YELLOWSTONE SCIENCE

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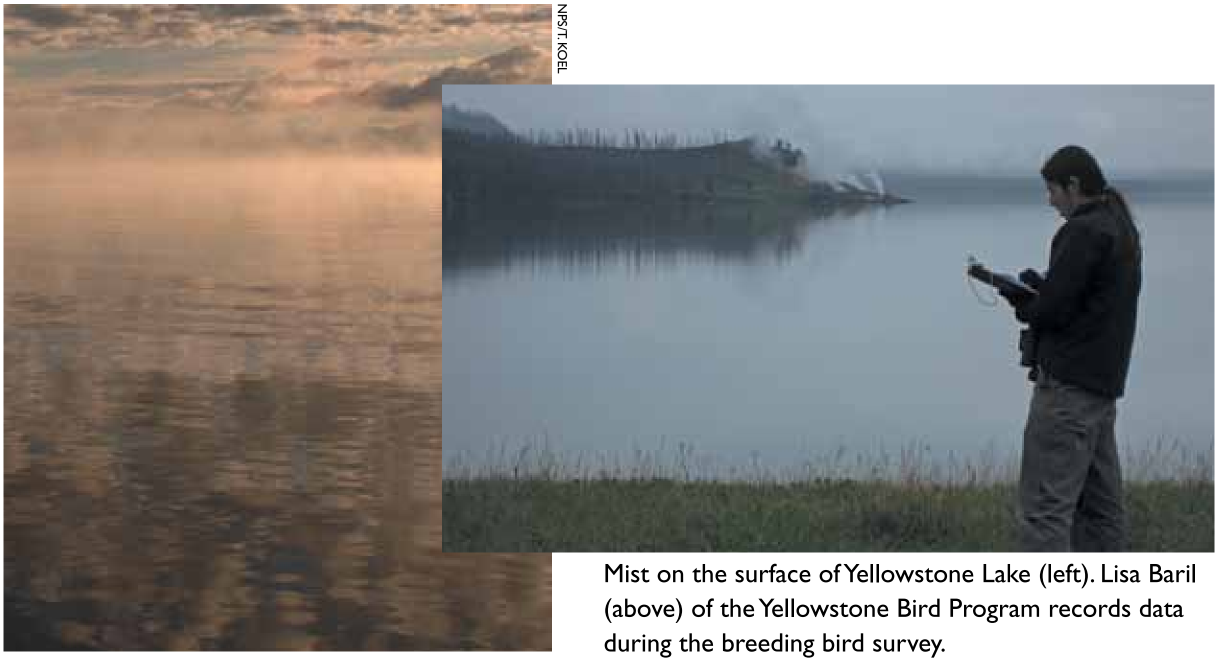
The Myriad Microfauna of Yellowstone Lake

Moss on a Hot Vent

Monitoring Birds of Prey


NPS/T. KOEL

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Mist on the surface of Yellowstone Lake (left). Lisa Baril (above) of the Yellowstone Bird Program records data during the breeding bird survey.

Observations Through a Lens

In this issue of *Yellowstone Science*, we decided to focus on the little things in life, or the smaller and unique resources found in the park. I find that visitors are often amazed by Yellowstone's charismatic megafauna such as bison, bears, or elk. They are awed by the beauty of the Grand Canyon of the Yellowstone and the park's ubiquitous geysers. Yet often, the small and sometimes invisible wonders of the park are overlooked.

We draw attention to a recent publication that examined the use of osteological remains of megafauna to establish records of species diversity and the use of landscapes from present to decades past. Josh Miller has summarized the article based on his research that was featured on the cover of the journal *Ecology*.

Articles by Mary Ann Franke and members of the Molecular All-Taxa Biodiversity Inventory Team highlight some of the smallest organisms found in the lake. Supported by the lake's unique geothermal input, these organisms form the building blocks for a complex food web important to higher forms of life. While small in size and largely invisible to the naked eye, the Bacteria, Archaea, and Eukarya of the lake make a sizeable contribution to its function and diversity. They also provide researchers with an opportunity to educate students and teachers about the park's hidden treasures.

We also take time to honor one of the world's most influential scientists, Carl Woese. Dr. Woese's discovery of Archaea and its subsequent classification as a new branch in the Tree of Life changed the study of microbiology. Carl's influence on how organisms are identified and classified has had a significant impact on microbiological research in Yellowstone, and beyond.

Lisa Baril and her colleagues write about another research effort the park has embarked on, the Yellowstone Raptor Initiative. This program is designed to inventory and track the status of birds of prey that are of special interest to the park. Lisa and her co-authors spend hours behind binoculars looking up, identifying and counting raptors on the wing, soaring high above where we tend to look for wildlife.

As the visiting editor of *Yellowstone Science*, I had the opportunity to work closely with an amazing group of writers and editors, including former editor Janine Waller, who is now applying her talents in Yellowstone's Resource Education and Youth Programs office. Janine's dedication to science education in the Greater Yellowstone Ecosystem is appreciated—her contributions to *Yellowstone Science* will be missed by staff and readers alike. Thanks Janine, and good luck at your new job!

We hope you enjoy the issue.

Christie Hendrix

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Cover photo:
A September sunrise on
Yellowstone Lake.
Photo by Brian Ertel.


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Doug Smith, Yellowstone Bird Program leader and participant in the Yellowstone Raptor Initiative, at a migratory bird site.

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An interdisciplinary team of researchers used molecular genetics to identify thousands of new species and determine their relationship to the lake's hydrothermal features. A project overview is followed by summaries of three of the papers that have been published as a result of this research.

Bacteria: Geochemistry and Community Analysis

Archaea: Comparing Extreme and Non-Extreme Environments

A Moss Colony: Survival in Geothermal Heat and Darkness

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This addition to the park's bird monitoring program is increasing our knowledge of eagles, hawks, falcons, and owls.

Lisa M. Baril, David Haines, Katharine E. Duffy, and Douglas W. Smith

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NEWS & NOTES

Workshop Examines Impact of Brucellosis on YNP's Bison

In February, Yellowstone National Park and Montana Fish, Wildlife and Parks conducted a three-day workshop that examined the science behind brucellosis, including the feasibility of and need for suppressing the disease in Yellowstone's bison population.

The workshop featured a panel of scientists with backgrounds in wildlife management and disease ecology who discussed current bison conservation and brucellosis management within the Interagency Bison Management Plan (IBMP). IBMP goals include conserving a free-ranging bison population of about 3,000 animals while minimizing the risk of brucellosis transmission to domestic cattle.

The panel considered public attitudes toward brucellosis, the nonnative bacterium that causes it, immunology, disease ecology, bison behavior and demographics, conservation biology, and transmission risk to other wildlife and cattle.

Key points of agreement reached by the panel include:

- Management to maintain separation between cattle and bison appears to have been effective at preventing transmission of brucellosis between these species.
- The best available data do not support that vaccination of wild bison with currently available vaccines would be effective in suppressing brucellosis to a level that would change bison management strategies.
- Control of the bison population size will likely continue to require culling or removal, along with hunting. Past and current culling practices have not had an apparent effect on reducing the prevalence of brucellosis in bison populations.
- Intervention through contraception is not needed to achieve the current goals of the IBMP. Contraception might assist in brucellosis suppression, but the available data are insufficient to make a judgment without further research.

The agencies will consider the panel's findings in the ongoing development of short- and long-term strategies consistent with the IBMP. The panel's final report is expected to be released in mid-2013.

Museum of the Rockies Plans Yellowstone Tourism Exhibit

A new exhibit about the history of tourism in Yellowstone National Park will open in September at the Museum of the Rockies in Bozeman, Montana. *Welcome to Yellowstone! Tourism in America's First National Park,* was developed by Museum of the Rockies staff and will explore how transportation, tour operators, park employees, and world events influenced park tourism through the late 19th and 20th centuries. The exhibit will run through January 2014.

Through a selection of artifacts and multimedia, *Welcome to Yellowstone!* will examine early tourist travel to the park, highlighting the early role of the Northern Pacific Railroad and later influence of automobiles on park accessibility and visitation. The exhibit will also focus on places people stayed in the park over time, from roadside tent-camps and rustic cabins to grand hotels like the Old Faithful Inn.

The *Welcome to Yellowstone!* exhibit will feature a variety of artifacts from the Hamilton–Povah Yellowstone Collection. Donated to the Museum of the Rockies in 2009, this extensive collection includes souvenirs sold in Yellowstone during the 20th century.

Yellowstone's Heritage and Research Center has loaned numerous pieces to the exhibit, including a US Cavalry sword and scabbard, a suitcase from the Shaw and Powell Camping Company, and other rare park souvenirs.

For more information, please visit www.museumoftherockies.org.

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Exhibit items include this Disneyland songbook and suitcase with souvenir stickers.

Oral History Symposium Records Recollections From 1995 Wolf Reintroduction

Many of the people who played key roles in the 1995 reintroduction of wolves to Yellowstone gathered at Mammoth Hot Springs, Wyoming, last October to share their personal experiences and perspectives as part of an oral history symposium.

Attended by about 40 managers, attorneys, biologists, historians and government wildlife trappers, the symposium documented the behind-the-scenes work of a significant conservation effort. For many participants, it was their first contact with each other since the reintroduction effort began.

Ed Bangs, the US Fish and Wildlife Service's wolf recovery coordinator at the time of the reintroduction, presented the keynote address. Yellowstone Wolf Project leader Douglas Smith, former Yellowstone Superintendent Bob Barbee, and former Yellowstone Wolf Project leader Mike Phillips, also gave presentations.

Symposium participants watched film taken by Bob Landis and others in 1995 and 1996 of the trapping of the wolves in Canada and their arrival, confinement, and eventual release in the park. During the screening, Landis filmed the audience as their recollections of the events were recorded. A copy of the film with that narration is available in the park library.


NPS/J. PEACO

About 40 people involved in the 1995 wolf reintroduction attended October's symposium.

The group participated in an early morning wolf-watching expedition. Douglas Smith, standing in the Lamar Valley, observed that "we tend to focus on biology and forget the human side of big events in Yellowstone. The Wolf Oral History Symposium was about the people, and reconvening this crowd was memorable."

The individual oral histories of wolf symposium participants will be collected throughout 2013 and archived with the conference video, transcripts of formal presentations, moderated discussions, and group interviews. For more information, contact charissa_reid@nps.gov.

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NPS/J. PEACO

Wolf symposium attendees and participants in the 1995 reintroduction of wolves to Yellowstone.

SHORTS

Elk Antler and Neonatal Remains Record Landscape Use

Miller, J.H. 2012. Spatial fidelity of skeletal remains: Elk wintering and calving grounds revealed by bones on the Yellowstone landscape. *Ecology* 93(11): 2474–2482.

Establishing how animals use their landscapes and how those needs change across seasons and years is essential for successful wildlife conservation and management. While species' landscape use (including home ranges, birthing grounds, and breeding areas) often varies across annual, decadal, and longer timescales, the available data with which we understand these patterns are often based on few individuals sampled across a restricted number of seasons and generations. Given the common absence of historical multi-season, multi-decadal population studies, other methods are needed to obtain extended temporal perspectives and more fully investigate the effects of climate change and other anthropogenic influences on animal communities.

Bones can persist on landscape surfaces for centuries in temperate settings and millennia in arctic latitudes. Landscape bone accumulations also faithfully record local diversity and past population changes. To discover whether bone accumulations additionally capture fine-scale patterns of season-specific landscape use, I compared the locations of shed elk antlers (which are dropped annually in late winter) and the skeletal remains of newborn elk calves (produced in spring) found across Yellowstone National Park's northern range to known bull elk wintering areas and elk calving grounds. To survey the bone accumulation, I sampled 10 plots in each of four habitats (grassland, forest, lake margin, and river margin) and logged the number of encountered shed antlers and the bones of newborns. The plots were 1 km in length and varied in width from 30 to 100 meters depending on habitat type. Using these data, I calculated the concentrations of antlers per square kilometers in each of the 40 sample plots and noted where neonatal remains were present or absent.


J. MILLER

A section of elk antler found on the park's landscape during Miller's research.

The concentrations of shed elk antlers tightly corresponded to areas used by bull elk as reported in late-winter aerial surveys; regions with higher antler concentrations had been used by higher concentrations of bull elk (bulls/km^2). Highlighting the long-term, multigenerational nature of landscape bone assemblages, antler concentrations were generally more faithful to decadally averaged patterns of bull elk landscape use than were individual aerial surveys themselves. Additionally, despite the susceptibility of newborn elk to bone loss due to weathering and consumption or dispersal by carnivores, they were found in all sampled calving areas and were not found in areas without documented use for calving and early rearing activity. These results indicate that bone accumulations can record highly faithful data on landscape use and that bone surveys offer a powerful new tool for providing historical perspectives on species' landscape use—data that are currently unavailable for most ecosystems.

—Joshua H. Miller, University of Cincinnati

Response of Lake Trout to 15 Years of Harvest in Yellowstone Lake

Syslo, J.M, C.S. Guy, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and T.M. Koel. 2011. Response of nonnative lake trout (*Salvelinus namaycush*) to 15 years of harvest in Yellowstone Lake, Yellowstone National Park. *Canadian Journal of Fisheries and Aquatic Sciences* 68(12):2132–2145.

Yellowstone National Park's lake trout (*Salvelinus namaycush*) removal program was initiated in 1995—a year after the nonnative species was verified to be present in Yellowstone Lake, which contains genetically pure Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*). This program, intended to provide protection for the Yellowstone cutthroat trout population, is the longest ongoing lake trout removal project in the Intermountain West. Using data from 1996 to 2009, including individual growth, body condition, fecundity, length and age at maturity, and mortality of lake trout, the authors evaluated the success of the removal program. They also applied a demographic model to simulate several scenarios of fishing mortality and the subsequent effects on population growth.

Models indicated the lake trout population was still increasing, even after more than a decade of removal efforts. It is likely that the drastic decline in the Yellowstone cutthroat trout population made more prey available for juvenile lake trout. At the same time, fewer cutthroat trout were available as prey for the older lake trout, contributing to slower growth in older lake trout.

Several population metrics indicated that lake trout have not reached carrying capacity in Yellowstone Lake. Population growth was most sensitive to reproduction, indicating that increasing the harvest of mature lake trout or targeting developing lake trout embryos may increase the effectiveness of lake trout suppression.

Lake trout suppression was initiated without knowledge of population size and harvest benchmarks that would lead to successful population decline. Consequently, the program proceeded for several years with insufficient harvest, and the lake trout population continued to increase. Harvest targets provided in this study will help inform lake trout suppression in the future. Lake trout suppression efforts in Yellowstone Lake highlight the necessity for baseline data, long-term planning, and a large amount of fishing pressure to substantially reduce a nonnative predator from a large, natural water body.

Responses of Benthic Macroinvertebrates to Geothermal Discharges

Clements, W.H., J.L. Arnold, T.M. Koel, R. Daley, and C. Jean. 2011. Responses of benthic macroinvertebrate communities to natural geothermal discharges in Yellowstone National Park, USA. *Aquatic Ecology* 41(1):137–149.


W. CLEMENTS

Geothermal discharge into local waterways is a factor influencing resident benthic communities.

The macroinvertebrates found in the sediments of streams, rivers, and lakes are often used in aquatic monitoring because they remain in one location during their lives and different taxa vary widely in their responses to various stressors. The abundance of certain taxa and specific traits such as body size and dispersal ability can serve as indicators of stream health. However, monitoring programs need to be able to distinguish the effects of stressors such as atmospheric deposition, road construction, wastewater, and climate change from the natural variations present in background conditions. In Yellowstone National Park, the background conditions often include geothermal influences such as high water temperature, high conductivity (indicative of the elevated concentration of trace elements and other dissolved ions), low pH, reduced dissolved oxygen, and the input of fine sediments.

The primary objectives of this research were to quantify the effects of geothermal discharges on benthic communities, develop a multimetric index that could be used to distinguish geothermal effects from anthropogenic stressors, and identify thresholds in the responses of benthic macroinvertebrates to gradients of geothermal influence. Data on water quality, habitat, and benthic macroinvertebrates were collected from 143 sites on 32 streams that were grouped into four categories based on their conductivity: non-geothermal (reference sites), low, moderate, and high.

