Wolf Diseases in Yellowstone National Park

Douglas W. Smith and Emily Almberg

Agate Creek pack wolf with abnormal teeth characteristic of distemper.

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).

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.
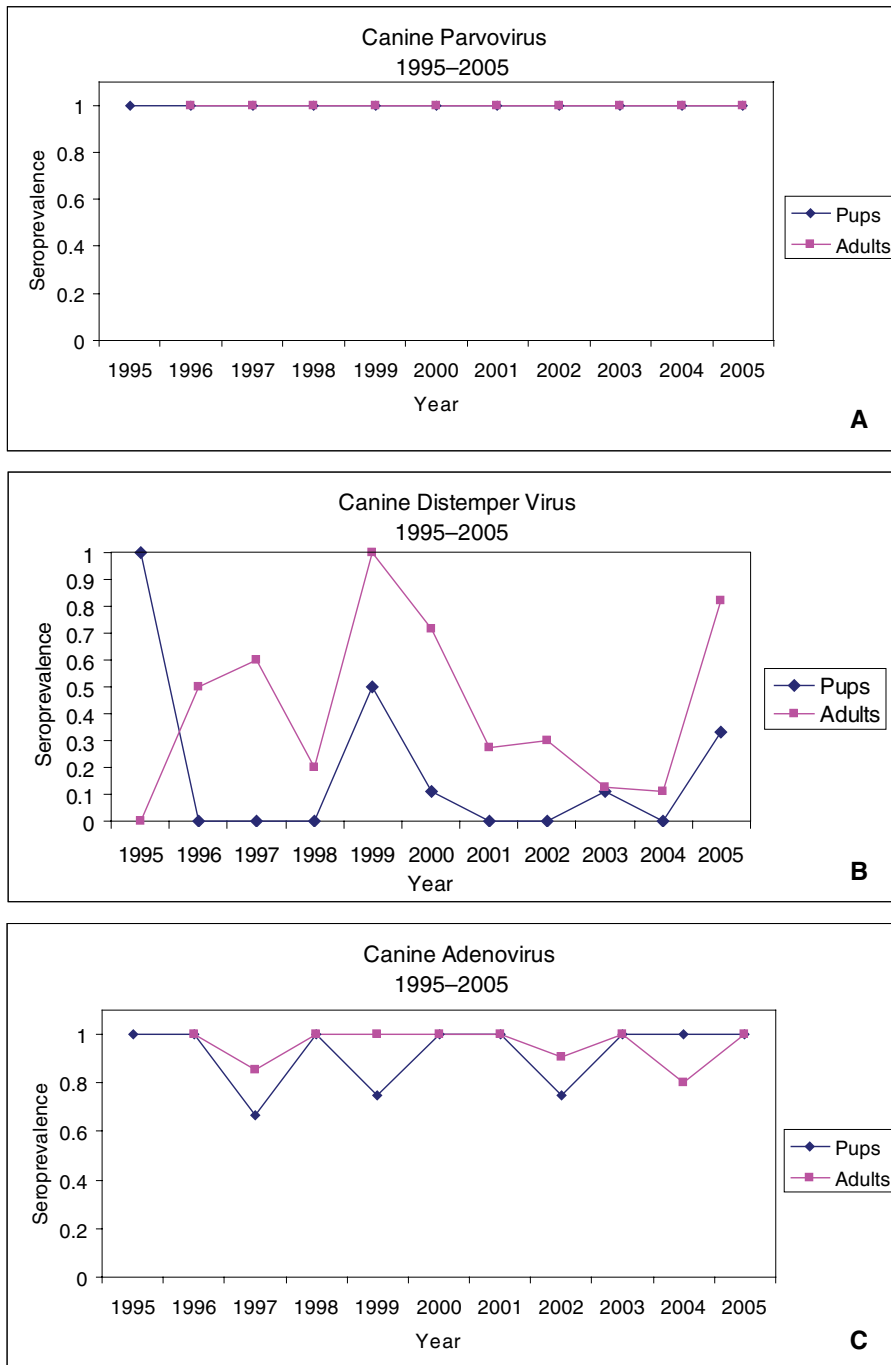

Figure 1. Seroprevalence of three canine diseases in Yellowstone National Park wolves. The vertical axis shows the proportion of animals that tested positive for exposure to parvovirus (A), distemper (B), and adenovirus (canine hepatitis) (C).

In 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

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.

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.

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.

