Survey for the pathogen, *Batrachochytrium dendrobatidis,* in Illinois

Project Number: T-56-R-1

Final Report

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By

Brooke L. Talley
Graduate Student Researcher, Ph.D. Candidate
Department of Zoology
Southern Illinois University
Carbondale, Illinois
(bltalley@siu.edu, bltalley@gmail.com)

Karen R. Lips
Primary Investigator
Department of Biology
University of Maryland
College Park, Maryland
(klips@umd.edu)

John Reeve
Fiscal Officer
Department of Zoology
Southern Illinois University
Carbondale, Illinois

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Executive Summary

The following is the final report for SWG project number T-56 R-1: Survey for the pathogen, *Batrachochytrium dendrobatidis,* in Illinois. The purpose of our project was to describe the distribution of *Batrachochytrium dendrobatidis* (*Bd*) within the state of Illinois. This project directly contributes to action items in the Illinois Wildlife Conservation Plan that covers the Wetlands Campaign, Invasive Species, Monitoring and Research of Wildlife Disease, and Monitoring and Research on Amphibian Species of Conservation Priority[1].

This project was initiated to assess the current and historic status of the invasive fungus *Bd* among amphibian species at selected sites throughout the state of Illinois. This information was intended to guide future management practices to minimize the spread of the fungus into new areas or new populations, if *Bd* were detected in some but not all populations. The goals of this project were achieved through four primary objectives:

Job 1. Determine the current distribution of Bd in Illinois.
We surveyed for Bd in Illinois because it was likely to be currently infecting amphibians, and to have caused declines in Illinois amphibians in the past. Yet, until this study, there was no database of infected sites, habitats, or anuran species. With the statewide survey, we provide distributional information on *Bd* needed for individual managers to determine whether they have a healthy or infected site, and how to either prevent introduction, or prevent future spread of this disease. This study would determine infection status of sites allowing the development and dispersal of guidelines on disease containment, prevention of spread, disease surveillance, and/or amphibian population monitoring.

> Objective 1: Survey protected areas of the State of Illinois for the presence of *Bd*, focusing on protected areas located within Natural Divisions (Northeastern Morrainal, Rock River Hill Country, Grand Prairie, Southern Till, Upper Mississippi / Illinois River Bottomlands, Wabash Border, Shawnee Hills, Coastal Plain, Middle Mississippi Border). (Spring and Summer 2008 & 2009, 31% of budget).

Job 2. Determine the time when Bd was first found infecting Illinois amphibians.
We conducted historic surveys to determine whether Bd has been present in Illinois for numerous decades or whether it arrived more recently. This information sheds light on whether this fungal agent is an ongoing threat of recent emergence (i.e., an epizootic disease) or whether anurans have been living with the disease agent for numerous decades (i.e., an enzootic disease).

> Objective 2: Use histology to survey for Bd in museum specimens collected prior to 1990 from the 9 Natural Divisions of Illinois. (Summer 2008-Summer 2012; <3% budget).

[1] http://dnr.state.il.us/ORC/WildlifeResources/theplan/final/

Job 3. Analyze field and histological data for the presence of Bd in current and historical populations, respectively.

Objective 3: Organize and analyze field survey data. (Summer 2008 – Summer 2012; 65% of budget)

Job 4. Communicate progress to the IDNR and develop a final report.

Annual reports have been provided to the IDNR regarding the project's progress, along with periodic updates when key research was completed. Any publications that result from this project will be made available.

Objective 4: Produce Reports and Maps. (Fall 2010-Spring 2013; <1% of budget)

The following report provides background on the Illinois *Bd* survey project. The tasks and deliverables of Jobs 1-3 are detailed in the report body and accompanying appendices. This final report fulfills the last objective of this project.

Introduction

Bd has contributed to global declines of amphibian populations

The disease agent, *Batrachochytrium dendrobatidis* (*Bd*), has been implicated in worldwide declines because it causes the disease chytridiomycosis in amphibians (Bosch et al. 2001, Muths et al. 2003, Lips et al. 2004, Wake and Vredenburg 2008). However, chytridiomycosis disease dynamics and impacts on amphibian populations differ around the globe, and among areas of the United States.

Bd is emerging in new areas and species, causing epidemics in many naïve populations of wild species and resulting in die-offs, population declines and even extinctions. *Bd* has infected over 200 species around the globe, and can potentially cause mortality of all amphibian species. The World Organization for Animal Health (the OIE) has begun the process to list *Bd* as a disease of global significance and is advising all nations to conduct surveys to determine the geographic and taxonomic distribution of this disease. This pathogen is of particular concern because it is an invasive species, it can infect and kill many species, it does not respect geopolitical boundaries, and it persists in the environment, thus limiting the effectiveness of traditional conservation tools such as reintroductions, translocations, and establishment of protected areas.

Bd is likely to be more widespread than currently described because of limited surveys in remote regions, high cost of analyses, and few historic surveys of museum specimens. Illinois is no exception as no large-scale surveys have been completed in the state, until this study. This fungus, as an emerging infectious disease, was first discovered in 1998 and much remains to be learned about its biology, how it is transmitted, its impacts on native amphibians, and its distribution in Illinois. This project provides information that may enable us to conserve amphibians in Illinois via specific management techniques despite the spread of this fungal infection.

The epidemiology of chytridiomycosis varies among geographic locations and populations because of variation in the interactions among the disease agent, host, and environment (Wobeser 2006). In the United States there are no reports of declines in some areas (e.g., Longcore et al. 2007) or some populations (e.g., *Pseudacris regilla*, Reeder et al. 2012) despite nearby declines in others (e.g., Bradley et al. 2002 & Schlaepfer et al. 2007; Vredenburg et al. 2005 & 2010). The differences in decline-related outcomes likely stem from a number of causes including time since first *Bd* infection to ascertain position along the epizootic wave (Lips et al. 2008), environmental factors like thermal regimes either exacerbating disease or preventing it (Piotrowski et al. 2004), and biological factors like environmental reservoirs that maintain zoospores in habitats (Reeder et al. 2012). In this study, we assessed numerous biotic and abiotic factors to determine which variables explained both measures of chytridiomycosis – *Bd* prevalence and intensity. By identifying key biotic and abiotic factors that regulate disease dynamics in natural populations, we can begin developing novel disease assessments and interventions (Hawley and Atizer 2011).

Bd is a generalist pathogen of amphibians

Most pathogens are capable of infecting a few closely related host species, affording ease of transmission and decreased likelihood of pathogen extinction (Woolhouse et al. 2001). *Bd* differs in that it is an extreme generalist (Wake and Vredenburg 2008), apparently capable of infecting all 7000+ species of amphibians. Although most species are susceptible to infection, species vary in response (Lips et al. 1998; Crawford et al. 2010; Gahl et al. 2011, Searle et al. 2010) because of underlying aspects like taxonomic relatedness (e.g. Corey and Waite 2008, Crawford et al), ecology (Lips et al. 2003; Rowley and Alford 2007; Brem and Lips 2008) and physiology (Harris et al. 2006, Longo et al. 2009, Ramsey et al. 2010, and Richards-Zawacki 2010). Infection with the chytridiomycete agent, *Bd* (Longcore et al. 1999), occurs after exposure to aquatic zoospores (Carey et al. 2006). Once an animal is infected with *Bd*, the mature zoosporangia release zoospores from keratinized tissues of the infected host that can then exist independently in the environment (Di Rosa et al. 2007, Walker et al. 2007), infect new hosts (Rachowicz and Vredenburg 2004), or re-infect the original host. Most amphibians are susceptible to *Bd* infection because they share the *Bd*-infected environment and most have keratinized skin.

Measuring disease impacts

Bd prevalence and intensity describe disease processes; prevalence describes how common the agent is in a population and intensity measures the infection burden on individuals. After a disease agent is introduced into a novel system, an epizootic wave will cause increased incidence among individuals that will then taper off to a predicable enzootic level (Wobeser 2006). Where we have documented *Bd*'s arrival into naïve host populations, we have seen epizootic wave where incidence and prevalence are high, many anurans die, and then prevalence falls to a level sustained by surviving members of the population (Lips et al. 2008). Whether or not an individual frogs dies from *Bd* infection depends upon the infection intensity (Briggs et al. 2010, Vredenburg et al. 2010). For *Rana sierrae* in California, average intensities exceeded 10,000 zoospores in declining populations (Briggs et al. 2010). Threshold levels for other areas or other species have not been determined.

Environmental factors affect disease prevalence and intensity

Disease ecology emphasizes the role of the environment in shaping individual responses and outcomes of host-pathogen interactions (Altizer et al. 2003). The thermal environment plays an important part for inhibitng and promoting *Bd*'s growth (Woodhams et al. 2003). Temperatures relating to seasonality and latitude affect *Bd* prevalence (e.g., Kriger and Hero 2006, Kriger et al. 2007, Lannoo et al. 2011, Ouellet et al. 2005). Under laboratory conditions, *Bd* grew best at temperatures between 17°C and 25°C (Piotrowski et al. 2004), a range coinciding with that of many upland neotropical (Ron 2005) and montane areas (Daszak et al. 1999) where *Bd* epizootic outbreaks have occurred (e.g., Berger et al. 1998, Lips 1998, Vredenburg et al. 2005).

Biological factors affect Bd disease prevalence and intensity

Disease epidemiology predicts that the disease host plays an important role in determining how diseases progess. And since *Bd* has such a broad rnage of hosts that means that species richness and abundance are important and may change outcomes when susceptibility varies among species. For example, the presence of reservoir species (e.g., *L. catesbeianus*, Daszak et al. 2004; *Pseudacris regilla*, Reeder et al. 2012) maintains *Bd* infections in populations. Amphibian density (Briggs et al. 2010) and species richness (Becker and Zamudio 2011, Searle et al. 2011) have been linked to variation in infection intensity. Anuran density best predicted probability of increased infection intensity in *R. sierrae,* and subsequent population crash (Briggs et al 2010). Density-dependent disease transmission suggests that below a host density threshold, the disease cannot invade or persist (McCallum, Barlow & Hone 2001; Lloyd-Smith et al. 2005a); the disease agent should go extinct before extirpation of the host population. But it may be maintained as long as susceptible host density within the community is sufficient (Ray and Collinge 2006). Species richness can affect disease transmission when susceptibility is not equal among species. For example, in a multi-host speciose system, infection would be matintained if reservoir species were present (Reeder et al. 2010) compared to a less speciose system absent the reservoir species. In human populations, superspreaders are individuals that infect unusually large numbers of secondary cases (Lloyd-Smith et al. 2005b).

Bd in the Midwest

In the United States, alone, the lack of catastrophic declines in some areas (e.g., Maine, Longcore et al. 2007) and species (e.g., *Pseudacris regilla*, Reeder et al. 2012) while being present in others (e.g., Arizona, Bradley et al. 2002 & Schlaepfer et al. 2007; California, Vredenburg et al. 2005 & 2010) indicates that chytridiomycosis epidemiology may vary among geographic locations and populations due to several reasons, all stemming from underlying factors associated with the complex interactions of disease agent, host, and environment (Wobeser 2006). The history of *Bd* in the Midwest is poorly known. There have been no reports of mass dieoffs or population extinctions in pristine areas, as seen in the *Bd*-Panama system. However, amphibian declines have occurred in the Midwest with several causative factors attributed (e.g., toxicants, habitat alterations, drought, etc.) (e.g., Beasley et al. 2005). In the face of *Bd*'s widespread presence in the US, there have been no large-scale attempts to ascertain the status of Chytridiomycosis in midwestern states even though this area has documented historic *Bd* presence (Ouellet et al. 2005), some widespread anuran population declines (e.g., Indiana (Brodman and Kilmurry 1998), Minnesota (Moriarty 1998), Wisconsin (Mossman et al. 1998)), and experiences environmental temperatures optimal for *Bd* growth (Piotrowski et al. 2004) at times simultaneous to anuran breeding seasons (Phillips et al. 1999).

While numerous midwestern species are known to carry *Bd* infections, the likelihood of experiencing death from exposure for some of these species remains uncertain (Gahl et al. 2011). *Bd* was reported in Illinois' *A. crepitans* collected in 1994 (Beasley et al. 2005, Pessier et al. 1999 Recent reports have indicated that *Bd* is present in *A. crepitans*' Midwestern populations (Steiner and Lehtinen 2008, Zippel and Tabaka 2008). Zippel and Tabaka (2008) found *Bd* in captive *A. crepitans* that came from wild

populations in Ohio, Missouri, and Michigan. Their results indicate that these animals may have entered into captivity with *Bd*, but did not address the infection status or vulnerability of the original wild populations. It has been present in the states surrounding Illinois since at least the 1980's (Indiana, Wisconsin) or 1990's (Missouri; Ouellet et al. 2005).

Bd in Illinois?

Multiple strains of *Bd* are present in the US (Morgan et al. 2007), and certain strains are more virulent than others, suggesting that even if *Bd* is present, continued management to minimize the spread may help prevent invasion by more lethal strains. Results from this project were meant to identify infected and uninfected area and to inform management actions to slow or prevent the spread of this fungus into healthy populations. *Bd* is naturally transmitted between individuals, or between infected environments (damp substrates, water) and amphibians. Sites that have not yet been affected may become infected in the future unless major management plans are developed. Amphibians infected with a less virulent strain might show greater losses if more virulent strains were introduced. How *Bd* is spread is still not fully known; once it invades a new strain cold infect surviving amphibians, new colonists, or reintroduced animals. Knowledge of infected sites should direct future reintroduction decisions.

Need

Bd has been detected in all regions of the US where field surveys have been conducted, often with very little effort (e.g., a few days, a few ponds, <100 animals), suggesting *Bd* is widespread in the US. Many Illinois amphibians species have been found infected with *Bd* elsewhere, including *Anaxyrus americanus*, *Ambystoma maculatum*, *Hyla versicolor*/*chrysoscelis*, *Notophthalmus viridescens*, *Pseudacris triseriata*, *Lithobates pipiens*, *L. sylvatica*, *L. palustris*, *L. clamitans*, and *L. catesbeiana* (Green et al. 2002). Terrestrial (Highton 2005) and stream (Banks et al. 2006) salamander populations have also declined throughout the eastern half of the US, including populations of *Plethodon cinereus* and *P. dorsalis* in Illinois, Indiana, and Missouri (Highton 2005). These populations have not been tested for *Bd*, but the timing of the declines was in the mid-1980's when *Bd* was known to be causing population declines in amphibians elsewhere in the US. *Bd* has been involved in many die-offs and declines in amphibian populations throughout the United States (e.g., California, Arizona, and Colorado).

Based on these reports, *Bd* is likely to be widespread in certain regions of Illinois presently. *Bd* has been reported from 8 Illinois individuals of *Acris crepitans* collected in 1996 (Pessier et al. 1999) and in larval *Rana clamitans* collected in 1998 (Lips unpubl. data), although there have been no large-scale surveys to determine its presence or prevalence among species or habitats. *Bd* has been present in the states that surround Illinois since at least the 1980's (Indiana, Wisconsin) or 1990's (Missouri; Ouellet et al. 2005), and may be causing population declines in Missouri hellbenders (Jeff Briggler pers. comm.). No retrospective surveys have been done to determine when the disease first appeared and its impact on the Illinois fauna. The status of Illinois amphibian populations has never been fully quantified, although there is anecdotal evidence that certain species are in decline in certain parts of the state. There are very few long-term

studies of amphibian populations in Illinois, which prevents any comparative studies of population trends. However, extensive collections of amphibians have been made over the decades by state herpetologists (e.g., Smith, Brandon, Phillips), which have produced an excellent understanding of the historic and current distribution of Illinois species.

We needed to survey for *Bd* in Illinois because it is likely to be infecting amphibians today, and to have caused declines in Illinois amphibians in the past; yet there is no database of infected sites, habitats, or species. We also needed to conduct historic surveys to determine when *Bd* arrived in the state.

Approach

We determined the distribution of *Bd* among amphibian populations in sites located within seven of the Natural Divisions of Illinois. We used field surveys to swab anurans for their current infection levels; we analyzed swabs with qPCR. We used histological techniques of museum specimens to ascertain historic infection levels. Because of histology's high false-negative rate, we followed up histological surveys with a new technique similar to that of the current samples. We swabbed museum samples and analyzed them with qPCR. Finally, we have shared updates and created annual reports for the IDNR via email and personal meetings. The following four objectives outline the specific approach for each job (outlined above), and how we completed and met each objective. Additionally, we provide the following table to illustrate when each objective was initiated and completed (Table 1):

Table 1. Project schedule for all objectives.

Objective	Sp 08	Su 08	F 08	W 08-09	Sp 09	Su 09	F 09	W 09-10	Sp 10	Su 10	F 10	W 10-11	Sp 11	Su 11	F 11	W 11-12	Sp 12	Su 12
Objective 1: Survey protected areas of Illinois for the presence of *Bd*	X	X			X	X												
Objective 2: Survey for *Bd* in museum specimens (histology)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Objective 3: Analyze (PCR) field samples			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Objective 4: Produce Reports and Maps, give presentations at meetings				X		X		X		X	X	X	X	X	X	X	X	X

Job 1. Determine the current distribution of *Bd* in Illinois.

Objective 1: Survey protected areas of the State of Illinois for the presence of *Bd*, focusing on protected areas located within Natural Divisions (Northeastern Morain, Rock River Hill Country, Grand Prairie, Southern Till, Illinois River Bottomlands, Wabash Border, Shawnee Hills) (Spring 2008 & 2009, 31% of budget).

1.1) Determine sites to be surveyed, choosing sites that encompass numerous natural divisions and those that are located in various latitudes.

We compiled species lists of study sites by consulting with IDNR, FWS and INHS staff and site managers and by conducting library and online research. We arranged site visits to speak with managers and determine whether appropriate habitat and sufficient abundance of focal species existed at each site and assessed feasibility of each site.

From these visits, we determined which sites would have adequate anuran population sizes among widespread species to yield sufficient power for estimating *Bd* prevalence. We sampled sites located in and adjacent to protected areas that covered all the primary natural divisions of Illinois (Schwegman 1973). We sampled 5 sites in 2008, and 17 different sites in 2009 (Figure 1).

