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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 (<i>Ambystoma tigrinum</i>)	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 (<i>Bufo boreas</i>)	Occurs throughout the GYE?	Present at 2–5% of potential breeding sites	Disease?	Corn et al. 2005
Boreal Chorus Frog (<i>Pseudacris maculata</i>)	Occurs throughout the GYE	Present at 32–43% of potential breeding sites		Corn et al. 2005
Northern Leopard Frog (<i>Rana pipiens</i>)	A few sites south of Jackson Lake	Extirpated?	Unknown	Koch and Peterson 1995; Patla and Peterson 2004
Columbia Spotted Frog (<i>R. luteiventris</i>)	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 of 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.



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.



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.

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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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*).

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