Most taxa were significantly less abundant at moderate geothermal sites than at reference sites, and some highly sensitive taxa (*Epeorus* sp., *Sweltsa* sp., *Zapada* sp., *Rhyacophila* sp.) were also reduced at low geothermal sites. However, certain damselflies (*Argia* sp.) and caddisflies (*Oxyethira* sp.) and the exotic New Zealand mudsnail (*Potamopyrgus antipodarum*) were found almost exclusively at geothermal sites. More generally, the benthic communities at the geothermal sites were dominated by organisms considered tolerant of anthropogenic stressors, including those found in streams impacted by mining activity. While factors other than geothermal characteristics likely influence the distribution and abundance of *P. antipodarum*, these data suggest that mudsnails are highly tolerant of the warm waters and elevated concentrations of trace elements in geothermal discharges**.**

Starting with a list of 21 possible metrics for benthic macroinvertebrates, the researchers arrived at five whose variation was most strongly correlated with the extent of geothermal influence at a site: percent tolerant species, the Hilsenhoff biotic index, the Shannon diversity index, the percent Ephemeroptera (mayflies) plus Plecoptera (stoneflies), and the richness of Ephemeroptera, Plecoptera, and Trichoptera (caddisflies). The resulting multimetric index showed a clearly defined threshold response to geothermal effects at very low levels of conductivity. However, this abrupt shift in community composition means that the magnitude of these effects must be quantified before the impacts of potential anthropogenic stressors can be assessed in streams with low geothermal input.

Although this multimetric index is specific to geothermal effects, the method used to develop it could also be applied to other systems in which the impacts of stressors must be assessed within the context of natural environmental gradients.

YS

Genetic Diversity in Yellowstone Lake:

The Hot and Cold Spots

Mary Ann Franke

NPS/J. PEACO

> *A lake is to the naturalist a chapter out of the history of a primeval time, for the conditions of life there are primitive,—the forms of life are, as a whole, relatively low and ancient, and the system of organic interactions by which they influence and control each other has remained substantially unchanged from a remote geological period. . . . Nowhere can one see more clearly illustrated what may be called the sensibility of such an organic complex,—expressed by the fact that whatever affects any species belonging to it, must speedily have its influence of some sort upon the whole assemblage. He will thus be made to see the impossibility of studying any form completely, out of relation to the other forms,—the necessity for taking a comprehensive survey of the whole as a condition to a satisfactory understanding of any part. If one wishes to become acquainted with the black bass, for example, he will learn but little if he limits himself to that species. He must evidently study also the species upon which it depends for its existence, and the various conditions upon which these depend and the entire system of conditions affecting their prosperity. . .*
>
> —*from* The Lake as a Microcosm, *by Stephen A. Forbes (1887)*

A pioneer in the field becoming known as ecology, Stephen Forbes conducted the first scientific inventory of invertebrates collected from Yellowstone Lake, "the aquatic headquarters of the region," as he called it in his 1893 report. "Our knowledge of the bottom fauna of Yellowstone Lake is based on the product of eleven dredgings, at depths varying from 15 to 198 feet. . . . In this deepest water the most abundant inhabitants of the bottom were long and slender annelid worms (*Oligochaeta*) not yet studied, blood-red larvae of *Ghironomus* of considerable size, and a small bivalve mollusk (*Pisidium*)." Forbes also noted that "the hot springs and geysers are found mostly on the western arm and at Steamboat Point in the northeastern part of the lake, but the amount of warm water contributed by them is quite insignificant for so large a lake."

What Forbes could not detect with his simple equipment was the effect of the far hotter fluids rising from vents in the lake floor. After six years of limnological studies starting in the mid-1950s, geothermal influences in the lake were still thought to amount to nothing more than a small influence in West Thumb (Benson 1961). But the irregular shape and temperature zones of Yellowstone Lake are the result of multiple volcanic eruptions, glacial advances and retreats, and hydrothermal activity that have been taking place over millennia (Morgan et al. 1977, 2007). The northern half of the lake lies within the crater left by the massive volcanic explosion 640,000 years ago; a smaller eruption approximately 174,000 years ago created the basin of the West Thumb. The swarms of earthquakes with epicenters beneath the lake floor have provided evidence of the underlying geothermal activity that is visible elsewhere in the park's springs, geysers and fumaroles.

Melting snow drained by more than 141 streams empties into Yellowstone Lake, most of which is covered by several feet of ice for half the year. Like most lakes in cold regions underlain by igneous rocks and filled largely by snowmelt, it is generally low in nutrients even in its photic zone (approximately the upper 10 m), where light is the main source of energy. With a surface area of 341 km^2 (131 mi^2) at an elevation of 2,357 m (7,730 ft), it is the largest high-altitude lake in North America and one of the few large freshwater bodies with geothermal inputs (Morgan et al. 2007). Others include Lake Baikal in Russia, Crater Lake in Oregon, Lake Taupo in New Zealand, and Lake Tanganyika in Tanzania (Buchholz et al. 1995).

Hydrothermal activity influences lake chemistry and therefore the food webs that are an essential part of the park's ecological processes.

More than One Food Chain in the Lake

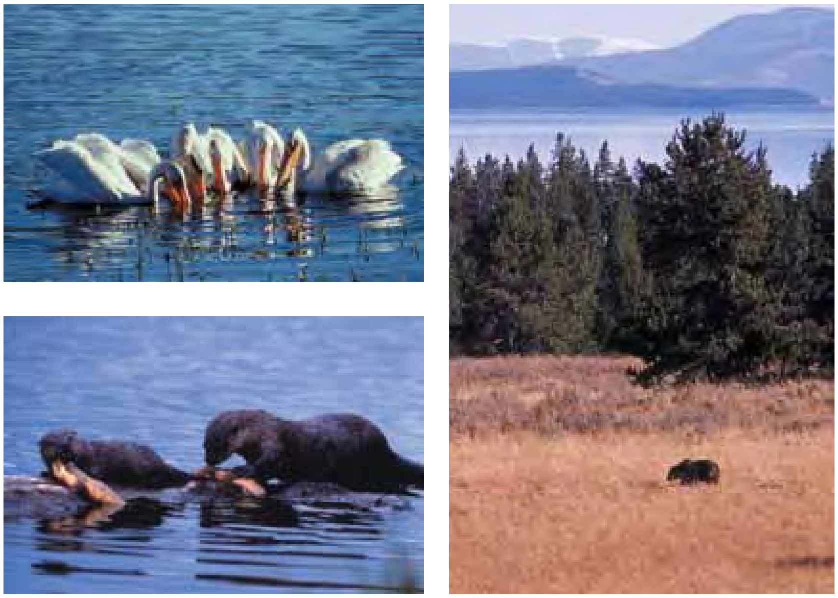
While the food chain in fresh water lakes typically starts with photosynthesis, which promotes the growth of microbes that feed zooplankton, geothermal vents release chemicals upon which other microbial species thrive. In 1984, observations of gas bubbles rising to the surface and "false bottoms" in the lake caused by sharp changes in density led to scuba explorations of the shallow, near-shore waters and the first reports of gas fumaroles surrounded by microbial and algal mats (Buchholtz et al. 1995). The University of Wisconsin researchers found that water samples from the lake's hydrothermal vents were rich in inorganic nutrients and their chemistry differed even within the same area of the lake (Klump et al. 1988).

Water coming out of a vent forms a plume that mixes with the cold water and transports vent material up through the water column. The influence and the size of the plume depend upon the amount and periodicity of flow coming out of the vent (Maki et al. 2002). Some vents emit methane and hydrogen sulfide that microbes use as an energy source in their aerobic metabolism; some release high amounts of arsenic and mercury that have shown up in the lake's trout and in bears and other animals that eat the trout. Each vent could provide a different habitat for different microbial communities. By adding heat and geochemical fluids to localized areas in the lake, the hydrothermal fields were creating organism-specific niches for significant biodiversity (Friend 2007).

High-resolution mapping of the lake, made possible by developments in global-positioning technology, sonar imaging, and a remotely operated submersible vehicle (ROV), helped confirm the unsurprising resemblance of the lake floor to other geothermally-influenced parts of the park. These surveys documented hydrothermal explosion craters, some as large as a football field, clustered along underwater faults, and more than 660 vents emitting fluids with temperatures up to 120°C (248°F) (Morgan et al. 2007).

This hydrothermal activity influences lake chemistry and therefore the food webs that are an essential part of the park's ecological processes (Clingenpeel et al. 2011). Microbial communities similar to those that had been found in the park's terrestrial hot springs might be living in or near the vents on the lake bottom. A century after Stephen Forbes, however, little study had been devoted to the species upon which the more visible animal life "depends for its existence, and the various conditions upon which these depend." As characterized by John Varley, senior research scientist at Montana State University's Institute on Ecosystems, "the known food chain was short" and largely limited to what the trout ate and what ate the trout (Jaeger et al. 2012).

During the 1990s, the lake's prokaryotes received their first major microbiological attention with "the characterization of a new thermophilic sulfate-reducing bacterium" that was named *Thermodesulfovibrio yellowstonii* (Henry et al. 1994), and the seasonal distribution patterns of the lake's major diatom species were documented (e.g., Interlandi et al. 1999). But little was known yet about other planktonic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Diverse species of fish, birds, and mammals in the Yellowstone Lake area depend on a complex food chain with planktonic bacteria and archaea at its foundation.

species smaller than rotifers (about 1 mm). Planktonic bacteria and archaea are regarded as the base of a pyramid that feeds a diverse array of bacteria-eating invertebrates and protists, which become food for rotifers, crustaceans, and insects, which are consumed by birds and fish, which are consumed by other bird and fish species, which provide food for bears, bald eagles, osprey, otter, and several dozen other species. "In short, the lake's bacterial and archaeal inhabitants are the unseen and silent foundation to a complex food chain," says Tim McDermott, of MSU's Thermal Biology Institute.

Although the first forms of life on Earth were single-celled microorganisms that appeared about 4 billion years ago, biological evolution has been more accurately depicted as a tree than a linear progression from the simple to the more complex. The "tree of life" proposed by Charles Darwin as a metaphor for the evolutionary relatedness among species has itself evolved over time and become the focus of dogged debate as to which taxa branched off from which, whether to include viruses, and the reliability of fossil records versus molecular techniques for comparing organisms. Most contemporary biologists assent to a tree in which life is categorized into three domains: Bacteria, Archaea, and Eukarya (fig. 1). For about 3 billion years, all organisms were microscopic, and most still are, but microorganisms include the smaller eukarya, such as protozoa, fungi, algae, and animals such as rotifers and planarians. While Bacteria and Archaea are both prokaryotes (with no true nucleus), rRNA evidence suggests that Archaea are more closely related to Eukarya (single-celled or multicellular organisms whose cells contain at least one distinct nucleus) than to true Bacteria (Sugden et al. 2003).

Microbial Biodiversity

Traditional microbiology and genome sequencing required the use of cultivated microbial isolates, but less than 1% of all microbial species present in an environmental sample have been successfully grown under laboratory conditions (Rusch et al. 2007). That is because using a particular growth medium selects for the organisms that can grow on that medium. During the last decade, however, advances in molecular genetics have provided a way to sequence microbial DNA without first isolating the individual organism. Metagenomics, as this field is called, is the study of metagenomes, or what John Varley refers to as "community DNA"—the genetic material recovered directly from the community of organisms within a sample.

These new techniques have shown that cultivation-based methods miss the vast majority of microbial biodiversity. They have also made possible rapid biodiversity assessments and examination of the often complex relationship between biochemical and genetic diversity. For example, distinguishing between bacterial and archaeal species is often based on the 16s ribosomal RNA gene, which codes for a portion of the ribosome, a cellular structure where protein synthesis occurs. Organisms in which the 16s rRNA gene sequences

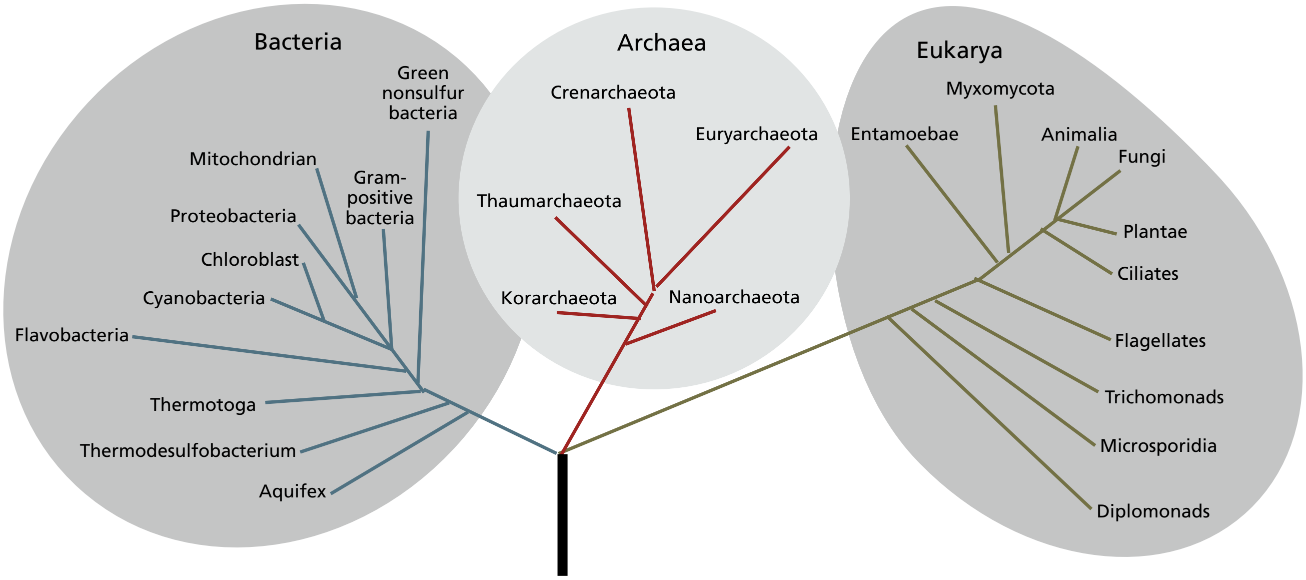

Figure 1. A simplified rendering of the phylogenetic tree ("tree of life") with some of the major taxonomic groups. The relative positions of each branch continue to be debated, along with the position of the root of the tree. Some of the emerging theories about horizontal gene transfer (of which Carl Woese became a strong proponent; see related story, page 13) suggest that a tree may not be the most appropriate way to represent genetic relationships. (Drawing by M.A. Franke based on Morgan et al. 2007.)

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During the pilot study, water samples were collected by divers in the shallow waters of the West Thumb area.

are at least 97% identical are commonly considered to be the same ribotype, which is generally understood to mean the same species. However, organisms that have at least 97% identical ribotypes may have very different physiology, biochemistry, and genome content (Rusch et al. 2007).

Using these new techniques, in 2003 the biologist and entrepreneur Craig Venter launched an effort on his personal yacht to assess the genetic diversity of marine microbial communities. In subsequent years, with funding from the Gordon and Betty Moore Foundation as well as the US Department of Energy, what became known as the Global Ocean Sampling Expedition (GOS) circumnavigated the planet collecting water samples. Of the 811 microbial ribotypes identified from water collected at 41 locations in 2003 and 2004, 52% were found to be novel at what is typically considered the species level, and 16% were different enough to constitute new families of Bacteria (Rusch et al. 2007).

Unlike most plant and animal species, microbes have evolved through metabolic diversification rather than increasing cell complexity and organism structure (Warren and Kauffman 2003). A microbe is expected to proliferate when the substrate suits its biochemical requirements, which should be predictable from its genomic profile or, if examining at the microbial community level, the metagenomic profile. "The enormous genomic diversity found in the GOS study presumably reflects environmental pressures," John Varley noted in a proposal to study the microbial life Yellowstone Lake, but "spatial and volumetric issues, and the homogeneity of marine surface waters" make that "difficult to establish" (Varley et al. 2007). John Varley and his colleagues pointed to evidence suggesting that the vents in Yellowstone Lake are analogous to marine hydrothermal systems while being far easier to sample and at one tenth the cost. "Given the importance of hydrothermal flux to its ecology, Yellowstone Lake offered a promising model for examining factors contributing to the gene diversity and community structure of aquatic microbial habitats" (Varley et al. 2007).

SUSAN KELLY

Scott Clingenpeel filters samples from Yellowstone Lake's deeper waters to extract organisms for DNA sequencing.

The Yellowstone Lake Pilot Study

Concerns about the loss of global biodiversity in the 1990s led to the development of the "all taxa biodiversity inventory" as a concentrated effort to document as many species as possible within a given area, including information about their distribution, abundance, habitat, symbiotic relationships, and predator–prey interactions. The project proposed for Yellowstone Lake was called a "molecular all-taxa biodiversity inventory" (MATBI) because the primary objective was to use gene sequences to identify previously undocumented organisms in the lake—the 16s rRNA genes for Archaea and Bacteria, and the analagous 18s rRNA genes for Eukarya. With funding from the Gordon and Betty Moore Foundation through the Yellowstone Park Foundation, an interdisciplinary team of university researchers, federal agencies, and nonprofit and for-profit groups conducted a four-day inventory in 2004.