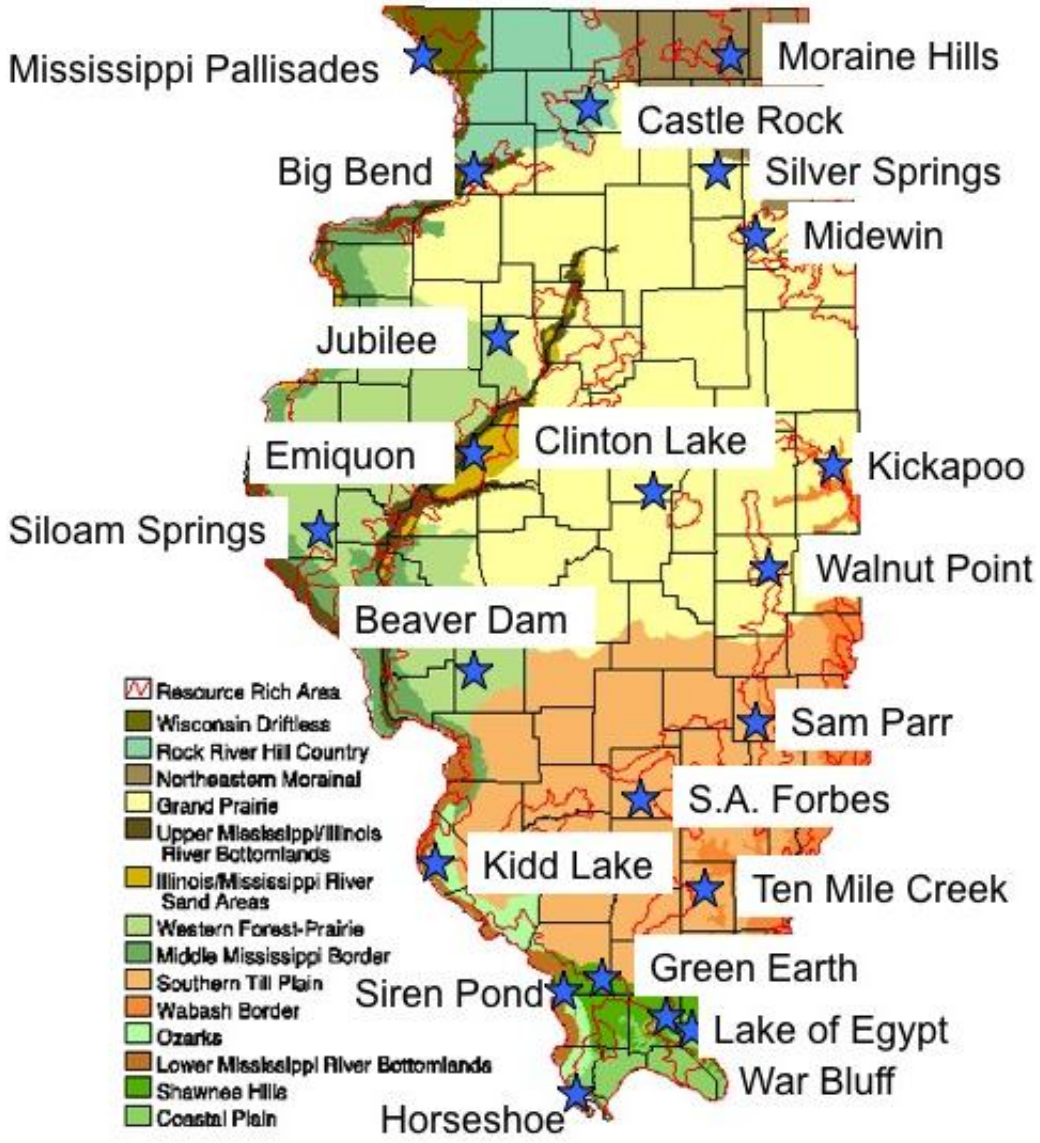


Figure 1. Blue stars indicate sample sites where we surveyed widespread anurans for current *Bd* infection levels in 2008 and 2009. Site names represent natural areas in the area. Color-coding on the map corresponds to Illinois' Natural Divisions (Schwegman 1973)[2].

[2] http://dnr.state.il.us/education/biodiversity/index.htm

We sampled 1–7 wetlands within each site to achieve minimum sample size (see 1.2 below) of anurans and to prevent resampling of individuals (Figure 2). We later tested both wetland- and site-level independent variables to determine if disease dynamics varied between spatial scales (see Appendix 4).

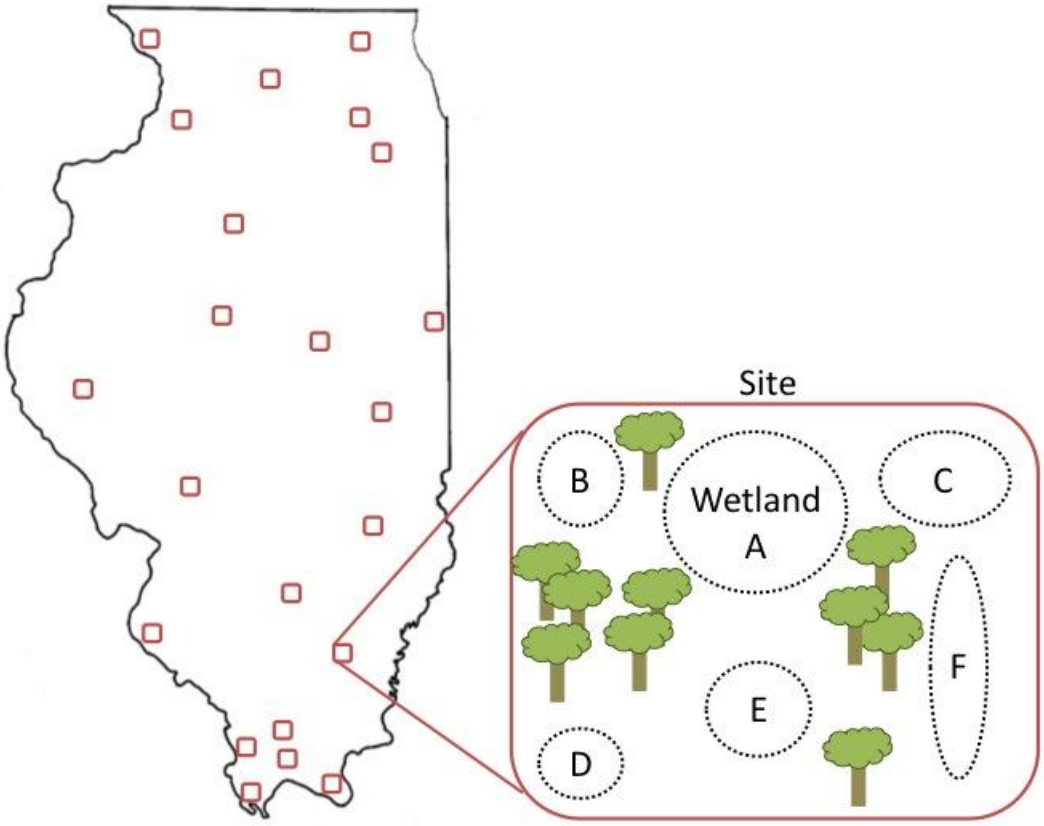
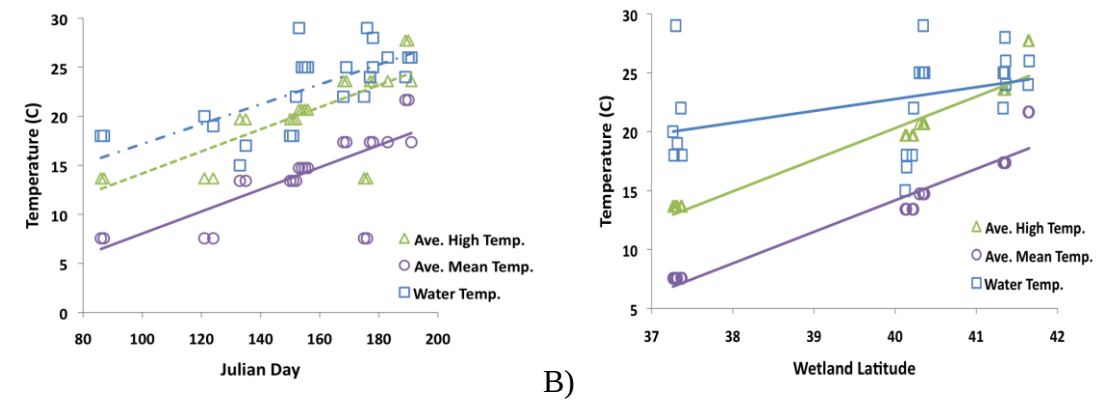


Figure 2. Sample sites from 2008 and 2009 are indicated with red squares. Numerous wetlands were sampled within each sample site (1–7 wetlands within each site).

Sampling dates were associated with latitude and temperature measurements at time of sampling because we starting sampling southern sites in the Spring, and moved northward to capture the peak breeding season at each location (Figure 3).



A) B)

Figure 3. We observed positive relationships between A) Julian day and temperature measures and B) latitude and temperature measures, which yielded from our sampling design along a South—North gradient.

1.2) Capture visually located amphibians. Swab captured amphibians to collect samples for Bd testing. Record data. Release all amphibians once swabbing completed.

A field crew of 2-3 SIUC students conducted surveys to establish the distribution of *Bd* among wetlands in 9 Natural Divisions (Coastal Plain, Middle Mississippi Border, Northeastern Morain, Rock River Hill Country, Grand Prairie, Southern Till, Upper Mississippi / Illinois River Bottomlands, Wabash Border, Shawnee Hills) (Figure 1). Multiple protected areas, and most species, including those of conservation priority (e.g., Bird-voiced treefrog, Crayfish frogs, Illinois chorus frogs) were included (Figure 4).

Figure 4. BLT swabs *H. avivoca* for *Bd* during a field survey.

We sampled during spring and early summer when amphibians are most abundant and when environmental conditions are within the optimal range for growth and survival of *Bd* and it is likely to be widely distributed among species and habitats.

In both years we attempted to swab 30 individuals per species at each wetland to have sufficient power to estimate prevalence for each species (DiGiacomo and Koepsell 1986, Hanley and Lippman-Hand 1983). These sample sizes were based on *a priori* estimates of 35% prevalence with 95% confidence (DiGiacomo and Koepsell 1986, Hanley and Lippman-Hand 1983). Not all species occurred at each site so in 2008 we haphazardly swabbed additional individuals until we reached 350 total swabs per site. Based on actual 2008 prevalence estimates, we reduced our 2009 target sample size to 96 samples per site, using 2008 estimates of 50% prevalence with 90% confidence.

We maximized our chances of finding *Bd* when present, by sampling species that have been shown to be infected elsewhere. We targeted two types of amphibians: widespread species and those of special interest. At each site, we sampled species that are distributed state-wide (e.g., *Acris crepitans, Anaxyrus americanus, A. fowleri, Hyla chrysoscelis/versicolor, Pseudacris crucifer, P. triseriata, Lithobates catesbeiana, L. pipiens,* and *L. sphenocephala)* to determine how local environments influence infection (Figure 5). All widespread sample species are known to carry *Bd* infections, based on previous published works (see Garner et al. 2006, Longcore et al. 2007, Ouellet et al. 2005, Steiner and Lehtinen 2008, Woodhams et al. 2008).

Figure 5. We sampled 12 widespread species for the presence of *Bd*. All sample species were known to carry *Bd* from previous studies.

We also sampled species that have previously declined (e.g., *A. crepitans*, *L. areolatus*) or are Threatened or Endangered (e.g., *P. streckeri*, *H. avivoca*) in 2008, 2009, and 2010. In 2010, we solely focused on sampling individuals of conservation concern (*H. avivoca*, *H. cinerea*, *P. streckeri*, *L. areolatus*, and *L. palustris*) because they had either limited geographic ranges, limited activity periods, limited scientific study, or are listed as Threatened or Endangered in Illinois. We sampled atleast 30 individuals of the aforementioned species types when there were enough animals present.

We obtained all animal care and scientific-use permits prior to surveying (Appendix 1).

We used standard field techniques to survey adult amphibians (Heyer *et al.* 1994). Teams of 2-3 researches walked along wetland edges and in emergent vegetation to visually search for frogs. Individuals were captured by hand, swabbed for *Bd* (Hyatt et al. 2006), toe-clipped for individual identification when necessary (i.e., when a single wetland was sampled on multiple nights), measured (SVL, mass) and then released (Figure 6). Each cotton tip used for swabbing was stored in ethanol (Lips et al. 2006).

Figure 6. BLT measures the mass of a *L. catesbeianus* during routine morphological assessment, prior to swabbing for *Bd*.

1.3) Measure microenvironmental and habitat variables, and obtain GPS location at each capture.

Field sampling occurred between 1900–0100 hours. At each sampling location we recorded water temperature 5 inches below water surface at the start of sampling and characterized wetland habitat type as pond, swamp, marsh, or roadside/agricultural ditch. At each wetland we determined latitude and longitude with a Garmin GPSMAP 60CSx Handheld GPS Navigator (Olathe, Kansas, USA). We calculated frog density as the number of of frogs·person^{-1}·hr^{-1}, and species richness as the number of species captured. We determined Shannon-Weiner diversity index (R package vegan, Oksanen et al. 2012), and calculated percentage of captures for each of the three families (Hylidae, Ranidae, and Bufonidae). We used the online Illinois State Water Survey[3] to determine average maximum and mean monthly air temperatures.

[3] www.isws.illniois.edu accessed July 23, 2011

Job 2. Determine the time when *Bd* was first found infecting Illinois amphibians.

Objective 2: Use histology to survey for *Bd* in museum specimens collected prior to 1990 from each of the Natural Divisions of Illinois (Summer 2008-Summer 2012; <3% budget).

2.1) Obtain list of all Illinois specimens (including species, collection date and locality data) deposited at SIUC INHS, UIUC, FMNH from curators.

We requested information from curators of SIUC, INHS, UIUC, and FMNH, and obtained listings of all Illinois specimens collected between 1950-1990 (Table 1). We summarized those lists to determine how many individuals of which species were available statewide in each decade from 1950-1990.

Table 1: List of specimen holdings for INHS, UIMNH, FMNH, and SIUC by decade for widespread species to be assessed for *Bd* using histologic procedures. Note: we did not analyze all museum holdings for *Bd* (See Appendx 8).

	Specimen Collection Period					
Species	**1950-59**	**1960-69**	**1970-79**	**1980-89**	**1990-99**	**Total**
A. crepitans	283	168	34	9	369	**863**
A. americanus	125	147	52	32	430	**786**
A. fowleri	160	192	32	9	382	**775**
H. chrysoscelis / versicolor	141	25	0	21	151	**338**
L. catesbeianus	86	67	10	14	287	**464**
L. pipiens	23	5	9	7	81	**125**
L. sphenocephalus	128	127	7	19	343	**624**
Total	**946**	**731**	**144**	**111**	**2043**	**3975**

We determined which species to sample by identifying those with widespread distribution (Figure 7) and equal effort on sampling from members of the families Bufonida, Hylidae, and Ranidae. Those species were *Anaxyrus americanus*, *A. fowleri*, *Acris crepitans*, *Hyla chrysoscelis/versicolor*, *Lithobates catesbeiana*, *L. pipiens*, and *L. sphenocephala*.

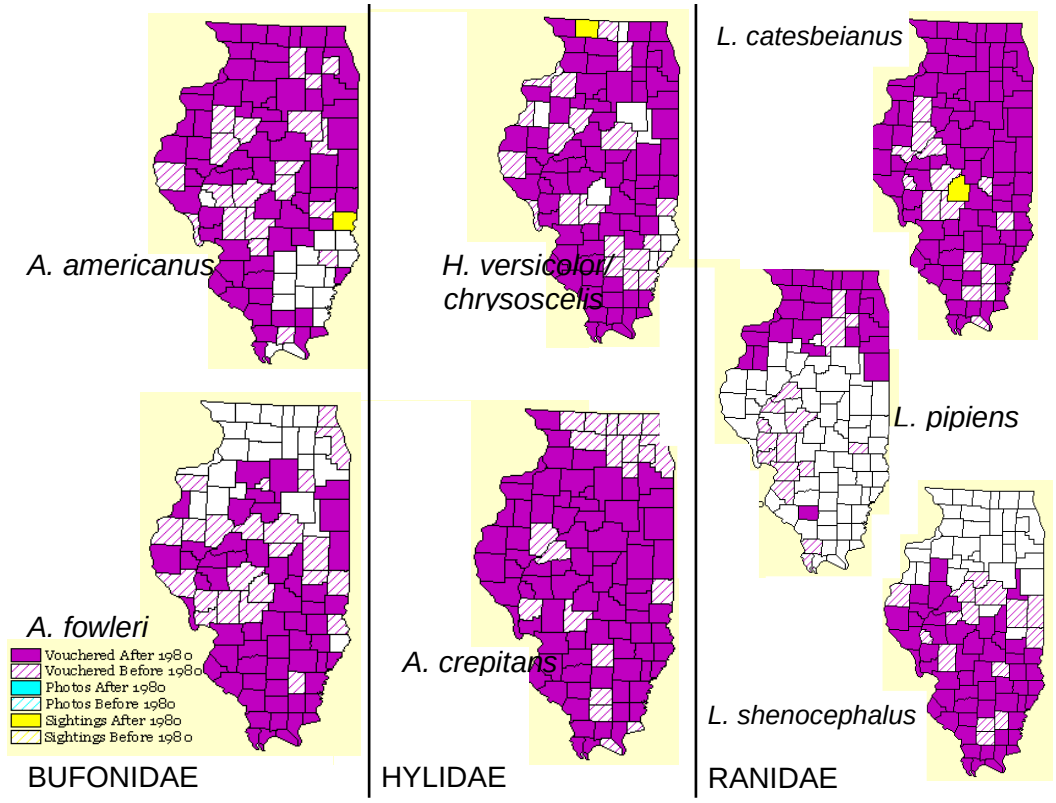


Figure 7. Geographic distributions of widespread focal species for histological examination. Range maps from Illinois Natural History Survey[4].

2.2) Remove a 4x4 mm snip of pelvic patch skin from at least 366 historical specimens collected prior to 1990 (the earliest record of Bd in the state) in each of the 9 natural divisions in the study area, choosing approximately 90 individuals from each decade (1950, 1960, 1970, 1980). Place collected tissue samples in alcohol to be transported to the SIUC Histology lab.

We visited SIUC, INHS, and UIMNH in 2008 and 2009 and completed sampling of the SIUC and UIMNH material. All adult specimens collected in Illinois between 1950 & 1989, were identified and selected for tissue samples to be taken. We removed a small (~4x 4 mm) piece of skin from the ventral side of the animal in the area of the pelvic patch for all animals fitting our criteria (Figure 8).

[4] http://www.inhs.uiuc.edu/animals_plants/herps/ilspecies.html; accessed January 2009, website last updated November 2008.

Figure 8. Removal of skin segment, used for histological examination for *Bd* among widespread anurans.

Material from FMNH was not used, as originally outlined, once we realized the time required to create histology slides (see 2.3 below) was greater than anticipated and that species resolution was representative with materials from SIUC, INHS, and UIMNH collections; the remaining 25% of materials we anticipated collecting from INHS were not obtained for the same reasons. We processed ~1,700 samples among (see 2.3 below).

2.3) Prepare histological slides of skin, examine under microscope for the presence of Bd. Record dates and locations of infection.

