in a web-accessible relational database, which would be one of the first of its kind to link microbial phylogenetic sequence data and geochemical measurements to spatial data (GIS). In addition to 16S rRNA genes, the project will screen sites for selected physiological genes, including sequences indicative of sulfate reduction, methane production, ammonium oxidation, and nitrogen fixation, which will be useful in further defining geochemical and phylogenetic co-relationships. This project will provide a baseline of the microbial diversity in the GYE that could be used for monitoring diversity and managing microbial resources.

Findings: Microbial mat and filaments, sediment, soil, water, or rock was collected from 86 thermal sites. The samples were collected from Mammoth Hot Springs, Washburn Hot Springs, Norris Geyser Basin, Lower Geyser Basin, Mud Volcano, Artist's Paint Pots, Gibbon Hill, Violet Springs, Highland Hot Springs, Glen Africa Basin, Coffee Pots Hot Springs, Joseph's Coat Hot Springs, Hot Spring Basin, Rainbow Hot Springs, Mary Bay, and the Mudkettles and Mushpots. The total microbial sample volume collected was approximately 0.75 liters. Water samples for geochemical analysis were also collected from 49 of the sites. The total water collected for geochemical analysis was approximately 49 liters. We have begun analysis of the microbial communities represented in the samples using DGGE or cloning and DNA sequencing. Our USGS collaborators have begun analysis of the geochemical samples. The results of these analyses will be posted at <http://www4.unm.edu/yellow/vesbachcfm/research/databas-

es.cfm>, and will be accessible to the public. Also, we have posted the Yellowstone Microbial Inventory Literature Database that was created by the Spatial Analysis Center of the Yellowstone Center for Resources. This database is available to the public and is searchable based on several variables including location, organism, researcher, etc.

Project title: Development of Harsh Environment Biosensors

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Co-investigators: William Apel, Kastli Schaller

Purpose: The objective of this study is to culture thermophilic microorganisms from Yellowstone hot springs. We are seeking bacteria that can grow under a variety of conditions such as high temperature, high or low pH, and/or high minerals content. We will culture water and sediment samples in culture media under the conditions we are interested in and incubate to see if growth occurs. When growth occurs, the bacteria will be screened for different types of enzymatic activities. Examples are catalase, urease, and xylanase activities. Bacterial strains that test positive will be grown in large quantities and harvested for enzyme purification. The cells are broken open and subjected to several chromatography steps for purification. Characterization of the purified enzymes will include kinetics, temperature and pH stability, stability against inhibitors, and temperature and pH optimum. These enzymes will then be used to develop biosensors for detection of things such as environmental pollutants. Since these biosensors will utilize thermostable enzymes, they should be much more stable and have longer lifetimes than conventional biosensors. Other applications for these enzymes will also be examined.

Findings: Environmental samples were obtained from two sampling trips to the Norris Geyser Basin of Yellowstone National Park. This geyser basin has numerous high temperature and acidic thermal features. The pools sampled were: Bathtub Spring (this pool was too deep to get accurate temperature and pH measurements, approximate temperature 96°C and approximate pH 4.0), Mud Springs (temperature 92.8°C and pH 4.4), Black Hermit Cauldron (temperature 85°C and pH 2.95), Branch Spring (temperature 81.3°C and pH 4.01), Son of Green Dragon (83°C and pH 3.3) and Yellow Funnel (89°C pH 3.4). Samples obtained from the hot springs were inoculated into a selective medium adjusted to pH 3.0 and supplemented with ground corncobs as the xylan carbon source. The cultures were incubated with shaking at 80°C and monitored periodically for growth. Growth was observed in cultures inoculated from Bathtub Spring, Mud Spring, and Black Hermit Cauldron samples. It was assumed that growth is indicative of the presence of a xylanase enzyme.

Project title: Ecology of Hot Spring Microbial Communities

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Report number: 27931
Co-investigators: Jess Allewalt, Mary Bateson, Devaki Bhaya, Fred Cohan, Arthur Grossman, John Heidelberg, Michael Madigan, Melanie Melendez, Marcel van der Meer

Purpose: (1) We continued two approaches to testing the hypothesis that cyanobacterial diversity in mats of alkaline siliceous hot spring results from adaptive evolutionary radiation. We published a manuscript on vertical distribution of oxygenic photosynthesis, light, oxygen and genetic diversity of cyanobacteria at 68°C in the Mushroom Spring. We characterized the adaptations of genetically relevant *Synechococcus* isolates from such mats to temperature and light. (2) We published a study of local to global geographic variation of hot spring *Synechococcus* (and other cyanobacteria), in an effort to understand whether geographic isolation could be another important driver of genetic diversification of microorganisms. (3) We continued research on the diversity of green sulfur-like bacteria in Yellowstone springs done in collaboration with Dr. Donna Bedard (RPI), who will report the results separately. (4) We continued organic geochemistry studies of hot spring microbial mats in order to cultivate and characterize major phototrophic microorganisms that are genetically relevant to the mats. We collaborated with Dr. Michael Madigan (So. Ill. Univ.) to try to cultivate the Yellowstone type-C organisms. We also continued organic geochemistry studies of hot spring microbial mats in order to understand how stable isotope signatures of organic compounds produced by mat phototrophs arise through different biochemical processes. In particular, we are trying to understand how autotrophic processes and sugar biosynthesis affect the stable carbon isotope signatures of organic compounds produced by cyanobacteria and GNSLB. (5) We initiated a major collaboration with Dr. Fred Cohan (Wesleyan Univ.), Dr. John Heidelberg (The Inst. for Genome Research) and Drs. Devaki Bhaya and Arthur Grossman (Carnegie Inst., Stanford) to employ genomic, microarray and high-resolution population genetics analysis in a detailed analysis of ecotypes within the mat community and their physiology.

Findings: (1) We characterized several *Synechococcus* isolates that we showed are genetically relevant compared to indigenous mat populations at the 16S rRNA and internal transcribed spacer levels of resolution. We conducted studies of temperature and light effects on growth rate and photosynthetic processes (using incorporation of 14C-labeled carbon dioxide). Genotypes B and A showed increasingly broader temperature optima and higher upper temperature limits (ca. 55–60°C, 60–65°C and 65–70°C, respectively), paralleling their distribution along thermal gradients in situ. There was less evidence of adaptation of the photosynthetic apparatus, with all genotypes exhibiting photosynthesis at 65°C and the A genotype still slightly active at 70°C. In preliminary experiments, genotypes B and A exhibited differences in growth rate at different light intensities that also parallel their vertical distribution in the 60°C mat. Genotype A grew faster at low light intensity, while genotype B grew faster at high light intensity. All genotypes show typical photosynthesis vs. intensity relationships, with rates increasing to an optimum intensity around 100–500 uE/m^2/sec, and evidence of partial photoinhibition at higher intensity. We also observed evidence of acclimation to low light intensity in some genotypes.

(2) A mini-review which is currently in press in *FEMS Microbiology Ecology,* which places these and other results in a theoretic context with regard to the importance of physical isolation in prokaryote evolution. (3) See Bedard, Donna, this report. (4) Through collaboration with Dr. Madigan, we succeeded in cultivating Yellowstone strains of *Roseiflexus,* a filamentous bacteriochlorophyll a-rich photoheterotrophic bacterium that is identical to the type-C filamentous bacteria dominating mats in alkaline Yellowstone springs. We have demonstrated that these isolates produce predominantly wax

esters typical of those predominating in these mats (including branched chain wax esters) and also smaller amounts of alkyl diol lipids. Mushroom Spring samples collected at regular intervals through a diel cycle were analyzed for polyglucose content. Polyglucose concentrations rose during the day and declined during the night. This trend was also observed in *Synechococccus* cells, which were separated from *Roseiflexus* filaments using Percol gradients. We have demonstrated that the Percol-separated fraction containing *Synechococcus* cells is nearly free of wax esters typical of *Roseiflexus*, although the fraction containing *Roseiflexus* still contains some evidence of *Synechococcus* lipids. Although the natural abundance of bulk mat material is similar in morning and afternoon, these Percol fractions exhibit differences and shifts during the day. *Roseiflexus*-dominated fractions are isotopically heavier than *Synechococcus*-dominated fractions, but the former is lighter the latter heavier in afternoon samples. These findings are consistent with our hypothesis that sugar biosynthesis in *Synechococcus* may impart a heavy isotopic signature that is transferred to *Roseiflexus*. 13C-labeling experiments provided evidence consistent with greater photoautotrophy by GNSLB during the morning and by *Synechococcus* in the afternoon. (5) We conducted a major collection at several temperatures and depths in the Mushroom Spring mat in support of our efforts to conduct environmental genomic, population genetic and gene expression studies. Preliminary genomic analysis revealed genomic clones with 16S rRNA sequences typical of mat microorganisms, but not *Synechococcus*, indicating lysis bias. We have developed a lysis protocol that should enable unbiased analysis of *Synecohcoccus* genomes. We have developed procedures for lysis of *Synechococcus* within gels, which is necessary for recovery of high-molecular-weight DNA for population genetic studies.

Paleontology

Project title: Recognizing the Signatures of Hyperthermophilic Biosignatures Within Hydrothermal Ecosystems and Their Fossilized Deposits

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Report number: 28490
Co-investigators: Jessica Goin, Rick Hugo, Niki Parenteau, Wendy Smythe

Purpose: (1) By identifying the processes by which hyperthermophilic biosignatures are formed and preserved in the geological record, we can establish a framework for recognizing hyperthermophiles in the geological record. This work has implications for improving our ability to recognize the presence and activities of high-temperature life in the geological record, whether on Earth or on other rocky planets such as Mars. Our aim is to determine how to detect and interpret the biosignatures microbial communities leave behind in the geological record. Our approach is to characterize the mechanisms and types of activities that result in the formation of microbial biosignatures by using a combination of field, experimental, and analytical techniques. (2) Develop technique for studying microbial biofilms in situ using a mobile biofilm unit, which allows us to study biofilm formation and mineralization on indigenous communities without collecting the natural hydrothermal precipitates they colonize.

Findings: (1) We are presently investigating the geochemical factors that enhance the formation of carbonaceous (aka permineralized or petrified) microfossils. These types of fossils are considered definitive evidence of life when found in the ancient geological record. The goal of this year's research was to characterize at the nanoscale the minerals associated with microbial cells. Field specimens were powdered for crystallographic studies using Energy Filtered Electron Diffraction (EFED) in the TEM. We found that the atomic structure of the primary precipitates do vary as a function of the precipitation conditions. Small but identifiable variations in the structure of the minerals associated with biosignatures may ultimately determine biosignature preservation. Once siliceous sinters precipitate, diagenetic processes cause the minerals to transform into more ordered phases, which affects microfossil fidelity. Research plans include an investigation of older, more ordered sinter deposits at Artist Point to compare the mineralogy and biofabrics with modern siliceous hot springs. This project will involve geological mapping, collecting samples along the thermal gradient (as evidenced by their biofabrics), and determining how mineralogy and sinter fabric vary with decreasing temperature and increasing silica concentrations. (2) Our mobile biofilm unit (MBU) provides a means of documenting the dynamics of indigenous biofilm formation in real time and at spatial and temporal scales relevant to geomicrobial processes under controlled hydrodynamic conditions. Biofilms will develop inside glass-enclosed flow cells attached to the MBU's inverted microscope stage when fluid from the hot spring is pumped through the system. The unit is solar powered, has a modular design, and can be deployed at field sites that can only be reached on foot. A digital camera connected to the microscope focuses on the inside surface of the flow cells and records a real-time stream of data. Inlet and outlet probes allow the flow and temperature to be recorded throughout the duration of each experiment. The unit is designed to allow surfaces colonized by the biofilms to be removed for time point characterizations using additional analytical techniques. Although flow cells are used to characterize the dynamics of laboratory grown, this instrument, the first of its kind, was developed to study the dynamics of natural biofilm formation in situ. Our initial set of questions will focus on whether the microbial communities that form in the MBU are the same as those forming in the natural hot spring ecosystem. The advantage of using this instrument to study microbial communities in hot springs is that we can allow populations and communities of organisms to colonize the flow cells of our MBU without having to disturb them in the natural environment. This is a non-destructive way of investigating these types of microbial communities, and we hope that it will serve as a model for other researchers working in pristine and unique environments, such as those found at Yellowstone.

Project title: The Search for Microbial Biomarkers in Terrestrial Deposits

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Co-investigators:

Purpose: The primary purpose of this investigation is to evaluate the fossilization process and the potential for a geologic record of the microbial life that exists associated with hot springs and their deposits. Basically, we are looking for biomarkers: indicators that microbes once existed as part of the hot spring environment. This will allow us to determine the likelihood of finding fossilized microbes in extraterrestrial bodies, e.g., Mars, as well as the nature of the most likely preserved material. For example, will we have a better chance of finding body fossils or geochemical indicators of former organisms? In order to carry out this investigation, we have and will continue to analyze the waters from which the mineral precipitates originate as well as the solid precipitates. It is our intent to search for mineralogical (crystal habit, size, etc.) and geochemical (major, minor, and trace elements as well as isotopic) differences between biotically induced and abiotic precipitates, as well as microbial remains (bacterial body fossils, biofilms, etc.). In the past, we have investigated carbonate and siliceous sinter deposits. We intend to modify this work to include Mn- and Fe-rich hot spring mineral deposits. Some hot springs at Yellowstone provide an ideal natural laboratory in which to conduct this avenue of research. Among the sites that we would like to visit to conduct this investigation include: Terrace Spring (near Madison), Pine Cone Geyser (Lower Geyser Basin), Black Warrior Basin area (especially Artesia Geyser), Locomotive Geyser and Black Sulphur Spring (Shoshone Geyser Basin), Fissure Group (Heart Lake Geyser Basin), White Creek area, Hot Lake area, and Chocolate Pots. All of these sites have been reported to have Mn- and Fe-rich mineral deposits. Recognition of biotically induced mineral precipitates will be based on macroscopic form of the precipitate, presence of microbial fossils, oxidation state of the metals, saturation of the waters with respect to the mineral precipitates, and isotopic fractionation of the metals in the precipitates. This avenue of research has not been previously conducted in Yellowstone. Laboratory and field investigations at universities and other field sites indicate that this area of research holds great promise of providing insights into biotic vs. abiotic mineral deposition. An area in which this research is relevant pertains to the search for life on Mars. Remote sensing of the Martian surface suggests that free water was present on the Martian surface, and that large areas may be covered by Mn- and Fe-rich spring-related deposits. Are these deposits biotic or abiotic, and how can we tell? Relevant data are likely to exist within the hot spring deposits in Yellowstone. Additionally, the origin of Mn- and Fe-rich deep sea nodules has long been debated; this research can shed light on that debate. This is a new, exciting area of investigation, and Yellowstone is the ideal site to perform the field work associated with this research.

Findings: No activity was conducted this report year.

Vegetation

Project title: Ecological, Physiological, and Molecular Biological Studies of Fungi from Geothermal Soils and Thermotolerant Plants in Yellowstone National Park

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Report number: 27465
Co-investigators: Jean Cronish, Joan Henson, Marshal Hoy, Regina Redman, Richard Stout

Purpose: The proposed research will provide information to increase our understanding of fungal and plant survival in unique environments, the roles of fungi in ecosystem dynamics, and the temporal and spatial scales of the micro-habitats that fungi occupy. Specifically, this work will provide information about: (1) how plants and symbiotic fungi survive in geothermal soils; (2) if fungal community structure changes in response to environmental conditions; (3) if fungi can alter between saprophytic and symbiotic lifestyles in response to environmental conditions; and (4) the scale of soil studies necessary to accurately assess the roles of these fungi in ecosystem dynamics. In addition, the feasibility of developing molecular biological tools will be determined for rapidly assessing (a) fungal community structure based on molecular biomass measurements; and (b) the occurrence of fungi in thermotolerant plants.

Findings: The findings of this study were very dramatic. The fitness of *Dichanthelium lanuginosum* under laboratory and field conditions was greater in symbiotic plants versus nonsymbiotic plants. In fact, at temperatures greater than 40°C nonsymbiotic died while symbiotic plants survived. This was the first demonstration that a fungal symbiont was responsible for thermotolerance in plants. More importantly, it suggests that symbioses, rather than plants alone, adapt to selective pressures. This may have significant impacts on habitat restoration strategies and provides a potential mechanisms to mitigate the impacts of global change. Additional studies have demonstrated that different fungal species occur in different members of the thermal–plant community. Although the significance of these fungi is unknown they will be investigated in the coming years. This work resulted in the following manuscripts: Redman, R.S., K.B. Sheehan, R.G. Stout, R.J. Rodriguez, and J.M. Henson. 2002. Thermotolerance generated by plant/fungal symbiosis. *Science* 298:1581; Rodriguez, R.J., R.S. Redman, and J.M. Henson. In press. The role of fungal symbioses in the adaptation of plants to high stress environments. *Mitigation and Adaptation Strategies for Global Change;* Rodriguez, R.J., R.S. Redman, and J.M. Henson. In press. Symbiotic lifestyle expression by fungal endophytes and the adaptation of plants to stress: unraveling the complexities of intimacy, in *The Fungal Community: Its Organization And Role In The Ecosystem,* J. Dighton, P. Oudemans, and J. White, eds.

Viruses

Project title: Isolation and Characterization of Thermophilic Viruses from Yellowstone National Park

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Report number: 26506
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Purpose: To isolate and characterize thermophilic viruses from the thermal features in Yellowstone National Park.

Findings: We have been monitoring three individual hot springs in three different locations around YNP for the past year. We have been studying the changing viral population and comparing it to the changing geochemistry of the pools. We have found that the viral population in these pools is quite diverse and changing, however, the geochemistry is not as variable as we would have thought, it seems to be mostly seasonal changes. We have also been trying to grow anaerobes from YNP, and have been somewhat successful.

Part II. Terrestrial Environment

Air Quality

Project title: Rocky Mountain Snowpack Chemistry Monitoring

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Co-investigators: Don Campbell, Craig McClure

Purpose: Monitor deposition to annual snowpacks of acidic compounds in the Rocky Mountain region, including Yellowstone National Park. Determine sources of regional and local emissions that result in deposition to sensitive mountainous areas including Class I Wilderness areas.

Findings: Snowpack chemistry results for 2003 are nearing completion of analytical quality control (as of 2/4/04). Preliminary results of two acidic compounds associated with snowmobile traffic indicate consistently higher levels of ammonium (NH_4+) and sulfate (SO_4–) at sampling sites with heavy snowmobile usage as compared to sampling sites at least 50 meters away from snowmobile routes. Average concentrations of NH_4+ and SO_4– at the West Entrance in the snowpacked roadway (in-road) (NH_4+, 22.0 microequivalents per liter (ueq/L); SO_4–, 12.6 ueq/L) were 50 to 60% higher than those off-road (NH_4+, 14.0 ueq/L; SO_4–, 7.4 ueq/L). Along the East Entrance Road near Sylvan Lake, in-road vs. off-road concentrations (NH_4+, 8.1 vs. 5.8 ueq/L; SO_4–, 4.6 vs. 3.2 ueq/L, respectively) were 40 to 50% higher, but noticeably lower than those detected for the West Entrance. Results from off-road Yellowstone snow-sampling sites may be compared to concentrations of ammonium and sulfate obtained from about 50 other snowpacks located in a network extending from northern New Mexico to northern Montana. Regional concentrations of ammonium and sulfate were represented at other locations in and near Yellowstone located away from snowmobile use including Canyon, Daisy Pass, Lake, Lewis Lake Divide, Old Faithful, and Twenty-one Mile. In general, ammonium concentrations (average of 5.4 ueq/L regionally, and average of 8.4 ueq/L at off-road sites in YNP) tended to be highest near West Yellowstone (14.0 ueq/L) and Targhee Pass (22.6 ueq/L, the regional maximum for 2003), and above the regional average elsewhere in Yellowstone. Sulfate concentrations at sites away from snowmobile traffic averaged 4.4 ueq/L and were generally similar to the regional mean of 5.2 ueq/L. Sampling at the sites described for 2003 is planned again for the 2004 snowpack.

Project title: Spatial Variation and Characteristics of Volatile Organic Compounds Associated with Snowmobile Emissions in Yellowstone National Park

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Report number: 27094
Co-investigators: Bruce Pape, David Shively

Purpose: We propose to perform an extensive investigation of the spatial variability of important atmospheric trace gases, including air toxics, in Yellowstone National Park (YNP) and the implications for air quality and regulations. As an extension to the first phase of this work performed in February 2002, we will continue our investigation on the effects of wintertime recreational vehicle regulation in the park by examining the sources and distributions of volatile organic compounds (VOCs). Sampling will be conducted on both low and high traffic days for the purpose of comparing differences in emissions. VOC measurements, including nonmethane hydrocarbons, halocarbons and alkyl nitrates, will be made from approximately 215 whole air samples collected within and adjacent to the park. Additionally, samples will be analyzed for carbon monoxide and methane. On the designated sampling days, whole air samples will be acquired in the early morning and in the early afternoon at each site in order to assess the relative impact of oversnow travel on VOC distributions within the park. Georeferenced observations will be obtained along the principal travel routes and in more remote areas of the park freely accessible to the public. Diurnal samples will also be obtained near Lake Ranger Station on both the low and high traffic days. The proposed sampling campaign will be more extensive than our first sampling endeavor conducted in February 2002. For each collection location, samples will be collected at distances of approximately 0.5 and 1.0 km upwind of the transportation routes to alleviate contamination of fresh exhaust plumes encountered on roadways. However, samples will also be collected at park entrances and on roadways in order to assess the impact of exhaust fumes and air toxics on park workers and visitors and to evaluate local transport and air mass mixing. Radiosondes will be launched twice a day during the collection periods to obtain local meteorological conditions. Exhaust samples will be collected from various two-stroke and four-stroke snow machines that are typically used by visitors in the park. A quantitative understanding of these compounds and their spatial variability will enhance the ability to predict potential outcomes of wintertime recreational vehicle regulation. Furthermore, the use of geographic information system (GIS) technology will permit the spatial analysis of these compounds with the aim of identifying spatial associations that have both process-based, ecological, and human risk-related implications. The proposed project will represent an extensive study with respect to regulation, and will be supplemented by follow-up post-treatment studies timed to coincide with the phasing in of regulations limiting oversnow traffic in the park.

Findings: A total of 218 whole air samples were collected and analyzed for 85 VOCs, carbon monoxide, and methane. The whole air samples were obtained at a variety of locations that included 21 sites selected to provide "parkwide snapshots" of air quality. Samples were acquired at these 21 sites in the early morning and early afternoon of February 12 and 15 in order to both observe any trends in local enhancements and for days that were characterized by differing levels of traffic. Additional samples were also collected in the community of West Yellowstone, Montana, in order to assess ambient air quality conditions on February 15 and 16. Exhaust samples were also acquired from representative snowmobiles, a snowcoach, and a snowplow in order to obtain “fingerprints” from each of these sources. Additionally, a full set of diurnal samples were acquired at Lake Ranger Station. Analytical instruments were placed at the National Park Service air quality monitoring site adjacent to the Station for measurements of particles, ozone, nitric oxide (NO), and total reactive nitrogen (NOy). Ten separate exhaust samples were collected from oversnow vehicles that are representative of the population used in YNP. The exhaust samples were used to compare the relative emission levels of VOCs from the two-stroke snowmobiles and from various four-stroke and diesel vehicles. The results indicate consistently higher emission levels for the two-stroke snowmobiles. In general, the relative contributions from two-stroke snowmobiles are 2–20 times greater than for four-stroke snowmobiles and snowcoaches, and significantly larger than those of the diesel snowcat. Additionally, the two-stroke engine types emitted much larger quantities of air toxics than the other engine types. The whole air samples were analyzed for 85 VOCs, and the results indicate that the majority of these compounds were derived from local sources with air masses ages on the order of 1–2 days. Furthermore, the results suggest that oversnow vehicle emissions represented the primary local source for the VOCs and carbon monoxide observed in the park. The data indicate significant enhancements in YNP above background levels commonly observed in northern hemispheric air masses, and even larger enhancements in the community of West Yellowstone.

The height of the atmospheric boundary layer increased on both February 12 and 15 during the daylight hours. Thus, dilution occurred during the daylight hours, decreasing the mixing ratios of most gases measured. However, certain aromatic VOCs that are major components of 2-stroke engine exhaust (i.e., benzene, toluene, ethylbenzene, xylenes) had median mixing ratios that were enhanced 2–55% during the afternoon hours which can only be attributed to large amounts of local emissions. The data also show that the increased levels of snowmobile traffic observed on February 15 as compared with February 12 generated higher VOC and carbon monoxide median mixing ratios in most locations. The abundance of gases in the park was also seen to vary spatially. For all gases that are indicative of two-stroke emissions, the highest levels were observed in those areas of the park that experienced higher levels of traffic. Near-road and off-road sample analysis indicated that the mixing ratios of all gases measured did decrease significantly as distance from the road increased. This suggests that the relatively still surface wind conditions present during sample periods did not serve to rapidly dilute emissions in a given area. Thus mixing ratios under such conditions are likely to be negatively correlated with distance from road. These findings have important implications for human and wildlife exposure to pollutants and air toxics.

Archeology

Project title: Archaeological Survey of the Shoreline of Yellowstone Lake

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Report number: 28725
Co-investigators:

Purpose: This survey will locate and record archaeological sites along the current shoreline of Yellowstone Lake. The primary purposes are to collect information on archaeological resources currently threatened by erosion and to provide a relatively controlled sample of the culture history of this portion of the park.

Findings: In 2003, we surveyed two portions of the lakeshore. The first begins on the hillside immediately north of Lake Hotel and extends along the old high beach line west of the old road that is now used as a pack trail. The main area surveyed in 2003 extended east from Fishing Bridge past the mouth of Pelican Creek and on to a point halfway across Mary Bay. We covered all of the present-day beach and the cut banks above it and extended the survey inland to higher ground to determine the extent of a site at Storm Point and to follow what appeared to be an old shoreline behind (north of) the causeway across the end of Mary Bay. During the course of the survey, we revisited four previously recorded sites, salvaging an eroding hearth from one of them and correcting the site boundaries for two others. We also recorded the presence of four new sites, two of which are potentially eligible for the National Register. Finally, we experimented with some new technology, specifically a hand-held computer with GIS software and a GPS antenna. This allowed us to record the positions of all of the artifacts and features that we encountered directly onto both the aerial photographs and topographic maps.

Project title: Archeological Investigations in Yellowstone National Park

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Report number: 27015
Co-investigators: Ann Johnson, Brian O.K. Reeves

Purpose: This project subsumes archeological inventory and testing done to insure agency compliance with cultural resource regulations and fieldwork done for research purposes, as well as research.

Findings: In 2003, the emphasis was on reporting of fieldwork. The FHWA inventories on Virginia Cascades Drive, Firehole Drive, and Firehole Lake Drive was completed. The FHWA report of testing nine sites between Mammoth and Gardiner was also completed. A draft was produced for the data recovery of the 9,400 year old Osprey Beach site on Yellowstone Lake. This report will be finished in 2004. Reports were begun on sections of Madison River and Yellowstone Lake shoreline inventories. It is anticipated more data recovery will occur at 24YE353 in the summer of 2004.

Project title: Miscellaneous Archeological Inventory and Site Evaluations

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Report number: 27183
Co-investigators: Paul H. Sanders

Purpose: n/a

Findings: A number of archeological projects were conducted in Yellowstone during 2003. Evaluative test excavations and a magnetometer survey was conducted for the proposed Mammoth Courthouse locale in Mammoth which was the former location of the Yellowstone Transportation Company's Mammoth operation (48YE1494), a portion of which burned down in March 1925. A number of building foundations were known to have occurred at one time within the project area, although our findings suggest that these foundations have been cleaned up. Some miscellaneous metal items were recovered. Class III cultural resource inventories of the Tower Campground road and Norris Geyser Basin road were conducted. No cultural resources were located. Evaluative test excavations were conducted at sites 48YE402 and 48YE14. Prehistoric and historic artifacts were noted at both sites. Finally, data recovery plans were completed for six prehistoric lithic scatters along the Mammoth to Norris Junction Road that passes by Obsidian Cliff National Historic Landmark.

Project title: Chemical Analysis of Obsidian Sources and Artifacts from the Northwest and Great Plains

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Report number: 28178
Co-investigators: Michael D. Glascock

Purpose: The major objective of this research is to establish a geochemical database of obsidian sources in the northwestern USA, including sources in Yellowstone National Park and adjoining areas.

Findings: No activity was conducted this report year.

Ecology

Project title: Building a Mechanistic Basis for Landscape Ecology of Ungulate Populations

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Co-investigators: James Forrester, Evelyn Merrill, Peter Turchin, Monica G. Turner, Nathan Varley

Purpose: Spatial dynamics are crucial for understanding population, community, and ecosystem processes. Two general problems in landscape ecology do not yet have adequate solutions: (1) translating observations taken at small spatial (and temporal) scales into expected patterns at landscape and geographic scales, and (2) understanding the influences of spatial heterogeneity on individual movements/demography, and on population distribution and dynamics. We propose to address these issues using a multi-faceted research program that integrates empirical data collection with spatial modeling and GIS analyses. The proposed experimental organism is the American elk. This species is of high conservation/natural management interest, and several elk reintroduction efforts in North America are currently in progress. However, little is known about how fast reintroduced elk populations may spread. Thus, by successfully completing the research goals outlined in this proposal we will not only be able to address questions of central importance to theoretical ecology, but will also obtain insights for improved management decisions.

