Yellowstone Science

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Disease Ecology and Wildlife Health

in the Greater Yellowstone Ecosystem

NPS

The Invisible Hand of Disease

HISTORICALLY, diseases were viewed as minor players in the ecosystem relative to predators, competitors, and resources. This is not surprising. In places like Yellowstone National Park, with intact predator and scavenger communities, disease impacts are often hard to detect because carcasses disappear so quickly. As a result, the observed effects of diseases have been limited to large, but sporadic mortality events. More and more studies, however, show that disease impacts may be both subtle and important. Pathogens (and the diseases they cause) may increase predation rates, decrease productivity, and alter competitive interactions. As a new member of the research community around the Yellowstone area, I am happy to serve as guest editor for this edition of *Yellowstone Science,* where we explore the role that pathogens play in the ecosystem dynamics of the Greater Yellowstone Ecosystem (GYE).

Some of these diseases, such as brucellosis in elk and bison, have a long history of research and management in the GYE. Many pathogens, however, have only recently been detected and/or studied. In this issue, we look at the current effects of brucellosis in bison, whirling disease in cutthroat trout, chytrid fungus in amphibians, distemper, parvovirus, and mange in wolves, and future potential plans to address chronic wasting disease in elk and mule deer.

The ability of managers to eliminate new invasive pathogens is often very limited, but new technologies and outlooks abound that may provide novel solutions to old problems. Research funding for ecological studies of disease has increased over the past decade due, in part, to the human health risks posed by many emerging infectious diseases in wildlife and domestic animals (e.g., hantavirus, West Nile virus, avian influenza, and severe acute respiratory syndrome [SARS]). Hopefully, we can translate this additional research into management solutions that will maintain healthy wildlife populations while minimizing human–wildlife conflicts. As part of that effort, Yellowstone National Park is in the early stages of a wildlife health initiative (described herein) that would be the first of its kind in the U.S. national park system.

I hope you enjoy the issue.

—Paul C. Cross
Disease ecologist, U.S. Geological Survey

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on the cover
Clockwise from top:
bull elk, NPS/Jim Peaco;
cutthroat trout, Paul Scullery;
bison calf nursing, NPS/Jenny Jones.

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News & Notes

NPS/ABBY NELSON

Glenn Plumb Receives Natural Resources Award

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Regional Director Mike Snyder (left) presents award to Glenn Plumb.

Dr. Glenn Plumb, Branch Chief of Natural Resources in the Yellowstone Center for Resources, received the Professional Excellence in Natural Resources Award at the George Wright Society meeting in St. Paul, Minnesota, on April 19, 2007. The award recognized Plumb for his leadership on the development of science and research agendas for national parks, especially in regard to wildlife health issues. Common to many of his accomplishments has been successful collaboration with domestic animal and public health organizations as well as academic institutions, with a focus on improvements in applied research and bio-medical technology to enhance wildlife health and develop fundraising partnerships to keep these initiatives moving forward.

For example, working with the U.S. Department of Agriculture Animal and Plant Health Inspection Service, Plumb organized an international symposium at the University of Wyoming in 2005 to discuss the fundamental technological gaps in the means available to manage brucellosis in greater Yellowstone. He has been appointed to a five-year term as Chair of the United States Animal Health Association Brucellosis Committee, which serves as a clearing house for brucellosis management proposals that have been submitted by state and federal agricultural officials, industry representatives, veterinarians, researchers, and wildlife interests.

Plumb, who joined the National Park Service in 1990, has been working at Yellowstone since 1998. Last year he developed the Yellowstone Wildlife Health Program, a partnership between the park and the University of California–Davis, Montana State University, and the Yellowstone Park Foundation to combine expertise from several disciplines to address existing and potential diseases in the park. Plumb was also on the team that developed the Greater Yellowstone Science Learning Center, a joint effort by Yellowstone and Grand Teton national parks, Bighorn Canyon National Recreation Area, the Greater Yellowstone Inventory and Monitoring Network, and the Rocky Mountains Cooperative Ecosystem Studies Unit at Montana State University to share information about resources and research in the parks with a variety of audiences using a web-based platform funded in large part by a grant from Canon U.S.A., Inc., through the Yellowstone Park Foundation.

U.S. Delegation Attends Disease Workshop in Kyrgyzstan

In December 2006, the U.S. State Department Bureau of International Security and Nonproliferation/Cooperative Threat Reduction (ISN/CTR) invited Dr. Glenn Plumb to join a delegation to Bishkek, Kyrgyzstan, to attend the Central Asian Disease Surveillance Workshop regarding development of a brucellosis management program for Kyrgyzstan. The ISN/CTR helps redirect former Soviet weapons experts, including scientists in the Kyrgyz Republic, toward peaceful, sustainable civilian research. This work includes engaging the ministries of health and agriculture in participating nations to develop projects that can meet nonproliferation goals and also combat global public health threats. The incidence of animal and human brucellosis in the Kyrgyz Republic and neighboring countries has increased dramatically in recent years, and the gravity of this situation led the Kyrgyz Deputy Minister of Health to request last summer that the U.S. bring over experts for consultations on how best to control it. Glenn and the delegation met with the Kyrgyz Deputy Minister of Health, Deputy Minister of Agriculture, and numerous other scientists, ministry officials, and members of the Kyrgyz National Academy of Sciences to review opportunities to help meet U.S. nonproliferation goals and for aiding the Kygryz Republic in its efforts to combat one of that country's most significant public health challenges.

Blood Test Required for Stock Entering Yellowstone

Beginning this year, owners of horses, mules, and burros must have proof that their animals have recently been tested for Equine Infectious Anemia (EIA) before bringing them into Yellowstone National Park.

The virus is spread from infected to healthy animals through large biting insects like horseflies. There is no vaccine, treatment, or cure. The disease can be fatal to members of the horse family.

The only way to know if an animal is infected with EIA is to conduct a Coggins Test, which checks for EIA

antibodies in the animal's blood. While proof that a negative Coggins Test has been conducted in the past 12 months must accompany every equine that enters the park, Yellowstone National Park does not require a Certificate of Veterinary Inspection or perform brand inspections on stock animals.

Late Winter Bison Count

Yellowstone National Park completed the 2007 late winter bison population estimate. Based on a late winter aerial survey, the late winter population was estimated to be 3,600 bison. The survey takes into account the 2006 late summer population estimate of 3,900 bison, known brucellosis risk management mortalities, and scientific estimates of over-winter mortality rates.

The population estimate is used to guide adaptive management strategies under the Interagency Bison Management Plan (IBMP). Specific management actions may be modified based on expected late winter population levels as corroborated by the annual late winter estimate. This is the seventh winter the IBMP has been used to guide brucellosis risk management actions.

The IBMP is a cooperative plan designed to protect Montana's brucellosis-free status while also conserving a viable, wild bison population. Protecting Montana's brucellosis-free status requires keeping bison from mixing with cattle grazing on land adjacent to the park.

The five cooperating agencies operating under the IBMP are the National Park Service, the U.S. Forest Service, the USDA Animal and Plant Health Inspection Service, the Montana Department of Livestock, and Montana Fish, Wildlife and Parks.

9th Biennial Scientific Conference on the GYE

The Greater Yellowstone Ecosystem biennial scientific conference series, initiated in 1991, is designed to encourage awareness and application of wide-ranging scientific work on the region's natural and cultural resources. These conferences, with the active involvement of professional societies and other institutions, provide a much-needed forum for knowledge-sharing among hundreds of researchers, park managers, and the general public.

The next conference, *The '88 Fires: Yellowstone and Beyond*, will remember the events of the greater Yellowstone area fires of 1988. As such, we are skipping a year in our regular conference schedule in order to hold the 9th conference September 7–13, 2008—the 20th anniversary of the 1988 fire season's end. This conference is being sponsored by the International Association of Wildland Fire and the Association for Fire Ecology in association with the 9th Biennial Scientific Conference on the Greater Yellowstone Ecosystem. It is expected to be much larger than previous conferences in the series, and will therefore be held at the Snow King Resort in Jackson Hole, Wyoming.

This conference will be both a scientific meeting and a homecoming for many people who were involved in the 1988 fires throughout the West. These history-making fires will provide springboards for discussions and presentations about lessons learned, ecological research related to the fires, fire effects, large fire management, wildland fire planning and policy, the use of fire as a management tool, human values and perceptions of fire, and many other issues.

The Call for Papers is expected to come out this October. For more information, visit http://www.iawfonline.org/conferences.shtml.

Heasler Appointed to Advanced National Seismic System Committee

Dr. Henry Heasler, Park Geologist in Yellowstone National Park's Center for Resources, has been appointed to

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serve on the Regional Advisory Committee for the Intermountain West Region of the Advanced National Seismic System (ANSS), which is part of the U.S. Geological Survey (USGS). The Yellowstone Volcano Observatory (http://volcanoes.usgs.gov/yvo/), a partnership between Yellowstone National Park, the USGS, and the University of Utah, contributes to the overall ANSS mission, which is to provide accurate and timely information on seismic events. Recent efforts of the Regional Advisory Committee have focused on developing a long-term strategic plan for seismic monitoring throughout the region and obtaining the resources needed to improve earthquake reporting. The committee is concerned that the existing broadband seismograph stations in the region are inadequate to meet ANSS minimum performance standards.

As one of the four at-large members on the Regional Advisory Committee, which also includes representatives from each of the eight states in the region, Heasler will not be representing a particular constituency, but he will be in a position to speak for the interests of Yellowstone National Park. Along with geologists, the committee is made up of other users of seismic information, such as engineers, emergency managers, utility operators, and transportation officials.

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COURTESY OF PAUL CROSS

Disease ecologist Paul Cross with an African buffalo.

Wildlife Health Initiatives in Yellowstone National Park

Paul C. Cross and Glenn Plumb

WILDLIFE AND THEIR PARASITES do not recognize political or jurisdictional boundaries and, as a result, national parks are not immune to the environmental changes occurring around them. Habitat fragmentation, habitat loss, introductions of invasive species, and climate change all have direct impacts on the many wildlife species that move across park boundaries. These disturbances are also likely to affect parasite communities and wildlife health. In particular, encroachment of native landscapes by people, pets, and livestock may increase the frequency with which alien invasive parasite species are introduced. Mange, canine parvovirus, brucellosis, chronic wasting disease, whirling disease, and chytrid fungus are all examples of the pathogen pollution that is occurring in the Greater Yellowstone Ecosystem (GYE). (A pathogen is any disease-producing agent, especially a virus, bacterium, or other microorganism.)

In this issue of *Yellowstone Science,* we highlight the ongoing work in Yellowstone National Park (YNP) and the surrounding region on wildlife health issues. Some of the diseases we cover in this issue have chronically infected the GYE for many years (e.g., brucellosis). Other diseases are relatively new to the system (e.g., canine parvovirus, chytrid fungus, whirling disease); while others are still on the horizon (e.g., chronic wasting disease). All of them will require novel management solutions that draw upon the expertise of many disciplines. The work detailed here is the beginning of a wildlife health program carried out by a collaborative group of researchers and managers from academia, federal and state government, and conservation organizations (*see inset page 7, The Yellowstone Wildlife Health Program*).

Wildlife and Human Health are Linked

Much of the interest in disease ecology and wildlife health has been prompted by the emergence, or resurgence, of many parasites that move between livestock, wildlife, and/or humans. Wildlife diseases are important because of their impact on both the natural ecosystem and human health. Many human diseases arise from animal reservoirs (WHO 2002). Hantaviruses, West Nile virus, avian influenza, and severe acute respiratory syndrome (SARS) are examples of disease issues that have arisen over the last decade. Indeed, nearly 75% of all emerging human infectious diseases are zoonotic (a disease that has spread to humans from another animal species). Many of these diseases have spilled over from natural wildlife reservoirs either directly into humans or via domestic animals (WHO/FAO/OIE 2004). Unprecedented human population abundance and distribution, combined with anthropogenic environmental change, has resulted in dramatic increases in human–animal contact, thus increasing the intimate linkages between animal and human health (Figure 1).

Linkage of human and animal health is not a new phenomenon, but the scope, scale, and worldwide impacts of contemporary zoonoses have no historical precedent (OIE 2004a). Zoonotic infectious diseases can have major impacts on wild and domestic animals and human health, resulting in

serious damage to the economies of developing and developed countries (WHO 2002, OIE 2004b). In wildlife and disease management there are many examples of well-intentioned ecological interventions going awry due to an incomplete understanding of all the ecological connections. In response to these realties, Conservation Medicine has arisen as a new field based at the crossroads of wildlife, human, and ecological health (Aguirre et al. 2002). Our ability to meet the disease management challenges of the twenty-first century will be greatly influenced by our ability to expand basic and applied disease research programs and communicate research results.

The Future of Wildlife Health Research and Management in the GYE

Here we suggest a number of new research directions that we hope will yield new insights and management options in the GYE. While some of these avenues echo those suggested in a recent text on the ecology of wildlife diseases (Hudson et al. 2001), others are particularly relevant to the GYE.

Predator and hunting effects. Recent theoretical work suggests that predators may have beneficial effects for prey populations by selectively preying upon diseased individuals, thus reducing the burden of disease in prey populations (Packer et al. 2003). Similar results may also be expected if diseased prey were more easily hunted by humans. So far, there are few tests of these hypotheses in large mammals, but with the potential spread of chronic wasting disease (CWD), the GYE may become a testing ground.

The potential for wolves to improve the health of elk herds depends upon a number of factors, many of which are poorly understood for CWD infections. First of all, selective predators are likely to have a greater impact on diseased prey than non-selective predators. Thus, we would expect wolves, which chase down weaker prey, to have a more beneficial effect than mountain lions, which are primarily stealth hunters and may be less selective. Secondly, the relative timing of the onset of symptoms and when individuals are infectious is likely to be very important (Figure 2). For example, wolves may have a much smaller impact upon disease prevalence if elk become infectious long before they show symptoms than if elk become symptomatic and are targeted by wolves before becoming infectious. For CWD, we still have little information on the relative timing of when individuals are symptomatic versus infectious. We also lack data on the overall survival rates of infected versus uninfected elk or deer. Clearly, more research needs to be done before we can understand the role of predation in GYE disease dynamics.