We worked under the guidance of Maureen Dornan in the SIUC Histology Lab to prepare slides for standard H&E histology (Puschendorf and Bolanos, 2006). This approach involves fixing the tissue in formalin, dehydrating the tissue in ethanol baths, embedding the tissue into paraffin, sectioning the skin to make very thin slices that are placed on a glass slide for staining. Hematoxylin and eosin (H&E) are the most commonly used stains in histology and histopathology. Hematoxylin colors nuclei blue; eosin colors the cytoplasm pink. To see the tissue under a microscope, the sections are stained with one or more pigments (Figure 9).

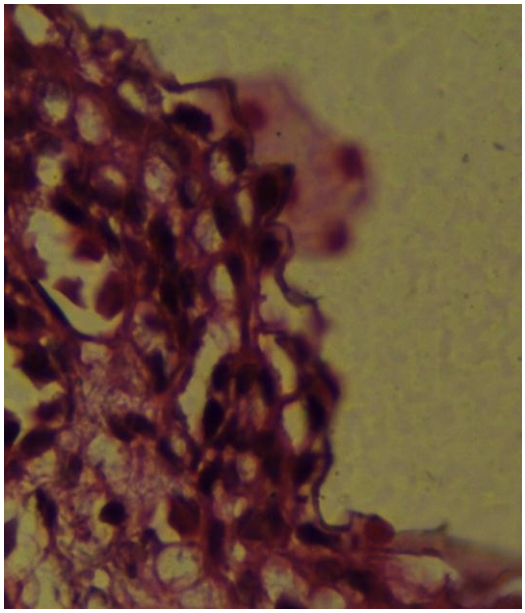


Figure 9. Suspect *Bd* positive sample with H&E staining. SIUC voucher #S3412, *Anaxyrus americanus*, Kane County, Illinois, 1988.

After we examined the first 100 specimens with the H&E technique, we became concerned that we were not finding any *Bd* positive individuals since we knew that current qPCR results yielded *Bd*-positive results (see Job 3, below), and that observer error might be to blame. Therefore, we used Gomori's Methenamine Silver (GMS) stain on a set of additional deceased anurans, which we found deceased in the field and collected. GMS is preferred for screening for fungi because it gives better contrast than H&E, and stains even degenerated and nonviable fungi. This is a more expensive staining process, and so we only used it to better train ourselves for identifying the *Bd* zoospore morphology of H&E-stained slides. Fungi stain black with GMS (Figure 10).

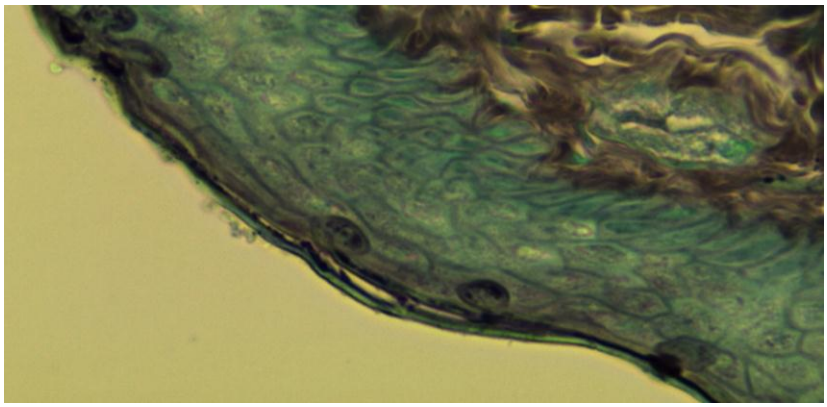


Figure 10. Suspect *Bd* positive samples with GMS staining (fungi stain black). This sample was collected when we were sampling in the field, from a deceased animal. SIUC voucher #S8737, *Scaphiopus holbrookii*, Horseshoe Lake, Alexander County, Illinois, 2008.

Approximately 1,700 specimens from the SIUC, UIMNH, and INHS collections were fixed onto microscope slides. We began examining all slides for evidence of *Bd* in museum specimens in Fall 2010. The examination process is very tedious; we completed slide examination in Spring 2012. We worked to complete as many slides as possible until the funding for undergraduate help expired. Our results show no evidence for historic *Bd* in the samples, BUT please see section 2.5 for results of the *Bd* positive genetic analyses of museum specimens and Appendix 8.

2.4) Enter date and GPS locality of each positive and negative record of Bd into database. Quantify degree and prevalence of infection by Bd among decades.

We plotted locations of all sampled amphibians with DIVA GIS (Appendix 8). We obtained geographic locality data for specimen locations from online resources[5] when it was not available from museums with specimen information. We used the Illinois Natural Resources Geospatial Data Clearinghouse online resources[6] for GIS layers (i.e., Illinois boundary, counties, wetlands, streams, municipalities, landcover).

Because we did not detect *Bd* in histologic samples, we rely on the results of the PCR analyses of museum specimens to calculate *Bd* infection prevalence and 95% Clopper-Pearson binomial confidence intervals for each decade (Appendix 8). These results should be assessed with caution because we are still in the process of using DNA sequencing to confirm that these are, in fact, *Bd*-positive samples.

2.5) Perform genetic analysis on suspect-positive and positive Bd slides to comfirm Bd's presence. Perform similar analyses on subset of negative slides.

New literature identifies methods of sampling museum specimens for *Bd* without performing histology, by using similar genetic techniques as those for living animals (Cheng et al. 2011). We analyzed our histological slides for *Bd* while performing genetic analyses on those that were positive or suspect-positive (see 2.3 above). Because we detected numerous positive samples with PCR, we continued swabbing even older museum specimens than we had taken skin samples from for histology. We sampled 1,012 individuals from 10 widespread species. They were originally collected 1892 – 1990. Positive samples were run in triplicate to ensure that they were positive (See Cheng et al. 2011 for methodology). See Appendix 8 for summary.

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[5] Carolina Herp Atlas <http://www.carolinaherpatlas.org/utmfinder/default.aspx>; Google Maps <http://maps.google.com>; <http://www.lat-long.com>; <http://itouchmap.com/latlong.html>

[6] Illinois Natural Resources Geospatial Data Clearinghouse <http://www.isgs.uiuc.edu/ndsihome/>

Job 3. Analyze field and histological data for the presence of *Bd* in current and historical populations, respectively

Objective 3. Organize and analyze survey data. (Summer 2008 – Summer 2012; 65% of budget)

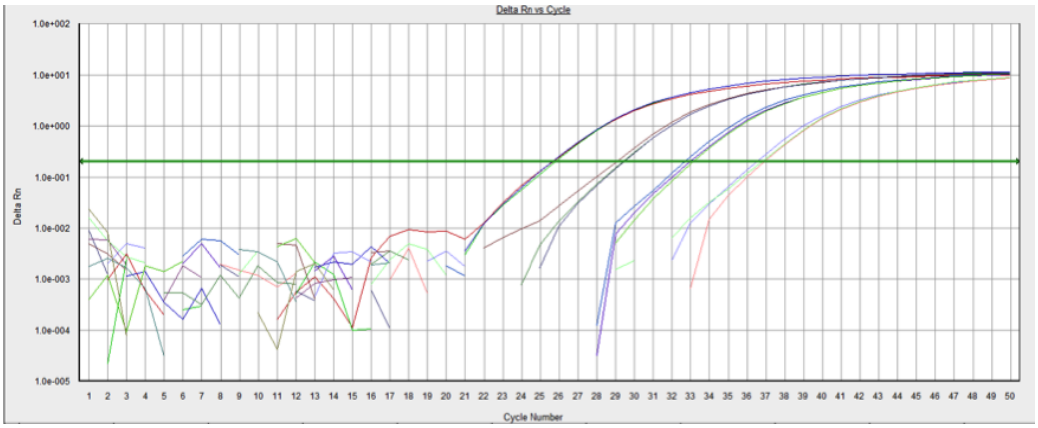
3.1) Analyze amphibian swabs from the current field study to determine the presence of Bd using PCR analysis.

Molecular methodology was learned under the guidance of Dr. Vance Vredenburg, at his lab in San Francisco State University (Figure 11), since the sensitivity of his qPCR test for *Bd* has been proven accurate and precise and no such analyses have been completed at SIUC.

Figure 11. BLT performs molecular analyses to look for *Bd* among Illinois anurans, specifically moving anuran DNA extracts into qPCR reaction tubes.

We used PrepMan® Ultra Sample Preparation Reagent (Applied Biosystems by Life Technologies Corporation, Carlsbad, California, USA) to prepare DNA of 2,690 samples from widespread species and an additional 485 from species of special concern. We used DNeasy Blood and Tissue Kit (Qiagen Incorporated, Valencia, California, USA) for the remaining 114 samples from widespread species with wood-shafted swabs to minmize any potential inhibition (Jacob Kerby, *pers. comm.).* We used qPCR to determine zoospore equivalents, using an ABI 7300 PCR machine (Figure 12). We used primers developed by Boyle et al. (2004), and ran DNA samples in singlicate. We assumed 1 genomic equivalent (GE) represented 1 *Bd* zoospore (Hyatt et al. 2007) and scored any swab that amplified as positive for *Bd*.

A



B

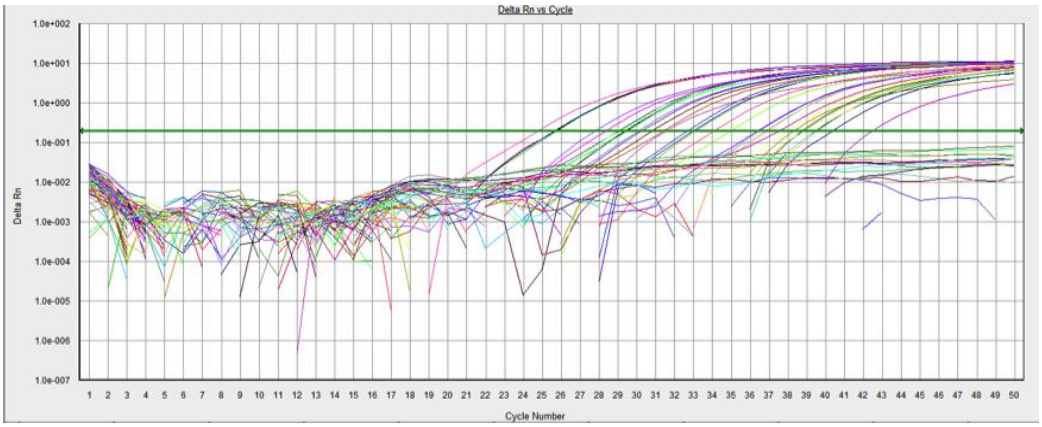


Figure 12. Spectral output from qPCR A) standards and B) samples. Colored lines represent quantity of *Bd* zoospores present in a single sample. Cycle number where sample amplifies above the threshold (solid green line) is calculated to ascertain the quantity of *Bd* zoospores in a sample; lower cycle number corresponds to a sample having more *Bd* zoospores.

3.2) Enter site data and GPS location into database

We collected environmental and biological variables at each wetland we sampled (Table 2) that we would later use to assess *Bd* prevalence and intensity levels among widespread species. The geographic positions of each wetland were also entered into the database (Table 3).

Table 2. Independent variables with ranges and decriptions, collected among Illinois wetlands. We tested these variables to see how they affected *Bd* prevalence and intensity in Illinois.

Variable Type	Independent Variable	Year	Range	Description
Environmental	**Latitude**	2008 2009	37.26 to 41.64 37.45 to 42.32	Decimal degrees of north latitude
	Longitude	2008 2009	- 90.12 to - 87.74 - 90.93 to - 88.03	Decimal degrees of longitude
	JDay	2008 2009	86 to 191 68 to 156	Day of the year specimen was sampled
	Wt.Water.Temp	2008 2009	17 to 29 9 to 30	Water temperature at the start of sampling (nightfall)
	Ave.High.Temp	2008 2009	14 to 28 8 to 23	Average high temperature approx. 30 days prior to sampling
	Ave.Mean.Temp	2008 2009	8 to 22 3 to 17	Average mean temperature approx. 30 days prior to sampling
Biological	**Wt.Shannon**	2008 2009	1 to 4.62 1 to 4.53	Shannon-Weaver diversity index of the wetland at time of sampling
	Wt.Frog.Density	2008 2009	3.18 to 106.00 0.24 to 29.30	Frog density index measured by the number of frog captures / person / hour
	St.Sp.Rich	2008 2009	1 to 8 2 to 7	Site species richness at the time of sampling
	Wt. Sp.Rich	2008 2009	1 to 5 1 to 6	Wetland species richness at the time of sampling
	St.Prop.Bufonidae	2008 2009	0 to 10.53 0 to 30.83	Proportion of animals at the site in the Bufonidae family
	St.Prop.Hylidae	2008 2009	0 to 93.02 21.05 to 91.59	Proportion of animals at the site in the Hylidae family
	St.Prop.Ranidae	2008 2009	3.10 to 100.00 6.25 to 65.35	Proportion of animals at the site in the Ranidae family
	Wt.Prop.Bufonidae	2008 2009	0 to 66.67 0 to 90	Proportion of animals in the wetland from the Bufonidae family
	Wt.Prop.Hylidae	2008 2009	0 to 100 0 to 100	Proportion of animals in the wetland from the Bufonidae family
	Wt.Prop.Ranidae	2008 2009	0 to 100 0 to 100	Proportion of animals in the wetland from the Bufonidae family

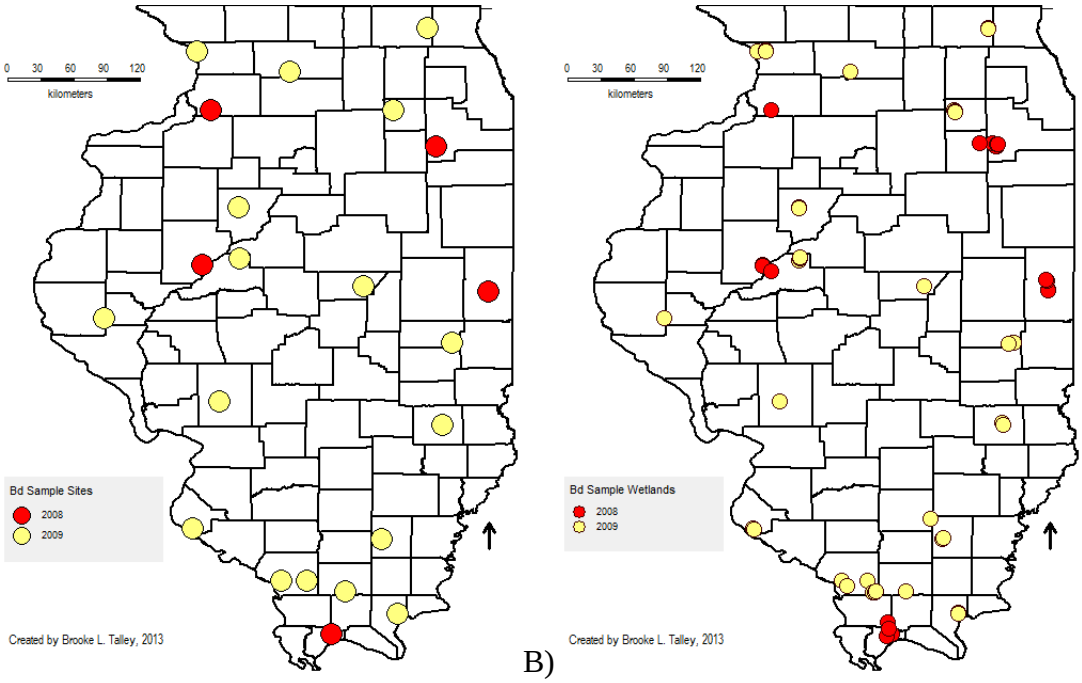
Table 3. Geographic location of each wetland where anurans were sampled. Site names are used to describe larger-scale management units for each wetland, typically named for nearby natural areas.

Sample Year	Site	Wetland #	Julian Day	Total Samples	Latitude (Degree Decimal)	Longitude (Degree Decimal)
2008	Big Bend	1	189	41	41.64	-90.04
2008	Cache	1	86	15	37.28	-89.05
		2	87	18	37.37	-89.08
		3	121	28	37.26	-89.09
		4	124	53	37.32	-89.07
2008	Emiquon	1	153	62	40.35	-90.11
		2	156	64	40.34	-90.12
		3	156	3	40.30	-90.04
	Kickapoo	1	135	53	40.14	-87.75
2008		2	151	1	40.14	-87.74
		3	150	2	40.21	-87.76
		4	152	122	40.23	-87.77
2008		1	168	49	41.33	-88.17
	Midewin	2	169	54	41.33	-88.18
		3	177	58	41.36	-88.21
		4	178	13	41.35	-88.17
		5	178	34	41.35	-88.17
		6	183	43	41.36	-88.31
		7	191	95	41.36	-88.31
2009	Beaver Dam	1	148	107	39.21	-89.98
2009	Castle Rock	1	152	103	41.95	-89.39
2009	Clinton Lake	1	139	120	40.17	-88.78
2009	Forest City	1	79	1	40.40	-89.80
		2	80	31	40.38	-89.82
		3	80	4	40.38	-89.82

		4	80	3	40.40	-89.81
		5	80	9	40.41	-89.81
2009	Green Earth	1	77	121	37.72	-89.24
		2	91	12	37.62	-89.21
		3	103	5	37.62	-89.18
		4	103	5	37.63	-89.17
		5	103	51	37.63	-89.17
2009	Jubilee	1	147	11	40.83	-89.81
		2	147	100	40.82	-89.82
2009	Kidd Lake Marsh	1	84	52	38.15	-90.20
		2	92	49	38.15	-90.19
2009	Lake of Egypt	1	74	34	37.63	-88.92
		2	74	72	37.63	-88.92
2009	Mississippi Pallisades	1	153	2	42.12	-90.16
		2	153	1	42.13	-90.09
		3	154	83	42.12	-90.09
		4	154	22	42.12	-90.09
2009	Moraine Hills	1	156	63	42.32	-88.24
		2	156	21	42.31	-88.25
2009	Rt3 Siren Pond	1	68	69	37.72	-89.46
		2	69	9	37.72	-89.46
		3	83	40	37.67	-89.42
2009	Sam Parr	1	133	41	39.01	-88.12
		2	134	24	39.03	-88.12
		3	133	61	39.02	-88.12
2009	Siloam	1	145	73	39.90	-90.93
		2	146	48	39.90	-90.93
2009	Silver Springs	1	131	1	41.63	-88.53
		2	131	16	41.63	-88.53
		3	132	20	41.62	-88.52

		4	132	20	41.62	-88.52
2009	Ten Mile Creek	1	112	42	38.06	-88.63
		2	112	24	38.08	-88.62
		3	118	16	38.23	-88.72
		4	118	31	38.23	-88.72
		5	118	10	38.23	-88.72
2009	Walnut Point	1	138	2	39.70	-88.04
		2	138	10	39.70	-88.03
		3	138	1	39.70	-88.03
		4	140	90	39.69	-88.07
2009	War Bluff	1	105	72	37.45	-88.49
		2	106	2	37.45	-88.49
		3	106	2	37.45	-88.49
		4	106	6	37.45	-88.49
		5	106	4	37.45	-88.49
		6	107	45	37.45	-88.49

All environmental, habitat variables, and GPS locations of current sample sites were entered into a database, and have been plotted into DIVA-GIS[7] (Figure 13).



