Fall 2017

Survey Of Anuran Chytrid (Batrachochytrium Dendrobatidis) In Kansas And The Influence Of Anuran Life History In Occurrence

Ariel Snyder
Fort Hays State University, a_snyder2@mail.fhsu.edu

SURVEY OF ANURAN CHYTRID (*BATRACHOCHYTRIUM DENDROBATIDIS)* IN KANSAS AND THE INFLUENCE OF ANURAN LIFE HISTORY IN OCCURRENCE

being

A Thesis Presented to the Graduate Faculty
of the Fort Hays State University in
Partial Fulfillment of the Requirements for
the Degree of Master of Science

by

Ariel Snyder
B.S., Fort Hays State University

Date _____ Approved_____
Major Professor

Approved_____
Chair, Graduate Council

This thesis for

The Master of Science Degree

By

Ariel Snyder

has been approved by

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Chair, Supervisory Committee

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Supervisory Committee

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Supervisory Committee

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Supervisory Committee

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Chair, Department of Biological Sciences

ABSTRACT

Amphibians are one of the most threatened groups of organisms worldwide. Introduction of non-native predators and habitat destruction, degradation, and fragmentation can be attributed to many declines. However, declines in protected areas might be due to the emergence of novel diseases such as ranavirus and chytridiomycosis. Chytridiomycosis has been implicated in the decline of many species world-wide, including the decline of Boreal Toads and Yellow-Legged Frogs in North America. Chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis*, or "chytrid". Chytrid has been detected in Colorado, Nebraska, and Oklahoma, and was first reported in two counties in south-central Kansas in 2014. The objectives of my study was to further assess the presence of chytrid throughout the state and assess aspects of anuran life history that might increase the potential for infection with chytrid. In cooperation with Kansas Department of Wildlife, Parks and Tourism, surveys were conducted spring 2015– spring 2017 to collect swab samples from anurans in Kansas. I sent samples to Research Associates Lab (Dallas, TX) for analysis by real-time PCR to detect the presence of chytrid in swab samples. Chytrid was detected at six sample locations across six species. I was unable to assess the potential influence of life history due to low frequencies of chytrid occurrence. I suggest continued monitoring of anuran populations to ensure population health into the future.

ACKNOWLEDGEMENTS

I thank the Kansas Department of Wildlife, Parks and Tourism for their interest and funding of this project. I also thank the many land managers who permitted me access to their property to collect samples; especially Brent Clark, Amy Coffman, Kristen Hase, Dan Mosier II and Luke Winge for their time and advice.

I thank the members of my graduate committee Dr. Mitchell Greer, Dr. Robert Channell, and Mr. Curtis Schmidt for their advice and support throughout this project. I also thank Dr. Yass Kobayashi for his assistance with molecular techniques during this project. I especially thank my advisor Dr. William Stark for his advice on this project and guidance in my graduate studies at Fort Hays State University.

I also thank field assistants Andrew Karlin and Vanessa Salazar, as well as the Ecological Services section of KDWPT for their time and effort collecting samples. I thank the numerous volunteers who helped me collect samples including, Holly Wilson, Allison Hullinger, Joshua Mead, Sean Rogers, Ernesto Flores, and Nicholas Bulthap. I especially thank Kaden Buer for his support in the molecular aspect of this project.

I thank my family and friends for supporting me during my academic career. I thank my parents Steve and Nancy Snyder for their continued love and support. I thank my sister, Hillary Snyder for taking time to edit this document and supporting me. I also thank my brother Eric for his love and support. I thank my fellow graduate students, past and present, for their support and encouragement. Without any of these people, this project would not have been possible, and for that, I am sincerely thankful.

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PREFACE

This thesis is written in the style of the Transactions of the Kansas Academy of Science. All anurans were handled in accordance with the Society for the Study of Reptiles and Amphibians. These methods were approved by the Institutional Animal Care and Use Committee of Fort Hays State University (IACUC 16-0001).

INTRODUCTION

Global biodiversity has been decreasing for the past 2,000 years. Based on the geological record, the current rate of extinction is at least several hundred times greater than the background extinction rate (Pimm and Brooks 1997). Biodiversity has importance for its intrinsic value, but it also serves to maintain ecosystem function (Ghilarov 2000). The relationships among components of an ecosystem are integral to the function of the ecosystem, but often not understood: removal of one component might affect another component (Godbold and Solan 2009). Biodiversity also has the potential for utilitarian uses, such as medicinal value yet to be discovered among unstudied species (Soejarto 1996). Global climate change threatens many species with extinction, thus reducing overall biodiversity (Thomas et al. 2004). Other threats to biodiversity include habitat degradation, fragmentation (Kruess and Tscharntke 1994), and destruction (Pimm and Raven 2000); overexploitation (Rosser and Mainka 2002); and introduction of non-native species (Hermoso et al. 2011; Clavero et al. 2009).

Amphibians are one of the most threatened groups of organisms worldwide (Stuart et al. 2004). According to the International Union for the Conservation of Nature (IUCN), 42% of amphibians for which there is sufficient data are listed as critically endangered, endangered, or vulnerable. Comparably, only 25% of mammals and 13% of birds are listed as such (IUCN 2016).

Amphibian populations can be monitored and used as a proxy for overall ecosystem health (Welsh and Ollivier 1998). Experimental evidence has shown that in some ecosystems, amphibians are a keystone species, meaning they have an influence on the ecosystem as a whole (Holomuzki, Collins, and Brunkow 1994; Wissinger et al. 1999). For example, both aquatic (Wissinger et al. 1999) and terrestrial amphibians help control insect populations (Beard et al. 2003). In other systems, amphibians play a role in nutrient cycling. Salamanders in deciduous forests prey on detritivorous insects within the leaf litter. Removal of salamanders causes increases in populations of these insects, which might influence carbon cycling in these ecosystems (Wyman 1998). Conservation of amphibian biodiversity also preserves the future utilitarian use of these organisms. For example, the skin secretions of waxy monkey frogs (*Phyllomedusa sauvagii*) produce a skin secretion with antibiotic properties against *Staphylococcus aureus* and could allow development of a prescription antibiotic for resistant *S. aureus* (Zhang et al. 2010).

Anecdotal evidence of declines in amphibian populations date to the 1970's when scientists noticed declines in populations of salamanders in Mexico and frogs in Australia, Brazil, Costa Rica, and the western United States. Many scientists reported dramatic declines in areas where amphibians were once abundant (Barinaga 1990; Blaustein and Wake 1990). Stochastic variation in populations might characterize the decline in some amphibian populations (Pechmann and Wilbur 1994), but many populations are experiencing anthropogenic-induced declines due to habitat destruction (Wyman 1990; Davidson, Shafffer, and Jennins 2002), fragmentation (Vos and Chardon 1998), and degradation (Delis, Mushinsky and McCoy 1996; Wyman 1990), as well as

the introduction of non-native predators (Moyle 1973; Bradford 1989). Declines in areas with little human-impact might be due to global climate change, pollution, ultraviolet radiation (Wyman 1990; Blaustein and Wake 1990), and the emergence of novel diseases such as ranavirus and chytridiomycosis (Daszak et al. 1999).

Chytridiomycosis has been implicated in the decline of over 200 species globally; specifically in Australia (Berger et al. 1998), Spain (Bosch, Martinez-Solano, and Garcia-Paris 2001), Mexico, and Guatemala (Cheng et al. 2011). It has been confirmed as a contributing factor in the extinction of multiple species including two gastric brooding frogs of Australia (Retallick, McCallum, and Speare 2004) 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 (Vredenburg et al. 2010) and Boreal Toad (*Bufo boreas*) in Colorado (Green & Muths 2005) has been attributed to chytridiomycosis.

Clinical signs of chytridiomycosis include excessive sloughing of skin (Berger et al. 1998), bloating (Parker et al. 2002), lethargy (Pessier et al. 1999), loss of righting ability (the ability to orient itself in a normal position if turned to the dorsum), reddening of the skin, and in rare cases gross skin lesions (Daszak et al. 1999). Two hypotheses as to how chytridiomycosis causes death have been proposed. One hypothesis is that molecular transport across the skin is inhibited (Pessier et al. 1999), therefore inhibiting osmoregulation, cutaneous respiration (Berger et al. 1998), and electrolyte balance (Voyles et al. 2007). The second hypothesis is that the pathogen that causes

chytridiomycosis might release a proteolytic enzyme that is then absorbed through the skin and causes tissue damage (Berger et al. 1998).

The causative agent of chytridiomycosis was first determined to be a fungus of the order chytridiales (chytrid fungi) in 1998 (Berger et al. 1998). This fungus was described in 1999 and named *Batrachochytrium dendrobatidis* (Longcore, Pessier and Nichols 1999). This pathogenic fungus is referred to as chytrid. A second pathogenic fungus, *Batrachochytrium salamandrivorans*, is now known to cause chytridiomycosis in salamanders, but was not the focus of this study (Martel et al. 2013).