Our specific approach to the two issues above will be based on developing a series of spatial models addressing a range of scales, from a microscale (tens of meters and hours) through a mesoscale (kilometers and weeks) to a macroscale (tens to hundreds of kilometers and years). At each scale we will parameterize the model using small-scale data on individual movements and demography, and test the model by predicting independent data on spatial redistribution of populations at broader (landscape and, ultimately, geographic) spatial scales. Data on individual movements will be obtained by following elk equipped with radiocollars at four different sites, varying in landscape heterogeneity and elk population density (two sites where elk have been long established, and two where they were recently introduced). Landscape heterogeneity will be quantified using a combination of remote sensing methods and detailed on-the-ground analyses of such variables as the distribution of winter and summer forage biomass and quality, land and habitat classification, and landscape structure. We will determine how accurately microscale model can predict mesoscale data, and mesoscale model can predict macroscale data. Furthermore, the macroscale predictions of the detailed, mechanism-based mesoscale model will be contrasted to the predictions from a more phenomoenological (but much less data-demanding) macroscale model. This comparison will allow us to determine whether it is essential to understand processes governing movement at multiple spatial scales in order to make meaningful predictions of population redistribution at larger (geographic) scales.

Findings: A study on habitat selection at different scales was recently completed by Boyce et al. (2003) in *Ecoscience,* "Scale and heterogeneity in habitat selection by elk in Yellowstone National Park," is an article in which results are reported on the resource selection at varying spatial scales. A summary follows: "Resource selection functions (RSF) can be used to explore the role of scale in determining patterns of habitat use. We estimated RSFs for 93 radiocollared adult female elk (*Cervus canadensis*) with resource availability defined at four spatial scales and two seasons in Yellowstone National Park. Habitat selection differed markedly among scales and seasonal ranges. During winter, elk moved to ranges at lower elevations where snow water equivalents were low and selected landscapes with a mix of forest and open vegetation at all spatial scales. Areas of high vegetation diversity were selected at large spatial scales during summer, whereas elk selected less diverse areas on winter range. During summer elk selected forests that burned 12–14 years earlier, but they used these burns less than expected by chance during winter. Habitat selection by elk occurred at multiple spatial scales; thus, we cannot prescribe a single scale as being best for modeling habitat use by elk. Instead, selection of an appropriate scale will vary depending on the research question or management issue at hand.

Project title: Causes for Habitat Selection of Uinta Ground Squirrel

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Report number: 26710
Co-investigators: Mitchel Hannon, Stephen Jenkins

Purpose: The Uinta ground squirrel represents a major prey item for predators in YNP, including coyotes, badgers, red foxes, felids, and many species of raptors. This ground squirrel is typically found in grasslands, but also lives in shrub communities, although at lower densities. It is commonly believed that the ground squirrels prefer areas of lower vegetation structure to make it easier to scan for predators, but there are benefits to living in shrub habitats. Animals in shrub habitats are harder for predators to catch because of the aboveground obstructions and the underground root systems that hinder excavation. Also, the foliage provides refuge from avian predators. Uinta ground squirrels will readily forage on grasses and shrubs, so sufficient forage does not seem to be a limiting factor on densities. So why do densities remain low in shrub habitats? One possibility could be that ground squirrels are "behaviorally" constrained from thriving in shrub habitats. This means that the behaviors they exhibit in shrub habitats have negative effects on their ability to survive (i.e., more time spent on the look out and less time spent eating). The investigators believe that low densities of Uinta ground squirrels in shrub habitats can be attributed to animal behavior. The project hopes to define the relationship between Uinta ground squirrels in three different habitat types (grassland, sage, sage burned in 1988) and the following: population density, vigilance, feeding (length of time), and stress hormone levels.

Findings: Analysis of the data appear to show that wariness of predation does not affect the distribution of ground squirrels. There appear to be correlations with vegetation composition and abundance however that will be further investigated over the next few months.

Project title: Northern Range Small Mammal Study: Populations Responding to Vegetation Change

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Report number: 26711
Co-investigators: Mitchel Hannon

Purpose: This study is primarily aimed at providing long-term monitoring of small mammal populations on the northern range. The long term studies will provide data for other past and concurrent studies. This study is a replication of a study performed from 1992–93, thus comparisons will be made on how small mammal populations change with respect to habitat changes over time. This will provide an assessment of habitat effects, species presence/absence in certain habitats and relative abundance of small mammals in the northern range.

Findings: No activity was conducted this report year.

Project title: Mapping Horizontal and Vertical Distribution of Fuel by Fusing High-Resolution Hyperspectral and Polarimetric SAR Data

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Purpose: The purpose of this study is to fuse hyperspectral (optical) imagery with polarimetric synthetic aperture radar (SAR) data to provide maps of the vertical and horizontal distribution of fire fuels by plant community type, critical information not available from today's fuel mapping methods. Current fuel maps have limited use for predicting fire behavior, analyzing fire hazard, or developing fuel management strategies. Thus a critical need exists for cost-effective remote sensing methodologies that render accurate, efficient fuel maps for landscape to regional scales. New remote sensing techniques are needed to accurately map wildland fuels according to specific vegetation types and the horizontal and vertical position of biomass, two factors that dramatically affect the intensity and spread of fires. The fusion of optical (especially hyperspectral) data with synthetic aperture radar (SAR) data has the potential to provide this much needed information. These two data types have complimentary strengths, enabling the creation of high-resolution maps describing the vertical and horizontal distribution of fuels by plant community or species.

Findings: This study has completed year two of a three-year study funded by the Joint Fire Science Program out of Boise, Idaho. Work on this study began January 1, 2002 and has included a great deal of effort during the first year. Major achievements include significant planning sessions and group meetings, clarification of the first two year's study site and a very successful collection of remote sensing data in year two (summer 2003), significant database development and calculation of field data, assembling and assessing the utility of existing remote sensing data, planning for collection of both hyperspectral and SAR data, and development of image analysis and data fusion algorithms and approaches. We are currently continuing data analysis from the first two field seasons, optimizing calculations to retrieve fuel parameters from field data, developing methods for image analysis and data fusion, and planning for a final year of laboratory and computer analysis.

Project title: Validation of High Resolution Hyperspectral Data for Stream and Riparian Habitat Analysis

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Purpose: The purpose of the Hyperspectral EOCAP Project are twofold. First, to test the application of airborne hyperspectral imagery to riparian and in-stream ecological and environmental studies and monitoring. Second, using experience gleaned from these application tests, we are defining the unique and common requirements of hyperspectral data for operational commercial and scientific uses in the area of stream and habitat analysis.

Findings: In 2003, hyperspectral data sets were collected in 1999–2000 collection areas (1 meter and 5 meter HSI). Extensive ground-truth data were collected in the fall of 2002 and were analyzed during 2003. The year 2003 effort consisted of change detection analysis between 1999 and 2003 data sets as well as between 1995 and 1999 high resolution data sets. Overall, the study examined seven main classes of ecological parameters that we seek to study and classify are: (1) stream morphological units, (2) steam depth and flow regime, (3) substrate particle size, (4) in-stream algae chlorophyll levels, (5) woody debris, (6) riparian vegetation discrimination including examining the spectral variability in relation to age of riparian woody species, and (7) riparian vegetation community mapping including individual species identification of willow, sedge, cottonwood, aspen, upland grasses, rushes, alder, sagebrush, and conifer species. These seven main classes of variables span the range from relatively easy to extremely difficult, in terms of hyperspectral measurement. Each ecological variable has its own degree of hyperspectral leverage, or observability in the hyperspectral data. Furthermore, key issues such as spatial and spectral resolution, noise level, geometric fidelity, geopositioning accuracy and timeliness of data delivery and processing affect each specific application differently. Using a multiple spatial and spectral resolutions, and multitemporal data sets, we are investigating and documenting the complex interplay between instrument and data parameters and the usefulness and accuracy of the derived ecological products. While spectral contrasts exist among classes and species of vegetation, and even exist among subclasses of a single type, they are subtle and change throughout the growing season. Unlike the small spatial scale and rapidly time-varying nature of the in-stream parameters, the riparian vegetation is distributed in broader units that generally persist from one season to the next.

Successful mapping of these plant species rests heavily on correlation of field spectrometry with airborne data. This particular application lends itself to a multi-temporal approach, leveraging the different spectral trajectories of the plant communities throughout the growing season. Initial investigations of the airborne data show tremendous spectral diversity in the riparian vegetation. Empirical spectral analysis indicates that more than a dozen spectrally unique vegetation classes can be mapped. Current efforts involve matching field mapping with the aircraft data results. Throughout our EOCAP project we are focusing on our dual hyperspectral objectives: developing convincing case study demonstrations of the hyperspectral measurement of important stream and riparian ecology parameters and documenting and developing the common and unique requirements of operational systems to perform these studies in the future. Specifically, we are collecting a laundry list of needs and requirements for commercial systems for hyperspectral stream studies. This list documents specific spatial, spectral, and radiometric design requirements. In addition we are addressing the more mundane, yet critical, aspects of operational acquisition and application including the timely delivery of data and products and its long-term use and archiving. Our initial results from our first field season are very encouraging and productive, both in terms of the development of tantalizing case studies and the frustration involved with finding and documenting technology gaps and shortcomings. Final report of Phase II is now available and we are working on numerous manuscripts. As of October 1, 2003, six have either been published or submitted for publication.

Project title: Specificity in Ectomycorrhizal Symbioses

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Purpose: Renewal will address questions of effects of dwarf mistletoe infection on ectomycorrhizal community structure and specificity patterns in forest adjacent to Yellowstone Lake, across a soil fertility gradient.

Findings: Findings indicate (1) that dwarf mistletoe significantly affects ectomycorrhizal communities in two soil types; (2) that it does so even in the presence of a second tree species; and (3) that soil functional diversity is affected as well, in pure pine. Manuscripts are in preparation.

Project title: Browsing Phenology of Willows, Cottonwoods, and Aspen on the Northern Range, Yellowstone National Park

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Report number: 26417

Purpose: Woody riparian species of the northern winter range are heavily browsed each winter and show strong hedging morphology, while those in other localities are also heavily browsed each winter but do not show obvious hedging. Apparently there are interactions between elk, woody riparian species, and other factors that are yet unknown and which need to be studied in order to answer questions regarding present range conditions on the northern winter range. It has been demonstrated that the northern range willows do not compensate for browsing as well as those in Rocky Mountain NP, probably because of differences in abiotic conditions. Climate is an abiotic factor that can cause differences on a short time scale, and casual observations over the years indicate that willows are browsed at different times in different years. Other studies of ungulate browsing have recorded browsing intensities in May for winter browsing and August for summer browsing, or ungulate presence in the willow stands on a monthly basis and utilization in spring. The purpose of this project is to document variation in the timing of winter browsing of woody riparian species and relate it to climatic conditions.

Findings: The time of browsing has followed the pattern exhibited in the previous years of the study. Early in the season there has been very light browsing, which we interpret as exploratory, followed by a short period of more intense browsing, terminated by a complete or nearly complete taking of at least the current year tips. This has occurred generally between late December and late January or late February. This year, the cottonwoods at a site that had been lightly browsed were browsed earlier than before. By the end of the winter, the stems in this stand were heavily browsed and had been severely thrashed and broken. During the summer there was considerable growth of new shoots originating from the broken and browsed stems, with lengths often exceeding one meter. At a second cottonwood stand, half the transect was heavily browsed but the other half was only browsed around the edges, leaving the central axis unbrowsed. Growth on the leaders of the central axes grew approximately 20–30 cm during the summer, but leader growth on the heavily browsed stems was 70–80+ cm. Cottonwood and aspen continue to be browsed later than the willows. We have been fortunate to have the services of a crew doing another willows study, and have instituted a leader length measurement protocol. In late November, the lengths of browsed and unbrowsed leaders were recorded. Length of browsed leaders will be followed to determine if the browsers continue to take more from browsed stems or just take previously unbrowsed leaders.

Project title: Geologic Controls on Ecology of the Greater Yellowstone Ecosystem, Particularly the Grassland–Forest Contrast

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Report number: 26418
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Purpose: The objectives are to document the relation between the ecologic distribution of plants and the surficial and bedrock geology of the Greater Yellowstone Area; to begin documenting the soil moisture distribution in grassland/forest contrast; and to test the hypothesis that the grassland/forest contrast is related to soil moisture distribution. This will be done through the following processes: (1) Compile and calculate the annual soil water distribution from existing data from 12 stations in the northern Greater Yellowstone Area that were monitored in the 1960s and 1970s; (2) Install TDR (time domain reflectometry) instrumented profiles each across the grassland/forest boundary will be used to follow soil moisture variation; and (3) Document the relation between vegetation and geology based on the statistical relation between vegetation and surficial geologic and bedrock geologic maps aided by ecologic and geologic knowledge accumulated over years of work, including fire history, glacial source areas and flow directions, deposition of thin, more nutrient-rich loess, and rapid snowmelt driving the annual soil-moisture cycle. We will particularly focus on the distribution of grassland and forest and the geologic and other factors that control this difference.

Findings: Information collected by the Soil Conservation Service was obtained, put into digital form, and is available for analysis. Two transects have been instrumented with moisture sensors at four depths (where possible), with four soil pits in each transect. The lower transect is located near Wraith Falls at approximately 2070 m, and the upper transect is approximately 4 km south of Tower Falls at 2219 m. These sensors have successfully recorded soil moisture since November 9, 2001. The record interval during winter months was twice daily and during spring and summer was six times per day. Snowmelt and rain events were recorded in the surface layer, and the wetting front was recorded as the moisture soaked into the profile. During the spring of 2002, it was apparent that the forest soils began their wetting cycle later in the season by a week or two. The moisture in the middle of the profile was wetter in the forested soils through the early growing season. Unfortunately, the box housing the recording instruments for the lower elevation transect flooded during the spring snowmelt, and autumn was well advanced before the damaged instruments could be replaced. The early part of spring wet-up was captured, but that was all. The instruments at upper transect malfunctioned from mid-April to early July, so the height of soil wet-up and much of the dry-down was missed. Hopefully, next spring will be better. Sand silt and clay content for each horizon of the soil profiles described during the sensor installation was determined in the laboratory as was water holding capacity for potentials of 0.1, 0.33, 0.5, 1, 5 and 15 atmospheres. These will aid in the calculation of soil water content for each horizon.

Project title: Effects of Predation by Bumblebee Wolves (*Philanthus Bicinctus, Sphecidae, Hymenoptera*) on Bee and Plant Biodiversity

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Purpose: The interactions between flowers and their pollinators are a classic model system in ecology and evolution, and recently have been closely examined by conservation biologists as well. Although predators can dramatically influence insect–flower interactions, this issue has been mostly ignored until recently. The best system for quantifying the effect of predators on pollinators is that of bumblebee wolves (*Philanthus bicinctus*), which are sphecid wasps endemic to the American Rockies, who prey almost exclusively on bumblebees (see photos at <http://psych.mcmaster.ca/dukas/pred&poll.htm>).

Relying on previous research, I found a large aggregation of these wasps at Yellowstone National Park and commenced research in summer 2003. Overall, my objective is to quantify the effects of bumblebee wolves on the diversity, density and behavior of bumblebees, and on bumblebee-pollinated plants. Specifically, I test the following hypotheses: (1) The number of bumblebees at flower patches within 1 km of a bumblebee wolf aggregation is lower than those farther than 4 km from the aggregation; (2) Seed production of plants specialized for large-bee pollination is lower within 1 km of a bumblebee wolf aggregation than farther than 4 km from the aggregation.

Findings: I counted the number of bumblebees visiting patches of Western rayless coneflower (*Rudbeckia occidentalis*), which was the most common plant in bloom at numerous natural forest-clearings. On average, the number of bumblebees observed within 4 km from the bumblebee wolf colony was 14 times lower than farther than 5 km from the colony. Similar dramatic differences in bumblebee abundance were also observed on Canada goldenrod (*Solidago canadensis*). Preliminary data also indicated approximately half as much seed production by the bumblebee pollinated Columbian monkshood (*Aconitum columbianum*) within 4 km than farther than 5 km from the bumblebee wolf colony. A series of experiments planned for summer 2004 will complete the data set from 2003 and critically test whether the bumblebee wolves are responsible for the observed differences in bumblebee abundance and monkshood seed set.

Project title: The Sustainability of Grazing Ecosystems

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Purpose: The goal of this project was to measure the effects of ungulates on grassland composition, production, and nutrient cycling. Composition, production, and nutrients were measured inside and outside 15×15m exclosures established in 1998 at 10 sites on the winter, transitional, and summer ranges. Aboveground production in grazed grassland was determined with temporary exclosures moved monthly. Root production was determined with minirhizotron tubes. Nitrogen net mineralization was measured with buried bags. The only field work performed in 2003 was to remove the exclosures.

Findings: Data analysis is ongoing. However, a small data set that has been written up and submitted for publication in 2003 explored the effect of grazers on grassland plant diversity. Results indicated that the response of plant diversity to herbivores was contingent on grassland production. Grazers increased plant diversity in moderately to highly productive grassland, and had no effect on plant diversity in relatively dry, low productive grassland.

Project title: Post-Burn Resource Selection, Physiological Condition, and Demographic Performance of Elk

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Purpose: The primary objective of this research is to evaluate the consequences of the 1988 fires on elk resource selection. Selection is being quantified for populations and individuals at multiple scales ranging from selection of patches within the landscape mosaic to selection of forages and plant parts within patches. The physiological and demographic consequences of observed resource selection strategies are being assessed through noninvasive urinary and fecal assays, and telemetry. Secondary objectives include basic research on forage plant chemical compositions, plant-animal interactions, and applied research to develop practical and rigorous management tools for population monitoring.

Findings: We have been successful in developing, testing, and applying a suite of research tools that is significantly enhancing our ability to address questions of animal resource selection and the physiological and demographic consequences of selection patterns. We have completed our twelfth field season of data collection and maintain an instrumented population of 25–35 cow elk. Most publications to date have focused on techniques including population estimation, pregnancy assessment, and nutritional indices. This year we completed a manuscript analyzing the demographic data collected during the first seven years of research (prior to wolf colonization of the study area), which was published in the *Canadian Journal of Zoology.* Adult survival and reproduction was near the biological maximum for the species, but recruitment was highly variable, being strongly influenced by environmental variation, primarily winter severity. Despite this variable recruitment, extensive Monte Carlo simulations indicate that the population has been relatively stable and regulated at approximately 600–800 animals. An additional manuscript, examining geo-chemical influence on elk demographics, was published in *Ecosystems.* Exposure to high concentrations of fluoride and silica result in aberrant tooth wear and result in the early onset of senescence, reduced life span, and an abbreviated age structure of elk in the Madison Drainage. We have generated a database of greater than 11,500 animal locations, and are exploring a variety of analytical tools for the analysis of these data. We continue to acquire and develop GIS data sets of landscape features for integration with all spatially-explicit data collected on this study, with two manuscripts. In collaboration with NASA scientists we have developed spatially and temporally explicit snowpack models for our area, as well as the first remotely sensed geothermal inventory of Yellowstone. A manuscript has been submitted to *Landscape Ecology* that integrates elk telemetry data with snowpack metrics generated from the models and landscape characteristics derived from the remote sensing mapping data. Results of recent work can be found at two web sites: <http://www.homepage.montana.edu/~rgarrott/centralyellowstone/index.htm> and <http://earthsystems.monterey.edu/~fwatson/projects/long/yellowstone/yellowstone.htm>.

Project title: An Analysis of the Relationship Between Species Composition And Ecosystem Function in Streams: Implications for Stream Bioassessment

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Purpose: This is a proposed addition to our application for a permit renewal (for the project, "Predictive modeling of benthic macroinvertebrate community composition in the streams of Yellowstone National Park"). The purpose of this study is three fold: (1) to determine whether stream classifications based on invertebrate species composition (which are used in predictive modeling approaches to bioassessment) scale to measures of ecosystem function. That is, are streams with similar community compositions similar with respect to ecosystem processes? (2) to determine whether deviations from the expected community composition (as determined using predictive modeling), resulting from anthropogenic impacts, are correlated with measurable changes in ecosystem processes. In other words, are streams with reduced biological integrity "impaired" with respect to ecosystem function? Essentially, all stream bioassessment methods make the implicit assumption that measured changes in community characteristics do reflect, if not actually cause, underlying changes in ecosystem function; and (3) to answer the more general question of how changes in biodiversity may affect ecosystem processes. This is an important and growing area of research, with broad implications in conservation biology. To date, however, most studies have addressed the issue in fairly simple plant communities. This project provides an opportunity to look at how biodiversity may affect ecosystem functions, as stream classes with similar community compositions also have similar biodiversity, whether measured at the species level, or in terms of functional classifications (e.g., trophic level, functional feeding group, etc.). In addition, we will have the ability to compare streams across a gradient of impairment, to address directly the question of how biodiversity may affect the ecosystem level response of a system to disturbance. The inclusion of Yellowstone NP in this study is important for several reasons. First, Yellowstone in general has a wide variety of stream types, most of which are relatively pristine. More particularly, Yellowstone has a large number of pristine low gradient meadow streams, which are underrepresented in the rest of the study area. In addition, this study provides an opportunity to begin to assemble a comprehensive picture of how stream ecosystems in the nation's premier national park function.

Findings: Due to unavoidable delays in the renewal of our research permit, we were not able to conduct most of the research that we planned. However, we continue to measure litter decomposition in several Yellowstone streams. Based on these data, in combination with decomposition data collected from a number of other Rocky Mountain streams, we are beginning to develop statistical models to predict ecosystem process rates in unimpaired streams. These models should allow us to use litter decomposition rates to detect ecosystem impairment in streams affected by anthropogenic activity. In addition, we are developing models to identify the relative importance of biotic and abiotic factors in determining ecosystem process rates in streams.

Project title: Evaluation of the Animal Detection System Located Along U.S. Highway 191 (between mile markers 28–29) in Yellowstone National Park

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Report number: 27022
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Purpose: Animal detection systems have the potential to reduce animal-vehicle collisions by about 80%. They also allow animals to continue to cross the road and move across the landscape. The purpose of the study is to evaluate the experimental animal detection systems located along U.S. Highway 191 in Yellowstone NP and determine whether such systems should be further developed and installed at other locations, too. The research focuses on three themes: (1) System reliability. (a) How many false negatives does the system produce? (b) How many false positives does the system produce? (c) Is there downtime for the system?; (2) System effectiveness. (a) Do the flashing warning lights result in lower vehicle speeds? (b) Does the warning system result in fewer animal–vehicle collisions?; and (3) System acceptance: (a) Document experiences and opinions of the traveling public (b) Document experiences with operation and maintenance.

Findings: 2003 was mostly devoted to getting the system operational. By the end of 2003, the system was performing much better, but the number of false positives was still too high. WTI/MSU started to investigate the reliability of the system. However, further modifications have been made since then. The evaluations up to date are aimed at helping the vendor improve the performance of the system rather than evaluating the final product.

Project title: Relating *Myxobolus Cerebralis* Infection in Native Yellowstone Cutthroat Trout and *Tubifex Tubifex* with Environmental Gradients at Multiple Spatial Scales

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Purpose: The objectives of the study are to: (1) Conduct a detailed examination of YCT infection risk in three spawning streams (Yellowstone River, Pelican Creek, Clear Creek) where past studies have shown varying disease risk in two hosts, YCT and *tubifex* worms; (2) Examine infection in spawning adult YCT to determine potential parasite spore loading of streams and in wild reared young to determine infection prevalence and severity in wild fish; (3) Examine the potential physical and chemical features of these three streams and determine which factors best explain infection risk, prevalence and severity in YCT and tubifex worms; (4) Determine the YCT life history patterns most likely to avoid infection by

M. cerebralis; (5) Continue long-term monitoring of fry in enclosures.

Findings: Polymerase chain reaction (PCR) analyses have detected *M. cerebralis*- (whirling disease) positive sentinel Yellowstone cutthroat fry from all study reaches in Pelican Creek, but no infection in sentinel or wild fry from the study reaches in each of the Yellowstone River and Clear Creek. Histological analyses of Pelican Creek sentinel Yellowstone cutthroat trout from the 2002 field season, and both PCR and histology analyses for sentinel and wild cutthroat fry from the 2003 field season, are currently underway. Although we were able to collect about 80–100 wild fry from the Yellowstone River and about 100–120 from Clear Creek, we found only 9 fry throughout Pelican Creek in 2003. Due to lack of personnel, we were not able to conduct any oligochaete (*T. tubifex*) or other invertebrate sample collections during the 2003 field season. Part of the environmental assessment data (e.g., sediments samples, pebble counts) from each of the reaches in each study stream will be processed during spring 2004. Some physico-chemical characteristics appear to be correlated to distribution and abundance of *M. cerebralis* in tributaries to Yellowstone Lake. For instance, in Pelican Creek (with highest disease severity) the conductivity and temperature were high, whereas discharge and dissolved oxygen were low in comparison to the Yellowstone River, Clear Creek, and others. In early June 2002, and in May and June 2003, Yellowstone National Park fisheries biologists attempted collection of adult Yellowstone cutthroat trout in lower Pelican Creek near the site of the historic spawning migration trap. At one time, this trap was used to collect thousands of upstream migrating cutthroat. Fewer than 20 adult Yellowstone cutthroat trout were found in Pelican Creek during what was once a seasonal period of intense use by these fishes. Although we need to examine Pelican Creek even more closely, evidence from fry and adult collections suggests that the Pelican Creek spawning population has decreased dramatically. Since *M. cerebralis* has been detected in adult Yellowstone cutthroat trout lake-wide, the potential exists for this parasite to cause similar declines in other tributaries. Quantification of environmental characteristics optimal for *M. cerebralis* in the Yellowstone Lake basin will assist fisheries managers in predicting probable areas with high risk of infection.

Project title: Ecology of Selected Habitats in Yellowstone National Park: A Wheaton College Science Station Course

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Report number: 26106
Co-investigators: Kenneth Petersen, Robert Reber

Purpose: The objective of this study was to introduce students enrolled in a General Ecology Course to the various ecosystems in Yellowstone National Park. Areas impacted by the 1988 fire were of particular interest. We compared lodgepole pine densities between areas that were burned only, to areas that were also impacted by the 1984 blowdown event. Finally, we investigated the bacteria, algal, and cyanobacteria communities at the West Thumb Geyser Basin, and related distributions of microbial communities to water temperature, as determined by color of the microbial mats. These studies were intended for educational use only and not for publication.

Findings: We found densities of lodgepole pine in burned only areas to be significantly greater (mean = 153.5/100 m^2) than densities in the blowdown and burned site (mean = 17.75/100 m^2, $t = -4.172$, $p=0.0016$). Plant species richness was slightly higher in the burn only site, however this difference was not significant ($t = -2.143$, $p = 069$). At West Thumb Geyser Basin, we found the water temperature to be much higher than in previous years, and students were able to relate temperature to color of bacterial mats. Water temperatures in the range of 160°F supported yellow mats. Orange mats were found when the water temperature was around 100°F, and when temperatures ranged from 80–90°F, the microbial mats were brown.

Project title: Yellowstone River Basin Study Unit, National Water Quality Assessment and Environmental Monitoring and Assessment Program

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Co-investigators: Greg Boughton, Myron Brooks, Peter Wright

Purpose: To collect baseline aquatic data for NAWQA and EMAP programs.

Findings: Aquatic ecological sampling was conducted on Cub Creek during September 2003. Samples were collected of algae, macro-invertebrates, and fish community. Habitat measurements also were made.

Project title: Browse History of Tree-Sized Aspen on Yellowstone's Northern Range

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Purpose: We propose to expose and examine the pith-revealed architecture of dead and downed aspen trees on Yellowstone's northern range to determine historic ungulate browse frequencies. Samples ($n = 3$) will be drawn from 112 previously identified aspen clones that were established for other monitoring efforts. Dead and downed tree butts will be extracted using a bow saw or a small pruning chainsaw, and the butts will be split, examined, and photographed. We wish to test the null hypotheses: (Ho1) Pith-revealed historic browse frequency of dead and downed aspen trees is not different from the current browse frequency of aspen suckers; (Ho2) The multi-hedged architecture of current aspen suckers as a result of repeated browsing is not evident in the pith trace of dead and downed aspen trees; (Ho3) There is no relationship between browse events and the height above ground at which the browse event occurred; and (Ho4) All recently fallen (post-1999) trees within the transect showing evidence for a browse event all date prior to 1920, a time period after which little recruitment into the aspen canopy has been observed. A more rigorous and broad-scale analysis of browsing history would contribute substantially to the complex and contentious debate surrounding aspen, and support elk management strategies that focus on functional outcomes rather than numeric responses.