They collected water samples from three habitats within the water column at eight locations: (1) the photic zone, (2) the heated water in hydrothermal sediments and fluids, and (3) the area where the heated water mixes with the colder lake water (fig. 2). Liquid and solid samples from the photic zone were collected using the same type of equipment and protocols as in the GOS expedition. The high elevation and extremes of cold and heat increase the risks of scuba diving in Yellowstone Lake where, for safety and visibility reasons, the activity is generally limited to the photic zone (Buchholz et al. 1995). The divers worked in pairs, equipped with thermometer at the end of a pole and another pole with cups at the end for scooping sediments, and syringes for drawing vent water. Some of the vent fields in the West Thumb are in water barely 15 feet deep and new vents have opened up since the first high-resolution mapping efforts began.

Most of the samples from the hydrothermal vents and the mixing zones were obtained using an ROV that has increased in sophistication since it was first lowered into Yellowstone Lake for mapping and sample collection decades ago. Dave Lovalvo of Eastern Oceanics, which specializes in underwater operations, developed the ROV and operated it from a National Park Service boat. Weighing about 200 pounds and powered by an electric motor with propellers, the ROV was equipped with lights, cameras, temperature sensors, and a mechanical arm that can probe and collect samples from vent openings. A suction tube in the sampling cup is connected to a vacuum device that passes water samples into a container with a 1-mm mesh cover that allows water to pass through while collecting solid material. A tube from the sampling cup to the boat allows for larger water samples to be brought to the surface via a peristaltic pump located on the boat deck. (Lovalvo et al. 2010).

In the laboratory over a six-month period, the samples were subjected to phylogenetic analysis using standard protocols of DNA extraction, PCR amplification, cloning, and sequencing. Comparing the DNA sequence of an unidentified microbe to that of known microbes can establish the relatedness of one to another, or to a range of microbes, and sometimes determine its identity (Maki et al. 2002). GenBank, a database maintained by the National Center for Biotechnology Information (part of the National Institutes of Health), contains the sequences for more than 100,000 organisms. But relatively few microbial species in the database have been identified based on traditional Linnaean taxonomy, and if a microbe's genetic sequence does not match any in the database, it is either "new to science" or cannot be identified.

Using molecular genetics, 232 different species were distinguished in the samples collected during the pilot inventory. The genetic signatures were not known for most of the 263 species that had previously been reported for the lake, so the extent of overlap between the two sets of records could not be determined. However, all three domains were represented in the pilot inventory, including many archaeal sequences new to science, both thermophilic and oligotrophic species, and species genetically similar to those found in locations as far apart as Antarctica and the Caribbean Sea.

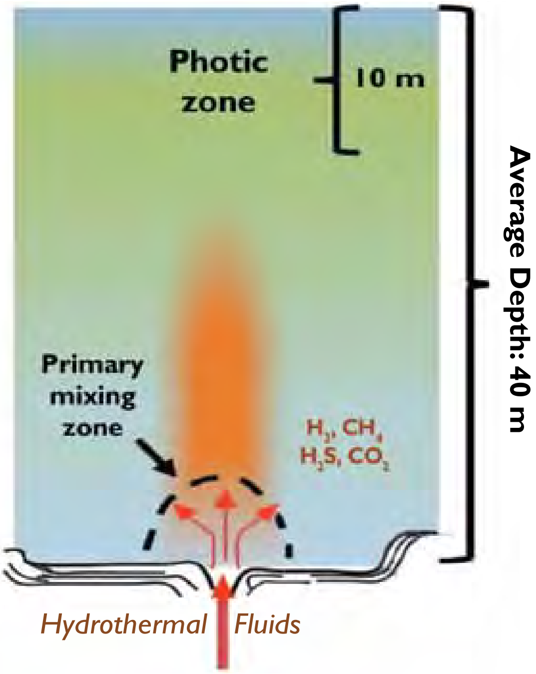


Figure 2. Schematic depicting a lake-floor hydrothermal vent and the impact on water column chemistry. Relatively shallow, high-output vents, such as those found in Yellowstone Lake's Inflated Plain, significantly influence the chemistry of the entire overlying water column. (Graphic courtesy of authors.)

Expanded Lake Survey

With the success of the pilot study, MATBI became a long-term project to characterize the diversity of species in Yellowstone Lake and their relationship to the lake's hydrothermal features. Additional funding from the Gordon and Betty Moore Foundation and a matching grant from National Park Service Centennial Challenge Initiative program supported a more extensive survey. New DNA sequencing technology allowed the examination of hundreds of thousands of 16S sequences, resulting in the identification of tens of thousands of species of Bacteria and Archaea throughout the lake.

The expanded survey also included a significant focus on Eukarya (e.g., microscopic fungi, protozoans, algae, nematodes, crustaceans, slime molds, and rotifers). "A top goal of this aspect of the project is to reduce a century of confusion over speciation of crustaceans in the lake," explains Stephanie McGinnis, now with MSU's Montana Watercourse. Researchers are looking at Cladocera, Copepod, and Amphipoda found in the lake to see if their genetic signatures reflect the morphological characteristics that have been used to differentiate species taxonomically (Varley and McGinnis, in prep.).

The project's technical partners have included Eastern Oceanics LLC, the J. Craig Venter Institute, the US Geological Survey, and the Thermal Biology Institute at

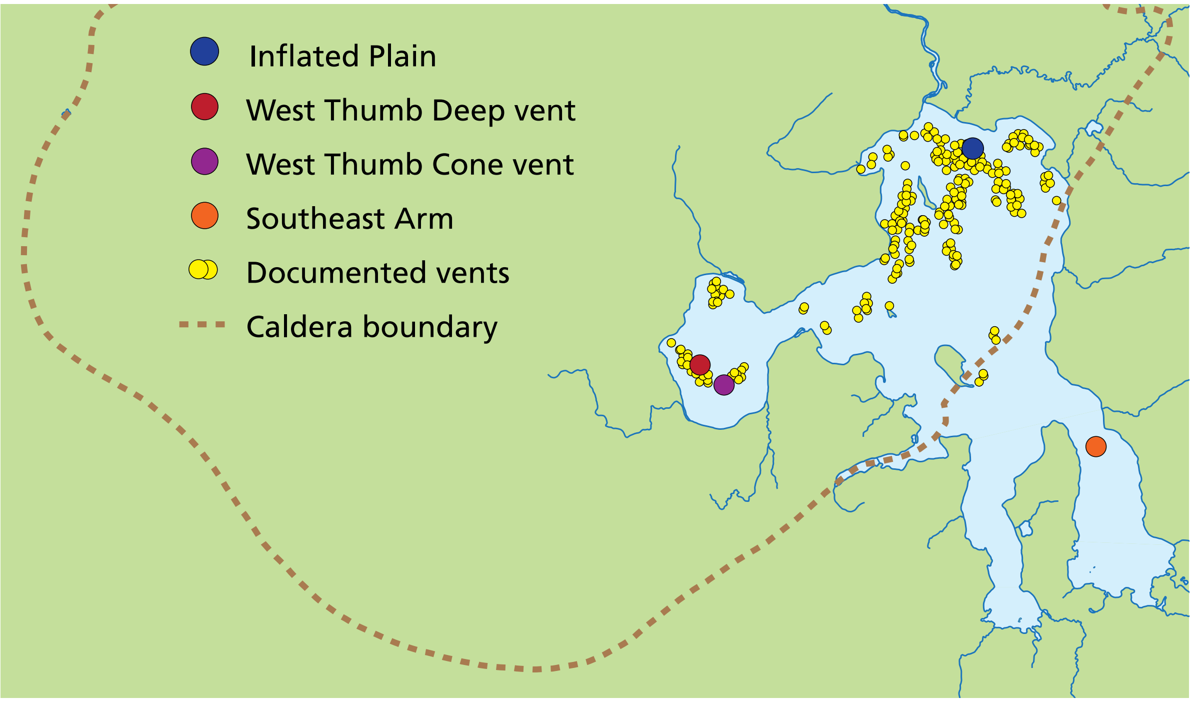


Figure 3. Four of the primary sampling locations in Yellowstone Lake. Areas where vents have been documented are also shown. (Drawing by M.A. Franke based on Morgan et al. 2007.)

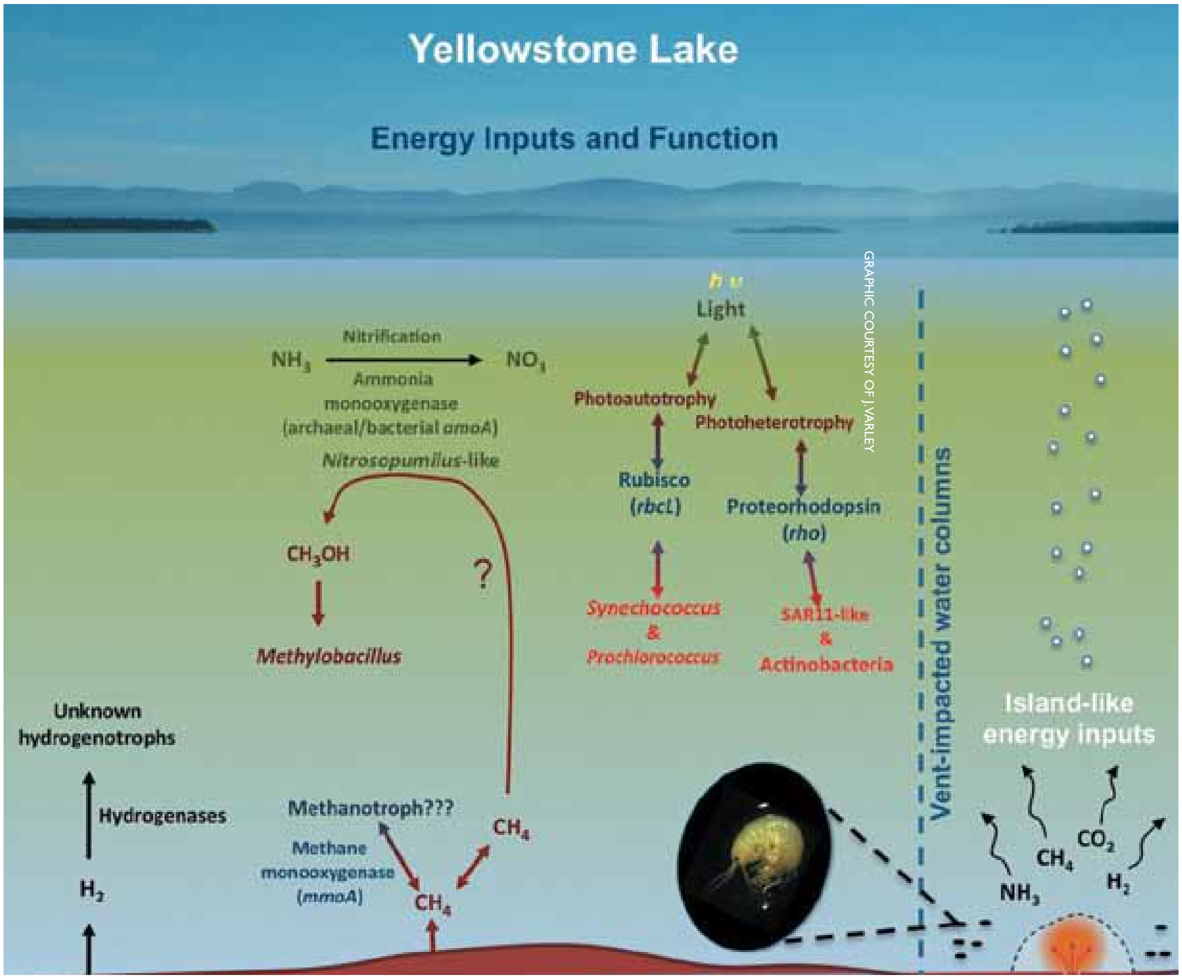
Montana State University (Varley et al. 2007). In September of 2007 and 2008, the researchers collected water samples from a total of 11 photic zone locations in three areas of the lake: the Southeast Arm, which is located outside the caldera boundary and appears to be lacking lake floor vent activity, and in the West Thumb and the Inflated Plain, where significant hydrothermal vent fields are known to occur (fig. 3) (Jaeger et al. 2012). The area referred to as "the inflated plain" is the largest hydrothermal dome in the lake, rising 30 m above the lake floor with a diameter of approximately 70 m (Morgan et al. 2007). The doming is believed to be caused by a buildup of steam, carbon dioxide, or other gases beneath a hydrothermal cap.

To determine the extent to which concentrations of hydrogen, carbon dioxide, and methane occur independently of lake floor vent emissions, photic zone samples were collected at 13 sites across a transect during a single day in 2008. From two sites in the West Thumb and one site in the Inflated Plain, the researchers also collected a total of 16 vent samples and six samples from mixing zones (Jaeger et al. 2012). Vents in the West Thumb caldera emit hydrothermal waters with distinctly different geochemical signatures from those at the other two sites, most likely due to separate subsurface magma chambers (Varley et al. 2007).

Functional Gene Diversity

In addition to identifying the microbes present in the lake, the researchers were interested in examining the microbes from the perspective of environmental selection pressures and how geothermal heat and energy flux had influenced functional gene diversity (Varley et al. 2007). "Functional genes are the ones that give microbes an observable function that varies from one species to another, like eating methane or using ammonium as a nitrogen source," explains Scott Clingenpeel, now with the Department of Energy Joint Genome Institute in California. "This is in contrast to 'housekeeping' genes, which perform activities that all microbes do, like copying DNA or making proteins." The researchers compared the functional genes found in samples collected at vents with different geochemical signatures and with photic zone samples to determine whether patterns in functional gene distribution could be correlated with the chemical and geological characteristics of the site.

When using standard statistical approaches, the researchers found no obvious relationship between functional gene occurrence and lake geochemistry, at least as it pertained to microbial energy sources, which were expected to be a major driver of the relationship (Jaeger et al. 2012). Although the high-output vents in the Inflated Plain created identifiable geochemical gradients in the overlying water column, a comparison of the vent emissions, microbial streamers, and the photic zone samples associated with one of these vents to the photic zone sample from the Southeast Arm indicated no statistically defensible correlation between H_2 or H_2S concentrations and the frequency of functional genes associated with their oxidation. For example, even though the H_2 concentration in the photic zone water over the Inflated



GRAPHIC COURTESY OF J. VARLEY

Figure 4. Model integrating environmental energy inputs, lake phylogeny and expected primary functional genes. Major environmental energy sources are H_2, CH_4, NH_3, and light. (Graphic courtesy of authors.)

Plain vent contained more than 20 times as much H_2 as the Southeast Arm photic zone sample, the occurrence of H_2 oxidation genes was only two times greater.

"Therein lies one of the challenges," Tim McDermott points out. "The search for relationships in huge data sets—and here we're working with approximately 6 billion base pairs of lake DNA sequence—requires the use of computers. In the Yellowstone lake environments studied, the concentrations of the microbial nutrients would exhibit wild swings, from barely detectable (as in the photic zone waters) to extraordinarily high levels (in the vent emissions) that are far above those required to support the microbial metabolism being examined. This makes it difficult to tease out significant correlations, but when the data sets are examined manually and subjected to step-by-step sorting, the relationship between microbial function and lake geochemistry emerges."

In addition, the genetic and metabolic diversity found in Yellowstone Lake is likely to blur distinct functional gene signals unless the completeness of the metagenome sequence is substantially greater than has been achieved there or in marine environments (Jaeger et al. 2012). The researchers therefore suggested that a metagenome approach to correlating functional genes with environmental factors in the marine setting seems unlikely, and that quantitative PCR of targeted functional genes, once workable primers are defined, is more likely to produce useful results.

Conceptual Model

The research led to development of a conceptual model (fig. 4) of Yellowstone Lake based on four sources of energy: (1) light for photoautotrophs and photoheterotrophs, (2) ammonia for bacterial and archaeal nitrifiers, (3) hydrogen for a variety of organisms (hydrogenotrophy is widespread throughout the phylogenetic tree), and (4) methane for methanotrophs, or indirectly, methylotrophs.