Presence of chytrid has been shown to vary seasonally (Berger et al. 2004) and geographically (Ron 2005), with the Great Plains of the United States predicted to exhibit low probability of occurrence (Olson et al. 2013). This is likely due to the low thermal tolerance of chytrid; temperatures above 30°C (86°F) cause death (Piotrowski, Annis, and Longcore 2004). Due to this low thermal tolerance, anuran die-offs often occur in cooler months when chytrid is able to proliferate (Berger et al. 2004; Bradley et al 2002). This leads to development of chytridiomycosis. Many chytrid fungi, including the species pathogenic to anurans, are closely associated with water (Sparrow 1960). Chytrid is subject to desiccation after one hour, suggesting hot, dry climates are at low risk for epidemics of chytridiomycosis (Johnson et al. 2003).

Chytrid infects the keratin in anurans (Berger et al. 1998), and thus infects only the mouthparts of larvae and is not often fatal (Marantelli et al. 2004). After

metamorphosis and keratinization of the skin (Fox 1994), infection with chytrid becomes widespread and leads to the development of chytridiomycosis (Berger et al. 1998).

Infection load (Berger, Speare, and Hyatt 1999) and virulence of the strain of chytrid infecting an individual anuran vary (Berger et al. 2005), and therefore chytrid might be present, without subsequent development of the disease chytridiomycosis. Variation in response to chytrid is also observed among species: American Bullfrogs (*Lithobates catesbeianus*) (Daszak et al. 2004), Wood Frogs (*Lithobates sylvaticus*), Northern Leopard Frogs (*Lithobates pipiens*), and Spring Peepers (*Pseudacris crucifer*) show some resistance to development of chytridiomycosis despite presence of chytrid. These species might act as vectors for spread of chytrid (Gahl, Longcore, and Houlahan 2011). While a host might not be susceptible to disease from a native strain of chytrid, introduction of a novel strain, a strain new to the area, might cause development of chytridiomycosis (Gahl, Longcore, and Houlahan 2011).

Differences in aspects of life history and ecology might result in differences in chytrid occurrence among species of anurans. These life history aspects include selection of breeding sites, time to metamorphosis, habitat selection, and annual active cycle. For example, spadefoots (*Spea*) breed only in ephemeral pools (Gilmore 1924), while bullfrogs breed in permanent water (Bragg 1940). Spadefoots spend nine months of the year in deep underground burrows and emerge occasionally during summer months to breed and feed (Ruibal, Tevis, and Roig 1969), while bullfrogs are restricted to permanent water sources (Bragg 1940). These differences might lead to differences in chytrid occurrence among species.

Diagnostic assays of chytrid on anurans include histological examination of skin scrapings or toe clips by light or scanning-electron microscopy (Berger et al. 1998) and DNA characterization of swab or skin samples by use of real-time Taqman polymerase chain reaction (real-time PCR) (Boyle et al. 2004). In a study conducted by Hyatt et al. (2007), real-time PCR was judged the superior method of analysis from both swab and skin samples, and each sample type yielded similar results. Real-time PCR detects low levels and early stages of chytrid infection on amphibians (Boyle et al. 2004).

Chytrid has been detected in the states surrounding Kansas, including in Colorado (Green & Muths 2005), Oklahoma (Marhanka et al. 2017), Nebraska (31%) (Harner, Merlino, and Wright 2013), and Missouri (Bondinof et al. 2011). It has recently been detected in Sedgwick and Kingman counties in south-central Kansas, where 72.6% of samples tested positive for chytrid (McTaggart et al. 2014).

The goal of my project was to assess the presence of chytrid on Kansas anurans. The objectives were 1.) to collect samples from throughout the state of Kansas to assess the presence of chytrid on anurans and 2.) to assess aspects of anuran life history, specifically their association with water, that might influence the potential for infection with chytrid. I hypothesize that chytrid will be widespread in the state because it was detected at high frequencies in the few samples tested within the state and has been detected in the surrounding states. I hypothesize that anurans with a close association with water will exhibit an increased rate of infection because chytrid is closely associated with water and is subject to desiccation outside of water.

METHODS

Project Design

To assess chytrid occurrence on anurans in the state of Kansas, samples were collected at 57 sample locations during three sample seasons (Appendix 1). In the course of other research, Kansas Department of Wildlife, Parks, and Tourism (KDWPT) collected samples opportunistically from 41 sample locations in the Arkansas River basin and southeastern region of the state in 2015. In the spring and summer of 2016, I collected samples from 13 sample locations including 12 public lands: Cimarron National Grasslands, Clark State Fishing Lake, Farlington Fish Hatchery, Kirwin National Wildlife Refuge, Marais Des Cygnes National Wildlife Refuge, Quirvira National Wildlife Refuge, Scott State Park, St. Francis Wildlife Area, and Tallgrass Prairie National Preserve (TPNP); and one private ranch north of Hays: Hadley Ranch. After limited sampling success at Scott State Park, I collected samples at Concannon and Finney State Fishing Lakes and Wildlife Areas. In the spring of 2017, I collected samples from five sample locations including Tuttle Creek Wildlife Area, Wichita State University (WSU)-Youngmeyer Ranch, Benedictine Bottoms Wildlife Area, Jamestown Wildlife Area, and TPNP. I resampled TPNP in 2017 because high temperatures during my survey in 2016 might have inhibited ability to detect chytrid (Table 1), and anurans exhibited clinical symptoms of chytridiomycosis. I chose these sample locations because they are accessible to the public, provided good habitat for anurans, and provided samples from a broad distribution of sample locations throughout the state. These sample

locations present a sample bias, as chytrid might occur at higher frequencies in areas of public access than private lands because chytrid could be introduced to public areas through foot-traffic, boats, on live bait, water in livewells, and fishing gear.

To assess the effect of anuran life history on presence of chytrid, anurans were separated into four life history groups: xeric, arboreal, semi-aquatic, and aquatic species. I placed species in these groups based on their association with water in their breeding habitat selection and general habitat selection. Xeric species are species that breed in ephemeral pools and are fossorial. These species are most often found far from permanent water outside of the breeding season. These included Plains Spadefoots (*Spea bombifrons*) and Western Narrow-Mouthed Toads (*Gastrophryne olivacea*) (Smith 1934; Ruibal, Tevis, and Roig 1969). Arboreal species are those that occur in trees and shrubs, except during breeding season, when they are observed calling from trees, logs, under rocks, or are partially submerged in water (Smith 1934). These included Gray Treefrogs (*Hyla chrysoscelis/versicolor*). Semi-aquatic species are those that breed in temporary or permanent water and inhabit floodplains. These included Great Plains Toads (*Anaxyrus cognatus*), Woodhouse's Toads (*A. woodhousii*), and American Toads (*A. americanus*) (Bragg 1940). Aquatic species are those that breed in permanent water and inhabit permanent water. These included Plains and Southern Leopard Frogs (*Lithobates blairi* and *L. sphenocephalus*), American Bullfrogs (*L. catesbeianus*), and Blanchard's Cricket Frogs (*Acris blanchardi*) (Bragg 1940; Ruibal, Tevis, and Roig 1969).

I focused on collecting 30 individual anurans at each sample location; 10 from each life history group, if present to address the overall survey. However, to meet the

requirements for statistical analysis, I needed 50 samples from each group. To reduce the effect of sample location on chytrid occurrence, I limited these samples to one sample location. However, arboreal species are restricted in range to the eastern one-third of the state and xeric species are limited, at least in high abundances, to the western two-thirds of the state (Collins, Collins, and Taggart 2010). For this reason, I collected 50 samples from arboreal species at Farlington Fish Hatchery in the eastern one-third of the state, and 50 samples from xeric, semi-aquatic, and aquatic species at Hadley Ranch in the western two-thirds of the state.

I collected samples from a specific site within each sample location until I had either collected samples from all individuals present or had reached my targeted sample size. If my target sample size was not met at the first site within a sample location, I moved to another site within the location and continued to collect samples until the target sample size was met or all suitable sites were assessed.