Viral tracking of hosts. Disease ecology requires the integration of many different disciplines, including immunology, genetics, veterinary science, microbiology, mathematics, epidemiology, and ecology. As no one person can excel in all of these fields, there is a strong need for collaborative research programs

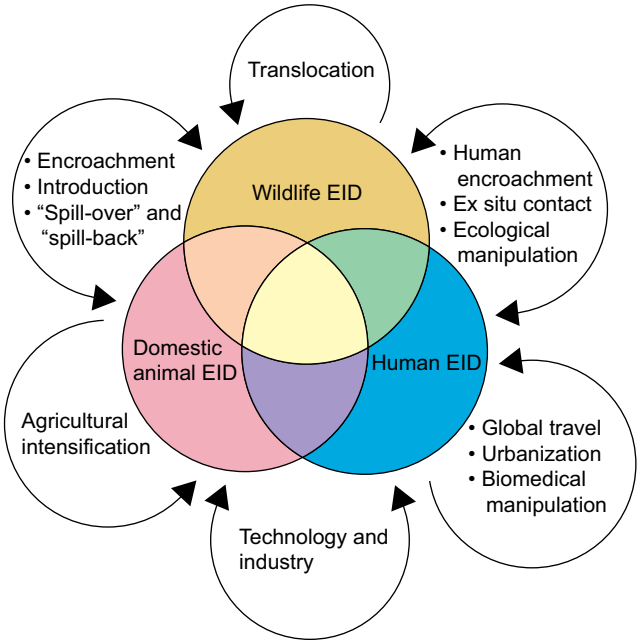

Figure 1. Many emerging infectious diseases (EIDs) are shared by wildlife, domestic animals, and/or humans. Arrows depict some of the key factors promoting disease emergence. Reprinted with permission from Dazsak et al. (2000).

incorporating experts from many different fields. One example that we think has great potential in the GYE is the use of parasite genetics to estimate the movement of a host species (the organism from which a parasite obtains its nutrition and/or shelter) among populations or the amount of transmission among different host species. Traditionally, population geneticists have looked at host genetics to investigate the amount of movement, or lack thereof, among populations. Since the generation time of large mammals is many years, however, host genetics typically inform researchers about historical connections. Parasites, and in particular viruses, have very short generation times, which may allow researchers a glimpse at contemporary movement patterns of the host. Roman Biek and Mary Poss have applied these techniques to Feline Immunodeficiency Virus to reveal the population structure of cougars across Montana (Biek et al. 2006). This approach may allow researchers to investigate the effects of recent land-use change on host connectivity. Data on the movement of hosts across the GYE will allow for the construction of detailed spatial disease models that can be used to address management issues.

Fire and land use. To date, we have found very few studies investigating the effects of fire and land use on disease processes, despite the importance of these factors to host dynamics, particularly in Rocky Mountain ecosystems. Many parasite species are either transmitted through the environment or have a free-living lifestage that may be susceptible to the effects of fire, and fires may increase in intensity or frequency due to

climate change (Westerling et al. 2006). We suspect that this may be an important avenue of future research.

Farnsworth et al. (2005) found that chronic wasting disease was more prevalent among mule deer in developed areas than in rural areas. At this point, it remains unclear whether this is due to a lack of hunting or increased aggregation in suburban neighborhoods. However, the fact that some of the regions surrounding YNP are among the fastest-developing in the nation along with the slow approach of chronic wasting disease from the south and east gives us substantial cause for concern.

Spill-over and spill-back. Many parasites are shared among livestock, wildlife, and humans. In several cases, such as brucellosis and tuberculosis, spill-over infections from livestock to wildlife led to disease epidemics within the new wildlife host. Subsequently, the disease was mostly controlled within livestock, but eradication efforts remain difficult due to potential "spill-back" infections from wildlife to livestock. In some cases, these spill-over infections from livestock to wildlife result in almost complete die-offs of the new wildlife host (e.g., rinderpest and African buffalo). In other cases, however, the impact of the disease has almost undetectable effects on population growth (e.g., brucellosis and bison). However, there have been very few cases where such diseases have been subsequently eradicated from the wildlife host. Thus, these new infections are continually added to the suite of challenges wildlife species face. Although any one disease may have a minor effect, collectively they may affect the resiliency of wildlife populations to natural and anthropogenic disturbances. Despite the important role of wildlife to the emergence and dynamics of disease in livestock, there have been very few studies in the GYE that have attempted to estimate the risk or actual amount of transmission between host species and between wildlife and livestock. Estimating disease transmission is not easy, particularly in wildlife systems. However, we believe new technologies in radio-tracking and genetics may be a powerful approach. Global Positioning System (GPS) collars now facilitate the collection of fine-scale data that can be used to estimate the amount of contact among different species, while genetic analyses of the parasites may allow researchers to estimate parasite transmission among host species. Finally, transmitters that record when they are in close proximity to one another will enable researchers to start looking at the rate of contact among individuals.

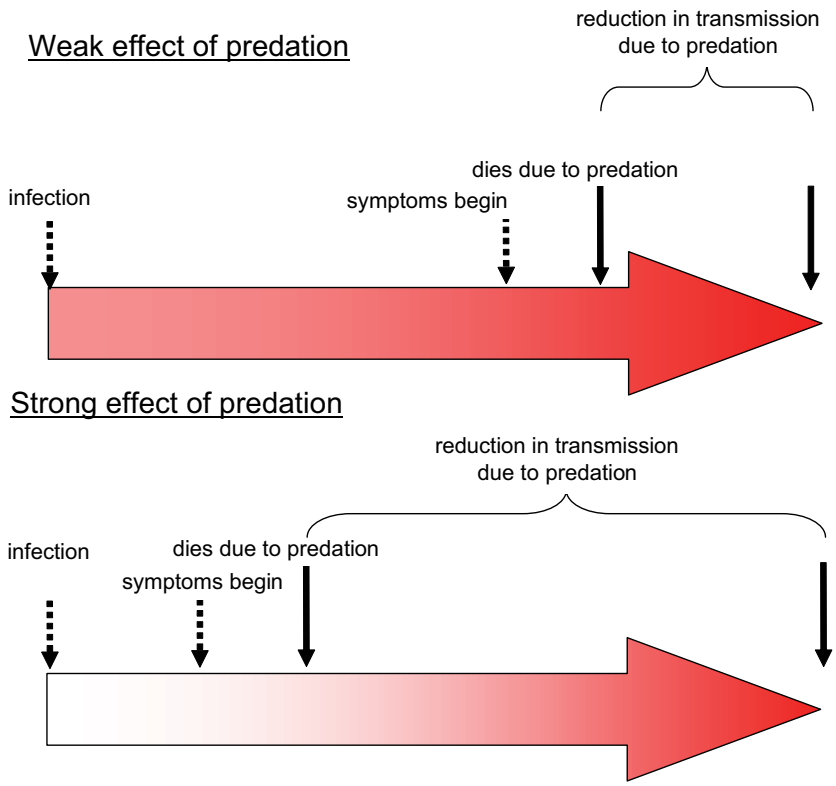

Figure 2. The effects of predation on disease. Each large arrow represents the course of disease in the prey species: the timing of infection, onset of symptoms, and when the individual would die from predation or disease (if predators were absent). The intensity of the red color indicates how infectious the individual is. Predators will have a weak effect if prey are infectious throughout the course of disease, but are only symptomatic later in life (A). Predators will have a strong effect upon disease if the prey are symptomatic earlier relative to when they become infectious (B).

Multi-host and multi-pathogen. Traditional wildlife biology began with an emphasis on the dynamics of single species. Over time, however, a more holistic research approach developed that incorporated the effects and interactions of competition, predation, and herbivory. The disease ecology field is undergoing a similar transition away from simple, single host–pathogen systems to embrace the complex interactions that occur in the more common multi-host and multi-pathogen ecosystems.

Recent immunological studies have shown that helminths (intestinal worms) and brucellosis can stimulate opposing sides of the immune system (Abbas 1996). As a result, individuals that are heavily infected with worms may be less able to control brucellosis and vice versa. Both bison and elk are likely to be infected with a diversity of helminths. Thus, there may be interactions between helminths and *B. abortus* that drive the dynamics of brucellosis in the elk and bison populations of YNP. Understanding how multiple parasites interact within a single host, and how alternative hosts affect disease dynamics in the primary host may lead to novel and effective management strategies.

The Yellowstone Wildlife Health Program

Yellowstone National Park recently signed a Memorandum of Understanding with Montana State University and the University of California–Davis School of Veterinary Medicine Wildlife Health Center to establish the Yellowstone Wildlife Health Program, focused on understanding and addressing priority wildlife disease and ecosystem health problems at Yellowstone National Park. Initial five-year funding is being provided by the Yellowstone Park Foundation.

The Yellowstone Wildlife Health Program goal is to design and implement a long-term wildlife health assessment program to monitor and evaluate wildlife diseases and health indicators as a subcomponent of the Greater Yellowstone Network Vital Signs Monitoring Program. Specific objectives of the program include:

- Facilitation of cooperation among scientists seeking competitive grant funds to investigate wildlife health issues.
- Development of an outreach program, including educational materials for field courses on wildlife health, that provides information for the public, faculty, and federal and private funding agencies.
- Development of on-site wildlife veterinary services, including veterinary support for animal handling activities, disease surveillance, and disease outbreak investigation, including field evaluation, necropsy, and specimen sampling.
- Establishment and coordination of on-site or cooperative wildlife disease diagnostics and field and laboratory research capacity.
- Facilitation of wildlife health professional capacity development, as well as research by veterinary students, graduate students, postdoctoral fellows, and post-graduate researchers.

An Ounce of Prevention

Although there are many cutting-edge research directions to pursue, a tremendous amount of basic research remains to be done in YNP. In 1995, Aguirre et al. argued for the collection of baseline data on wildlife diseases in national parks. Over a decade later, we have yet to reach that goal. Currently, only two full-time wildlife veterinarians work for the NPS across the national park system of 390 units covering more than 84 million acres. Wildlife diseases have traditionally been very difficult to eradicate in natural settings, and although any one particular disease may have minor effects upon a host population, the cumulative effects of many new parasite species may threaten the resilience and persistence of the many wildlife species we appreciate in national parks.

The logistics and consequences of disease control or eradication efforts in NPS areas have been and will continue to be challenging. Due to the difficulties inherent in eradicating or controlling wildlife diseases in a field setting, we call attention to the need for more work on the prevention of disease. As a prerequisite for this work, active surveillance is needed on a multitude of wildlife species, which would allow for the detection of new parasites as well as changes in the intensity or prevalence of infection of pre-existing parasite species. As Yellowstone National Park is home to one of the most intact remaining wildlife ecosystems, it is fitting that the park should serve as a proving ground for the problem-oriented, basic, and applied disease research that will be necessary to conserve these wildlife resources for future generations.

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Dr. Paul Cross is a disease ecologist with the U.S. Geological Survey and a faculty affiliate with Montana State University. Previously, he worked on bovine tuberculosis in African buffalo and now works on brucellosis and chronic wasting disease. He holds a PhD in Environmental Science, Policy, and Management from the University of California–Berkeley, and a BA in Environmental Science from the University of Virginia–Charlottesville. **Dr. Glenn Plumb** is the Branch Chief of Natural Resources in the Yellowstone Center for Resources, Yellowstone National Park.

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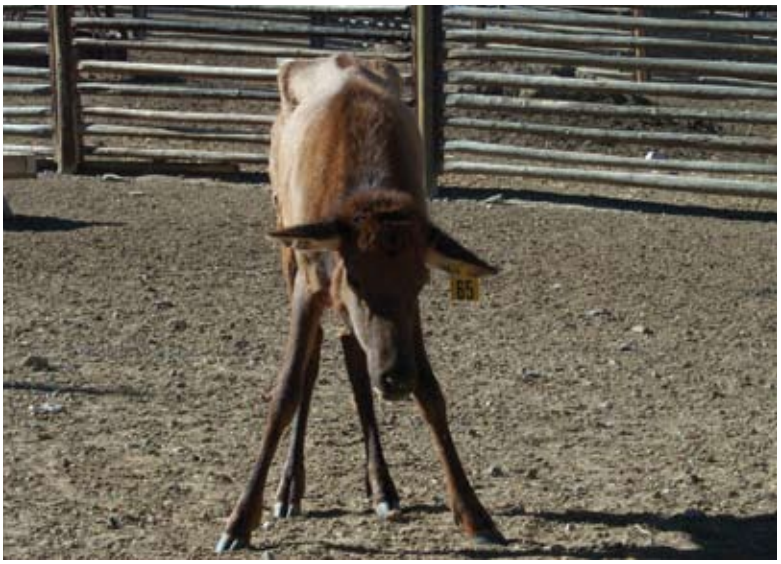
Chronic Wasting Disease
Planning for an Inevitable Dilemma

P. J. White and Troy Davis



TERRY KREEGER/WYOMING GAME AND FISH DEPARTMENT

An infected elk showing exaggerated wide-legged posture, lowered head, and bony frame typical of chronic wasting disease.

THE HIGH MOUNTAINS AND PLATEAUS of Yellowstone National Park (YNP) provide summer range for an estimated 20,000–30,000 deer (*Odocoileus* sp.) and elk (*Cervus elaphus*) from at least eight herds, most of which winter at lower elevations outside the park. These world-renowned herds provide significant visitor enjoyment and revenue to local economies through guiding and sport hunting. Elk are the most abundant ungulate in the park and constitute a foundation species that has strong, ramifying effects on other species and processes in the ecosystem. For example, elk comprise approximately 85% of kills made by wolves (*Canis lupus*) during winter and are an important source of protein for black and grizzly bears during spring and early summer (Smith et al. 2004; Barber et al. 2005). They also provide an important source of energy for mountain lions and at least 12 species of scavengers, including bald eagles and coyotes (Wilmers et al. 2003; Ruth 2004). In addition, elk browsing and nitrogen deposition can have significant effects on vegetative production, soil fertility, and plant diversity (Frank and McNaughton 1992). Thus, changes in elk abundance over space and time can alter species abundance, community composition, nutrient concentrations of plants, and the physical structure of vegetation in YNP.