A) B)

Figure 13. A) Sample sites and B) sample wetlands for widespread anuran survey from 2008 and 2009 (see Appendix 2 for results). Wetlands were nested within sample site for all analyses (see Figure 2 for layout).

GPS locations of museum specimens used in the histologic analyses are reported in Appendix 8.

3.3) Quantify degree and prevalence of infection by Bd among amphibian species, sites, and habitat types. Determine current Bd distribution: ubiquitous, patchily distributed (by region, habitat, or species), or not present.

We used results of the qPCR assay to calculate *Bd* infection prevalence and 95% Clopper-Pearson binomial confidence intervals for each widespread species at each site, in each wetland, and among species (Appendix 2). We detected *Bd* at every site, suggesting that *Bd* is ubiquitous. Furthermore, *Bd* was present in every wetland except for those sampled with very few anurans present. Similarly, we detected *Bd* in all widespread species except those when few animals were sampled.

[7] DIVA-GIS is a free computer program for mapping and geographic data analysis (a geographic information system) <http://www.diva-gis.org/>

We also analyzed the *Bd* data among species of special conservation concern to identify whether these species are infected with *Bd* (Appendix 3). These species had either limited geographic ranges, limited activity periods, limited scientific study, or are listed as Threatened or Endangered in Illinois: *H. avivoca*, *H. cinerea*, *P. streckeri*, *L. areolatus,* and *L. palustris*.

Using data entered in Obj. 3.2, we identified environmental and biological cofactors (e.g. Witte 2005) of infection (Appendix 4, Appendix 5). This assessment was covered in the manuscript that is currently under review by co-authors. We anticipate submitting this manuscript to Ecology, Spring 2013.

Job 4. Communicate progress to the IDNR and develop a final report.

Objective 4. Produce Reports and Maps. (Fall 2010-Spring 2013; <1% budget)

4.1) Produce maps of current and historic dates and locations of Bd distribution.

Bd prevalence and intensity among widespread anuran species is geographically displayed in Appendix 6 and Appendix 7. Appendix 6 reports site-level analyses while Appendix 7 reports wetland-level analyses.

We are currently using DNA sequencing to examine the genetic code among a handful of museum specimens who tested positive for Bd in the PCR analyses (see Appendix 8). Once those data are available we will produce maps of the historic geographic distribution of *Bd*.

4.2) Develop management plans to assist land managers based on presence/absence of Bd across state, in particular habitats, and in particular species.

Because of *Bd*'s ubiquitous distribution in Illinois we recommend that managers minimize the spread of *Bd* by educating site users and providing bleaching stations. There are multiple strains of *Bd* known to date (Morgan et al. 2007), some more lethal than others, so even though a site is infected we recommend minimizing additional introductions. We also recommend molecular analyses to identify the distribution of different strains throughout the state to better manage amphibian populations. In addition, we do not recommend reintroduction of threatened and endangered species anywhere in Illinois until methods are developed to eradicate *Bd* from the wild. Furthermore, we recommend determination of the population status and changes thereof for all wild populations, those that are of special conservation concern, and those species with restricted ranges. Thorough mark-recapture studies of Illinois amphibians will help identify whether they are declining, stable, or still recovering and how that relates to infection status.

4.3) Produce progress reports for IDNR and FWS.

We've used the following IDNR guidelines to create performance reports:

> *"(i) A comparison of actual accomplishments to the objectives established for the period. Where the output of the project can be quantified, a computation of the cost per unit of output may be required if that information will be useful.*
> *(ii) The reasons for slippage if established objectives were not met.*
> *(iii) Additional pertinent information including, when appropriate, analysis and explanation of cost overruns or high unit costs."*

Annual reports have been provided to the IDNR regarding the project's progress (Table 4), along with periodic updates via email[8] when key research was completed.

Table 4. Reports outlining progress, submitted to the IDNR.

Report Type	Date Submitted[9]	Study Year	Report Period
Annual Report	July 28, 2009	1	May 24 2008 – May 23, 2009
Annual Report	August 11, 2010	2	May 24 2009 – July 25, 2010
Annual Report	July 15, 2011	3	July 25, 2010 – July 15, 2011
Annual Report	August 15, 2012	4	July 15, 2011 – August 15, 2012
Final Report	February 1, 2013	1 – 4	May 24 2008 – August 15, 2012

Any publications that result from this project will be made available. Thus far, two publications in peer-reviewed journals have been published, with additional newletter contributions (see below). We are working on additional publications, which will also be the focus of BLT's PhD dissertation.

[8] Emails were sent to Scott Ballard <Scott.Ballard@illinois.gov> and/or Jody Shimp <Jody.Shimp@illinois.gov>.

[9] All reports submitted to Jody Shimp, IDNR, via email <Jody.Shimp@illinois.gov>

Peer-reviewed Publications:

Miller, D.A.W., B.L. Talley, K.R. Lips, and E.H.C. Grant. 2012. Estimating patterns and drivers of infection prevalence and intensity when detection is imperfect and sampling error occurs. Methods in Ecology and Evolution (*in press).*

Methods in Ecology and Evolution

Methods in Ecology and Evolution doi: 10.1111/j.2041-210X.2012.00216.x

Estimating patterns and drivers of infection prevalence and intensity when detection is imperfect and sampling error occurs

David A. W. Miller[1]*, Brooke L. Talley[2], Karen R. Lips[3] and Evan H. Campbell Grant[1]

[1]*U.S. Geological Survey – Patuxent Wildlife Research Center, 12100 Beech Forest Road, Laurel, MD 20708, USA;* [2]*Department of Zoology, Southern Illinois University, LS II 333, Carbondale, IL 62901, USA; and* [3]*Department of Biology, University of Maryland, 1210 Biology-Psychology Bldg, College Park, MD 20742, USA*

Summary

1. Epidemiological studies are crucial for understanding the distribution and dynamics of emerging infectious diseases. To accurately assess infection states in wild populations, researchers need to account for observational uncertainty. We focus on two sources of uncertainty when estimating epidemiological parameters: nondetection of infection in sampled individuals and sampling error when quantifying infection intensity for infected individuals.

2. We developed new analytical methods to simultaneously estimate prevalence and the distribution of infection intensities based on repeated sampling of individuals in the wild. The methods are an extension of those used for occupancy estimation and address both sources of observation error. At the same time, we account for heterogeneity in detection probability that results from individual variation in infection intensity.

3. We use two estimation approaches to account for detection. The first is to use the complete likelihood in a hierarchical Bayesian model and fit using Markov chain Monte Carlo sampling. The second is to estimate the detection relationship using a mark–recapture abundance estimator and use those results to calculate weighted estimates for prevalence and mean infection intensities.

4. We use data from a field survey of *Batrachochytrium dendrobatidis* in Illinois amphibians to test these methods. We show that detection probability using quantitative PCR is strongly related to infection intensity, measured in zoospore equivalents. Sites in the study varied greatly in estimated prevalence and to a lesser extent in mean infection intensities of infected individuals. We did not find evidence of a relationship of snout-vent-length to infection intensity or prevalence. Naïve estimates of prevalence that do not account for detection were less than estimates for either of our methods, which yielded similar prevalence values for most sites.

5. Uncertainty when assessing disease state is a characteristic of most diagnostic tests. The estimators presented here account for this uncertainty and thus can improve accuracy when assessing the relationship of ecological factors to prevalence and infection intensity.

Key-words: *Batrachochytrium dendrobatidis*, chytridiomycosis, detection, disease, occupancy, prevalence, sensitivity, specificity

Introduction

Increased interest in the dynamics of emerging wildlife diseases and zoonotics has spurred a need for field investigations to study the dynamics of diseases in wild animals (Daszak, Cunningham & Hyatt 2000). Scientists and wildlife managers need accurate assessments of current disease states and infection to understand disease dynamics in wild populations. However, methods that do not incorporate the effects of imperfect detection ignore a serious source of potential bias when estimating disease parameters (Jennelle *et al.* 2007; Conn & Cooch 2009; Murray *et al.* 2009; McClintock *et al.* 2010; Beyer *et al.* 2011). Recently developed inferential methods that account for incomplete detection in epidemiological studies have the potential to improve the quality of inferences researchers make from field data (reviewed in Cooch *et al.* 2011).

*Correspondence author. E-mail: davidmiller@usgs.gov

Talley, B.L., K.R. Lips, S.R. Ballard. 2011. *Batrachochytrium dendrobatidis* in *Siren intermedia* in Illinois, USA. Herpetological Review 42(2):216–217.

Herpetological Review, 2011, 42(2), 216–217.

Batrachochytrium dendrobatidis in *Siren intermedia* in Illinois, USA

We report the first case of *Batrachochytrium dendrobatidis* (*Bd*) in a wild-caught *Siren intermedia* from Illinois, USA. We add to the growing list of amphibian species susceptible to *Bd* infection in North America (Adams et al. 2007; Longcore et al. 2007; Ouellet et al. 2005; Pearl et al. 2007). Examples of fully aquatic salamanders with *Bd* originate from wild populations (*Cryptobranchus alleganiensis*, Briggler et al. 2007, 2008; *Andrias japonicus*, Goka et al. 2009), the pet trade (*Necturus maculosus* and *Siren lacertina*, Speare and Berger 2000), and zoos (*C. alleganiensis*, Briggler et al. 2007; *A. japonicus*, Goka et al. 2009). Internal parasitic helminth infections (McAllister et al. 1994) and two external copepod parasites (Frick 1999; Graham and Borda 2010) have been reported for Sirenidae, but our report is the first infectious disease of any kind reported in a wild-caught animal for this family (Hendricks 2005; Leja 2005; Moler 2005a, 2005b).

We sampled in a roadside ditch along Route 3 in Jackson County, Illinois (37.7241°N, 89.4634°W) on 9 March 2009 (1800–1830 h). Emergent vegetation and accumulated sediments were present, typical of *Siren* habitat (Petranka 1998; Wells 2007). Temperatures of the sampling date varied from 0°C (daily low) to 18°C (daily high); water temperature at time of sampling was 17°C. We captured two *S. intermedia* (SVL_1 = 132.1 mm, $Mass_1$ = 18.5 g; SVL_2 = 75.4 mm, $Mass_2$ = 3.9 g).

We handled sirens with latex powder-free gloves, and kept them in clean plastic sandwich bags filled with on-site water until we completed all data collection on site at which point we released all animals. We gently wiped each animal with a standard rayon-bud swab along their body lengths' ventral surfaces, as well as the front limbs (Hyatt et al. 2007). We stored the swab at room temperature in 70% ethanol until we ran real-time quantitative PCR analysis for *Bd*.

We used standard *Bd* DNA extraction and real-time PCR protocols (Hyatt et al. 2007), except we ran single samples instead of in triplicate to reduce costs (Kriger et al. 2006). Running single samples instead of triplicate is currently accepted as a reliable practice for *Bd* assessment (e.g., Vredenburg et al. 2010; Kriger et al. 2006). We used standards (i.e., 100, 10, 1, and 0.1 zoospore equivalents) and negative controls to ensure repeatability and continuity between runs.

The larger of the two *S. intermedia* that we captured tested positive for *Bd* (3.14 zoospore equivalents) while the other had no infection.

Because of their secretive nature (Petranka 1998) and the lack of population information (Leja 2005), the effect of *Bd* on Sirenidae populations is not known. Previous research on populations of fully aquatic salamanders in natural habitats is limited to *A. japonicus* in Japan (Goka et al. 2009) and *C. alleganiensis* in the Ozark Highlands, USA (Briggler et al. 2008). The former represents a commensal relationship that may have co-evolved with *Bd* over a long time period (Goka et al. 2009), while the latter identifies populations that have experienced declines likely resultant from synergistic negative effects of widespread *Bd* and other biotic and abiotic factors (e.g., habitat degradation, chemical contamination, introduced species, and commercial exploitation) (Briggler et al. 2008). We show that an individual *S. intermedia* carries a low-level *Bd* infection in Illinois. Future research should focus on if the fatal effects of chytridiomycosis are realized in Sirenidae.

Acknowledgments.—We thank S. N. Becker and two reviewers for comments on an early draft of this manuscript, and Erin and Edmund Tucker and T. Hill for field data collection. The laboratories of E. Heist (Southern Illinois University–Carbondale) and V. Vredenburg (San Francisco State University) were used during sample processing and qPCR, and J. Shimp (Illinois Department of Natural Resources) provided logistical support for all aspects of research. Funding by the Illinois Department of Natural Resources, through a State Wildlife Grant (#T-56 R-1), is very much appreciated. Authors have complied with all applicable institutional Animal Care guidelines (Institutional Animal Care and Use Committee Protocol #08-014), and all required state and federal permits have been obtained (IDNR Research Permit #SS09-21).

BROOKE L. TALLEY*
Southern Illinois University – Carbondale
Department of Zoology, Carbondale, Illinois 62901, USA
KAREN R. LIPS
University of Maryland,
Department of Biology, College Park, Maryland 20742, USA
SCOTT R. BALLARD
Illinois Department of Natural Resources,
9053 Route 148, Suite B, Marion, Illinois 62959, USA

** Corresponding author, e-mail: btalley@siu.edu*

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Newsletter Contributions:
Outdoor Illinois Magazine. April 2010.
Green Earth News. 2009. Vol 23. Issue 1.
Chicago Wilderness Habitat Project: *The Habitat Herald.* 2009. Vol 10. Issue 3.

4.4) Give presentations at scientific meetings

We presented fourteen presentations and three posters at scientific meetings (2009 – 2012) (See below).

Posters:

B.L. Talley and K.R. Lips. 2011. High levels of *Bd* prevalence and intensities in Illinois Amphibians. Ecology and Evolution of Infectious Diseases. Santa Barbara, CA.

B.L. Talley and K.R. Lips. 2010. Cache River State Natural Area Retains Population of Disease-Free State-Listed Treefrogs. Cache River Joint Venture Partnership & Southern Illinois University-Carbondale's 2010 Cache River Symposium. Vienna, IL.

Becker, S.N., B.L. Talley, and K.R. Lips. 2010. Familial Variation in Ecological Factors Influencing Anuran Body Temperatures. Joint Meeting of Icthyologists and Herpetologists. Providence, RI.

Presentations:

K.R. Lips. 2012. Untangling the Complexity of Amphibian Population Declines. SESYNC Seminar Series, Annapolis, MD 18 Sept. 2012.

K.R. Lips. 2012. Exploring the complexity of amphibian population declines: a tale of three studies. Forestry and Natural Resources Graduate Seminar Series, Purdue University, West Lafayette, IN, 28 February 2012.

B.L. Talley, K.R. Lips, and V. Vredenburg. 2012. Untangling the effects of environmental and biological factors on the intensity and prevalence of chytridiomycosis in Illinois amphibians. World Congress of Herpetology. Vancouver, Canada.

K.R. Lips. 2011. Emerging Infectious Disease and the Loss of Amphibian Biodiversity. Sigma Xi Speaker, Lehigh University, Lehigh PA, 14 April 2011.

B.L. Talley, K.R. Lips, and V. Vredenburg. 2011. Weighing the Odds: Environmental and Biological Factors Predicting Prevalence and Intensity of Disease in Illinois. Integrated Research Challenges in Environmental Biology (IRCEB), Emerging Wildlife Diseases: Threats to Amphibian Biodiversity. Tempe, AZ.

B.L. Talley and K.R. Lips. 2011. Heavy infections in Illinois anurans: Rethinking

enzootic disease status. Midwest Ecological and Evolution Conference. Carbondale, IL.

S.N. Becker, B.L. Talley, and K.R. Lips. 2011. Impacts of environmental factors on anuran body temperature and repercussions for disease dynamics. Ecological Society of American. Austin, TX.

B.L. Talley, K.R. Lips, and V. Vredenburg. 2011. High levels of disease prevalence and intensity in Illinois amphibians: Rethinking enzootic infections. Ecological Society of America. Austin, TX.

K.R. Lips. 2010. Emerging Infectious Disease and the Loss of Amphibian Biodiversity. Smithsonian Center for Conservation Biology, Washington DC, 3 December 2010.

B.L. Talley and K.R. Lips. 2010. A systematic survey of variation in the intensity & prevalence of Bd infection across Illinois: wetlands, sites, and species. Integrated Research Challenges in Environmental Biology (IRCEB), Emerging Wildlife Diseases: Threats to Amphibian Biodiversity. Tempe, AZ.

S.N. Becker, B.L. Talley, and K.R. Lips. 2010. Relating body temperature and substrate temperature to intensity of infection by *Bd* in Illinois anurans. Integrated Research Challenges in Environmental Biology (IRCEB), Emerging Wildlife Diseases: Threats to Amphibian Biodiversity. Tempe, AZ.

B.L. Talley and K.R. Lips. 2010. Patterns, prevalence, and intensity of infection of *Batrachochytrium dendrobatidis* across Illinois. Joint Meeting of Icthyologists and Herpetologists. Providence, RI.

Talley, B.L. and K.R. Lips. 2009. Bird-voiced treefrogs and statewide monitoring of chytridiomycosis in Illinois amphibians. The Midwest Fish and Wildlife Conference. Springfield, IL.

Talley, B.L. and K.R. Lips. 2009. From the mountains to the plains: Statewide surveys of *Bd* in Illinois. Integrated Research Challenges in Environmental Biology (IRCEB), Emerging Wildlife Diseases: Threats to Amphibian Biodiversity. Tempe, AZ.