Sample collection

At each sample location, I located anurans by call or by focusing on appropriate habitat such as streams and ponds. I used a standardized protocol for chytrid sampling, developed by Brem, Mendelson III, and Lips (2007), with modified swab preservation based on the recommendations of Hyatt et al. (2007). A field assistant captured individual anurans by hand or by use of a dip-net. Next, I swabbed the anuran with a 70-mm non-woven polyester swab (Grainger Inc.), focusing on areas of likely infection, including the fore feet, hind feet, thighs, and venter. I rubbed the swab five times across

each area: the fore foot, hind leg and foot, along the sides and around the cloaca. I placed the swab in a 15 ml polypropylene centrifuge tube (Grainger Inc.) without alcohol, as recommended by Hyatt, et al. (2007), and placed the anuran in a sterile, individual container to prevent re-sampling. My assistant and I then sterilized our hands with hand sanitizer. We used new latex gloves to handle each anuran. Swabs were stored at room temperature until analysis could be completed. At the conclusion of a sampling effort, I released anurans to the pond or stream from which they were captured. Then according to decontamination protocols, I removed mud from sampling equipment and vehicles. To kill chytrid and prevent spreading it between sample locations, I cleaned field equipment including nets, boots, waders, and containers in a 1:9 bleach solution and soaked it in the bleach solution overnight (Brem, Mendelson III, and Lips 2007).

Laboratory Analysis

I sent swab samples to Research Associates Laboratory (Dallas, TX) for real-time PCR analysis. This technique amplifies template deoxyribose nucleic acid (DNA) (in this case, chytrid DNA) by repeating the following three steps: 1.) denaturation: in which the solution is heated, and double-stranded template DNA is denatured and separated into two single strands. 2.) annealing: in which a primer, a short segment of complementary DNA, aligns to the template strand of DNA, and 3.) extension: in which a polymerase capable of withstanding extreme temperatures extends the primer to complement the template DNA. This process is repeated and results in many strands of the target DNA (Mullis and Faloona 1987). Real-time PCR adds a fluorescent dye, which binds only to double-stranded DNA, and is emitted after a single template strand has completed

extension (Heid et al. 1996). The fluorescent dye does not bind to single-stranded DNA, and because the solution is heated, only DNA which has been replicated during the reaction is double-stranded. This allows detection of target DNA after the reaction has been completed. Because a single template strand of DNA can be amplified using this technique, low levels of chytrid infection can be detected (Boyle et al. 2004). The lab provided me with positive or negative results for each sample.

RESULTS

Kansas Department of Wildlife, Parks and Tourism collected 409 samples from 41 sample locations in 2015. I collected 393 samples from 16 sample locations between 9 April 2016 and 6 July 2016. In 2017, I collected 133 samples from five sample locations between 8 April 2017 and 24 May 2017. In total, 935 samples were collected. Of these, 560 samples were analyzed from 30 sample locations across 12 species in Kansas (Appendix 1). I chose these samples for analysis based on location and species composition to provide results that were widely distributed across the state.

In total, 24 of 560 samples representing six of 28 sample locations tested positive for the presence of chytrid. Positive samples were taken from Marais Des Cygnes National Wildlife Refuge, Farlington Fish Hatchery, Hadley Ranch, Quivira National Wildlife Refuge, Tuttle Creek Wildlife Area, and Jamestown Wildlife Area (Figure 1, Table 2). Across species, chytrid was detected in Gray Treefrogs, Boreal Chorus Frogs, Woodhouse's Toads, Blanchard's Cricket Frogs, Plains Leopard Frogs, and American Bullfrogs. It was not detected in American Toads, Great Plains Toads, Plains Spadefoots, Southern Leopard Frogs, or Western Narrow-Mouthed Toads (Table 3). Of note, chytrid was not detected in either of two samples collected and analyzed from Spring Peepers at Crawford State Park. This species is listed as a Species In Need of Conservation (SINC) in Kansas. Across all samples, chytrid was detected in 4.3% of samples.

Among the four life history groups, in samples collected from Farlington Fish Hatchery and Hadley Ranch, chytrid was detected once in arboreal species at Farlington

Fish Hatchery and once in aquatic species at Hadley Ranch (Table 4). Chytrid was not detected among samples representing xeric or semi-aquatic species. Statistical analysis of a possible relationship between life history and occurrence of chytrid was not possible due to low chytrid occurrence (1%) at these sample locations.

DISCUSSION

Sample Efforts

Rainfall in the past three years impacted sample efforts. Prior to 2015, western Kansas experienced a severe drought (USDA, NDMC, and NOAA 2016). After increased rainfall in 2015 and 2016, xeric species were abundant during May and June in western Kansas, including at Hadley Ranch and Concannon Wildlife Area. Sample effort at Concannon Wildlife Area was improved by precipitation during the evening, and I was able to collect samples from Plains Spadefoots, whose emergence was triggered by rainfall. Increased rainfall may have hindered sample efforts at Clark State Fishing Lake and Quivira National Wildlife Refuge where anurans were detected calling, but could not be located because high water levels caused individuals to be widely dispersed or individuals were in water too deep to sample.

Chytrid Occurrence

I detected chytrid at six sample locations in Kansas. Among the four life-history groups, I expected aquatic species to exhibit an increased rate of chytrid occurrence because chytrid is closely associated with water. Due to the low occurrence of chytrid in samples collected to assess life history, I could not test this hypothesis. At sample locations where chytrid was detected, frequency of occurrence ranged from 0.7% to 90%. As such, location, instead of life history, might be a better predictor of chytrid occurrence in Kansas. However, due to limited sample collection from each location, I do not have data to test this observation. One sample location within the Gerber Preserve in

Sedgwick County, where chytrid was documented previously (McTaggart et al. 2014), but chytrid was not detected from this location during this study.

The Great Plains of the United States, including Kansas, were predicted to exhibit a low probability of chytrid occurrence (Olson et al. 2013). With an overall occurrence of 4.3%, my data support this hypothesis. While chytrid was detected at multiple sample locations in Kansas, I did not observe clinical symptoms of the disease chytridiomycosis or dead anurans at any of these sample locations. This indicates that while chytrid occurs in Kansas, I do not have evidence that it has a substantial negative impact on populations of anurans in Kansas at this time.

Anurans at multiple sample locations appeared in poor health. At Finney State Fishing Lake, many individuals were bloated and lethargic. These are symptoms of chytridiomycosis, however chytrid was not detected at this location. Anurans at Tallgrass Prairie National Preserve also appeared in poor health; many were lethargic, had macroscopic ectoparasites parasites, or had not developed both hind legs. Chytrid was also not detected at this location. In North America, abnormal limb development in anurans has been associated with trematode infections (Johnson et al. 1999) and this might be the cause of limb abnormalities at Tallgrass Prairie National Preserve. At Marais Des Cygnes National Wildlife Refuge two of twelve individuals in which chytrid was detected also had parasite infections. Parasites might have caused an increase in stress in these individuals and subsequently decreased immune response, resulting in continued presence of chytrid.

With increased rainfall in May 2015, anurans have had the opportunity to disperse. In the course of this study, I observed recent dispersal as the presence of Gray Treefrogs at Jamestown Wildlife Area, where this species had not previously been documented. Anuran dispersal might spread chytrid to locations where it has previously been absent, or introduce a strain to which the local population has no natural resistance. Chytrid was detected at Jamestown Wildlife Area in 2017 on one Boreal Chorus Frog and two Blanchard's Cricket Frogs. While chytrid was not detected in samples collected from Gray Treefrogs at this location, continued presence of Gray Treefrogs at a location from which chytrid is known to occur might allow this species to contract chytrid. As Gray Treefrogs and other species continue to disperse, they might act as a vector for spread of chytrid to surrounding areas.

Human-mediated dispersal of anurans might also impact chytrid occurrence in Kansas. Larval amphibians are often used as bait by fishermen. If these amphibians are infected with chytrid, its dispersal seems likely. Chytrid might also disperse through foot-traffic and boat traffic. Chytrid decontamination protocols are not followed by the public, and as such, boats, waders, boots and fishing gear could harbor chytrid and allow chytrid to disperse.

As the global climate changes, future environmental conditions might support increased chytrid occurrence on Kansas anurans. Global climate change is predicted to increase surface temperatures and increase the number of extreme precipitation events per year (IPCC 2014). Extreme precipitation events or drought could affect the presence of chytrid in the environment, as chytrid is closely associated with water. Drought might

decrease the size or abundance of water resources and suitable habitat for chytrid in an area, but also result in increased densities of anurans at remaining water sources. This might lead to increased interaction between individual anurans, and increased potential for anurans to contract chytrid. Anurans might also develop chytridiomycosis as stress from factors such as drought or parasite infection decreases their natural resistance to chytrid.

Real-time PCR is not always 100% accurate. Though it is unlikely that the molecular assay will give a false positive, a false positive might arise from sample contamination during laboratory analysis, technician error, or contamination during sample collection (Brem, Mendelson, and Lips 2007). Quality control of PCR analysis in a study concerning hepatitis suggested that false positives occur with a frequency of 6.8×10^{-3} (Bogard et al. 1997). In a study concerning tuberculosis, false positives were detected at 0 – 40% (Noordhoek, Embden, and Kolk 1996). There is an overall lack of studies regarding the incidence of false positives (Borst, Box, and Fluit 2004) and information regarding frequency of false positives in chytrid surveys was not available. At each location with a low frequency of occurrence of chytrid in my study, chytrid was detected in only one sample. As such, this might indicate inaccuracies in PCR analysis. Hadley Ranch (relative frequency (f) = 0.7%, sample size (n) = 147), Farlington Fish Hatchery (f = 2.2%, n = 45), and Tuttle Creek Wildlife Area (f = 3.1%, n = 31) might have falsely tested positive for chytrid, and continued research should be conducted to ensure the accuracy of occurrence.