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Brucellosis in Yellowstone Bison
Implications for Conservation Management

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

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
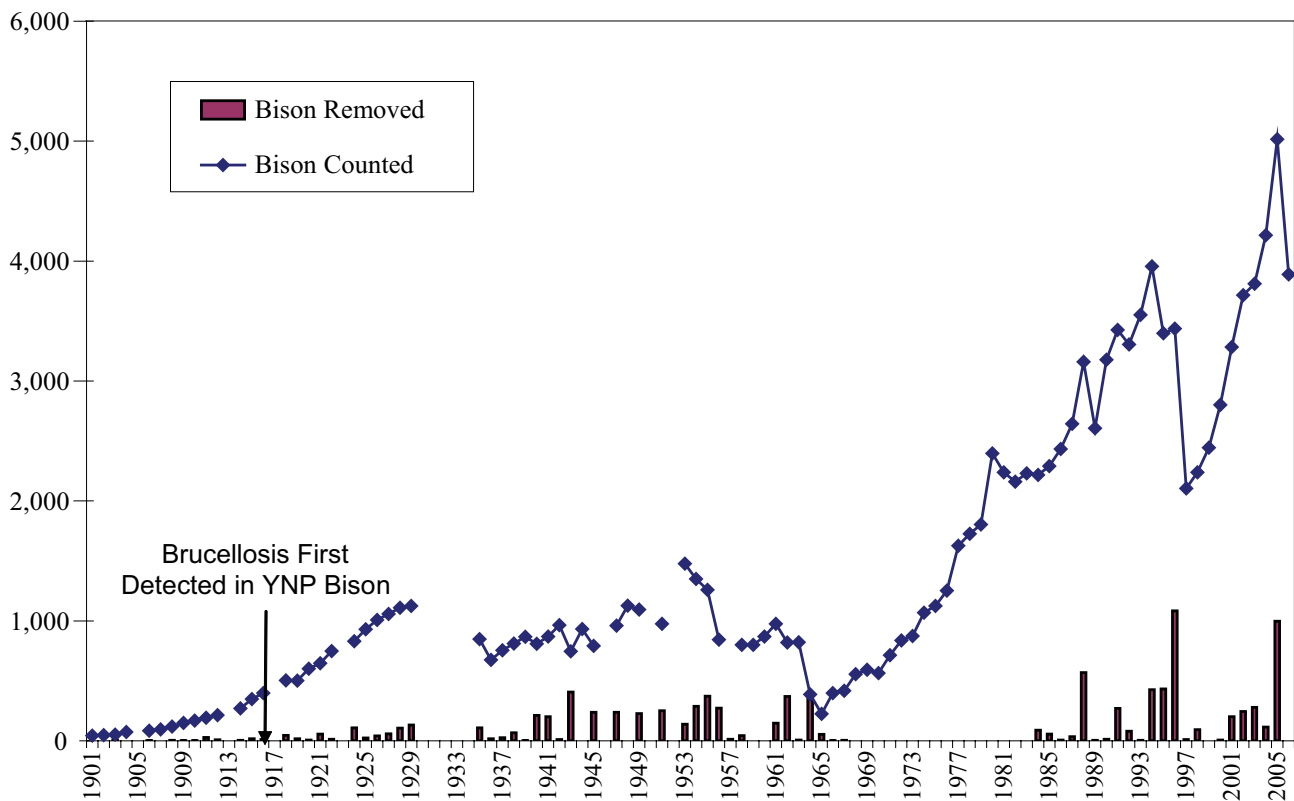

Figure 1. Bison population estimates and management removals since 1901. The YNP bison population has steadily increased despite brucellosis and management removals.

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*
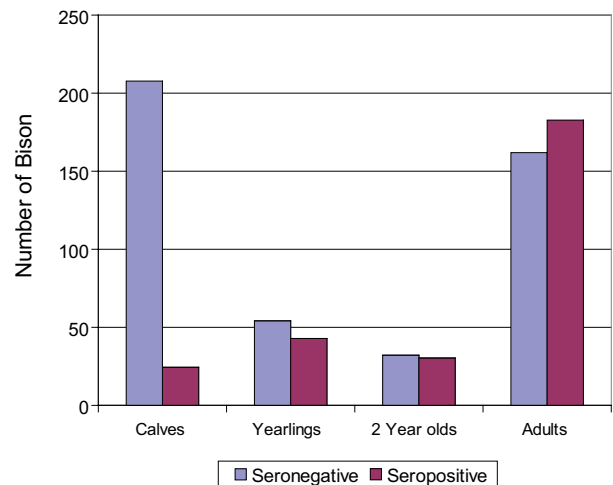

Figure 2. Brucellosis seroprevalence in bison age classes. A high proportion of bison show an antibody response to brucellosis in early age classes. Total bison of known age classes removed during the winter of 2005–06 was 736.

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.

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.