Citizen Scientists

The matching grant from the National Park Service Centennial Challenge added to the project an educational dimension that was directed by Susan Kelly, an outreach and

Carl Woese Redrew the Tree of Life

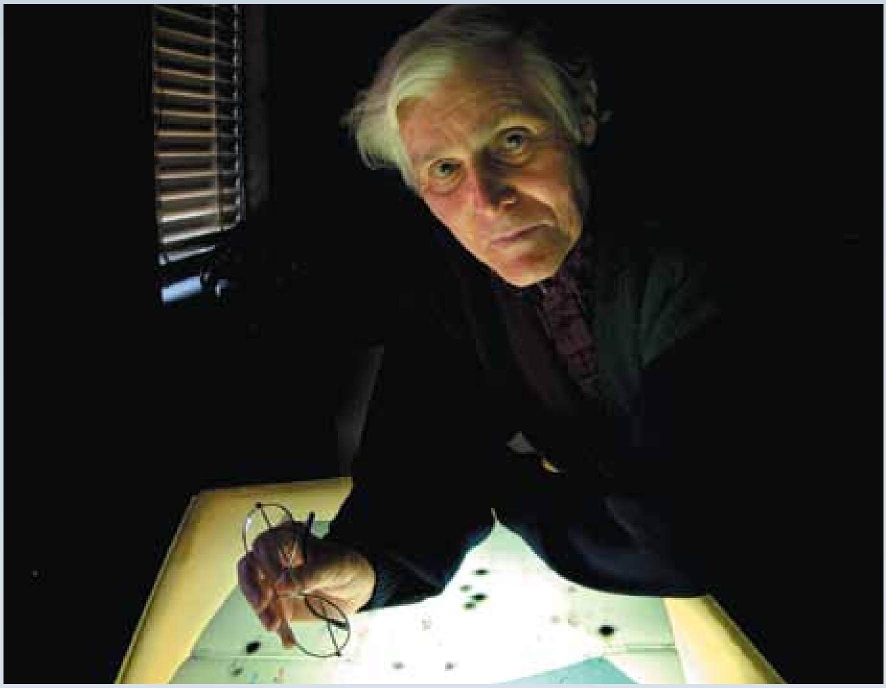


PHOTO COURTESY/SCHOOL OF MOLECULAR & CELLULAR BIOLOGY, UNIV OF ILLINOIS URBANA-CHAMPAIGN

Shortly before his death last December at age 84, Carl Woese and his colleagues at the University of Illinois, Urbana-Champaign, received a five-year, $8 million grant from NASA's Astrobiology Institute to research "the fundamental principles underlying the origin and evolution of life." It was a fitting acknowledgement of Woese's contributions to addressing the most fundamental questions of microbiology.

Until his research convinced biologists otherwise, the consensus was that all living things were either Eukaryotes, whose cells contain a nucleus, or Prokaryotes, which included bacteria and other single-celled organisms without a nucleus. When the first Archaea were discovered in extreme environments like Yellowstone's hot springs, they were classified as bacteria. Woese, who thought the division of organisms into two groups relied on differences of little evolutionary significance, began examining the genetic sequences of archaeal methane producers and concluded that they belonged to a third branch on the tree of life.

This idea, which Woese and George E. Fox presented in 1977 in the Proceedings of the National Academy of Sciences, met with extensive resistance and even ridicule until the 1990s. The use of the polymerase chain reaction (PCR) greatly advanced Woese's painstaking method of gene sequencing and led to widespread acceptance of the three domains he had proposed: Eukarya, Bacteria, and Archaea.

In the 1980s, Woese came to Yellowstone to assist two of his University of Illinois students, David Stahl and Gary Olsen, with their research on Archaea in Octopus Spring. But it was his use of ribosomal RNA, which became the standard approach to identify and classify all organisms, that had the most far-reaching implications for microbiology in Yellowstone and elsewhere. In 1996, Woese, Olsen, and researchers from the Institute for Genomic Research in Rockville, Maryland, published the first complete genome structure of an archaeon, *Methanococcus jannaschii,* providing further evidence that Archaea are, in some ways, more closely related to *Homo sapiens* than to Bacteria.

Woese's research was a manifestation of his quest for a deeper understanding of the process of evolution, including how it might have occurred before genes even existed. He suggested that the "universal ancestor" was not a single organism but a collective of RNA that evolved into cells.

"The origin of life is about the sharing of genetic information between organisms," explains Bruce Fouke, whose career as a geologist shifted course in 1997 when Woese encouraged him to investigate the role of microbes in sedimentation and mineral precipitation. Fouke, who went on to study how microbial mat communities provide substrates for the travertine deposits at Mammoth Hot Springs, is now director of the Roy J. Carver Biotechnology Center at the University of Illinois. "Woese provided us with a profoundly different way to understand and conceptualize the rise of life on planet Earth," Fouke recently told *Yellowstone Science*. "His work provided the first gene sequence-based quantitative evaluation of all living organisms, thus emphasizing the universality of the emergence of life that is central to understanding life in Yellowstone."

In a 2004 article for *Microbiology and Molecular Biology Reviews*, Woese described the field of biology as being "at a crossroads":

> *"Biology has a choice to make, between the comfortable path of continuing to follow molecular biology's lead or the more invigorating one of seeking a new and inspiring vision of the living world, one that addresses major problems that molecular biology could not handle and so avoided. The former course… is certain to turn biology into an engineering discipline. The latter holds the promise of making biology an even more fundamental science, one that, along with physics, probes and defines the nature of reality. This is a choice between a biology that solely does society's bidding and a biology that is society's teacher."*

YS

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Early surveyors with the Hayden Expedition in the first boat ever launched on Yellowstone Lake, 1871.

education specialist at the Institute on Ecosystems at MSU. This included participation in Yellowstone's 2009 bioblitz, when John Varley and Stephanie McGinnis joined park staff and other researchers in the area to inventory as many living organisms as possible during a 24-hour period. The team collected and identified stream macroinvertebrates, contributing valuable taxonomic and genetic data.

Since 2010, the team has also been offering a one-week graduate course through the Masters of Science in Science Education (MSSE) program at Montana State University. The course covers the unique ecology of Yellowstone Lake and allows the students, who are science teachers, to play an active role in the research. They have collected samples from the lake's lagoons and deltas, which are areas not included in the previous inventories, identified organisms taxonomically using microscopes in a field lab, and then returned to MSU for the molecular genetics analyses. Many of the specimens were fairly common in the park, but much of their genetic information was new to science.

In 2011, for example, the teachers discovered at least one Cladoceran (*Eurycercus lamellatus*), two Copepoda (*Macrocyclops* sp. and *Diaptomus nudus*), and an Ostracoda crustacean (Cypridae) not previously reported from Yellowstone Lake. In other categories, they confirmed the presence of Collembola (springtails) in the lake, plus numerous insects that have yet to be analyzed and added to the MATBI (Citizen Scientists 2011).

The Future of MATBI

All of the identified gene sequences from this study have been and will continue to be added to the GenBank database, and consonant portions will be deposited in the Global Oceanic Survey database to help further its goals and discovery.

The cataloging of the biodiversity of all national park units is mandated by federal statute. MATBI scientists have sought to use 21st century technology to accelerate progress toward this goal in one small but highly important part of Yellowstone National Park. "We hope that our example at Yellowstone Lake will inspire others to use Linnaean taxonomy together with molecular genetics to advance the inventory of flora and fauna in natural areas in this country, and around the globe," says John Varley, noting that the technology is becoming simpler to use and much less expensive. "Before molecular genetics were used, there were roughly 250 known species. The MATBI pilot study doubled that number to around 500, and when all is said and done, the current study is expected to double or triple that number."

"The results of the Molecular All-Taxa Biological Inventory have whetted the appetite of the scientists working on Yellowstone Lake," Tim McDermott says. "Efforts on the drawing board as well as currently underway include a closer examination of the genetics of the Cladocerans, the Copepods, and other microcrustacea." They are a keystone species in the ecology of Yellowstone Lake and probably most of the park's other lakes, so information about their distribution and diversity can lead to an understanding of their stability and resilience during historical times and before. These scientists would like to find out how invasive species such as lake trout may have upset the microcrustacea's normal representation and function in the food chain, which in turn would affect the bacteria, archaea, and tiny eukarya that they feed upon.

"These smallest of the smallest are largely responsible for the lake ecosystem functioning in a balanced fashion so if predation patterns are altered, the normal biogeochemical cycling and lake energy webs become the first casualty," McDermott points out. "How does this affect functional genes, lake geochemistry, and topics such as greenhouse gases? Stay tuned—discoveries are emerging regularly."

Editor's Note

Analysis of the water and soil samples and the thousands of specimens collected during the Yellowstone Lake inventories has led to a number of publications in scientific journals. Some of the findings on Bacteria, Archaea, and a moss colony found growing near a hydrothermal vent are summarized on the following pages. A consolidated listing of the literature referenced in preparing the MATBI article and related summaries is presented on page 22.

Bacteria

Geochemistry and Community Analysis

This article summarizes some of the results of the genetic analysis of samples collected during the Molecular All Taxa Inventory of Yellowstone Lake in 2007 and 2008. It was adapted from information presented in "Yellowstone Lake: High energy geochemistry and rich bacterial diversity," by S. Clingenpeel, R. Macur, J. Kan, W. Inskeep, D. Lovalvo, J. Varley, E. Mathur, K. Nealson, Y. Gorby, H. Jiang, T. LaFracois, and T. McDermott in Environmental Microbiology *(2011), 1462–2920. The two lead authors are affiliated with the Thermal Biology Institute at Montana State University.*

Yellowstone Lake is central to the balanced functioning of the Yellowstone ecosystem, yet little is known about the microbial component of its food chain. To the authors' knowledge, their research was the first attempt at deep sequencing characterization of freshwater Bacteria diversity. Deep sequencing involves examining the DNA sequence of a particular gene from a very large number of organisms in order to capture a picture of the entire community including the rare members.

The environments studied in Yellowstone Lake for this research included the hydrothermal vents in the lake floor and near-surface photic zones that vary with respect to the extent to which they are influenced by vent emissions. The authors concluded that Yellowstone Lake is rich in multiple energy sources and supports significant bacterial diversity, particularly associated with the hydrothermal vents.

Geochemical Analysis

The influence of hydrothermal emissions on the overlying water column chemistry was evident. Both H_2 and CH_4 occur at microbially relevant concentrations throughout the lake, but both gases were enriched in vent emissions, which also contained substantial H_2S and CO_2. The H_2 and CH_4 concentrations in the Inflated Plain surface waters located 30–32 m above the vents and directly within a significant gas plume (evident from profuse bubbles rising to the surface) were significantly greater than in surface waters at the Southeast Arm. The H_2 and CH_4 concentrations in the Inflated Plain surface waters were also greater than those in the water over the West Thumb vents, which were deeper than those in the Inflated Plain, contained lower levels of all gases, and did not cause surface bubbling.

IMAGE COURTESY OF R. MACUR/W. INSKEEP

Diatomaceous sediment containing a variety of minerals including aluminosilicates and silica.

Analysis of the lake floor emissions revealed that these vents derive from different geothermal sources than those found elsewhere in the park. Most notable in this regard were the H_2 measurements, which ranged from 34 to 7524 nM, depending on the vent, which is significantly higher than those of any terrestrial vent that has been documented in the park. Hot springs in Yellowstone support significant hydrogenotrophic microbial populations and activity with H_2 concentrations ranging from 10 to 20 nM (Spear et al. 2005; D'Imperio et al. 2008).

However, the H_2 and CH_4 measurements in the lake indicated that these energy-yielding substrates are not necessarily associated with vents. The H_2 and CH_4 not attributable to vent emissions most likely derive from benthic fermentation, as is commonly found in freshwater lakes. Other potential sources of aquatic H_2 include nitrogenase activity and eukaryotic algae.

Microbial Community Analysis

Based on their laboratory analysis of the samples collected from the lake, the authors made a detailed characterization of the microbial community in four environments referred to as the West Thumb Deep Vent, the West Thumb Otter Vent, the Inflated Plain Surface Photic, and the Southeast Arm Surface Photic. Their analysis began with an assessment of total cell counts, which were two- to threefold greater in the surface water samples than in the vent samples, and decreased as a function of nominal filtration size class, particularly for the surface waters. The virus particle/bacteria ratio varied from 13.4 to 38.6.

Diversity analysis in this study was linked to an ongoing metagenomic sequencing effort that has intentionally avoided the large genomes of eukaryotic organisms. Consequently, this analysis was limited to organisms in the > 0.1 to < 0.8 µm size class, accounting for approximately 18–21% of the total cell counts for the vent samples and 10–15% of the surface samples.

Post-sampling gas analysis suggested that microorganisms associated with the Inflated Plain vents would be of particular interest because of the elevated H_2S, H_2, and CH_4 levels. However, the high H_2S content in these samples resulted in significant precipitation of elemental sulfur while they were being transported to the shore lab, rendering filtration for biomass collection impossible.

Phylotypes

The authors detected some of the same phylotypes throughout the lake, presumably as a result of wind-driven and other mixing currents. A few thermophile signatures could be detected at very low levels across the lake, similar to evidence found of surface-generated mesoscale eddies transporting hydrothermal vent emissions away from the northern East Pacific Rise (Adams et al. 2011). However, despite what appeared to be a sizeable representation of Actinobacteria in the West Thumb Deep Vent, thermophiles do not proliferate in this cold lake. The influence of floor vents is restricted to the immediately surrounding area, resulting in gradients that span from oligotrophic (Southeast Arm) to mesotrophic (Inflated Plain region).

They found the greatest phylotype diversity and richness in the two West Thumb vents that were sampled. Although there was a sizeable contingent of apparent nitrifiers associated with West Thumb Deep Vent emissions (60–66°C), bacterial nitrifier signatures were not found elsewhere in the lake. This vent was also associated with a significant enrichment (≥10-fold relative to the other three environments) of a *Polynucleobacter*-like organism, though *Polynucleobacter* is a freshwater heterotroph not known to be thermophilic.

Traditionally it has been thought that the highest microbial diversity is found in soils, while that of lakes and other bodies of fresh water were expected to be orders of magnitude lower. Yet Yellowstone Lake data showed similar bacterial diversity to soil samples.

Phylogenetic analysis of both traditional full-length PCR and pyrosequencing reads indicated that in many ways the bacterial composition of Yellowstone Lake is very similar to that reported for other freshwater lakes. Among the 26 phyla found in the lake, Actinobacteria were dominant (~40–62% of the total reads), followed by Proteobacteria (~20–43% of the reads), Bacteroidetes, and Cyanobacteria. Some of the dominant surface water phylogroups are globally distributed, including the Actinobacteria group acI, specific phylotypes within Bacteroidetes, and Cyanobacteria.

Given the highly localized impact of the lake floor hydrothermal vents, it may generate unusual food chain inputs. The prominent geochemical signatures of the different vents are easily quantified and may create focused chemosynthesis zones, offering an oasis of high-energy compounds and carbon for chemolithoautotrophs—bacteria and archaea that obtain energy from the oxidation of inorganic compounds and carbon from the fixation of carbon dioxide. In the Mary Bay region, clouds of the tiny crustaceans known as copepods were observed swarming around a relatively cool vent (23°C at ~51m depth compared to 8°C for the lake). Where vent emissions mix with lake water, chemolithoautotrophs (mesophiles in this case) would be favored to flourish due to the continuous flux of high energy e-donors and carbon sources (H_2, H_2S, CO_2) mixing with the energetically favorable e-acceptor O_2. This provides a rich food source that may not involve phototrophs such as algae or cyanobacteria, or require the copepods to migrate daily through the water column to feed on plankton.

Table 1. Estimates of bacterial diversity found in Yellowstone Lake based on the first 242,410 sequences, expressed as the number of operational taxonomic units (OTUs).

Location	**OTUs**
West Thumb Deep Vent	4,715 ± 82
West Thumb Otter Vent	2,610 ± 41
Inflated Plain Photic Zone	1,631 ± 21
Southeast Arm Photic Zone	3,439 ± 74

Despite the high CH_4 levels found in the lake, however, recognizable methanotrophs were nearly absent in the pyrosequencing data set. This could be because of the way in which samples were prepared. Although phototrophy presumably is an important driver of primary productivity in photic zone waters, chemosynthetic hydrogenotrophy and methanotrophy are likely important components of the lake's food chain.

Marine Environments

Direct microbial and virus counts of the ≤ 20 mm filtrate revealed total cell counts and virus estimates to be similar to those typically reported for marine environments. Samples from the lake's surface waters were dominated by two phylotypes: the Actinobacteria freshwater acI group and an a-proteobacterium that is closely tied to an uncharacterized freshwater clade that has been found from the Arctic to Panama, and which is most closely related (88–90% identity) to a heterotrophic a-proteobacterium *Pelagibacter ubique*, a cultured representative of the SAR-11 clade, which are the most common bacteria in ocean waters (Morris et al. 2002).

From one near-surface photic zone of the lake, the authors recovered full-length clones that shared 97–100% identity with *Prochlorococcus marinus,* a dominant cyanobacterium and photosynthesizer in marine waters (Partensky et al. 1999). This was the first time the species had been documented in fresh water.


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West Thumb thermal area, Yellowstone Lake.

Archaea

Comparing Extreme and Non-Extreme Environments

> *Although well-known from the park's hot springs, Archaea were not documented in Yellowstone Lake until the 2004 pilot study. Subsequent sampling and analysis in multiple research efforts has led to some unexpected findings.*

When characterization of microbes depended on the cultivation of specimens, Archaea were thought to be primarily "extremophiles"—organisms found in high temperature, acidic, hypersaline, or strictly anoxic habitats (Woese et al. 1990). This was the kind of microbe that researchers expected to find, and did find, in a place like Yellowstone's hot springs. The archaea cultivated from samples collected from Obsidian Pool in the early 1990s may have tripled the archaeal phylogeny documented at the time (Barns et al. 1994). Since then, however, molecular tools have revealed that Archaea are present in a broad range of habitats, including soils, marshlands, the human colon, and especially oceans.