While a false positive might occur, it is far more likely that a false negative occur. False negatives occur when swab samples are not collected from all anurans at a sample location and infected individuals are not sampled, the infected area of an individual anuran is not swabbed during sample collection, or chytrid present on a swab fails to be included in the solution used for PCR. At each sample location, it is possible I did not collect a swab sample from an individual that does have chytrid. It is also possible I did not swab the infected area of the amphibian. Lastly, due to laboratory procedures, it is possible chytrid present on a swab does not become part of the solution used for PCR. During DNA extraction, swab samples are dipped in a buffer solution and this solution is heated. Then a small portion of this solution is used for PCR. It is therefore possible chytrid present on a swab sample is not in the portion of solution used for PCR. This is particularly likely at low levels of chytrid infection.

Future Research

Continued monitoring of Kansas anurans is needed. Sample locations at which chytrid has been detected should be monitored annually by use of anuran call surveys to assess populations. Population declines might indicate the presence of chytrid and its negative impact, particularly during summer months when chytrid is not likely to be detected due to high temperatures. Sample locations at which populations were in poor health should also be monitored for continued abnormalities, as continued stress might decrease natural resistance to the development of chytridiomycosis, should chytrid occur

within these populations. A swab survey should be conducted at a sample location if population declines are noted through anuran call surveys. When conducting research at sample locations from which chytrid is known to occur, chytrid decontamination protocols should be maintained to prevent possible spread of chytrid, even if chytrid surveys are not conducted.

Swab surveys for chytrid should be conducted systematically to monitor chytrid presence throughout the state and ensure the health of populations in the future. Priority should be on 1.) sample locations at which chytrid was detected with low frequency, 2.) sample locations at which chytrid was detected and in the surrounding area, and 3.) remaining previously sampled locations.

Sample locations with low frequency of occurrence include Farlington Fish Hatchery, Hadley Ranch, and Tuttle Creek Wildlife Area. Low frequency of chytrid occurrence might indicate inaccurate PCR results, and these sample locations should be resampled to ensure presence of chytrid.

In my study, sample location appeared to be an important variable in chytrid occurrence. The remaining areas where chytrid was detected include Jamestown Wildlife Area (f = 9.4%, n = 33), Quivira National Wildlife Refuge (f = 24%, n = 25), and Marais des Cygnes National Wildlife Refuge (f (2015) = 33%, n = 9; f (2016) = 90%, n = 10). These sample locations, and the closely surrounding area should be monitored for changes in occurrence of chytrid.

Continued sample collection at all previously sampled locations might allow statistical analysis to determine if location influences the occurrence of chytrid. This would also allow comparison between surveys to monitor changes in occurrence.

Sample collection should be conducted in spring and fall, when chytrid is more likely to be detected rather than during summer when high temperatures might inhibit or eliminate chytrid infection on anurans. If new sample locations are to be assessed, areas of public access should be targeted for sample location. Areas of public access might have an increased likelihood of chytrid occurrence because boats, bait, boots, and waders might act as vectors for chytrid movement. Sample locations where chytrid was detected with high frequency (9% or greater) were all wetlands. This indicates wetland habitat might present an increased occurrence of chytrid. As such, wetland habitat should also be targeted for chytrid surveys.

Conclusions

As predicted in previous studies, my data suggest chytrid occurrence in Kansas is low. Overall, chytrid was detected in 4.3% of samples. At sample locations where chytrid was detected, I did not observe anurans that exhibited clinical symptoms of chytridiomycosis. Despite the presence of chytrid, I do not have evidence that it is negatively impacting populations of anurans in Kansas at this time. As global climate changes, chytrid might pose a stronger threat to Kansas anurans. I suggest monitoring for signs of chytridiomycosis and systematic surveys for chytrid to ensure the effective conservation of anurans in Kansas.

LITERATURE CITED

Baringa, M. 1990. Where have all the froggies gone?. Science 247:1033+.

Beard, K.H., Eschtruth, A.K., Vogt, K.A., Vogt, D.J., and Scatena, F.N. 2003. The effect of the frog *Eleuthrodactylus coqui* on invertebrates and ecosystem processes at two scales in the Luquillo Experimental Forest, Puerto Rico. Journal of Tropical Ecology 19:607-617.

Berger, L., Speare, R., Daszak, P., Green, E.D., Cunningham, A.A., Goggin, L.C., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., and Parkes, H. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Population Biology 95:9031-9036.

Berger, L, Speare, R., and Hyatt, A. 1999. Chytrid fungi and amphibian declines: overview, implications and future directions. pp.23-33 in Campbell, A (ed.), Declines and Disappearances of Australian frogs, Environment Australia, Commonwealth of Australia.

Berger, L., Speare, R., Hines, H.B., Marantelli, G., Hyatt, A.D., McDonal, K.R., Skerratt, L.F., Olsen, V., Clarke, J.M., Gillespie, G., Mahony, M., Sheppard, N., Williams, C., and Tyler, M.J. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Australian Veterinary Journal 82:31-36.

Berger, L., Marantelli, G., Skerratt, L.F., and Speare, R. 2005. Virulence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* varies with the strain. Diseases of Aquatic Organisms 68:47-50.

Blaustein, A.R. and Wake, D.B. 1990. Declining amphibian populations: a global phenomenon?. Trends in Ecology and Evolution 5:203-204.

Bogard, M., Buffet-Janvresse, C., Cnataloube, J.F., Biagini, P., Duverlie, G.,Castelain, S., Izopet, J., Martinot-Peignous, M., Halfron, P., Gerolami, V., Frangeul, L., Pawlotsky, J.M., Roudot-Thoraval, F., Dussaix, E., Loiseau, P., Ravera, N., Lewin, P., Lamoril, J., Lerable, J., Bourchardeau, F., Laperche, S., Mariotti, M., Lefrere, J.J., Nicot, T., Denis, F., Gassain, M., Merel, P., Trepo, C., Thiers, V., Brechot, C., Ferec, C. Mercier, B., Stoll-Keller, F., Palmer, P., and Lebon, P. 1997. GEMHEP multicenter quality control study of PCR detection of GB virus c/hepatitis G virus RNA in serum. Journal of Clinical Microbiology 35:3298-3300.

Bondinof, C.M., Briggler, J.T., Duncan, M.C., Beringer, J., Millspaugh, J.J. 2011. Historic occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in hellbender *Cryptobranchus alleganiensis* populations from Missouri. Diseases of Aquatic Organisms 96:1-7.

Borst, A., Box, A.T.A., and Fluit, A.C. 2004. False-Positive results and contamination in nucleic acid amplification assays; suggestions for prevent and destroy strategy. European Journal of Clinical Microbiology and Infectious Diseases 23:289-299.

Bosch, J, Martínez-Solano, I., and García-París, M. 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. Biological Conservation 97:331-337.

Boyle, D. G., Boyle, D. B., Olsen, V., Morgan, J.A.T., and Hyatt, A.D. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Diseases of Aquatic Organisms 60: 141-148.

Bradford, D.F. 1989. Allotopic distribution of native frogs and introduced fishes in high Sierra Nevada lakes of California: implications of the negative effect of fish introductions. Copeia 1989:775-778.

Bradley, G.A., Rosen, P.C., Sredl, M.J., Jones, T.R. and Longcore, J.E. 2002. Chytridiomycosis in Native Arizona Frogs. Journal of Wildlife Diseases 38:206-212.

Bragg, A.N. 1940. Observations on the ecology and natural history of anura. iii. the ecological distribution of anura of Cleveland County, Oklahoma, including notes on the habits of several species. The American Midland Naturalist 24:322-335.

Brem, F., Mendelson III, J. R., and Lips, K.R. 2007. Field-sampling protocol for *Batrachochytrium dendrobatidis* from living amphibians, using alcohol preserved swabs. Version 1.0 (18 July 2007), 10pp. Conservation International, Arlington, Virginia, USA. URL [http://www.amphibianark.org/pdf/Field%20sampling%20protocol%20for%20amphibian%20chytrid%20fungi%201.0.pdf] Date accessed [March 2015].

Cheng, T.L., Rovito, S.M., Wake, D.B., and Vredenburg, V.T. 2011. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. Proceedings of the National Academy of Sciences 108:9502-9507.

Clavero, M., Brontons, L., Pons, P., and Sol, D. 2009. Prominent role of invasive species in avian biodiversity loss. Biological Conservation 142:2043-2049.