Findings: During 2003, we visited 74 (66%) of the 112 aspen sites. A total of 336 candidate trees was initially sampled, resulting in 158 suitable aspen trees for analysis of pith architecture. Based on strong pith deflections and/or the presence of a preserved and imbedded woody stub, 128 of 158 trees (81%) showed evidence of previous terminal browsing while 30 trees (19%) showed no apparent browsing. A total of 241 browse events were observed in the lower 1.5m of the tree bole among the 128 trees showing historic browsing. The terminal stem was browsed 3 or more times in 21% (n=33) of the 158 trees, while 60% (n=95) were browsed 1–2 times. Overall, the proportion of sample trees showing evidence of previous browsing is consistent with the proportion of browsed aspen suckers recently reported from these sites as well as browsed proportions reported by other investigators from other aspen sites throughout the northern range. We were able to obtain the targeted 3 tree samples for analysis of pith architecture from 43 (58%) of the 74 visited sites. Ten (14%) and nine (12%) of the 74 sites produced only 2 and 1 suitable sample(s), respectively. We were unable to obtain suitable trees for analysis from 13 (18%) of the 74 sites. The inability to obtain the desired number of sample trees per site was due to either a limited number of dead trees from which to sample or, more commonly, advanced stages of decay and rot among the available downed trees. One hundred percent of the samples were previously browsed in 38 (51%) of the 74 sites, while 33–66% of the samples were previously browsed in 22 (30%) of the sites. In only 2 (3%) of the sites, both of which produced only 1 sample tree, could we not find evidence of previous browsing. We are continuing with dating historic browsing from 4 aspen tree samples of known death date. We are also pursuing slight permit modification to include aspen contained within the range exclosures as well as opportunistically sampling and dating aspen trees that have recently died and fallen.

Project title: Aspen–Migratory Bird Relationships in the Northern Yellowstone Ecosystem

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Co-investigators: Jeff Hollenbeck

Purpose: Our objectives are to determine the relationships of aspen patch characteristics and stand composition with migratory and resident birds. Previous research at lower elevations in the western U.S. suggests that migratory birds may respond to landscape level attributes of aspen stands (patches) when settling on their breeding range. We wish to determine if the relationships found between patch characteristics and breeding bird abundance at lower elevations occur more widely. Other researchers have found relationships between local level (habitat) attributes and bird abundance or diversity. We also wish to confirm these relationships for the northern Yellowstone ecosystem. We measured several landscape and local level characteristics for 32 aspen patches within the northern ungulate winter range during 2001, and conducted point counts in each patch during June 2001, 2002, and 2003.

Findings: We detected 2,648 birds, representing 54 species, in 32 northern range aspen patches. Additionally, we established and sampled 6 conifer-dominated sites for stand characteristics and bird abundance and diversity. Analyses in progress: Landscape scale. Current analyses suggest patch area is moderately related to bird diversity but not abundance. This relationship appears to be greater for migratory birds than resident. Patch shape and orientation (relative to migration direction) do not seem related to relative bird abundance or diversity. Matrix effect analyses are planned for 2004. Local scale. Preliminary analyses suggest some habitat attributes, such as stand density, size class distribution of aspen stems, and understory characteristics are weak to moderately related to bird abundance or diversity. We found a moderate negative relationship between habitat heterogeneity, measured as ratio of conifer to aspen, and bird diversity. This relationship is stronger for migratory birds than resident. We did not find evidence of increased bird diversity with the presence of conifers in aspen dominated stands.

Project title: Aspen Regeneration in Northern Yellowstone National Park

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Purpose: Our continuing objective is to measure aspen regenerative success and the status of other woody browse species on YNP's northern range. Based on trophic cascade theory, we hypothesize that wolves may displace elk from some areas of the northern range, thus allowing more robust aspen regeneration in areas of higher wolf presence. Permanent, 1×20 m aspen belt transects (plots) were established in 1999, marked with both a metal identification tag on a large-stemmed aspen tree and nails in the ground at 3, 5, 10, and 20 m from the starting point. Subsequently, we have monitored aspen growth and ungulate browsing intensity in our 112 permanent aspen plots.

Findings: A fourth year of field data was collected from our permanent aspen plots during July and August 2003. Aspen overstory density and diameter at breast height (DBH) were recorded and compared to data from previous years. Information describing aspen ramet density, height, and browsing intensity was obtained and compared to data from previous years. The number of ungulate pellet groups present was recorded for each plot. Our data continues to demonstrate an overall trend of high ungulate browsing pressure on most upland aspen stands on the northern range. We are combining our aspen plot results with other sources of data to develop a paper concerning the possible effects of a three-level trophic system (wolves, elk, and aspen) on the regeneration of aspen and other woody browse species. In 2003, we published "Aspen age structure in the Northern Yellowstone Ecosystem, USA"in the journal *Forest Ecology and Management* (179: 469–82), summarizing some of our research in the Yellowstone area. We also published a related paper in the same journal in 2003, "Wolf reintroduction, predation risk, and cottonwood recovery in Yellowstone National Park," (184: 299–313).

Project title: Cougar–Wolf Interactions in Yellowstone National Park: Competition, Demographics, and Spatial Relationships

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Purpose: Successful restoration of large carnivores in the Northern Rockies and the concomitant increase in carnivore abundance and distribution will challenge humans as human development increases throughout the West. If restored wolves limit cougar populations in number or distribution, this limitation may have synergistic effects with current relaxation of cougar hunting regulations and rapid development. An added stress such as low prey availability (e.g., caused by hard winter or disease) could further impact populations. Understanding competitive relationships between large carnivores and the role that habitat and prey availability play is paramount to predicting and preparing for changes in the Greater Yellowstone region. In order to assess population-level effects of wolf (*Canis lupus*) re-establishment on cougars (*Puma concolor*) in and near Yellowstone National Park, we initiated a Phase II study of YNP cougars in 1998. The study is designed to examine the characteristics of the cougar population including: sex and age structure, density, reproductive and survival rates, dispersal and recruitment events, rate of predation on prey, and spatial and temporal movements. These parameters will be compared with analogous estimates made prior to the wolf restoration event in 1995 and similar parameters documented for the wolf population to assess competition and resource partitioning between the two species. In particular, the project is working to: (1) Document characteristics of the cougar population, including predation rates, population size, survival, cause-specific mortality, and natality, and to compare these with analogous estimates made prior to wolf restoration; (2) Assess competition and resource partitioning between cougars and wolves by comparing spatial, temporal, habitat, and food use characteristics of the two species; (3) Quantify spatial interactions between cougars and wolves; (4) Assess the effects of cougars on elk and mule deer populations as influenced by the presence of wolves; and (5) Communicate research findings to state and federal agencies and the general public through annual technical reports, research updates, and presentations.

Findings: During 1998–2003, 65 cougars were captured in and adjacent to areas used by 35–88 wolves within 3–5 wolf packs on the Northern Yellowstone Study Area, Montana and Wyoming. Since the study began, our researchers have documented 19 litters produced by 9 females with an average litter size of 2–3 kittens. About 50% of these kittens survive to dispersal age where they leave the protected area of the park in search of a home range of their own. Our data continue to suggest that female and male cougars that have the greatest success of producing offspring are those that live the longest lives. During aerial search flights covering a 200-mile radius, Wildlife Conservation Society researchers continue to monitor the movements of offspring as they disperse from the study area. However, successfully locating dispersers is difficult due to their rapid and long-distance movements and the high cost of flying large areas to search for them. This past year, 6 cougars that had been missing for 6 months to 1.5 years after their dispersal were located because they were killed during the hunting season. These individuals ranged in age from 14 months to 3.5 years old and were located as far away as Ennis, Montana, and Dubois, Wyoming. Lead field scientist Toni Ruth summarized data on solitary and maternal females for a paper on the presence and movements of lactating and maternal female cougars and the implications of these data for state hunting regulations. Our data suggest that female cougars are solitary (without dependent young) for 18 to 35% (95% CI) of their reproductive life, and with dependent young for 65 to 82% (95% CI) of their reproductive life (calculations based on a 10.5 year reproductive life assuming survival to 13 years of age). Although female cougars may care for young for 44–82% of their reproductive life, detection of kitten tracks with the mother is low. Kittens traveled with their mother proportionally less during their first year of life than during their second year of life and detection of kittens is often possible only through extensive backtracking. Although estimates likely differ for hunted cougar populations, we suggest this type of information for various geographic regions should be provided to hunters and state game managers and that conservative approaches in setting sport hunting regulations should be considered.

We quantified predation rates and prey selection by cougars on Yellowstone's northern range prior to (Phase I) and post wolf (Phase II) re-establishment. During Phase II, cougars spent an average of 3.7 days at kills and 4.4 days between each kill. The mean annual rate of cougar predation in Phase I was 9.4 (SD=4.0; 95% CI=7.8 to 11.0) days per ungulate kill, and 10.9 (SD=8.5; 95% CI=6.7 to 15.1) days per ungulate kill in Phase II. Rate of predation varied by cougar social class. When converted to biomass killed per day, cougars averaged 12.2 kg per day during Phase I and 12.9 kg per day during Phase II. We documented a total of 306 and 256 positive and probable cougar kills during Phase I and Phase II, respectively. During Phase II, 70% (n=179) of cougar kills were elk, 17% (n=43) were mule deer and 13% (n=34) were other prey. During both Phase I and II more than 50% of cougar kills were elk calves, with cow elk making up the next largest category. During Phase I, cougar predation was neither a major source of mortality nor a significant factor limiting the numbers or growth rates of elk and mule deer populations in northern Yellowstone. Cougars present on the study area killed 2–3% of the elk and 3–5% of the mule deer estimated to be available during five years, spanning the Phase I study. We are continuing our data collection and analyses and plan to: (1) compare cougar and wolf per capita rate of predation; (2) contrast femur marrow fat content of cougar and wolf kills, by season killed and prey age; and (3) compare yearly off-take of elk and mule deer by cougars and wolves.

Project title: Natural Experiments with Height Releases of Willows

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Purpose: A research proposal to address the hypothesis of multiple factor controls over willow persistence has been funded for 2001–2004 by USGS-NRPP and the Yellowstone National Park Center for Resources. However, limited NRPP funding allows pursuit of only two of our three principal hypotheses. This supportive proposal focuses primarily on our third hypothesis: the relation of willow persistence to patterns of ungulate herbivory, by using natural experiments with height-released willows. We propose to document willow height and growth response, and relate them to herbivory levels by elk, water table height, and possibly wolf distributions. Preliminary observations suggest this release may be patchy, spatially and temporally, and may be limited to only some willows, and occurring some years, but not others. Limited observations in 2000 and 2001 suggest some (perhaps < to 1/3) of willow patches were released about 1997 or 1998 (wolves were reintroduced in 1995) due to much lower winter browse levels and are now 2–4 times taller than in pre-wolf years. Three interrupted time series analyses, i.e., opportunistic or natural experiments, will be conducted: (1) comparisons of consumption levels on willows pre-1995 (pre-wolf) to post-1997 (post-wolf), on long-term plots; (2) comparison of willow growth parameters at the same permanent marked plots for those two time periods; and (3) comparison of elk distributions pre- and post-1995 from annual aerial elk surveys. This project requires only a resampling in 2001 and 2002 of the marked plots that were sampled in 1986–1992 (pre-wolf) and analysis of other already existing data. No new markers and no manipulations are requested, although a handful of stems and will need to be sampled to verify the interpretation of the timing of height releases from leaf node scars.

Findings: During this reporting year, we continued data collection on 18 of 20 long-term willow transects on the northern range. Data collection included status of willow community (suppressed, height released, or escaped beyond height of browsing), annual production, and summer and winter offtake. We also established additional sites in four willow community strata (suppressed, releasing, escaped/thickets, and sites that were tall prior to wolf reintroduction). Willow production and offtake data were collected at all of these sites. Surveys were conducted to map and characterize all willow patches along Blacktail, Geode, Crystal, and Lower Slough Creeks. Using annual elk winter census data collected for 8 years before wolf reintroduction and five years after wolf reintroduction, we have analyzed changes in count unit density and attempted to correlate significant changes with wolf activity areas. We are currently comparing elk habitat selection, including variables of group size, topography, and snow water equivalents between pre- and post-wolf reintroduction. We measured length of annual growth increments of willows in releasing and escaped willow stands and we will correlate years of release and location of releasing stands with wolf activity.

Initial findings indicate that elk density has decreased in several count units that have experienced heavy winter wolf activity in the past five years. The distribution of elk over the winter range also appears to have decreased since wolf re-introduction. Preliminary analysis of height increment data indicates two periods of height release of willows on the northern range 1996–1997 and 1999–2000. We have found willow productivity to be significantly greater in those stands where willows have reached heights that escape elk browsing compared to suppressed or releasing willow stands. Winter offtake rates on willow were significantly higher in suppressed and releasing stands than in escaped stands or those tall prior to wolf reintroduction. Further analysis is currently being conducted on all aspects of this study.

Project title: Persistence of Willow in Yellowstone National Park: Interactive Effects of Climate, Hydrology, and Herbivory

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Purpose: Determine the impacts that climate change, hydrologic modification, and elk herbivory have on willows' ability to recruit new generations. Determine historic presence of beaver within the northern range in order to understand long-term dynamics of willow recruitment. Propose treatment methods that will sustain a healthy, reproducing willow population.

Findings: The study areas consist of the floodplains and channels along reaches of four streams: Elk Creek, Lost Creek, and the West and East Forks of Blacktail Deer Creek. The design of the overall experiment is a 2×2 factorial randomized complete block with two levels of water table treatment (dammed and undimmed), and two levels of herbivory (exclosed and unexclosed).
We excavated a total of 80 willows at four study sites. The recruitment of willows occurred steadily over the 85 years since the oldest willow established in 1917. The longest gap in recruitment during this time period is five years long, from 1984 to 1988; the next largest gap occurred from 1931 to 1934. From 1935 to 1957, at least one willow established in every year. Willow establishment frequency and timing at our study sites are not correlated with patterns of hydrologic disturbance associated with our reconstruction of annual peak discharge, nor with annual average discharge or total annual precipitation for the current year, or previous years. However, the four-year gap in willow establishment occurred during the 1930s, the decade with the driest extended period of below average annual streamflow in the past 300 years. The largest, five/seven-year establishment gap came in the 1980s, which was the eleventh-driest decade in the last three centuries.

We compared the year and elevation of willow establishment between sites to verify that similar establishment processes operate along the study streams. No significant differences were found when comparing establishment elevation by species, establishment year by species, or elevation by site. During the period of highest mean establishment elevation, in the mid-1940s to 1950s, willows were beginning to establish on coarser-grained surfaces. The 39 willows that established on pond sediments have a mean establishment year of 1946.18 and the 31 that established on alluvium have a mean of 1968.94. The mean establishment elevation relative to the current channel is 1.25 m on pond sediment and 0.82 m on alluvium. Our dams had a significant effect on water table depth, causing an increase of .35 to .51 meters in late season water table depth. This was not due to interannual variation, as undammed plots experienced no similar increase.

Willows appear to compensate for herbivory by producing additional aboveground biomass. This effect is more pronounced with increased water availability; the greatest growth was seen in the plots that were browsed and water elevated. We are currently in the process of calculating mean growth per plot for the 2003 data. In order to gain a more precise understanding of growth response to water table depth, we also performed a regression analysis on growth versus water table depth at the location of individual plants, regardless of treatment. Growth was positively correlated with water table depth. We expected that a greater proportion of variation would be explained by water table depth than was actually seen. Nutrient limitation may account for some of the unexplained variation in the data. Compensation for herbivory was quite dramatic in aboveground shoot length. Total plant height at the end of the 2002 growing season was not significantly different inside versus outside of the exclosures, even though the height of two-year-old growth, an indicator of the previous winter's browse height, was an average of 27 cm higher inside exclosures. Exclosures have a slight but significant effect on both predawn and midday water potentials, as measured by a pressure bomb, with lower water potentials, indicating a greater degree of water stress, inside exclosures. Seed production in 2003 was again much higher inside exclosures than outside, as has been seen in previous studies.

Project title: Rocky Mountain Field Ecology

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Purpose: The purpose of this course is to introduce students to the ecology of the Rocky Mountains through field investigations of plant and animal interactions in Yellowstone National Park. It is also intended to introduce advanced ecology students to non-invasive sampling methods for animal behavior and plant ecology.

Findings: As before, we began with a tour of the park, noting the major landforms, prominent wildlife, management and conservation issues, and other points. We gave a number of formal evening lectures on history, climate, wildlife, fire ecology, the northern range controversy, and wolf reintroduction. We also had several guest lectures, as before, and visited several park interpretive trails at Canyon, Norris Geyser Basin, Midway Geyser Basin, Lower Geyser Basin, Mud Volcano, Tower Fall, and Rose Creek. The main portion of the course was the field exercises. We repeated two of the exercises of the previous year: a comparison of elk vigilance in low and high wolf predation sites, and a comparison of lodgepole density in moderate and severe burn sites, monitoring the long-term study sites established by Dr. Jay Anderson. We also added two new exercises: a study of osprey behavior at high nesting densities (in the canyon) compared with low densities elsewhere, and a comparison of vegetation on north and south facing slopes at mid-elevation, mostly conducted outside the park on U.S. Highway 212 toward the end of our visit. We began these exercises as a group, then in the final week of the visit, students formed small groups that continued each of the exercises, culminating in a Powerpoint presentation of the entire research project with analyzed results on the final evening of the course. Students also submitted a written report within two weeks of their return to Clemson, allowing them time to go to the library to add references and context to their work. As before, it was a spectacular success for the students, who prized the opportunity to learn about research under such realistic and spectacular conditions. We believe we achieved our objectives well, and provided the students a once-in-a-lifetime learning opportunity as well.

Project title: Developing Effective Ecological Indicators for Watershed Analysis

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Purpose: The objective of this study was to identify attributes of river and riparian systems that might be used as indicators of watershed condition, and whether these relationships are spatially scale-able and remotely sensible. Study sites were established in watersheds within the northern Greater Yellowstone Area, Cache Creek, Soda Butte Creek, Pebble Creek, and Tom Miner Creek. Using traditional stream and riparian ecological measurements, this assessment examined the effects of natural and anthropogenic disturbances to stream ecosystems. This study also examined the cumulative effects of natural disturbance, fire, on an entire watershed.

Findings: River Indicators. River attributes related to watershed characteristics included factors associated with stream power. Primary watershed condition related to stream power was percentage burned by 1988 Yellowstone fires. Regression models suggested that 12–13 years post-fire, channels with a greater percentage of burned watershed were associated with higher stream power, low w/d ratios, and lower bank failure rates. Because stream power integrates several elements of fluvial processes, it may serve as a good indicator of watershed response to changing fire management procedures. Riparian Indicators. Riparian attributes related to watershed characteristics included vegetation community structure and composition. Fluvial processes produce heterogeneous conditions for riparian vegetation creating different vegetation patch types: herb, deciduous, and coniferous. Watershed characteristics including elevation, subwatershed size, and land cover types accurately classified differences among these riparian patch types. In most watersheds, floodplain vegetation heterogeneity resulted from different magnitudes of stream power related to flood intervals. Riparian vegetation integrates outputs of watershed alteration. Validation of Watershed Condition. Water quality results show that all three watersheds were different. The best predictors for each watershed were: for Cache Cr. (% burned % forest, bankfull depth; for Soda Butte Cr. (% water class, % barren class, perimeter of barren class, % south aspect); for Tom Miner Cr. (% north aspect, patch area of range class, % n-w aspect, perimeter of deciduous trees). Among watersheds, macroinvertebrate presence/absence was the best biological predictor. Within watersheds, macroinvertebrate presence/absence data and relative abundance data predicted equally well. Remote Sensing. Hyperspectral imagery "identified" several features within and adjacent to streams (i.e., depth, river habitats and woody debris). Spectral response of fluvial characteristics on Soda Butte Creek indicated that all stream characteristics except cobble size were significantly related to spectral response. With further development, specific river indicators may be identified and quantified using hyperspectral imagery. Hyperspectral imagery has been shown to identify different riparian types. Integration of Indicators. Stream power and vegetation patch type were used for integration of indicators for watershed assessment. Cache Creek was used because it had more overlap in watershed drivers and a gradient in percent burn from 1988 fires. A simple predictive model was developed from regression equations tying the two indicators together using the herb patch type and relating them to the percentage of watershed burned, showing potential use of these indicators for highly altered watersheds. Scalability of Indicators. Assessment of several watersheds showed that each had its own set of attributes that influenced river and riparian processes. Consequently, river and riparian indicators of watershed attributes or conditions will usually be different between and among watersheds which reduces the possibility of cross-regional scalability of indicators. On the other hand, remote sensing techniques developed for river and riparian attributes may be scalable across watersheds.

Project title: The Ecology of Arbuscular Mycorrhizae in Yellowstone's Thermal Areas

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Purpose: *Arbuscular mycorrhizae* (AM) are a plant–fungus symbiosis, in which the plant provides the fungus with a carbon source, while the fungus increases nutrient availability (especially phosphorus, P) and water, protecting the plant from pathogens. Previous research on AM shows that the effect of this symbiosis on the host plant can range from positive (mutualistic) to negative (parasitic). Our research focuses on AM in plants growing in the thermal areas of Yellowstone National Park. This research is important because we know very little about this symbiosis in extreme environments. Our research addresses the following questions: (1) Does the AM symbiosis function differently in extreme environments as compared to what has been documented in non-extreme sites? (2) Are AM fungal species native to thermal soils unique species, unique ecotypes, or no different from AM fungal species on non-thermal soils? 3) Are mycorrhizal fungi specifically adapted to thermal site conditions, or does the range of genetic variability found in AM fungi naturally enable them to inhabit a broad range of environments, including thermal soils? The research we propose for the 2003 summer includes two field components: assessing the identity of AM fungi present in thermal soils, and completion of a field test of the hypothesis that AM increase plant fitness in thermal soils.

Because AM fungi are obligately symbiotic, the establishment of single species cultures requires growing fungi in pot cultures with a host plant. Plants are grown in the greenhouse in field soil for approximately 12 weeks, before they are allowed to die back, causing the AM fungi to produce spores. After a 30-day resting period, spores are isolated, and separated into species, as indicated by spore morphology. Then either single-spore cultures, or single-morphotype cultures using multiple spores, can be started. Single species cultures would be used for future greenhouse experiments to determine whether AM fungi from thermal soils are unique ecotypes, and whether they function differently than AM fungi isolated from non-thermal soils.

Field experiments. We are examining the role of mycorrhizae in enhancing plant growth on thermal soils in the field by comparing the growth of mycorrhizal and non-mycorrhizal plants growing at different temperatures. In the Rabbit Creek drainage, we are growing *Mimulus guttatus* plants either with or without AM fungi in growth chambers placed in both high and low temperature soils. The plants were propagated by direct seeding into the pots with seed collected from Rabbit Creek in July 2002. Our growing chambers are made of perforated PVC pipe, 4×6″ deep and lined with nitrocellulose membrane (0.45 mesh) to allow water and solute movement, but not AM hyphae from outside the chamber. Soils from the field were removed to place the chambers in the ground flush with the surface, and then the chambers were refilled with the soil. For the chambers with the non-mycorrhizal treatment, soil was autoclaved at MSU laboratory facilities, prior to returning it to the chambers. The mycorrhizae we are testing in this experiment are from the site, so we have not introduced any non-native or non-local soil microbes. We have a total of 24 chambers in the field, which includes 2 chambers at 12 locations across a temperature gradient. In addition, we have 2 dataloggers to monitor soil temperatures in each growing chamber. The dataloggers are enclosed in a protective case and hidden from direct view, with pieces of wood from the site. Additionally, two deep cycle marine batteries, enclosed in battery cases, and one solar panel are also in the field to power the dataloggers. The *M. guttatus* plants will be harvested in July 2003 and the chambers will be completely removed by September 2003, and soils replaced.

Findings: The two main objectives for our 2003 field season were (1) to assess the identity of AM fungi present in thermal soils, and (2) to complete a field test of the hypothesis that AM increase plant fitness in thermal soils. The identity of AM fungi can be identified morphologically, based on spore wall composition, or using molecular methods. For the former, spores are either collected from field soils or fungi are grown in the greenhouse in a plant-culture, and spores are extracted from the soils after *mycorrhizae sporulate*. Spores from field soils change color as they age, compromising the use of color as an identifying characteristic. Based on spore morphology, we have identified the following species as present in these thermal soils: *Paraglomus occultum, Glomus mosseae,* and *Acaulospora delicata. Acaulospora delicata* has previously been reported only in southern Arizona and Costa Rica.

Molecular identification of AM in roots of plants collected in the Rabbit Creek area show the presence of *Glomus intraradices*-like sequences. In fall 2003, we collected additional samples, and Dirk Redecker, University of Basel, did the molecular analysis of AM fungal tissues. The AM community in root samples of *Dichanthelium lanuginosum* (hot springs panic grass) showed a relatively unusual composition: 2 of 3 root samples contained a previously unknown sequence type of *Archaeospora,* one of them in addition a previously unknown deeply diverging member of *Glomus* group A. The third root sample contained *Paraglomus occultum,* which is also found in other habitats. Some *Paraglomus* isolates were isolated from mine spoils in West Virginia, but they can also be fierce greenhouse contaminants. These data suggest that *D. lanuginosum* has uncommon AM fungal associates (dominated by *Archaeospora*), that may be specialized for the extreme conditions. We are planning to continue this work in the upcoming year.

Last August, we collected soil samples at 18 sites within the Rabbit Creek thermally active drainage. Soil and roots from below *Mimulus guttatus, Agrostis scabra,* and *D. lanuginosum* were collected in areas with soil temperatures above 30°C at 10 cm below the surface (range of 30.2–46.2°C). Samples were mixed with autoclaved sand in preparation of “trap cultures,” which were seeded with ~80 sterilized sudan grass (Sorghum bicolor) seeds and grown for no less than four months. We modified previous greenhouse methods used in 2002 experiments to incorporate the thermal properties of Rabbit Creek soils. Trap cultures were grown on a 2×4′ heat blanket at 40°C. Soil temperatures varied from the soil surface to the pot base (24°C at 3 cm; 47°C at 8 cm) mimicking actual conditions in Rabbit Creek as documented with the datalogger currently in the field. Twenty-four pots are currently in a “resting” stage which triggers the mycorrhizae to produce spores. Spores will be extracted from the soil and identified by morphotype. These spores will be transferred to sudan grass seedlings in the establishment of single species monocultures.

Our second objective, to test the effects of AM fungi on plant growth in field soils was completed last June. We grew *M. guttatus* and *D. lanuginosum* in pots in the field. Because Rabbit Creek is not accessible until June 1, we were not able to measure plant growth in all of the pots. The pots growing in soils at the highest temperature had gone to seed before we were able to access the field site. Therefore, we were not able to collect useful data to address this hypothesis. We conducted a greenhouse experiment using heat blankets to warm soils in pots, and are just analyzing the data, which compares plant growth with and without AM fungi when plants are grown at elevated or ambient temperatures. The data analysis will be completed during summer 2004.

EXOTIC SPECIES

Project title: Influence of Biopollution on Ecosystem Processes: the Impact of Introduced Lake Trout on Streams, Predators, and Forests in Yellowstone National Park

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Purpose: Our objective is to examine the links between spawning cutthroat trout and stream and terrestrial processes near Yellowstone Lake. Given that cutthroat trout may decline because of predatory lake trout, how will systems outside of the lake respond? We will examine how trout affect nitrogen cycling and productivity in streams. We will also examine otter populations and transfer of fin nitrogen to terrestrial forests.

Findings: Our project has two components: investigating the transport of nitrogen by spawning fish from Yellowstone lake to tributary streams, and examining responses of otters to variation in fish abundance. The recent decline in YCT may decrease the amount of nutrients transported to streams. To estimate the amount of nutrients YCT transport to streams, we measured YCT excretion rates and collected water samples weekly on Clear Creek from June through August 2003. About four thousand YCT migrated up Clear Creek in 2003, excreting 5.9 mg NH_4 fish-1 hr-1 on average. We estimated the amount of ammonium taken up by stream biota by subtracting exported ammonium at the stream outlet from YCT transported ammonium. YCT excreted >400 g NH_4 d-1 (>0.0057 g NH_4 m-2 d-1) and stream biota took up 200 g NH_4 d-1 (0.0029 g NH_4 m-2 d-1) on average during spawning. YCT transport less ammonium into streams compared to historical levels when >50,000 YCT migrated up Clear Creek and excreted >7,000 g NH_4 d-1 (>0.10 g NH_4 m-2 d-1). Surveys for river otter latrine (scent-marking) sites were conducted from May 23 to August 15, 2003. Areas surveyed included: Yellowstone River inlet, Yellowstone River from the outlet to the Lower Falls; Pelican, Sedge, Cub, Clear, Columbine, Bridge, and Arnica Creeks, and the perimeter of Yellowstone Lake, excluding non-motorized zones. Total stream length surveyed was 52.8 km, and total length of lake shoreline surveyed was 203 km. Latrine site density was 0.68 sites/km on steams and 0.15 sites/km on Yellowstone Lake. These latrine densities were low compared with similar surveys on the Green and Colorado rivers. On streams, otters selected for high presence of shade and large rocks with shallower, accessible stream banks in choosing latrine sites. On Yellowstone Lake, latrines commonly had a spruce overstory and were associated with lagoons, tributary streams, and large rocks. Our results support the hypothesis that river otters transport aquatically-derived N onto latrine sites, and in turn, fertilize some plants at these locations. Tissues in six of eight plant species were significantly enriched in d15N and percent N on latrine sites compared to random sites. We are analyzing tree cores of Engelmann spruce for d15N in order to relate temporal changes in nitrogen isotope values to historical otter activity. There was a general trend towards increased otter use of cutthroat trout spawning streams, and decreased use of Yellowstone Lake during the height of spawning season, with a return to elevated otter activity on the lake after spawning had ended. A comprehensive diet analysis of otter scats is being performed to further investigate the seasonal importance of cutthroat to otters.