Yet of the three widely accepted phylogenetic domains, Archaea remains the least understood in terms of its diversity, physiology, and ecological context (Barns et al. 1994). Archaea are single-celled microorganisms that have no cell nucleus or any other membrane-bound organelles within their cells. Classification has been difficult because the only a tiny portion of archaeal species have been studied in the laboratory, and they can be identified only by analyzing their nucleic acids. Crenarchaeota and Euryarchaeota are the most widely accepted and intensively studied Archaea phylum-level sub-classifications, but archaea have been found that do not fit obviously into either of those groups.

Having both hydrothermal vents and traits associated with marine surface waters, Yellowstone Lake became an obvious place to extend the search for Archaea. Analysis of the microbial samples taken from the lake in 2007 and 2008 revealed more Bacteria than Archaea, which is consistent with what has been found in other lakes (Kan et al. 2011). But more than 100 archaeal sequences were documented, including several clusters of closely related species that were new to science.

Extreme and Non-Extreme Environments

The samples selected for archaeal analysis came from four sites with differing water chemistry. The two vents in the West Thumb were similar in temperature and concentrations of H_2, but differed significantly in pH and the presence of potential important microbial nutrients such as CO_2, CH_4, and NH_4—differences that were expected to exert selective effects on the types of physiology that the microbes found there would have (Kan et al. 2011). The gas composition of these vent waters was significantly greater than that which has been reported for Yellowstone's terrestrial hot springs.

The two photic zone locations, in the Inflated Plain and the Southeast Arm, differed in their proximity to hydrothermal vents and to the lake's largest tributary, the Yellowstone River. The Inflated Plain surface water samples were obtained directly within a gas plume that was evident in the profuse bubbles rising to the surface; their H_2, CH_4, and CO_2 levels were significantly greater than those from the Southeast Arm surface waters (Kan et al. 2011).

Despite of the frequent winds that generate surface currents which mix the lake's waters, about 78% of the "operational taxonomical units" (established at 97% sequence identity) were found associated only with vent emissions (Kan et al. 2011). Exclusive vent associations that could be linked to characterized thermophiles involved organisms belonging to a Crenarchaeota group closely related to the thermophilic nitrifier *Candidatus* Nitrosocaldus yellowstonii (Kan et al. 2011). However, no evidence was found of Thermoprotei, a common Crenarchaeon in thermophilic environments, including Yellowstone's hot springs.

The presence of some predominantly photic zone clones and non-thermophilic microorganisms in vent emissions could have resulted from the mixing of lake water with vent water. Rocks surrounding the vents made it difficult, or sometimes impossible, for the ROV sampling cup to form a tight seal around the vent orifice (Clingenpeel et al. 2011a).

As expected, the archaea in the samples from the vents differed from those in the photic zone samples, the samples from the two vents differed from each other, and most of the archaeal diversity was associated with the vents (Kan et al. 2011). However, the archaeal populations in some areas not directly influenced by hydrothermal vents contain thermophilic species such as the Thermoplasmatales, Thermoproteales, and Desulfurococcales lineages. This suggests that although the surface sediment temperature at some

Inflated Plain sites is relatively cold, and water immediately above the substrate may be 4–13°C (39–55°F), heat flow from the caldera provides habitats in which thermophiles may remain viable for a while, and could eventually proliferate if they reach a warmer area conducive to growth (Varley et al. 2005).

Similarity to Marine Environments

Yellowstone Lake's microbial community was found to bear a "striking phylogenetic similarity" to that described for marine environments, and the parallels between Yellowstone Lake archaea and marine microorganisms can provide opportunities to compare "evolutionary tracks between freshwater and marine lineages" (Kan et al. 2011). Most of the clones retrieved from the lake were vent associated and related to Marine Group 1 (proposed as phylum Thaumarchaeota), *Candidatus* Nitrosocaldus, or unclassified and miscellaneous Crenarchaeal groups.

The Inflated Plain and Southeast Arm photic zones were dominated by clone group YLCG-1.1, which was 96–97% identical to the cultured organism *Nitrosopumilus maritimus,* a Thaumarchaeal marine nitrifier (Könneke et al. 2005). This suggests that phylogenetically close archaeal organisms have adapted to very different environments and reflects the broad distribution of Marine Group 1 in environments as varied as mines, freshwater, saltwater, drinking water plants, soils, and sponge symbionts (Kan et al. 2011).

The Euryarchaeota signatures were most prevalent in vent fluids, especially in the West Thumb Otter Vent. For example, clone YLA099 was abundant in Otter Vent, but nearly absent at the other sites, suggesting that the organism represented by phylotype YLA099 is a thermophile even though its closest known phylogenetic neighbor is an environmental clone from a hypersaline mat. Most of the Euryarchaeota clones appeared to be related to the Deep Sea Euryarchaeal Group or the Deep Sea Hydrothermal Vent Euryarchaeal Group 6 (Kan et al. 2011).

Nanoarchaeota

The German scientists who analyzed the first and, so far, only cultivated specimen of Nanoarchaeota, which came from a hydrothermal vent off the coast of Iceland, named it *Nanoarchaeum equitans* (Huber et al. 2002). Based on its ribosomal RNA, they concluded that it belonged to a newly discovered archaeal phylum, but the Nanoarchaeota's position on the phylogenetic tree remains a matter of debate; some consider it a member of the phylum Euryarchaeota (Casanueva et al. 2011).

An anaerobic hyperthermophile, *N. equitans* has the smallest cell size (about 400 nm in diameter) of any known archaeon, and the smallest genome size (0.5 megabases), which puts the organism "close to the theoretical minimum genome size calculated for a living being" (Huber et al. 2002). It survives only while in contact with a growing hyperthermophilic Crenarchaeon, *Ignicoccus hospitalis*, the first reported parasitic or symbiotic relationship between archaea. *N. equitans* lacks the genes for lipids, amino acids, and nucleotide biosynthesis yet, unlike many parasitic microbes, *N. equitans* can carry out DNA repair and replication (Stetter 2006).

Nanoarchaeum were subsequently found attached to *Ignicoccus* in the chimneys over hydrothermal vents in the East Pacific Rise (McClement et al. 2006). Microorganisms removed from the Uzon caldera in Kamchatka, Russia, and the Obsidian Pool in Yellowstone National Park were considered to be "a distinct family within the Nanoarchaeota" because their RNA sequences showed an 83% similarity to *N. equitans* (Hohn et al. 2002). These Nanoarchaeota were tiny cocci about the size of *N. equitans*, but they were attached to a different host (Stetter 2005), one that appeared to be more closely related to *Pyrobaculum*, another hyperthermophilic Crenarchaeon (Stetter 2005). Using Nanoarchaea-specific primers, PCR amplicons of a *Nanoarchaeum*-like organism that appeared to be free-living were detected in samples from a New Zealand hot spring (Casanueva et al. 2008). Nanoarchaeal phylotypes have also been found in mesophilic, hypersaline conditions in Inner Mongolia and South Africa, suggesting that Nanoarchaeota are not strictly hyperthermophilic, are not dependent on hyperthermophilic hosts, and may be found in a wide range of environmental conditions (Casanueva et al. 2008).

These results were supported by the discovery of Nanoarchaeota in Yellowstone Lake in 2004 by the MATBI pilot study (Varley et al. 2005). Given the lake's hydrothermal vents, the presence of Nanoarchaeota in the lake was not itself surprising, but they were found associated with microbial morphotypes that do not resemble the only known host, *Ignicoccus*. This visual evidence was consistent with the follow-up work conducted in 2007 and 2008, when Nanoarchaeota were found throughout the lake, although *Ignicoccus* was absent (Clingenpeel et al. 2011b).

Future Archaeal Research in Yellowstone Lake

Research efforts around the world during the last decade have demonstrated the ubiquitous nature of Archaea in both extreme and non-extreme environments, so the microbial inventory of the hydrothermally active Yellowstone Lake was designed with the expectation of finding archaeal specimens there too. What could not be anticipated, however, was the extent of archaeal novelty and diversity concealed within the lake. Future research on the lake's archaea may focus on quantitative assessments of specific taxa to gain a better understanding of archaeal abundance, population dynamics and functional roles (Kan et al. 2011).


COUTESRY STEPHANIE MCGINNIS

Composite image (not to scale) of invertebrates found near the moss: *Hyalella azteca, Polycelis*, and *Gammarus lacustris*.

A Moss Colony

Survival in Geothermal Heat and Darkness

This article was adapted from information presented in "A geothermal-linked biological oasis in Yellowstone Lake" by D. Lovalvo, S.R. Clingenpeel, S. McGinnis, R.E. Macur, J.D. Varley, W.P. Inskeep, J. Glime, K. Nealson, and T.R. McDermott, in Geobiology *8:327-336 (2010). Lovalvo is affiliated with Eastern Oceanics in Redding, Connecticut, and Clingenpeel with the Thermal Biology Institute at Montana State University.*

One of the more remarkable discoveries made during the inventory of Yellowstone Lake in September 2008 was a robust colony of moss, worms and crustaceans that encircled one of the hundreds of geothermal vents on the lake's floor.

The moss was identified as *Fontinalis*, previously known only as a cold-water bryophyte. The local water temperature considerably exceeded the bryophyte's known temperature tolerance, and the light level was below optimal conditions. A beige-colored silica and aluminum mineral emitted by the vent settles on the moss, which presumably reduces its ability to acquire the sunlight necessary for photosynthesis. Yet the moss was only found close to the mouth of the vent, indicating that the conditions created by the vent were apparently responsible for the moss being able to live in what the human eye perceives as total darkness.

The moss was colonized by metazoa, including the crustaceans *Hyalella* and *Gammarus*, a flatworm identified as *Polycelis*, and a segmented worm in the Lumbriculidae family. The presence of these invertebrates suggests a highly localized food chain that is adapted to the habitat created by the geothermal inputs, creating a unique oasis of life on the lake floor.

Living Conditions at the Vent

The vent is located approximately 0.5 km offshore from the West Thumb geyser basin and 28 m underwater. When the samples were collected, the temperature was 34.6°C in the vent, much lower than most vents described for this lake (Morgan et al. 2007), and 32.3°C within the moss adjacent to the vent, much greater than the sustained temperature range of the cold-water *Fontinalis* (Glime 1987). As a group, bryophytes are tolerant of shade (Martin and Churchill 1982), and *Fontinalis* is known to occur at depths of up to 120 m in Crater Lake, where the water clarity allows light to penetrate to considerable depths (Hasler 1938). In Yellowstone Lake, however, almost daily afternoon winds generate currents that stir lake sediments, creating turbidity that reduces light penetration. Light levels were also reduced by the constant emission of solid phase material from the vent, clouding the water and covering the leaf surfaces, which apparently can photosynthesize using very low levels of light.

The large volumes of water emitted by the vent contain toxins such as arsenic and cadmium, but are also super-saturated with carbon dioxide, hydrogen, and other gases. The vent water was acidic (pH 5.5), but contained some important macronutrients to provide significant levels of available nitrogen, sulfur, and potassium, although total phosphorus was below detection.

Species Identification

In addition to making it possible to observe the moss from the safety of a boat, the remotely operated vehicle was used to obtain samples of the vent emission sediment, the moss, and the metazoa attached to its leaves and branches. The moss samples were stored frozen on the boat and samples of the four mesofauna species were preserved in 80% ethanol. Traditional Linnaean taxonomy was used to make an initial identification of the plant and the metazoa found with it, then this assessment was confirmed by molecular phylogeny.

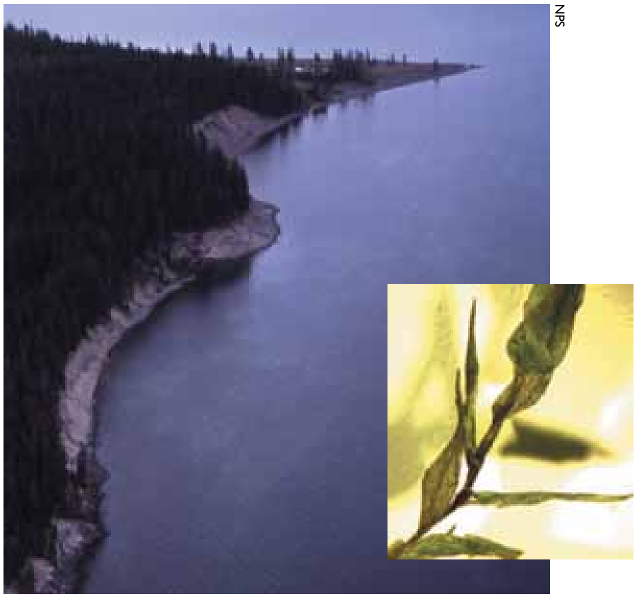
The two crustaceans were both amphipods. *Gammarus lacustris,* first reported in the lake by Forbes (1893), was


NPS/J. ARNOLD

A full moon forms patterns in gold on the surface of Yellowstone Lake.

> *The moss was colonized by metazoa, including the crustaceans* Hyalella *and* Gammarus*… The presence of these invertebrates suggests a highly localized food chain that is adapted to the habitat created by the geothermal inputs, creating a unique oasis of life on the lake floor.*

confirmed by Benson (1961), who also made the first report of a less common amphipod, *Hyalella azteca*. The sexual immaturity of the segmented annelid worm in the 2008 sample limited visual identification to the family Lumbriculidae, the first reported in the lake. Suctioning the flatworm specimen from the moss apparently damaged it, but it was tentatively identified as *Polycelis coronata.* Phylogenetic analysis confirmed that it was a *Polycelis*, one of half a dozen annelid worms that have been found in the lake. Phylogenetic identification for the *Polycelis* and the Lumbriculidae worm was constrained by the limited number of sequences for these taxa in the GenBank database. (GenBank, which contains all publicly available nucleotide sequences and their protein translations, is maintained by the National Center for Biotechnology Information.)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COURTESY OF AUTHORS

Shoreline of West Thumb (above). The moss *Fontinalis* (inset), discovered near a geothermal vent on the lake's floor approximately 0.5 km offsore from the West Thumb geyser basin.

Geothermal Eukaryotes

How frequently the *Fontinalis* moss may be associated with lake vents is not known, but it was not seen at any of the dozens of other vents that were inspected during sampling trips in 2007 and 2008. Relationships of geothermal features with eukaryotic organisms are far less common than those with microbial communities. Bryophytes have been reported growing in hot springs—*Bryum japanense* can grow at 40°C, and *Philonotis laxiretis* and *Bryum cyclophyllum* at 38°C (Watanabe 1957)—but this may be the first documentation for a freshwater habitat.

The respiration rate of plants increases as the temperature rises, and *F. antipyretica* has been shown to experience a net carbon loss at 20°C (Carballeira et al. 1998). At the temperatures observed at the Yellowstone Lake site, the CO_2 lost to respiration would be expected to greatly exceed the photosynthetically captured carbon. However, the moss's RuBisCO, an enzyme used when plants convert carbon dioxide to sugar during photosynthesis, may be constantly saturated by the high concentration of CO_2(aq) in the vent water and the area immediately around the vent. This could enable the moss to efficiently capture and fix carbon at rates that exceed respiratory costs, enabling biomass accumulation and growth. This geothermal, vent-associated *Fontinalis* would presumably also benefit from the availability of substantial fixed nitrogen that is present in the vent water.

The mesofaunal species found with the moss typically live near the lake shore or in shallower waters. In the vent environment, which is deeper and darker than their usual habitats, they also experience acidic pH, depleted oxygen, potentially toxic levels of aluminum and arsenic, and significantly higher temperature and CO_2 concentrations than would be optimal for them. Although motile, these animals are subject to lake currents that could carry them in and out of the vent water plume where they would be exposed to these extreme conditions for at least brief periods. It is not known how they persist in that environment.

The vent-water properties represent a paradox with respect to bryophyte photosynthesis, but they nevertheless appear to support a food web in which *Fontinalis* provides a habitat for metazoa that in turn are known to be consumed by fish. This linkage between a food web and raw geochemical inputs from the Yellowstone caldera parallels that documented for deep marine vents, where geochemical energy is converted to biochemical energy that sustains complex communities. Ecologically, these deeply submerged hydrothermal vents are essentially islands with distinctive chemistry and conditions that provide oases from which biological diversity emerges. Future research may look for evidence of genetic communication between those islands.