Collins, J.T., Collins, S., Taggart, T. 2010. Amphibians, reptiles, and turtles in Kansas. Eagle Mountain Publishing, LC, Eagle Mountain, Utah.

Daszak, P., Berger, L., Cunningham, A.A., Hyatt, A.D., Green, D.E., and Speare, R. 1999. Emerging infectious diseases and amphibian population declines. Emerging Infectious Diseases 5:735-748.

Daszak, P., Strieby, A., Cunningham, A.A., Longcore, J.E., Brown, C.C., and Porter, D. 2004. Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, and emerging fungal disease of amphibians. Herpetological Journal 14:201-207.

Davidson, C., Shaffer, H.B., and Jennins, M.R. 2002. Spatial tests for the pesticide drive, habitat destruction, UV-B, and climate change hypotheses for California amphibian declines. Conservation Biology 16:1588-1601.

Delis, P.R., Mushinsky, H.R., and McCoy, E.D. 1996. Decline of some west-central Florida anuran populations in response to habitat degradation. Biodiversity and Conservation 5:1579-1595.

Fox, H. 1994. The structure of the integument. pp. 4 in Heatwole, H., Barthalamus, G.T., and Heatwole, A.Y. (eds.), Amphibian Biology: The integument, Surrey Beatty & Sons PTY Limited, Chipping Norton, New South Wales, Australia.

Gahl, M.K., Longcore, J.E., and Houlahan, J.E. 2011. Varying responses of Northeastern North American amphibians to the chytrid pathogen *Batrachochytrium dendrobatidis*. Conservation Biology 26:135-141.

Ghilarov, A.M. 2000. Ecosystem functioning and intrinsic value of biodiversity. Oikos. 90:408-412.

Gilmore, R.J. 1924. Notes on the life history and feeding habits of the spadefoot toad of the Western Plains. Colorado College Publication Science Series 13:1-12.

Green, D.E. and Muths, E. 2005. Health evaluation of amphibians in and near Rocky Mountain National Park (Colorado, USA). Alytes 22:109-129.

Godbold, J.A. and Solan, M. 2009. Relative importance of biodiversity and the abiotic environment in mediating an ecosystem process. Marine Ecology Progress Series 396:281-290.

Harner, M.J., Merlino, J.N., and Wright, G.D. 2013. Amphibian chytrid fungus in Woodhouse's Toads, Plains Leopard Frogs, and American Bullfrogs along the Platter River, Nebraska, USA. Herpetological Review 44:469-461.

Heid, C.A., Stevens, J., Livak, J.J., and Williams, P.M. 1996. Real time quantitative PCR. Genome Research 6:986-994.

Hermoso, V., Clavero, M., Blanco-Garrido, F., and Prenda, J. 2011. Invasive species and habitat degradation in Iberian streams; an analysis of their role in freshwater fish diversity loss. Ecological applications 21:175-188.

Holomuzki, J.R., Collins, J.P., and Brunkow, P.E. 1994 Trophic control of fishless ponds by tiger salamander larvae. Oikos 71: 55-64.

Hooper, D.U., Chapin III, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A.J., Vandermeer, J., and Wardle, D.A. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75:3-35.

Hyatt, A.D., Boyle, D.G., Olsen, V., Boyle, D.B., L. Berger, Obendorf, D., Dalton, A., Kriger, K., Hero, M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Gleason, F., and Colling, A. 2007. Diagnostic assays and sampling protocols for

the detection of *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 73:175-192.

IPCC, Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change-Core Writing Team. 2014. Climate Change 2014: Synthesis Report. R.K. Pachauri and L.A. Meyer (eds.), Geneva, Switzerland, 151 pp.

IUCN. 2016. Summary statistics. URL [http://cmsdocs.s3.amazonaws.com/summarystats/2016-3_Summary_Stats_Page_Documents/2016_3_RL_Stats_Table_1.pdf]. Date accessed [6 April 2017].

Johnson, M.L., Berger, L., Philips, L., and Speare, R. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 57:255-260.

Johnson P.T.J., Lunde, K.B., Ritchie, E.G. and Launer, A.E.1999. The effect of trematode infection on amphibian limb development and survivorship. Science 284:802-804.

Knapp, R.A. and Matthews, K.R. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. Conservation Biology 14:428-438.

Kruess, A., and Tscharntke, T. 1994. Habitat fragmentation, species loss, and biological control. Science 264: 1581-1584.

Longcore, J.E., Pessier, A.P., and Nichols, D.K. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219-227.

Marantelli, G., Berger, L., Speare, R., and Keegan, L. 2004. Distribution of the amphibian chytrid *Batrachochytrium dendrobatidis* and keratin during tadpole development. Pacific Conservation Biology 10:173-179.

Marhanka, E.C., Watters, J.L., Huron, N.A., McMillin, S.L., Winfrey, C.C., Curtis, D.J., Davis, D.R., Farkas, J.K., Kerby, J.L., and Siler, C.D. 2017. Detection of high prevalence of *Batrachochytrium dendrobatidis* in amphibians from southern Oklahoma, USA. Herpetological Review 48:70-74.

Martel, A., Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M.C., Woeltjes, A., Bosman, W., Chiers, K., Bossuyt, F., and Pasmands, F. 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. Proceedings of the National Academy of Sciences 110:15325-15329.

McTaggart, A.L., Eberl, T., Keller, O. and Jameson, M.L. 2014. First report of *Batrachochytrium dendrobatidis* associated with amphibians in Kansas. Herpetological Review 45:439–441.

Moyle, P.B. 1973. Effects of introduced bullfrogs, *Rana catesbeiana*, on the native frogs of the San Joaquin Valley, California. Copeia 1973:18-22.

Mullis, K.B., Faloona, F.A. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods in enzymology 155:335-350.

Noordhoek, G.T., Embden, J.D., Kolk, A.H.J. 1996. Reliability of nucleic acid amplification for detection of *Mycobacterium tuberculosis*: an international collaborative quality control study among 30 laboratories. Journal of Clinical Microbiology 34:2522-2525.

Olson, D.H, Aanensen, D.M., Ronnenberg, K.L., Powell, C.I., Walker, S.F., Bielby, J., Garner, T.W.J., Weaver, G., the *Bd* mapping group, and Fisher, M.C. 2013. Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. PLoS One 8:1-13.

Parker, J.M., Mikaelian, I., Hahn, N., and Diggs, H.E. 2002. Clinical diagnosis and treatment of epidermal chytridiomycosis in African Clawed Frogs (*Xenopus tropicalis*). Comparative Medicine 52:265-268.

Pechmann, J.H.K. and Wilbur, H.M. 1994. Putting declining amphibian populations in perspective: natural fluctuations and human impacts. Herpetologica 50:65-84.

Pessier, A.P., Nichols, D.K., Longcore, J.E., and Fuller, M.S. 1999. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's trees frogs (*Litoria caurulea*). Journal of Veterinary Diagnostic Investigation. 11:194-199.

Pimm, S.L. and Brooks, S.M. 1997. The sixth mass extinction; how large, where, and when?. pp. 46-62 in Raven, P.H. and Williams, T. (eds.), Nature and human society; the quest for a sustainable world, National Academy Press, Washington, DC.

Pimm, S.L. and Raven, P. 2000. Extinction by the numbers. Nature 403:843-845.

Piotrowski, J.S., Annis, S.L., and Loncore, J.E. 2004 Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9-15.

Retallick, R.W.R., McCallum, H., and Speare, R. 2004. Endemic infection of the amphibian chytrid fungus in a frog community post-decline. PLoS Biology 2:11.

Ron, S.R. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the new world. Biotropica 37:209-221.

Rosser, A.M. and Mainka, S.A. 2002. Overexploitation and species extinctions. Conservation Biology 16:584-586.

Ruibal, R., Tevis Jr., L, and Roig, V. 1969. The terrestrial ecology of the spadefoot toad *Scaphiopus hammondii*. Copeia 1969:571-584.

Skerratt, L.F., Berger, L., Speare, R., Cashins, S., McDonald, K.R., Phillott, A.D., Hines, H.B., and Kenyon, N. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125-134.

Smith, H.M. 1934. The amphibians of Kansas. The American Midland Naturalist 15:377-527.

Soejarto, D.D. 1996. Biodiversity prospecting and benefit-sharing; perspectives from the field. Journal of Ethnopharmacology 51:1-15.

Sparrow, F. 1960. Introduction. pp 16-21. in Aquatic Phycomycetes, The University of Michigan Press, Ann Arbor, Michigan.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., and Waller, R.W. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783-1786.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., Ferreira de Siqueira, M., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L., and Williams, S.E. 2004. Extinction risk from climate change. Nature 427:145-148.