Project title: Food Web Impacts of Exotic New Zealand Mudsnails in Rivers in Yellowstone National Park

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Report number: 28036
Co-investigators: Mark Dybdahl, Lusha Tronstad, Maria VanderLoop

Purpose: Our objective is to continue summer biomass and production estimates of exotic New Zealand mud snails and native invertebrates in Gibbon and Firehole rivers. We hypothesize that annual pattern of snail populations compared with native invertebrates will allow one way of estimating impact of snails.

Findings: Estimate impact of exotic *Potamopyrgus antipodarum*, New Zealand mud snails, on native invertebrates by examining response of native invertebrates to inter-annual population variation in mud snails. During summer 2002 and 2003, we observed low densities of mud snails in Firehole River, and we want to use this natural variability in snail abundance and biomass to estimate native invertebrate population response. We sample during July, August and September, when mud snail densities are typically highest. We are currently counting and processing these invertebrate samples.

Project title: The Invasiveness of the Exotic New Zealand Mud Snail in the Greater Yellowstone Ecosystem

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Report number: 28660
Co-investigators: Robert Hall

Purpose: (1) Assessing abiotic and biotic limits on mud snail invasion success, and (2) Measuring impact of mud snails on population, community and ecosystem processes.

Findings: In the summer of 2003, we studied positive and negative effects of snail consumers on their resource to determine if positive consumer effects may be facilitating invasion. Consumer–resource interactions often focus on losses to the resource, even though the resource might benefit if consumers recycle nutrients. The New Zealand mud snail, *Potamopyrgus antipodarum*, an exotic in western U.S. rivers, attains high densities and dominates macroinvertebrate communities. In one well-studied river, it consumes the majority of primary productivity, cycles most nitrogen, and can grow faster at higher densities. In field experiments, we tested the hypothesis that this invasive grazer stimulates algal growth via nitrogen excretion, which might explain its self-facilitation and invasiveness. Using in-stream cages subdivided into "with snails" and "without snails" sections, we examined the response of periphytic algae to snail grazing and excretion and snail excretion alone at various levels of snail biomass. We found that chlorophyll and GPP (gross primary productivity) decreased as the biomass of snails increased in the grazed sections. Snail excretion, in the absence of grazing, increased both chlorophyll and GPP, demonstrating a positive effect of snails on the resource, consistent with the nutrient recycling and enrichment hypothesis. We found no evidence for increased algal growth at intermediate snail densities in grazed treatments, as predicted by the Herbivore Optimization Curve hypothesis. However, the difference in chlorophyll between "with snails" and "without snails" treatments increased as snail biomass increased. This suggests that snail compensation of the resource, through excretion, decreases at extremely high levels of grazing pressure and the net effect of snail grazing becomes negative. Together, these results suggest that invasiveness in some rivers may be fostered by this self-facilitation and recycling of essential nutrients.

Project title: Gypsy Moth Detection

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Report number: 26443
Co-investigators: Aimee Tallian, YELL North District Resource Management Team

Purpose: Detect and capture any gypsy moths, which are non-native to Yellowstone National Park, and eliminate them before they damage the tree foliage of the area.

Findings: Caught one gypsy moth within the commercial campground at Fishing Bridge and one at Madison.

Project title: Assessment of the New Zealand Mud Snail, *Potamopyrgus antipodarum*, as a Potential Fish Disease Vector

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Report number: 26623
Co-investigators: Crystal Hudson, Billie Kerans, Beth MacConnell

Purpose: (1) Collect New Zealand mud snails (NZMS) from diverse environments and screen for parasites; (2) Collect wild fish and observe tissues grossly and histologically for parasites; and (3) Expose parasite free cutthroat trout to NZMS collected in the wild to determine if parasite transmission would occur from the snails to the fish.

Findings: In total, 6,910 snails were analyzed during this project for the presence or absence of digenetic trematodes. New Zealand mud snail populations, shell size, and snail stream location fluctuated from June to October. New Zealand mud snails sampled were not observed to be intermediate hosts for digenetic trematodes. However, organisms were observed to be utilizing the external shell of the mud snails, i.e., algae, *Epistylis sp., Trichodina,* and diatoms. Midway Geyser Basin had the greatest overall population of snails compared to the Upper Gibbon (above the falls), Nez Perce Creek, Lower Gibbon (Madison Junction), and the Madison River outside the park above Hebgen Lake and Darlington Ditch (near Three Forks, Montana).

Project title: Weed Inventory for the Northern Range of Yellowstone

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Report number: 26747
Co-investigators: Frank Dougher, Bruce Maxwell, Amanda Morrison

Purpose: The objective of the project is the creation of a non-indigenous plant species inventory for the northern range. Approximately 62 non-indigenous plant species are being targeted including; those listed as noxious weeds in adjacent states, species that have invaded or been introduced to the park, and species that are a potential threat but have not yet been recorded in the park. Maps will be generated of observed and predicted locations of non-indigenous species.

Findings: In the 2003 season, 121 transects were completed that covered 212,315 m × 10 m. Again, the 62 species were targeted, but only 16 of these species were recorded in the transects. Seven of these species were recorded with occurrence rates of more than 1% over the study area. *Phleum pratense* had a percentage occurrence of 32.1%, which was more than any other species but not surprising considering that it was intentionally introduced to the park in the early 1900s. *Poa pratensis* occurred over 14.1% of the surveyed area, *Alyssum desertorum* occurred just over 5%, *Cirsium arvense* occurred 4.7%, *Bromus tectorum* occurred over 3.1% of the studied area, and *Bromus inermis* and *Trifolium hybridium* had occurrences of 2.8 and 1% respectively during this study year. Percentage occurrence over the infested area was generally considerably less than 1% for all other species. Infestation length and width measures were estimated by pacing or visual determination from a central location within the patch when the patch size was small enough. When the length of the patch was too large to visually perceive or pace from a single location, the start and end of the patch length along the transect was recorded with GPS. The total length was determined by data analysis in the field. (Patch widths were estimated up to a width of 64 m). Transects were allocated to ensure that each ended at least 2,000 m from a road, trail or road and trail. Once entered into the GIS, distance from road and trails was re-calculated for each transect and the distance partitioned into 10 m intervals from both roads and trails for further analysis.

The patterns observed for those species with more than 1% occurrence have been plotted and show a decline with distance from road/trail.

Fire

Project title: Fire Effects

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Report number: 27670
Co-investigators: Shawn Jackson, Sean McEldery, Emily Moss, Andy Oestreich, Becky Seifert

Purpose: Monitor the effects of prescribed fire, hazard fuels treatment, and wildland fire in Yellowstone.

Findings: In late winter, Miller collaborated with Mack McFarland (GRTE) to develop methods for monitoring hazard fuel reduction projects (31 backcountry cabins and 5 frontcountry areas). These methods are still being refined and focus on canopy, ladder, and surface fuel loadings. Forty-one pilot plots were installed and eight were resampled post-treatment. The resulting information was presented at the Fire Management Office and will be used to modify hazard fuel reduction methods of future projects to accommodate esthetics and effectiveness of the treatments. Effects of canopy fuel loading on canopy fire behavior is not well understood and will not be well understood until treatment areas are exposed to crown fire. To this end we are beginning to incorporating canopy fuel loading measurements in our monitoring plots installed ahead of naturally occurring wildfires. Forty-nine Composite Burn Index (CBI) plots were sampled on the 2002 Broad and Phlox Fires. Yellowstone currently has sufficient CBI plots to begin an analysis. We plan to combine the Yellowstone dataset with that of Grand Teton to increase the sample size and better understand what the Normalized Burn Ratio data actually represent on the ground. We have completed the fuel load sampling phase of our early post-fire lodgepole pine (LP0) custom fuel model and photoseries. We were assisted by students from Brigham Young University and the University of Idaho's Upward Bound Program. We will disseminate the photoseries as a web-style, browsable document available online or compact disc.

Although the fuel load sampling phase has been completed, we will continue to add fire weather and fire behavior information to the model as opportunities arise. In conjunction with the LP0 fuel modelling project, we established two field sites to monitor the moisture of LP0 foliage, meadow herbs and heavy fuels through the season. This information will fill out the picture of how fuel moisture controls fire behavior in the custom LP0 fuel model. Nine FMH plots were installed or resampled. A fire use plot was installed on the Grizzly Fire but it did not burn. Phlox, Crevice, Stone, Sulphur, Two-Smokes, Deaf Jim, Electric Peak, and two plots at the Boundary Fire were sampled. These data continue to provide baseline ecological data on the effects of fire on Yellowstone's forested ecosystems. We plan to focus future plot monitoring efforts on the Douglas-fir and whitebark pine cover types which are less well represented than lodgepole pine. We relocated two of Despain's fire plots that did not burn in 1988 and have not been revisited in 15 years. These plots are in Douglas-fir (Buffalo), and whitebark pine cover types (Observation Peak). These plots will be resampled in 2004 and used as control plots to Despain's other 11 burned plots that were resampled in 2001. We hope to get the entire set of fire plots resampled in 2004. Two permanent fire weather log stations were established to monitor fuel moisture over the winter to address the question of how drought and low 1,000-hour fuel moisture carries over from one season to the next. Our Fire History project is mostly completed. This is an accurate, GIS dataset (point and polygon) that ranges back to 1931 and sporadically to the late 1800s. Error checking and data clean-up remain.

Project title: Study of the Effects of the 1988 Wildfires on Yellowstone Stream Ecosystems

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Report number: 28408
Co-investigators: Robert Crabtree, Andrew Marcus, Duncan Patten

Purpose: This project examines the processes of stream ecosystem recovery after a large-scale disturbance (fire). Changes are being monitored in the chemical properties of water, physical habitat conditions, and structure of biotic communities, which include primary producers (algae) and secondary consumers (macroinvertebrates). The results accrued over an extended period of time (14–20 years) will be used to determine mid-range effects of wildfire on stream ecosystem recovery.

Findings: No new data were collected this report year. The results of the first ten years of study and a synthesis of the macroinvertebrate responses to fire were published. Also, analysis of the 2002 collections and data was completed and a final report is nearing completion. Publications: Minshall, G. W. 2003. Responses of stream benthic macroinvertebrates to fire. *Forest Ecology and Management* (17) 178:155–161; Minshall, G. W., K. E. Bowman, and C. D. Myler. 2003. Effects of wildfire on Yellowstone stream ecosystems: a retrospective view after a decade. Proceedings of Fire Conference 2000: The First National Congress on Fire Ecology, Prevention, and Management. Miscellaneous Publication No. 13, Tall Timbers Research Station, Tallahassee, Fla. pages 164–173.

Project title: A Comparison of Fire Regimes and Stand Dynamics in Whitebark Pine Communities in the Greater Yellowstone Ecosystem

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Report number: 26888
Co-investigators: James R. (Randy) Walsh

Purpose: The purpose of this study is to reconstruct fire history and stand age structure in whitebark pine forests of two contrasting locations. One location is outside Yellowstone National Park, in the Centennial Mountains just west of the park. The other location is in Yellowstone Park, within the perimeter of the Arthur fire that burned near the East Entrance in 2001.

Findings: We sampled three old-growth whitebark pine stands in 2003. One was located within YNP (near Avalanche Peak); the others were on National Forest lands close to YNP. Fire scars were scarce in all three stands, indicating that low-severity fires have not been prevalent in these stands during the last three centuries. Tree age structure indicated episodic recruitment of whitebark pine. We plan to sample an additional 6–7 stands in 2004 to more fully characterize fire history and stand dynamics of whitebark pine forests in and around YNP.

Project title: How Do Disturbance-Generated Patterns Influence the Spatial Dynamics of Ecosystem Processes?

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Report number: 27728
Co-investigators: William H. Romme, Daniel B. Tinker

Purpose: Our studies following the 1988 Yellowstone fires demonstrated that succession was surprisingly more variable in space and time than even current theory would have suggested, and that initial spatial patterns of disturbance may persist to produce long-lasting changes in vegetation. Our focus now is on explaining the spatial and temporal patterns of succession and understanding how these patterns influence ecosystem function. The most interesting new questions revolve around the degree to which the spatial variation in postfire vegetation in particular, the six orders of magnitude variation in pine sapling density, ranging from 0 to greater than 500,000 saplings/ha controls the spatial variability in ecosystem processes across the landscape. We are exploring four major questions: (1) Do the enormous differences in postfire tree density produce differences in carbon and nitrogen availability across the landscape? Or, is nutrient availability governed largely by broad-scale abiotic gradients (e.g., climate, substrate) and/or fine-scale (i.e., less than 10 cm) heterogeneity in resources or the microbial community, such that nutrient variability is not sensitive to the spatial variation in plant community structure? (2) Does the disturbance-created mosaic leave a persistent functional legacy? What mechanisms in vegetation development may contribute to convergence (or divergence) in ecosystem structure and function across the landscape as succession proceeds? (3) How does the spatial pattern of coarse woody debris vary across the post-1988 landscape, and what is the importance of this variation for ecosystem function? Are patterns of coarse woody debris abundance related to both prefire stand structure and postfire sapling density? (4) Does the spatial heterogeneity of processes such as ANPP, nitrogen mineralization, and decomposition change with time since fire? How quickly do spatial patterns in processes develop following a large fire?

Findings: During the summer of 2003, our field studies focused on questions associated with the ecological role of postfire coarse woody debris and with convergence or divergence in ecosystem structure and function through time. We initiated studies in 2002 of the influence of postfire coarse woody debris on soil nutrient dynamics and decomposition in three locations burned in the 1988 fires under the leadership of co-Principal Investigator Dr. Daniel B. Tinker and postdoctoral associate Dr. Kristine Metzger. At each site, we collected soil samples from positions located under trees that have fallen since the 1988 fires, under wood that was down prior to the 1988 fires, under lodgepole pine saplings, and out in the open. The effect of coarse woody debris on nitrogen availability was measured using one-year incubations of ion exchange resin placed in soil cores at each site during summer 2002. Cores were removed during summer 2003, and integrated measure of nitrate and ammonium production will be obtained for a yearly time step. In addition, we are characterizing the microbial community composition of the soil, enzyme activity levels, and gross nitrogen mineralization in the laboratory. Analyses are currently underway. In addition, decomposition studies were also initiated in 2002 by placing litterbags containing herbaceous litter or conifer litter in each of the positions described above. These decomposition studies are continuing for two years. Half the bags were retrieved during summer 2003, and the remainder will be collected during summer 2004; this study will form the basis of an MS thesis for Alysa Darcy, student at University of Wisconsin. In addition to obtaining mass loss estimates, the microfauna associated with decomposition are being quantified from the litterbags retrieved in 2003. Monitoring of microclimate conditions as they vary with treatment is also continuing at all sites. To explain and predict variation in the rates of treefall and abundance of postfire coarse woody debris, extensive sampling was begun during summer 2002 to quantify downed wood throughout the area burned by the 1988 fires. This sampling was continued during summer 2003, and the combination of the intensive process-based measurements with the broad-scale analysis of coarse woody debris these effects at landscape scales. This study is the basis of a MS thesis for Heather Lyons, student at Colorado State University, which should be completed during 2004. Alysa Darcy also placed litterbags in 20 stands, all burned in 1988 but varying in their abundance of coarse woody debris, to determine whether there were stand-level effects of the postfire treefall on decomposition. These will also be retrieved in 2004.

During summer 2003, we also re-sampled 16 0.25-ha plots within the 1988 burn from a larger sample of 90 plots that were sampled during summer 1999. Our goals were to re-estimate aboveground net primary production and leaf area and to characterize gross nitrogen mineralization and microbial community composition in the soil, to determine whether there is a detectable effect of lodgepole pine density. In addition, we sampled the abundance of cones on the lodgepole pine saplings in each of the stands, and also harvested some of the 15-year old pine saplings to test the allometric relationships used to predict ANPP and leaf area. Finally, in September 2003, we initiated studies of nitrogen mineralization rates and vegetation in areas that burned during the summer of 2003. Previously, our measurements of ecosystem process rates began a year or more following fire; these new studies will permit the immediate responses of soil processes to fire to be characterized. These areas will be re-sampled during summer 2004.

Project title: The Status of Whitebark Pine Regeneration in the Greater Yellowstone Area Following the 1988 Fires: Burned vs. Unburned Forests and Mesic vs. Xeric Conditions; Assessment of Blister Rust Infection in Seedlings

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Report number: 28197
Co-investigators: Phillip E. Farnes, Anna W. Schoettle

Purpose: In 1990, Tomback established 125 permanent plots in the subalpine zone on Mt. Washburn to study patterns of whitebark pine regeneration following the 1988 fires. The plots were measured from 1990 to 1995 and then again in 2001 by Tomback and Schoettle for further studies. We plan to remeasure the plots again in 2005, using the data collectively to address the following: (1) What are the current regeneration densities of whitebark pine and other conifers for the following ecological conditions on Mt. Washburn: xeric, burned (50 plots); mesic, burned (50 plots); mesic, mixed severity burned (25 plots). What can we learn about the timeframe for regeneration? (2) What is the percentage cover and composition of understory dominants on each plot? (3) Based on seedlings known to be alive in 1995, what are the survival rates to 2005? (4) Given that blister rust is present at low to moderate levels in unburned whitebark pines stands throughout the Greater Yellowstone, how prevalent is blister rust on seedlings on the plots? We also plan to address the following related questions: (5) Are there differences in spring snowpack depth and melt-out dates between the xeric burned and mesic burned treatments? 6) Are there detectable differences in water availability for soil subsurface that might account for differences in regeneration densities between these two treatments? 7) Are there differences from plot to plot in snowpack depth and water availability within a treatment that might correlate with differences in either whitebark pine regeneration density or survival?

Findings: The focus of the current research has been to investigate differences in snow depth, temperature, and soil moisture between the two main ecological conditions, xeric, burned and mesic, burned, and among plots as predictors of regeneration density and survival. Snow depth measurements were taken twice near the time of snow melt-out on all permanent plots in on the xeric, burned and mesic, burned study "treatments." Three data loggers were reinstalled in the Mt. Washburn study area on each of the same treatments in order to record continuous data on air temperature, relative humidity, and soil moisture throughout the summer. Soil moisture was measured periodically on each of the plots in these treatments throughout the summer using time domain reflectometry. In addition, soil moisture measurements were taken on several categories of micro-sites, which reflect the types of sites that whitebark pine seedlings commonly grow in. PVC pipes over plot markers were used to mark plot centers. These were dismantled after snow melt-off and put back in place in September 2003. It is anticipated that additional snow depth measurements will be taken in spring 2004.

Fish

Project title: Le Hardys Rapids Yellowstone Cutthroat Egg Collection for the Development of Species Specific Brood Stock for Drainage Restoration

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Co-investigators: Pete Feck, Brian King, Paul Krestchmar, Tom Lorenzen

Purpose: To collect and fertilize eggs from Yellowstone cutthroat trout pairs to develop a captive broodstock program for purposes of restoration efforts in Wyoming. Eggs will be collected from the population that inhabits the Yellowstone Lake to the Upper Falls. The primary capture location will be LeHardys Rapids, although other sites may be considered if catch rates do not meet objectives. The original objective each year was to collect 25 pairs for consecutive years (1993–1996) for the purpose of stock recruitment of a broodstock to be held at Clark's Fork Fish Hatchery. This broodstock will provide drainage restoration of the endemic range of the Yellowstone River in Wyoming and will also assist in the restoration in Montana.

Findings: Eggs were collected on two occasions (6/12/03, 6/19/03)

6/12/03
Spawned 19 pairs (5,950 eggs)
Water Temp = 47°F
Air Temp = 65°F
Eggs shipped to Tillett Isolation Hatchery

6/19/03
Spawned 19 pairs (7,140 eggs)
Water Temp = 55°F
Air Temp = 65°F
Eggs shipped to Tillett Isolation Hatchery

Project title: Cutthroat Trout Egg and Sperm Collection

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Report number: 25701
Co-investigators: Gary Bertellotti

Purpose: To succesfully manage Montana's fishery resources needed to maintain our hatchery broodstocks with a wide genetic diversity. These broodstocks should mirror their wild ancestors as closely as possible. The original gametes for our Yellowstone Cutthroat Trout Broodstock came from McBride Lake in Yellowstone National Park in 1969. The last time gametes were taken from the lake to supplement the broodstock was 1987. To once again infuse our broodstock with new genetic material we want to again collect gametes from McBride Lake for three consecutive years beginning in 2001. Each year of the three year program would require eggs and sperm be taken from the number of pairs of spawning fish needed to acquire 20,000 green eggs. This would require at least 15 females and 15 males per year. These fish will be lethally sampled to assure protocols are met for genetic testing an disease certification. Means of collecting the fish would be by electrofishing or netting.

Findings: No activity was conducted this report year.

Project title: Demonstration of Airborne Fish Lidar for Locating Spawning Lake Trout at Yellowstone Lake

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Co-investigators: James Churnside

Purpose: We propose to conduct demonstration flights of an airborne lidar (laser radar) system in a small airplane over and around Yellowstone Lake during the middle part of September 2003. The overall purpose of the study is to demonstrate the use of airborne lidar for locating Lake trout during spawning season. A secondary objective, which is key to the main purpose, is to measure the optical properties of the water in Yellowstone Lake, which will allow further design and analysis for future research projects. The water is expected to extremely clear, but lidar analysis and design requires a quantitative characterization of water clarity. Approximately 10–20 flight hours will be conducted, during day and night.

Findings: Airplane problems prevented us from accomplishing flights in the 2003 lake trout spawning season. Planning is underway for flights during the 2004 spawning season (approximately mid-September 2004).

Project title: Assessment of Tributary Potential for Wild Rainbow Trout Recruitment in Hebgen Reservoir, Montana

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Report number: 27901
Co-investigators: Scott Barndt, Pat Clancey, Ryan White

Purpose: The conversion of trout stocking to self-sustaining wild trout populations has been a cornerstone of fisheries management in Montana rivers for the past 30 years. However, trout fisheries in Montana reservoirs are almost entirely maintained by stocking hatchery fish. Due to the presence of apparently high quality spawning tributaries, in 1979, Montana Fish, Wildlife and Parks initiated a wild trout management program for Hebgen Reservoir with the purpose of establishing a wild self-sustaining trout fishery. Since 1979, stocks of DeSmet and Eagle Lake strains of rainbow trout have established wild spawning runs in several tributaries. Montana Fish, Wildlife and Parks continues to stock 100,000 fingerling Eagle Lake rainbow trout annually to maintain the Hebgen Reservoir fishery. However, spring gill net catch rates of rainbow trout have been unexpectedly low from 1989 to 2001. A lack in natural recruitment may be reflected in these catch rates. The objective of this study is to assess reservoir potential for wild rainbow trout recruitment among tributaries of Hebgen Reservoir and to identify potential limiting factors. Primary objectives in 2003 included: (1) estimating the relative contribution of tributary spawning to the total rainbow trout production of Hebgen Reservoir, (2) assessing the quantity and quality of tributary habitat associated with wild reproduction and recruitment and, (3) determining life history characteristics for juvenile rainbow trout outmigrating to Hebgen Reservoir. This study will provide management agencies with an assessment of local habitat conditions, habitat limitations and enhancement suggestions, as well as an evaluation of recruitment and potential for recruitment in Hebgen Reservoir. This study will also provide the framework for assessing potential for wild trout recruitment in other reservoir systems.

Findings: Field investigations and monitoring in 2003 included basin redd surveys, tributary habitat inventories, egg-pocket core sampling, and juvenile outmigrant trapping. Adult trap data from 2002, redd survey totals from 2002 and 2003, and substrate quality averages were combined to estimate yearly tributary fry production. Basin-wide redd surveys were repeated during the 2003 field season. Redd surveys were conducted post runoff (late June through mid-August) and were executed in the following order: Gibbon River, Firehole River, Madison River, Duck Creek, Cougar Creek, South Fork Madison River, Grayling Creek, Trapper Creek, Watkins Creek, Red Canyon Creek, Cherry Creek, and Rumbaugh Creek. The distance surveyed in 2003 was approximately 170 stream km. The total number of redds counted in 2003 (1,293) was far below that of 2002 (4,394). However, the proportion of redds encountered between tributaries in 2003 was similar to 2002. Duck Creek (Upper Duck Creek within YNP) and the South Fork Madison River (including Black Sand Spring) contained nearly 75% of the basin estimate. A large proportion of redd construction was observed in late May 2003, during peak runoff, and many redds may have been obscured and difficult to identify in 2003. Mean daily water temperature, from May through July, again varied widely among tributaries (5.6 to 16.5°C) while most spawning production (90%) was associated with temperatures from 8.5–13°C. Characteristics and quantities of tributary habitats associated with wild reproduction and recruitment were assessed through basin wide habitat inventories in 2003. Total available spawning and rearing habitat were estimated for each tributary inventoried. Areas of potential spawning gravel, patches of substrate at least 0.25 m^2 in area with gravel from 6–128 mm in diameter, were measured in meters and expressed as a percent of total stream surface area. Total area of rearing habitat was quantified as the sum of all slow water habitat units. Basin inventory totals are as follows: Total length (171 km), Total area (3,421.24 km^2), Total available spawning habitat (70,262.6 m^2), Total available rearing habitat (785,126.7 m^2). Spawning habitat quality of the Hebgen Basin was assessed (in relation to fine sediment accumulation in egg pockets of redds) and compared between tributaries by sampling with a modified McNeil hollow-core sampler. Egg pocket cores, 10 samples or 1% of all redds inventoried per tributary (which ever was greater) were randomly collected after the observed spawning period. Samples were taken from the front one third of the tailspill at egg depths recorded during redd surveys (~14–20 cm) and processed in the field by the wet sieve method. Substrate composition was expressed as the percent fine sediment less than 6.35, 2.36, and 0.85 mm in diameter. Survival to emergence estimates (STE), of rainbow trout fry, were made for all tributaries based on percent fine sediment accumulation (particles less than 9.5 and 0.85 mm). Spawning substrates were of moderate to high quality, with an average STE estimate of 38%, and no significant difference in egg pocket substrate composition was detected between tributaries. Numbers of outmigrant rainbow trout fry (age-0) and juveniles (age-1 and 2) from Duck Creek and South Fork Madison River were estimated in 2003 using a series of drift and screw traps. Traps were operated from April through August 2003 and were located downstream of the majority of redds constructed during both study years. Outmigrant life history patterns varied markedly between tributaries. High numbers of outmigrant fry (n=8,950) and moderate numbers of juvenile outmigrants (n=341) were detected on the South Fork Madison River. No fry were collected at the Duck Creek trap sites while high numbers of outmigrant juveniles (n=1,734) were captured. Fry production per tributary was estimated by taking the total number of redds within a tributary multiplied by the egg deposition per-female (obtained from adult female rainbow trout collected on Duck Creek in 2002) and then by tributary estimates of fry survival to emergence. The total estimate of tributary fry production for Hebgen Basin in 2002 and 2003 is over 3 million.

Project title: The Spatial and Temporal Spawning Distributions of Yellowstone Cutthroat and Rainbow Trout in the Upper Yellowstone River Drainage

Principal investigator: Dr. Alexander Zale
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Address: Montana Cooperative Fisheries Research Unit, Montana State University, PO Box 173460, Bozeman, MT 59717
Report number: 26151
Co-investigators: James N. De Rito, Bradley B. Shepard

Purpose: To determine where and when spawning occurs for Yellowstone cutthroat trout, rainbow trout, and hybrids of the two species, what types of habitats they are selecting for spawning, and the mechanisms leading to reproductive isolation or overlap between the three study groups.

Findings: No fish were radio-tracked into the park during the 2003 field season.

Geochemistry

Project title: Geochemical Baselines in the Greater Yellowstone Area

Principal investigator: Dr. Maurice Chaffee
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Address: U.S. Geological Survey, Federal Center MS 973, Denver, CO 80225-0046
Report number: 28358
Co-investigators: Harley D. King

Purpose: (1) Provide objective, unbiased geochemical baseline data for about 50 chemical elements determined in samples of rock, active stream sediment, water, plants, and animal scat collected from scattered localities throughout Yellowstone National Park and the adjacent U.S. Forest Service lands. Baselines to include raw data and interpretive reports. (2) Identify the sources of anomalous concentrations of selected elements, such as geothermal features, past mining, and recreation. (3) Determine the chemistry of selected elements in the food chain and how these elements may impact the health of wildlife in the park. (4) Publish results of the investigations.

Findings: Field work for this project has been completed. Two reports based on the sample analyses are nearing completion for inclusion in a U.S. Geological Survey Professional Paper on Yellowstone National Park. See previous year's reports for some of the conclusions of the study.

Project title: Geochemical and Geophysical Investigations of Mine Impacts and the Soda Butte Creek Watershed

Principal investigator: Dr. James Harris
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Address: Millsaps College, Dept. of Geology, 1701 N. State Street, Jackson, MS 39210
Report number: 27977
Co-investigators: Lori Eversull

Purpose: The fundamental goals of this project are to contribute to understanding of the complex geochemistry of the Soda Butte Creek watershed, and to investigate the impact of mining activities in the creek's watershed. This is accomplished through: (1) developing a long-term database documenting seasonal/annual variations in stream chemistry and metal concentrations in stream sediments, and (2) delineating shallow subsurface features in the Soda Butte Creek floodplain.

