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Article *in* Herpetological Review · January 2013

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Amphibian Chytrid Fungus in Woodhouse's Toads, Plains Leopard Frogs, and American Bullfrogs along the Platte River, Nebraska, USA

We recently documented the first published record of the fungal pathogen *Batrachochytrium dendrobatidis* (chytrid) in Nebraska, USA, where we detected a high prevalence of chytrid in American Bullfrogs (*Lithobates catesbeianus*) in off-channel aquatic habitats on an island of the Platte River (Harner et al. 2011). This pathogen causes the disease chytridiomycosis (Longcore et al. 1999), and widespread amphibian mortality from infection is contributing to declines and regional extirpation of amphibians worldwide (Berger et al. 1998; Collins 2010; Daszak et al. 1999; Lips et al. 2006). We were concerned by the high prevalence of chytrid in American Bullfrogs on this island because American Bullfrogs have been introduced to the region (Fogell 2010), and they may negatively affect native amphibians through both direct (e.g., predation) and indirect (e.g., competition, disease transmission) interactions. Loss of native amphibians is of conservation concern for a number of reasons, notably the loss of biodiversity (e.g., Collins 2010) and alterations to ecosystem structure and function (Whiles et al. 2006). Along the Platte River, amphibian declines also have potentially negative consequences for an endangered migratory bird, the Whooping Crane (*Grus americana*), which relies on the river and surrounding wetlands for critical habitat during spring and autumnal migrations (USFWS 1978) and preys upon frogs during migratory stops (Geluso et al., *in press*).

Objectives of our study were to spatially extend sampling for chytrid to other sites and species along the central Platte River. In our prior survey we also sampled native Plains Leopard Frogs (*Lithobates blairi*) and Woodhouse's Toads (*Anaxyrus woodhousii*) and did not detect chytrid, but our sample sizes were low (N = 20 and 21, respectively) and from a small geographical area, so we may have failed to detect infection (Skerratt et al. 2008). Therefore, in this survey we focused on obtaining sample sizes of greater than 40 individuals for each species and sampled several locations to determine whether chytrid was present in native species and at multiple locations along the central Platte River in Nebraska.

We sampled amphibians along a 100-km reach of the Platte River in central Nebraska (Fig. 1) on Jeffrey Island (40.6901°N, 99.5956°W) and Cottonwood Ranch (40.6868°N, 99.4784°W) in Dawson County; Blue Hole East (40.6836°N, 99.3758°W) in Kearney County; Dippel (40.7036°N, 98.7950°W) in Buffalo County; and Shoemaker Island (40.7920°N, 98.4620°W) in Hall County. Property ownership was as follows: Jeffrey Island (Central Nebraska Public Power and Irrigation District), Cottonwood Ranch and Blue Hole East (Nebraska Public Power District), and Dippel and Shoemaker Island (The Crane Trust). Sampling effort was more extensive on Jeffrey Island to compare this more western portion of the river reach to the previously sampled Shoemaker Island (Harner et al. 2011).

Amphibians were sampled from a variety of habitats including river channels, sloughs (natural and created), ponds, borrow pits, stock tanks, and uplands. Sampling occurred in autumn 2011 (Shoemaker Island) and spring-summer 2012 (all sites).