These magnificent herds may soon be infected with chronic wasting disease (CWD), which was detected approximately 130 miles from the park in the Bighorn Basin area of Wyoming during 2003. Chronic wasting disease is a fatal neurologic disease of elk, moose (*Alces alces*), mule deer (*O. hemionus*), and white-tailed deer (*O. virginianus*) from the family of diseases known as transmissible spongiform encephalopathies or prion diseases. Other diseases in this family include scrapie in sheep, bovine spongiform encephalopathy (i.e., "mad-cow disease") in cattle, and Creutzfeldt-Jacob disease in humans. Chronic wasting disease attacks the brains of infected animals, causing them to become emaciated, display abnormal behaviors (e.g., "zoned-out" appearance, aimless wandering), lose bodily functions (i.e., excessive salivation, drinking, and urination), and eventually die (Williams et al. 2002). Infections may occur at any time of year and sexes appear to be equally susceptible.

Prevalence or susceptibility to CWD appears higher in mule deer and white-tailed deer than in elk in endemic areas of Colorado and Wyoming (Miller et al. 2000). Bighorn sheep (*Ovis canadensis canadensis*), bison (*Bison bison*), mountain goats (*Oreamnos americanus*), and pronghorn (*Antilocapra americana*) appear to be resistant or at least much less susceptible to the disease than deer and elk. There is no evidence that CWD is naturally transmissible to humans or domestic livestock (Food and Drug Administration 2001). However, a related animal disease, bovine spongiform encephalopathy (BSE), has been causally linked to the human form of that disease known as variant Creutzfeldt-Jakob disease (vCJD). While current evidence indicates that the differences between BSE/vCJD and CWD are significant, there is still ongoing research to establish whether CWD can cross the human species barrier. Thus, health experts (e.g., World Health Organization 2000) warn that no part or product of any animal with evidence of CWD should be fed to any species (human or any domestic or captive animal).

Chronic wasting disease is contagious and transmissible

by direct animal–animal contact or indirectly through the environment (Miller et al. 2004; Johnson et al. 2006). Human activities have exacerbated the distribution and prevalence of the disease by translocation of infected deer and elk between game farms, research facilities, and zoological parks and also by concentration of wildlife through artificial feeding, loss of habitat, and changes in movement patterns due to fragmented landscapes. Chronic wasting disease and associated control actions have substantially decreased deer and elk populations in some areas where outbreaks occurred (Williams et al. 2002). Similar population reductions in YNP could indirectly alter the structure and function of this ecosystem, adversely affect species of predators and scavengers (including the federally listed bald eagle [*Haliaeetus leucocephalus*], grizzly bear [*Ursus arctos*], lynx [*Lynx canadensis*], and wolf), and have serious economic effects on the recreation-based economies of the area (Duffield and Neher 1996). Thus, the early detection and management of CWD is recommended to stabilize or reduce the proportion of infectious individuals and avoid a precipitous decrease in the population growth rate (Gross and Miller 2001).

In 2004, we began collaborating with Montana Fish, Wildlife and Parks to test for CWD in northern Yellowstone elk. We focused on these elk because they migrate throughout park and surrounding lands during summer, coming into contact with deer and elk from other herds. Thus, they are likely to be exposed to CWD soon after it arrives at the park. Also, the highest densities of deer and elk in the park occur on the northern range, making these animals at relatively higher risk for CWD infection. State biologists collected brain stem and lymph node tissue samples from animals harvested during the Gardiner Late Elk Hunt. These samples were sent to Colorado State University Veterinary Diagnostic Laboratory in Fort Collins for testing with an enzyme-linked immunosorbent assay (ELISA) developed by Bio-Rad Laboratories, Inc. Tissue samples from 703 elk harvested adjacent to YNP's northwest boundary from the 2004 and 2005 late hunts were tested and found negative for CWD (Anderson and Aune 2004; Anderson and Southers 2005).

During winter 2006–07, we also collected and tested tissue samples from deer or elk killed by vehicle collisions in or near YNP. Data from Colorado indicate that CWD-infected mule deer were more vulnerable to vehicle collisions than otherwise healthy deer (Krumm et al. 2005). Thus, testing tissue samples from vehicle-killed deer and elk could enhance our ability to detect CWD. We will also attempt to collect samples from wolf-killed deer and elk as part of ongoing ungulate and wolf programs. If wolves detect and select deer and elk infected by CWD, then testing tissue samples from these animals could enhance our ability to detect CWD.

Even if this surveillance is successful at detecting CWD in or near YNP when prevalence is low (~1%), however, there would already be approximately 100–150 infected animals in the population. At this point, it would be extremely difficult, if not impossible, to eliminate CWD from the population because the best available evidence suggests eradication of CWD in free-ranging deer and elk is unrealistic and culling efforts to reduce prevalence have been relatively unsuccessful (Williams et al. 2002; Conner et al. 2007). Thus, we developed a management plan for CWD that was approved by YNP's superintendent during 2006. Under this plan, we will take actions to stabilize the prevalence and reduce the spread of CWD if it is detected in or near the park, while ensuring management actions (or inaction) do not harm the integrity of park resources or values. We will implement regular surveillance of deer and elk in the infected population(s) using ground and aerial surveys and remove any animals with clinical signs of CWD by shooting.

We will also respond to reports of "sick" deer, elk, and moose to evaluate them for clinical signs of CWD, remove clinical animals by shooting, and test tissue samples for CWD. If necessary, we will randomly cull animals from certain populations to assess the prevalence and spread of the disease. However, we hope to avoid large-scale culling operations or population reductions because they would remove many more healthy animals than infected animals and substantially reduce the prey base for predators and scavengers. Likewise, we will not use culling to assess the prevalence and distribution of CWD in small populations (e.g., the Madison–Firehole elk herd) because even the removal of 50 animals would constitute a 20% reduction in an already decreasing population.

In addition, we will evaluate if selective predation by wolves and other predators on CWD-infected elk reduces transmission rates and numbers of infected animals. Unlike other areas where CWD outbreaks have occurred, YNP supports an intact large-predator complex including black bears (*Ursus americanus*), coyotes (*Canis latrans*), grizzly bears, humans, mountain lions (*Puma concolor*), and wolves. Wolves are highly selective for elk throughout the year and bears are highly selective of neonatal elk during summer (Smith et al. 2004; Barber et al. 2005). If predators can detect CWD-infected animals, then

NPS PHOTO/ROCKY MOUNTAIN NATIONAL PARK

This elk, photographed in Rocky Mountain National Park, displays clinical signs of CWD, such as lowered head and ears and emaciated body.

selective predation and quick removal of carcasses by scavengers could reduce CWD transmission rates and, in turn, the prevalence and spread of the disease. Wolves could also reduce the risk of transmission by dispersing deer and elk. Model simulations based on conditions at Rocky Mountain National Park (i.e., high elk density) suggest wolves could have "potent effects" on the prevalence of CWD (Wild and Miller 2005). Also, compensatory and density-related effects could result in less net mortality than rates of infection and death from CWD would suggest. Thus, the net effect of CWD on the abundance, reproduction, and survival of deer and elk could be less than predicted based on data collected in areas with few large predators.

We are confident the selective culling of infected animals by park staff, in conjunction with selective predation on CWD-infected animals, will slow disease transmission while removing relatively few healthy animals and not substantially reduce population growth rates. However, the success of this approach depends, in part, on the vigilance of park staff and visitors at identifying and reporting animals that display repetitive behavior (e.g., moving in a set pattern) or abnormal behavior such as staggering, standing with an exaggerated wide-legged posture, or carrying their heads and ears lowered. Infected animals become emaciated and sometimes stand near and consume large amounts of water. Drooling or excessive salivation may also be apparent. Please accurately document the location of any animal that shows CWD symptoms and immediately contact P. J. White (307-344-2442 or pj_white@nps.gov) or Troy Davis (307-344-2218 or troy_davis@nps.gov). Do not attempt to touch, disturb, kill, or remove the animal. Your assistance will help preserve the diverse and intact predator–prey complex of YNP for the enjoyment of future generations.

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Boreal toad (*Bufo boreas*) at High Lake.

Amphibians and Disease

Implications for Conservation in the Greater Yellowstone Ecosystem

Paul Stephen Corn

THE DECLINE OF AMPHIBIAN populations is a worldwide phenomenon that has received increasing attention since about 1990. In 2004, the World Conservation Union's global amphibian assessment concluded that 48% of the world's 5,743 described amphibian species were in decline, with 32% considered threatened (Stuart et al. 2004). Amphibian declines are a significant issue in the western United States, where all native species of frogs in the genus *Rana* and many toads in the genus *Bufo* are at risk, particularly those that inhabit mountainous areas (Corn 2003a,b; Bradford 2005).

As is true for most of the cold and dry Rocky Mountains, relatively few amphibian species are native to the Greater Yellowstone Ecosystem (GYE; Table 1). One of the five native species, the northern leopard frog (*Rana pipiens*), may have been extirpated. Except for a photograph of leopard frogs taken near Flagg Ranch in 1995 and an occasional unsubstantiated report, this species has not been observed during recent surveys. The remaining four species are distributed throughout the GYE, and their ranges do not appear to have retreated from historical coverage of the landscape. However, some or all of these species are declining or have declined in some portions of the GYE. Although we lack the historical data to judge whether the current percentages of potential breeding sites occupied represent declines for most species, it is likely that boreal toads have declined to some extent in the GYE. This species has declined severely in the southern Rocky Mountains (Colorado, northern New Mexico, and southeast Wyoming), and occupancy of 5% of potential breeding sites (based on sampling at selected water catchments) in the GYE is lower than the 7–15% occupancy observed in similar surveys in Glacier National Park.

Habitat degradation or loss, predation by alien species, over-exploitation, climate change, pollution, emerging infectious diseases, and complex interactions among two or more factors are demonstrated or hypothesized causes of amphibian declines. Here, I review the role disease may play in amphibian declines in the GYE, but note that other causes have been identified (Table 1) and may be as important as or more important than disease.

A variety of pathogens and their relationships to amphibian declines have been studied in recent years (reviewed by Daszak et al. 2003). These include viruses, fungi, protists (single-celled or multi-cellular organisms that are neither plants nor animals, but which may show characteristics of both), and more complex parasites such as trematodes (*Ribeiroia* sp.). Trematodes are now known to be the primary cause of the occurrences of frogs with extra limbs and other deformities that received considerable public and scientific attention in the late 1990s (Souder 2000). Water molds are oomycete protists that may infect and kill the embryos in amphibian egg masses. Although water molds can kill large numbers of embryos at some locations and populations afflicted with deformities may have high mortality of young frogs after metamorphosis, neither water molds nor trematode parasites have been shown to affect the persistence of populations and they are unlikely to have caused widespread declines.

Ranaviruses

Viruses are another group of virulent pathogens that so far have had primarily local effects (Daszak et al. 2003). Ranaviruses are a large complex of related viruses in the Family Iridoviridae that infect reptiles, amphibians, and fish. Ranaviruses are not novel pathogens for amphibians. Different strains have coevolved with their amphibian host populations and typically attack stressed individuals. Ranavirus infections are more likely to occur when hosts are in dense aggregations that sometimes occur as temporary ponds dry before metamorphosis can be completed. Tiger salamander larvae may suffer catastrophic mortality from ranavirus infection, and such episodes can recur year after year in the same population. Ranaviruses do not survive outside their hosts and are transmitted via direct contact. Some individuals survive the infection and may carry the virus back to the breeding pond in subsequent years or serve as a means of transmitting the pathogen to new sites (Brunner et al. 2004).

Although ranaviruses are part of the natural life history of amphibians, human activities may be disrupting this system and creating situations where ranavirus should be considered as the agent of emerging infectious disease. Specifically, the transportation of tiger salamander larvae (Figure 1) around western North America for use as fishing bait appears to have exposed

Table 1. Distribution and status of amphibians in the GYE.

Species	Distribution	Status	Causes of decline	References
Tiger Salamander (*Ambystoma tigrinum*)	Occurs throughout the GYE	Present at 18–24% of potential breeding sites; genetic evidence for historic and recent declines in the northern range	Fish stocking (historic) and disease (recent)	Corn et al. 2005; Spear et al. 2006
Boreal Toad (*Bufo boreas*)	Occurs throughout the GYE?	Present at 2–5% of potential breeding sites	Disease?	Corn et al. 2005
Boreal Chorus Frog (*Pseudacris maculata*)	Occurs throughout the GYE	Present at 32–43% of potential breeding sites		Corn et al. 2005
Northern Leopard Frog (*Rana pipiens*)	A few sites south of Jackson Lake	Extirpated?	Unknown	Koch and Peterson 1995; Patla and Peterson 2004
Columbia Spotted Frog (*R. luteiventris*)	Occurs throughout the GYE	Present at 14–26% of potential breeding sites; declines of Lodge Creek populations	Development (road building, employee housing, spring diversion)	Patla 1997; Patla and Peterson 2004; Corn et al. 2005

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Figure 1. Tiger salamander (*Ambystoma tigrinum*) larvae (waterdogs) have been transported around the West as live bait. This practice may expose populations to novel pathogens.

salamander populations to novel virus strains (Jancovich et al. 2006). Live salamanders are used as bait mainly in warm-water fisheries and transmission of ranavirus via live bait is unlikely to be a problem in the GYE, but managers should be aware of the issue. Ranaviruses have been detected in tiger salamanders in the GYE, and may have been the cause of a mortality event in Columbia spotted frogs downstream of a sewage treatment plant near Fishing Bridge in 2002 (Patla and Peterson 2004).

Bd and Chytridiomycosis

The chytrids (Chytridiomycota) are an ancient group of saprophytic fungi, likely the sister group to all other true fungi. They cause a variety of important plant diseases and blights. Longcore et al. (1999) described *Batrachochytrium dendrobatidis* (Bd) as the chytrid responsible for chytridiomycosis in amphibians. There are two stages in the life cycle of Bd: a zoosporangium that invades the keratinized outer layers (epidermis) of frog skin, causing chytridiomycosis; and a flagellated zoospore, produced asexually in the zoosporangia and released through a characteristic discharge tube, which is the means of infecting other amphibians (Berger et al. 2005). The zoospore typically requires an aquatic environment, meaning that transmission of Bd is thought to occur mainly among tadpoles or adults in breeding aggregations. However, zoospores have survived up to three months in moist, sterile river sand and remained viable on feathers after one to three hours of drying (Johnson and Speare 2005). This suggests that a site may remain infective for a time in the absence of amphibians and, more importantly, that Bd could be transported among sites by birds or in sediments (e.g., mud on ungulate feet or fishermen's boots).

