Fungus and Frogs: Preliminary Analysis of a Survey of Chytrid in Kansas

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ABSTRACT: Amphibians are one of the most threatened groups of organisms worldwide, per the International Union for Conservation of Nature. A disease known as chytridiomycosis has been linked to population declines worldwide. Incidence of the fungus has recently been reported at three locations in south-central Kansas. The objective of our study was to assess the status of chytrid throughout the state of Kansas. In 2015-2016 we conducted surveys at multiple locations. We used real-time PCR to analyze swabs collected at each location. Preliminary results show chytrid present at 4 locations in Kansas. We will continue to analyze samples to determine aspects of life history that might increase the potential for infection.

Introduction

Amphibians are one of the most threatened groups of organisms worldwide. Per the Global Amphibian Assessment conducted by the International Union for the Conservation of Nature (IUCN), 32% of described amphibians are listed as vulnerable, endangered, or critically endangered and 43% are in decline. One major threat to amphibians is the introduction of novel diseases such as ranavirus and chytridiomycosis (Daszak et al., 1999). Chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis* known as chytrid. Chytridiomycosis has been confirmed as a contributing factor in the extinction of multiple species including two Gastric-brooding frogs of Australia and the Golden Toad of Central America (Daszak et al. 1999). Within the United States the decline of mountain yellow-legged frogs (*Rana mucosa* and *Rana sierra*) in California and Boreal Toads (*Bufo boreas*) in Colorado has been attributed to the disease. Recently, chytrid has been detected in Sedgwick and Kingman counties in south-central Kansas, where 45 of 62 individuals tested positive for presence of chytrid (McTaggart et al. 2014).

Our objectives were as follows:

1.) To survey populations of frogs across Kansas and determine the proportions that are affected or at least test positive for exposure to chytrid.
2.) To investigate the life history characteristics that might increase the potential for infection with chytrid by surveying species that exhibit diverse life histories.

Methods

Sample Collection

Within a sample site, we targeted 30 individuals, 10 within each of four life history groups including xeric, arboreal, semi-aquatic, and aquatic (Figure 1). We located amphibians by searching appropriate habitat such as streams or ponds, and by listening for frog calls. We followed a standardized chytrid sampling protocol. We captured amphibians by hand and swabbed each individual five times across the venter, each hind leg, and each foot (Figure 2). To prevent resampling, we placed the amphibian in an individual container until we completed sampling at a location.

To prevent cross-contamination and spread of the fungus, we handled each amphibian with a fresh pair of disposable gloves. We scrubbed and soaked all sampling equipment including waders, boots, and dip nets in a 1:9 bleach solution. We also scraped mud from vehicles and scrubbed tires with the bleach solution.

Lab Analysis

Swab samples were sent to Research Associates Laboratory for analysis. Samples were analyzed by using real-time polymerase chain reaction (rt-PCR) to detect presence or absence of the fungus in our samples. This process allowed us to detect low levels of chytrid present on an individual.

Figure 1: Examples of species from four life history groups. From left to right: Plains Spadefoot (a xeric species), Gray Treefrogs (an arboreal species), American Toad (a semi-aquatic species), and American Bullfrog (an aquatic species).

Figure 2: Sample collection. One person holds the frog while a second person swabs the venter of the organism.

Results

We collected 408 samples from 10 species in 2015. In 2016 we collected samples from 387 individuals across nine species. Over the course of the two-year sampling effort, we collected samples from 795 individual amphibians across 12 species. Preliminary analysis of 200 samples yielded 18 samples that tested positive for the presence of chytrid. The fungus was detected at four of the 16 locations we have results for at this time (Figure 3). Locations where chytrid was detected include Quivira and Marais des Cygnes National Wildlife Refuges, Farlington Fish Hatchery, and a location south of Nashville, KS.

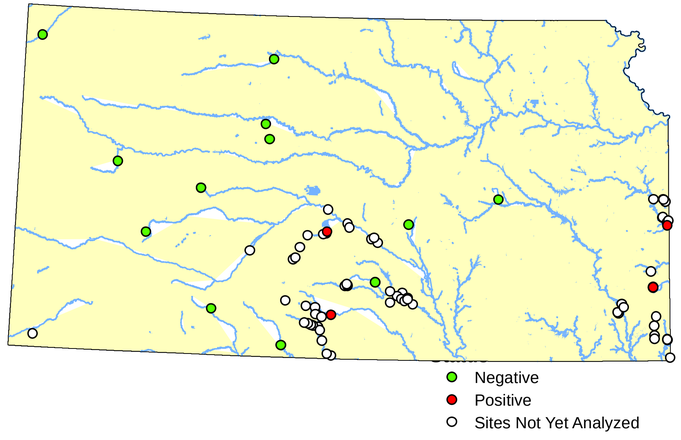

Figure 3: Sample locations throughout Kansas

Discussion

Chytrid was detected at four of the 16 locations we have currently analyzed. Among the four life history groups there might be an association between aquatic frogs and chytrid, as 17 of the 18 individual that tested positive for the presence of chytrid were aquatic species. Chytrid is closely associated with water and quickly susceptible to desiccation in dry, warm environments. The physiology of chytrid is such that optimal growth rates are achieved at 17-25° C, and temperatures above 28°C result in death of the fungus. For these reasons, it is hypothesized the central plains of the United State might have a low susceptibility to the presence of chytrid (Aanensen & Fisher 2015). Our preliminary data support this hypothesis. We are currently working to continue analysis and will collect more samples in the spring of 2017.

	Species	Positive	Total Sampled	Frequency +
Xeric Frogs	Plains Spadefoot	0	11	0
	Western Narrow-Mouthed Toad	0	15	0
Arboreal Frogs	Gray Treefrog	1	30	0.03
	Spring Peeper	0	2	0
Semi-aquatic Frogs	Woodhouse's Toad	0	30	0
	Great Plains Toad	0	1	0
	Toad Species	0	2	0
Aquatic Frogs	Blanchard's Cricket Frog	13	31	0.42
	Plains Leopard Frog	1	50	0.02
	American Bullfrog	3	28	0.11
	TOTAL	18	200	0.09

Table 1: Results of rt-PCR analysis of 200 samples across 9 species from 2015-2016. Preliminary analysis suggests that aquatic frogs might be more susceptible to chytrid.

Literature Cited

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