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

Remote vaccination delivery system for ballistic vaccination.
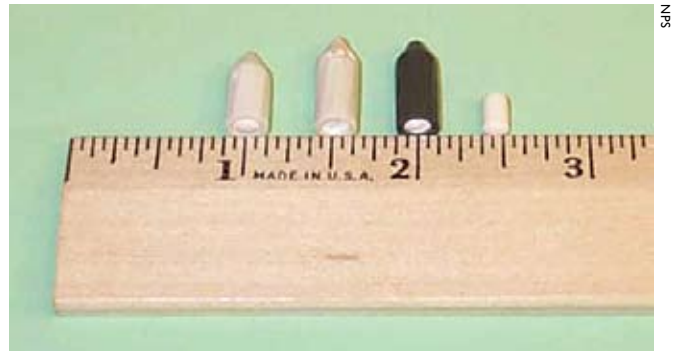
Biobullets used for delivering the encapsulated vaccine.

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 brucel-

losis 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.

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,
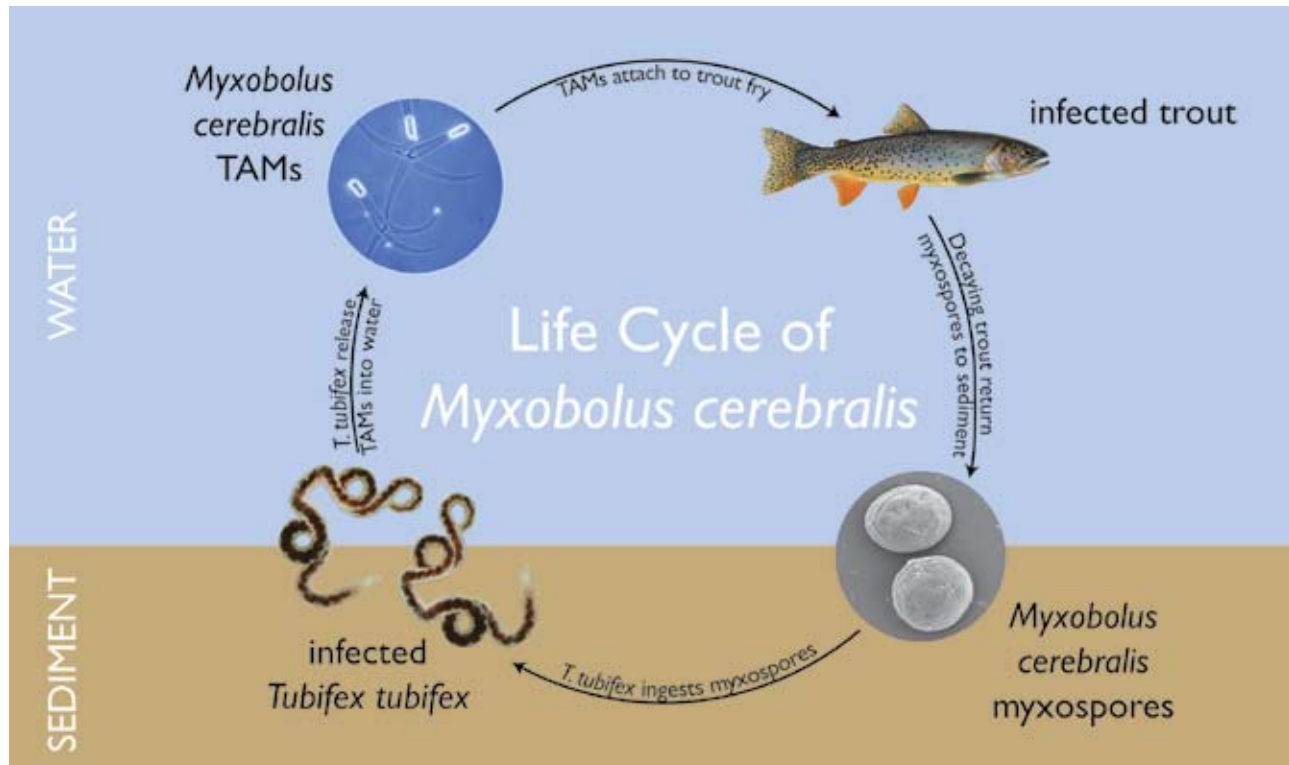*Crystal J. Hudson, Silvia Murcia, and Billie L. Kerans*