The mechanism by which chytridiomycosis kills its amphibian host is not yet known. Hypotheses include toxins released by the zoosporangia or disruption of the animal's ability to regulate body fluids and ion concentrations. Adult and metamorphic amphibians infected by Bd may show inflammation of the skin, particularly on the legs and pelvic region, frequent shedding, sometimes with a buildup of dead skin, and behavioral changes such as lethargy and loss of righting ability. Tadpoles lack keratin in their skin, and Bd infections are mainly found on their mouth parts (external tooth rows and jaw sheaths). Presence of Bd is associated with abnormalities of the oral disk and loss of keratinized parts in tadpoles of some species. Tadpoles infected with Bd do not appear to develop chytridiomycosis and usually metamorphose normally.

Bd is often highly virulent. In the laboratory, healthy individuals may die within a few days after being infected. However, in wild populations there may be a variety of outcomes from the presence of Bd. Depending on the biology of Bd, the biology of the host amphibian, and potential external factors, there may be no effect from the presence of Bd; infection and mortality of some individuals but no effect on persistence of the population; significant mortality with population crashes but development of resistance to Bd and subsequent recovery; or lasting declines and extinction (Daszak et al. 2003; DEH 2006b). Because of several examples of the latter scenario, chytridiomycosis is currently receiving the most attention among diseases as a cause of amphibian decline.

Chytridiomycosis is the likely cause of the extinction of at least one Australian frog, the sharp-snouted torrent frog (*Taudactylus acutirostris*), and possibly other Australian species now considered extinct, including the two gastric brooding frogs (*Rheobatrachus* sp.). Several other Australian frogs have been extirpated from significant portions of their ranges by chytridiomycosis but have not yet been driven to extinction (DEH 2006b). In Central and South America, up to 30 species of harlequin frogs (*Atelopus* sp.) are feared to be extinct, an additional 12 species have undergone declines of at least 50%, and only 10 of 113 species surveyed are thought to have stable populations (La Marca et al. 2005).

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Boreal chorus frog (*Pseudacris maculata*) in a wetland area near High Lake.

Closer to the GYE, the Wyoming toad (*Bufo baxteri*), a glacial relict species endemic to the Laramie Basin in southeast Wyoming, began declining around 1970 due to chytridiomycosis, and would certainly now be extinct without a captive breeding program begun in 1988 (Odum and Corn 2005). Boreal toad populations in southeast Wyoming, northern New Mexico, and throughout the mountains in Colorado have also been in severe decline for the last four decades. Chytridiomycosis is the likely cause and was associated with the collapse of one the few remaining robust populations in the mid-1990s in Rocky Mountain National Park (Muths et al. 2003).

The origin of chytridiomycosis is an interesting question that has implications for managing the consequences for amphibian populations. Bd may be a novel pathogen, recently evolved and spread around the world by human actions, or it may be an endemic pathogen that has been present but has undergone a recent increase in pathogenicity (Rachowicz et al. 2005). Genetic evidence and the apparent sudden outbreaks of chytridiomycosis on five continents within the last 30 years argue for the novel pathogen hypothesis (Daszak et al. 2003; Rachowicz et al. 2005; Lips et al. 2006). However, the presence of Bd over large areas and in species that have not declined (Daszak et al. 2005; Ouellet et al. 2005; Longcore et al. 2007) is more suggestive of an endemic pathogen. Pounds et al. (2006) hypothesized that climate warming was creating a more favorable environment for growth of Bd at middle elevations in the mountains of Central and South America and was responsible for the recent crash of harlequin frog species. Climate warming is a mechanism for the emergence of an endemic pathogen, but it could also be a synergistic factor in the spread of a novel pathogen. If Bd is a novel pathogen, efforts at control should emphasize limiting transmission into uncontaminated areas, but if Bd is endemic, then control requires dealing with the environmental factors affecting pathogenicity (Rachowicz et al. 2005). Either alternative is a huge challenge.

Chytridiomycosis in the GYE. Both Bd and chytridiomycosis have been recorded at several locations in the GYE. Columbia spotted frogs that died in 2002 near Fishing Bridge were diagnosed with chytridiomycosis in addition to the presence of ranavirus (Patla and Peterson 2004). Chytridiomycosis has also been detected in the spotted frogs studied by Debra Patla at Lodge Creek (David E. Green, USGS National Wildlife Health Center, Madison WI, unpublished data). PCR tests found Bd present on 12 of 17 live Columbia spotted frogs from Schwabacher Landing in Grand Teton National Park in 2004 (Spear et al. 2004), but necropsies of eight dead frogs from Schwabacher Landing failed to detect evidence of chytridiomycosis (D. E. Green, unpublished). Chytridiomycosis was detected in 2001 in a boreal toad from Nowlin Creek on the National Elk Refuge and from 6 of 13 toad carcasses from an oxbow of the Buffalo Fork (Figure 2) near the Black Rock Ranger Station, east of Moran Junction (Patla and Peterson 2004; D. E. Green, unpublished). Erin Muths (USGS Fort Collins Science Center, CO), David Pilliod (USGS Forest and Rangeland Ecosystem Science Center, Boise, ID), and I have been studying the boreal toads at Black Rock (Figure 3). This is a robust population, with at least 250 adult toads marked during each breeding season annually since 2003. The presence of Bd is robust in this population also. PCR testing each year of sub-samples of marked toads consistently yields high rates (up to 50%) of samples with Bd present. However, we have not seen any indications of mortality caused by chytridiomycosis since 2001.

Bd was studied in greater detail in Grand Teton National Park and the Rockefeller Parkway in 2004 by Spear et al.

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Figure 2. Oxbow pond near the Black Rock Ranger Station, Bridger Teton National Forest, Wyoming. Four species of amphibians breed at this site, and it is one of the most productive amphibian breeding ponds known in the GYE.

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Figure 3. Two male and one female boreal toad (*Bufo boreas*) in a mating ball at the Black Rock breeding site. Male toads in dense populations, such as the one at Black Rock, often encounter intense competition during breeding. Dense populations may provide greater opportunities for transmitting pathogens among hosts.

(2004). Boreal toads from breeding aggregations in beaver ponds at Schwabacher Landing and a gravel pit near Flagg Ranch had high incidence of Bd in late May (15 of 18 and 6 of 20, respectively). However, when radios were attached to 12 toads from Schwabacher Landing in mid July, Bd was present on only four (33%), and no Bd was detected when radios were removed in September. The seasonal variation in Bd infection and the apparent ability of individual animals to clear Bd infection have also been observed in Stony Creek frogs (*Litoria wilcoxii*) in Australia (Kriger and Hero 2006).

At least two hypotheses can be generated to explain the current coexistence of Bd and non-declining populations of boreal toads in the GYE. The strain of Bd present in the GYE might be less pathogenic than other strains of the chytrid. Alternatively, the relative scarcity of boreal toads and the effects of chytridiomycosis on toads farther south in the Rocky Mountains suggest the possibility that boreal toads in the GYE have already undergone a cycle of decline and recovery. We currently lack the data to distinguish between these hypotheses, but research is continuing. I am collaborating with Idaho State University faculty Sophie St.-Hilaire, Peter Murphy, and Chuck Peterson, and graduate student Sarah Bruer on a study in 2006 and 2007 (Figure 4) which is gathering further information on the distribution of Bd in Grand Teton National Park. It includes laboratory experiments to compare the pathogenicities of Bd cultured from the Black Rock site and a strain of Bd from Colorado known to be highly virulent. The results of this study should provide considerable insight into the magnitude of the threat from chytridiomycosis to amphibians in the GYE.

CHARLES R. PETERSON

Figure 4. Sophie St.-Hilaire (left) and Debra Patla, Idaho State University, collect amphibians at the National Elk Refuge to test for the presence of the chytrid fungus *Batrachochytrium dendrobatidis*.

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Columbia spotted frog (*R. luteiventris*).

Managing chytridiomycosis. The Australian government has prepared a threat abatement plan for dealing with chytridiomycosis (DEH 2006a) which recognizes that eradication of the disease is not currently possible. Instead, the plan focuses on control, based on the assumption that Bd is a novel pathogen. It emphasizes the need to limit the spread of Bd into uninfected areas and the need for additional research and monitoring. Unfortunately, beyond prohibiting transport of frogs known to be infected with Bd and mandating strict biosecurity measures for laboratories conducting research on Bd, it is not clear that methods to control the spread of Bd are effective. Field studies of amphibians are now conducted using methods intended to prevent the transport of pathogens among study sites (DEH 2006b). However, these procedures, which mainly involve washing and disinfection of equipment (waders, nets, etc.), are not used for other human activities, such as recreational boating or fishing, that are as likely as researchers to transport Bd among sites. If Bd is routinely transported by animals, then biosecurity measures imposed on humans are unlikely to have a significant effect on the spread of Bd.

If a species is declining toward extinction, captive breeding may be the only means of preservation in the short term (Mendelson et al. 2006). This solution is being pursued for boreal toads in Colorado and for the Wyoming toad. If the cause of the decline is chytridiomycosis and there is no potential site free of Bd for reintroducing the species, then it is difficult to be optimistic about the ultimate success of the effort. Surveys for the presence of Bd in the Rocky Mountains (E. Muths and D. Pilliod, unpublished data) have found it to be largely endemic. If captive breeding were to become necessary for any of the amphibians in the GYE, finding sites free of Bd for reintroduction may prove difficult. The current best alternatives for managing chytridiomycosis in the GYE are to continue to monitor the status and health of amphibian populations, refine our knowledge of the distribution of Bd, and continue research into the biology of Bd and its effects on amphibians.

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YS

Wolf Diseases in Yellowstone National Park

Douglas W. Smith and Emily Almberg

Agate Creek pack wolf with abnormal teeth characteristic of distemper.

NPS PHOTOS

Doug Smith and Debra Guernsey collect a blood sample during capture operations. Blood serum is used to check for exposure to diseases.

FORTY-ONE WOLVES were reintroduced to Yellowstone National Park (YNP) between 1995 and 1996. The population has since thrived, reaching a high of 174 wolves in 2003. In 2004, wolf numbers were similar (169), but in 2005 the population declined by 30%, to 118 wolves. This sudden population drop led park biologists to suspect disease as the cause, because population declines resulting from other causes are generally more gradual. Wolf numbers also declined in 1999, a year preceded and succeeded by years of rapid population growth fueled by abundant prey. Were these population declines caused by disease? Which diseases affect wolves in the park, and how do wolves contract them? How will diseases affect the wolf population in the future? Wolf Project staff hope to address these questions through wolf studies in the park, and they are the subject of Emily Almberg's PhD dissertation at the University of Minnesota.

Disease monitoring in park wolves to date has relied heavily on one technique: extracting and analyzing blood samples. Serum, the clear fluid that remains after blood clots, contains a record of diseases to which a wolf has recently been exposed. If a wolf survives an infection, the wolf's immune system produces antibodies that can be detected and measured in the serum. These antibodies are unique for each disease, and thus act as records of previous disease exposure. Extracting blood is a priority during annual capture operations, when Wolf Project staff handle 25–30 wolves as part of the collaring program. When feasible, staff also collect dead wolves and conduct necropsies in order to document disease and determine cause of death.

In 1999 and 2006, the Yellowstone Wolf Project sent approximately 222 serum samples, collected and banked from wolves captured since 1995, away for analysis. In 1999, we screened for four diseases: canine parvovirus, canine distemper virus, rabies, and *Brucella canis*, a type of brucellosis that affects canids. In 2006, we screened for parvovirus, distemper, and infectious canine hepatitis. For financial reasons, we decided not to screen for rabies and brucellosis again because the 1999 results did not show any exposure to those diseases. Over the years and throughout our collaring efforts, we have also been on the lookout for sarcoptic mange, a disease that is easy to identify during a physical examination of a wolf by its visible symptoms (e.g., characteristic hair loss).

These screenings yielded some interesting results. In addition to learning that rabies was not an issue for YNP wolves,

and that they did not appear to contract and spread canine brucellosis, we found that they were commonly exposed to parvovirus, distemper, and hepatitis. Seroprevalence (the proportion of positive test results for exposure to a given disease) was 100% for parvovirus, and nearly 100% for hepatitis; distemper seroprevalence was extremely variable and peaked in 1999 and 2005 (Figure 1).