Findings: Sediments and waters of Soda Butte Creek were sampled in the last week of May 2003. Field measurements of pH, total dissolved solids (TDS), conductivity, and temperature were made at nine sites along Soda Butte Creek from the Lamar confluence to the tailings pile east of Cooke City. Findings are consistent with data collected in previous years under high stream flow conditions. Stream water was slightly alkaline; recorded pH ranged from 8.0 to 8.8. TDS in Soda Butte Creek varied from 53 to 69 ppm, but the small hydrothermal creek near Soda Butte Mound was significantly higher in dissolved solids: 800 ppm. Streambed sediment was collected at five sites within Yellowstone National Park. Metals analysis of the fine-sediment fraction is pending. Also initiated in 2003: design of a database and web page. The database will incorporate field data as well as data generated from laboratory analysis of sediment and stream water. Both projects are in progress; completion is expected in 2004.

Geography/Geographic Information Science

Project title: The Effects of Forest Fires on the Radiation Balance of Yellowstone National Park

Principal investigator: Dr. Mark Hildebrandt
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Address: Department of Geography, So. Ill. Univ., Edwardsville, IL 62026
Report number: 27048
Co-investigators: Michael J. Starr

Purpose: The goal of the project was to teach undergraduate college students about the positive and detrimental effects of forest fires on the natural environment. All goals were met.

Findings: On two occasions, students performed research on surface temperatures in burn and non-burn/old burn areas. Qualitative and quantitative analyses suggested that areas of recent burns are significantly warmer than non-burn/old burn areas. This difference in surface temperature is possibly due to the lack of tree canopy and darker, exposed soils in areas of recent burn activity. The goals of this projected were completed in a timely manner, and no plants or animals were affected by our study whatsoever.

Project title: The Wilderness Experiences of Yellowstone National Park's Tourism Service Employees

Principal investigator: Mr. Jamie LeDent
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Report number: 28529
Co-investigators:
Purpose: n/a
Findings: n/a

Project title: GIS Mapping of Geothermal Features at Ragged Hills, Norris Geyser Basin, Based on Ground Survey and Aerial Photographs

Principal investigator: Dr. Britta Planer-Friedrich
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Report number: 26407
Co-investigators: Juliane Becker, Beate Bohme

Purpose: n/a

Findings: From September 29–October 3, 2003, two overflights with a Cessna 172 and two balloon surveys from the ground were conducted at Ragged Hills area, Norris Geyser Basin. While the pictures from the overflight at 2,000 ft produced a good overview of the area, resolution was too low for the required detailed study of the geothermal features. The pictures taken from the helium balloon at ca. 50 m altitude with a digital camera, set on autoshot (one picture every minute), however, showed an excellent resolution (ca. 5 cm). Wind conditions had a severe effect on the balloon efficiency, more helium than the 2m3 used in this study is recommended for future survey. With the help of the 102 reference crosses marked on the ground in a 28×28 m raster and surveyed with differential GPS and theodolith the pictures taken from the balloon were georeferenced and combined to a mosaic with the GIS program TNTmips (Microimages). The total area of approximately 450×150 m was created from approximately 40 single pictures. Additionally, thermal pictures were taken on the ground and from the airplane at 2,000 ft as an overview. To georeference those pictures, normal digital pictures were taken parallel from a camera mounted on the same axis as the thermal camera. Ground survey included a detailed description of approximately 100 features, which will be linked to the thermal features digitalized from the created mosaic. All maps and information will be combined in a digital atlas and published together with a written report about the processing of the pictures, evaluation of the balloon method and recommendations for future use in Juliane Becker's master thesis (to be completed July 2004).

Project title: Remote Sensing of Non-Forest Vegetation in the Northern Range of Yellowstone National Park

Principal investigator: Ms. Shannon Savage
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Address: PO Box 168, Yellowstone National Park, WY 82190
Report number: 27551
Co-investigators: Rick Lawrence, Ann Rodman

Purpose: There is a need for a vegetation map of rangeland in the northern range of Yellowstone National Park that is more detailed than the current parkwide information. In addition, this map must be accurate and more flexible than the current map. The intended results of this project will be a detailed current rangeland/riparian vegetation map meeting USGS mapping accuracy standards. The map will be attributed in a hierarchical manner to enable flexible outputs meeting the needs of diverse research and management interests. In addition, a method for easily updating this map with any changes on an annual basis (using Landsat satellite imagery) will be developed. These data will be available to researchers and managers interested in the northern range. Also, change detection from as far back as 20 years ago might assist analysis of changing vegetation patterns and assist modeling potential vegetation changes in the future.

Findings: The majority of the activity for this report year was in literature reviews and research into remote sensing methods that would best accomplish the objectives of the study. Some progress was made in determining which methods will not work, and identifying methods that will potentially work. No new final data were created, nor was any field work done.

Geomorphology

Project title: Holocene and Modern Geomorphic Response to Fires, Floods, and Climate Change in Yellowstone National Park; Natural and Anthropogenic Influences on Stream Systems

Principal investigator: Dr. Grant Meyer
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Report number: 27161
Co-investigators: Lyman Persico, Paula M. Watt

Purpose: To provide a long-term perspective on the geomorphic impacts of the 1988 Yellowstone fires, we are investigating Holocene sedimentation in northeast Yellowstone, using post-1988 fire-related events as a guide for interpreting alluvial fan stratigraphy. Comparison of the timing of fire-related events with climate proxy records elucidates the relative controls of climate, fire, and intrinsic geomorphic thresholds on alluvial systems. We are documenting extreme floods of the last ~300 years and their effects on valley floor landscapes of northeast Yellowstone. Recent changes in stream channels seen through analysis of air-photos, historical photos, floodplain stratigraphy, and resurveying are evaluated in the context of flood history, riparian vegetation, ungulate browsing, and intrinsic characteristics of basins and channels. A related component study uses the record of beaver activity and stream dynamics contained within radiocarbon-datable beaver pond sediments to understand how Holocene environmental changes have affected beavers and their stream habitats in the area of Yellowstone's northern range. Part of this effort lies in quantification of both the geomorphic context of past beaver dam sites (e.g., valley width, slope; contributing basin area; stream power) as well as the geomorphic effects of beaver activity (e.g., thickness of postglacial fill; texture and composition of beaver-related floodplain sediments; channel characteristics in beaver-influenced vs. non-influenced reaches, including changes after abandonment of dams and ponds. We are also studying a 1950 dam failure at Cooke City, Mont. that deposited acidic, metals-rich mine tailings along the Soda Butte Creek floodplain.

Findings: Field work in 2003 consisted primarily of reconnaissance work on the beaver pond geomorphology study, including location of past dam sites and pond sediment stratigraphic exposures along Tower, Lost, Elk, Yancey's, Oxbow, Geode, and Blacktail Deer Creeks for future detailed study.

Project title: Quaternary Geology and Geoecology of the Greater Yellowstone Area

Principal investigator: Dr. Kenneth Pierce
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Report number: 28867
Co-investigators: Don Despain, John Good, Joe Licciardi

Purpose: To document and determine the Quaternary geology and history of Yellowstone, and the relation between the Quaternary geology and ecology of Yellowstone, and neotectonic features of Yellowstone, including faulting and caldera unrest based on Yellowstone Lake and River level changes.

Findings: A field trip and publication titled "Quaternary Geology and Ecology of the Greater Yellowstone Area" was led as part of the International Union for Quaternary Research (INQUA) meeting in Reno, Nevada. Seven leaders spent seven days leading the field trip, summarized in a 31-page field guide. A major theme was the synthesis of the glacial geology of the greater Yellowstone area, particularly the Pleistocene glacial flow northward down the Yellowstone Vallley past Chico Hot Springs, west to the West Yellowstone basin (Pinedale and Bull Lake) and south into Jackson Hole (Pinedale and Bull Lake). The relation of the surficial geology to the ecology was interpreted at most stops, primarily by Don Despain. For a volume on the Quaternary Geology of the United States, a chapter titled "Pleistocene Glaciations of the Rocky Mountains" was published, summing knowledge of the age and dynamics of the greater Yellowstone glacial system, including the problem that cosmogenic ages indicate a chronology several thousand years younger than radiocarbon ages. The report "Post-Glacial Inflation-Deflation Cycles, Tilting, and Faulting in the Yellowstone Caldera Based on Yellowstone Lake Shorelines" was returned from editing, and is nearer publication. as a chapter in a USGS professional paper.

Herpetology

Project title: Amphibian and Reptile Inventory and Monitoring: Greater Yellowstone Network

Principal investigator: Dr. Charles Peterson
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Report number: 27623
Co-investigators: Paul Bartelt, Char Corkran, P. Stephen Corn, Erin Muths, Debra Patla, David Pilliod

Purpose: Assess amphibian status, distribution, and trends in YELL and GRTE. This project is jointly supported by USGS Amphibian Research and Monitoring Initiative and NPS GRYN Inventory and Monitoring Project. (1) Continue testing and establishment of an amphibian monitoring program on Department of Interior lands in the Greater Yellowstone Ecosystem (YELL and GRTE), to be part of a transect of USGS monitoring areas in the Rocky Mountains, from Glacier National Park to Rocky Mountain National Park. Provide baseline and inventory data for NPS Inventory & Monitoring Program. (2) Collect data for evaluation of the Proportion of Area Occupied estimator, which is an integral part of assessing amphibian population status and trends. (3) Continue long-term monitoring of a Columbia Spotted Frog population at Lodge Creek. (4) Continue monitoring at a subset of previously-identified Boreal Toad breeding sites. (5) Conduct targeted surveys for rare reptile species such as the Eastern Yellow-bellied Racer. (6) Model terrestrial habitats of amphibians in Yellowstone using physical models to measure thermal and evaporative gradients among four different habitats (willow, sedge/tall grass, sagebrush, and forest) in the Lamar Valley.

Findings: (1) We conducted amphibian visual encounter surveys in YELL within four catchments (watershed units) in 2003. Since this type of monitoring began in 2000, we have conducted surveys in 19 catchments at 466 sites in YELL. At least one amphibian (of any life stage) was found at approximately 70% of the sites surveyed (all years summed). Amphibians were found in all catchments. The most widely distributed species (2000–2003) was the Columbia Spotted Frog, found breeding in 89% of the catchments (17 of 19) and present in all 19 catchments. This was followed by the Boreal Chorus Frog (breeding in 74% of the catchments, present in 84%); the Blotched Tiger Salamander (breeding in 58% of the catchments, present in 68%), and Boreal Toad (breeding in 26% of the catchments, present in 37%). In all four years, relative abundance of the numbers of active breeding sites found per species was quite consistent despite the great variety of terrain sampled each year, with Boreal Chorus Frog breeding sites the most numerous, and Boreal Toad the least. In three of the four years, more Columbia Spotted Frog breeding sites were found than Blotched Tiger Salamander breeding sites. (2) PAO (proportion of area occupied) methodology provides estimates of occupancy rates (based on breeding sites) that are unbiased by the variable detectability of species. Boreal Chorus Frog breeding sites had the highest PAO for both years in YELL: 52 % and 37% in 2002 and 2003; Blotched Tiger Salamander PAO: 21%

and 28% in 2002 and 2003; Columbia Spotted Frog PAO: 27% and 15% in 2002 and 2003; Boreal Toad PAO: 4% and 2% in 2002 and 2003. (3) Columbia Spotted Frog population, Lodge Creek: The number of egg masses was higher than previous years, due mainly to a large reproductive effort at the lagoon at the mouth of Lodge Creek. Of three breeding sites: one produced metamorphs; one experienced total loss of the tadpole population when the wetland dried up in July; and one (the lagoon) produced a large number of abnormal metamorphs, with swellings, ulceration, and bloating. Preliminary diagnosis for the abnormalities (provided by USGS National Wildlife Health Center) is encysted parasites, family *Diplostomatidae*. (4) Boreal Toad monitoring in 2003: nine previously identified breeding sites were checked; seven were active. One new (previously undocumented) breeding population was detected during surveys. (5) Reptiles: Searches for Northern Sagebrush Lizard, Rubber Boa, Eastern Yellow-Bellied Racer Bullsnake, and Prairie Rattlesnake have been conducted (2000–2003), concentrated in lower elevation portions of YELL (northwest corner) and thermal areas, in the vicinity of historical and more recent observations. Observations were documented for all these species except racers. Wandering Gartersnakes and Valley Gartersnakes were documented during amphibian surveys. (6) Using the physical models, we found that sagebrush had the greatest evaporation rates; models in this habitat evaporated about 60 g (shaded) to 80 g (exposed) of water per day. Exposed models in the sedge/grass habitats evaporated about 35 g of water per day, and models in the open forest evaporated about 25 g of water per day. Shaded models in the willow habitat had the smallest rates of evaporation; these models evaporated about 15 g of water per day. These data were critical for calibrating the mechanistic model for amphibians against variations in vegetative cover across the Yellowstone landscape.

Project title: Gene Flow among Tiger Salamander Populations (*Ambystoma tigrinum melanostictum*) at a Local Spatial Scale in Yellowstone National Park

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Address: Biological Sciences, Campus Box 8007, Idaho State University, Pocatello, ID 83209
Report number: 28710
Co-investigators: Denim Jochimsen, Stephen Spear, Andrew Storfer

Purpose: The main purpose of this study is to investigate population structure in tiger salamanders across the northern part of Yellowstone National Park. This will be done by estimation of gene flow using microsatellite markers. Additionally, I will examine whether landscape characteristics are correlated with salamander gene flow.

Findings: The effect of landscape variables on the genetic structure of tiger salamander populations in northern Yellowstone was studied based on samples taken from ten sites in 2002 and 2003. Measures of genetic diversity and population subdivision were inferred from eight microsatellite loci. Genetic diversity was measured using allelic richness, expected heterozygosity, and the inbreeding coefficient, FIS. The level of diversity was also used to test for evidence of recent population declines. Population subdivision was determined through statistics such as FST and RST, analysis of molecular variance (AMOVA), and assignment tests. Landscape variables such as topographical distance, wetland likelihood, rivers and cover type, as well as presence of perennibranchiate individuals were then tested for correlations with the genetic data using a regression approach. Specifically, we tested the correlation of landscape with inbreeding and with gene flow. For the gene flow analysis, we created five different hypothetical routes to connect each pair of sites. These routes were straight-line, stepping-stone, least slope, maximum wetland likelihood, and combination slope/wetland likelihood. Overall, there was low genetic diversity and high population subdivision across the study area. Allelic richness ranged from 1.86–2.26 per site (averaged over eight loci) and expected heterozygosity ranged from 0.21–0.37 per site. Inbreeding varied among sites, with sites in the Gardiner and Mount Everts areas having significant levels of inbreeding. Seven of the ten sampled sites demonstrated evidence of a recent decline in effective population size, based on the genetic data. All pairwise site comparisons greater than one kilometer were genetically differentiated from one another, and the global; was 0.24, indicating high population structuring. Also, sites in the Gardiner area had very high levels of genetic differentiation compared to the rest of the sites in the study area. The landscape variables accounted for a great deal of the variation in gene flow. The best model was the route representing the straight-line path, which had a model of 0.828. This model included topographical distance, elevational difference, fire-regenerated open shrub cover, and river crossings as significant variables. Topographical distance and elevational difference were negative predictors of gene flow, whereas fire-regenerated open shrub and rivers were positive predictors. However, none of the landscape variables we tested were significantly correlated with differential levels of inbreeding among sites.

INTERPRETATION

Project title: Collection for Interpretive Educational Programs

Principal investigator: Ms. Judith Knuth Folts
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Address: PO Box 168, Yellowstone National Park, WY 82190
Report number: 27021
Co-investigators:

Purpose: This permit would: (1) provide visitor education on cultural and natural history interpretive themes at Yellowstone National Park through the use of interpretive props by the Division of Interpretation. Collected items for use may include rocks, antlers, bones, fur, feathers, scat, etc. (2) allow greater opportunities for interpretive park rangers to connect with visitors on park critical resources and management activities thus engendering a greater understanding and appreciation of the park's significance.

Findings: During 2003, the Division of Interpretation completed 5,857 formal interpretive programs, 196 curriculum-based education programs, and 13,990 hours of critical resource roves. More than 524,000 visitors attended the walks, talks, hikes, evening campfire programs, and curriculum-based education programs, or they talked to interpretive park rangers on informal rove assignments at campgrounds, pullouts, or wildlife jams. 2,610,285 visitors were contacted at the park's nine visitor centers/contact stations and four winter warming huts. All interpretive park rangers used various field specimens as props for these programs. These tangible aids assisted visitors in learning about the park's resources, protection methods, and visitor/wildlife interactions. Props are critical to our ability to engender a stewardship for America's national parks.

Project title: Xanterra Road Base Tours: Douglas-fir Cone Story

Principal investigator: Ms. Madeline Savko
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e-mail:
Address: PO Box 2061, Yellowstone National Park, WY 82190-2061
Report number: 28907
Co-investigators: Leslie Quinn

Purpose: Through interpretation and storytelling, the investigator wishes to demonstrate and teach visitors how to identify Douglas-fir pine cones. Also wishes to wishes to use other natural resource specimens as teaching aids.

Findings: No activity was conducted this report year.

INVERTEBRATES

Project title: Butterflies of Yellowstone and Grand Teton National Parks (also *odonata*)

Principal investigator: Mr. Richard Lund
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Report number: 27699
Co-investigators: Mardell Moore

Purpose: To produce field guides about the insects of Yellowstone and Grand Teton National Parks. Photographs need to be taken of all species in the parks. Only data and photos need be obtained. No specimens are collected. All specimens are safely netted, photographed, and released live in the area of the park where they were discovered by the researchers.

Findings: In addition to photographing adult butterflies, dragonflies, and damselflies, researchers photographed dragonfly and damselfly nymphs or larvae. A simple strainer was used to sample the cold water creeks that flow into Lake Yellowstone, for example, and several photographs were taken of damselfly and dragonfly nymphs in various instars. Researchers were surprised to find few nymphs in thermal fed creeks as adult dragonflies and damselflies were often active and numerous in this area during the summer season. Slides will be made available to the park upon the opening of the museum at its new location.

Project title: Parasites of Mosquitoes in Yellowstone National Park

Principal investigator: Dr. Edward G. Platzer
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Report number: 26795
Co-investigators: Bradley C. Hyman

Purpose: The purpose of this project is to investigate the characteristics of mermithid nematodes reported by previous investigators in mosquitoes of Yellowstone National Park. In 1988 through 1991, Blackmore (*Canadian J. Zoology* 67:1725, 1988), Blackmore and Charnov (*American Naturalist* 134:817, 1989), Blackmore and Nielsen (*J. American Mosquito Control Association* 6:229, 1990), and Blackmore (*Zoolische Anzieger* 226:319, 1991) reported the presence of a species of *Romanomermis* in several mosquito species from snowmelt pools from Grebe Lake and DeLacy Creek in Yellowstone National Park. Although the nematodes were morphologically similar to *Romanomermis nielseni* described from mosquitoes from Lonetree, Wyoming, the authors were uncertain about the specific designation for these nematodes. During past several years, Dr. Bradley Hyman and I have initiated a study of the biogeography of mermithid nematodes in mosquitoes of North America. Earlier, we described the unique mitochondrial DNA from *R. culicivorax* (*Current Genetics* 11:71, 1986) and are currently investigating the relationships in the genus *Romanomermis* with the use of mitochondrial DNA and ribosomal DNA. We have compared *R. culicivorax* from Louisana and *R. nielseni* from Lonetree, Wyoming, and found unique differences on the basis of molecular properties. The possibility of obtaining further populations of *R. nielseni* or a related species from Yellowstone National Park that have been reproductively isolated for long periods of time has the potential to aid enormously in the interpretation of the evolution of this interesting parasite of mosquitoes. This mermithid nematode is parasitic only in the larval stages of the mosquito host so this places severe constraints on the distribution on the parasite. At this juncture, we believe that the nematode distribution is dependent on either flooding or transport by water birds. In the case of the Yellowstone populations, the two known sites are separated by the continental divide and, if occupied by the same species, then it is implicit that transport must have involved the mediation of water birds. We hope to test this hypothesis, in part, through both morphologic and molecular studies.

The study will be carried out by collecting infected mosquitoes and developing adult nematodes from snowmelt pools at Grebe Lake and DeLacy Creek. We plan to visit the sites during June through early July for the collection of larval mosquitoes. Larval mosquitoes will be collected with the use of a standard mosquito dipper and transported in a refrigerated container to our laboratories at the University of California, Riverside, where the mosquitoes will be dissected to recover the nematodes. In addition, we plan to visit the sites in later parts of the summer to recover developing adult stages of the nematodes from the soil at the bottom of the snowmelt pools. This will be done with the use of small soil samplers and approximately 100 milliliters of soil will be taken per sample. We estimate that 5–10 samples will be taken per site. The nematodes will be extracted from the soil samples at our laboratories. Nematodes will be studied by both morphologic and molecular procedures. For morphology, the nematodes will be preserved in fixatives for both light and scanning electron microscopy. For molecular studies, DNA will be extracted and used as a template for molecular probes designed in Dr. Hyman's laboratory for mitochondrial and ribosomal DNA.

Findings: No activity was conducted this report year.

Natural Resource Monitoring

Project title: A Remote Sensing and GIS-Based Model of Habitat as a Predictor of Biodiversity

Principal investigator: Dr. Diane Debinski
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Report number: 27127
Co-investigators: Kelly Kindscher

Purpose: The major objectives of the research are to (1) Quantify the spatial and temporal variability in montane meadows; (2) Develop a spectrally-based spatially-explicit model for predicting plant and animal (butterflies and birds) species diversity patterns in montane meadows; and (3) Test the spectrally-based spatially explicit model developed in Objective 2 for predicting plant and animal species diversity patterns in montane meadows.

Findings: No surveys were conducted in Yellowstone National Park sites during 2003 due to limited funding. Surveys were conducted in Grand Teton National Park sites.

Project title: Quantification of Non-Invasive Population Sampling Biases: A Comparison of Genetic and Independent Estimates for Cougars in Yellowstone National Park

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Purpose: Many carnivores, including cougars (*Puma concolor*), are difficult to study due to their low densities and secretive nature. Estimating population size is important to the conservation and management of most carnivore species. Currently, no reliable methods of estimating cougar population size exist other than radio collaring, which is intrusive and expensive. Non-intrusive genetic sampling (NGS) has great potential as a tool for population enumeration and monitoring, but has not been adequately developed to date for cougars. The purpose of this study is to quantify the biases associated with non-intrusive genetic sampling of cougars by comparing results from genetic sampling with results obtained through capture work and track surveys. Two methods of sampling will be used to collect hair samples: hair snagging and backtracking to find bed sites. Scat will be collected by backtracking to find kill sites. Cost/effort of these methods will also be evaluated. The overall goal of the study is to provide YNP (and wildlife managers elsewhere) with a reliable long-term population monitoring tool for cougars. Specific objectives include: (1) Compare two non-invasive sampling techniques for their potential to yield samples and amplify micro satellites for fingerprinting individual cougars and provide, in combination or in exclusion of each method, an estimate of cougar population size as compared to a known population. (2) Evaluate the assumptions associated with, and efficacy of, fecal and hair DNA sampling technology by quantifying the effect of sampling intensity, rates of dropout and misprint genotyping errors and influence of such errors on estimates of population size and trend, rates of dropout and misprint genotyping errors and influence of such errors on estimates of population size and trend, movement information on assumptions of geographic and demographic closure, and biases associated with sex/age heterogeneity of "capture." (3) Evaluate the influence of resources (cost and effort) on population estimation results for standard grid, double intensity grid and each sampling method (hair snares and fecal/ bed site hair sampling). (4) Provide results and recommendations to Yellowstone National Park as potential monitoring technique for cougars, and potentially bobcats, and to state agencies across the west to evaluate, modify, and implement current and future monitoring for cougars and other carnivore species. Other study objectives include: (1) evaluating the effect of varying grid size for these hair-snagging stations on population estimates, (2) quantifying genotyping error rates by comparing non-intrusively collected samples to blood and tissue samples taken during capture, and (3) analyzing hair and scat (DNA) samples to identify species and individual-specific information on cougars. In addition to oversight and some field effort provided by the principal investigator, this project will require one technician (Master's student) and three volunteers.

Our study is designed with the following products in mind: provide scientific information regarding population monitoring of carnivores which addresses sampling assumptions and biases that influence resulting inferences (this information could have global application to carnivores); evaluation of capture-recapture models based on sampling methods, sampling intensity, and influence of biases; and provide YNP and state agencies with an assessment of and potential method (or methods) for monitoring cougar population size and trend. If we produce a population estimate that is comparable to our independent population estimate, the information may be used as an index of comparison for future genetic sampling efforts. The final products will be important information disseminated to park managers, planners, Montana Fish, Wildlife and Parks, annual and final reports, and popular and technical publications. This study could provide a potentially low-cost, long-term, population-monitoring tool for YNP and other wildlife managers, thus reducing the need for capturing and collaring cougars and consequently reducing park over-flights. The results could also have global application for non-intrusive monitoring of elusive carnivore species, although they will not be applicable everywhere.

Findings: The Yellowstone Cougar Project has radio-marked approximately 87% of the resident adult cougar population and has collected blood from all captured individuals (n=68 as of winter 2002–2003). Therefore, the YNP Cougar Project provides a unique situation in which to test and develop NGS methods. In January 2003, we initiated a study to test and develop NGS methods. Backtracking was used to find scat at kill sites and hair at bed sites. Hair was also collected through the use of hair-snagging stations. During the first sampling period, January–March 2003, field crews established and maintained 365 hair-pad stations and conducted track surveys covering over 1,200 km. During this time, 51 hair samples and 16 scat samples were collected. Twenty-five hair samples were collected through capture efforts, 7 were collected opportunistically, 22 samples were collected from hair-pad stations, and 12 samples were collected through snow backtracking. Of the 16 scat samples collected, 4 were collected through snow backtracking.

Ornithology

Project title: Monitoring Wyoming's Birds

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Co-investigators: Ken Behrens, Bill Reddinger

Purpose: To date, resource managers have relied on data derived from the Breeding Bird Survey (BBS), currently the best and most extensive bird-monitoring program, to monitor bird populations. However, many species and habitats are inadequately sampled by the BBS. Additionally, the design and implementation of the BBS is such that results generated from these efforts are often inconclusive due to the difficulty associated with interpreting index counts and numerous confounding variables. For these reasons, BBS data are generally insufficient to guide local or regional management decisions. Several authors have suggested implementing regional habitat-based bird monitoring programs to complement data generated by BBS. Rocky Mountain Bird Observatory along with its partners have developed a successful program in Colorado which has been adopted for Wyoming. This program is Monitoring Wyoming's Birds (MWB). MWB is designed to provide population trend or status data on all regularly-occurring breeding species in the state. A total of 246 species of birds has bred in Wyoming. The first phase of MWB is to ensure that count-based data are obtained for all species that can be monitored effectively through a habitat-based approach, and that species-specific tracking or census programs are employed for those species requiring more specialized techniques.

Findings: This report provides information on birds observed on surveys conducted within Yellowstone National Park as part of the larger statewide program, Monitoring Wyoming's Birds. Habitats. Within Yellowstone National Park, we sampled bird populations from two habitats: Mid-elevation Conifer and Montane Riparian. Below are descriptions for these two habitats based on GAP coding and the Wyoming Bird Conservation Plan. Mid-elevation Conifer. This habitat generally contains several conifer species in either pure or mixed stands. Tree species include Douglas-fir (*Pseudotsuga menziesii*), blue spruce (*Picea pungens*), lodgepole pine (*Pinus contortal*), limber pine (*Pinus flexilis*), ponderosa pine (*Pinus ponderosa*), and occasionally has an aspen component. This is the dominant forest habitat in Wyoming and occurs in all major mountain ranges, except in the far northeast corner of the state. GAP codes: 42003, 42004, 42009, 42016, 42001 (between 7,000 and 8,500 feet). Montane Riparian. This habitat is associated with higher-elevation rivers and streams where willow is the dominant woody cover. This habitat's transects focus on the suite of bird species dependent on willows as a nesting substrate. However, these areas tend to be linear and narrow in nature, so the surrounding forest type usually influences species recorded. GAP codes: 61001, 62001, 62003 (above 7,500 feet). Methods.

We conducted point transects according to protocol established by Leukering et al. Point transects consisted of 15 point counts spaced 250 meters apart. At each point count, all birds detected were recorded, as well as an estimated distance from point to each bird in the five-minute count period. Transect Selection. Stand selection depended on initial GAP Analysis Land Cover data with secondary ground-truthing during the field season by the technician responsible for that transect.

Transect Name	**Location**	**Habitat**
MC02	Kepler Cascades	Mid-elev. Conifer
MC50	Cascades Picnic Ground	Mid-elev. Conifer
MR22	Slough Creek Trail	Montane Riparian
MR97	Grayling Creek	Montane Riparian
MR98	Grassy Lake Reservoir	Montane Riparian

We conducted a total of 75 point counts along 5 point transects in two habitats. We detected a total of 400 birds of 54 species. Ruby-crowned Kinglet had the most detections with 49 individuals recorded. Only four species were recorded on each transect: American Robin, Yellow-rumped Warbler, Chipping Sparrow, and Lincoln's Sparrow.