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APPENDICES

APPENDIX 1
Animal Care and Scientific Permits

Animal care and scientific permits held during the course of this survey:

2008 –
IDNR Research Permit **#SS08-17**, Issued 28 March 2008
IDNR Scientific Permit for Possession of Endangered of Threatened Species **#NH08.5157**, Issued 17 March 2008

2009 –
IDNR Research Permit **#SS09-21**, Issued 12 March 2009
IDNR Scientific Permit for Possession of Endangered of Threatened Species **#NH09.5157**, Issued 3 March 2009

2010 –
IDNR Research Permit **#SS10-04**, Issued 1 January 2010
IDNR Scientific Permit for Possession of Endangered of Threatened Species **#NH10.5157**, Issued 31 December 2009
USFWS Research/Monitoring Permit **#1018-0102**, Issued 24 March 2010

2011 –
IDNR Research Permit **#SS11-12**, Issued 1 March 2011
IDNR Scientific Permit for Possession of Endangered of Threatened Species **#NH11.5157**, Issued 22 February 2011

2012 –
IDNR Research Permit **#SS12-04**, Issued 27 January 2012

IACUC:
#08-014, covers 1 May 2008 – 1 May 2011 (Cover Page below)

Southern Illinois University Carbondale

SOUTHERN ILLINOIS UNIVERSITY AT CARBONDALE
Animal Care & Use Protocol
Section 1 - Basic

FOR OFFICE USE ONLY		
Protocol #:	Date received:	☐ Animal Welfare Act covered species
Date of Final Approval:	Date of Expiration:	☐ IACUC Approved Exception
Comments		

Part A. Administrative Data

1. **Research protocol** **Revision/Modification** Replacement for protocol # (if applicable): **08-014**
2. Additional sections completed: ☐ Section 2 - Surgery ☐ Section 3 - Breeding ☐ Additional Personnel
3. Protocol Title: **Survey for the pathogen, Batrachochytrium dendrobatidis, in Illinois**
4. Proposed starting date: **1 May 2008** Proposed completion date: **1 May 2011**
   (Start date should be after anticipated protocol approval date. Total period should not exceed three years. If the entire project is expected to last > 3 years, describe only those activities expected to occur during the next 3 years.)
5. Funding Source: IDNR State Wildlife Grant Effective Date: **1 May 2008** Expiration Date: **1 May 2011**
6. If externally funded, list the exact application title (same title)
7. Date submitted to agency (current)
8. Are the contents of this protocol and the proposal animal care and use section the same **yes**
9. If not, explain
10. If this project is externally funded you **MUST** complete an External Funding Form.
11. University Account Number: **IDNR (pending)**
12. Provide the following information for **all** individuals authorized to conduct procedures involving animals under this proposal. (Only personnel current on employee health program requirements and listed on an IACUC approved animal use protocol will be provided access to the Laboratory Animal Program animal housing facilities. All personnel must provide assurance they are qualified to perform the procedure(s) indicated by describing their experience &/or training.)

Principal Investigator: **Karen Lips** Department: **Zoology**
- Work Tele. #: **618-453-4117** Home Tele. # (for after hour emergencies).
- Completed appropriate employee health program: **Yes**
- Procedures performed on animals as part of this proposal:
  ☒ Basic animal handling ☐ Husbandry ☐ Surgery ☐ Drug administration ☐ Euthanasia
  If other, explain:
- Describe specific animal related experience ensuring this individual is qualified to perform the procedures above on animals. For personnel without prior relevant experience, state how the person will be trained and who will do the training and the qualifications of the trainer. **Over 20 years of ecological field research experience**
- Have viewed the following SIUC IACUC web training modules and successfully passed the corresponding quizzes:
  ☐ Mice ☐ Rats ☐ Rabbits ☐ Guinea Pigs ☐ Hamsters ☐ Swine ☐ Fish
  ☒ Humane Care & Use ☐ Surgery ☐ Occ. Health & Safety ☐ Anesthesia & Anesthetic

Name **Brooke Talley** Status **Graduate Student**
- Work Tele. #: **618-453-4117** Home Tele. # (for after hour emergencies).
- Completed appropriate employee health program: **Select**
- Procedures performed on animals as part of this proposal:
  ☒ Basic animal handling ☐ Husbandry ☐ Surgery ☐ Drug administration ☐ Euthanasia
  If other, explain:
- Describe specific animal related experience ensuring this individual is qualified to perform the procedures above on animals. For personnel without prior relevant experience, state how the person will be trained and who will do the training and the qualifications of the trainer. **Over 6 years of ecological field research experience**
- Have viewed the following SIUC IACUC web training modules and successfully passed the corresponding quizzes:
  ☐ Mice ☐ Rats ☐ Rabbits ☐ Guinea Pigs ☐ Hamsters ☐ Swine ☐ Fish
  ☒ Humane Care & Use ☐ Surgery ☒ Occ. Health & Safety ☐ Anesthesia & Anesthetic

Figure A1.1 IACUC coversheet proposal; entire document presented to SIU IACUC committee by BLT and KRL on 23 April 2008.

APPENDIX 2
Overview of *Bd* Among Widespread Illinois Species

We found *Bd* in all sites, wetlands within sites, and species that had reliably large sample sizes. *Bd* intensity and prevalence estimates are reported in the following three tables:

Table A2.1 Site-level results for *Bd* prevalence and intensity. N represents the number of *Bd*-positive individuals at each site.

Year	Site	N / Total	Prevalence % (95% CI)	Intensity (Zoospore Equivalents)	
				Mean (± 95% CI)	Range
2008	Big Bend	3 / 41	7.32 (25.2 – 19.4)	18.8 ± 24.6	0.3 – 42.8
	Cache	69 / 114	60.5 (51.4 – 69.0)	2,241.3 ± 1,543.3	0.04 – 35,896.0
	Emiquon	22 / 129	17.1 (11.5 – 24.5)	717.1 ± 879.6	0.1 – 8,744.0
	Kickapoo	90 / 204	44.1 (37.5 – 51.0)	2,497.4 ± 1,921.1	0.3 – 57,020.0
	Midewin	90 / 345	26.1 (21.7 – 31.0)	1,007.2 ± 547.0	0.2 – 20,852.0
2009	Beaver Dam	92 / 107	86.0 (78.2 – 91.3)	2,076.3 ± 737.7	0.4 – 17,267.2
	Castle Rock	65 / 103	63.1 (53.5 – 71.8)	1,742.0 ± 1,892.7	0.2 – 58,361.6
	Clinton Lake	78 / 120	65.0 (56.1 – 72.9)	1,840.7 ± 1,435.4	0.2 – 53,040.8
	Forest City	15 / 48	31.2 (19.9 – 45.3)	1,995.5 ± 2,798.2	0.01 – 21,074.4
	Green Earth	132 / 194	68.0 (61.2 – 74.2)	2,082.5 ± 1,956.3	0.2 – 130,307.2
	Jubilee	64 / 111	57.7 (48.4 – 66.4)	4,588.8 ± 2,749.2	0.04 – 68,722.4
	Kidd Lake Marsh	50 / 101	49.5 (40.0 – 59.1)	1,127.3 ± 1,178.7	0.01 – 29,168.8
	Lake of Egypt	51 / 106	48.1 (38.8 – 57.5)	257.2 ± 295.5	0.03 – 7,482.4
	Mississippi Palisades	98 / 108	90.7 (84.0 – 94.9)	6,254.5 ± 3,187.1	1.2 – 98,176.0
	Moraine Hills	21 / 84	25.0 (17.0 – 35.2)	1,758.2 ± 2,227.4	0.2 – 21,549.6
	Siren Pond	61 / 118	51.7 (42.8 – 60.5)	267.9 ± 170.1	0.02 – 4,096.8
	Sam Parr	57 / 128	44.5 (36.2 – 53.2)	827.02 ± 498.3	0.02 – 8,401.6
	Siloam Spring	58 / 121	47.9 (39.2 – 56.8)	2,878.3 ± 2,784.4	0.01 – 76,483.2

Silver Springs	35 / 57	61.4 (48.4 – 72.9)	262.5 ± 180.2	0.02 – 2,298.7
Ten Mile Creek	28 / 123	22.7 (16.2 – 30.9)	2,417.6 ± 2,582.6	0.08 – 35,728.8
Walnut Point	65 / 103	63.1 (53.5 – 71.8)	1,154.4 ± 1,216.7	0.07 – 35,568.0
War Bluff	63 / 131	48.1 (39.7 – 56.6)	1,298.6 ± 1,148.8	0.01 – 32,718.4

Table A2.2 Wetland-level prevalence and intensity results for all animals. N represents the number of *Bd*-positive individuals in each wetland.

Year	Wetland ID	Latitude (Decimal Degrees)	Longitude (Decimal Degrees)	Habitat	N / Total	Prevalence % (95% CI)	Intensity (Zoospore Equivalents)	
							Mean ± 95% CI	Range
2008	Big Bend A	41.64	-90.04	ditch	3 / 41	7.32 (2.52 – 19.4)	18.8 ± 24.6	0.3 – 42.8
	Cache B	37.28	-89.05	wetland	13 / 15	86.7 (62.1 – 96.2)	405.2 ± 486.7	0.2 – 3,324.0
	Cache C	37.37	-89.08	wetland	8 / 18	44.4 (24.6 – 66.2)	140.1 ± 114.1	0.04 – 408.0
	Cache D	37.26	-89.09	wetland	13 / 28	46.4 (29.5 – 64.2)	305.1 ± 201.8	6.9 – 1,128.0
	Cache E	37.32	-89.07	wetland	35 / 53	66.0 (52.6 – 77.3)	4,122.8 ± 2,922.5	0.3 – 35,896.0
	Emiquon A	40.35	-90.11	wetland	3 / 62	4.84 (1.66 – 13.3)	6.04 ± 1.9	4.2 – 7.5
	Emiquon D	40.34	-90.12	pond	19 / 64	29.7 (19.9 – 41.8)	829.4 ± 1,012.7	0.1 – 8,744.0
	Emiquon E	40.30	-90.04	ditch	0 / 3	0 (0 – 56.1)	na	na
	Kickapoo D	40.14	-87.75	pond	6 / 13	46.2 (23.2 – 70.9)	117.6 ± 106.9	5.8 – 352.6
	Kickapoo E	40.14	-87.75	pond	20 / 36	55.6 (39.6 – 70.5)	528.3 ± 410.9	0.3 – 3,844.0
	Kickapoo F	40.14	-87.75	pond	2 / 4	50.0 (15.0 – 85.0)	607.6 ± 1,129.8	31.2 – 1,184.0
	Kickapoo G	40.14	-87.74	pond	1 / 1	100 (5.12 – 100)	24.6	na
	Kickapoo H	40.21	-87.76	wetland	1 / 2	50.0 (2.56 – 97.4)	6.8	na
	Kickapoo I	40.21	-87.76	wetland	10 / 25	40.0 (23.4 – 59.3)	45.4 ± 49.9	0.9 – 264.8
	Kickapoo L	40.23	-87.77	wetland	50 / 122	41.0 (32.7 – 49.9)	4,235.9 ± 3,391.5	0.3 – 57,020.0
	Midewin A	41.33	-88.17	pond	19 / 49	38.8 (26.4 – 52.8)	209.1 ± 143.5	2.8 – 1,140.0
	Midewin B	41.33	-88.18	pond	37 / 54	68.5 (55.3 – 79.3)	2,009.3 ± 1,242.6	0.2 – 20,852.0
	Midewin C	41.36	-88.21	pond	3 / 58	5.17 (1.77 – 14.1)	7.4 ± 5.5	3.8 – 12.9

	Midewin D	41.35	-88.17	pond	3 / 13	23.1 (8.18 – 50.3)	1,310.04 ± 2,108.4	6.1 – 3,444.0
	Midewin E	41.35	-88.17	pond	6 / 34	17.6 (8.35 – 33.5)	80.5 ± 81.8	0.4 – 246.4
	Midewin F	41.36	-88.31	wetland	18 / 43	41.9 (28.4 – 56.7)	244.04 ± 186.3	9.2 – 1,584.0
	Midewin H	41.36	-88.31	wetland	4 / 95	4.21 (1.65 – 10.3)	876.0 ± 1,667.3	15.0 – 3,428.0
2009	Beaver Dam A	39.21	-89.98	wetland	92 / 107	86.0 (78.2 – 91.3)	2,076.3 ± 737.7	0.4 – 17,267.2
	Castle Rock A	41.95	-89.39	wetland	65 / 103	63.1 (53.5 – 71.8)	1,741.9 ± 1,892.7	0.2 – 58,361.6
	Clinton Lake A	40.17	-88.78	pond	78 / 120	65.0 (56.1 – 72.9)	1,840.7 ± 1,435.4	0.2 – 53,040.8
	Forest City A	40.40	-89.80	field	1 / 1	100 (5.12 – 100)	3.4	na
	Forest City B	40.38	-89.82	field	3 / 31	9.68 (3.34 – 24.9)	1.5 ± 1.8	0.3 – 3.2
	Forest City C	40.38	-89.82	ditch	1 / 4	25.0 (1.28 – 69.9)	0.4	na
	Forest City D	40.40	-89.81	field	2 / 3	66.7 (20.8 – 98.3)	9.6 ± 17.5	0.7 – 18.6
	Forest City E	40.41	-89.81	ditch	8 / 9	88.9 (56.5 – 99.4)	3,738.1 ± 5,078.5	0.01 – 21,074.4
	Green Earth A	37.72	-89.24	wetland	90 / 121	74.4 (65.9 – 81.3)	2,269.3 ± 2,844.3	0.7 – 130,307.2
	Green Earth B	37.62	-89.21	wetland	7 / 12	58.3 (32.0 – 80.7)	522.6 ± 902.3	6.4 – 3,281.6
	Green Earth C	37.62	-89.18	ditch	1 / 5	20.0 (1.03 – 62.4)	148.8	na
	Green Earth D	37.63	-89.17	wetland	5 / 5	100 (56.6 – 100)	1,204.7 ± 868.8	52.2 – 2,446.4
	Green Earth E	37.63	-89.17	ditch	29 / 51	56.9 (43.3 – 69.5)	2,097.5 ± 1,225.0	0.2 – 14,526.4
	Jubilee A	40.82	-89.82	wetland	60 / 100	60.0 (50.2 – 69.1)	4,744.3 ± 2,918.5	0.04 – 68,722.4
	Jubilee B	40.83	-89.81	wetland	4 / 11	36.4 (15.2 – 64.6)	2,257.4 ± 4,415.5	0.4 – 9,016
	Kidd Lake Marsh A	38.15	-90.20	wetland	26 / 52	50.0 (36.9 – 63.1)	693.6 ± 614.6	0.01 – 7,725.6
	Kidd Lake Marsh B	38.15	-90.19	wetland	24 / 49	49.0 (35.6 – 62.5)	1,597.2 ± 2,377.1	0.1 – 29,168.8
	Lake of Egypt A	37.63	-88.92	field	2 / 34	5.88 (1.63 – 19.1)	11.0 ± 21.1	0.2 – 21.760
	Lake of Egypt B	37.63	-88.92	wetland	49 / 72	68.1 (56.6 – 77.7)	267.2 ± 307.3	0.03 – 7,482.4
	Miss.Palisades A	42.12	-90.16	ditch	0 / 2	0 (0 – 65.8)	na	na
	Miss.Palisades B	42.13	-90.09	wetland	0 / 1	0 (0 – 94.9)	na	na
	Miss. Palisades C	42.12	-90.09	wetland	78 / 83	94.0 (86.7 – 97.4)	4,119.3 ± 2,046.3	2.7 – 44,344.8
	Miss.Palisades D	42.12	-90.09	wetland	20 / 22	90.9 (72.2 – 97.5)	14,581.8 ± 13,050.3	1.2 – 98,176.0
	Moraine Hills A	42.32	-88.24	wetland	17 / 63	27.0 (17.6 – 39.0)	2,170.3 ± 2,728.9	0.2 – 21,549.6
	Moraine Hills B	42.31	-88.25	wetland	4 / 21	19.0 (7.67 – 40.0)	6.5 ± 9.8	0.9 – 21.4
	Siren Pond A	37.72	-89.46	ditch	52 / 69	75.3 (64.0 – 84.0)	274.5 ± 190.6	0.02 – 4,096.8
	Siren Pond B	37.72	-89.46	ditch	1 / 9	11.1 (0.57 – 43.5)	0.2	na
	Siren Pond C	37.67	-89.42	wetland	8 / 40	20.0 (10.5 – 34.8)	258.5 ± 408.5	0.1 – 1,685.6

Sam Parr B	39.01	-88.12	ditch	13 / 43	30.2 (18.6 – 45.1)	92.9 ± 91.0	0.7 – 585.6
Sam Parr C	39.03	-88.12	wetland	17 / 24	70.8 (50.8 – 85.1)	1,127.0 ± 1,061.9	0.1 – 8,191.3
Sam Parr R	39.02	-88.12	ditch	27 / 61	44.3 (32.5 – 56.7)	991.6 ± 799.9	0.02 – 8,401.6
Siloam A	39.90	-90.93	wetland	30 / 73	41.1 (30.5 – 52.6)	3,975.3 ± 5,109.6	0.01 – 76,483.2
Siloam B	39.90	-90.93	wetland	0 / 1	0 (0 – 94.9)	na	na
Siloam C	39.90	-90.93	wetland	12 / 24	50.0 (31.4 – 68.6)	103.8 ± 116.2	0.1 – 648.8
Siloam D	39.90	-90.93	wetland	16 / 23	69.6 (49.1 – 84.4)	2,902.2 ± 3,176.3	0.02 – 24,876.8
Silver Springs A	41.63	-88.53	wetland	1 / 1	100 (5.12 – 100)	147.2	na
Silver Springs B	41.63	-88.53	wetland	12 / 16	75.0 (50.5 – 89.8)	357.9 ± 313.7	7.6 – 1,727.68
Silver Springs C	41.62	-88.52	wetland	10 / 20	50.0 (29.9 – 70.1)	421.9 ± 489.9	0.02 – 2,298.72
Silver Springs D	41.62	-88.52	wetland	12 / 20	60.0 (38.7 – 78.1)	44.0 ± 54.7	0.04 – 343.200
Ten Mile Creek A	38.06	-88.63	wetland	1 / 42	2.38 (0.12 – 12.3)	14.2	na
Ten Mile Creek B	38.08	-88.62	wetland	12 / 24	50.0 (31.4 – 68.6)	4,334.8 ± 5,898.7	0.1 – 35,728.8
Ten Mile Creek C	38.23	-88.72	field	9 / 16	56.3 (33.2 – 76.9)	1,531.5 ± 1,293.6	9.36 – 5,761.6
Ten Mile Creek D	38.23	-88.72	field	6 / 31	19.3 (9.19 – 36.3)	312.7 ± 519.1	0.2 – 1,631.3
Walnut Point A	39.70	-88.04	wetland	1 / 2	50.0 (2.56 – 97.4)	311.3	na
Walnut Point B	39.70	-88.03	wetland	3 / 10	30.0 (10.8 – 60.3)	46.7 ± 78.5	0.4 – 126.4
Walnut Point D	39.70	-88.03	wetland	1 / 1	100 (5.12 – 100)	55.0	na
Walnut Point E	39.69	-88.07	wetland	42 / 67	62.7 (50.7 – 73.3)	1,756.2 ± 1,865.5	0.1 – 35,568
Walnut Point F	39.69	-88.07	wetland	18 / 23	78.3 (58.1 – 90.3)	42.9 ± 37.7	0.2 – 299.2
War Bluff A	37.45	-88.49	wetland	35 / 72	48.6 (37.4 – 59.9)	1,279.4 ± 997.0	0.01 – 15,767.2
War Bluff B	37.45	-88.49	wetland	1 / 2	50.0 (2.56 – 97.4)	3,502.0	na
War Bluff C	37.45	-88.49	wetland	2 / 2	100 (34.2 – 100)	4.2 ± 7.3	0.5 – 7.984
War Bluff D	37.45	-88.49	wetland	5 / 6	83.3 (43.6 – 99.1)	6,560.6 ± 12.817.1	0.01 – 32,718.4
War Bluff E	37.45	-88.49	ditch	3 / 4	75.0 (30.1 – 98.7)	5.6 ± 7.5	0.6 – 13.04
War Bluff F	37.45	-88.49	pond	17 / 45	37.8 (25.1 – 52.4)	41.5 ± 47.1	0.1 – 330.4

Table A2.3 We detected *Bd* in all species except those with small sample sizes, and found high levels of prevalence and intensity among species.