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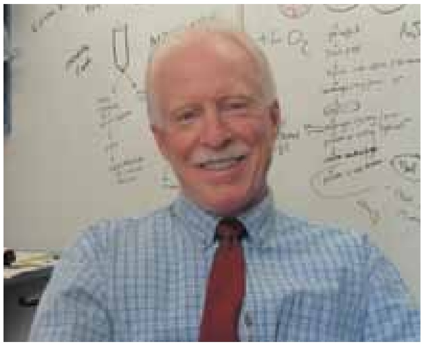
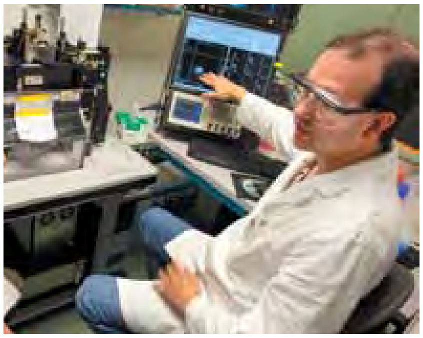
Mary Ann Franke is an unrepentant English major and liberal arts generalist with an MS in journalism. She has enjoyed translating the foreign language of science as a writer-editor for the Yellowstone Center for Resources since 1996.



Tim McDermott is a professor of soil and environmental microbiology at Montana State University. He is particularly interested in microbial ecophysiology pertaining to biogeochemical cycling in nature and looks forward to future research in picoplankton participation in Yellowstone Lake nutrient cycling and food webs.

Dave Lovalvo is the founder and owner of Eastern Oceanics. Dave has spent 28 years exploring, filming, and mapping Yellowstone Lake and continues to support projects in Yellowstone and many other locations around the world. While Dave has maintained two major science ROVs under his own company, he is currently heading the new deep-ocean ROV systems for NOAA's Office of Ocean Exploration and Research.



Scott Clingenpeel is a project scientist at the Department of Energy Joint Genome Institute in California, where he uses single cell genomics to figure out the role microbes play in the environment. He is currently applying this form of DNA analysis to study bioremediation and bacteria that help plants grow better.

Stephanie McGinnis is currently the Coordinator of Education and Outreach for Montana Watercourse. Her research interests are focused on the conservation and restoration of freshwater ecosystems.

John Varley holds degrees in zoology and entomology from the University of Utah. He spent the first 20 years of his career as a fisheries researcher and manager, including a nine-year assignment with the US Fish and Wildlife Service. He served for ten years as chief of research in Yellowstone and later was appointed the founding director of the Yellowstone Center for Resources. Now retired from the NPS, John serves as a Senior Research Scientist for Montana Institute on Ecosystems.

Literature Cited

Adams, D.K., D.J. McGillicuddy, Jr., L. Zamudio, A.M. Thurnherr, X. Liang, O. Rouxel, C.R. German, and L.S. Mullineaux. 2011. Surface-generated mesoscale eddies transport deep-sea products from hydrothermal vents. *Science* 332:580–583.

Barns, S.M., R.E. Fundyga, M.W. Jeffries, and N.R. Pace. 1994. Remarkable archaeal diversity in a Yellowstone hotspring environment. *Proceedings of the National Academies of Sciences USA* 91:1609–1613.

Benson, N.G. 1961. Limnology of Yellowstone Lake in Relation to the Cutthroat Trout. Research Report 56, US Fish and Wildlife Service, Washington. D.C. 33 pp.

Buchholz, L.A., P.D. Anderson, R.L. Cuhel, J.V. Klump, J.E. Kostka, R.W. Paddock, C.C. Remsen, J.S. Maki, and D. Lovalvo. 1995. Employment of ROV techniques and Scuba in Yellowstone Lake. Pages 1–7 *in* D.E.J. Harper, editor. Diving for Science. Nahant, MA: American Academy of Underwater Science.

Carballeira, A.D. S. Díaz, M.D. Vásquez, and J. Lopez. 1998. Inertia and resilience in the responses of the aquatic bryophyte *Fontinalis antipyretica* Hedw. to thermal stress. A*rchives of Environmental Contamination and Toxicology* 34: 343–349.

Casanueva, A., M. Tuffin, and D.A. Cowan. 2011. The Nanoarchaeota: Physiology, Genomics and Phylogeny. Pages 63–174 *in* S.Y. Katô, editor) Archaea: Structure, habitats and ecological significance. New York: Nova Science Publishers.

Casanueva, A., N. Galada, G.C. Baker, W.D. Grant, S. Heaphy, B. Jones, M. Yanhe, A. Ventosa, J. Blamey, and D.A. Cowan. 2008. Nanoarchaeal 16S rRNA gene sequences are widely dispersed in hyperthermophilic and mesophilic halophilic environments. *Extremophiles* 12:651–656.

Citizen Scientists in the Molecular-All-Taxa-Biodiversity Inventory of Yellowstone Lake. 2011. *Yellowstone Science* 19(3):2.

Clingenpeel, S., R. Macur, J. Kan, W. Inskeep, D. Lovalvo, J. Varley, E. Mathur, K. Nealson, Y. Gorby, H. Jiang, T. LaFracois, and T. McDermott. 2011. Yellowstone Lake: High energy geochemistry and rich bacterial diversity. *Environmental Microbiology* 2011: 1462–2920.

Clingenpeel, S.R., J. Kan, R.E. Macur, D. Lovalvo, J. Varley, W.P. Inskeep, K. Nealson and T.R. McDermott. 2011b. Yellowstone Lake reveals evidence of non-extreme Nanoarchaeota and ecotype diversity. The 11th International Conference on Thermophiles Research, Program and Abstracts, p. 84. Big Sky, Montana.

D'Imperio, S., C.R. Lehr, H. Oduro, G. Druschel, M. Kühl, and T.R. McDermott. 2008. Relative importance of H_2 and H_2S as energy sources for primary production in geothermal springs. *Applied and Environmental Microbiology* 74:5802–5808.

Forbes, S.A. 1893. A preliminary report on aquatic invertebrate fauna of the Yellowstone National Park, Wyoming and of the Flathead Region of Montana. *Bulletin of the US Fish Commission* 11:207–256.

Friend, T. 2007. The Third Domain. Washington, D.C.: Joseph Henry Press.

Glime, J.M. 1987. Phytogeographic implications of a *Fontinalis* (Bryopsida) growth model based on temperature and flow conditions for six species. *Memoirs of the New York Botanical Garden* 45: 54–170.

Hasler, A.D. 1938. Fish biology and limnology of Crater Lake, Oregon. *Journal of Wildlife Management* 2:94–103.

Henry, E.A., R. Devereux, J.S. Maki, C.C. Gilmour, C.R. Woese, L. Mandelo, R. Schauder, C.C. Remson, and R. Mitchell. 1994. Characterization of a new thermophilic sulphate-reducing bacterium. *Archives of Microbiology* 61(1):62–89.

Hohn, M.J., B.P. Hedlund, and H. Huber, H. 2002. Detection of 16S rDNA sequences representing the novel phylum "Nanoarchaeota": Indication for a wide distribution in high temperature biotopes. *Systematic and Applied Microbiology* 25:551–554.

Huber, H., M.J. Hohn, R. Rachel, T. Fuchs, V.C. Wimmer, and K.O. Stetter. 2002. A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature* 417(6884), 63–7.

Interlandi, S.J., S.S. Kilham, and E C. Theriot. 1999. Responses of phytoplankton to varied resource availability in large lakes of the Greater Yellowstone Ecosystem. *Limnology and Oceanography* 44:668–682.

Jaeger, N., Varley, J., T. McDermott, W. Inskeep, S. Clingenpeel, and R. Macur. Model study to ascertain new biodiversity in Yellowstone Lake and to correlate metagenomics with environmental conditions. Report to Big Sky Institute, University of Montana. December 27, 2012.

Kan, J., S.R. Clingenpeel, R. Macur, W.P. Inskeep, D. Lavalvo, J. Varley, K. Nealson, and T.R. McDermott. 2011. Archaea in Yellowstone Lake. I*nternational Society for Microbial Ecology Journal* 5(11):1784–1795.

Kelly, S., L. Morgan, and S. McGinnis. 2011. Fire and brimstone continues to shape Yellowstone Lake: Connecting geology and past climates with today's biodiversity. *The Earth Scientist* 27(3):23–28.

Klump, J.V., C.C. Remsen and J.L. Kaster. 1988. The presence and potential impact of geothermal activity on the chemistry and biology of Yellowstone Lake, Wyoming. Pages 81–98 *in* M.P. DeLuca and I. Babb. editors. Global venting, midwater, and benthic ecological processes. National Undersea Research Program Research Report 88.

Könneke, M., A. Bernhard, J.R. de la Torre, C.B. Walker, J.B. Waterbury, and D.A.Stahl. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437:543–546.

Maki, J.S., C.M. Schroeder, J.C. Bruckner, C. Wimpee, A. Weir, C.C. Remsen, C. Aguilar, and R.L. Cuhel. 2002. Investigating the microbial ecology of Yellowstone Lake. Pages 101–113 *in* R.J. Anderson and D. Harmon, editors. Yellowstone Lake: Hotbed of chaos or reservoir of resilience? Proceedings of the 6th Biennial Scientific Conference on the Greater Yellowstone Ecosystem. Yellowstone National Park, Wyoming.

Martin, C.E., and S.P. Churchill. 1982. Chlorophyll concentrations and a/b ratios in mosses collected from exposed and shaded habitats in Kansas. *Journal of Bryology* 12:297–304.

Morgan, L.A. W.C. Shanks, III, K.L. Pierce, D.A. Lovalvo, G.K. Lee, M.W. Webring, et al. 2007. The floor of Yellowstone Lake is anything but quiet—new discoveries from high resolution sonar imaging, seismic-reflection profiling, and submersible studies. Pages 95-126 *in* L.A. Morgan, editor. Integrated geoscience studies in the greater Yellowstone area: Volcanic, tectonic, and hydrothermal processes. US Geological Survey Professional Paper 1717, .

Morgan, L.A., D.D. Blackwell, R.E. Spafford, and R.B. Smith. 1977. Heat flow measurements in Yellowstone Lake and the thermal structure of the Yellowstone caldera. *Journal of Geophysical Research* 82:3719–3732.

Morris, R.M., M.S. Rappé, S.A. Connon, K.L. Vergin, K.L., W.A. Siebold, C.A. Carlson et al. 2002. SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* 420:806–810.

Partensky, F., W.R. Hess, and D. Vaulot. 1999. Prochlorococcus, a marine photosynthetic prokaryote of global significance. *Microbiology and Molecular Biology Reviews* 63:106–127.

Rusch, D.B., A.L. Halpern, G. Sutton, K.B. Heidelberg, S. Williamson, S. Yooseph, et al. 2007. The Sorcerer II global ocean sampling expedition: Northwest Atlantic through eastern tropical Pacific. *PLOS Biology* 5:398–431.

Spear, J.R., J.J. Walker, T.M. McCollom, and N.R. Pace. 2005. Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. *Proceedings of the National Academy of Sciences USA* 102:2555–2560.

Stetter, K. O. 2006. Hyperthermophiles in the history of life. *Philosophical Transactions of the Royal Society* 361B:1837–1843.

Stetter, K.O., M.J. Hohn, H. Huber, R. Rachel, E. Mathur, B. Hedlund, and U. Jahn. 2005. A novel kingdom of parasitic Archaea. Pages 249–259 *in* W.P. Inskeep and T.R. McDermott, editors. Geothermal biology and geochemistry in Yellowstone National Park, Proceedings of the Thermal Biology Institute Workshop, Yellowstone National Park, Wyoming. Bozeman: Montana State University Publications.

Sugden, A.M., B.R. Jasny, E. Culotta, and E. Pennisi. 2003. Charting the evolutionary history of life. *Science* 1300 (5626):1691.

Varley, J. et al. Yellowstone Lake: Genetic and gene diversity in an aquatic, vent-impacted system. Proposal to the Gordon and Betty Moore Foundation. June 27, 2007.

Varley, J.D., C. Chang, L. Christoffersen, G. Toledo, J. Ritter, and M. Podar. 2005. A Proof-of-Concept Molecular All-Taxa Biodiversity Inventory in Yellowstone National Park: Microbial and small metazoan diversity survey in Yellowstone Lake using ribosomal gene sequences. Report submitted to the Gordon and Betty Moore Foundation by the Yellowstone Park Foundation, April 11, 2005.

Warren, L. and M.E. Kauffman. 2003. Microbial geoengineers. *Science* 299(5609):1027–1029.

Watanabe, R. 1957. On some mosses growing in hotspring water. *Miscellanea Bryologica et Lichenologica* 13: 2.

Welch, W.H. 1960. A monograph of the Fontinalaceae. Dordrecht: Nijhoff.

Woese, C.R., O. Kandler, M.L. Wheelis. 1990. Towards a natural system of organisms-proposal for the domain Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences USA* 87: 4576–4579.

The Yellowstone Raptor Initiative

Lisa M. Baril, David Haines, Katharine E. Duffy, and Douglas W. Smith

B. ERTEL

A golden eagle with an unidentified waterfowl as its prey.

It was the first backpacking trip of the season, our first time hiking this trail in its entirety, and the first season of a new program designed to document raptor populations in Yellowstone National Park. As we (Lisa Baril and David Haines) tightened our boots and cinched the straps of our backpacks loaded with tripods, spotting scopes, and enough food to last three days, we headed down the trail just after dawn on a blue-bird day in June. Our task was to survey this 18-mile stretch of trail for raptor activity and breeding territories. We were especially interested in golden eagles and red-tailed hawks, but documented all raptor species observed along the way. We gave cliff faces particular attention since this is the preferred nesting habitat for golden eagles, peregrine falcons, prairie falcons, and occasionally red-tailed hawks. Using spotting scopes we scanned the cliffs for the tell-tale signs of raptor activity—whitewash (the highly acidic avian waste material) and the stick nests created by golden eagles and red-tailed hawks. Whitewash often shows up as long, vertical streaks for falcons and shorter, wider streaks for other raptors.

A golden eagle came into view and began an undulating flight display—a graceful plunging dive with wings tucked in from hundreds of meters above the ground, then pulling up and climbing back into the sky for a repeat performance—a signal to other golden eagles (and us) that this territory was occupied. A second golden eagle, the female, flew in front of the cliff face and landed in a large stick nest. Squinting into our spotting scopes from half a mile away, we could make out a small chick, about 5 or 6 weeks old, still covered in downy white feathers, wobbily negotiating its home on the rocks. Elated with this find within the first few hours of the trip, we took careful notes and photographs to document the exact location for future reference and headed on our way. It was an auspicious start for the Yellowstone Raptor Initiative—a trip that would reveal the locations of two prairie falcon territories, four golden eagle territories, a bald eagle nest, and countless red-tailed hawk and American kestrel observations.

Five-Year Initiative

Established in 2011 and funded in annual increments by the Yellowstone Park Foundation, the Yellowstone Raptor Initiative (YRI) is a five-year project to collect baseline information on certain raptor species in the park. It was designed to complement Yellowstone's existing bird monitoring program, which includes trumpeter swans (*Cygnus buccinator)*, common loons (*Gavia immer*), colonial nesting species, songbirds, and three raptor species: bald eagles (*Haliaeetus leucocephalus*), ospreys (*Pandion haliaetus*), and peregrine falcons (*Falco peregrinus*). Although Yellowstone's existing raptor monitoring program has provided more


NPS/J. PEACO

An immature great horned owl.


USFWS

A Swainson's hawk, among raptor species prioritized by YRI.

than 20 years of information regarding raptor population changes—including the recovery of the bald eagle and peregrine falcon following the 1972 restrictions on DDT use—an additional 19 species of hawk, eagle, falcon and owl species breed in Yellowstone, and another 14 currently use or have moved through the park seasonally. We have had very limited or no information on most of these species, several of which are of growing conservation concern throughout the western United States as residential development and other human activity increases across the region. With such a large preserved habitat, Yellowstone provides an ideal location to increase our understanding of raptors and their ecology throughout the Northern Rocky Mountain ecosystem.

However, given the large number of raptor species in the park and the limited staff and funding, the YRI's priorities are species of conservation concern, species with high visibility, and the development of techniques that can be used to survey multiple species. The YRI is focused on gathering baseline information on golden eagles (*Aquila chrysaetos*), red-tailed hawks (*Buteo jamaicensis*) and Swainson's hawks (*B. swainsoni*) with less intensive surveys for American kestrels (*F. sparverius*), prairie falcons (*F. mexicanus*), and owls.

Although important to understanding the role of raptors in the park, sharp-shinned hawks (*Accipiter striatus*), Cooper's hawks (*A. cooperii*), and northern goshawks (*A. gentilis*) will not be surveyed during the initial five-year period because of the intensity of the surveys needed to obtain adequate sample sizes. Yellowstone has habitat suitable for a large and healthy population of these forest-dwelling hawks and if the YRI continues beyond this initial five-year period, accipiters will be added to the program.

The YRI will distribute the results of this research in internal reports, peer-reviewed publications, and popular literature, and provide public outreach and education opportunities to highlight the ecological importance of aerial predators throughout the Yellowstone landscape. The YRI has also solicited raptor observations from park employees and visitors to augment field work done by YRI staff. In this article we highlight some of the accomplishments made during the first two years of the YRI.