Project title: Birds of Forested Landscapes

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Co-investigators: Eric Hendrixson

Purpose: Check for presence of breeding populations and habitat requirements of Veery, Lewis's, Woodpecker, Williamson's Sapsucker, Willow Flycatcher, Olive-Sided Flycatcher.

Findings: None of the target species were observed.

Paleoecology

Project title: Niche Partitioning in Late Pleistocene Mammals of North America: A Test Combining Isotopic and Morphologic Data

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Purpose: Niche partitioning influences ecosystem diversity by affecting competition and allowing for sympatry of species. This project will examine the extent to which niches, defined by body size, shape, and diet, change on the thousand year to million year time scale for certain ungulates. The project will test three main hypotheses: (1) the niches of ungulates are climatically controlled; (2) niche relationships between taxa are unvarying over time; and (3) niche overlap increased from the last glacial maximum to the Holocene influencing the extinction of taxa. The study will focus on specimens from Rancho La Brea (Calif.; ~30ka–11ka), four sites over 2 million years old from Florida and California, and Yellowstone National Park (modern). Morphological and dietary data will be gathered from taxa including *Bison, Equus,* and *Cervus* by taking cranial, post-cranial, and stable isotope measurements. The project differs from other studies in that morphology and diet will be analyzed simultaneously, identifying niche space over time, and the interpretation of fossil data will be buttressed by a detailed study of modern analogs. Besides addressing the hypotheses above, the study is significant in providing detailed paleoecological information for five important fossil localities, and in providing new teaching tools and research applications for the uses of isotopes in vertebrate paleoecology.

Findings: Fecal material was collected for five of the seven large ungulate genera within Yellowstone National Park during August 2003. Included were *Bison* (bison, ~20 samples), *Antilocapra* (antelope, ~20 samples), *Odocoileus* (deer, ~15 samples), *Cervus* (elk, ~20 samples), and *Alces* (Moose, 1 sample), while *Oreamnos* and *Ovis* could not be found. Most of these samples were fresh to insure correct identification. These samples are currently being freeze dried and prepared for isotopic analysis as of March 5, 2004. Carbon and nitrogen isotope data should be available by late spring 2004. Many of the samples were collected in the northern portion of the park (northern range). However, the deer samples were mainly taken from the Lake and Fishing Bridge areas. Antelope samples were mainly taken from Mt. Everts and the surrounding areas. Bison samples were mainly taken from the Hayden Valley area. Elk was collected all over the park. These samples will be compared to a sample collected in summer 2002 to determine if there is any variation in diet from year to year being picked up by the carbon and nitrogen isotopes. The summer samples will also be compared to a winter sample of the same animals to be collected during winter 2004.

Analyses on the 2002 samples yielded data after the 2002 IAR was reported. Results showed that stable carbon isotopes showed significant differences among many of the large herbivores. Both inter-generic and intra-generic comparisons were made using the fecal and tooth enamel isotope values. The carbon isotope values suggest that the tooth enamel and scat samples faithfully record predominantly C3 forage for the five ungulate genera. Genera that live in a more closed habitat, such as *Cervus* and *Odocoileus,* appear to have more negative carbon isotope values than do open habitat animals such as Bison signifying that resource partitioning in either forage or habitat is possible to ascertain. This study suggests that resource partitioning among ungulate genera is possible to distinguish in predominantly C3 environments by the analysis of stable isotope values. This study can be used as a model for understanding resource use and partitioning among fossil taxa in predominantly C3 environments such as those prior to the C4 global carbon shift that occurred 7 million years ago worldwide.

Similar analyses will be performed on the samples collected in summer 2003, and will be reported on in the IAR for 2004. As stated above, these results provide a base-line for comparison to fossil localities. Currently, four different fossil localities are being analyzed to determine whether similar patterns of diet among ungulates is observed on evolutionary time scales. The fossil sites, two from California and two from Florida, are all over 2 million years old. The Yellowstone sample provides invaluable information as a modern comparison to this study.

Project title: Evolution and Ecology of Vertebrates of Yellowstone National Park

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Purpose: Our objective was to examine the influence of parent material on soil and ecosystem properties. Specifically, we addressed the question, do rocks provide a majority of cations to ecosystems on 10ky and 150ky substrates in continental montane environments, or does atmospheric deposition swamp bedrock inputs during this time?

Findings: The development of soils plays an important role in the structure and function of ecosystems. However, most chronosequences that have been used to elucidate changes in soils with time have intentionally been located on a single rock substrate. Unfortunately, this precludes examination of the influence of parent material on soil and ecosystem properties in most continental settings. On the continents, long-term atmospheric inputs are difficult to detect because atmospheric inputs can be similar in chemistry and mineralogy to bedrock. To address this problem, we established a chronosequence of bedrock contacts in and around Yellowstone and Grand Teton national parks. We compared sites glaciated during the Bull Lake glaciation (150ky) and not reglaciated to sites that were glaciated during the Pinedale glaciation (10ky). Selecting these sites allowed us to address the following question: do rocks provide a majority of cations to ecosystems on 10ky and 150ky substrates in continental montane environments, or does atmospheric deposition swamp bedrock inputs during this time?

This question has been difficult to answer, because atmospheric inputs on continents are largely influenced by continental material nearby. Thus, tracing granite dust that has blown from California to Wyoming is difficult if the bedrock in Wyoming is also granite. Furthermore, the variety of potential dust sources on a continent make spatial separation (almost a necessity for a chronosequence that substitutes space for time) problematic. The selection of bedrock contacts allowed us to circumvent this problem. By examining plants on either side of bedrock contacts (but within a few meters of each other), we were able to ensure that the ecosystems on either side of the contact was receiving the same atmospheric inputs. By examining a variety of such contacts in a 10ky ecosystem (Lamar Valley, Yellowstone National Park) and a 150ky ecosystem (Sleeping Indian Range, Jackson, Wyoming), we were able to ascertain the relative importance of rocks and atmospheric inputs to ecosystems properties in different aged landscapes. We collected small rock samples from three locations in the Lamar Valley: the top of Junction Butte, upstream from the Pebble Creek campground, and near the confluence of Soda Butte Creek and Lamar River.

We used strontium (Sr) isotopes as a tracer of cation provenance. Because of its similar charge and size, Sr is a useful tracer for calcium, and has been used as a rough proxy for other putatively rock-derived elements. The Sr isotopic signature of a rock is a function of its age and composition. Because strontium is not measurably fractionated by biological properties, the strontium signature of a plant will reflect the available strontium in the soil. This, in turn, will be a mixture of bedrock and atmospherically-derived strontium. By examining plants on both sides of a bedrock contact, we were able to determine the relative importance of rock vs. atmospheric strontium without knowing a priori the Sr isotopic signature of the atmospheric endmember.

We hypothesized that rock-derived Sr would dominate the signature of plants on 10ky substrate. Thus, plants in this landscape would have a Sr isotopic signature (expressed as 87Sr/86Sr) very similar to the rock on which they are growing and very different from other plants on other nearby substrates. However, we reasoned that in an older (150ky) landscape, atmospheric inputs would have begun to swamp the rock signature. As a result, the 87Sr/86Sr of plants in this aged landscape would be different from the rocks on which they were growing, but similar to other plants on similar aged substrate. Although our data at this point are very preliminary, it appears that these hypotheses were correct. The one exception is plants going on granite in the 10ky landscape, and this is likely to be the result of deposition of glacial till on the granite substrate.

Project title: A Continuing Investigation of the Eocene Palynoflora of the Yancey Creek Drainage Basin, Yellowstone National Park, Wyoming

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Purpose: The purpose of this study is to further examine the palynoflora of the Eocene Sepulcher Formation from a location near Yancey Creek, in the northern part of Yellowstone National Park. This is a continuation of a preliminary investigation that hopes to document the presence of fossil pollen and spores at this site. The long range goal of this project includes extensive sampling resulting in more detailed floristic, paleoecological and stratigraphic analysis then is presently known.

Findings: On June 25, 2003, seven undergraduate students in the Eastern Illinois University Geology Field Camp accompanied the Principal Investigator into the Yancey Creek drainage basin, near Lost Lake, northern Yellowstone National Park. Four small, fist size, rock samples were collected for palynological analysis. The brown volcaniclastic sandstone samples were obtained from float and an outcrop of the Eocene Sepulcher Formation. These specimens are on the Eastern Illinois University campus and are awaiting processing to extract the fossil pollen. Once slides containing the pollen and spores are made, the grains will be identified and the palynomorph assemblage floristically interpreted and paleoclimatic inferences determined.

Project title: Climate Effects on Small Mammals: A Multi-scale Approach to the Study of Mammalian Response to Global Climate Change

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Purpose: Our ability to predict the effects of global warming on biotic communities ultimately depends on our understanding of how individual species track specific climate variable at multiple scales. The purpose of this study is to gain insight into biotic response to climate change by characterizing the effects of climate on ground squirrel distribution, body size and population dynamics. Specifically, I hypothesize that temperature limits the distribution of Sp*ermophilus armatus.* To test this I will examine both present and historical patterns of distribution. First, I will use GIS data in conjunction with digitized locality data to perform statistical correlation analyses to determine if temperature correlates with the present distributional boundaries of the species. Second, to determine if there has been local extirpation and /or species replacement in response to the climate fluctuation of the late Holocene, I will perform phylogenetic analyses of ancient DNA sequences from specimens excavated from Lamar cave, a paleontological site in Yellowstone National Park. I also hypothesize that the effects of temperature will affect ground squirrel biology at the population level. I predict that in addition to range shifts, one of the way I which ground squirrels respond to changes in temperature is by proximate an local changes in body size. I will test this in two ways. First I will examine the population level, phenotypic response of *S. armatus* to changes in the regional climate during the late Holocene by tracking changes in the body size of specimens from Lamar Cave. I will also study the distribution of body size along an elevational gradient. Changes in body size have direct and predictable effects on life history characteristics and thus on population dynamics. In order to gain insight into the mechanistic processes that explain these broader patterns of response I will develop and test a model that examines how the energetics of the individual are effected by the local thermal environment. Ground squirrel of the genus *Spermophilus* are particularly useful for studying biotic response to climate change because they are obligate hibernators and they show sensitivity to environment cues such as temperature. In addition, ground squirrels do not migrate therefore changes in abundance, distribution or the timing of life history events reflect response to local climate phenomena. Ground squirrels are also a vital link in terrestrial trophic interactions. As such, changes in ground squirrel abundance and distribution are likely to affect other species that prey on them. Thus, ground squirrels may prove to be a useful indicator species for tracking the effects of current climate change at the local level and for predicting the effects of climate change on the community.

Findings: As part of an effort to better understand how microclimate affects the body size, distribution, and population density of small mammals, we trapped, measured and marked individual ground squirrels from four sites along an elevational (and thus temperature) gradient. Our field research in Yellowstone National Park was conducted intermittently from April 6 to August 25, 2003. Three of our sites were within the boundaries of the park. At each site we set up a trapping grid of 150×245' and laid out 21–40 Sherman folding live traps (XLF 15). Each captured animal was given a unique ear tag number. Sex, estimated age, and standard body size measurements including body mass, total length, tail length, hind foot, ear from notch, forearm length and zygomatic width were taken for each animal captured. Body mass was recorded every time an animal was captured. Microclimate data was collected at each site using a Hobo Pro Series Temp/RH. Climate data collected included ambient air temperature six inches above the ground level (ground squirrel height), and relative humidity. We also collected genetic samples from ear snips of every individual captured to use in a genetic analysis of the genetic diversity across the elevation gradient. Ear snipping is a low-impact, non-harmful method of genetic sampling that is widely used among mammalogists. The genetic data allow us to calculate effective population size, assess the level of genetic diversity presently represented at the sites and establish how much gene flow there is between populations. We conducted vegetational sampling to determine if resource availability differed between the sites and to quantify how much it changed throughout the active season within a site. The biomass of several vegetational characteristics was estimated for 50 vegetation plots along a transect that ran diagonally through the trapping grid. Three vegetational transects were conducted at each site during the active season: just after adult and yearling ground squirrel emergence, during the birthpulse, and just prior to immergence of the adult ground squirrels.

The following is a summary of our 2003 research with a brief description of our preliminary findings. Site one (Gardiner) is located about 0.5 miles from the park's North Entrance. The first ground squirrel emerged at this site on April 12. Over the course of the active season we caught 14 adults and yearlings and 15 juveniles. We trapped for a total of 5,280 trapping hours. We did not work at Site 2 (Lamar) this year. Site three (Lamar South) is located along the Lamar River near Bison crossing about one mile north of the northeast entrance road if you park near Junction Butte. The first ground squirrel emerged on April 6. We trapped for a total of 2,089 trapping hours and caught 56 adult and yearlings and 18 juveniles. To compare the morphologic measurements between sites, I performed a series of One-Way ANOVAs to test for significant differences in body size between the sites. Preliminary analysis of the morphologic data indicates that there is significant difference in the average body mass of ground squirrels between the four sites. Population density, sex ratio, and the overall length of the active season also differed between the sites. The genetic analyses indicate that all populations are *Spermophilus armatus* and that there is a large amount of genetic differentiation between the populations. The vegetational and microclimate analyses are still in progress.

This report summarizes what I have found from the last of three planned field seasons. I have completed the field work portion of my research and will spend the next year analyzing and writing up the results of the data I have collected. I will submit a complete and final report that includes all analyses related to the research I have done in Yellowstone National Park from June of 2001 through

August of 2003 by March 2005.

Project title: Postglacial Fire History and Its Relation to Long-Term Vegetational Changes in Yellowstone National Park

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Report number: 26523
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Purpose: The objective of this project is to study the climate, fire, and vegetation history of Yellowstone National Park. A network of pollen and plant macrofossil records from lakes and wetlands, spanning the last 14,000 years, has been examined from different environmental settings within the park. These data are used to reconstruct past changes in vegetation and climate. Information on past fire frequency is obtained from an analysis of particulate charcoal and lithologic variations in lake sediment cores. Interpretation of the paleofire records is based on a study of modern charcoal deposition into lake sediments and a comparison of charcoal and dendrochronological records for the last 750 years. In 2003, the primary objective was to analyze the environmental history of the last 2,000 years at Crevice Lake in northern Yellowstone Park. Crevice Lake has annually laminated sediments, and thus offers the opportunity to reconstruct past variations in fire, climate, vegetation, and limnology with high temporal precision. The Crevice Lake project involves scientists from the University of Oregon, University of Nebraska, and the U.S. Geological Survey.

Findings: Sediment cores from Crevice Lake were collected from the ice surface in February 2001. The cores, ca. 5–6 m in length, were taken from three locations at water depths of 27.5 m, and were transferred to University of Nebraska for refrigeration. Cores were sliced longitudinally and photographed. The lithology consists of laminated gyttja, with layers of marl and coarse sand. A volcanic ash, attributed to the eruption of Mount Mazama in southwestern Oregon, was present at 2.92 m depth. The bottom sediments consist of gravels and clays of late-glacial age. Distinctive sedimentary layers allow us to correlate among cores by tracing individual laminations. The similarity of the lithologic records taken from locations that are 50 m apart is quite impressive.

Six radiocarbon dates on plant macrofossils were submitted for age determinations. The results are: 1.15 m depth had a date of 2,999 cal yr BP; 1.374 m depth dates to 3,420 cal yr BP; 1.528 m depth dates to 3,759 cal yr BP; 1.635 m depth dates to 4,887 cal yr BP; 2.105 m dates to 5,544 cal yr BP; and 4.935 m dates to 11,426 cal yr BP. An age–depth model based on these dates suggests that the laminations present in most of the cores and <1 mm thick represent annual deposition. Sampling efforts are focusing on contiguous samples every 4–8 years.

This year, scientists on the project are working on the analysis of the pollen, charcoal, diatoms, stable isotopes, and sediment properties of the last 2,000 years. These data sets will be used to reconstruct past variations in vegetation, fire activity, paleolimnology, and erosion. We expect to have the first set of results in early summer. The fire and vegetation research is taking place at the University of Oregon, and we are currently processing pollen and charcoal samples spanning the last 2,000 years. Sediment analyses at the U.S. Geological Survey on samples spanning this same period are nearly finished. Diatom and isotopic analyses are being conducted at University of Nebraska. Other accomplishments of note are publication of papers on the relevance of long-term fire records for ecosystem management, and a paper describing current research and directions in fire history studies. The manuscript describing the Holocene fire, vegetation, and climate history of Yellowstone National Park, based on records at Cygnet and Slough Creek lakes, should be published this year in a long-awaited volume edited by Linda Wallace.

Vegetation

Project title: Ecology and Community Dynamics of Remnant Grasslands, Western Montana

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Purpose: One purpose of this study is to reinvestigate study plots that have been described in literature 20 or more years ago. We want to assess the amount and trajectory of vegetation change over that time period in the dry, mid-elevation grasslands of the intermountain region of western Montana. Changes will be assessed in relation to human development and to fire history in each plot. Therefore, our study design includes plots in both developed areas and natural areas, and burned and unburned areas throughout western Montana.

Findings: During the last year, we have been working on describing how *Pseudoroegneria spicata* and *Festuca spp.* bunchgrass communities have changed across western Montana during the past 30 years. We have compared plant community data collected in YNP in 2002 and data collected in Glacier NP from 1999 to 2001 with data collected by Mueggler and Stewart's field crews between 1971 and 1973. The frequencies of occurrence of bunchgrass plant species have been compared at several different spatial scales to determine (1) if change has occurred over the past 30 years, (2) if the scale of analysis affects perceptions of change, and (3) if the factors driving change differ across western Montana. Several different analytical tools have been used to explore whether these communities have changed significantly over time. Two-sample Wilcoxon rank sum tests were used to test whether there were significant differences between historic plots and contemporary plots in the frequency of occurrence of introduced species, annuals, grazing indicators, life forms, and diversity indices. Non-metric multidimensional scaling (NMS) was used as a multivariate analysis technique to integrate all composition factors and see if historic and current plots plotted in different areas of dimensional space and, if so, why. Factors that correlated with differences in position between historic and contemporary plots in dimensional space were analyzed using linear regression on the NMS scores. The south region, which includes YNP and the surrounding area, shows significant changes in community composition over the 30 years using both the two-sample tests and NMS. The changes are mainly attributed to highly significant increases in several species of shrubs over the time period ($p<0.01$), as well as less significant ($p<0.05$) increases in introduced species. This region may have the closest correspondence between the predicted effects of climate warming and community change for the 30-year period. The region has experienced a slight decrease in grass and perennials, a highly significant loss of litter coverage, and a highly significant increase in bare ground and exposed rock. Species with drought-resistant physiology, like cacti and sedum, have doubled in frequency in 30 years. Several species of shrubs have also increased significantly in frequency during the time. Precipitation was significantly less in all seasons examined except for spring in the high elevations and winter in the low elevations. Temperatures were warmer for all seasons in 2002 than they were in 1971–1973 and, in most cases, they were also warmer than the 30-year mean. Although both bison and observed elk have increased significantly in the park itself since 1971, the grazing indicators for the region show minimal differences from past conditions. Invader shrubs are the only indicators that occur significantly more frequently than in the past. We are currently in the process of an internal review of a paper that describes the changes over 30 years in these bunchgrass communities in all four of the ecoregions examined and for the whole western Montana area. We have also started to examine the changes that have occurred in bunchgrass community composition at the individual plot scale using time-series analysis. The time-series analyses only include sites that were examined during the 2002 field season and that have good historic data at several intervals over a span of at least two decades. They should give a different perspective on *Pseudoroegneria spicata* and *Festuca spp.* community stability than this regional study, which only compared two points in time.

Project title: Cottonwood Age and Diameter Relationships in Yellowstone's Northern Range

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Report number: 26899
Co-investigators:

Purpose: To establish quantitative age vs. diameter relationships for Cottonwoods in the Lamar and Soda Butte Creek valleys of the northern range. Such information is needed to understand long-term stand dynamics and the influence of various factors on cottonwood recruitment. In 2003, measurements of cottonwood height vs. diameter and seedling/sucker establishment will be also be undertaken in selected locations within the study area.

Findings: The initial phase of this study has focused on evaluating cottonwoods (*Populus spp.*) within a 9.5 km^2 portion of the Lamar Valley (elevation ~2,000 m) in the northern range of northeastern Yellowstone National Park. At this site, the diameter at breast height (DBH) of all cottonwood (*Populus spp.*) greater than 5 cm in diameter have been measured. A total of 700 trees have been measured, of which 71% were narrowleaf cottonwood (*P. angustifolia*) and 29% were black cottonwood (*P. trichocarpa*). Tree diameters for both species ranged mostly between 30–110 cm with a nearly total absence of cottonwoods between 5–29 cm in diameter. Age vs. diameter relationships from previous studies outside and within the northern range were utilized as a basis for estimating establishment dates for all narrowleaf cottonwoods. These relationships, in conjunction with the DBH data, indicated an absence of cottonwood recruitment (i.e., growth of seedlings/suckers into greater than 5-cm-diameter trees) over approximately the last 60 years. Collection of increment cores for cottonwood trees has been undertaken to better clarify age vs. diameter relationships for northern range cottonwoods. Additional data collection (i.e., diameters and increment coring) has been undertaken for cottonwood groves at Buffalo Ranch, in the Soda Butte Creek Valley, and outside the northern range to provide additional insights regarding cottonwood stand dynamics over time. The number of mature trees is being annually reinventoried. Over a two-year period, approximately 10% of the Lamar Valley cottonwoods have been lost primarily due to channel erosion. Cottonwood establishment data have also been collected at the Lamar Valley study site. An estimated 150,000 young cottonwoods between 0.2 and 3 m in height are currently present indicating a high recruitment potential for the coming years.

Project title: White Pine Blister Rust Pest Trend-Impact Plots

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Report number: 28494
Co-investigators:

Purpose: White pine blister rust disease (WPBR), caused by the fungus, *Cronartium ribicola,* is a devastating, exotic, and invasive disease of five-needle pines. This disease is severely impacting whitebark pines (*Pinus albicaulis*) and limber pines (*P. flexilis*) in the northern Rocky Mountains, and is well established in five-needle pine stands of Wyoming forests. The USDA-Forest Service, Rocky Mountain Region, Forest Health Management established permanent plots in five-needle pine stands with the disease, representing a wide range of stand conditions. These plots were designed to collect long-term data on this disease. The objective of this study was to remeasure the WPBR permanent plots in the Rocky Mountain Region in 2003. Results will be used to calibrate rust disease models for the prediction of WPBR disease impacts and spread, and to report historical trends in the continuing impact of this important disease. The final report will include a summary of all 22 plots in Wyoming and South Dakota, and will be out later in 2004. It is hoped that this study will continue for several years, with remeasurements and reports every three to six years.

Findings: There are 14 whitebark pine trees (DBH >4 inches) in three plots in Yellowstone National Park. One branch canker was observed in 2003, which was the same number observed in 2000. No stem cankers were observed on trees in the park. However, mountain pine beetle recently killed six of the trees. Seedling (height <4.5 feet) regeneration within the plot consisted of 16% whitebark pine and 84% subalpine fir, with 92 total seedlings. Saplings (DBH between 0.1 and 3.9 inches) regeneration consisted of 43% whitebark pine and 57% subalpine fir, with 14 total saplings. None of the seedlings or saplings were infected with WPBR.

Project title: Parkwide Seedbank

Principal investigator: Mrs. Eleanor Clark
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Report number: 26916

Co-investigators: Paul Anderson, Stephanie Cochrane, Lori Gruber, Orvin Loterbauer, Sam Reid

Purpose: Revegetation.

Findings: Will submit a seed collection technical report to have on file.

Project title: Vascular Flora of the Greater Yellowstone Area

Principal investigator: Mr. Erwin Evert
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e-mail:
Address: 1476 Tyrell Ave., Park Ridge, IL 60068
Report number: 27900
Co-investigators:

Purpose: To collect vascular plant specimens as vouchers for distribution maps to be included in the investigator's *Flora of the Greater Yellowstone Area.*

Findings: One taxon previously unknown from the Park was collected: *Urtica dioica var. occidentalis.* Several other species of interest were collected: *Oxalis dillenii, Hieracium caepitosum, Selaginella selaginoides, Luzula glabrata var. hitchcockii, Carex livida, C. dioica, C. buxbaumii, Eriophorum viridicarinatum,* and *Drosera anglica,* among others.

Project title: Pollination and Reproductive Ecology of *Abronia ammophila*, a Rare Plant Endemic to Yellowstone National Park

Principal investigator: Dr. Sedonia Sipes
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Report number: 28141
Co-investigators: Elizabeth Saunders, Jennifer Whipple

Purpose: Yellowstone sand verbena (*Abronia ammophila*) is a highly restricted endemic occurring only along the shoreline of Yellowstone Lake. A 1998–99 field survey, which was partially funded by the National Fish and Wildlife Foundation, found a total of 8,325 plants along the Yellowstone Lake shoreline. When combined these plants occupy an area of 2.5 acres. The need to gather information on the basic biology of rare species is of twofold importance. First, and most obviously, we must know something about a species's biology so that land managers can take steps to insure the continued existence of particular species of concern. Second, in order to examine patterns of rarity in a broader sense, or implement community-level conservation plans, we must first have a foundation of species-specific knowledge. We propose to investigate the reproductive ecology and demographic trends of the rare endemic Yellowstone sand verbena, *A. ammophila.* Currently, it is not known whether or not this species is dependent upon animal pollinators for reproduction and survival, and it is not known whether populations are declining, stable, or increasing. We will describe the breeding system of *A. ammophila* using a combination of hand-pollination experiments and observational data. These studies will determine if *A. ammophila* has an autogamous, outcrossing, or mixed breeding system. We will also describe the floral phenology and the timing of stigma receptivity and pollen viability. Additionally, we will examine which animal pollinators, if any, are necessary for *A. ammophila* to reproduce; the floral morphology of this species, and some of its congeners, suggest that moths may play an important role in pollination. We will examine the relative importance of floral visitor taxa by observing visitation rates and the foraging behavior of visitors. To investigate the relative importance of nocturnal/crepuscular visitors versus diurnal visitors, we will conduct a day/night caging experiment. Finally, we will re-census long-term demographic plots established in 1998 in order to measure the reproduction of *A. ammophila,* and to measure demographic trends. These data will assist managers in developing a long-term community-level conservation plan for the Yellowstone Lake area.

Findings: We studied the reproductive output, breeding system, and pollinators of the rare Yellowstone sand verbena (*Abronia ammophila*) from June–August 2003. We found that this species does reproduce sexually: seeds were produced throughout the growing season, but seed set dropped in some sited late in the summer, presumably from drought conditions. Hand pollinations performed on plants that had been caged to exclude pollinators revealed that *A. ammophila* is capable of automatic self-pollination. In other words, this plant can produce seeds even in the absence of pollinators. This feature contrasts with some other species of *Abronia* that have been found to be obligate outcrossers. Seeds were also set when flowers were artificially cross-pollinated with other plants. Flowering plants were observed to be visited by several species of bumblebees during the day *(Bombus spp.),* and by noctuid moths *(Noctuidae)* and hawk moths *(Sphingidae)* in the evening. Grizzly bear activity at the study site impaired evening and early morning observations; however, our limited evening observations suggest that the moths are the most abundant floral visitors, and may be more important pollinators than the diurnal visitors. The reproduction, breeding system and pollinator data are still being analyzed. Additionally, we are investigating the viability of seeds produced naturally as well as via the artificial pollination treatments. A report will be submitted to Yellowstone National Park by May 31, 2004. However, it appears at this point that *A. ammophila* does reproduce sexually, that it reproduces by a mixed mating system (self-pollination and cross-pollination), and that moths are the primary pollinators.

Project title: Physiology of Thermotolerant Plants in Yellowstone Park

Principal investigator: Dr. Richard Stout
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Report number: 26590
Co-investigators:
Purpose: Little is known about plants adapted to grow and reproduce in surface geothermal environments. Such plants have likely produced thousands of generations under selection pressure from these harsh environments. Over the past few years, we have been studying plants growing on the hot ground adjacent to surface geothermal features in Yellowstone National Park. Very few flowering plant species are found on geothermally heated soils in Yellowstone. In such areas the perennial grass

Dichanthelium lanuginosum (a.k.a., *Panicum thermale* and hot springs panic grass) is often the only vascular plant present, with some individuals able to tolerate constant rhizosphere temperatures above 45°C for weeks to months. This plant's physiological and cellular responses to short-term (hours) versus long-term (weeks to months) high temperature stress are currently being studied along with attempts to identify genes that may help to confer heat tolerance. How this plant copes with acidic soil pH (greater than 4) and the consequences of this (e.g., toxic levels of metals and poor nutrient availability) is also an area of study in which we are interested. Finally, we are also interested in plant-microbe interactions in this unusual environment, especially with regard to how such interactions may contribute to the plant's ability to withstand such extreme heat and soil conditions characteristic of geothermal areas.

Findings: During 2003, we have focused primarily on the effects of the fungal symbiont *Curvularia* with *Dichanthelium lanuginosum* on the thermotolerance of this grass commonly found in Yellowstone's geothermal soils. We collected specimens of *D. lanuginosum* along thermal gradients in geothermal areas to assess the relative levels of infection of the plant by the fungus. No relationship between soil temperature and degree of fungal infection has been demonstrated to date. We also participated in experiments aimed at elucidating the cellular mechanism of fungal-induced heat tolerance in the grass. Early results indicate that fungal infection may elicit chemical signals, which trigger systemic stress response mechanisms in the plant. Although plant specimens were collected at Amphitheater Springs and Rabbit Creek areas, as allowed in our permit, these plants were destroyed after analysis. Most of our experiments, however, were conducted in the lab with plants grown from seed, either collected in Yellowstone or harvested from plants grown in the greenhouse.