United States Department of Agriculture (USDA), National Drought Mitigation Center (NDMC), and National Oceanic and Atmospheric Association (NOAA). 2016. United States Drought Monitor. Accessible at http://droughtmonitor.unl.edu/Home.aspx. Accessed 12 September 2016.

Vos, C.C. and Chardon, J.P. 1998. Effects of habitat fragmentation and road density on the distribution pattern of the moor frog *Rana arvalis*. Journal of Applied Ecology 35:44-56.

Voyles, J., Berger, L., Young, S., Speare, R., Webb, R., Warner, J., Rudd, D., Campbell, R., and Skerratt, L.F. 2007. Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. Diseases of Aquatic Organisms 77:113-118.

Vredenburg, V.T., Knapp, R.A., Tunstall, T.S., and Briggs, C.J. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proceeding of the National Academy of Sciences 107:9689-9694.

Welsh, H.H. and L.M. Ollivier. 1998. Steam amphibians as indicators of ecosystem stress: a case study from California's redwoods. Ecological Applications. 8:1118-1132.

Wissinger, S.A., Whiteman, H.H., Sparks, G.B., Rouse, G.L., and Brown, W.S. 1999. Foraging trade-offs along a predator-permanence gradient in subalpine wetlands. Ecology 80:2102-2116.

Wyman, R.L. 1990. What's Happening to the amphibians?. Conservation Biology.

Wyman, R.L. 1998. Experimental assessment of salamanders as predators of detrital food webs; effects on invertebrates, decomposition, and the carbon cycle. Biodiversity and Conservation 7:641-650.

Zhang, R., Zhou, M., Wang, L., McGarth, S., Chen, T., Chen, X., and Shaw, C. 2010. Phylloseptin-1 (PSN-1) from *Phyllomedusa sauvagei* skin secretion: a novel broad-spectrum antimicrobial peptide with antibiofilm activity. Molecular Immunology 47:2030-2037.

TABLES

Table 1: Dates of sample collection at sample locations during spring 2016- spring 2017 to assess the presence of anuran chytrid in Kansas, with maximum day time temperatures as recorded by the National Weather Service.

Sample Location	Date	Recorded High Temperature from Day (°C)
Quivira National Wildlife Refuge	09 April 2016	23.9
Hays	16 April 2016	16.7
Marias Des Cygnes National Wildlife Refuge	09 May 2016	No Record
Marias Des Cygnes National Wildlife Refuge	20 May 2016	No Record
Marias Des Cygnes National Wildlife Refuge	21 May 2016	No Record
Scott State Park	25 May 2016	30.0
Scott State Park	26 May 2016	30.6
Saint Francis Wildlife Area	27 May 2016	17.2
Fort Hays State	28 May 2016	25.6
Hadley Ranch	31 May 2016	22.8
Hadley Ranch	01 June 2016	23.9
Hadley Ranch	02 June 2016	26.7
Quivira National Wildlife Refuge	03 June 2016	30
Quivira National Wildlife Refuge	04 June 2016	28.9
Kirwin National Wildlife Refuge	14 June 2016	26.1
Farlington Fish Hatchery	16 June 2016	No Record
Clark State Fishing Lake	21 June 2016	27.2
Cimarron National Grasslands	22 June 2016	28.9
Finney County State Fishing Lake	23 June 2016	30.0
Concannon State Fishing Lake	23 June 2016	30.0
Clark State Fishing Lake	28 June 2016	27.2
St. Francis Wildlife Area	29 June 2016	32.2
Tallgrass National Prairie Preserve	06 July 2016	32.8
Tuttle Creek Wildlife Area	08 April 2017	26.7
KHS	22 April 2017	17.2
Tallgrass National Prairie Preserve	23 April 2017	20.6
Youngmeyer Ranch	22 May 2017	28.9
Tallgrass National Prairie Preserve	23 May 2017	27.8
Benedictine Bottoms	23 May 2017	18.9
Warknock Lake- Forest of Friendship	24 May 2017	20.6
Jamestown Wildlife Area	24 May 2017	19.4

Table 2: Results of real-time polymerase chain reaction (PCR) analysis of swab samples collected in Kansas spring 2015- spring 2017 and tested for presence of anuran chytrid.

Field Season/ Sample Location	Number Positive	Sample Size	Percent Positive (%)
2015 Field Season	**3**	**110**	**2.8**
Byron Walker Wildlife Area	0	32	0
American Bullfrog	0	8	0
Blanchard's Cricket Frog	0	16	0
Plains Leopard Frog	0	8	0
Crawford State Park	0	28	0
American Bullfrog	0	1	0
American Toad	0	8	0
Blanchard's Cricket Frog	0	5	0
Gray Treefrog Complex	0	9	0
Plains Leopard Frog	0	1	0
Spring Peeper*	0	2	0
Southern Leopard Frog	0	2	0
Marais Des Cygnes Wildlife Refuge	3	9	33.3
Blanchard's Cricket Frog	3	9	42.9
Medicine Lodge River	0	9	0
Blanchard's Cricket Frog	0	7	0
Plains Leopard Frog	0	2	0
Mule Creek	0	5	0
Woodhouse's Toad	0	5	0
Neosho State Fishing Lake	0	6	0
American Toad	0	2	0
Blanchard's Cricket Frog	0	1	0
Southern Leopard Frog	0	3	0
Neosho Wildlife Area	0	2	0
Blanchard's Cricket Frog	0	1	0
Southern Leopard Frog	0	1	0

Table 2. (continued)

Field Season/ Sample Location	Number Positive	Sample Size	Percent Positive (%)
NF Walnut Creek	0	5	0
Great Plains Toad	0	1	0
Plains Leopard Frog	0	3	0
Woodhouse's Toad	0	1	0
Rattlesnake Creek	0	5	0
Plains Leopard Frog	0	5	0
Smoots Creek, WSU Gerber Preserve	0	1	0
Blanchard's Cricket Frog	0	1	0
South of Nashville	0	2	0
Blanchard's Cricket Frog	0	1	0
Plains Leopard Frog	0	1	0
Spring Creek	0	6	0
Blanchard's Cricket Frog	0	5	0
Plains Leopard Frog	0	1	0
2016 Field Season	**17**	**320**	**5.3**
Cimarron National Grasslands	0	7	0
American Bullfrog	0	4	0
Woodhouse's Toad	0	3	0
Clark State Fishing Lake	0	9	0
American Bullfrog	0	9	0
Concannon State Fishing Lake and Wildlife Area	0	18	0
Great Plains Toad	0	4	0
Plains Spadefoot	0	11	0
Woodhouse's Toad	0	3	0
Farlington Fish Hatchery	1	45	2.2
Gray Treefrog Complex	1	45	2.2
Finney State Fishing Lake	0	10	0
Plains Leopard Frog	0	9	0
Woodhouse's Toad	0	1	0

Table 2. (Continued)

Field Season/ Sample Location	Number Positive	Sample Size	Percent Positive (%)
Hadley Ranch	1	147	0.7
Blanchard's Cricket Frog	1	3	33.3
Boreal Chorus Frog	0	1	0
Plains Leopard Frog	0	40	0
Plains Spadefoot	0	6	0
Western Narrow-Mouthed Toad	0	55	0
Woodhouse's Toad	0	42	0
Hays	0	2	0
Great Plains Toad	0	1	0
Plains Spadefoot	0	1	0
Kirwin National Wildlife Refuge	0	12	0
Blanchard's Cricket Frog	0	10	0
Woodhouse's Toad	0	2	0
Marais De Cygnes National Wildlife Refuge	9	10	90.0
Blanchard's Cricket Frog	9	10	90.0
Quivira National Wildlife Refuge	6	25	24.0
American Bullfrog	3	10	30.0
Blanchard's Cricket Frog	1	1	100.0
Great Plains Toad	0	1	0
Plains Leopard Frog	1	8	12.5
Woodhouse's Toad	1	5	20.0
Scott State Park	0	10	0
American Bullfrog	0	10	0
St. Francis Wildlife Area	0	10	0
American Bullfrog	0	1	0
Plains Leopard Frog	0	9	0
Tallgrass Prairie National Preserve	0	15	0
American Bullfrog	0	2	0
Blanchard's Cricket Frog	0	2	0
Gray Treefrog Complex	0	5	0
Plains Leopard Frog	0	6	0

Table 2. (Continued)