Golden Eagle

The golden eagle, considered one of the quintessential eagles of the world, is found throughout most of the northern hemisphere with ecological equivalents throughout the southern hemisphere. Driven by prey availability, golden eagles primarily occupy open habitat that allows for hunting small mammals such as rabbits, hares, and ground squirrels. Cliffs serve as their primary nesting substrate, but tree nesting is common in locations that provide high prey density and limited cliffs. Golden eagles have long been observed and recorded in the park, especially during the winter feeding at carcasses. Although some are park residents, many of the golden eagles observed during winter are likely migrants from Alaska and Canada. Studies of several radio-tagged birds show that golden eagles from as far north as the Brooks Range in Alaska migrate to areas in and around Yellowstone for the winter, while others pass through on their way to wintering grounds in Colorado, New Mexico, and as far east as Arkansas (R. Domenich, raptorview.org, pers. comm.; Carol McIntyre, Denali National Park, pers. comm.).

Prior to the YRI, there were no documented records of breeding golden eagles in Yellowstone despite ample nesting habitat and a suspected large population size. Throughout the western U.S., concerns about wind energy development, agriculture, and increased urbanization have stimulated research on how these changes are affecting golden eagle populations (Kochert et al. 2002). The portion of the park's population that is migratory may be exposed to some or all of these threats when leaving the park. Increasing what is known about golden eagles in Yellowstone, where the species' ecology is largely unaltered by human activities, can help inform management decisions, such as the location of wind farms, throughout the northern Rockies.

For the golden eagle, the objectives of the YRI are to locate and document all breeding territories in the park, determine the breeding population density across the park, evaluate annual measures of reproductive success, and determine nesting chronology. The YRI also collects prey remains and eggshell fragments from nests in order to identify breeding season prey selection and the presence of chemical contaminants that affect eggshell thickness and therefore reproductive success. We will also collect golden eagle biological specimens shed in the nest area for a DNA study examining territory turnover rates and genotypic variation across a broad region. The YRI is working with researchers in Wyoming and Montana to better understand the impacts of land-use types on golden eagle occupancy and reproduction, and how its breeding season diet varies across the landscape.

Methods. Although most searches for territorial golden eagles and observations of active nests were made from the ground during the 2011 and 2012 breeding seasons (April to July), several flights were conducted to assess the effectiveness of using fixed-wing airplanes in locating active territories. We also followed up on reported sightings and areas where birds were opportunistically observed during general bird surveys in Yellowstone's core bird program. Exploratory searches were conducted in areas park-wide where the habitat was considered suitable for golden eagle occupancy (i.e., cliffs near open habitat for foraging). Although golden eagles may build nests in trees, we concentrated our efforts on cliff-nesting birds since they are more easily found. More focused efforts to locate tree-nesting eagles will be made after most of the suitable cliffs have been surveyed.

We determined occupancy by the presence of a mated pair of adult birds, a single bird showing territorial behavior, nest structures that contained new material and fresh greenery, or by other reproduction-related activities. We observed suspected territories during a minimum of two four-hour visits from April through July. If there was no evidence of occupancy during these visits, we considered the territory unoccupied. If a territory was occupied, we determined if the pair was actively nesting as indicated by evidence of egg-laying, the presence of nestlings, or an adult in incubation posture in the nest. Golden eagles commonly forgo breeding in years when food has been limited during the prior months, and as a result not all pairs occupying a territory lay eggs. The proportion of pairs that lay eggs in a given year can therefore be an important measure of a population's response to changing food supplies (Steenhof and Newton 2007), but this information is often difficult to obtain because nests may fail before they have been observed.

If a nest was active, we monitored the nest until it failed or the nestlings fledged. Locating young after they fledge is difficult, so nests were considered successful if at least one nestling reached 52 days old (~80% of fledging age) since nestlings at this stage have a high probability of fledging. To assess the reproductive health of the population, we calculated productivity as the number of fledged young per occupied territory. Careful notes and photographs were taken of the location of occupied territories and all nests within a territory. In August 2011, after the eagle chicks had fledged, we entered three of the five nests to collect eggshell fragments and prey remains.

Results. Although aerial surveys can be an economical way to survey a large number of territories, they yielded little return on finding new territories in Yellowstone. Golden eagle nests in Yellowstone tend to blend in well with the rock, making it difficult to survey from a plane. With their ability to remain stationary, helicopters would be a more effective aerial survey method, but are cost prohibitive. Future surveys will therefore be done on foot.

From previous observations by core bird program staff we knew of three golden eagle territories that had not been documented. There had also been reports of multiple sightings by staff and visitors at three other locations that were later identified as breeding areas. YRI staff discovered an additional 12 not previously reported territories.

J. KEEL

A golden eagle observed from the ground by survey participants.

Table 1. Golden eagle territories surveyed in Yellowstone National Park, 2011 and 2012.

Year	Occupied Territories	Active Territories	Successful	Fledglings	Productivity†
2011	15	5	4	5	0.56
2012	16	5	0	0	0

† Estimate is based on 9 occupied territories for 2011 and 13 for 2012 because these are the territories for which enough information was collected to calculate productivity estimates.

We documented a total of 18 territories and 44 golden eagle nests during the 2011 and 2012 breeding seasons. A possible 19th territory was located, but could not be confirmed. Like many raptors, golden eagles build and maintain several nests within a territory. Maintaining multiple nests within a territory is thought to "hedge" against destruction of one of the nests so that the pair may quickly re-nest if it is early enough in the breeding season to allow for completion of the nesting cycle. Multiple nests may also provide an alternative if the parasite load in a favored nest becomes high and serve as a territorial warning to other golden eagles.

In 2011, we confirmed nesting in five of the nine territories for which sufficient observations were made. Observations were made at an additional seven territories, but because of the time of year they were visited and/or remoteness we were unable to make definitive assessments of nesting behavior and occupancy at these sites. Four of the five nests fledged a total of five young for a productivity of 0.56 young produced per occupied territory (table 1). In 2012, we monitored 14 of the 18 known sites for nesting activity and confirmed occupancy at an additional two sites, but only five pairs nested and none were successful. The nesting status of the remaining four territories could not be determined.

When visiting a nest that had young, we determined the approximate age of nestlings using a descriptive guide to nestling development (Driscoll 2010). Using the approximate age of the nestlings, we back-calculated to the estimated date of egg-laying and hatching based on published estimates of the average incubation period. We also forward-calculated fledge dates by using published estimates of the average time it takes young to fledge. We estimate that the golden eagles on average laid eggs from March 28 to April 1, and that the chicks fledged from July 18 to 22. Analysis of eggshell thickness revealed that eggshells were within the normal historical range, indicating little, if any, contaminant loads. Prey remains collected from the three nests revealed a high proportion of birds were fed to nestlings, including common ravens, black-billed magpies, several species of duck, and even a Swainson's hawk. Mammal remains included the skull of a deer, ground squirrels, and a yellow-bellied marmot. Fewer mammal remains were found than bird remains, but this may be because shed feathers are more likely to remain in the nest than mammal bones after the female removes the carcass.


C.R. PRESTON

A golden eagle chick peers from its nest.

Conclusions. Although we have only two years of data, it appears that while Yellowstone supports a sizeable breeding population of golden eagles, few are able to produce young. As mentioned above, golden eagles may forgo breeding in years when prey is limiting, particularly during late winter and early spring. Food-stressed females are unable to increase their body mass enough to allow for egg-laying. In Idaho, the percent of females that lay eggs is related to jackrabbit abundance and inversely related to winter severity (Kochert et al. 2002), and nesting success in Alaska has shown direct correlations with the cyclic nature of snowshoe hares (McIntyre and Schmidt 2011). Yellowstone's winter–spring prey base may be limited with a patchily distributed acyclic snowshoe hare population (Hodges et al. 2009), a restricted white-tailed jackrabbit population (Gunther et al. 2009), and high variability in the distribution of wolf- and winter-killed carcasses on the landscape depending on winter severity (Wilmers et al. 2003). Only long-term monitoring of both golden eagles and their prey will reveal patterns of reproduction as related to prey density and variation in weather.

Although our sample size was small, the nesting dates for golden eagles in Yellowstone are later on average than has been reported for eastern Wyoming (Kochert et al. 2002) and central Montana (V. Slabe-Catena Consulting, personal comm.), presumably because of the difference in elevation.


C.R. PRESTON

Red-tailed hawk.


NPS/N. BOWERSTOCK

Red-tailed hawk nest with adult and one chick.

As more territories become known and monitored, more precise information on nesting chronology will be gathered. Knowledge of how the timing of nesting relates to environmental factors will augment reproductive data and help us to better understand the effects of climate change on golden eagle ecology in Yellowstone.

Red-tailed Hawk Monitoring

Look up into the sky in Yellowstone and you are likely to see a red-tailed hawk, by far Yellowstone's most observable raptor species. Named for the rusty-red tail feathers of the adults, red-tails are raptors of open country, preferring to forage for ground squirrels and other small mammals in grasslands and sagebrush, while primarily nesting in trees on the periphery of their hunting grounds. Like most raptors, red-tails build and maintain multiple nests in a single territory, but only choose one per season for egg-laying. Despite the ubiquitous nature of red-tails in Yellowstone, little is known about their population size, habitat use, diet, or reproduction. To start filling this information gap we initiated a monitoring program for which the goals are to locate and document their nesting territories, determine their reproductive success and annual variations in nesting chronology, and estimate their population size on the northern range.

Methods. Survey methods for red-tails largely followed those used for golden eagles. We conducted one flight during May 2011 to determine whether aerial surveys were an effective way to locate and monitor red-tailed hawk nests. Most nest searching and monitoring, however, was done from the ground by looking for red-tails exhibiting territorial or nesting behavior (e.g., undulating flight, carrying nesting material or food, copulation). Once a territory was identified we monitored the hawks' behavior to locate a nest. In many cases we found nests by searching for them in trees within the territory. As with the golden eagles, active nests were monitored until the nestlings fledged or the nest failed. Nests were considered successful if at least one nestling reached 34 to 37 days old (~80% of fledging age) since nestlings at this stage have a high probability of fledging and locating young after they fledge is difficult. To assess the reproductive health of the northern range population, we calculated productivity as the number of fledged young per occupied territory. Careful notes and photographs were taken of the location of territories and all nests within a territory.

Results. The aerial surveys were not effective in searching for or monitoring red-tailed hawk nests due to the large amount of nesting habitat available on the northern range and the difficulty of observing nests in conifers. During 2011, we documented 33 locations where we had observed red-tail pairs. Within those territories we located 18 nests, 14 of which were active. Twelve of the nests were successful, with a minimum of 19 fledglings. We wondered if this high rate of nesting success (86%) may have been inflated by the small sample size and our locating some nests relatively late in the season. The nestlings are older then and more likely to fledge, and we were unlikely to find nests that had already failed.

By the end of the 2012 breeding season, however, we had documented 48 nests in 34 territories on the park's northern range. We monitored 26 active nests, most of which were found at the start of the nesting season in May, and 24 of

Table 2. Red-tailed hawk nesting chronology, 2011 and 2012.

Nesting Chronology	2011 Average (n=10)	2012 Average (n=24)
Mean Incubation	May 8–10	May 6–8
Mean Hatching	June 8–10	June 2
Mean Fledging	July 22–24	July 13–17

them fledged a total of 47 young, resulting in a nesting success rate of 88% and a productivity estimate of 1.7 young produced per occupied territory. The breeding chronology for red-tailed hawks on the northern range varied slightly from 2011 to 2012 (table 2). The average lay, hatch, and fledge dates were approximately one week earlier in 2012 than in 2011, which could be attributed to the milder winter conditions. Ground squirrels, a primary prey resource, come out of hibernation earlier when conditions are milder. Continued monitoring over the next three years may indicate if these slight shifts are a function of spring conditions.

Conclusions. The high density of ground squirrels coupled with ample nesting habitat appears to have allowed for a high density of nesting red-tails on the northern range with some active nests spaced as little as 0.66 km apart. The high nesting success rates we observed are unusual and indicate a healthy population that may be a source for less productive populations in and around Yellowstone. Since great horned owls (*Bubo virginianus*) and great gray owls (*Strix nebulosa*) will use old red-tail nests or compete with red-tails for nesting space, the high density of red-tails may aid owl populations. Our goal is to monitor at least 30 nests a year distributed across the northern range to obtain annual estimates of nesting success and productivity for this region. Although we restricted our surveys to the northern range for this five-year project, a future goal may be to compare reproductive rates and nesting densities to those of other areas such as Yellowstone Lake or the Thorofare region where the red-tail population is suspected to be lower.

Raptor Roadside Survey

The purpose of the roadside survey for red-tailed hawks, Swainson's hawks, and American kestrels on the northern range is to provide an annual index of population abundance for these species and long-term data for detecting population changes. These surveys are designed to be accurate, rapid, cost-effective for limited staffing, and repeatable, with a protocol that complements nesting surveys for these species. The roadside surveys will continue beyond the initial five-year period as staffing allows.

Methods. We developed a point count survey method based on other survey protocols with similar goals and the advice of several experts in the field. We treated the 2011 season as a pilot year to evaluate the method for accomplishing our goals in an accurate and timely manner. We used the road corridor from Swan Lake Flats to the northeast entrance as a transect and placed 100 stop points approximately 0.8 km apart. The transect was split into two routes and one observer completed each route. During 2011, each point was surveyed for 10 minutes during which the observer recorded all raptors heard and observed onto a topographic map along with information on species, sex if possible, behavior, color morph where applicable, and abundance. Surveys began by 0900 and were completed by 1800.

Table 3. The percentage and abundance of raptors observed during the northern range roadside survey, 2011 and 2012.

	% (Abundance)	
Species	**2011**	**2012**
Red-tailed hawk	64% (67)	72.5%(153)
American kestrel	11% (11)	8.1%(17)
Swainson's hawk	1% (1)	7.1%(15)
Golden eagle	6% (6)	3.8%(8)
Bald eagle	1% (1)	2.8%(6)
Osprey	4% (4)	2.8%(6)
Sharp-shinned hawk	2% (2)	0.9%(2)
Turkey vulture	3% (3)	0.5%(1)
Cooper's hawk	1% (1)	0.5%(1)
Northern goshawk	—	0.5%(1)
Prairie falcon	4% (4)	0.5%(1)
Unknown buteo	NA (7)	—
Unknown raptor	NA (11)	—
Total birds observed	**122**	**215**

Based on data gathered during the pilot season and expert opinion, in 2012 we increased the distance between points to 1.6 km and doubled the survey time at each point. Increasing the distance between points reduced the likelihood of double-counting raptors and reduced the number of stops from 100 to 38. Lengthening the time at each point increased the likelihood of detection. Whereas each point was surveyed only once during the breeding season in 2011, during 2012 each point was surveyed once in May and once in June, to account for only the breeding population, avoiding young of the year and migrants. After the survey, all data collected at each point are entered into the geographic information system (GIS) to spatially analyze detections.

Results. During 2011, we recorded 122 detections of 11 species. Of the detections identified by species, red-tailed hawks accounted for 64% followed by American kestrels (11%) and golden eagles (6%) (table 3). In 2012, when we visited each point twice, we recorded 215 detections. As in 2011, we recorded 11 species, and red-tailed hawks were once again the most numerous (73%), followed by American kestrels (8%), next were Swainson's hawks (7%).

Conclusions. Due to modifications of the protocol and only two years of data, it is too soon to estimate population sizes or breeding densities for the three target species on the northern range. By the end of 2015, however, the YRI plans to have an estimate of the adult red-tailed hawk, Swainson's hawk, and American kestrel populations for this region, including an estimate of the number of breeding pairs. This five-year dataset will serve as baseline information to which


DEPARTMENT OF THE INTERIOR

A ranger-led program on raptor migration, September 2011.

future studies may be compared and, if continued beyond the initial five-year period, this survey may reveal population changes and trends over time.

Autumn Migration in Hayden Valley

Allowing a brief glimpse into the wild world around us, the spectacle of raptor migration has fascinated humans for centuries. Ordinarily difficult to study because of their secretive and wide-ranging nature, most raptors become highly visible during migration. Because migration is energetically costly raptors reduce the need for flapping flight by using either thermal or slope soaring techniques to aid southward (autumn) and northward (spring) movements (Duerr et al. 2012). Thermal soaring takes advantage of warm rising air masses. On days with good thermal lift, raptors can quickly gain elevation then glide to the next thermal pocket to make forward progress. Slope soaring occurs when raptors take advantage of air masses in lower elevation areas coming into contact with mountains. This causes the air mass to rise and raptors can gain lift in this way. Slope soaring is common in the Rocky Mountains because of the numerous mountain ranges present. Specific points along these mountain corridors provide areas from which to observe large numbers of raptors during the migration period. Surveying raptors from migration sites is a cost-effective and efficient way to gather data on multiple species over a short period. Data gathered during raptor migration over the long term have led to important discoveries, including the effects of DDT on raptor populations (McCarty and Bildstein 2005). In addition to indicating population trends, information gathered from migration sites has increased our understanding of the phenomenon of migration itself, encompassing routes, phenology, flight dynamics, and other aspects of raptor behavior (Bildstein et al. 2007).