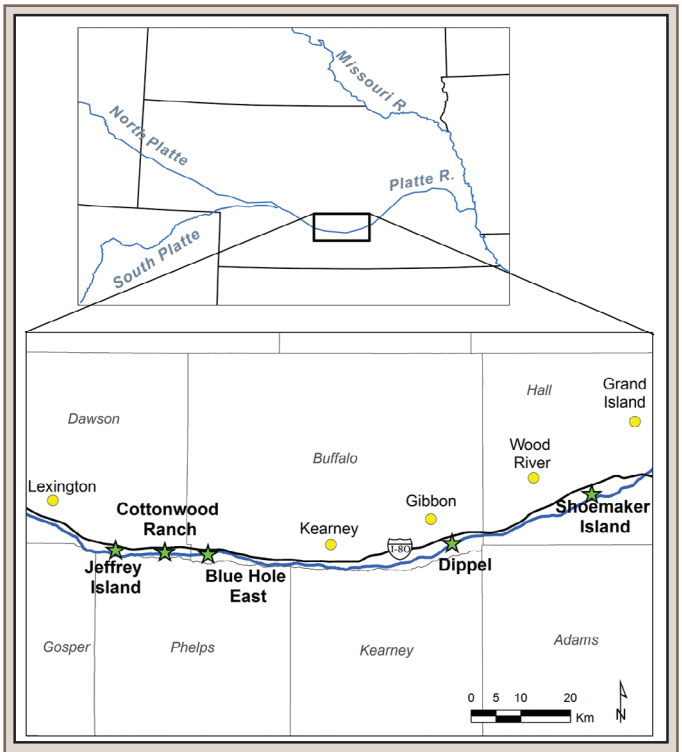

FIG. 1. Location of sites sampled (denoted by green stars and bold text) for amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, along the Platte River in central Nebraska, USA. Detailed image also includes county names (italics), select towns (yellow circles), the Platte River (blue line), and interstate highway (dark black line).

Individuals were captured by hand, and researchers wore disposable gloves to minimize contamination and changed gloves between each capture. After capture we rubbed a sterile swab (Fisherfinest® Dry Transport Swab; Fisher HealthCare, USA) across the abdomen, throat, inner thighs, webbing of toes, and mouthparts. Swabs were air-dried, stored frozen, and shipped frozen to the University of South Dakota for testing.

Samples were analyzed for chytrid with real-time Taqman PCR assay. DNA was extracted from swabs using Qiagen DNEasy Blood and Tissue spin column kits according to manufacturer's protocol. Pathogen load per swab was determined with quantitative PCR on a StepOnePlus machine (Applied Biosystems). Reactions were run based on a standard protocol described in Kerby et al. (2013). Each plate contained a standardized dilution

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series of quantified zoospore numbers to use as a standard curve. A negative control also was included on every plate. Samples were run in triplicate, and positive values were averaged to determine a final estimated pathogen load for each swab.

The study area experienced prolonged drought in 2012 with an onset of abnormally dry conditions by late May that transitioned to extreme drought by the end of August (droughtmonitor.unl.edu; accessed 30 November 2012). As a result, virtually no temporary ponds were present, hindering our ability capture any Plains Spadefoots (*Spea bombifrons*) and restricting our captures of Boreal Chorus Frogs (*Pseudacris maculata*) to only three. Both species rely on temporary ponds and both are known from the region (Ballinger et al. 2010, Fogell 2010).

Table 1. *Batrachochytrium dendrobatidis* (*Bd*) prevalence (no. *Bd*-positive individuals/total no. individuals sampled) at sites along the central Platte River, Nebraska, USA.

Date	Site	Woodhouse's Toad	American Bullfrog	Plains Leopard Frog
Oct 2011	Shoemaker Island	—	—	1 /5
May 2012	Jeffrey Island	0/2	6/18	7/11
	Dippel	—	1/3	10/13
	Shoemaker Island	—	2/8	4/5
Jun 2012	Jeffrey Island	0/5	0 /11	1/8
Jul 2012	Jeffrey Island	10/17	—	0/8
	Cottonwood Ranch	4/14	0/3	3/14
Aug 2012	Jeffrey Island	—	1/12	0/2
	Blue Hole	0/2	—	—
	TOTAL	14/40 (35%)	10/55 (18%)	26/66 (39%)

We sampled a total of 163 individuals of four species for chytrid (Table 1). Five Plains Leopard Frogs were sampled from Shoemaker Island on 25 October 2011 prior to winter, whereas all other individuals were sampled from 9 May to 21 August 2012. Four Plains Leopard Frogs were metamorphs, and all other individuals were adults.

Across sites, prevalence of chytrid was 35% for Woodhouse's Toads, 18% for American Bullfrogs, and 39% for Plains Leopard Frogs (Table 1). None of the Boreal Chorus Frogs tested positive. One Plains Leopard Frog metamorph tested positive. For positive samples, the pathogen load (number of zoospore equivalents per swab) ranged from 0.87-6560 (median = 51) in Woodhouse's Toads; 0.02-84 (median = 2) in American Bullfrogs; and 0.04-8139 (median = 27) in Plains Leopard Frogs.