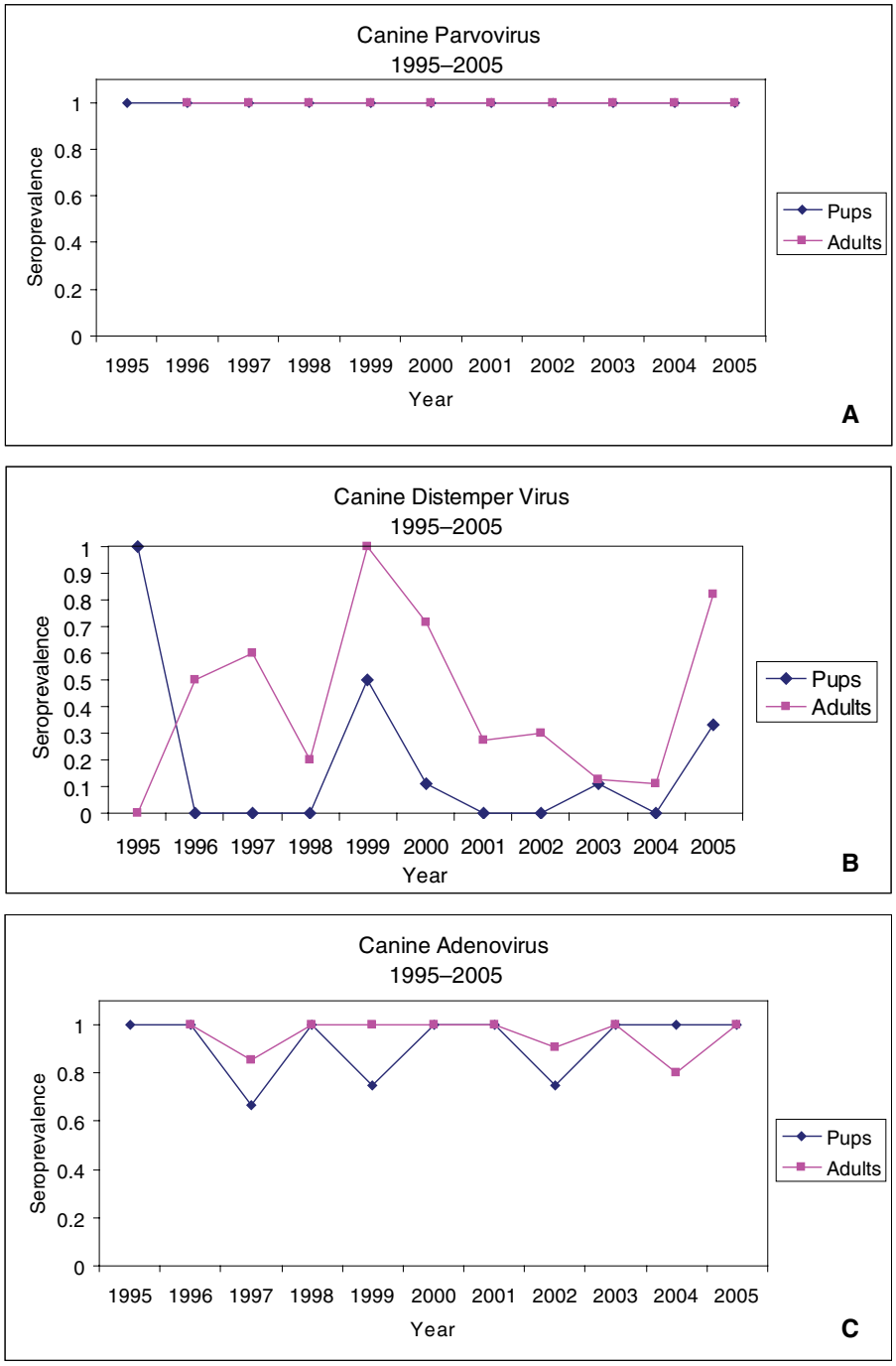


Figure 1. Seroprevalence of three canine diseases in Yellowstone National Park wolves. The vertical axis shows the proportion of animals that tested positive for exposure to parvovirus (A), distemper (B), and adenovirus (canine hepatitis) (C).

In 2005, Wolf Project staff initially suspected that parvovirus had been a factor in the population decline of Yellowstone wolves in both 1999 and 2005. Parvovirus is known to cause high pup mortality in domestic dogs and was suspected to be the cause of a significant wolf population decline on Isle Royale in 1980–82. In accordance with what we know about parvovirus, most of the mortalities that occurred during those two years were among pups. In 1999, we documented that only 21 of 43 pups born (49%) survived, and that in 2005, only 22 of 69 pups born (32%) survived. However, pups are among the most vulnerable members of the population to a large number of potentially lethal diseases, including distemper, canine herpesvirus, canine caronavirus, and a variety of intestinal parasites. Furthermore, the high and relatively constant exposure to parvovirus and hepatitis over time makes it difficult to draw any conclusions about observed patterns of pup mortality. Distemper, which appears to have peaked in prevalence in 1999 and 2005, may very well have been partially responsible for the high pup mortality.

During the 2005 capture season, we caught a wolf pup in the Agate Creek pack with extremely abnormal teeth. After consulting with several wildlife veterinarians, we concluded that the abnormalities were likely due to a previous distemper infection. Distemper can disrupt the production of enamel on the erupting teeth of young animals, and result in discoloration, pitting, or malformations of the teeth. However, a year later that pup is still alive and thriving in its pack. In early 2007, following a summer of high pup survival, we caught another wolf, probably a yearling, with teeth indicating distemper. It is unclear how long distemper can persist in the wolf population and why there are discrepancies in observed patterns of pup mortality. The tooth damage documented on the yearling caught in 2007 is much more severe than that of the pup caught in 2005, so we do not expect it to survive as long.

In 2006, 60 of 77 pups (78%) survived until winter. Some believe that high survival during non-outbreak years compensates for cases of sporadic, disease-induced mortality. However, only long-term monitoring of disease and survival will shed light on this question. This will be an important area of research in the future.

Although park policy dictates non-intervention unless an organism is deemed exotic or non-native to the park ecosystem, in many cases it is difficult to classify diseases as native or exotic. New strains of virus and bacteria are constantly evolving, and it is often difficult to know whether a particular strain is of "native" origin or introduced from domestic species. Distemper is extremely old and likely originated from the European continent, yet it has been circulating on the North American continent since at least the early 1800s. Parvovirus appears to have mutated from feline panleukopenia virus and was first discovered in wild and domestic canids in the mid-1970s.

NPS PHOTOS

Oxbow Creek pack yearling #588 with severe tooth damage indicating possible distemper.

Nine-year-old male wolf and former alpha of Mollie's pack with severe case of mange.

This wolf, also from Mollie's pack, has only minor evidence of mange but appears otherwise healthy.

Regardless of the classification, the practical considerations for managing parvovirus, hepatitis, or distemper are extensive. Disease management in Yellowstone wolves would require a thorough understanding of transmission dynamics, environmental reservoirs, and alternate hosts including coyotes, fox, weasels, badgers, and perhaps even cougars and bears. Vaccinations of pups at den sites would be intrusive, and the multiple doses and visits required would make it impractical.

Sarcoptic mange presents different considerations. Mange is caused by a mite that burrows into the skin and causes uncontrolled itching that leads to hair loss and secondary skin infections. Beause mange was intentionally introduced in the early twentieth century to reduce wolf and coyote populations, the park may consider future treatment for extreme cases of infection. In January 2007, we documented mange in a Yellowstone wolf pack for the first time. Mange has existed in many of the packs surrounding Yellowstone, but has remained outside of the park until recently. We believe the reason for this distribution pattern is that wolf movement has primarily been from inside the park to areas outside of the park; since 1995, we have documented 78 radio-collared wolves permanently dispersing from the park, but no radio-collared wolves from outside the park moving into and residing inside the park.

Mange has now been documented in two wolves in the Mollie's pack (see photos), a pack that lives in the interior of the park. The wolf with the more severe case is one of the oldest known animals in the population (9 years old). Another wolf exhibited only minor evidence of mange (see photo) but otherwise looked healthy (he's one of the largest wolves ever handled in the park). Researchers have recorded severe and persistent outbreaks of mange in the wolf population in Sunlight Basin, an area east of the park and not too distant from Pelican Valley. An uncollared wolf from Sunlight Basin could have moved into or spent time with Mollie's pack, or Mollie's pack could have contracted mange during a territorial foray to the east.

We will continue to monitor the Yellowstone wolf population with an awareness of disease as a potentially important factor in population dynamics. We hope to identify, describe, and monitor the diseases of importance for Yellowstone wolves, understand long-term patterns of disease as they relate to wolf survival and reproduction, and begin to understand the role of multiple hosts in the spread and persistence of diseases in the Yellowstone ecosystem.

YS

Douglas W. Smith is Yellowstone National Park's Wolf Project leader. He holds a PhD in Ecology, Evolution, and Conservation Biology from the University of Nevada at Reno. Smith has been with the Yellowstone Wolf Project from its beginning in 1994. **Emily Almberg**, a long-time employee on the Wolf Project, is a doctoral student at the University of Minnesota where she is exploring the role of diseases in wolf population ecology.

Brucellosis in Yellowstone Bison

Implications for Conservation Management

John J. Treanor, Richard L. Wallen, David S. Maehr, and Philip H. Crowley

NPS

Bison held at Stephens Creek capture facility in Yellowstone National Park prior to being shipped to slaughter during the winter of 2005–06.

WILDLIFE CONSERVATIONISTS have traditionally identified habitat loss as the primary cause of species decline. The expansion of humans into declining habitat has also resulted in an increasing number of infectious diseases shared by wildlife, domestic animals, and humans. Impacts to human health, resulting from animal diseases transmissible to humans, have become an additional obstacle to wildlife conservation. Because these emerging diseases pose a threat to public health, disease management efforts are largely focused on controlling infection and outbreaks in the wildlife hosts. The difficulty and cost of eradicating infectious agents from wildlife reservoirs have resulted in methods such as intensive culling practices that are largely unacceptable to the concerned public. This problem exposes a need for acceptable approaches that combine wildlife conservation with concerns over the health of humans and domestic animals. Suitable approaches, however, are often few and far between. In Yellowstone National Park (YNP), wildlife managers have been dealing with the disease brucellosis in YNP bison for decades. Brucellosis in YNP bison is a problem that demonstrates the challenges of addressing an infectious disease established in a wildlife species of conservation concern (Plumb et al. 2007).

In the early part of the twentieth century, brucellosis, a non-native disease, was discovered in YNP's bison population. Although the disease is not currently considered a threat to the long-term survival of the YNP bison herd, the risk of brucellosis transmission to cattle on lands adjacent to the park has been and continues to be a contentious issue. As a result, bison have been subjected to lethal control when they migrate beyond YNP's boundaries. Conflicts between state and federal agencies and public concern over the treatment of bison demonstrated the need for a comprehensive bison management plan. In 2000, Yellowstone National Park, U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service, USDA Forest Service, Montana Fish, Wildlife and Parks, and Montana Department of Livestock agreed to jointly implement the Interagency Bison Management Plan (IBMP) to "maintain a wild, free-ranging population of bison and to

address the risk of brucellosis transmission to protect the economic interests and viability of the livestock industry in the state of Montana" (NPS 2000).

The objective of the IBMP is not to eradicate brucellosis, but to manage the risk of transmission of brucellosis from bison to cattle. Controlling brucellosis transmission risk is implemented in three successive steps involving management at YNP's boundaries. Each step requires specific actions (expiration of cattle grazing leases, development of vaccination programs, and brucellosis research) that ultimately will result in allowing a limited number of untested bison outside YNP's boundaries. Boundary management is focused on preventing the commingling of bison and cattle. Under the IBMP, bison outside the park that cannot be hazed back across YNP's boundaries may be captured and tested for brucellosis. Positive reactions on blood tests result in those bison being shipped to slaughter. Brucellosis vaccination was identified as a way to control transmission risk by reducing infection within bison, which would also result in fewer test-positive bison shipped to slaughter.

Immunization is an attractive conservation approach for addressing the brucellosis problem. Any vaccination program for wild bison will require an understanding of the associations between the disease pathogen and the host. A vaccination program also requires an efficient delivery method for a safe and effective vaccine, as well as accurate diagnostics for measuring the program's effectiveness. This paper summarizes the relevant information on brucellosis in bison and how this information may aid in implementing the IBMP.

Background: Brucellosis in YNP Bison

The disease brucellosis in YNP bison is caused by *Brucella abortus,* a bacterial organism transmitted through ingestion of infected birth tissues or infected milk. *B. abortus* is not native to North America and was most likely introduced to YNP bison by European cattle. The disease was first detected in YNP's bison herd in 1917 and is not considered a major factor regulating bison abundance (Figure 1). *B. abortus* is usually found in the reproductive system prior to being shed into the environment. Both male and female bison can become infected, but brucellosis transmission appears to depend exclusively on females. The shedding of *B. abortus* occurs during infectious births and abortions. These events attract other bison in the herd, resulting in disease transmission when they come in contact with infected tissues.

Brucellosis transmission usually follows two events: 1) *B. abortus* is shed via infectious births or abortions, and 2) susceptible individuals consume infectious material. A key component of transmission is the number of exposures that

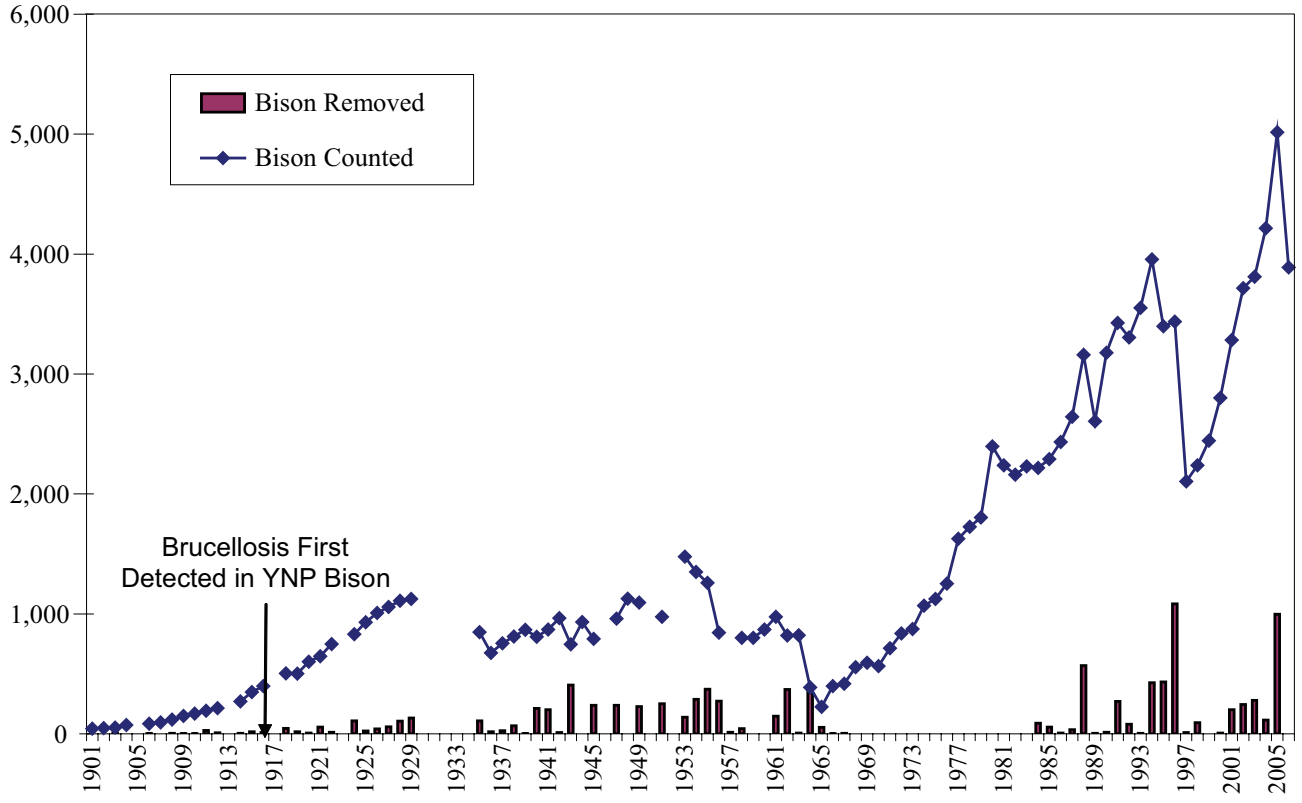


Figure 1. Bison population estimates and management removals since 1901. The YNP bison population has steadily increased despite brucellosis and management removals.