Field Season/ Sample Location	Number Positive	Sample Size	Percent Positive (%)
2017 Field Season	**4**	**133**	**3.0**
Benedictine Bottoms	0	31	0
American Toad	0	1	0
Blanchard's Cricket Frog	0	12	0
Boreal Chorus Frog	0	7	0
Gray Treefrog Complex	0	10	0
Plains Leopard Frog	0	1	0
Jamestown Wildlife Area	3	32	9.4
American Bullfrog	0	1	0
Blanchard's Cricket Frog	1	4	25.0
Boreal Chorus Frog	2	14	14.3
Gray Treefrog Complex	0	9	0
Plains Leopard Frog	0	3	0
Woodhouse's Toad	0	1	0
Tallgrass Prairie National Preserve	0	18	0
Blanchard's Cricket Frog	0	18	0
Tuttle Creek Wildlife Area	1	31	03.2
Blanchard's Cricket Frog	1	5	20.0
Boreal Chorus Frog	0	25	0
Gray Treefrog Complex	0	1	0
WSU- Youngmeyer Ranch	0	21	0
Blanchard's Cricket Frog	0	20	0
Western Narrow-Mouthed Toad	0	1	0
Grand Total	**24**	**563**	**4.3**

* Species In Need of Conservation (SINC) in Kansas

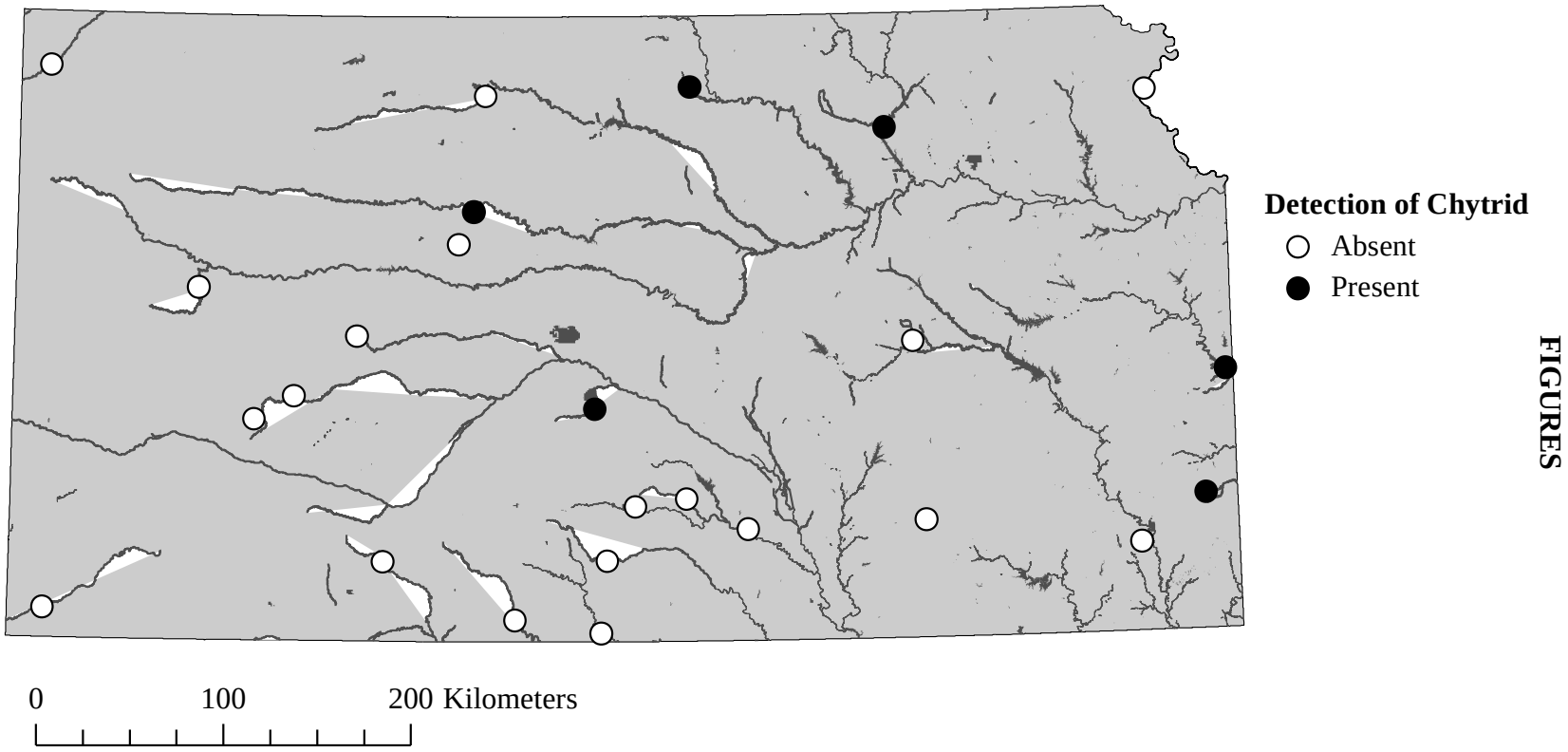
Table 3: Results of anuran chytrid samples collected from 12 species of anurans in Kansas during spring 2015- spring 2017 and analyzed by use of real-time PCR.

Species	Number Positive	Sample Size	Percent Positive (%)
American Bullfrog	3	46	6.5
American Toad	0	11	0
Blanchard's Cricket Frog	16	129	12.4
Boreal Chorus Frog	2	47	4.3
Gray Treefrog Complex	1	79	1.3
Great Plains Toad	0	7	0
Plains Leopard Frog	1	96	1.0
Plains Spadefoot	0	18	0
Southern Leopard Frog	0	6	0
Spring Peeper	0	2	0
Western Narrow-Mouthed Toad	0	56	0
Woodhouse's Toad	1	63	1.6
Total	**24**	**560**	**4.3**

Table 4. Results of real-time PCR analysis across six species exhibiting four distinct life histories. Samples were collected from Hadley Ranch and Farlington Fish Hatchery during spring 2016.

Life History Group/Species	Number Positive	Sample Size	Percent Positive (%)
Xeric Species	**0**	**61**	**0**
Western Narrow-Mouthed Toad	0	55	0
Plains Spadefoot	0	6	0
Arboreal Species	**1**	**45**	**2.2**
Gray Treefrog Complex	1	45	0.022
Semi-aquatic Species	**0**	**42**	**0**
Woodhouse's Toad	0	42	0
Aquatic Species	**1**	**43**	**2.3**
Blanchard's Cricket Frog	1	3	0.333
Plains Leopard Frog	0	40	0
Total	**2**	**191**	**1.0**

Figure 1: Detection of chytrid in samples analyzed by use of real-time PCR at 25 sample locations in Kansas.



APPENDICES

Appendix A. Global Positioning System (GPS) coordinates for sample locations at which chytrid samples were collected spring 2015 – spring 2017 to assess the presence of anuran chytrid in Kansas.

Sample Location	Site Name	Latitude	Longitude	Site Status
Amber Creek	AMCR-001	37.388	-98.595	NA
Amber Creek	AMCR-002	37.447	-98.617	NA
Arc River Hutchinson	LAH-001	38.027	-97.926	NA
Arc River Hutchinson	LAH-002	38.057	-97.994	NA
Arc River Hutchinson	LAH-003	38.071	-97.967	NA
Arkansas River	ARK11-001	38.194	-98.271	NA
Bagdad Road	BARD-001	37.025	-94.620	NA
Benedictine Bottoms	Forest of Friendship	39.533	-95.149	Negative
Benedictine Bottoms	South of Office	39.593	-95.077	Negative
Byron Walker Wildlife Area	BWWA-001	37.645	-98.287	Negative
Byron Walker Wildlife Area	BWWA-002	37.646	-98.255	Negative
Byron Walker Wildlife Area	BWWA-003	37.650	-98.258	Negative
Cimarron National Grasslands	Cimarron Recreation Area	37.136	-101.825	Negative
Clark State Fishing Lake	Creek	37.406	-99.784	Negative
Clark State Fishing Lake	Outflow	37.381	-99.783	Negative
Clearwater Creek	CLCR-001	37.562	-97.634	NA
Clearwater Creek	CLCR-002	37.576	-97.635	NA
Clearwater Creek	CLCR-003	37.591	-97.635	NA
Concannon State Fishing Lake and Wildlife Area	AOR1	38.067	-100.557	Negative
Concannon State Fishing Lake and Wildlife Area	AOR2	38.076	-100.554	Negative
Concannon State Fishing Lake and Wildlife Area	AOR3	38.137	-100.554	Negative
Concannon State Fishing Lake and Wildlife Area	AOR4	38.057	-100.555	Negative
Concannon State Fishing Lake and Wildlife Area	AOR5	38.046	-100.555	Negative
Concannon State Fishing Lake and Wildlife Area	AOR6	38.031	-100.555	Negative
Concannon State Fishing Lake and Wildlife Area	Waterhole	38.062	-100.572	Negative
Crawford State Park	CSP-003	37.648	-94.805	Negative
Crawford State Park	CSP-004	37.644	-94.805	Negative
Crawford State Park	CWA-001	37.649	-94.806	Negative
CREP Ellinwood	CREPE-001	38.313	-98.496	NA
CREP Kinsley	CREPK-001	37.930	-99.374	NA
East Pleasanton Lake	East Pleasanton Lake	38.190	-94.694	NA