			Intensity (Zoospores, GE)	
Species	**# Infected / Total**	**Prevalence % (95% CI)**	**Mean ± 95% CI**	**Range**

	2008	2009	2008	2009	2008	2009	2008	2009
Acris crepitans	110 / 244	360 / 570	45.1 (39.0 – 51.4)	63.2 (59.1 – 67.0)	3,152 ± 1,554	3,002 ± 845	0.172 – 45,872	0.022 – 76,483
Anaxyrus americanus	14 / 53	64 / 155	26.4 (16.4 – 40.0)	41.3 (33.8 – 49.2)	92 ± 62	732 ± 540	0.331 – 326	0.007 – 13,076
Anaxyrus fowleri	0 / 3	12 / 66	0 (0 – 56.1)	18.2 (10.7 – 29.1)	na	15 ± 19	na	0.0200 – 104
Hyla chrysoscelis / versicolor	33 / 152	161 / 329	21.7 (15.9 – 28.9)	48.9 (43.6 – 54.3)	2,939 ± 3,444	3,694 ± 2,057	0.121 – 57,020	0.018 – 98,176
Pseudacris crucifer	17 / 37	103 / 193	45.9 (31.0 – 61.6)	53.4 (46.3 – 60.3)	649 ± 481	845 ± 653	0.324 – 3,844	0.016 – 29,169
Pseudacris feriarum	---	7 / 10	---	70.0 (39.7 – 89.2)	---	4,637 ± 3,431	---	148.8 – 11,614
Pseudacris triseriata	0 / 4	16 / 51	0 (0 – 49.0)	31.4 (20.3 – 45.0)	na	355 ± 535	na	0.330 – 4,317
Lithobates blairi	1 / 2	14 / 17	50.0 (2.56 – 97.4)	82.3 (59.0 – 93.8)	3	289 ± 204	na	0.80 – 998
Lithobates catesbeianus	50 / 138	164 / 303	36.2 (28.7 – 44.5)	54.1 (48.5 – 59.6)	321 ± 387	768 ± 365	0.94 – 9,912	0.011 – 21,074
Lithobates clamitans	26 / 63	---	41.3 (30.0 – 53.6)	---	263 ± 276	---	1.472 – 3,428	---
Lithobates pipiens	5 / 106	9 / 11	4.71 (2.03 – 10.6)	81.8 (52.3 – 94.9)	76 ± 110	55 ± 72	0.303 – 299	0.035 – 343
Lithobates sphenocephalus	18 / 30	123 / 158	60.0 (42.3 – 75.4)	77.8 (70.8 – 83.6)	367 ± 363	2,046 ± 2,153	0.036 – 3,324	0.054 – 130,307

APPENDIX 3
Bd Among Species of Special Conservation Concern

Summary:
We sampled species of special conservation concern to ascertain whether these populations harbor *Bd* infections. These anuran species included those that have declined for unknown reasons (i.e., *L. areolatus*), are state-listed as threatened or endangered (i.e., *P. streckeri, H. avivoca*) or those that have limited geographic distributions (i.e., *H. cinerea* and *L. palustris*). Many of the aforementioned species fall within multiple categories of conservation concern previously mentioned, along with limited activity periods and limited scientific study in Illinois. We sampled atleast 30 individuals of the aforementioned species types when there were enough animals present.

Results:

Table A3.1 *Bd* prevalence and intensity among species of special concern.

Species	# Bd Positive	Total	Prevalence (%)	Prevalence 95% CI	Intensity (μ Genomic Equivalents)
Hyla avivoca	4	93	4.3	1.7 – 10.5	0.47
Hyla cinerea	4	89	4.5	1.8 – 11.0	1.18
Lithobates areolatus	34	107	31.8	23.7 – 41.1	589.34
Lithobates palustris	0	20	0	0 – 16.1	---
Pseudacris streckeri	52	176	29.5	23.3 – 36.7	37.47

Discussion:
L. areolatus and *P. streckeri* are the species of greatest conservation conern, regarding their *Bd* prevalence levels (~30%) and mean *Bd* intensity. While their *Bd* prevalence and intensity levels fall within the normal range of widespread species in Illinois (see Appendix 2), we suggest further monitoring of these species to ascertain how population levels respond to *Bd* infection. The high level of *Bd* intensity among *L. areolatus* is particularly concerning because chytridiomycosis is a load-based disease, suggesting that a heavier burdens of *Bd* zoospores will make an individual sicker.

The low prevalence and intensity levels of the remaining species of special conservation concern indicate that these animals either (1) exist in habitats or wetlands that are not infected with *Bd* at the time of study, (2) fight-off infection with a species-based trait, (3) or quickly succomb to chytridiomycosis and are, therefore, unavailable for study (i.e., dead frogs are quickly resorbed into the environment, and not typically available for *Bd* infection). Further research into these questions will help us understand *Bd*-related risk for these species.

Future Products:
We plan to further analyze these data and publish the results in the near future.

APPENDIX 4
Independent Variables Explaining *Bd* Prevalence and Intensity

The following tables display results of all the models used in explaining prevalence and intensity of *Bd* infection among widespread species. They are arranged by univariate results used to build multivariate models, which follow. Summary of top models is displayed in Table A4.7, at the end of this Appendix.

Table A4.1 Univariate results of site-level Bd prevalence in 2008. Because we could not construct multivariate models of factors controlling site-level Bd prevalence in 2008, we rely on the following univariate tests of relationships among environmental and biological variables to help guide our assessments. Bolded numbers indicate significant relationships ($p < 0.05$) between model and Bd prevalence based on analysis of maximum likelihood estimates. Models are ranked in descending order based on AIC score. Wt = wetland-level factor, St = site-level factor, Prop = proportion.

2008 Univariate Site-Level Prevalence Models	K	AIC	Delta AIC	AIC Wt	Cum Wt	Estimate	St. Error	Z value	No. Obs.	No. Groups	p-value
JDay	3	964.8956	0.0000	0.6427	0.6427	-0.037182	0.0066830	-5.563	833	5	**0.0000000**
Wt.Prop.Hylidae	3	966.5366	1.6411	0.2829	0.9256	0.012343	0.0023890	5.167	833	5	**0.0000002**
Wt.Frog.Density	3	969.3893	4.4938	0.0679	0.9935	-0.035256	0.0073780	-4.778	833	5	**0.0000018**
Wt.Prop.Ranidae	3	974.7221	9.8265	0.0047	0.9982	-0.010723	0.0024640	-4.352	833	5	**0.0000135**
Wt.Water.Temp	3	976.8079	11.9123	0.0017	0.9999	-0.19375	0.0365200	-5.306	833	5	**0.0000001**
St.Prop.Bufonidae	3	984.4331	19.5375	0.0000	0.9999	0.28132	0.0643100	4.375	833	5	**0.0000122**
Ave.Mean.Temp	3	985.9670	21.0714	0.0000	1.0000	-0.19296	0.0472200	-4.086	833	5	**0.0000438**
Wt.Prop.Bufonidae	3	986.4549	21.5593	0.0000	1.0000	-0.01907	0.0070100	-2.72	833	5	**0.0065300**
Ave.High.Temp	3	986.4681	21.5726	0.0000	1.0000	-0.19101	0.0493200	-3.873	833	5	**0.0001080**
Latitude	3	987.3888	22.4932	0.0000	1.0000	-0.5372	0.1660000	-3.236	833	5	**0.0012100**
Longitude	3	988.3354	23.4398	0.0000	1.0000	1.0364	0.4202000	2.466	833	5	**0.0136000**
St.Sp.Rich	3	993.1564	28.2608	0.0000	1.0000	0.2114	0.1892000	1.118	833	5	0.2637000
Wt.Sp.Rich	3	993.1738	28.2782	0.0000	1.0000	0.07297	0.0657800	1.109	833	5	0.2673000
St.Prop.Ranidae	3	993.7840	28.8884	0.0000	1.0000	-0.01057	0.0138600	-0.762	833	5	0.4460000
Wt.Shannon	3	993.9979	29.1024	0.0000	1.0000	-0.07612	0.1211900	-0.628	833	5	0.5300000
St.Prop.Hylidae	3	994.0826	29.1871	0.0000	1.0000	0.007583	0.0144880	0.523	833	5	0.6010000

Table A4.2 Constructing multivariate models that best describe site-level Bd prevalence in 2009. (a) Univariate tests of relationships among environmental and biological variables. Bolded numbers indicate significant relationships ($p < 0.05$) and those used to build more complex multivariate models ($p < 0.25$) between model and Bd prevalence. (b) Multivariate models of relationships among environmental and biological variables on site-level *Bd* prevalence. P-values are based on analysis of maximum likelihood estimates between model and prevalence. Models are ranked in descending order based on AIC score. Wt = wetland-level factor, St = site-level factor, Prop = proportion.

(a)

2009 Univariate Site-Level Prevalence Models	K	AIC	Delta AIC	AIC Wt	Cum Wt	Estimate	St. Error	Z Value	No. Obs.	No. Groups	p-value
Wt.Water.Temp	3	2292.4570	0.0000	1.0000	1.0000	0.10063	0.0849900	1.184	1797	17	**0.2360000**
Wt.Prop.Ranidae	3	2348.8400	56.3828	0.0000	1.0000	0.011999	0.0020940	5.73	1863	17	**0.0000000**
Wt.Prop.Bufonidae	3	2351.6660	59.2091	0.0000	1.0000	-0.01403	0.0026010	-5.393	1863	17	**0.0000001**
Wt.Shannon	3	2368.0160	75.5589	0.0000	1.0000	0.3065	0.0814900	3.761	1863	17	**0.0001690**
JDay	3	2374.1920	81.7350	0.0000	1.0000	-0.0259	0.0072600	-3.5730	1863	17	**0.0003530**
Wt.Sp.Rich	3	2375.9650	83.5083	0.0000	1.0000	0.1335	0.0534000	2.5	1863	17	**0.0124000**
St.Prop.Hylidae	3	2377.4490	84.9927	0.0000	1.0000	0.020485	0.0087620	2.338	1863	17	**0.0194000**
St.Prop.Bufonidae	3	2378.0690	85.6119	0.0000	1.0000	-0.03034	0.0140400	-2.162	1863	17	**0.0307000**
Longitude	3	2379.4410	86.9840	0.0000	1.0000	-0.4081	0.2417000	-1.6890	1863	17	**0.0913000**
Wt.Frog.Density	3	2380.0570	87.6002	0.0000	1.0000	-0.0229	0.0154600	-1.481	1863	17	**0.1385000**
St.Sp.Rich	3	2380.9420	88.4850	0.0000	1.0000	-0.1492	0.1297000	-1.151	1863	17	**0.2500000**
Wt.Prop.Hylidae	3	2380.9630	88.5064	0.0000	1.0000	-0.002092	0.0018630	-1.123	1863	17	0.2620000
Latitude	3	2381.0380	88.5809	0.0000	1.0000	0.1333	0.1228000	1.085	1863	17	0.2780000
St.Prop.Ranidae	3	2381.0970	88.6398	0.0000	1.0000	-0.01318	0.0123200	-1.07	1863	17	0.2850000
Ave.High.Temp	3	2382.0280	89.5717	0.0000	1.0000	0.01999	0.0476000	0.42	1863	17	0.6740000
Ave.Mean.Temp	3	2382.1020	89.6452	0.0000	1.0000	0.01531	0.0478700	0.32	1863	17	0.7490000

(b)

2009 Multivariate Site-Level Prevalence Models	K	AIC	Delta AIC	AIC Wt	Cum Wt
Wt.Water.Temp+Wt.Prop.Ranidae+Wt.Prop.Bufonidae	5	2241.847	0.0000	0.9997	0.9997
Wt.Water.Temp+Wt.Prop.Ranidae	4	2259.357	17.5098	0.0002	0.9999
Wt.Water.Temp+Wt.Prop.Bufonidae	4	2260.151	18.3039	0.0001	1.0000
Wt.Water.Temp+Shannon+J.Day	5	2273.262	31.4151	0.0000	1.0000

Wt.Water.Temp+Shannon	4	2273.968	32.1203	0.0000	1.0000
Wt.Water.Temp+J.Day	4	2285.449	43.6013	0.0000	1.0000
Wt.Water.Temp+Wt.Frog.Density+J.Day	5	2285.728	43.8808	0.0000	1.0000
Wt.Water.Temp+Wt.Frog.Density+Wt.Sp.Rich	5	2286.229	44.3820	0.0000	1.0000
Wt.Water.Temp+Wt.Sp.Rich	4	2286.439	44.5912	0.0000	1.0000
Wt.Water.Temp+St.Prop.Hylidae	4	2289.934	48.0861	0.0000	1.0000
Wt.Water.Temp+St.Prop.Bufonidae	4	2290.443	48.5955	0.0000	1.0000
Wt.Water.Temp+Longitude	4	2291.399	49.5515	0.0000	1.0000
Wt.Water.Temp+Wt.Frog.Density	4	2291.417	49.5698	0.0000	1.0000
Wt.Water.Temp	3	2292.457	50.6093	0.0000	1.0000
Wt.Water.Temp+St.Sp.Rich	4	2293.096	51.2484	0.0000	1.0000
Wt.Prop.Ranidae+Wt.Prop.Bufonidae	4	2334.823	92.9760	0.0000	1.0000
Wt.Prop.Ranidae+Shannon	4	2339.455	97.6079	0.0000	1.0000
Wt.Prop.Ranidae+Wt.Sp.Rich	4	2343.535	101.6880	0.0000	1.0000
Wt.Prop.Ranidae+J.Day	4	2345.619	103.7712	0.0000	1.0000
Wt.Prop.Ranidae+Wt.Frog.Density	4	2346.193	104.3458	0.0000	1.0000
Wt.Prop.Ranidae	3	2348.840	106.9922	0.0000	1.0000
Wt.Prop.Bufonidae	3	2351.666	109.8184	0.0000	1.0000
Shannon	3	2368.016	126.1682	0.0000	1.0000
Shannon+Wt.Frog.Density	4	2369.351	127.5035	0.0000	1.0000
J.Day	3	2374.192	132.3444	0.0000	1.0000
J.Day+Wt.Frog.Density	4	2375.089	133.2418	0.0000	1.0000
Wt.Sp.Rich	3	2375.965	134.1177	0.0000	1.0000
Wt.Sp.Rich+Wt.Frog.Density	4	2376.510	134.6628	0.0000	1.0000
St.Prop.Hylidae	3	2377.449	135.6020	0.0000	1.0000
St.Prop.Bufonidae	3	2378.069	136.2212	0.0000	1.0000
Longitude	3	2379.441	137.5934	0.0000	1.0000
Wt.Frog.Density	3	2380.057	138.2095	0.0000	1.0000
St.Sp.Rich	3	2380.942	139.0943	0.0000	1.0000

Table A4.3 Constructing multivariate models that best describe wetland-level Bd prevalence in 2008. Each model was run with a random wetland variable nested within site. (a) Univariate tests of relationships among environmental and biological variables. Bolded numbers indicate significant relationships ($p < 0.05$) and those used to build more complex multivariate models ($p < 0.25$) between model and Bd prevalence. (b) Multivariate models of relationships among environmental and biological variables on wetland-level *Bd* prevalence. P-values are based on analysis of maximum likelihood estimates between model and prevalence. Models are ranked in descending order based on AIC score. Wt = wetland-level factor, St = site-level factor, Prop = proportion.