Project title: Sagebrush Ecology and Ungulate Relationships

Principal investigator: Dr. Carl Wambolt
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Report number: 27322
Co-investigators:

Purpose: (1) Determine the current status of the sagebrush-shrub community on the northern Yellowstone mule deer winter range. (2) Determine the importance of the sagebrush–shrub community to wintering mule deer and elk. (3) Describe the effect of man-caused and natural fire, including interactions with browsing, on sagebrush ecology on the northern Yellowstone winter range. (4) Determine what management techniques can be implemented to preserve or enhance mule deer and elk habitats associated with sagebrush–shrub communities.

Findings: Mule deer and elk utilize the several sagebrush habitat types on the northern range as key wintering types. They use the four woody sagebrush and three rabbitbrush heavily as browse, although they display a decided preference among taxa on winter range. Data collection is ongoing to complement previous findings.

Project title: Alpine Vegetation of Yellowstone National Park in a Mountain Goat Context

Principal investigator: Dr. T. Weaver
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Report number: 28234
Co-investigators: Ken Aho

Purpose: (1) To characterize the alpine vegetation of volcanic substrates in YNP by sampling five environments (talus, S-slopes, ridges, N-slopes, and late melt sites) in the alpine landscape. Two reps on each of ten mountains will be studied. Physical/chemical qualities of the environments are being measured on one mountain, Mt. Washburn. (2) To describe vegetation and environments of alpine/subalpine cliffs. (3) To provide preliminary information on impacts of exotic mountain goats by comparing the vegetation of goat used and unused mountains. (4) To permanently mark the sample plots as a baseline for later measurement of change that may be associated with changes in environment, such as climate or goat numbers.

Findings: Classified alpine vegetation and reported. Analyzing cliff vegetation data. Gathered environmental data and began analysis.

Project title: Yellowstone Flora

Principal investigator: Ms. Jennifer Whipple
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Report number: 26763
Co-investigators:

Purpose: n/a

Findings: Ongoing inventory of vascular plants and collections for the Yellowstone National Park Herbarium [YELLO]. Five taxa of native vascular plants previously not reported for Yellowstone were discovered: Teton anemone (*Anemone tetonensis*), Kotzebue's grass-of-parnassus (*Parnassia kotzebuei),* Columbian stickwort (*Minuartia austromontana*), bristly mousetail (*Myosurus apetalus var. borealis*), and lanceleaf grapefern (*Botrychium lanceolatum*). Several other species were also confirmed to occur in the park, including Wyoming paintbrush (*Castilleja linariifolia*), alpine springbeauty (*Claytonia megarhiza*), thickleaf draba (*Draba crassa*), beavertip draba (*Draba globosa*), and Letterman's needlegrass (*Achnatherum lettermanii*). One new exotic species was located, euclidium (*Euclidium syriadcum*) at a hitching post at a trailhead.

Water Resources

Project title: Reference Stream Monitoring: Long-Term Trend Sites

Principal investigator: Mr. Scott Collyard
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Report number: 26245
Co-investigators: Jason Martineau, Jeremy Zumberge

Purpose: Collect long-term water quality, biological, and stream habitat data at least-impacted streams in Yellowstone National Park. These data will be used as benchmarks for assessing water quality, biological, and habitat conditions at test streams within the Middle Rockies ecoregion.

Findings: Ongoing monitoring of long-term reference sites with no final report available at this time.

Project title: Water Sample Collection: Yellowstone Lake

Principal investigator: Dr. James Gibson
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Address: Dept. of Biology, Chadron State College, NE 69337
Report number: 26100
Co-investigators: Mike Leite

Purpose: Collect water samples at 12 depths via Kemmerer sampler for instructional purposes. Standard limnological chemistry tests will be carried out on shore.

Findings: Course cancelled - no work carried out in park.

Project title: The Biogeochemistry of Sublacustrine Geothermal Vents in Yellowstone Lake

Principal investigator: Dr.Val Klump
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Milwaukee, WI 53204
Report number: 26154
Co-investigators: Pat Anderson, David Lovalvo, Robert Paddock, R.T. Short, James T. Waples

Purpose: To explore and understand the extent and influence of hydrogeothermal activity within Yellowstone Lake on the biogeochemistry, biology, and ecology of the system.

Findings: Harsh environment mass spectrometry is an emerging science with significant potential. In aquatic systems, this technology will eventually enable researchers to make in situ measurements of an incredible array of compounds ranging from dissolved gases to DNA fragments. When married to an ROV or AUV platform, underwater mass spectrometry will ultimately be able to map chemical (e.g., petroleum) and biological (e.g., bacterial populations) plumes in three-dimensional space in real time. In this project, Principal Investigators at the University of Wisconsin-Milwaukee and the University of South Florida intend to refine work already begun on the development of an integrated, high-resolution sampling/sensor system featuring a 200-amu underwater mass spectrometer (UMS) on an ROV platform. The PIs of this proposal successfully carried out the first integrated ROV/UMS dive in Yellowstone Lake during the summer of 2002. This site was chosen primarily because previous direct observations of the bottom of Yellowstone Lake revealed extensive hydrothermal activity in both the shallow nearshore lakebed and in the deep profundal basins in the lake. The release of hot anoxic hydrothermal solutions (>100°C) into cold well-oxygenated bottom waters causes large and very sharp gradients in dissolved gas concentrations (e.g., O_2, CO_2, CH_4, 222Rn), which provides an excellent environment to test both the sensitivity and response time of the underwater mass spectrometer. The major goal of this project is to explore the utility of new in situ sampling and analytical techniques to the problem of tracing hydrogeothermal plumes, and the processes that control them. Specific objectives include: (1) Design, fabricate and deploy in situ instrumentation and sampling gear package for high resolution analysis of hydrothermal plumes in a large sublacustrine hydrothermal vent system in Yellowstone Lake, including: (a) a new state-of-the-art in situ underwater mass spectrometer system developed by the Center for Ocean Technology at the Univ. of South Florida; (b) an ROV deployable in situ sample collection system, capable of collecting up to 32 individual water samples suitable for dissolved gas analysis; (c) a gas equilibrator system; (d) a conventional CTD system; and (e) an in situ methane sensor (CAPSUM Technologie GmbH). (2) Demonstrate the utility of this instrument package to map plume morphologies, mixing rates and non-conservative behavior of reactive components. (3) Use these sampling tools as "sniffers" for determining the potential presence of geothermal and hydrothermal activity within the lake. (4) Develop preliminary whole lake/basin estimates of geothermal impact on lake biogeochemistry.

Project title: Trophic Classification of Selected Lakes in Yellowstone National Park

Principal investigator: Dr. Woodruff Miller
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Report number: 26775
Co-investigators: Brad Miller

Purpose: Our purpose is to evaluate the trophic state of lakes in YNP as we have done over the past four years. We have previously studied Lewis, Heart, Riddle, Shoshone, Duck, Ice, Wolf, Grebe, and Cascade lakes. This coming year we would like to study smaller, more accessible lakes like Slide, Phantom, Swan, Beaver, Lemonade, Twin, Nymph, Harlequin, Goose, Hot, Turbid, and Lake of the Woods. We want to take a one liter water sample for analysis three or four times during the year from some, if not all, of these lakes.

Findings: No activity was conducted in Yellowstone this reporting year.

Project title: Assessment of Volatile Metalloids

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Report number: 26295
Co-investigators: Juliane Becker, Beate Bohme, Manja Seidel

Purpose: Geothermal waters within Yellowstone National Park typically contain high concentrations of dissolved metal(loid)s at low pH and low redox conditions. For the first time ever volatile metal(loid)s were detected in summer 2002 by the principal investigator both in the gas phase and dissolved in water from hot springs at Norris Geyser Basin and Hazel Lake. Advantage was taken of a fast screening method developed by the principal investigator, where all volatile metal(loid)s are dissolved and oxidized to their aqueous species and detected by multi-element analyses. Application for a research and sampling permit for summer 2003 is motivated by trying to achieve a higher spatial resolution of occurrence of volatile metal(loid)s by sampling more locations with the fast, effective, and already approved screening method. This screening is completed by species selective sampling by means of a new technique of solid phase micro extraction. Detailed investigations on horizontal and vertical spatial variations of volatile species as well as correlations between volatile metal(loid)s and hydrochemical parameters will help to understand the occurrence of volatile metal(loid)s at different locations, concentrations, and in species.

Findings: In 2003, two sampling campaigns were conducted (June 15–July 16 and September 2–October 12). In total, the following water and gas samples were collected: Gibbon Geyser Basin 10 water/15 gas samples, Hazel Lake 5/7, Lower Geyser Basin 3/4, Nymph Lake 12/14, Ragged Hills (Norris Geyser Basin) 29/40. High total arsenic concentrations in the thermal waters of several mg/L already found in previous studies were confirmed. Arsenic speciation undertaken by means of ion exchangers revealed a clear predominance of As(III) (except for Hazel Lake, where dimethylated arsenic prevailed). Volatile As was found at all the sampling sites, concentrations however varying significantly over space and time. Concentrations range from 1 to 100 ug/L in 100 mL oxidizing solution (NaOCl) with an average exposure of 3–4 days in the field. Speciation of the volatile As was done via enrichment on solid phase micro extraction fibers in the field and subsequent analysis via GC-MS. Predominant species were dimethylarsine, methyldichloroarsine, dimethylchloroarsine. Additionally, a new volatile As species was found, so far only created in the lab ($(CH_3)_2AsSCH_3$). Interpretation on correlations between water and gas chemistry are still ongoing. Results will be published around July 2004 in the PI's Ph.D. thesis.

Project title: Field trip to Yellowstone National Park, Water Sampling

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Report number: 27000
Co-investigators:

Purpose: Investigation of water composition at a variety of park locations in an effort to correlate the origin, fate, and history of the solutions.

Findings: New focus on humic substances in the fire hole river foam have shown exceptional purity of these organic compounds.

Wildlife

Project title: A Proposal to Cooperatively Monitor and Assess Wolf–Ungulate Interactions and Trends in Selected Locations within the Greater Yellowstone Ecosystem of Montana

Principal investigator: Dr. Scott Creel
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Report number: 26200
Co-investigators: Dave Christianson, Robert Garrott, Stewart Liley, Amanda Morrison, Chris Wallstrum

Purpose: The wolf population reintroduced into Yellowstone National Park has grown steadily since 1995, expanding in both numbers and geographic distribution. With this growth has come recolonization by wolves of areas outside of Yellowstone National Park. The effect of wolf recolonization on the numbers, distribution, and behavior of elk will be a contentious issue in the statewide management of both wolves and elk. In anticipation of federal delisting of the wolf, data on wolf–elk interactions in areas outside of YNP will be critical. This study will collect data on wolf–elk interactions from five sites in the Greater Yellowstone Ecosystem (one in YNP in the Madison–Gibbon–Firehole area, and four outside the park). Data collection will include (1) monitoring trends in elk population sizes and recruitment, (2) quantifying offtake by wolves, (3) examining interactions between the distributions and movements of elk and wolves, and (4) examining behavioral responses of elk to the risk of predation. In addition, we will use noninvasive physiological assays of pregnancy rates and stress hormone levels in elk, to test for sublethal effects on fitness. Analysis will include comparisons among the five sites, which differ with respect to fundamental variables expected to affect the rate of predation (e.g., snow depth, herd size), and comparisons within sites of pre-wolf and post-wolf data on population size and demography. Pre-wolf data extend back as far as 75 years BP for some sites. This project is a collaborative effort among scientists at several agencies, including Montana State University, Montana Fish Wildlife and Parks, the U.S. Fish and Wildlife Service, and Yellowstone National Park. Ongoing research on wolf–elk interactions in the Madison–Firehole site have been permitted by YNP in a permit issued to Robert Garrott. Research at three sites does not overlap with YNP. The research proposed for the Gallatin Canyon site is not yet permitted by YNP, but this site will straddle the park boundary. As described in detail in the proposal, the Gallatin Canyon site includes four subsites, three of which are outside YNP (the Porcupine, Taylor and Teepee drainages) and one of which is inside the park (the Daly drainage). Because wintering elk in this population move freely among the subsites, it is critical that the entire site be treated as a single unit. We (MSU and Montana FWP) have discussed methodology in detail with YNP biologists and administrators, and the proposed work is tailored to meet YNP guidelines. For example, radiocollars for elk will be colored to be inconspicuous, and will be equipped with drop-off mechanisms. Finally, the work is designed to complement ongoing research and monitoring by the YNP wolf project in YNP's northern range, in working toward a broad understanding of wolf-elk dynamics in southwest Montana.

Findings: To date, we have been successful at documenting behavioral responses of elk to variation in the risk of predation by wolves. We have also detected substantial changes in physiology and demography, though more years of data are needed for these analyses. Using GPS collars that we custom built, we have collected 18,317 unbiased locations for adult elk in four drainages of the Greater Yellowstone Ecosystem. These data reveal significant changes in use of the landscape on days that wolves are present within a drainage, relative to days when wolves are absent. Changes in landscape use are driven primarily by changes in the behavior of bulls, while cows show no significant response. This sex-difference is as predicted, because analysis of 51 wolf kills on our site reveals that bulls and calves are taken significantly more often than predicted if prey were selected at random, while cows are taken less often than expected (chi sq = 39.21, df = 2, $P < 0.001$).

Elk, particularly bulls, are significantly more likely to use high elevations and wooded areas when wolves are present, and move to grassland sites at lower elevations when wolves are absent. Herd sizes also shift on days that wolves are in a drainage, dropping significantly ($F = 33.48$, $P < 0.0001$) from a 95% CI of 18.8–23.2 elk/herd in the absence of wolves to 7.9–13.4 elk/herd in the presence of wolves. This suggests that grouping in response to predation risk does not benefit elk (for example via the "many eyes" effect). Rather, the bulk of our data suggest that behavioral responses by elk are aimed at reducing the likelihood of being detected by wolves, rather than altering the outcome following detection. This interpretation is reinforced by data on herd size in relation to distance to obstructive cover. In general, herd size increases as distance to cover increases (ANOVA, $F = 13.22$, $P < 0.0001$), as expected under the many eyes hypothesis. However, there is a significant interaction between the distance to cover and the presence of wolves in their effects on herd size. Aggregation far from cover occurs only when wolves are absent. When wolves are present, elk remain in small herds at all distances to cover. An apparent pattern strongly suggests that aggregation on open (grass) communities is a foraging response during periods of low predation risk, rather than an antipredator response.

The behavior of individual elk also responds to the presence of wolves within a drainage. Using scan sampling to record 10,642 observations, contingency tables reveal that the presence of wolves within a drainage on the day of observation is associated with an increase in vigilance (chi sq = 113.78, df = 1, $P < 0.001$), with a decrease in foraging (chi sq = 16.64, df = 1, $P < 0.001$) and a decrease in movement (chi sq = 29.46, df = 1, $P < 0.001$). The proportion of time bedded did not change significantly (chi sq = 0.10 , df = 1, NS), probably in part because this was not a common behavior. In aggregate, these responses reveal that elk behave quite differently on days that wolves are present within their drainage, but they do not demonstrate that these changes carry costs. Trends in population size (by aerial total count) and recruitment (from ground and aerial classification counts) suggest that predation (both direct and indirect effects) may be altering elk demography and dynamics. Recruitment in early winter has been at or below 20 calves:cow on five of six winters with data since colonization by wolves, compared to one of 13 winters prior to wolf colonization (Fisher's exact test, $P = 0.12$). Similarly, population size has been below 1,500 elk in six of seven winters since wolf colonization, compared to 16 of 41 winters prior to wolves (Fisher's exact test, $P = 0.17$).

Project title: Predator–Prey Dynamics in a Wolf–Ungulate System

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Report number: 26896
Co-investigators: Matthew S. Becker, L. Lee Eberhardt, Claire Gower, Christine L. Kenyon, Andrew Pils, Jennifer Pils

Purpose: The objective of this study is to examine prey selection of wolves on the ungulate populations in the Madison, Firehole, and Gibbon drainages of Yellowstone National Park. Specifically, studying predation rates, prey selection, and wolf movements according to landscape attributes and prey abundance and distribution patterns. Aspects of prey selection being studied include species, sex, and age class, condition of prey, and landscape features and snow conditions of encounter and kill sites. The data collected will be used to help predict impacts of wolf predation on the prey populations.

Findings: Data collection for the 2003 field season was initiated in mid-November 2002 and extended through mid-May 2003. Utilizing a combination of radio-telemetry, snow-tracking, and opportunistic sightings, wolves were detected in the study tract on 149 of 170 days during the season. Three main packs comprised the bulk of the wolf presence, namely the Nez Perce pack, the Cougar pack, and the Gibbon pack, with detections on 102, 51, and 55 days respectively. Multiple packs were detected in the study area on 55 occasions, a fivefold increase from the 2002 season. Seventy-five total wolf kills were found, of which 61 were elk and 14 were bison. Field methods remained unchanged from the start of the predator–prey study in 1998, and analyses of wolf movement data and spatial response to landscape variables were completed and submitted for publication. Further investigations and analyses of predation rate variables were initiated and will be completed in summer 2007.

Project title: Intraspecific Variation in *Microtus Montanus* in Response to Climatic Change in the Yellowstone Ecosystem

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Report number: 28250
Co-investigators: Judsen Bruzgal, Yvonne Chan, Maneesh Kumar, Kim O'Keefe, Paula Spaeth

Purpose: The goal of this collecting season is to determine the feasibility of a more intensive study that seeks to investigate how animals respond to climatic change. By coupling modern spatial genetic, morphologic, and habitat data with temporal genetic data and morphologic data from Lamar Cave and Waterfall Locality, this study will increase our understanding of mammalian adaptation and migration, and their dynamics in response to environmental change.

Findings: This report summarizes the work conducted in Yellowstone National Park from July 19 to August 13, 2003, by Yvonne Chan and her field assistant, Maneesh Kumar. In an effort to better understand mammalian response to environmental change, we set out to collect 20–30 genetic samples from *Microtus montanus* in locations of differing elevation and habitat throughout Yellowstone National Park.

Eight sites were chosen: Hayden Valley, Junction Butte, Lamar Valley, Northeast, Ojo Caliente, Rescue Creek, Soda Butte, and Slough Creek, and a total of 21 trapping nights conducted (see Appendix 1 for dates at each site). At each location, 100 traps were set and baited with oatmeal and peanut butter as close to sunset as possible (between 9 and 10 p.m.) and checked the following morning. All captures were identified, sex and age determined, and basic morphological measurements taken (total length, hindfoot, tail length, ear, weight). If a *Microtus* species was captured, zygomatic arch was also taken, the individual was tagged with a unique ear tag combination, and a small genetic sample was obtained from an ear clip. Over the 21 trapping nights there were 358 captures of five different species: *Peromyscus maniculatus* (new = 217, recapture = 7), *Microtus* species (new = 109, recapture = 11), *Clethrionomys gapperi* (new = 4, recapture = 2), *Zapus princeps* new = 6, recapture = 1), and *Neotoma cinera* (new = 1). There were 11 mortalities (~3%), all *Microtus* species (Lamar Valley = 2, Northeast = 6, Soda Butte = 3), Every effort was made to reduce mortalities, and towards the end of the summer we decided that the best combination was to place a small piece of fleece with cotton balls in the trap. All mortalities were prepared and have been deposited at the Museum of Vertebrate Zoology, University of California, Berkeley, under the care of Dr. Christopher Conroy. We are currently in the process of sequencing the genetic samples taken from this summer. This report summarizes what was conducted for the first preliminary field season. We had set out to capture 20–30 individuals per site and accomplished that goal at three sites (Lamar Valley, Northeast, and Soda Butte). We would like to return this coming season (2004) to complete the genetic sampling of several sites where we were not able to obtain adequate sample sizes.

List of sites, dates trapped, site abbreviations, and number of captures at each site by species.

Site	**Dates**	**Abbrev.**
Hayden Valley	7/29, 7/31	HV
Junction Butte	7/18, 7/19, 8/9	JB
Lamar Valley	7/20, 8/7, 8/8, 8/10	LV
Northeast	7/24, 7/26, 7/27	NE
Ojo Caliente	8/1	OC
Rescue Creek	8/3	RC
Soda Butte	7/21, 7/22, 7/23, 7/28, 8/6	SB
Slough Creek	8/11, 8/12	SC

	Microtus sp.		***P. maniculatus***		***C. gappperi***		***Z. princeps***		***N. cinera***	**Total**
Abbrev.	N	R	N	R	N	R	N	R	N	
Total	109	11	217	7	4	2	6	1	1	358

Total captures by site

HV	2
JB	58
LV	72
NE	69
OC	13
RC	37
SB	71
SC	36

Project title: Evaluating the Relative Abundance and Distribution of Snowshoe Hares in Yellowstone National Park

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Report number: 27611
Co-investigators: L. Scott Mills

Purpose: Our primary research goals in 2003 were to evaluate annual differences in snowshoe hare and red squirrel distribution, to expand our spatial and habitat type coverage within Yellowstone by surveying new places, and to obtain sufficient vegetative information to link stand structure to snowshoe hare and red squirrel relative abundance. We continued to focus on four major stand types (1988 burn, two classes of mature lodgepole, and spruce-fir), but also sampled three other stand types (Douglas-fir, aspen, willow riparian). We greatly increased our vegetation sampling to include information on overstory stocking, canopy closure, understory cover, sapling density, and coarse woody debris. Our specific field objectives were: (1) to live-trap snowshoe hares on a subset of the sites that were trapped in 2002; (2) to use pellet counts to resurvey some of the snowshoe hare sites that were surveyed in 2003 to enable annual comparisons; (3) to use pellet counts to survey sites in new areas and habitat types; and (4) to survey for red squirrels on all sites by counting their middens along long transects within each site.

Findings: We trapped for snowshoe hares on four sites (20 hectares each) that we had also trapped in 2002. We trapped one site each in the four major habitat types suspected to be important for snowshoe hares: 1988 burn, lodgepole 2, lodgepole 3, and spruce-fir. Our trapping results were similar between years, and very low compared to snowshoe hare populations elsewhere. To date we have captured 23 snowshoe hares in Yellowstone; one individual was captured in both 2002 and 2003. Our site with the highest numbers of hares had six individuals. In total, we conducted pellet and squirrel surveys with complete vegetative surveys on 36 sites in seven habitat types. This numbers includes revisiting 11 of our 13 3002 trapping sites. We were not able to survey the remaining two sites: Frank Island burned, and our site along the Mesa road near Madison Junction was being trapped for grizzly bears. The Frank Island and East fires burned three of our former trapping sites and two of our pellet sampling sites. Two of the trapping sites had been surveyed for pellets prior to the East fire. One of the pellet-only sites, Lake Butte, was a Douglas-fir site that had some of the highest numbers of pellets recorded in 2003. The pellet surveys show similar patterns of habitat preference for the four primary habitats surveyed as were detected in 2002, but we also documented snowshoe hares using some Douglas-fir areas and willow riparian areas (which were not surveyed in 2002). Our 2003 results confirm and extend our conclusions from 2002. Yellowstone forests represent marginal habitat for snowshoe hares. Snowshoe hares are very patchily distributed within the park, and clearly appear to be concentrated in habitats with denser than usual understory. Some of our highest recorded densities have been in sites that burned in 1988, showing that hares are definitely able to travel between habitats to colonize these areas. Red squirrels are also patchily distributed in Yellowstone, and appear primarily in mature forests with many cone-bearing trees.

Project title: Bat Inventories in the Greater Yellowstone Network

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Co-investigators: Gary Beauvais, Hamilton Smith

Purpose: The National Park Service (NPS) is undertaking a nationwide effort to inventory and monitor the biological resources within its management areas. Recognizing the need for a cross-boundary, ecosystem approach to natural resource management, the system of national parks has been grouped into Cooperative Ecosystem Units to facilitate inventory, monitoring, and subsequent management decisions in ecologically meaningful areas. The Greater Yellowstone Network (GYN) includes Yellowstone and Grand Teton national parks (YELL and GRTE) and Bighorn Canyon National Recreation Area (BICA). A combined effort of biologists from these parks and regional wildlife experts resulted in the recent release of a study plan for the GYN inventory and monitoring efforts. This document identified significant gaps in information on the species richness, abundance, and distribution of bat species within all GYN parks and terrestrial mammals in BICA. They have therefore proposed that the NPS conduct a comprehensive inventory of bats throughout GYN and terrestrial mammals in BICA to establish a benchmark for future monitoring efforts and management actions. The specific goals of these inventory efforts, as stated in the GYN Study Plan are as follows: (1) To document, through existing, verifiable data and targeted field investigations, the occurrence of at least 90% of the species of vertebrates and vascular plants currently expected to occur in Bighorn Canyon National Recreation Area, Grand Teton National Park, and Yellowstone National Park. (2) To describe the distribution and relative abundance of species of special concern, such as threatened and endangered species, non-native species, and other species of special management interest occurring within park boundaries. (3) To provide the baseline information needed to develop a general monitoring strategy and design that can be implemented by parks once inventories have been completed, tailored to specific park threats and resource issues. (4) To make information easily available to park managers, resource managers, scientists, and the public.
Findings: Field technicians searched each of the focal areas to identify specific sites that were suitable for conducting mist net and acoustic monitoring activities. Approximately 170 sites were evaluated in this way, and mist netting was conducted on just over 30 of these. Over 36 nights of mist netting, we captured 527 bats of 12 species. The most species rich area was BICA, which had nearly twice the amount of species in less than 9% and 1% of the land area of GRTE and YELL respectively. YELL had the greatest number of captures per unit effort, largely because of locally high concentrations of little brown bats (*Myotis lucifugus*). In general, GRTE and YELL were very similar in the apparent composition of their bat fauna and collectively they were much different that BICA. The question of how close we are to documenting the complete suite of bats in each park is more difficult to answer. Expert opinion suggests that we are very close to the full complement of bats in GRTE. However, BICA and YELL may yield more species with additional effort. A more quantitative estimate of progress can be roughly approximated by using rarefaction or species accumulation curves. We developed species accumulation curves for each park unit. Although the effort to date is insufficient for formal analysis of richness predictions, we can gain an idea of our progress by studying the curves. The curve for GRTE clearly shows an asymptote at six species, which was reached early in our efforts. However, YELL and BICA seem to

exhibit curves that have not clearly reached a maximum value. In the case of YELL there are two species that are likely to occur there but were not conclusively documented. We believe that both small-footed myotis (*Myotis volans*) and Townsend's big-eared bat (*Corynorhinus townsendii*) occur in YELL, but have not yet been captured in this study.

Project title: Document the Presence and Distribution of Lynx in Yellowstone National Park

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Report number: 26624
Co-investigators: Heidi Anderson, Nathan Berg, Debbie Berg, Kerry Gunther, Justin Hadwen, James Halfpenny, Margot Higgins, Tildon Jones, Emily Klein, Peter Lundberg, Mike McCain, Elliot Pickering, Tiffany Potter, Andy Weidman

Purpose: Document the presence and distribution on lynx in Yellowstone National Park.

Findings: In 2000, the U.S. Fish and Wildlife listed the Canada lynx as a threatened species in the contiguous 48 states. After completing design work from 1999–2000, Yellowstone staff initiated a three-year survey project in 2001 to document lynx presence and distribution in the park. The survey includes three summer field seasons using the U.S. Forest Service lynx detection protocol (hair snares and laboratory-based DNA extraction), with work focused primarily on the east side, but opportunistically in other parts of the park as well. To date, we have collected 516 total hair, bone, and fecal samples along 151 transects (typically five hair snare stations per transect), detecting one female lynx on the east side of the park in summer 2001. The survey also includes three winters of ski-based and snowmobile-based snow-tracking effort in habitats dominated by mid-aged (> 50 years), mature, and old-aged stands of lodgepole, spruce, and subalpine fir widely distributed in the park. To date we have completed 243 different transects totaling 1,388 miles in length and ranging from 1 to 32 miles. Surveys have been conducted mostly in soft snow that readily showed fresh animal tracks. We have detected one possible, two probable, and one definite lynx track, including a female traveling with a male kitten whose presence was confirmed by extraction of a DNA from a hair (bed site) and fecal sample. We have detected nearly all other Yellowstone carnivores, including tracks of wolverines (three observations), cougars and bobcats in the park interior during winter, and river otter. No fishers have been detected by any method. The detection of the female lynx with kitten was unique in that it was the only confirmed observation of lynx reproduction in park history. During winter 2004, we will focus a fourth survey on the east side of the park where the family was documented, attempting to confirm the residence of the adult female and/or her kitten and learn about their travel patterns and food habits.

Project title: Natural Resource Education and Monitoring

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Report number: 27091
Co-investigators: Patrick White, YELL Wildlife Biologists

Purpose: To collect natural objects to use in education presentations.

Findings: Collected bighorn sheep fecal specimens for analysis.