Sample Location	Site Name	Latitude	Longitude	Site Status
Elm Creek	ELCR-001	37.276	-98.573	NA
Elm Creek	ELCR-002	37.388	-98.610	NA
Elm Creek Trib.	ELCT-002	37.367	-98.538	NA
Elm Creek Trib. Medicine Lodge Park	ELCT-001	37.278	-98.575	NA
Farlington Fish Hatchery	FFH1	37.648	-94.805	Positive
Farlington Fish Hatchery	FFH2	37.650	-94.807	Negative
FHSU	Animal House	38.873	-99.355	NA
FHSU	Ephemeral Pool	38.874	-99.348	NA
Finney State Fishing Lake	Boat Ramp	38.174	-100.333	Negative
Hadley Ranch	DR	39.071	-99.238	Negative
Hadley Ranch	HR1	39.095	-99.238	Positive
Hadley Ranch	HR2	39.091	-99.231	Negative
Hadley Ranch	HR3	39.088	-99.231	Negative
Hadley Ranch	HR4	39.086	-99.233	Negative
Hadley Ranch	HR5	39.079	-99.237	Negative
Hadley Ranch	HR7	39.046	-99.238	Negative
Hadley Ranch	RoadsD	39.064	-99.239	Negative
HAYS	Hays	38.914	-99.192	Negative
HAYS	Hays	39.002	-99.188	NA
HAYS	Hays	38.914	-99.201	NA
HAYS	Hays	38.913	-99.215	Negative
Hollister Wildlife Area	HWA-001	37.785	-94.827	NA
Jamestown Wildlife Area	Gunclub Marsh	39.661	-97.900	Positive
Kirwin National Wildlife Refuge	Bow Creek	39.620	-99.166	Negative
La Cygne Burned Site	LCBS-001	38.399	-94.651	NA
La Cygne Wildlife Area	LCWA-001	38.418	-94.675	NA
La Cygne Wildlife Area	LCWA-002	38.400	-94.652	NA
La Cygne Wildlife Area	LCWA-003	38.416	-94.674	NA
Marais Des Cygne Wildlife Area	MDCWA-001	38.262	-94.686	NA
Marais Des Cygne Wildlife Area	MDCWA-001	38.260	-94.686	NA
Marais Des Cygne Wildlife Area	MDCWA-002	38.261	-94.685	NA
Marais Des Cygne Wildlife Area	MDCWA-002	38.263	-94.684	NA
Marais Des Cygne Wildlife Refuge	MDCR-001	38.231	-94.618	Positive
Marais Des Cygne Wildlife Refuge	MDCR-002	38.217	-94.637	Negative
Marais Des Cygnes National Wildlfe Refuge	Oxbow	38.245	-94.680	Positive
Marais Des Cygnes National Wildlfe Refuge	State Line Pond	38.229	-94.619	NA
Marais Des Cygnes National Wildlfe Refuge	Swan Marsh	38.240	-94.656	Positive
Marais Des Cygnes National Wildlfe Refuge	Tureky Foot Pond	38.217	-94.627	NA
Marais Des Cygnes National Wildlfe Refuge	Zenor Road	38.192	-94.631	Positive
Medicine Lodge River	MLRI-001	37.025	-98.420	Negative
Medicine Lodge River	MLRI-002	37.039	-98.470	Negative

Sample Location	Site Name	Latitude	Longitude	Site Status
Medicine Lodge River	MLRI-003	37.156	-98.529	NA
Medicine Lodge River	MLRI-004	37.287	-98.633	NA
Medicine Lodge River	MLRI-005	37.293	-98.659	NA
Medicine Lodge River	MLRI-006	37.305	-98.686	NA
Medicine Lodge River	MLRI-007	37.313	-98.731	NA
Medicine Lodge River Trib.	MLRT-001	37.249	-98.551	NA
Miami State Fishing Lake	MSFL-001	38.422	-94.787	NA
Mined Lands-8	MLWA8-001	37.390	-94.772	NA
Mule Creek	ZBAR1	37.104	-98.987	NA
Neosho State Fishing Lake	NSFL-001	37.418	-95.197	Negative
Neosho State Fishing Lake	NSFL-002	37.420	-95.195	NA
Neosho State Fishing Lake	NSFL-003	37.427	-95.206	Negative
Neosho State Fishing Lake	NSFL-003	37.427	-95.206	Negative
Neosho State Fishing Lake	NSFL-004	37.428	-95.206	Negative
Neosho Wildlife Area	NWA-001	37.501	-95.162	NA
Neosho Wildlife Area	NWA-001	37.501	-95.162	Negative
Neosho Wildlife Area	NWA-002	37.501	-95.159	NA
Neosho Wildlife Area	NWA-003	37.427	-95.206	NA
NF Walnut Creek	NESS1	38.464	-99.954	Negative
Ninnescah River	NIRI-001	37.562	-97.691	NA
Ninnescah River	NIRI-002	37.538	-97.644	NA
Ninnescah River	NIRI-003	37.518	-97.608	NA
Ninnescah River	NIRI-004	37.491	-97.515	NA
Ninnescah River Trib.	NINT-001	37.548	-97.573	NA
North Trib to Marsh	BWWA5	37.661	-98.265	NA
Northeast Elm Creek	NECR-001	37.456	-98.718	NA
Peace Creek	PECK-001	38.159	-98.247	NA
Quivira National Wildlife Refuge	ANNWO1	38.211	-98.473	Positive
Quivira National Wildlife Refuge	Kid's Fishing Pond	38.074	-98.494	Positive
Quivira National Wildlife Refuge	QNWR2	38.093	-98.478	Negative
Quivira National Wildlife Refuge	R3	38.078	-98.485	Negative
Quivira National Wildlife Refuge	R4	38.104	-98.489	Negative
Quivira National Wildlife Refuge	RSC	38.105	-98.509	Negative
Quivira National Wildlife Refuge	Sandy Pond	38.115	-98.501	Positive
Quivira National Wildlife Refuge	Unit29	38.150	-98.500	Negative
Quivira National Wildlife Refuge	Windmill Pond	38.125	-98.492	Negative
Rattle Snake Creek, Camel Pasture	Rattle3	37.867	-98.878	NA
Rattle Snake Creek, Jordan Pasture	Rattle2	37.881	-98.853	NA
Rattlesnake Creek	QNWR1	38.101	-98.508	Negative
Rattlesnake Creek	RATTLE5	37.971	-98.807	NA
Rattlesnake Creek	RATTLE6	38.080	-98.718	NA

Sample Location	Site Name	Latitude	Longitude	Site Status
Rattlesnake Creek	RATTLE9	38.093	-98.546	NA
Road Crew	RDCR-001	37.221	-94.796	NA
Road Crew	RDCR-001	37.194	-94.796	NA
Road Crew	RDCR-001	37.308	-94.796	NA
Sand Creek	SACR-001	37.503	-97.771	NA
Scott State Park	Barrel Springs	38.665	-100.917	Negative
Scott State Park	Barrel Springs	38.665	-100.917	Negative
Scott State Park	Elm Grove	38.667	-100.918	Negative
Scott State Park	Elm Grove	38.667	-100.918	Negative
Scott State Park	Outlfow	38.692	-100.925	Negative
Smoots Creek, WSU Gerber Preserve	WSU4	37.681	-97.946	Negative
South Fork Ninnescah River	SFNR-001	37.601	-97.773	NA
South of Nashville	SAND1	37.386	-98.429	Negative
Spring Creek	SPCR-001	37.533	-97.575	Negative
Spring River Wildlife Area	SRWA-001	37.182	-94.648	NA
Spring River Wildlife Area	SRWA-002	37.186	-94.649	NA
Spring River Wildlife Area	SRWA-003	37.190	-94.651	NA
Spring River Wildlife Area	SRWA-004	37.183	-94.649	NA
St. Francis Wildlife Area	North Sand Pit	39.741	-101.873	Negative
St. Francis Wildlife Area	South Fork Republican	39.741	-101.867	Negative
Tallgrass Prairie National Preserve	Amphibian Pond	38.422	-96.556	Negative
Tallgrass Prairie National Preserve	FishPond1	38.413	-96.505	Negative
Turkey Creek	TKCR-001	37.499	-98.949	NA
Tuttle Creek Wildlife Area	Creek	39.452	-96.698	Positive
Tuttle Creek Wildlife Area	pond1	39.451	-96.699	Negative
Tuttle Creek Wildlife Area	pond2	39.450	-96.701	Negative
Youngmeyer Ranch	Pond	37.564	-96.503	Negative
Youngmeyer Ranch	Robey Ranch	37.568	-96.445	Negative
Youngmeyer Ranch	Robey Ranch	37.581	-96.453	Negative

NA= Not Analyzed