(a)

2008 Univariate Wetland-Level Prevalence Models	K	AIC	Delta_AIC	AIC	Cum. Wt	Estimate	St. Error	Z Value	No. Obs.	No. Groups	p-value
JDay	17	915.77	0.00	0.30	0.30	-0.04	0.01	-5.24	833	22	**0.0000002**
Wt.Water.Temp	17	916.44	0.67	0.21	0.51	-0.19	0.04	-4.63	833	22	**0.0000038**
Ave.Mean.Temp	17	917.38	1.61	0.13	0.64	-0.22	0.05	-4.81	833	22	**0.0000015**
Ave.High.Temp	17	917.88	2.11	0.10	0.74	-0.22	0.05	-4.78	833	22	**0.0000017**
St.Prop.Bufonidae	17	918.00	2.22	0.10	0.84	0.29	0.06	4.70	833	22	**0.0000026**
Wt.Frog.Density	17	918.78	3.01	0.07	0.91	-0.03	0.01	-3.87	833	22	**0.0001100**
Latitude	17	920.18	4.41	0.03	0.94	-0.32	0.12	-2.63	833	22	**0.0085000**
St.Sp.Rich	17	921.21	5.44	0.02	0.98	-0.53	0.20	-2.67	833	22	**0.0076600**
Longitude	17	921.20	5.42	0.02	0.96	0.87	0.20	4.42	833	22	**0.0000097**
St.Prop.Ranidae	17	923.91	8.14	0.01	0.98	-0.03	0.01	-3.95	833	22	**0.0000780**
Wt.Sp.Rich	17	924.71	8.94	0.00	0.99	-0.10	0.10	-0.95	833	22	0.3430000
St.Prop.Hylidae	17	924.77	9.00	0.00	0.99	0.03	0.01	3.99	833	22	**0.0000666**
Wt.Prop.Ranidae	17	925.29	9.52	0.00	0.99	0.00	0.00	-0.57	833	22	0.5700000
Wt.Prop.Bufonidae	17	925.32	9.55	0.00	1.00	0.00	0.01	0.48	833	22	0.6321000
Wt.Prop.Hylidae	17	925.45	9.68	0.00	1.00	0.00	0.00	0.35	833	22	0.7260000
Wt.Shannon	17	925.54	9.77	0.00	1.00	0.02	0.19	0.11	833	22	0.9150000

(b)

2008 Multivariate Wetland-Level Prevalence Models	K	AIC	Delta_AIC	AICWt	Cum.Wt
J.Day+Ave.Mean.Temp+Ave.High.Temp	19	912.75	0.00	0.15	0.15
Latitude+Longitude	18	912.91	0.16	0.14	0.28
Wt.Water.Temp+Ave.High.Temp+Ave.Mean.Temp	19	913.18	0.43	0.12	0.40
J.Day+Wt.Frog.Denisty	18	914.08	1.34	0.08	0.48

J.Day+Latitude+Ave.Mean.Temp+Ave.High.Temp	20	914.51	1.76	0.06	0.54
J.Day+Wt.Water.Temp+Ave.Mean.Temp+Ave.High.Temp	20	914.52	1.78	0.06	0.60
J.Day+Wt.Water.Temp	18	915.73	2.99	0.03	0.64
J.Day+St.Prop.Bufonidae	18	915.75	3.00	0.03	0.67
J.Day	17	915.77	3.03	0.03	0.70
J.Day+Wt.Frog.Density+St.Sp.Rich	19	916.00	3.26	0.03	0.73
St.Prop.Bufonidae+Wt.Frog.Density+J.Day	19	916.07	3.32	0.03	0.76
Wt.Frog.Density+St.Sp.Rich	18	916.18	3.44	0.03	0.79
J.Day+Wt.Water.Temp+Ave.Mean.Temp	19	916.33	3.59	0.02	0.81
Wt.Water.Temp	17	916.44	3.70	0.02	0.83
J.Day+Latitude	18	916.49	3.75	0.02	0.86
J.Day+Wt.Frog.Density+St.Sp.Rich+Ave.Mean.Temp+Ave.High.Temp	21	916.63	3.89	0.02	0.88
J.Day+Wt.Water.Temp+Ave.High.Temp	19	916.72	3.97	0.02	0.90
Ave.Mean.Temp	17	917.38	4.64	0.01	0.91
J.Day+Latitude+Wt.Water.Temp	19	917.70	4.96	0.01	0.92
Wt.Frog.Density+St.Sp.Rich+Latitude	19	917.72	4.97	0.01	0.94
Ave.High.Temp	17	917.88	5.14	0.01	0.95
J.Day+Wt.Frog.Density+St.Sp.Rich+Ave.Mean.Temp+Ave.High.Temp+Wt.Water.Temp	22	917.95	5.21	0.01	0.96
St.Prop.Bufonidae	17	918.00	5.25	0.01	0.97
St.Prop.Bufonidae+Wt.Frog.Density	18	918.77	6.02	0.01	0.98
Wt.Frog.Density	17	918.78	6.03	0.01	0.98
St.Prop.Ranidae+St.Prop.Hylidae	18	918.93	6.18	0.01	0.99
Latitude	17	920.18	7.43	0.00	0.99
Longitude	17	921.20	8.45	0.00	1.00
St.Sp.Rich	17	921.21	8.47	0.00	1.00
St.Prop.Ranidae	17	923.91	11.17	0.00	1.00
St.Prop.Hylidae	17	924.79	12.05	0.00	1.00

Table A4.4 Univariate results of wetland-level Bd prevalence in 2009. Because we could not construct multivariate models of factors controlling wetland-level Bd prevalence in 2009, we rely on the following univariate tests of relationships among environmental and biological variables to help guide our assessments. Bolded numbers indicate significant relationships ($p < 0.05$) between model and Bd prevalence based on analysis of maximum likelihood estimates. Models are ranked in descending order based on AIC score. Wt = wetland-level factor, St = site-level factor, Prop = proportion.

2009 Univariate Wetland-Level Prevalence Models	K	AIC	DeltaAIC	AICWt	CumWt	Estimate	St. Error	Z Value	No. Obs.	No. Groups	p-value
Wt.Water.Temp	155	2442.54	0.00	1	1	0.22	0.08	2.76	1797	53	0.00583
Latitude	155	2534.88	92.34	0	1	0.13	0.05	2.79	1863	55	0.00535
Wt.Prop.Bufonidae	155	2536.24	93.70	0	1	-0.01	0.00	-2.16	1863	55	0.03106
Wt.Frog.Density	155	2536.38	93.84	0	1	-0.06	0.02	-2.91	1863	55	0.00361
Wt.Prop.Ranidae	155	2537.04	94.50	0	1	0.21	0.08	2.51	1863	55	0.0119
Longitude	155	2537.66	95.12	0	1	0.15	0.08	1.83	1863	55	0.0671
St.Sp.Rich	155	2537.93	95.39	0	1	-0.15	0.06	-2.32	1863	55	0.02036
St.Prop.Hylidae	155	2537.98	95.44	0	1	0.01	0.00	1.39	1863	55	0.163
St.Prop.Bufonidae	155	2538.37	95.83	0	1	-0.02	0.01	-2.53	1863	55	0.0114
St.Prop.Ranidae	155	2538.40	95.86	0	1	0.00	0.00	-1.05	1863	55	0.2956
Ave.Mean.Temp	155	2538.70	96.16	0	1	0.02	0.02	1.09	1863	55	0.277
Ave.High.Temp	155	2538.71	96.17	0	1	0.02	0.02	1.10	1863	55	0.27
Wt.Sp.Rich	155	2538.87	96.33	0	1	-0.08	0.06	-1.24	1863	55	0.2142
JDay	155	2539.13	96.59	0	1	0.00	0.00	0.51	1863	55	0.612
Wt.Shannon	155	2539.18	96.64	0	1	-0.09	0.13	-0.69	1863	55	0.492
Wt.Prop.Hylidae	155	2539.23	96.69	0	1	0.00	0.00	0.06	1863	55	0.951

Table A4.5 Constructing multivariate models that best describe Bd infection intensity in 2008. Univariate tests of relationships among all environmental and biological variables were run assuming (a) all fixed effects and with (b) mixed models, where each model was run with a random wetland variable nested within site. Bolded numbers indicate significant relationships ($p < 0.05$) and those used to build more complex multivariate models ($p < 0.25$) between model and Bd intensity. (c) Multivariate mixed effects models of relationships among environmental and biological variables on wetland-level *Bd* prevalence. Models are ranked in ascending order based on AIC score. Wt = wetland-level factor, St = site-level factor, Prop = proportion.

(a)

2008 Univariate Intensity Fixed-Effects Models	**K**	**AIC**	**DeltaAIC**	**AICwt**	**CumWt**	**Estimate**	**St. Error**	**F statistic**	**df**	**p-value**
Wt.Sp.Rich	3	846.77	0.00	0.29	0.29	0.16	0.06	6.83	1, 272	**0.009458**
Wt.Prop.Hylidae	3	847.74	0.97	0.18	0.47	0.00	0.00	5.85	1, 272	**0.01625**
Wt.Prop.Ranidae	3	847.76	0.98	0.18	0.65	0.00	0.00	5.83	1, 272	**0.01641**
St.Prop.Bufonidae	3	849.94	3.16	0.06	0.71	0.06	0.03	3.63	1, 272	**0.05784**
St.Sp.Rich	3	849.97	3.20	0.06	0.77	0.10	0.05	3.60	1, 272	**0.05889**
Wt.Frog.Density	3	850.06	3.29	0.06	0.83	-0.01	0.01	3.50	1, 272	**0.06236**
Wt.Longitude	3	850.89	4.12	0.04	0.86	0.15	0.09	2.67	1, 272	**0.1033**
St.Proportion.Hylidae	3	851.60	4.82	0.03	0.89	-0.01	0.00	1.97	1, 272	**0.1621**
Wt.Shannon	3	851.63	4.86	0.03	0.92	0.10	0.07	1.93	1, 272	**0.1659**
St.Prop.Ranidae	3	851.94	5.17	0.02	0.94	0.00	0.00	1.62	1, 272	**0.2041**
Wt.Water.Temp	3	852.97	6.19	0.01	0.95	0.02	0.02	0.60	1, 272	0.4397
J.Day	3	853.45	6.68	0.01	0.96	0.00	0.00	0.11	1, 272	0.7349
Wt.Prop.Bufonidae	3	853.52	6.75	0.01	0.97	0.00	0.01	0.05	1, 272	0.829
Wt.Latitude	3	853.55	6.78	0.01	0.98	0.01	0.04	0.02	1, 272	0.8893
Ave.High.Temp	3	853.56	6.78	0.01	0.99	0.00	0.02	0.01	1, 272	0.9148
Ave.Mean.Temp	3	853.57	6.79	0.01	1.00	0.00	0.04	0.00	1, 272	0.9457

(b)

2008 Univariate Intensity Mixed-Effects Models	**K**	**AIC**	**DeltaAIC**	**AICwt**	**CumWt**	**Estimate**	**St Error**	**t-value**
Wt.Prop.Ranidae	18	867.51	0.00	0.22	0.22	-0.01	0.00	-2.75
Wt.Prop.Hylidae	18	867.82	0.30	0.19	0.40	0.01	0.00	2.72
Wt.Shannon	18	868.69	1.17	0.12	0.52	0.18	0.08	2.41

Wt.Sp.Rich	18	868.75	1.33	0.12	0.64	0.18	0.07	2.39
St.Prop.Bufonidae	18	868.99	1.48	0.10	0.74	0.08	0.03	2.33
St.Prop.Hylidae	18	870.81	3.30	0.04	0.78	-0.01	0.00	-1.66
St.Prop.Ranidae	18	870.99	3.48	0.04	0.82	0.01	0.00	1.62
Wt.Frog.Density	18	871.26	3.84	0.03	0.85	-0.01	0.01	-1.66
Ave.Mean.Temp	18	871.61	4.09	0.03	0.88	-0.04	0.02	-1.74
Ave.High.Temp	18	871.72	4.21	0.03	0.91	-0.04	0.02	-1.70
Latitude	18	872.17	4.65	0.02	0.93	-0.08	0.05	-1.55
Longitude	18	872.52	5.00	0.02	0.95	0.15	0.10	1.55
Wt.Prop.Bufonidae	18	872.93	5.42	0.01	0.96	0.01	0.01	1.19
J.Day	18	872.95	5.43	0.01	0.98	0.00	0.00	-0.55
St.Sp.Rich	18	873.17	5.66	0.01	0.99	0.08	0.07	1.09
Water.Temp	18	873.53	6.01	0.01	1.00	-0.02	0.03	-0.83

(c)

2008 Multivariate Intensity Mixed-Effects Models	**K**	**AIC**	**Delta_AIC**	**AICWt**	**Cum.Wt**
Wt.Prop.Ranidae+Wt.Prop.Hylidae+St.Prop.Hylidae+St.Prop.Ranidae	21	865.77	0.00	0.22	0.22
Wt.Prop.Ranidae+Wt.Prop.Hylidae+Wt.Shannon	20	867.38	1.60	0.10	0.31
Wt.Prop.Ranidae	18	867.51	1.74	0.09	0.40
Wt.Prop.Hylidae	18	867.82	2.04	0.08	0.48
Wt.Prop.Ranidae+Wt.Prop.Hylidae+Wt.Frog.Density+Wt.Shannon	21	868.26	2.48	0.06	0.54
Wt.Frog.Density+Wt.Sp.Rich	19	868.48	2.71	0.06	0.60
Wt.Shannon	18	868.69	2.91	0.05	0.65
Wt.Sp.Rich	18	868.75	2.97	0.05	0.70
St.Prop.Bufonidae	18	868.99	3.22	0.04	0.74
Wt.Prop.Ranidae+Wt.Prop.Hylidae+Wt.Sp.Rich	20	869.19	3.41	0.04	0.78
Wt.Prop.Ranidae+Wt.Prop.Hylidae+Wt.Shannon+Wt.Sp.Rich	21	869.37	3.60	0.04	0.81
Wt.Prop.Ranidae+Wt.Prop.Hylidae	19	869.39	3.62	0.04	0.85
Wt.Prop.Ranidae+Wt.Prop.Hylidae+Wt.Frog.Density+Wt.Sp.Rich	21	869.63	3.86	0.03	0.88
Wt.Prop.Ranidae+Wt.Prop.Hylidae+Wt.Frog.Density	20	870.06	4.28	0.03	0.91
Wt.Shannon+Wt.Sp.Rich	19	870.15	4.38	0.02	0.93
Wt.Frog.Density+Wt.Sp.Rich+Wt.Shannon	20	870.20	4.42	0.02	0.95
St.Prop.Hylidae	18	870.81	5.04	0.02	0.97

St.Prop.Ranidae	18	870.99	5.22	0.02	0.99
Wt.Frog.Density	18	871.36	5.58	0.01	1.00

Table A4.6 Constructing multivariate models that best describe Bd infection intensity in 2009. Univariate tests of relationships among all environmental and biological variables were run assuming (a) all fixed effects and with (b) mixed models, where each model was run with a random wetland variable nested within site. Bolded numbers indicate significant relationships ($p < 0.05$) and those used to build more complex multivariate models ($p < 0.25$) between model and Bd intensity. (c) Multivariate mixed effects models of relationships among environmental and biological variables on wetland-level *Bd* prevalence. Models are ranked in ascending order based on AIC score. Wt = wetland-level factor, St = site-level factor, Prop = proportion.

(a)

2009 Univariate Intensity Fixed-Effects Models	**K**	**AIC**	**Delta_AIC**	**AICwt**	**Cum. Wt**	**Estimate**	**St. Error**	**F statistic**	**df**	**p-value**
Wt.Frog.Density	3	3408.51	0.00	1	1	0.04	0.01	19.97	1, 973	**<0.0001**
Wt.Water.Temp	3	3553.48	144.97	0	1	0.04	0.01	13.96	1, 1004	**<0.0001**
St.Prop.Hylidae	3	3605.90	197.38	0	1	0.02	0.00	57.46	1, 1031	**<0.0001**
St.Prop.Ranidae	3	3625.51	217.00	0	1	-0.02	0.00	36.99	1, 1031	**<0.0001**
St.Sp.Rich	3	3630.08	221.57	0	1	-0.18	0.03	32.27	1, 1031	**<0.0001**
J.Day	3	3639.25	230.74	0	1	0.01	0.00	22.87	1, 1031	**<0.0001**
St.Prop.Bufonidae	3	3640.33	231.81	0	1	-0.02	0.00	21.78	1, 1031	**<0.0001**
Wt.Prop.Hylidae	3	3643.00	234.48	0	1	0.01	0.00	19.06	1, 1031	**<0.0001**
Wt.Longitude	3	3648.38	239.87	0	1	-0.21	0.06	13.60	1, 1031	**0.0002379**
Ave.High.Temp	3	3648.82	240.31	0	1	0.03	0.01	13.16	1, 1031	**0.0003004**
Ave.Mean.Temp	3	3651.78	243.27	0	1	0.03	0.01	10.17	1, 1031	**0.001471**
Wt.Prop.Bufonidae	3	3652.08	243.57	0	1	-0.01	0.00	9.87	1, 1031	**0.00173**
Wt.Latitude	3	3652.11	243.60	0	1	0.08	0.03	9.83	1, 1031	**0.001762**
Wt.Prop.Ranidae	3	3652.35	243.84	0	1	0.00	0.00	9.60	1, 1031	**0.002002**
Wt.Sp.Rich	3	3657.65	249.14	0	1	0.06	0.03	4.27	1, 1031	**0.03906**
Wt.Shannon	3	3661.86	253.35	0	1	-0.01	0.04	0.06	1, 1031	0.811

(b)

2009 Univariate Intensity Mixed-Effects Models	**K**	**AIC**	**Delta_AIC**	**AIC_wt**	**Cum. Wt**	**Estimate**	**St Error**	**t-value**

Wt.Frog.Density	139	3600.71	0.00	1	1	0.01	0.01	0.62
Water.Temp	156	3771.50	170.79	0	1	0.05	0.01	3.41
St.Prop.Hylidae	156	3858.15	257.45	0	1	0.02	0.00	7.18
St.Sp.Rich	156	3865.68	264.97	0	1	-0.29	0.04	-7.27
Wt.Prop.Hylidae	156	3865.84	265.13	0	1	0.01	0.00	3.36
Ave.High.Temp	156	3866.51	265.81	0	1	0.03	0.01	2.28
Ave.Mean.Temp	156	3866.84	266.14	0	1	0.03	0.01	2.19
J.Day	156	3867.46	266.75	0	1	0.00	0.00	1.88
St.Prop.Ranidae	156	3867.75	267.04	0	1	-0.01	0.00	-1.95
Wt.Prop.Bufonidae	156	3868.29	267.59	0	1	0.00	0.00	-1.63
Wt.Shannon	156	3868.53	267.83	0	1	0.17	0.06	2.62
Wt.Sp.Rich	156	3869.03	268.32	0	1	0.07	0.05	1.37
St.Prop.Bufonidae	156	3869.03	268.33	0	1	-0.03	0.00	-6.55
Wt.Prop.Ranidae	156	3869.30	268.60	0	1	0.00	0.00	-1.57
Latitude	156	3869.49	268.79	0	1	0.04	0.04	0.98
Longitude	156	3876.82	276.11	0	1	-0.36	0.08	-4.46

(c)

2009 Multivariate Intensity Mixed-Effects Models	K	AIC	Delta_AIC	AICWt	Cum.Wt
Wt.Frog.Density+Wt.Water.Temp+St.Prop.Hylidae+St.Prop.Ranidae	142	3494.74	0.00	0.29	0.29
Wt.Frog.Density+Wt.Water.Temp+St.Prop.Hylidae	141	3495.25	0.51	0.22	0.51
Wt.Frog.Density+Wt.Water.Temp+St.Prop.Hylidae+St.Prop.Ranidae+Ave.Mean.Temp+Ave.High.Temp	144	3495.32	0.58	0.23	0.77
Wt.Frog.Density+Wt.Water.Temp+St.Prop.Hylidae+Ave.High.Temp	142	3495.96	1.22	0.17	0.94
Wt.Frog.Density+Wt.Water.Temp+Ave.High.Temp+Ave.Mean.Temp	142	3499.18	4.44	0.03	0.97
Wt.Frog.Density+Wt.Water.Temp+Ave.Mean.Temp	141	3500.82	6.08	0.01	0.98
Wt.Frog.Density+Wt.Water.Temp	140	3501.48	6.74	0.01	1.00
Wt.Frog.Density+Wt.Water.Temp+Wt.Prop.Hylidae	141	3503.32	8.58	0.00	1.00
Wt.Frog.Density+Wt.Water.Temp+Ave.High.Temp	141	3507.91	13.17	0.00	1.00
Wt.Frog.Density+St.Prop.Hylidae+St.Prop.Ranidae	141	3588.37	93.63	0.00	1.00
Wt.Frog.Density+St.Prop.Hylidae	140	3589.18	94.44	0.00	1.00
Wt.Frog.Density+Wt.Prop.Hylidae+St.Sp.Rich	141	3595.73	100.99	0.00	1.00
Wt.Frog.Density+St.Sp.Rich	140	3596.00	101.26	0.00	1.00
Wt.Frog.Density+Wt.Prop.Hylidae	140	3597.87	103.13	0.00	1.00

Wt.Frog.Density	139	3600.71	105.97	0.00	1.00
Wt.Water.Temp+St.Prop.Hylidae	157	3765.24	270.50	0.00	1.00
Wt.Water.Temp+St.Prop.Hylidae+St.Prop.Ranidae	158	3765.81	271.07	0.00	1.00
Wt.Water.Temp+Wt.Prop.Hylidae+St.Sp.Rich	158	3767.23	272.49	0.00	1.00
Wt.Water.Temp	156	3771.50	276.76	0.00	1.00
Wt.Water.Temp+J.Day	157	3773.13	278.39	0.00	1.00
St.Prop.Hylidae	156	3858.15	363.41	0.00	1.00
St.Sp.Rich	156	3865.68	370.94	0.00	1.00
Wt.Prop.Hylidae	156	3865.84	371.10	0.00	1.00
Ave.High.Temp	156	3866.51	371.77	0.00	1.00
Ave.Mean.Temp	156	3866.84	372.10	0.00	1.00
J.Day	156	3867.46	372.72	0.00	1.00
St.Prop.Ranidae	156	3867.75	373.01	0.00	1.00

Table A4.7 Summary of components in the top models that best explain prevalence and intensity levels. UME = Univariate mixed effects model, MME = Multivariate mixed effects model, UFE = Univaraite fixed effects model.