Our findings add to the geographical extent and list of species infected by chytrid in central Nebraska along the Platte River. Results confirm presence of chytrid in invasive American Bullfrogs as well as in two native species, Woodhouse's Toads and Plains Leopard Frogs, across this 100-km reach of river. Prevalence of chytrid was moderate (30%) across species sampled, but pathogen load did not exceed levels reported as a threshold for mass mortality in other species (~ 10,000 zoospore equivalents per swab; Vredenburg et al. 2010). Prevalence of chytrid was lower in American Bullfrogs from Shoemaker Island in this study (25%) compared to our 2010 survey (41%; Harner et al. 2011). This difference may be linked to climatic differences between years, as 2010 had above-average precipitation (4th wettest over 74-year record), whereas 2012 had below-average precipitation (4th driest over 74-year record) measured at Grand Island, NE (www.ncdc.noaa.gov; accessed 30 November 2012). The influence of drought on chytridiomycosis is currently debated, but some argue that dry conditions may decrease prevalence and severity of chytrid (Kriger 2009). Regardless, presence of chytrid in invasive American Bullfrogs is a concern because they are nonclinical carriers of chytrid (Daszak et al. 2004; Garner et al. 2006; Schloegel et al. 2009).

Seasonality of chytrid infection was not a focus of this study, but trends emerged that warrant further investigation. Prevalence of chytrid in Plains Leopard Frogs from Shoemaker Island went from 1 in 5 to 4 in 5 individuals between autumn and spring, and prevalence of chytrid was higher in May than subsequent months in 2012 across sites. These trends potentially suggest that Plains Leopard Frogs may be at an elevated risk in spring. Other studies have observed peaks in chytrid prevalence and pathogen load in early spring (Gaertner et al. 2012; Kriger and Hero 2007a; Retallick et al. 2004), as well as a negative relationship between temperature and chytrid prevalence (e.g., Kriger and Hero 2007a). From a conservation standpoint, availability and abundance of Plains Leopard Frogs in March and April may be important for foraging Whooping Crane during migratory stopovers in the region (Geluso et al., *in press*).

There is need for ongoing monitoring of chytrid along the central Platte River to capture variation across habitats as well as through time. Along with the seasonal patterns noted above, we saw a trend of varying incidences of infection among different habitats (data not presented due to small sample size), though more systematic sampling is required to draw inferences about prevalence among different habitats. Other studies have detected an effect of local environmental conditions on chytrid, such as greater prevalence in permanent compared to ephemeral water bodies (Kriger and Hero 2007b). A better understanding of the seasonality and influence of habitat on the pathogen may help resource managers target conservation measures to reduce the threat of chytrid to native amphibians along the central Platte River, and thus help maintain the natural structure of the interconnected aquatic and terrestrial food webs along these flood plains.

Acknowledgments.—We thank Central Nebraska Public Power and Irrigation District (CNPPID) and the Crane Trust for funding; Mark Peyton (CNPPID) and Jim Jenniges (Nebraska Public Power District) for providing information about and access to the western study sites; the laboratory of Jacob Kerby at the University of South Dakota for conducting analyses; Keith Geluso for comments on an earlier version of the manuscript; and Alexandra Frohberg, Jamie Jones, Liz McCue, Evan Suhr, and Angelina Wright for assistance with sampling. Amphibians were studied under authorization of the Nebraska Game and Parks Commission (Scientific and Educational Permit issued to Keith Geluso).

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Herpetological Review, 2013, 44(3), 461–464.

Amphibian Populations in Brazos River Basin, Texas, Show No Evidence of *Bd* Infection

Discovered and described only a decade ago (Longcore et al. 1999), amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has rapidly expanded its range, and new geographic distribution reports are being added frequently (http://www.bd-maps.net/). In the United States, *Bd* infection has been documented in amphibian populations in nearly all of the states (Olson et al. 2013). However, except for two studies, the state of Texas remains under-surveyed for this pathogen. Gaertner et al. (2009) reported *Bd* occurrence in individuals of five salamander species along parts of the Colorado and Guadalupe River basins in Central Texas. Saenz et al. (2010) reported incidences of *Bd* infection in five anuran and one plethodontid species *s*pread across forested areas in Eastern Texas. To address the lack of surveys in the Brazos River basin of Texas, we sampled amphibians for *Bd* from two disjunct areas along the watershed (Fig. 1).

In August–September 2011 and April–May 2012 we sampled from urban modified playa wetlands within the city of Lubbock, located in northwest Texas near the upper reaches of the Brazos River basin. Lake Waco wetlands, situated in the central portion of the Brazos River basin in central Texas, were sampled in May–July 2012. Areas of study were under severe drought in the year 2011. However, sampling in 2012 for Lubbock and Lake Waco wetlands (sampled only in 2012) was carried out earlier in the year when temperatures were cooler and rain events facilitated breeding aggregations.

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