NPS/JENNY JONES

Bison cow with newborn calf and afterbirth along the Madison River.

occur during each infectious event. This depends partly on the behavior of the cow at the time of parturition (the act of giving birth). Bison may or may not calve or abort in close proximity to other group members. It is the behavior of the birthing cow and the proportion of susceptible neighbors that determines the likelihood of transmission. Interactions of herd members with the newborn calf, including licking and nudging, have been observed in YNP bison (Aune et al. 1998). Bison social behavior largely contributes to within-group transmission and, hence, to the maintenance of *B. abortus* within the herd. This oral route of exposure is believed to be the most important method of transmission.

B. abortus also infects bison mammary glands and can be transmitted from bison cow to calf through infected milk (Olsen and Holland 2003). Although this route of transmission has been documented in experimental studies, milk infection in wild YNP bison is estimated to be rare (Rhyan 2000). Brucellosis testing on bison captured outside the park indicates that bison encounter *B. abortus* early in their lives (Figure 2). Curiosity of young bison drawn to births and abortions may influence contact with *B. abortus* and explain the high level of young infected. Identifying all the routes of brucellosis transmission is essential for understanding how the disease is maintained within the bison population. Any effort to reduce brucellosis prevalence in YNP bison will require understanding the events leading to transmission and infection.

B. abortus succeeds by hiding from its host's immune system. Brucellae are intracellular pathogens that persist in white blood cells of the host. Infected white blood cells provide protection for the bacteria to replicate prior to being shed during reproductive events. *Brucella* bacteria also replicate in placental cells during the middle and late stages of host gestation. This incubation period, the time between the entry of the pathogen in the host and first expression of infection, complicates the identification of infected bison because standard tests are not available to detect *B. abortus* during early infection.

Following extensive replication in placental cells, *B. abortus* induces the synthesis of specific hormones, thereby creating conditions within the host that mimic those present at the initiation of parturition. The resulting abortions and premature births are highly infectious because of the large number of bacteria on the aborted fetus, placenta, and birth fluids.

The pathogen's ability to persist undetected by the host's immune system results in a class of individuals called latent carriers. Latently infected bison are problematic for disease management because infected immature animals can initially test negative but shed *B. abortus* when reproductively mature. Female bison are assumed to abort their pregnancy at a high rate subsequent to infection. Steve Olsen and colleagues from USDA found that 96% (26 of 27) of non-vaccinated bison experimentally challenged with *B. abortus* aborted their pregnancy. Some bison may eventually clear the bacteria and no longer be infectious, but latency can extend beyond the abortive stage of infection and infected animals may shed bacteria during future pregnancies.

Recrudescence (the relapsing of latent animals to the infectious state) is a concern with YNP bison, and makes effective control difficult. The rates of recrudescence are not known for bison, but may be important for understanding how *B. abortus* is maintained in the population. It is the combination of the bacteria's ability to persist undetected for long periods (latency) and then rapidly replicate during the favorable conditions of late pregnancy that leads to the state of chronic infection observed in the YNP bison population.

B. abortus can persist in young animals until reproductively mature, as well as in mature animals that have shown symptoms of infection. In YNP bison, chronic infection results in an unknown proportion of infected bison shedding *B. abortus*

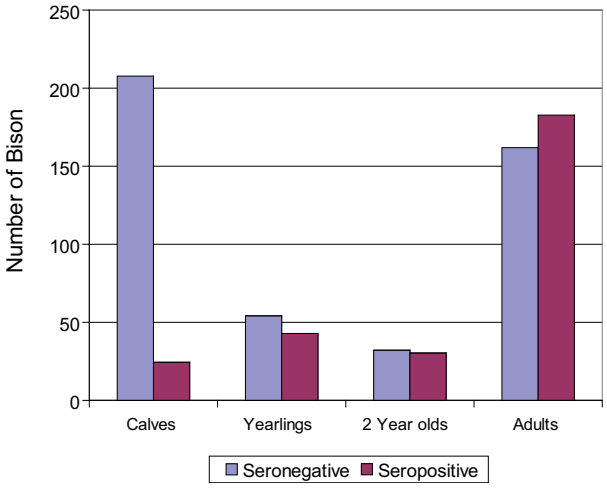


Figure 2. Brucellosis seroprevalence in bison age classes. A high proportion of bison show an antibody response to brucellosis in early age classes. Total bison of known age classes removed during the winter of 2005–06 was 736.

in a given year. Yearly reactivation of infection within a portion of latent carriers creates a state of chronic infection where the proportion of infected females is always greater than the number shedding bacteria (Cheville et al. 1998). The inability of some bison to completely recover from brucellosis complicates diagnoses and, consequently, identifying the state of infection for the entire herd.

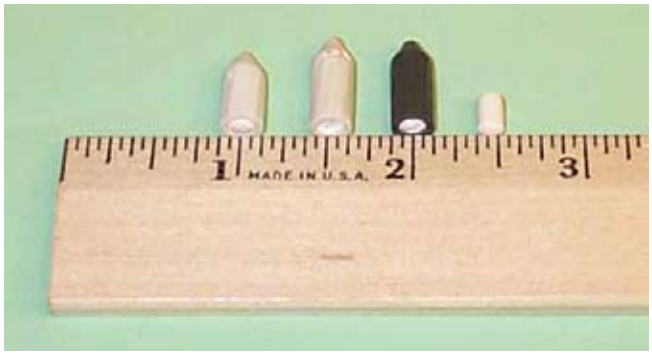
NPS

Remote vaccination delivery system for ballistic vaccination.

Diagnosis

Blood tests provide indirect evidence of infection because they detect antibodies (responses to infection), not living bacteria, in serum. However, such serologic tests are not sensitive enough to detect low levels of antibodies in the early stages of infection (Cheville et al. 1998); negative-testing animals may be infected (false negatives). Likewise, antibodies can be long-lived, producing positive test results in otherwise recovered bison (false positives).

Bacterial cultures are better for identifying infected animals, but require live bacteria collected from infected tissues. The ability of *B. abortus* to persist in small numbers obscures diagnosis. Culture tests examine specific tissues collected primarily from dead animals and test results can take longer than a week to obtain. For this reason, culture analyses cannot be used in the field when rapid diagnoses are needed. Culture tests, however, can be used to estimate the reliability of serology results when both tests are done on the same animals. Roffe et al. (1999) were able to culture *B. abortus* in almost half (46%) of seropositive female bison. Because serologic tests can be misleading, there is a need for reliable culture data to validate serologic testing. Improving diagnostic tests is essential for implementing a vaccination program. Identifying infected bison allows for monitoring the success of managing the risk of brucellosis transmission.



NPS

Biobullets used for delivering the encapsulated vaccine.

Reducing Brucellosis Infection

The available vaccine for YNP bison is a live, attenuated vaccine called Strain RB51 derived from a virulent strain of *B. abortus*. Attenuation is a process in vaccine development that reduces virulence, the ability of *B. abortus* to cause disease, while still producing an immune response in the bison host. Immunizing bison with this vaccine imitates the processes of natural exposure and the development of antibodies. Vaccination operates by stimulating the immune system and thus preparing it for future exposure. As more bison are immunized, the reduction in the number of susceptible individuals leads to a decrease in transmission. SRB51, the official livestock brucellosis vaccine for cattle in the U.S., provides effective protection for cattle, but there have been conflicting experimental results regarding its effectiveness in bison.

The efficacy of SRB51 is determined by its ability to provide protection from infection and abortion following experimental exposure with *B. abortus*. SRB51 has been demonstrated to offer protection from abortions, placental infection, and transmission to calves via infected milk (Olsen et al. 2003). However, Davis and Elzer (1999) concluded that SRB51 did not confer significant protection in vaccinated bison despite intensive vaccination efforts. Discrepancies between the two studies' results are indicative of the uncertainty in the level of protection offered by SRB51, and further research is needed to address these uncertainties.

The safety of SRB51, defined as its influence on host survival and reproductive potential, has also been addressed in experimental studies that have presented differing results. Palmer et al. (1996) demonstrated that SRB51 has an affinity for placental tissues and can induce abortions in pregnant bison. Davis and Elzer (1999), however, found multiple infections of SRB51 to be safe in pregnant bison with no abortions observed. A more recent study found that bison vaccinated with SRB51 during calfhood may be safely booster-vaccinated during their first pregnancy (Olsen and Holland 2003). Vaccinating pregnant bison presents a safety concern, but these experimental studies suggest that appropriate vaccination strategies can be designed to avoid harmful effects to the population.

Despite discrepancies that underscore the uncertainty in the effectiveness and safety of the vaccine, SRB51 can be

distinguished from field strain *B. abortus*. This important characteristic of the vaccine allows managers to monitor vaccination status in free-ranging bison. Vaccination with SRB51 does not cause bison to test positive on standard serologic tests, although antibody responses specific to SRB51 can be detected with the appropriate analysis (Olsen et al 1998).

Simply identifying whether bison have received the vaccine does not guarantee that they will be protected from subsequent infection throughout their lives. The duration of protection provided by a single SRB51 dose is unknown, but, because of the longevity of female bison, cows may need booster vaccinations (Olsen and Holland 2003). For YNP bison, this creates the need to develop an effective remote delivery system that can be used on many animals each year.

Vaccine delivery is a challenge in free-ranging wildlife. An additional benefit of SRB51 is that it can be packaged into biodegradable projectiles (biobullets) and delivered to free-roaming YNP bison using an air rifle. The immune responses to SRB51 encapsulated biobullets appear similar to hand injections (Olsen et al. 2006). This makes remote vaccine delivery an attractive strategy for immunizing YNP bison because it does not require capture and handling of animals. Vaccine encapsulated biobullets are manufactured in a lab, which eliminates handling live vaccine for dart or syringe delivery. The biobullet is delivered into the muscle tissue where it breaks down and no delivery vessel is left behind. This remote delivery approach provides safety to staff handling the vaccine and would be largely unnoticed by park visitors.

Conclusion

The threat of diseases that can infect humans, domestic animals, and wildlife is a recurring challenge for wildlife conservation. Human and livestock health concerns often result in conflicts with conservation efforts. Although brucellosis has not regulated the growth of the bison population, disease risk management remains an important issue to resolve. Social and economic concerns require an acceptable solution to reduce brucellosis infection. In the past, YNP bison have been an important source for bison conservation efforts throughout North America. Today, YNP bison represent the largest wild and genetically important source population for conservation of the species. In order to use this valuable resource for future conservation of bison, brucellosis will have to be substantially reduced. Vaccination appears to be a promising alternative to large-scale culling of infected animals. By identifying the processes leading to infection, we increase our understanding of brucellosis dynamics in the population. This knowledge will aid in developing an effective immunization strategy for reducing brucellosis infection and increase the likelihood of a lasting solution.

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John Treanor (above left) is a biologist in YNP's bison ecology and management office and a doctoral student at the University of Kentucky. John is studying nutritional aspects of brucellosis dynamics in Yellowstone bison and modeling vaccination strategies. **Rick Wallen** (above right) is the team leader for YNP's bison program and has been involved with ungulate management projects in Grand Teton, Redwood, and Bryce Canyon national parks prior to coming to YNP. **David S. Maehr** is professor of conservation biology at the University of Kentucky, where he studies the ecology, conservation, and restoration of large vertebrates. **Dr. Phil Crowley** is an evolutionary ecologist and mathematical biologist who has worked on a diverse array of research systems and questions. He especially enjoys graduate training and collaborating on modeling projects.

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Whirling Disease and Native Cutthroat Trout of the Yellowstone Lake Ecosystem

Todd M. Koel, Daniel L. Mahony, Kendra L. Kinnan, Charlotte Rasmussen, Crystal J. Hudson, Silvia Murcia, and Billie L. Kerans

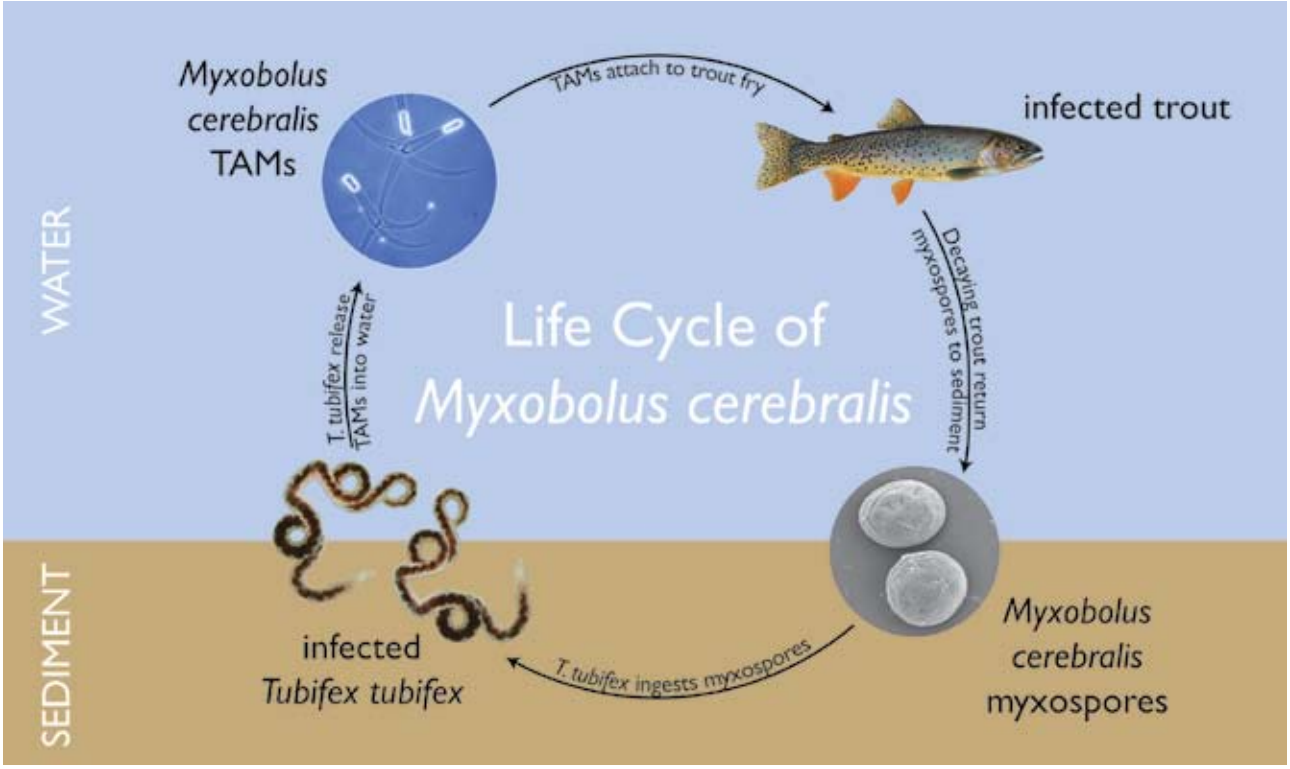

Figure 1. The life cycle of *Myxobolus cerebralis*, the causative agent of whirling disease in fish of the family Salmonidae. *M. cerebralis*, a microscopic parasite, infects two hosts, including native Yellowstone cutthroat trout, and *Tubifex tubifex*, a common aquatic worm found in the park. The primitive life forms of *M. cerebralis* include a triactinomyxon (TAM), which is a relatively fragile, free-floating form carried by water currents, and a myxospore, which is a highly resistant form that may remain viable within sediments of aquatic systems for decades. (Adapted from M. El Matbouli et al., 1992, *Annual Review of Fish Diseases* 3:367–402; TAM and myxospore images by Ron Hedrick, University of California–Davis, *Tubifex tubifex* photo by Kendra Kinnin.)