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
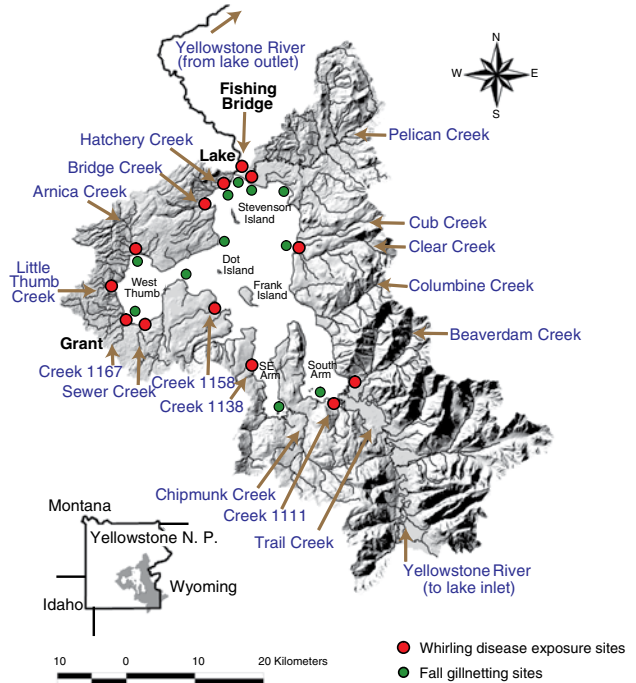

Figure 2. Map of Yellowstone Lake and the upper Yellowstone River drainage within Yellowstone National Park, showing the locations of the 11 cutthroat trout lake gill-netting sites, the 12 streams, and the Yellowstone River near Fishing Bridge, where Yellowstone cutthroat trout sentinel fry exposure 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*
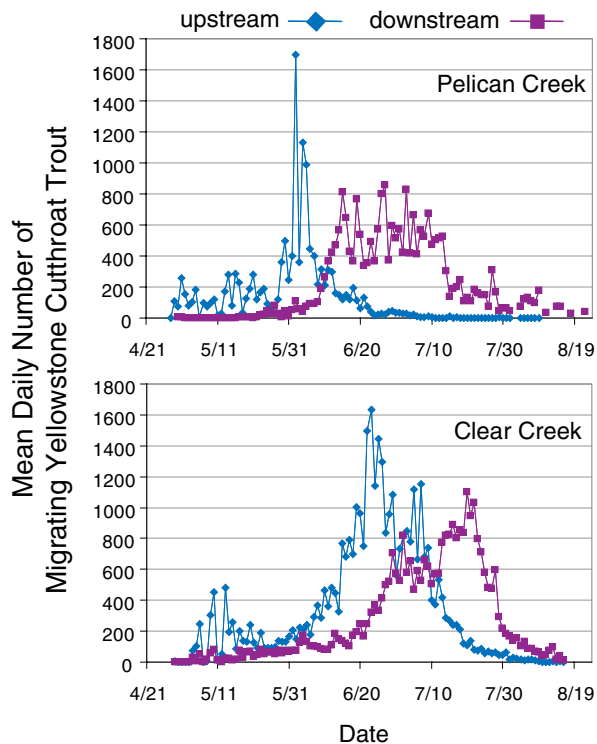
Figure 3. The mean daily number of Yellowstone Lake adfluvial Yellowstone cutthroat trout that were enumerated at migration traps while moving upstream or downstream in Pelican Creek (1964–1983) and Clear Creek (1977–2001).

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 138ºC 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.

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

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.*

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 Inter-mountain 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 triactino-myxons 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 geneti-cally similar to laboratory strains known to produce moderate to high levels of triactinomyxons (Kerans et al. 2004), suggest-ing that the establishment of whirling disease in the Yellow-stone *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.

Pelican Creek whirling disease site.

Evidence from this study suggests that *M. cerebralis* toler-ates higher mean water temperatures than have been docu-mented for most other systems. The unique geothermal influ-ences of Pelican Creek have perhaps concentrated tubificids and *M. cerebralis* infection. Many areas upstream of the expo-sure 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 pre-dicting 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 dur-ing the emergence of Yellowstone cutthroat trout fry in Peli-can 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 cut-throat trout are indicating a substantial decline in this spawn-ing 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 popula-tion 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 contin-ued. We have determined that infection prevalence and sever-ity of exposed fry extends to the upper reaches of the Pelican Creek watershed, suggesting that whirling disease risk for Yel-lowstone 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* infec-tion in tubificids from sites sampled throughout the water-shed, 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.

This article has been adapted with permission from the American Fisheries Society. It was originally published as "Myxobolus cerebralis in Native Cutthroat Trout of the Yellowstone Lake Ecosystem" by Todd M. Koel, Daniel L. Mahony, Kendra L. Kinnan, Charlotte Rasmussen, Crystal J. Hudson, Silvia Murcia, and Billie L. Kerans in the Journal of Aquatic Animal Health 18(3):157–175 (September 2006).

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