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Chytrid Fungus in American Bullfrogs (*Lithobates catesbeianus*) along the Platte River, Nebraska, USA

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Chytridiomycosis is an emerging infectious disease in amphibians that was discovered in the late 1990s (Berger et al. 1998; Longcore et al. 1999), with retrospective surveys indicating isolated cases dating to 1902 in Japan (Goka et al. 2009), 1938 in Africa (Weldon et al. 2004), and 1961 in North America (Ouellet et al. 2005). This disease is caused by the pathogen *Batrachochytrium dendrobatidis* (*Bd*), a species of chytrid fungus, and it is hypothesized to contribute to amphibian declines worldwide (Berger et al. 1998; Daszak et al. 1999; Lips et al. 2006; Collins 2010). *Bd* has been detected in amphibians on all six continents inhabited by amphibians, and large numbers of *Bd*-positive samples have been collected from various sites across North America (www.Bd-maps.net; accessed 7 June 2011). The central United States, however, has not been sampled extensively for *Bd*. To date, no published accounts of *Bd* exist for amphibians from Nebraska, USA, although two localities with *Bd* in Nebraska are noted online (www.Bd-maps.net; J. Krebs, Henry Doorly Zoo, Omaha, Nebraska, and Zimmerman Ranch, Dunning, Nebraska). Our objective was to determine whether *Bd* is present along the Platte River in south-central Nebraska.

We sampled anurans along the Platte River in Hall County, Nebraska, on land managed by Platte River Whooping Crane Critical Habitat Maintenance Trust, Inc., located on Shoemaker Island (40.7884°N, 98.4650°W). We prioritized capturing *Lithobates catesbeianus* (American Bullfrog) because they are nonclinical carriers of *Bd* (Daszak et al. 2004; Garner et al. 2006), are abundant in Nebraska (Fogell 2010), and are a concern for wildlife management worldwide due to their invasiveness (Ficetola et al. 2007). We targeted sampling along two sloughs (linear, water-filled depressions) surrounded by mesic, tall-grass prairie (Fig. 1; Slough 1 start: 40.7921°N, 98.4628°W; end: 40.7939°N, 98.4584°W; Slough 2: start: 40.7959°N, 98.4444°W, end: 40.7989°N, 98.4421°W). We also opportunistically collected *Anaxyrus woodhousii* (Woodhouse's Toad) and *Lithobates blairi* (Plains Leopard Frog) from these sloughs, as well as nearby point locations across the island, including isolated ponds, puddles, and roads (Fig. 1). Samples were collected 28 April, 5 June, 9 and 10 July, and 3 and 6 September 2010.

To minimize contamination, we captured amphibians by hand while wearing disposable vinyl gloves that we changed between each capture. We also captured some Plains Leopard Frogs with nets from ponds and puddles. Each animal captured was kept individually in plastic or cloth bags until processed in the field and then released; plastic bags were disposed after one usage whereas cloth bags were washed between samples. For each individual, we determined sex and visually examined for wounds and other abnormalities. We sampled for *Bd* following protocols of the Amphibian Disease Laboratory, Institute for Conservation Research at the San Diego Zoo and used their preferred sampling kits, comprised of plastic-handled, fine tip cotton swabs (Dryswab™; MW113; Medical Wire & Equipment Co. LTD., England) and screw-top storage tubes (Cryogenic Vials, Nalge Nunc International, New York). We swabbed the skin on ventral surfaces, targeting the pelvic patch, thighs, and toe webbing, making 5 passes on each surface with a single swab for each animal. The swab was air-dried, and the tip placed in an individually marked vial.

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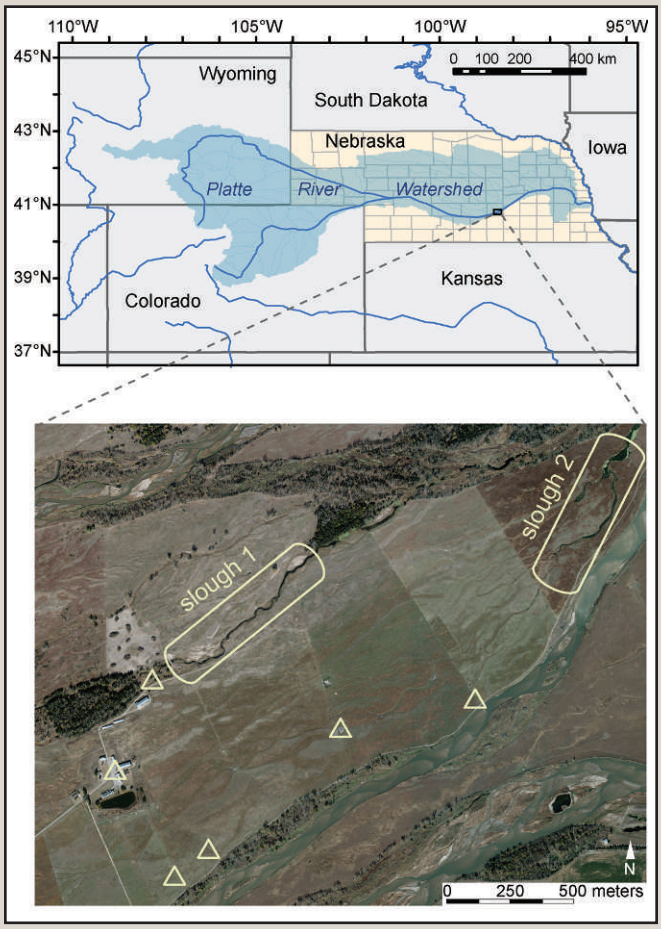

FIG. 1. Location of sampling sites along the Platte River in central Nebraska, USA. Locator map shows the study region within the Platte River watershed. Aerial photograph depicts Shoemaker Island (Hall County, Nebraska) where anurans were sampled. Most sampling occurred in sloughs 1 and 2, and opportunistic captures were collected from point locations (denoted by triangles). Aerial photograph was taken in October 2010.