WHIRLING DISEASE, caused by the exotic parasite *Myxobolus cerebralis*, is responsible for severe declines in wild trout populations in the Intermountain West (Bartholomew and Reno 2002). In Colorado (Nehring and Walker 1996) and other states where infection has been severe, whirling disease has had a significant negative economic impact on the recreational fishing industry. In Montana, the number of wild rainbow trout (*Oncorhynchus mykiss*) in the Madison River declined 70–90% after the introduction of *M. cerebralis* (Vincent 1996). The parasite has spread to many other drainages in the western part of the state, resulting in population-level effects (E. R. Vincent, personal communication). The parasite was first documented in Wyoming waters in 1988 and has spread to at least seven river drainages there.

In Yellowstone National Park (YNP), examination of wild trout for whirling disease began in earnest in 1995 through the U.S. Fish and Wildlife Service's Wild Fish Health Survey. *Myxobolus cerebralis* was first detected in the park in 1998 in native Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvierii*) collected from Yellowstone Lake. The Yellowstone cutthroat trout is considered a keystone species in the Greater Yellowstone Ecosystem. It provides a significant source of protein for the grizzly bear (*Ursus arctos*) during the spring and midsummer (Reinhart and Mattson 1990; Gunther 1995). The diet of the threatened bald eagle (*Haliaeetus leucocephalus*) in the park consists of about 25% fish (Swenson et al. 1986).

Many other avian and terrestrial species in the Yellowstone Lake ecosystem use Yellowstone cutthroat trout as an energy source (Schullery and Varley 1995).

The life cycle of *M. cerebralis* involves two hosts, including fish from the family Salmonidae and an aquatic worm (the oligochaete *Tubifex tubifex;* Wolf et al. 1986; Gilbert and Granath 2003; Figure 1). Myxospores within the infected salmonid become available and are ingested by *T. tubifex* upon a fish's death and decay. Within *T. tubifex*, the parasite proliferates and assumes a second form, known as a triactinomyxon (TAM). The TAMs are released by tubificids into the water column where they suspend and are carried by the current. Upon contact with a salmonid, the TAMs attach and infect them, completing the parasite's life cycle. Compared to other species, Yellowstone cutthroat trout appear to be highly susceptible to whirling disease when challenged with triactinomyxons in the laboratory (Hedrick et al. 1999) and in the field (Murcia et al. 2006). Recent studies have indicated that whirling disease susceptibility of *T. tubifex* varies among genetically distinct strains (Kerans et al. 2004). However, no previous studies have examined the genetic composition of *T. tubifex* in the park, but samples from the Madison River in Montana indicated the presence of a clade that is moderately susceptible to the transmission of whirling disease (Kerans et al. 2004).

The waters of YNP provide a unique opportunity to study whirling disease in native cutthroat trout. The spawning streams vary widely in their thermal, hydrological, and geological characteristics within a relatively undisturbed region that is free from the confounding effects of land use. We hypothesized that *M. cerebralis* infection prevalence and severity in the upper Yellowstone Lake basin (above the upper falls of the Yellowstone River), would be related to Yellowstone cutthroat trout life history strategies; the presence, abundance, and infection of tubificid oligochaetes (worms); and stream environmental gradients. The overall goal of this study was to describe patterns in infection risk of Yellowstone cutthroat trout. Specific objectives were to (1) determine the prevalence and spatial extent of *M. cerebralis* infection in Yellowstone cutthroat trout within Yellowstone Lake, (2) assess the *M. cerebralis* infection risk of age-0 Yellowstone cutthroat trout in spawning tributaries, (3) determine the relative abundance, phylogeny, and *M. cerebralis* infection of tubificid oligochaetes in spawning tributaries, and (4) relate the *M. cerebralis* infection risk to basic environmental characteristics of spawning tributaries. Improving our understanding of relationships among whirling disease infection and ecological factors will allow resource managers to focus efforts and funding on waters that have high disease potential.

Study Area

At an elevation of 2,357 m, Yellowstone Lake is the largest high-elevation lake in North America. Yellowstone cutthroat trout of the upper Yellowstone Lake basin primarily exhibit

Figure 2. Map of Yellowstone Lake and the upper Yellowstone River drainage within Yellowstone National Park, showing the locations of the 11 cutthroat trout lake gill-netting sites, the 12 streams, and the Yellowstone River near Fishing Bridge, where Yellowstone cutthroat trout sentinel fry exposure and tubificid studies were conducted, 1999–2001.

an adfluvial life history strategy (Gresswell and Varley 1988), although other movement patterns exist (Kaeding and Boltz 2001). Spawning has been documented in 68 tributaries, but 16 of them are used only during years with above-average stream discharge (Jones et al. 1987; Gresswell et al. 1997). Many tributary basins have been influenced by natural fire disturbance (Farnes 1996), potentially influencing their suitability for *M. cerebralis* through nutrient (nitrogen and phosphorus) enrichment (Brass et al. 1996; Robinson and Minshall 1996) or changes in retention of organic matter (McIntyre and Minshall 1996).

Methods

Infection prevalence in Yellowstone Lake. Juvenile and adult Yellowstone cutthroat trout were collected from 1999 to 2001 using gill nets set overnight in September at 11 sites located throughout the lake in waters 2–6 m deep (Figure 2; Koel et al. 2005). Yellowstone cutthroat trout that were incidentally killed during gill netting for lake trout in waters primarily 45–50 m deep were also examined for *M. cerebralis* (Bigelow et al. 2003). Each Yellowstone cutthroat trout was screened by the pepsin–trypsin digest (PTD) method for the presence of myxospores (Andree et al. 2002). Because another *Myxobolus*

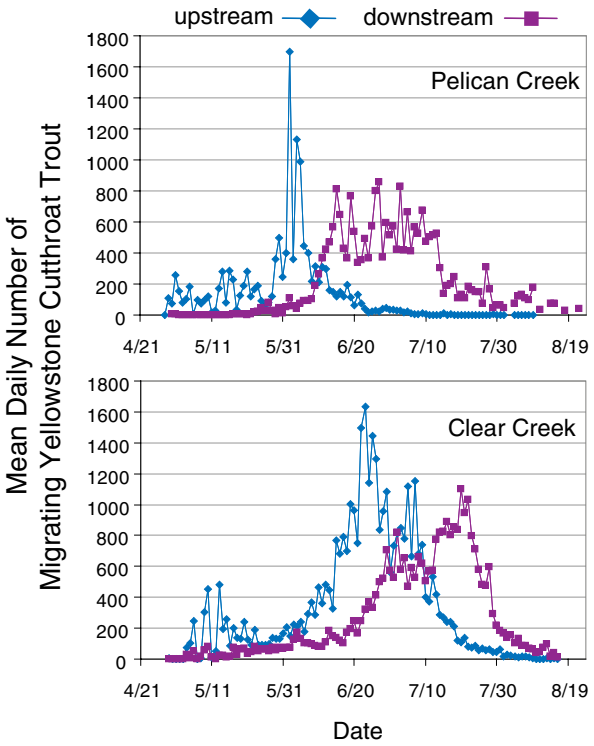

Figure 3. The mean daily number of Yellowstone Lake adfluvial Yellowstone cutthroat trout that were enumerated at migration traps while moving upstream or downstream in Pelican Creek (1964–1983) and Clear Creek (1977–2001).

species is also known to infect Yellowstone cutthroat trout, if *M. cerebralis* was suspected by PTD screening, the spore digest suspension was used for confirmation of *M. cerebralis* by the nested polymerase chain reaction (PCR) technique (Andree et al. 1998).

Infection risk in tributary streams. Information on basin size, aspect, slope, precipitation yield, stream order and length at specific elevations, geological characteristics, and forest composition was compiled for 54 known Yellowstone cutthroat trout spawning tributaries to Yellowstone Lake. We conducted principal components analysis (PCA; Krebs 1999) to determine similarities among spawning stream watersheds and selected 12 tributaries and the Yellowstone River near the lake outlet (Figure 2) to best represent the ranges in large-scale environmental gradients (including temperature and flow), given the logistical challenges of conducting sentinel fish exposures in remote areas of the Yellowstone Lake basin.

The daily mean numbers of upstream- and downstream-migrating Yellowstone cutthroat trout spawners were compiled for Pelican and Clear creeks from National Park Service historical records (migrating Yellowstone cutthroat trout were counted daily at fixed weirs located near the stream mouth during 1964–1983 at Pelican Creek and 1977–2001 at Clear Creek; Figure 3). Potential fry emergence dates were estimated based on known incubation periods for Yellowstone cutthroat trout at various temperatures. Sentinel fish exposure periods from mid-July through mid-October were selected to encompass the times when the fry would be emerging and vulnerable to *M. cerebralis* infection in tributary streams.

Sentinel cage exposures were conducted on the selected streams during 1999–2001 (Figure 2) using cylindrical enclosures constructed of 5-mm galvanized wire mesh. Yellowstone cutthroat trout fry (60–80 per cage) obtained from the Wyoming Game and Fish Department broodstock (LeHardys Rapids, Yellowstone River origin) were exposed during 10-day periods within each study stream starting after peak flows and spawning. After the exposure periods, the fry were maintained in aquaria at 138°C for 90 days (El-Matbouli et al. 1999) and then lethally sampled after anesthetization. Half of the head of each fish was preserved for histological analysis to describe pathology associated with the presence of *M. cerebralis* in cranial cartilage (Baldwin et al. 2000). The other half was screened for *M. cerebralis* following procedures similar to those used for the fish sampled from Yellowstone Lake (described above).

Tubificid and actinosporean examination. Each exposure stream was sampled for live tubificids three times during 2001. To the extent possible, collections occurred when Yellowstone cutthroat trout fry were being held in sentinel cages. Oligochaetes were collected by sieving sediments within 30 m upstream of each cage location. If an oligochaete was detected within one hour, the collection effort would persist for one additional hour or until 300 oligochaetes were collected, whichever occurred first. If no oligochaetes were detected within one hour, a 30-m reach downstream of the sentinel cage was sampled.

The collected oligochaetes were examined under a microscope. Those with an external morphology similar to that of *T. tubifex* (with hair chaetae; Kathman and Brinkhurst 1998) were placed into wells of tissue culture plates and periodically examined for seven days for actinospore production. (Actinospores include triactinomyxons and other kinds of parasitic spores released by aquatic invertebrates.) Tubificids that produced actinospores, the actinospores themselves, and randomly selected non-actinospore-producing tubificids from each collection site were prepared for DNA extraction and *M. cerebralis* PCR analysis.

Environmental characteristics of tributaries. In 2001, water temperatures were recorded hourly to determine daily and seasonal thermal regimes near the sentinel cages of each exposure stream. Recent and historical hydrological characteristics of the Yellowstone Lake basin were assessed based on information provided by the U.S. Geological Survey stream discharge gauge on the Yellowstone River downstream of the lake outlet. Habitat assessments were conducted by assessing the relative quantity and quality of natural structures that could provide ecological niches (Barbour et al. 1999).

Water samples were collected from the lower reaches of all exposure streams during July and September 2001. Analyses to determine nutrient and other chemical characteristics of waters

were conducted by the Great Lakes Water Center, University of Wisconsin–Milwaukee. Measurements of dissolved oxygen concentration, percent oxygen saturation, and specific conductance were collected at each sentinel cage site during the exposure periods in 2001.

Results

Infection prevalence. Of the 453 juvenile and adult Yellowstone cutthroat trout collected from within Yellowstone Lake by gillnetting in 1999–2001, 89 were infected by *M. cerebralis*. In general, these infected fish showed no significant external signs of disease and otherwise appeared healthy.

Yellowstone cutthroat trout fry exposed in spawning tributaries that tested positive for the presence of *M. cerebralis* were first obtained from the Yellowstone River in August 1999 (Table 1). None of the other 12 exposure streams examined that year showed evidence of the parasite. In 2000, *M. cerebralis* was found in Pelican Creek (strong infection) and Clear Creek (weak infection). Infection was not found in fish in the Yellowstone River or the other four streams tested that year, even though multiple exposure periods were used in an attempt to span peak infection periods. The 2001 results provided further evidence of a severe infection in Pelican Creek; infection was found during all exposure periods and the fry showed clinical signs of the disease. All of the fish exposed during the mid-July period were infected; the mean histological ranking of severity was 4.00 (maximum of 5.00) on the MacConnell–Baldwin scale (Table 1). A weak infection was found in the Yellowstone River, but none of the other streams examined in 2001 showed evidence of the parasite.