Most hawk watch sites in North America are located in the eastern United States where tens of thousands of hawks, eagles, and falcons migrate along the Appalachian mountains and eastern coastline each autumn. The western United States presents more of a challenge in determining the paths of migrating raptors because of the abundant mountain ranges and competing ridgelines that can spread the migrants out over a large area and reduce concentrations in any one particular spot.

The site closest to Yellowstone is located north of the park in the Bridger Mountains near Bozeman, Montana. More than 2,000 raptors, many of them golden eagles, pass through the Bridgers on their way to wintering areas in the southern United States and Central, and South America. With Yellowstone located along the same flight path, we wondered if there was a similar corridor in the park. During the fall of 2010 we scouted several potential areas and found a promising site in Hayden Valley. Although Hayden Valley is not a traditional migration site, it does provide a location for thermal uplift as raptors move across a large and mostly forested plateau. Despite the differences between the landscape features of Hayden Valley and traditional monitoring sites, we observed significant numbers of migrating raptors there. During our one-week pilot monitoring effort at the Hayden Valley site in 2010, we counted 737 migrating raptors of 10 species in just 20 hours of observation. Most (81%) of the birds identified to species were Swainson's hawks, which have one of the longest migrations of any raptor—8,000 to 10,000 km from breeding areas across western North America to wintering grounds in central Argentina. The results from 2010 were exciting and prompted us to increase our monitoring effort the following year.

Methods. During the 2011 and 2012 observation periods, a total of 16 people participated in the counts with an average of 2 or 3 observers at the site each day. We observed for approximately six hours a day (less if limited by weather) on 35 days from September 13 to October 27 in 2011 and on 38 days from September 4 to October 26 in 2012. Observers scanned the entire northern portion of the sky and recorded all raptors seen moving past the site by species and, when possible, by age. Weather data, including sky condition, wind speed and direction, temperature, barometric pressure, and overall visibility were collected at hourly intervals for the duration of observations. We also record the thermal lift index, which is based on the amount of sunlight, wind, and raptor behavior (e.g., birds circling up in elevation indicates good thermal lift whereas birds having to flap frequently indicates poor thermal lift).

The area where we established our original count site in 2010 was located on a hilltop several miles south of Canyon Village that was closed during 2011 as a result of two fatal grizzly attacks in the area. We moved the count site to a lower elevation area at the northern end of the valley and then back to the original location in 2012. To determine if there were differences in the number of raptors detected between count sites, we conducted one day of simultaneous counts at both stations during 2012.

Results. In 2011, observers recorded 1,846 raptors of 16 species; in 2012, 953 raptors of 17 species; with a two-year total of 2,799 migrating raptors belonging to 17 species. Overall, red-tailed hawks, Swainson's hawks and golden eagles were the most abundant species; peregrine falcon, prairie falcon (*Falco mexicanus*) and turkey vultures (*Cathartes aura*) were the least abundant.

Despite the slightly greater effort in 2012 (194 hours) than during 2011 (177 hours), far fewer birds were recorded. We observed 526 Swainson's hawks in 2010, compared to 357 in 2011 and 46 in 2012, despite increasing effort over the three years. Variability in Swainson's hawk numbers is most likely a result of their notoriously unpredictable migration routes. Increases from 2011 to 2012 were seen in rough-legged hawks (*Buteo lagopus*) abundance, which nearly doubled, and turkey vultures, which did double. Observers at each count station during the simultaneous count recorded nearly the same number of each species indicating that although differences exist between the count stations in terms of visibility, observers are able to detect a similar number of birds. Although uncommon in the western US, we observed seven broad-winged hawks (*Buteo platypterus*) in 2012. A common migrant through the east, groups containing hundreds of broad-winged hawks, sometimes totaling tens of thousands in a single day, can be seen at migration sites in places like Duluth, Minnesota, and along the western shore of Lake Erie, Michigan.

Conclusions. The results of the 2012 season were drastically different from those of the previous year. For the observers, the major difference in 2012 was the reduced visibility on 16 of the 38 observation days due to smoke from fires burning in and outside the park. We strongly believe that this was the primary factor responsible for reducing the total count. Although the simultaneous count results indicated that a similar number of raptors could be observed at both locations, we acknowledge that this test was done for just a single day. A more robust assessment would have been to do simultaneous counts over multiple days, but due to the numerous days with low visibility and low daily counts comparisons between sites would have been difficult, if not impossible. Regardless of the lower counts in 2012, observations during the last few years have indicated that migrant raptors are moving across the Yellowstone plateau, and that Hayden Valley may be serving as a key geographic feature along their route. Only continued surveys will confirm this. The YRI will continue to explore locations throughout the park and determine if other features may produce comparable or higher concentrations. Over time, we hope to contribute to a large data set that will help track population trends along the Rocky Mountain Flyway.

Citizen Science and Education Programs

In 2010 the YRI initiated a program to involve the public and park staff in projects that not only raise awareness and appreciation of these top avian predators, but contribute to our understanding of raptor distribution in the park. Yellowstone's interpretive staff played an integral role by encouraging visitors to submit their sightings. To aid in the identification of raptors, we developed and made available at all visitor centers and ranger stations a handbook of raptor photos and identification tips for commonly observed species. All raptor sightings are entered into a GIS database.

Since the raptor sightings program began, we have received more than 1,000 sightings (fig. 1). They included 22 raptor species of which just three accounted for nearly half of all observations: 314 red-tailed hawks (23%), 174 bald eagles (13%), and 165 ospreys (12%). Observers ranged from park staff to visitors who were in the park for only a day. Report forms assisted in narrowing YRI staff efforts to locate breeding raptors and contributed to locating several of the red-tailed hawk nests that were monitored. In general, owls and

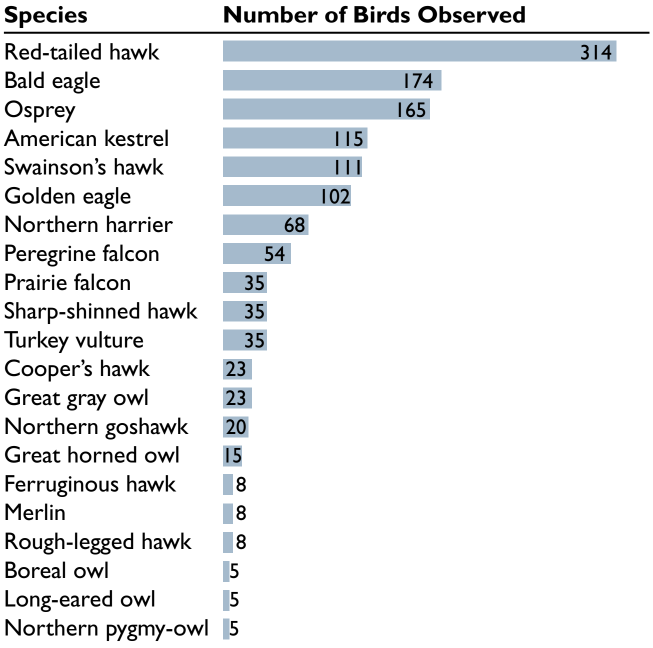


Figure 1. Raptor observations reported by staff and visitors, 2010–2012 (does not include unidentified raptor species).

accipiters (forest hawks) are infrequently documented largely because of their secretive nature and nocturnal behavior (most owls). Sightings of these species are especially important since little is known about their distribution and abundance in Yellowstone. For example, we received two reports of the rarely observed short-eared owl, a species known to breed here but with no known nesting territories. Reports like these are critical to increasing our awareness of how raptors are distributed across the Yellowstone landscape.

For the past three years, Education Ranger Katy Duffy has led migratory raptor ecology and identification field trips in which more than 180 people have participated. After meeting at the Fishing Bridge Museum to learn about raptor ecology and identification using mounts of raptors, participants go to Hayden Valley where Duffy points out migrating raptors and discusses identification tips and the ecology of migration. Duffy also teaches a raptor ecology and identification class for the Yellowstone Association each fall and was a guest speaker at the Winter Speaker Series in West Yellowstone in 2012, where she discussed the ecology of raptors in Yellowstone. On January 2012 and 2013 the YRI hosted a survey in which 25-30 volunteers annually helped us document winter use by bald and golden eagles throughout the park, Gardiner, and Paradise Valley. The YRI also presented a poster at the 11th biennial conference on the Greater Yellowstone Ecosystem on October 8-10, 2012.

Swainson's Hawk, American Kestrel, and Prairie Falcon

Although our primary nest searching and monitoring efforts are focused on golden eagles and red-tailed hawks, we are also interested in knowing more about the reproductive success of Swainson's hawks, American kestrels, and prairie falcons in the park. While the road-based survey described above will provide a northern range population estimate for Swainson's hawks and American kestrels, it will provide little information regarding reproduction. In the past, nest searching for these species has been largely opportunistic. All locations where territorial behavior was observed have been carefully marked and mapped so that more focused efforts in these areas can be made in the future. Since we have met our goal of monitoring 30 red-tailed hawk territories and identified 18 golden eagle territories, we will be able to devote more time to Swainson's hawks, American kestrels, and prairie falcons beginning with the 2013 breeding season.

USFWS/S.HILLEBRAND

Male American kestrel.

Our American kestrel nest searching efforts will be primarily focused on the northern range because of the relatively high density observed there. For Swainson's hawks, however, we will focus on other areas where they are more abundant. More than 13 territories in the Yellowstone Lake area and Hayden Valley have been identified and volunteer Jack Kirkley, Ph.D. with the University of Montana-Western, has located two nests. An additional 20 territories were documented by YRI staff for future studies, but many of them are located in the Mirror Plateau and the Thorofare region where nest searching and monitoring is impractical due to the difficulty of accessing these areas on a regular basis to check nests.

YRI staff located six prairie falcon territories primarily while searching cliffs for peregrine falcons and golden eagles. Due to staffing limitations we monitored only two territories during 2012, but both produced fledglings, a total of at least four young. Our goal for 2013 is to monitor all six prairie falcon territories.

The Future of the YRI

Because of Yellowstone's long-term managed and preserved habitat, the park serves as a living laboratory for understanding ecology, ecosystem maintenance, predator/prey relationships, and raptor conservation. While the ecological contribution of predators such as grizzly bears, wolves, and coyotes has been well documented in Yellowstone, the landscape scale effects attributed to these keystone terrestrial predators do not encompass the aerial niche held by raptors. Awareness of raptors as an ecosystem driver has long been overlooked, and is only now perceived in other habitats as a primary or secondary contributor to top-down trophic cascades and the ecological functioning of Rocky Mountain systems. The YRI is designed to fill this gap in knowledge and to provide baseline information for several species of raptor not previously studied in Yellowstone.

The information gathered during the first two years of the YRI has stimulated questions for future research that we hope to be able to address with additional funding. For example, our observations of many raptors foraging in Hayden Valley just prior to fall migration indicates that it may be a stopover site for migrating raptors. We also are curious about the inter- and intra-seasonal movements of golden eagles and what proportion of the breeding population remains in the park during winter. Yellowstone's northern range may be a source for red-tailed hawks, but we have no information on juvenile dispersal. Are red-tailed juveniles coming back to the park to breed or are they colonizing areas outside the park? Only banding and radio-tagging birds will enable us to answer these questions.

The more we learn about how raptors are using the Yellowstone landscape, the better prepared we will be to address conservation issues such as climate change and increasing anthropogenic effects. For example, a solid estimate of the current red-tailed hawk population could be used as a benchmark for future monitoring. In the late winter/early spring of 2013 we began preliminary surveys to learn more about the distribution and habitat associations of select owl species in Yellowstone. We plan to continue, and perhaps increase, the role of citizen science in acquiring knowledge of Yellowstone's raptors. We will also continue educating the public concerning raptor ecology through interpretive programs, park publications, the park website and other social media.

We are excited to continue our efforts over the next three years and look forward to all we will learn about the raptors of Yellowstone National Park. The future of the Yellowstone Raptor Initiative beyond the five-year period is uncertain, but the data collected thus far has provided a glimpse into raptor ecology in Yellowstone. YS

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Lisa Baril began her career as an ornithologist in 2000 banding songbirds in Yosemite National Park. In 2009, Lisa recieved her Master's Degree in Ecology at Montana State University in Bozeman studying willow-songbird relationships and joined the Yellowstone Bird Program in 2008. In 2011, Lisa transitioned to the newly developed YRI.

David Haines has worked as a wildlife biologist for over ten years specializing ornithological studies. He has focused much time studying birds a prey throughout the western United States. Formerly of the Santa Cruz Predatory Bird Research Group David joined the YRI at its inception in 2011.

Katy Duffy has banded diurnal raptors, owls and songbirds in Wyoming, Montana, and New Jersey since 1980 and has been a volunteer for Yellowstone's bird program since 2008. Katy has worked as an education ranger in Yellowstone since 1999; she is currently the park's interpretive planner.

Douglas W. Smith, Yellowstone National Park's Senior Wildlife Biologist, holds a PhD in Ecology, Evolution, and Conservation Biology from the University of Nevada at Reno. He has led YNP's Wolf Project since 1994 and the Bird Program since 2008.

Literature Cited

Bildstein, K.L., J.P. Smith and R. Yosef. 2007. Migration counts and monitoring. *In* D.M. Bird and K.L. Bildstein (eds). Raptor research and management techniques. Hancock House Publishers, Blaine, WA.

Driscoll, D.E. 2010. Protocol for golden eagle occupancy, reproduction, and prey population assessment. American Eagle Research Institute, Apache Jct., AZ. 55pp.

Duerr, A.E., T.A. Miller, M. Lanzone, D. Brandes, J. Cooper, K. O'Malley, C. Maisonneuve, J. Tremblay, and T. Katzner. 2012. Testing an emerging paradigm in migration ecology shows surprising differences in efficiency between flight modes. *PLoS ONE* 7(4): e35548.doi: 10.1371/journal.pone.0035548.

Gunther, K.A., R.A. Renkin, J.C. Halfpenny, S.M. Gunther, T. Davis, P. Schullery, and L. Whittlesey. 2009. Presence and distribution of white-tailed jackrabbits in Yellowstone National Park. *Yellowstone Science* 17(1):24–32.

Hodges, K.E., L.S. Mills, and K.M. Murphy. 2009. Distribution and abundance of snowshoe hares in Yellowstone National Park. *Journal of Mammalogy* 90(4):870–878.

Kochert, M.N., K. Steenhof, C.L. McIntyre, and E.H. Craig. 2002. Golden eagle (*Aquila chrysaetos*). *In* A. Poole and F. Gill (eds). The Birds of North America, No. 684. Philadelphia: The Birds of North America Inc.

McCarty, K. and K.L. Bildstein. 2005. Using autumn hawk watch to raptor migration and monitor populations of North American birds of prey. USDA Gen. Tech. Rep. PSW-GTR-191.

McIntyre, C. and J. Schmidt. 2011. Ecological and environmental correlates of territory occupancy and breeding performance of migratory golden eagles (*Aquila chrysaetos*) in interior Alaska. *Ibis* 153:1–12.

Steenhoff, K. and I. Newton. 2007. Assessing nesting success and productivity. Pages 81-192 *in* D.M. Bird and K.L. Bildstein (eds). Raptor Research and Management Techniques, Hancock House Publishers, Blaine, WA U.S.A.

Wilmers, C.C., R.L. Crabtree, D.W. Smith, K.M. Murphy, and W.M. Getz. 2003. Trophic facilitation by introduced top predators: Grey wolf subsidies to scavengers in Yellowstone National Park. *Journal of Animal Ecology* 72:909–916.

From the Archives

FROM THE LINK FAMILY PAPERS, YELLOWSTONE NATIONAL PARK ARCHIVES

Millions of park visitors have browsed the Old Faithful Lower Hamilton Store unaware of the Million Dollar Room overhead. It was created by Charles A. Hamilton, who had an apartment and office over the store that he purchased and named for himself in 1905. Today only his office remains, its walls covered with $1.8 million in cancelled checks—including two he wrote to buy the store (*inset*, courtesy Yellowstone National Park curator's Million Dollar Room project file). With funding provided by the Yellowstone Park Foundation, preservation of Hamilton's eccentric office is expected to be completed by the end of this year. To learn more about Hamilton and the Million Dollar Room project, go to: www.ypf.org/site/News2?page=NewsArticle&id=6402.

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Double rainbow over Yellowstone Lake.

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