Project title: Multi-trophic Level Responses to the Addition of a Top Carnivore

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Purpose: This project seeks to find out how wolves change the ecosystem of the Northern Range. The researchers have defined several specific changes that they can measure and study: (1) Does the number of wolves depend strictly on the number of elk? If there are more elk are there more wolves? (2) What controls how many elk are killed per wolf? Is it how many elk cluster together in a group? Is it what other prey are available? Is it where the elk choose to live? Is it some combination of those factors? (3) Do wolves affect recruitment (amount of calves that reach adulthood) in elk? (4) Will aspen and willow grow robustly as in historical photos if there are fewer elk? (5) Are moose numbers affected by elk numbers? Will there be more moose if there are fewer elk? (6) What is more important for aspen and willow growth—browsing by elk, or soil/climate/topography/fire/etc.? To answer these questions, the researchers will begin by studying the elk. They will characterize resource selection by determining where elk reside in relation to known centers of wolf activity, vegetation types, precipitation and snow condition pattern, topography, season, and distance to roads. In the next phase of the study, researchers will identify locations where presence of wolves is likely to reduce use by elk. These locations will be used in the future vegetation studies. The researchers also propose to make a virtual model of the elk population that can be used to estimate relative sizes of annual cohorts. This work will especially address the question of recruitment. The cause of death and age at death are used to build this mathematical model. Some data useful to this study has been collected since 1996 and will be used in the current work. Researchers expect to obtain data useful for a plethora of analyses. The data collected in this first phase of work will be used later to establish the number of prey killed per predator (functional response) in late fall/early winter and in late winter/early spring. Data will be available to identify such patterns as intergenerational nutrition effects, herd organization, habitat use, genetic relationships, age-specific productivity, activity patterns and other behavior.

Findings: Among the accomplishments of this research project in 2003 is the completion of two graduate degrees and the associated value of the two degree dissertations. Julie Mao, graduate student under Mark Boyce, completed her M.S. thesis, published the dissertation, and submitted an article for publication in the *Journal of Wildlife Management.* Her citation and abstract follow: Mao, J. S. 2003. Habitat selection by elk before and after wolf reintroduction in Yellowstone National Park, Wyoming. M.S. thesis, U. of Alberta, 69 pp. Prey species select habitat in an attempt to obtain necessary resources (food, mates, shelter, etc.) while also avoiding predation. I examined whether habitat selection by elk (*Cervus elaphus*) changed following the reintroduction of wolves (*Canis lupus)* into Yellowstone National Park in 1995. Using conditional fixed-effects logistic regression to build habitat selection models, I compared seasonal habitat selection by elk before and after wolf reintroduction based on weekly elk radiolocations taken in 1985–1990 (prewolf) and 2000–2002 (postwolf). In summer, when wolf activity was centered around dens and rendezvous sites, elk avoided wolves by selecting higher elevations, less open habitat, more burned forest, and, in high wolf density areas, steep slopes. In winter, elk did not spatially separate themselves from wolves on a weekly timescale and, compared to the pre-wolf period, they selected for more open habitats after wolf recovery. Elk appear to use habitat selection to avoid wolves in summer, but must rely on behavioral anti-predator strategies such as grouping in winter.

Another graduate student, Greg Wright, under Principal Investigator Dr. Rolf Peterson, completed his degree. The citation is as follows: Wright, G. J. 2003. An analysis of the northern Yellowstone elk herd: population reconstruction and selection of elk by wolves and hunters. M.S. Thesis, Michigan Technological University, Houghton, MI. 124 pp. This work compared the age classes of elk that were (1) killed by wolves and (2) killed by hunters adjacent to the park and explored the implications of these two distributions on the future demographics of the northern range elk herd. Other work continues in 2004, including the completion of another M.S. thesis by Shaney Evans under the guidance of Dr. L. David Mech. This thesis looks at mortality factors for adult female elk in the northern range herd, and associated topics. Field data has been collected and analysis continues, a draft of one chapter is being reviewed by the PIs. A demographics model as discussed in the objectives of the IAR was begun in 2003, and is nearing completion in 2004. This model simulates the population response to wolves, harvest and climate of the northern range elk herd. A finished report on the model will be submitted to NPS personnel at Yellowstone early in 2004. Field data continues to be collected. There are still ~40 elk with active VHF collars and six with GPS collars that are being monitored this winter. Further investigations into the ecology of the elk as it is influenced by wolves is being investigated. Separate research permits have been issued for this ongoing work.

Project title: The Behavioural-Ecological Role of Wolf Howling

Project title: Population Dynamics of the Yellowstone Grizzly Bear
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Report number: 25503
Co-investigators: Chad Dickinson, Mark Haroldson, Craig Whitman

Purpose: To describe the population trend from threatened status to recovery and approximate stability.

Findings: These data include information collected by the Interagency Grizzly Bear Study Team (members include U.S. Geological Survey, National Park Service, Wyoming Game and Fish, Idaho Fish and Game, Montana Fish, Wildlife and Parks, U.S. Fish and Wildlife Service, U.S. Forest Service) for the entire Greater Yellowstone Ecosystem. Data obtained within Yellowstone National Park is not broken out separately. Forty-four individual grizzly bears were captured a total of 54 times during the 2003 field season in the Greater Yellowstone Ecosystem (GYE). Thirty captures were new individuals that had not been previously marked. Thirteen captures of 13 bears were the result of management trapping efforts. Twelve of these instances resulted in relocation of the nuisance bears, 1 individual was removed from the population. A total of 716 aerial radio-locations were obtained from 80 individual grizzly bears radio-monitored during all, or a portion of the 2003 field season. Twenty-five of the grizzly bears radio-monitored were adult females. Two rounds of observation flights were conducted as part of our effort to count unduplicated females with cubs-of-the-year and document distribution of females with young (cubs, yearlings, or 2-year-olds). The first round of flights began June 12. One-hundred seventy grizzly bears were observed in 109 groups during 78 hours of flying. The second round of flights began on July 11. A total of 131 grizzly bears in 93 groups were observed during 76 hours of flying. Fifty-two females with young were observed during observation flights; 23 of these were initial observations of unduplicated females with cubs-of-the-year. Thirty-eight unduplicated females with cubs were identified during 2003. A total of 75 cubs were observed during the initial sightings of unduplicated females. Six single cub litters, 27 litters of twins, and 5 litters of triplets were observed. Mean litter size was 1.97. Unduplicated females with cubs were observed in 14 of 18 Bear Management Units (BMU) within the grizzly bear recovery zone. Females with young (cubs, yearlings or 2-year-olds) were documented in 16 of 18 BMUs. We documented 12 known and 1 possible human-caused grizzly bear mortalities in the GYE during 2003. Eleven human-caused mortalities, including 6 females, occurred within the USFWS Recovery Zone and 10-mile perimeter. Two losses were from management removals, both in Wyoming. Three natural mortalities (probable) of cubs from two radiomarked females were documented. An additional two grizzly bears that died from unknown causes were documented during 2003.

Project title: Food Habits and Habitat Use of the Yellowstone Grizzly Bear

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Report number: 25504
Co-investigators: Mark Haroldson, Shannon Podruzny

Purpose: To determine habitat requirements for the Yellowstone grizzly bear.

Findings: These data include information collected by the Interagency Grizzly Bear Study Team (members include U.S. Geological Survey, National Park Service, Wyoming Game and Fish, Idaho Fish and Game, Montana Fish, Wildlife and Parks, U.S. Fish and Wildlife Service, U.S. Forest Service) for the entire Greater Yellowstone Ecosystem. Data obtained within Yellowstone National Park is not broken out separately. Surveys to determine an index of spring ungulate carcass availability were conducted during May. Approximately 300 km of transect routes were surveyed in five different ungulate wintering areas. A total of 23 elk, 3 bison, and 1 mule deer carcasses were observed for a rate of 0.10 ungulate carcasses/km. These results indicate a relatively small number of winter-killed ungulates were available to bears during the spring of 2003. Surveys of 19 whitebark pine cone productivity transects distributed throughout the GYE were completed during July. Mean cones per tree for the read transects were 29.5. Cone production was good throughout the ecosystem. A total of 208 grizzly bear observations, in 132 groups, were recorded at 25 of 50 army cutworm moth aggregation sites identified through 2002. Grizzly bears were observed digging in talus, presumably for moths, at five additional, previously unknown sites during 2003.

Project title: Black Bear Demographics in Yellowstone National Park: Their Interrelationship to Other Carnivores, Habitats, and Humans

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Report number: 25506
Co-investigators: Kerry Gunther, Mark Haroldson, Glenn Plumb

Purpose: To determine patterns of habitat use, food habits, activity patterns, movements, and home range size for a sample of randomly captured black bears.

Findings: One adult male black bear was captured during the 2003 field season and instrumented with a GPS collar. The GPS collar of an adult male black bear that failed was found and turned into YNP staff. Location data was successfully retrieved from this collar.

Project title: Yellowstone Gray Wolf Restoration Project

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Report number: 28632
Co-investigators: Debra Guernsey, Daniel Stahler, YELL Wolf Project volunteers

Purpose: To monitor wolves and conduct research on their behavior and ecology in Yellowstone National Park.

Findings: See 2003 Yellowstone Wolf Project Annual Report.

Project title: The Behavioural-Ecological Role of Wolf Howling

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Report number: 26664
Co-investigators: Mary Theberge

Purpose: To understand the behavioural and ecological role of wolf howling. Wolf howling is one means of social coordination in wolf packs, one way that helps packs function as biological units. This study is designed to test the extent to which the forgoing statement is true. To qualify, howling must be shown to alter behaviour of the pack or individuals in the pack. That alteration must assist pack fitness by helping it accomplish a task of biological necessity such as procurement of food, raising young, defending territory. If the beneficiary of howling is the individual, then howling can be interpreted as a trait arising from natural selection. But, if the beneficiary is also the pack, then howling can be interpreted, as well, as assisting in group selection. Group selection is debated in the biological literature as both common among social species, or, as non-existent. Proponents of the latter position invoke an interpretation of cooperative behaviour with advantage to the individual for displays of mutual benefit. Where group advantage has been shown, often interpretations involve kin selection. The tests of group selection include a sharing to some degree of a common fate, a division of labour, and competition between groups. Superficially, wolf packs seem to qualify. The most fit group may be the one that survives and leaves the most offspring. We have discussed this in our book, *Wolf Country: Eleven Years Tracking Algonquin Wolves,* 1998. McClelland & Stewart, Toronto (Chapter 9, Foys Lake Pack Supra-organism?). The packs at Yellowstone offer an opportunity to compare the use of howling in packs whose unfolding history is, and will continue to be known. While many factors undoubtedly contribute to individual and group success, these packs may provide information on differences in their use of howling. Hypothesis 1. Howling plays a role in group cohesion. Prediction: There will be more howling in both the season of highest group cohesion (most pack members together or close to one another), and the pack that shows the highest cohesion. Possible outcomes: (1) Howling high when cohesion high, and howling low when cohesion low. (Not a definitive outcome because other factors could be responsible.) Howling high when cohesion low, or vice versa. Disproves hypothesis. (2) There will be a set of observations of cases where howling of distant pack members brings them together, versus a set of observations where howling does not bring them together. (3) Howling will be more frequent when some pack members are known to be absent versus all present, or when alphas are absent. Hypothesis 2. Howling acts to coordinate pack movements. Prediction: There will be a set of observations of wolves bringing pack members together either when the pack is travelling, or to initiate travel, or at a kill or rendezvous site or den. Hypothesis 3. Howling acts to identify pack members to each other versus non-pack members. Prediction: Set of observations of: wolf howling as wolf comes in to rendezvous site, den, or kill where the rest of the pack is present; ambivalent behaviour of approaching wolves broken with a howl (pack members show acceptance, non-pack members show avoidance); packs near each other, or aware of each other, separate more after a howl. Hypothesis 4. Group howling aids in social bonding/social partitioning. Prediction: Set of observations associated with play or group social display (before play or to initiate it, part of it, terminate a bout of it) or when pack members arrive or leave the rest of the pack. Hypothesis 5. Howling aids in territorial defense. Prediction: (1) Sets of observations of packs aware of each other, howling (versus packs not howling). (2) Group howling more common (howls per minute of observation) at kills near territorial boundaries than distant.

Findings: We recorded wolf howls on 10 occasions over the six days. Distance made most of the recordings faint. Experiments to analyze these howls with computer software indicate that we need more volumn to look at characteristics other than length, highest and lowest pitch. Accordingly, we have modified a commercial parabola to make better recordings in the future. Subjectively, we identified qualitatively different howls from the same wolves in two contexts. One context we interpret as anxiety, when cars on the road were blocking the passage of one pup to reach the rest of the pack. The other context was a more normal back-and-forth between adjacent groupings of wolves. We are working with a re-designed data sheet to quantify the social context of howls in a database of observations made year-long by naturalist-interpreter Rick McIntyre, who is a cooperator in our study.

Project title: Spatial Dynamics of the Central Yellowstone Bison Herd: Integration and Visualization of Large Spatial Databases

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Report number: 28776
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Purpose: The purpose of this project is to integrate data available from past bison research projects that have identified patterns of spatial distribution exhibited by bison and seek to explore more specifically how bison move across the landscape between areas of high and low occupancy of habitats. Managers at Yellowstone National Park need to understand these patterns of movement relative to the ecology of the species for implementing the Interagency Bison Management Plan. An understanding of how and when bison are likely to move through the landscape may help predict when animals are likely to move towards and outside park boundaries. In addition, understanding movement patterns will provide a mechanism for choosing locations to implement a remote vaccination program that delivers vaccines to a higher proportion of eligible individuals. Finally, the park needs to understand the physical distribution of bison travel network to objectively compare how it interfaces with human travel networks (primarily the road network used by motor vehicles). The product of this proposal will provide the park with a powerful data set to educate the staff and visiting public about the ecological dynamics of bison population movements across the landscape of central Yellowstone National Park, and about the relationship between snowpack dynamics and the propensity for animals to wander. This display of information will be invaluable to park staff trying to inform and interpret the Joint Bison Management Plan. Several management questions will be addressed by this project: (1) What is the probability that bison will move toward and across the west boundary of the park? (2) Are there specific constriction points in the landscape that funnel bison travel corridors sufficiently to enable Yellowstone National Park to implement a highly efficient remote vaccination protocol? The two factors of efficiency would be high probability of most vaccination eligible animals wandering by a select few locations and a short period of time to deploy field technicians delivering vaccines. (3) Can YNP quantify by seasons of the year what percent of bison travel is conducted along roadways? Likewise, can YNP quantify the percent of the road system that overlaps with the bison travel network? (4) Can a system for classifying age and sex composition of the bison population be established along the bison migration corridor using the camera stations established for monitoring group movements?

Findings: To gather spatial distribution information, 11 ground-based bison surveys were conducted that recorded the locations of 614 groups while classifying 9,381 bison. Relevant to determining the bison travel network, both road travel and trail use data were collected. Our road use surveys documented 312 traveling bison groups totaling 4,099 animals while our trail monitoring efforts recorded 4,226 bison events on the Nez Perce monitor, 3,042 events at the Gneiss Creek site, and 2,201 events at Mary Lake. Using our 2002–03 results, along with an examination of our long-term database, we are beginning to understand some of the potential ecological driving forces that induce bison movement across a range of spatio-temporal scales. From ground surveys it is evident that the meadow complexes in the middle and upper portions of the Firehole drainage form the principal winter range, while both the Madison and Gibbon River valleys play ancillary roles. The trend in population increase within the Madison–Gibbon–Firehole for the 2002–03 season was similar to that seen in previous years; that is, growth in a sigmoidal manner with an interval of rapid increase from mid-January to mid-March. With this pattern we can propose three stages in the migration that are each hypothesized to be influenced by differing ecological driving forces: snowpack dynamics, population size, and plant phenology. The tendency in bison road travel appears to be lower intensity of use from November through February; a peak in travel in March, followed by a period of reduced use with magnitudes that are still high relative to the earlier portion of the season. Within the Firehole drainage two important segments of road, the Gibbon Bridge–Freight Road and Firehole Lake Drive–Biscuit Basin sections, were identified as significant in the bison travel network. Essential road portions within the Madison and Gibbon drainages include the Harlequin Lake–Cougar Meadows and Tuff Cliffs–Artist Paint Pot segments, respectively. In 2002–03 at the Mary Lake trail monitor there were periods of oscillating travel intensity similar to that observed at the Nez Perce Creek monitor with a peak occurring in April, suggesting a dual influence of snowmelt and vegetation emergence as groups move back-and-forth between the Firehole drainage and meadows along Nez Perce Creek. Travel along the Gneiss Creek trail and use of Cougar Meadows peaked in fall, was minimal throughout mid-winter as the snowpack accumulated, and increased dramatically as soon as snowmelt began for all years.

A model of snow accumulation and melting processes using reference data from snotel sites in Yellowstone National Park has been completed. The model is currently being used to support investigations of wildlife habitat use (at MSU), and as one of the elements of an integrated visualization of the Yellowstone ecosystem. The model is presently being documented for publication. During the fall of 2003, the pilot study to test prototype anemometers was conducted in the Hayden Valley area. Data were collected successfully and the field worthiness of the anemometers has been validated. These equipment will be deployed at future times to assist in refining the details of the snow model for Hayden Valley.

During fall 2003, fifteen bison were randomly selected from the central Yellowstone Bison Population and fitted with radio transmitting collars. Capture operations were conducted between November 7 and December 18. Bison are being monitored for locations at least two times per month. The collars are due to be removed in December of 2004 for retrieval of all GPS data stored on board the collars.

Project title: Wildlife Response to Winter Human Use in Yellowstone National Park (FY 2003 Monitoring Plan)

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Purpose: During FY 2003, we will continue to implement the pilot monitoring program of wildlife responses to winter human use. The FY 2003 pilot program will involve the establishment of partnerships with other federal, state, and/or private organizations to gain needed multidisciplinary expertise and matching funds. We will document human activity levels, human-wildlife interactions, wildlife distribution patterns, and an index of wildlife stress in relation to winter road corridors in Yellowstone National Park. Three two-person crews will use snowmobiles or wheeled vehicles to randomly sample eight road segments repeatedly during December 26, 2002, through April 4, 2003. Observers will document the responses of bald eagles, bison, elk, trumpeter swans, and wolves to over-the-snow vehicles (i.e., snowmobiles and snow coaches) and associated human activities. This FY 2003 monitoring will specifically address two specific management-related questions posed by the park's Division of Planning and Compliance: (1) do the responses of wildlife to snowmobiles and snow coaches differ; and (2) are the levels of human activities and behavioral responses of wildlife different between commercially guided and unguided groups of snowmobiles? The FY 2003 monitoring will also enable us to address sampling concerns (e.g., statistical power to detect differences and make inferences) and test sampling designs and protocols. In combination with similar data collected during recent winters by Montana State University (1998–99, 1999–2000) and the Resource Management Office (2001–02), the proposed monitoring during FY 2003 would result in up to four winters of data regarding the responses of bison and elk to human winter use. During FY 2003, we will analyze these data in collaboration with a behavioral ecologist and/or biometrician contracted by the National Park Service to: (1) evaluate variances in winter use by humans and responses by wildlife; (2) identify and quantify conditions leading to responses by each species of wildlife; and (3) estimate the inferential power to detect statistically significant changes in response variables following the implementation of proposed changes in winter use during the winter of 2003–04. Based on these analyses, we will develop and implement a long-term monitoring program for quantitatively evaluating the effects of human winter use on wildlife in Yellowstone National Park that can be implemented beginning in FY 2004.

Findings: Staff from the Yellowstone Center for Resources and the Resource Management and Visitor Protection Office monitored wildlife responses to motorized winter recreation during December 16, 2002 through April 18, 2003. The purpose of this monitoring was to collect baseline information on existing conditions for comparison to data collected after the implementation of changes in winter use management during winter 2004. Such comparisons will enable us to evaluate the effectiveness of changes in management at attaining desired conditions. The winter of 2003 was relatively mild in terms of snow pack and temperatures. As a result, visitor over-the-snow vehicle traffic was relatively low in comparison to previous winters. We used snowmobiles and wheeled vehicles to conduct repeated surveys of wildlife responses to motorized winter use vehicles and human activities along eight groomed or plowed road segments in areas of both low and high intensity human and wildlife use. Our sampling unit was the interaction between motorized winter use and an observed group of wildlife within 500 meters of the road. We focused our efforts on monitoring the responses of bison, elk, and trumpeter swans to motorized winter use vehicles owing to the proximity and/or perceived sensitivity of these species to motorized recreation activities during winter.

Overall, the responses of wildlife to over-the-snow vehicles and associated humans was typically minor, with 61% of the observed responses by groups of bison, elk, and swans categorized as no apparent response, 23% look/resume, 5% attention/alarm, 8% travel, 2% flight, and 1% defense. Wildlife responses to motorized winter use were species dependent and the likelihood of observing an active response by bison and swans (but not for elk) increased as the numbers of snowmobiles in a group increased. Also, the likelihood of observing an active response by elk and swans (but not for bison) increased as the numbers of snow coaches in a group increased. The likelihood of a response by each species decreased as distance from the road increased. Trends in the abundance of bison and elk populations since the onset of motorized winter use in Yellowstone National Park provide no evidence of population-level effects to ungulates from motorized winter use, with the abundance of bison and elk either increasing or remaining relatively stable prior to wolf restoration. Thus, any adverse effects of motorized winter use to ungulates have apparently been compensated for at the population level.

Based on monitoring results during winter 2003, we recommend that training for guides, park staff, and concessionaires include the following voluntary recommendations: (1) stop at distances >100 meters from groups of wildlife, when possible; (2) reduce the frequency of multiple groups of motorized vehicles stopping in the same area to observe wildlife; and (3) reduce the number of stops to observe wildlife and human activities away from vehicles during these stops. We are currently analyzing data collected during 1999–2003 to evaluate potential indicator variables of wildlife responses to human winter use, identify key conditions leading to responses, quantify variations in the frequencies of responses, and estimate thresholds for important disturbance factors. These analyses should help us refine our recommendations for adaptive management of motorized winter use to minimize the frequency of possible disturbances to wildlife. This monitoring is scheduled to continue during winter 2004.

Project title: Demographic Monitoring Program for Yellowstone Pronghorn

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Purpose: Yellowstone pronghorn (*Antilocapra americana*) face a serious risk of extinction because isolation, low abundance and recruitment, and reductions in the quantity and quality of available winter range have increased their susceptibility to random, naturally occurring catastrophes such as severe winter weather, droughts, or disease epidemics. We propose to implement a rigorous monitoring program of key vital rates (e.g., adult fecundity and survival, recruitment) that are indicative of pronghorn population dynamics and continue the monitoring program through FY 2010. Key vital rates that will initially be monitored include the following: (1) annual estimates of cause-specific mortality and survival rates for adult females; (2) annual estimates of recruitment and reproductive rates; (3) annual estimates of population size and associated variance; (4) estimates of age structure; and (5) influence of environment and weather on vital rates. Remaining fee-demo funds designated for pronghorn conservation (approximately $60,000) would be used to establish this monitoring program during FY 2003. We will also solicit matching funds from partnerships to implement the following necessary conservation actions and research: (1) study of cause-specific mortality and survival rates of young (i.e., assess the possible regulatory role of predation); (2) monitoring of distribution, movements, and behavior; (3) periodic monitoring of genetic heterozygosity; (4) habitat restoration; (5) development of contingency plans; (6) periodic assessments of resource availability and use, nutrition, and physiological condition; and (7) periodic assessments of parasites and diseases. The proposed monitoring program requires that capture operations be conducted periodically to maintain a sample of >20 radio-collared adult females in the population. Because pronghorn are extraordinarily difficult to capture and immobilize, there is the risk of animal deaths during capture operations. We have developed a helicopter net-gunning capture plan to minimize this risk, and will ensure that the population is not adversely affected by the proposed monitoring and research program. The proposed monitoring and research program will benefit the park by: (1) enabling resource managers to respond quickly and effectively to future, possible decreases in abundance of Yellowstone pronghorn; (2) addressing key recommendations of the National Research Council's three-year review of ungulate management and the pronghorn expert panel convened in Yellowstone National Park during January 2002; (3) providing essential information for analyzing proposed alternatives for the possible re-alignment of the north entrance road; and (4) implementing the Network Inventory and Monitoring initiative by providing, testing, and reporting on a monitoring program of key vital rates.

Findings: We continued to monitor the demographics and migratory movements of Yellowstone pronghorn fit with VHF telemetry collars during 1999 and 2000. We obtained locations, group composition, behavior, and other information from approximately 650 groups (>1 animal) of radiocollared pronghorn during January through December 2003. We also conducted a spring population count, summer classification, and two aerial surveys during their spring and autumn migration periods. The annual spring count of the Yellowstone pronghorn population was conducted on March 25, 2003, using a Supercub airplane and a single observer. In addition, during the survey flight a seasonal wildlife biologist used telemetry to independently locate 14 radio-collared pronghorn from the ground. These ground locations of radio-collared animals were obtained to provide an assessment of the effectiveness of the aerial observer at detecting and accurately counting pronghorn. The minimum count of Yellowstone pronghorn based on combined aerial and ground surveys was 246 pronghorn. This total includes 196 pronghorn counted by the aerial observer in the Gardiner basin, 23 pronghorn missed by the aerial observer but detected by the ground observer in the Gardiner basin, and 27 pronghorn observed in the Carbella area of Paradise Valley on March 20, 2003. This count is similar to annual counts obtained during 1995 to 2002 (range = 204–242 pronghorn), suggesting that the abundance of the Yellowstone pronghorn population has remained approximately constant during this time period. The annual summer classification of Yellowstone pronghorn was conducted on July 14, 2003, using a SuperCub airplane and a single observer. We classified 113 pronghorn (73 adult females, 11 adult males, and 29 fawns) in 19 groups. Observed sex and age ratios for these pronghorn were 40 fawns per 100 adult females and 15 adult males per 100 adult females. These results suggest that fawn survival and recruitment may be somewhat higher this year than during the previous five years. Thirty-five percent of the observed adult pronghorn were non-migratory residents of the Gardiner basin, whereas 65% of the adults were migratory and summered in areas east of Mammoth. Likewise, 65% of radiocollared adult female pronghorn were migratory during 1999–2001. More fawns were observed in migratory groups (n = 22 fawns) than non-migratory groups (n = 7). However, the ratio of fawns per 100 adult females was similar between non-migratory (32 fawns per 100 females) and migratory (43 fawns per 100 females) groups. Migratory pronghorn "stage" on the flats and lower elevation slopes at the northwest end of Mount Everts for approximately one to two weeks prior to their migratory movements. The majority of spring migratory movements occur during April and early May, while autumn movements occur during August through early November. Pronghorn appear to use at least three migratory routes over Mount Everts to travel to and from their summer range. Migratory pronghorn summer in widely dispersed areas, including the Blacktail Deer Plateau, Oxbow Creek slopes, Hellroaring slopes, Specimen Ridge, and Lamar Valley. Location and attribute data for observed Yellowstone pronghorn have been entered into an Excel database and Geographic Information System (GIS). Monitoring will continue during FY 2004, and we intend to capture and radiocollar additional pronghorn during winter 2004.

Project title: Multi-Trophic Level Ecology of Wolves, Elk, and Vegetation in Yellowstone National Park: Calf Elk Mortality Study

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Purpose: In March 2002, the National Research Council completed a three-year review of ungulate management on the northern range of Yellowstone National Park. The Council concluded that information regarding the extent of predation by wolves (*Canis lupus*) on elk (*Cervus elaphus*) that is additive rather than compensatory to historic patterns of mortality (e.g., winterkill, other predators) was critical for the effective management of this area in the future. Approximately 12,000 elk winter on the northern range and constitute the primary prey for wolves (>85% of winter kills) residing in this area (approximately 77 wolves during 2002). NRPP funds will be used to implement a three-year study (FY 2003–05) of northern Yellowstone elk that focuses on determining cause-specific elk calf mortality and evaluating the degree to which wolf predation is additive or compensatory to other forms of mortality. Each year, a sample of 35–50 calves that are <3 days old will be captured and fitted with ear tag transmitters that enable us to quickly and conveniently monitor their survival on a daily basis. Our sampling areas and methods will be similar to those used during a study of elk calf mortality conducted in Yellowstone National Park during 1987–1990 (i.e., prior to the restoration of wolves). That study estimated the timing of births, mass of neonates, calf survival, cause-specific calf mortality, recruitment, population size, and environmental conditions (e.g., summer and winter precipitation). These data provide a baseline for comparison with post-wolf recovery data and assessment of the degree to which wolf predation is compensatory or additive to other forms of mortality.

Findings: During May 2003, the Yellowstone Center for Resources, U.S. Geological Survey, and University of Minnesota initiated a three-year study (FY 2003–05) of mortality in northern Yellowstone elk calves. The primary objectives of the study were to: (1) estimate the relative causes and timing of calf deaths; (2) estimate calf survival rates; and (3) evaluate factors that may predispose calves to death. During May and June 2003, a total of 51 calves <6 days old were captured and fit with ear tag transmitters. These calves were subsequently monitored on approximately a daily basis. During May through December 2003, 34 instrumented calves died (31 predation, 3 other causes) and one transmitter ceased functioning. Preliminary determinations of causes of death for instrumented calves were 19 killed by bears, 5 killed by wolves, 3 killed by coyotes, 2 killed by either wolves or bears, 1 killed by a mountain lion, 1 killed by a wolverine, and 3 non-predation deaths due to unknown causes. Monitoring of instrumented calves will continue through winter 2004, and the next capture is scheduled for May and June 2004.

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