Samples were shipped to the San Diego Zoo for processing where they were analyzed for *Bd* using real-time (Taqman) PCR to amplify the ITS1 region (Boyle et al. 2004). Results were reported as positive or negative. We examined associations among *Bd* presence, sex, sampling date, and sampling location with Pearson Chi-square tests in PASW Statistics version 18 (Chicago, Illinois, USA).

We captured a total of 118 amphibians, including 76 adult American Bullfrogs, 1 American Bullfrog tadpole, 20 Plains Leopard Frogs, and 21 Woodhouse's Toads. We detected *Bd* only in American Bullfrogs, with 31 adults (41%) testing positive. We did not detect an association between *Bd* infection and sex (χ^2 = 1.14, df = 1, P = 0.28) or sampling location (χ^2 = 1.81, df = 2, P = 0.40), but we found an association between *Bd* infection and sampling date (χ^2 = 14.12, df = 2, P = 0.001), with 67% of American Bullfrogs testing positive in April, 0% testing positive in June, and 39% testing positive in July. We observed infected American Bullfrogs in both sloughs; the one American Bullfrog sampled from an isolated pond located 600 m from the sloughs tested negative. No infected individuals were detected in the other two species, even in sloughs with infected American Bullfrogs (N = 2 Woodhouse's Toads collected from Slough 1, N = 18 Plains Leopard Frogs collected from Slough 1, and N = 1 Plains Leopard Frog from Slough 2).

We detected high prevalence of *Bd* (41%) in American Bullfrogs along the Platte River in central Nebraska. Other central USA accounts of *Bd* include surveys in the north-central United States (Iowa, Wisconsin, and Michigan), where *Bd* was detected in nine species, including American Bullfrogs (Sadinski et al. 2010); surveys in Colorado and Wyoming, where *Bd* was widely distributed (51% of sites examined) in Boreal Toads (*Bufo boreas boreas*), Western Chorus Frogs (*Pseudacris triseriata*), Northern Leopard Frogs (*Lithobates pipiens*), and Wood Frogs (*Lithobates sylvaticus*) (Young et al. 2007); and surveys along the Rocky Mountains, where *Bd* was detected in six species (Muths et al. 2008). Additional surveys of *Bd* throughout the central United States are warranted to gain baseline information about the presence of this pathogen, especially as it relates to species that have declined in recent decades for unknown reasons (e.g., Hayes and Jennings 1986).

Accumulating evidence supports the "novel pathogen hypothesis" to explain the recent worldwide invasion of *Bd* (Rosenblum et al. 2009). This specifically suggests that human-mediated movement of infected frogs, including the American Bullfrog, precipitated *Bd* range expansion (Fisher and Garner 2007; Schloegel et al. 2009a,b). In light of this, our results are a special concern for the region as American Bullfrogs were introduced to waterways throughout Nebraska in the 20th century and appear to be expanding their distribution (Fogell 2010). American Bullfrogs were not documented in surveys of herpetofauna in 1980 on an island adjacent to our study site (Jones et al. 1981), but they were abundant in our 2010 surveys. Expansion of American Bullfrogs could result in the introduction of *Bd* to the native amphibians in the region, notably Plains Leopard Frogs, Woodhouse's Toads, and Boreal Chorus Frogs. *Bd* infections already have been reported in Chorus Frogs (*Pseudacris maculata* and *P. triseriata*) and Northern Leopard Frogs (*Lithobates pipiens*) in Colorado and Wyoming (Young et al. 2007; Muths et al. 2008). Although we did not detect *Bd* in Plains Leopard Frogs or Woodhouse's Toads, a number of factors may have prevented this. Our sample size was small (Skerratt et al. 2008), and studies have shown lower prevalence of *Bd* during the summer (Longcore et al. 2007; Retallick et al. 2004; Voordouw et al. 2010). In our study, most positive detections of *Bd* were at the earliest sampling date (April) in American Bullfrogs, but we captured most Plains Leopard Frogs and Woodhouse's Toads at later sampling dates, thus we may have missed a seasonal peak in infection. In addition, recent work suggests that our collection method (swabbing) is less sensitive than other methods (toe clips and bag rinses) for detection (Voordouw et al. 2010).

Additional surveys of native anurans need to be conducted at this site to monitor potential cascading effects of transmission of *Bd* from invasive American Bullfrogs. Prevalence of *Bd* varies among American Bullfrog populations, but it is frequently high (Garner et al. 2006), and American Bullfrogs are often nonclinical carriers (Daszak et al. 2004; Garner et al. 2006). Despite high genetic similarity of isolates, different strains of *Bd* differ in virulence (Berger et al. 2005; Fisher et al. 2009; Rosenblum et al. 2009), yet there appears to be little host specificity (James et al. 2009; Rosenblum et al. 2009). Thus, *Bd* strains carried by American Bullfrogs likely are easily spread to other native species. Therefore, characterization of the strain of *Bd* in this region is needed because American Bullfrogs occur in the same water bodies with many native species and may threaten them, especially if carrying a lethal strain.

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