Tubificid and actinosporean examination. A high number of tubificids was found in Beaverdam, Sewer, and Little Thumb creeks and Creek 1167, especially in late August and early September of 2001. Few of the 3,037 collected tubificids were sexually mature, making morphological identification difficult. The mature oligochaetes with hair chaetae were identified as *T. tubifex*, *Ilyodrilus templetoni*, and individuals of the genus *Rhyacodrilus*. The 17 mature *T. tubifex* were found in three geographically distant streams (Pelican and Beaverdam creeks and Creek 1167; Figure 2).

Only 20 of the collected tubificids produced actinospores during the 7-day observation periods. Arnica Creek exhibited the highest prevalence of infection: 7.50–9.43% of the 93 observed tubificids (Table 2). Repeated nested PCR assays did not detect *M. cerebralis* in any of the infected or immature worms or any actinosporean preparations. (We were able to detect *M. cerebralis* in actinosporean-producing

Stream	Year	Period	Dates	Prev (%)	Severity
Pelican Creek	2000	1	09/12–09/23	94	2.76
	2001	1	07/12–07/23	100	4.00
		2	08/07–08/17	75	1.00
		3	08/29–09/07	94	2.72
Clear Creek	1999	1	08/12–08/23	0	0.00
	2000	1	09/12–09/23	2	0.02
		2	09/25–10/05	0	0.00
		3	10/09–10/19	0	0.00
	2001	1	07/12–07/23	0	0.00
		2	08/07–08/17	0	0.00
		3	08/29–09/07	0	0.00
Yellowstone River	1999	1	08/12–08/23	14	0.20
	2000	1	09/12–09/23	0	0.00
		2	09/25–10/05	0	0.00
		3	10/09–10/19	0	0.00
	2001	1	07/14–07/23	20	0.40
		2	08/07–08/17	7	0.07
		3	08/29–09/07	0	0.00

Table 1. Results of sentinal fry exposure studies from streams in which Yellowstone cutthroat trout fry tested positive for *Myxobolus cerebralis* during 1999–2001. Prevalence (prev) is the proportion of individuals examined that were infected. Severity is the average histological score from laboratory examination and is based on a scale of 0–5 (5 = the most severe infection). Pelican Creek was not tested in 1999. A single exposure period occurred on all tested streams in 1999 and on Pelican Creek in 2000.

Orientation Stream	Tubificids to cage	Producing per hour	Number (%) of Tubificids: Observed	*Mc* positive Actinospores	by PCR
Pelican Creek	upstream	94.5	189	0	1
	downstream	28.0	28	0	0
Clear Creek	upstream	0.0	0	0	0
	downstream	0.3	1	0	0
Beaverdam Creek	upstream	259.0	777	1 (0.13)	0
	downstream	ns	ns	ns	ns
Creek 1111	upstream	13.3	40	0	0
	downstream	ns	ns	ns	ns
Creek 1138	upstream	84.3	253	0	0
	downstream	ns	ns	ns	ns
Creek 1158	upstream	47.0	141	0	0
	downstream	ns	ns	ns	ns
Sewer Creek	upstream	0.0	0	0	0
	downstream	200.0	200	1 (0.50)	0
Creek 1167	upstream	139.0	278	1 (0.36)	0
	downstream	132.0	264	0	0
Little Thumb Creek	upstream	40.0	80	1 (1.25)	0
	downstream	196.0	392	1 (0.26)	0
Arnica Creek	upstream	17.7	53	5 (9.43)	0
	downstream	40.0	40	3 (7.50)	0
Bridge Creek	upstream	61.3	184	6 (3.26)	0
	downstream	51.0	51	0	0
Hatchery Creek	upstream	0.0	0	0	0
	downstream	10.0	10	0	0
Yellowstone River	upstream	17.0	34	1 (2.94)	0
	downstream	22.0	22	0	0
Total			**3,037**	**20**	**1**
Mean		**64.6**	**38**	**1**	**0**

Table 2. Numbers of tubificids with hair chaetae selected from bulk live oligochaete samples taken near Yellowstone cutthroat trout cage sites over three time periods and observed for actinospore production for seven days. Areas not sampled are indicated "ns". Triactinomyxon-type actinospores were produced by all infected tubificids except those isolated from Beaverdam Creek, which produced synactinomyxon-type actinospores. The diagnostic *Myxobolus cerebralis* (*Mc*) nested polymerase chain reaction test was used to assay for *Mc* infection.

tubificids collected from other *M. cerebralis* endemic areas and in our positive plasmid controls.) However, one sexually mature *T. tubifex* collected from Pelican Creek in early July that was not shedding triactinomyxons tested positive for *M. cerebralis* by PCR analysis, indicating the presence of infected worms in that stream. The mature *T. tubifex* were most abundant in early summer and genetically homogenous, belonging to an mtDNA lineage that has been associated with high levels of whirling disease (Beauchamp et al. 2002).

Discussion

Biological aspects of* Myxobolus cerebralis *infection risk. The Yellowstone cutthroat trout fry in exposure cages in Pelican and Clear creeks and the Yellowstone River were infected by *M. cerebralis* during at least one exposure period. Whereas the infections at the Yellowstone River site (1999 and 2001) and Clear Creek (2000) were relatively light, the fish exposed at Pelican Creek were severely infected and showed clinical signs of whirling disease in laboratory aquaria. These streams are located along the north and east-central shores of Yellowstone Lake; the other exposure streams tested negative for *M.*

cerebralis. The higher infection prevalence in the northern and central sections of the lake in 1999 may have been due to Pelican Creek and, to a lesser extent, the Yellowstone River and Clear Creek as sources of *M. cerebralis*.

Only two of the 89 infected fish detected during the 1999–2001 study period within Yellowstone Lake were found in 2001; the reason for this significant temporal variation is not known. Our results suggest at least some resilience of this cutthroat trout subspecies to whirling disease; a significant number have evidently been surviving and recruiting to the spawning population even though infected (perhaps at an older age) by *M. cerebralis*. Population-level declines have only recently been noticed, and it is likely that *M. cerebralis* has only recently invaded this system. A serious concern is the potential for this parasite to increase in its prevalence, further diminishing the ability of Yellowstone cutthroat trout to survive to spawning age.

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Fisheries technician Scott Favrot checks a sentinel cage holding cutthroat trout fry on Clear Creek. After exposure to the creek for 10 days, the fry were examined for *Myxobolus cerebralis.*

Recent studies in Idaho and Colorado have suggested a relationship between infection risk in salmonids and the abundance of *T. tubifex* (Hiner and Moffitt 2001; Nehring and Thompson 2003) and between infection risk and the density of infected worms (Krueger 2002). However, our results from Pelican Creek may indicate that even low numbers of tubificids can support severe infection of native Yellowstone cutthroat trout. Alternatively, the distribution of infected tubificids may be clumped or the infection source may exist some distance upstream from our exposure site. The finding of an infected tubificid from Pelican Creek that belongs to an mtDNA lineage associated with high salmonid infection levels in whirling disease endemic regions (Beauchamp et al. 2002) is consistent with severe infection rates in Pelican Creek.

Actinosporean production was low among streams except Arnica Creek, where prevalence was relatively high at 7.5–9.4% of the 93 tubificids observed. Low infection rates have also been reported in Montana (2.6%, Rognlie and Knapp 1998) and Colorado (0.4–1.5%, Beauchamp et al. 2002). None of the actinosporeans examined by this study tested positive for the presence of *M. cerebralis* genes. The stocking of non-native fishes early in the history of Yellowstone National Park (Varley 1981), before *M. cerebralis* was introduced in the United States, could have contributed to the introduction of relatively unknown myxozoans.

Environmental aspects of* Myxobolus cerebralis *infection risk. Pelican Creek, where infection was most severe, is a fourth-order stream and the largest tributary to Yellowstone Lake in terms of stream length (53.5 km), total drainage size (17,656 ha), and precipitation yield. It also has more length at lower elevations (<2,396 m) than the other exposure streams. Chemical analysis of surface waters indicated that Pelican Creek generally had much higher concentrations of ammonium, chloride, sulfate, and phosphorus than did the other streams, suggesting that Pelican Creek had the highest potential for biological productivity. Specific conductivity was also much higher in Pelican and Beaverdam creeks and may indicate the higher overall productive potential of these streams. This parameter has been significantly correlated with *M. cerebralis* infection prevalence in Oregon, where specific conductivities were in the same range as those of Yellowstone Lake tributaries (Sandell et al. 2001).

During the first exposure period in 2000, a single Yellowstone cutthroat trout fry at Clear Creek was lightly infected by *M. cerebralis*, but the parasite was not detected in this stream in 2001. Peak spawning migrations at Clear Creek took place several weeks after those at Pelican Creek. The emergence of fry much later in the season and the environmental setting at Clear Creek may be somewhat incompatible with successful *M. cerebralis* life cycle establishment.

In Montana (Baldwin et al. 2000) and Oregon (Sandell et al. 2001), the prevalence of infection has varied seasonally and was significantly higher later in the calendar year. In Yellowstone Lake tributaries, however, sentinel fry infection was most prevalent and severe early in the season (mid-July). The fry infection did not seem to correlate with tubificid abundance at exposure sites, as most tubificids were collected in late August and early September, and only one tubificid, collected in early July, tested positive for *M. cerebralis* infection.

Interpretation of stream characteristics in 2001 must take into consideration the conditions of extreme drought present in Yellowstone National Park that year. Many tributary streams decreased to zero or near-zero surface flow and became disconnected from the lake. Peak discharge was 46% below

the long-term average. Although other studies in the Intermountain West have demonstrated the relationship between *M. cerebralis* infection and stream temperature (Baldwin et al. 2000; Hiner and Moffitt 2001; Krueger 2002), mean water temperatures during the exposure periods were 6.2–10.8°C. The water in Pelican Creek, which has a drainage aspect largely to the south, warmed to above 20°C in June 2001, and elevated temperatures (>15°C) remained through early September. The first of the three exposure periods in Pelican Creek had the highest mean temperature (18.1°C) and the highest infection severity. A temperature of 15°C has been considered optimal for triactinomyxon development, but an increase to 20°C has stopped the production of *T. tubifex* in laboratory incubations (El-Matbouli et al. 1999). Pelican Creek was well above 20°C during parts of most days of exposure periods 1 and 2. However, tubificids in Pelican Creek could be releasing triactinomyxons during the night, when water temperatures declined somewhat.

Conclusions

Pelican Creek, which once supported nearly 30,000 upstream-migrating Yellowstone cutthroat trout (Jones et al. 1982), now appears to be the center of *M. cerebralis* infection in the upper Yellowstone Lake basin. There has been significant variation in infection prevalence and severity in the exposed fry at Pelican Creek and other infected streams, and the host–parasite and ecological interactions in this system have been unclear. The *T. tubifex* strain found during this study is genetically similar to laboratory strains known to produce moderate to high levels of triactinomyxons (Kerans et al. 2004), suggesting that the establishment of whirling disease in the Yellowstone *T. tubifex* populations poses a substantial threat to the Yellowstone cutthroat trout. Moreover, other myxozoans that exist in the lake basin are infecting tubificids and unknown fish hosts.

NPS/TODD KOEL

Pelican Creek whirling disease site.

Evidence from this study suggests that *M. cerebralis* tolerates higher mean water temperatures than have been documented for most other systems. The unique geothermal influences of Pelican Creek have perhaps concentrated tubificids and *M. cerebralis* infection. Many areas upstream of the exposure reach are thermally heated and remain without ice cover throughout the winter. Management action to reduce *M. cerebralis* infection risk in this stream could be taken if more information about infected tubificid locations in Pelican Creek were obtained, especially if the distribution is highly clumped. The temperature effects on *M. cerebralis* in both hosts are of interest, and studies aimed at relating infection prevalence in *T. tubifex* and Yellowstone cutthroat trout to temperature and other environmental characteristics would be useful for predicting the risk of whirling disease establishment in other park watersheds.

Vincent (2001) predicted population-level losses of wild rainbow trout in systems with histological infection grades exceeding 2.5. This study has shown that infection rates during the emergence of Yellowstone cutthroat trout fry in Pelican Creek have the potential to significantly affect this fishery. Angler survey data from throughout the stream and recent efforts to capture upstream-migrating adult Yellowstone cutthroat trout are indicating a substantial decline in this spawning population; the Yellowstone Lake population overall is currently at extremely low levels (Koel et al. 2004, 2005). The establishment and the potential proliferation of *M. cerebralis* add a significant threat to a Yellowstone cutthroat trout population that is already imperiled due to predation and competition by non-native lake trout. Although laboratory challenges and previous field studies have suggested that Yellowstone cutthroat trout are only moderately susceptible to *M. cerebralis* infection, the results of our research indicate that this subspecies may be very susceptible. Additional work should be done to compare the *M. cerebralis* resistance among potentially unique cutthroat trout from isolated populations. Perhaps inherent resistance to this parasite exists and could be used to support ongoing broodstock development programs for conservation efforts in Yellowstone National Park and the surrounding region.

Authors' Note

During the years 2002–2007, our collaborative research on whirling disease in Yellowstone National Park has continued. We have determined that infection prevalence and severity of exposed fry extends to the upper reaches of the Pelican Creek watershed, suggesting that whirling disease risk for Yellowstone cutthroat trout extends far upstream in the valley. In contrast, however, Montana State University PhD student Julie Alexander has observed patchy patterns of *M. cerebralis* infection in tubificids from sites sampled throughout the watershed, suggesting that for *T. tubifex,* at least, the risk of whirling disease varies spatially. Additional work has confirmed the

presence of *M. cerebralis* in the Yellowstone River proper in its Hayden Valley reach, and in the lower reaches of several tributaries there. What remains uncertain are the mechanisms responsible for 1) dissemination of *M. cerebralis* among waters, and 2) allowing *M. cerebralis* to persist in these habitats and proliferate, causing losses of cutthroat trout. The highly variable patterns of oligocheates and abundance of infected *T. tubifex* relative to habitat types warrants further research. Potential vectors of whirling disease dissemination are also being investigated, particularly the role of avian piscivores such as American white pelican (*Pelicanus erythrorhynchos*), great blue heron (*Ardea herodius*), and double-crested cormorant (*Phalacrocorax auritus*). In the pristine environment of Yellowstone National Park, improving our understanding of *M. cerebralis* ecology and life history strategies should increase our ability to mitigate for this harmful disease in the future.

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