		Prevalence		**Intensity**			
		2008	2009	2008			2009
Variable Type	**Independent Variable**	MME	UME	UFE	UME	MME	MME
Environmental	Latitude	X					
	Longitude	X					
	JDay	X					
	Wt.Water.Temp	X	X				X
	Ave.High.Temp	X					X
	Ave.Mean.Temp	X					X
Biological	Wt.Shannon				X	X	
	Wt.Frog.Density	X					X
	St.Sp.Rich						
	Wt. Sp.Rich			X	X		
	St.Prop.Bufonidae				X		
	St.Prop.Hylidae					X	X
	St.Prop.Ranidae					X	X
	Wt.Prop.Bufonidae						
	Wt.Prop.Hylidae			X	X	X	
	Wt.Prop.Ranidae			X	X	X	

APPENDIX 5
Graphical display of variables explaining *Bd* prevalence and intensity among widespread species in 2008 and 2009.

See Appendix 4 for model-selection analyses.

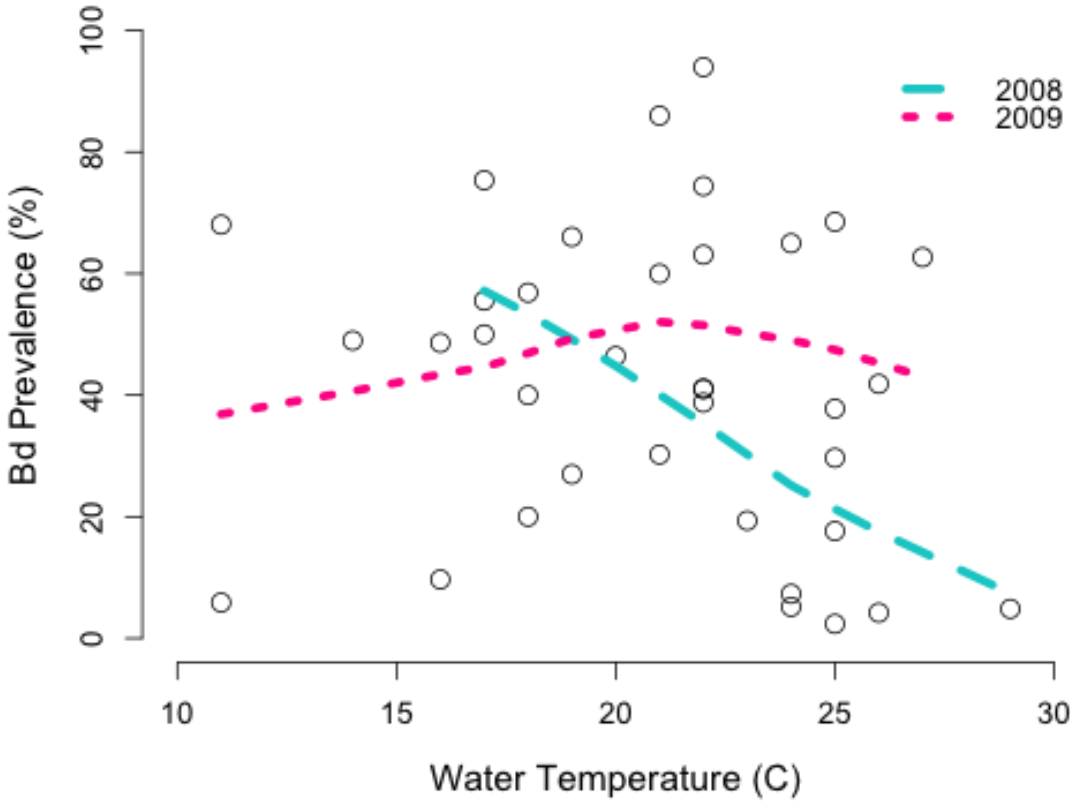
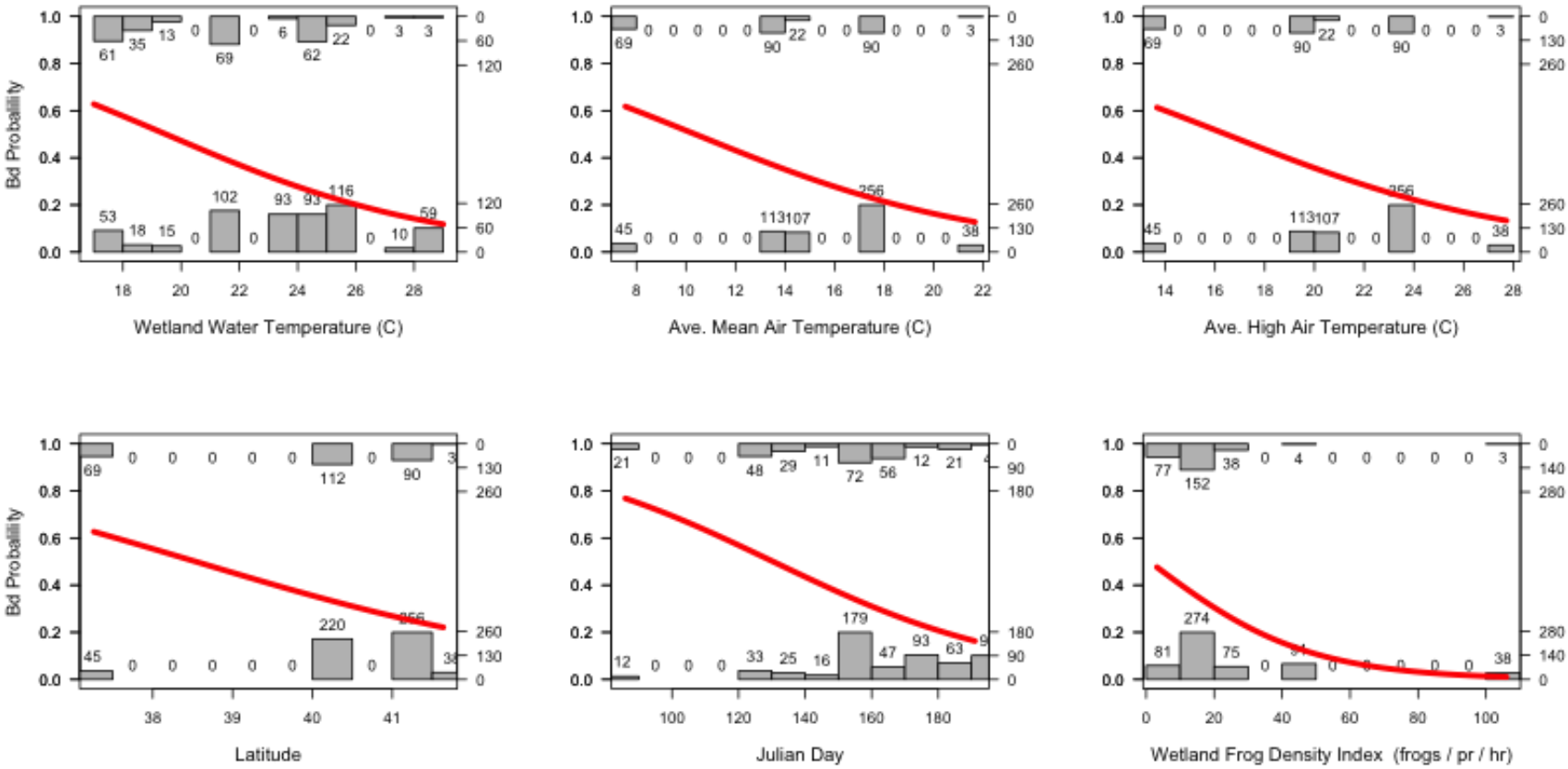
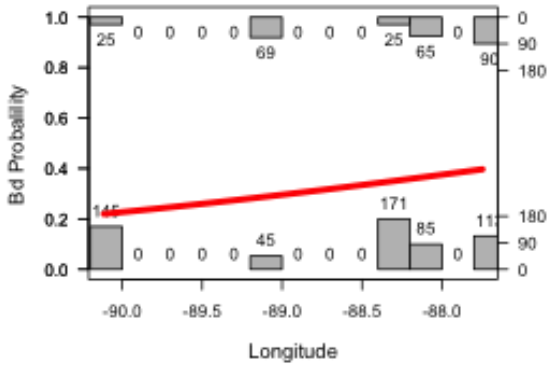


Figure A5.1 Of the environmental variables analyzed against *Bd* prevalence, wetland water temperature best explained prevalence levels. In 2009, we took samples from the coolest wetlands. When considering lowess curves in both years, we see that prevalence generally begins low at the coldest temperatures, increases to the optimal temperature for *Bd* growth range, and then decreases above the optimal growth temperatures.

(a)


Bd Probability
Wetland Water Temperature (C)
Ave. Mean Air Temperature (C)
Ave. High Air Temperature (C)
Latitude
Julian Day
Wetland Frog Density Index (frogs / pr / hr)



(b)

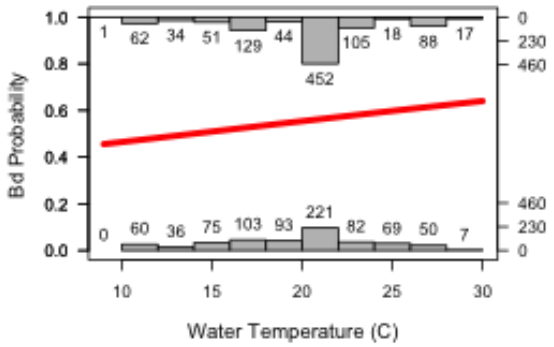
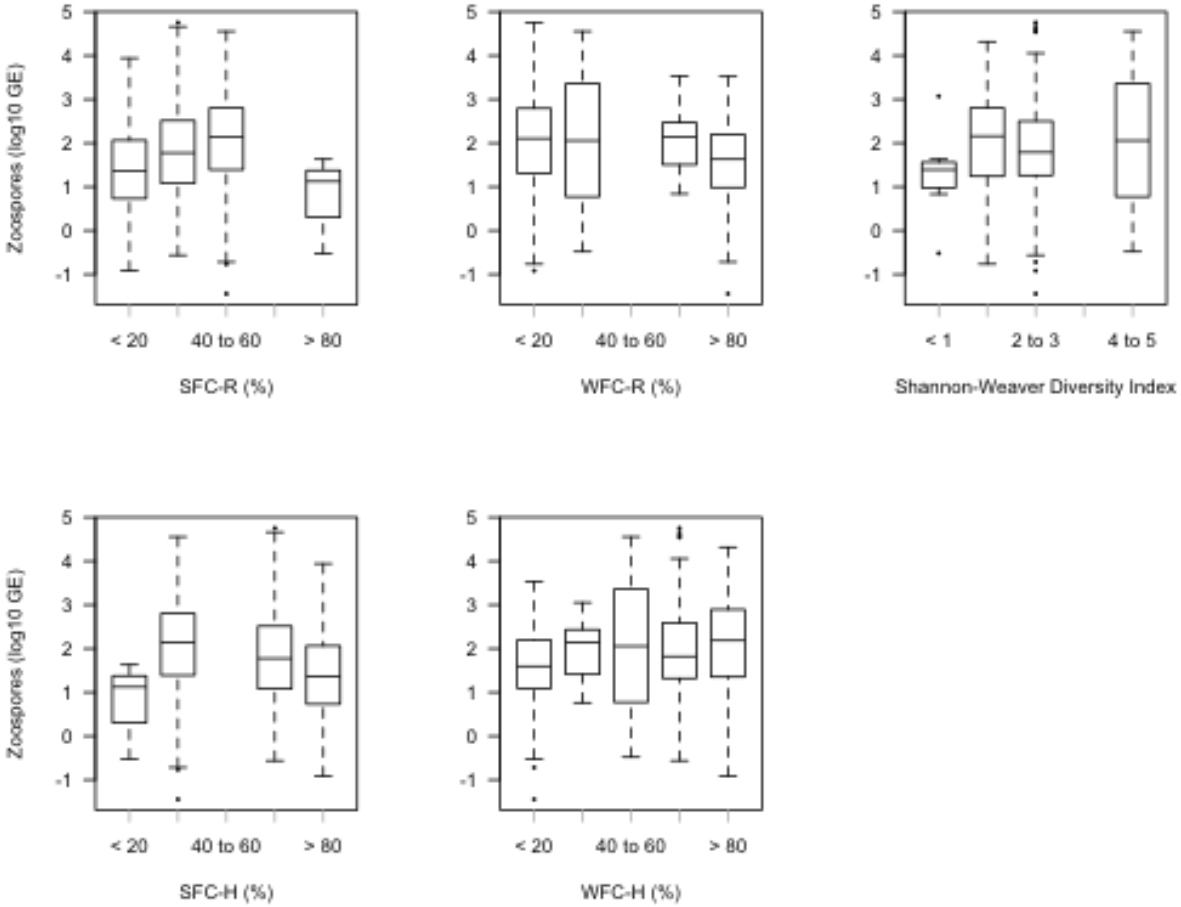


Figure A5.2 Components of best-fitting multivariate factors for (a) 2008 and top univariate model for (b) 2009 *Bd* prevalence. Factors are displayed as the univariate factors of interest versus *Bd* probability. (Note: see Tables A4.3 and A4.4 for results of univariate and multivariate analyses for wetland-level prevalence). Zero *Bd* probability = uninfected individual; 1.0 *Bd* probability = infected individual; values above histograms = number of anurans; red trend line = fitted logistic regression curve. Graphics based on de la Cruz Rot (2005) and Smart et al. (2004), using R package 'popbio' (Stubben and Milligan 2007).

(a)


Zoospores (log10 GE)
5
4
3
2
1
0
-1
< 20
40 to 60
> 80
SFC-R (%)
WFC-R (%)
< 1
2 to 3
4 to 5
Shannon-Weaver Diversity Index
SFC-H (%)
WFC-H (%)

(b)

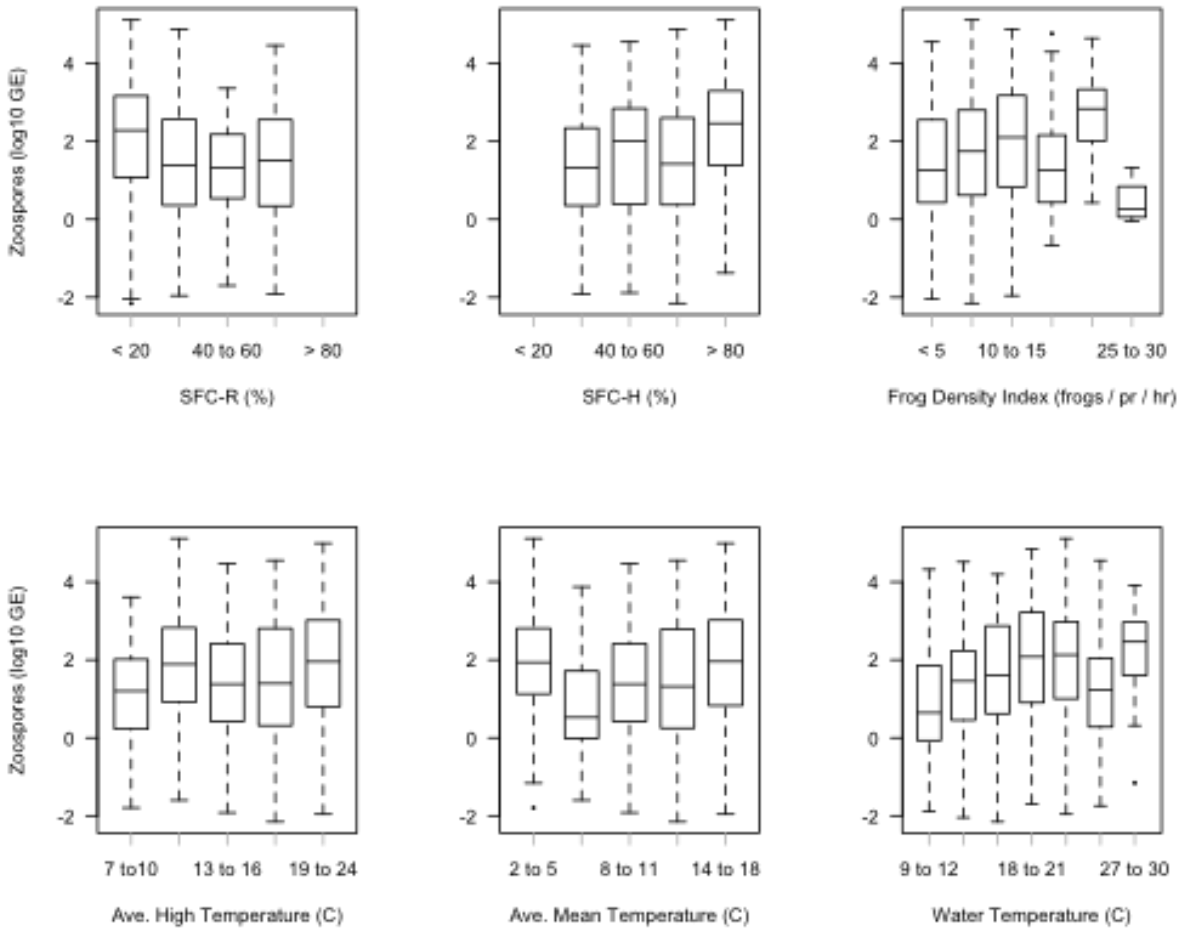


Figure A5.3 Components of best-fitting multivariate factors (see Tables A4.5 & A4.6 for multivariate results) for (a) 2008 and (b) 2009 *Bd* intensity, displayed as the univariate factors of interest versus *Bd* intensity.

APPENDIX 6
Site-level analyses of current *Bd* distribution among widespread anurans.

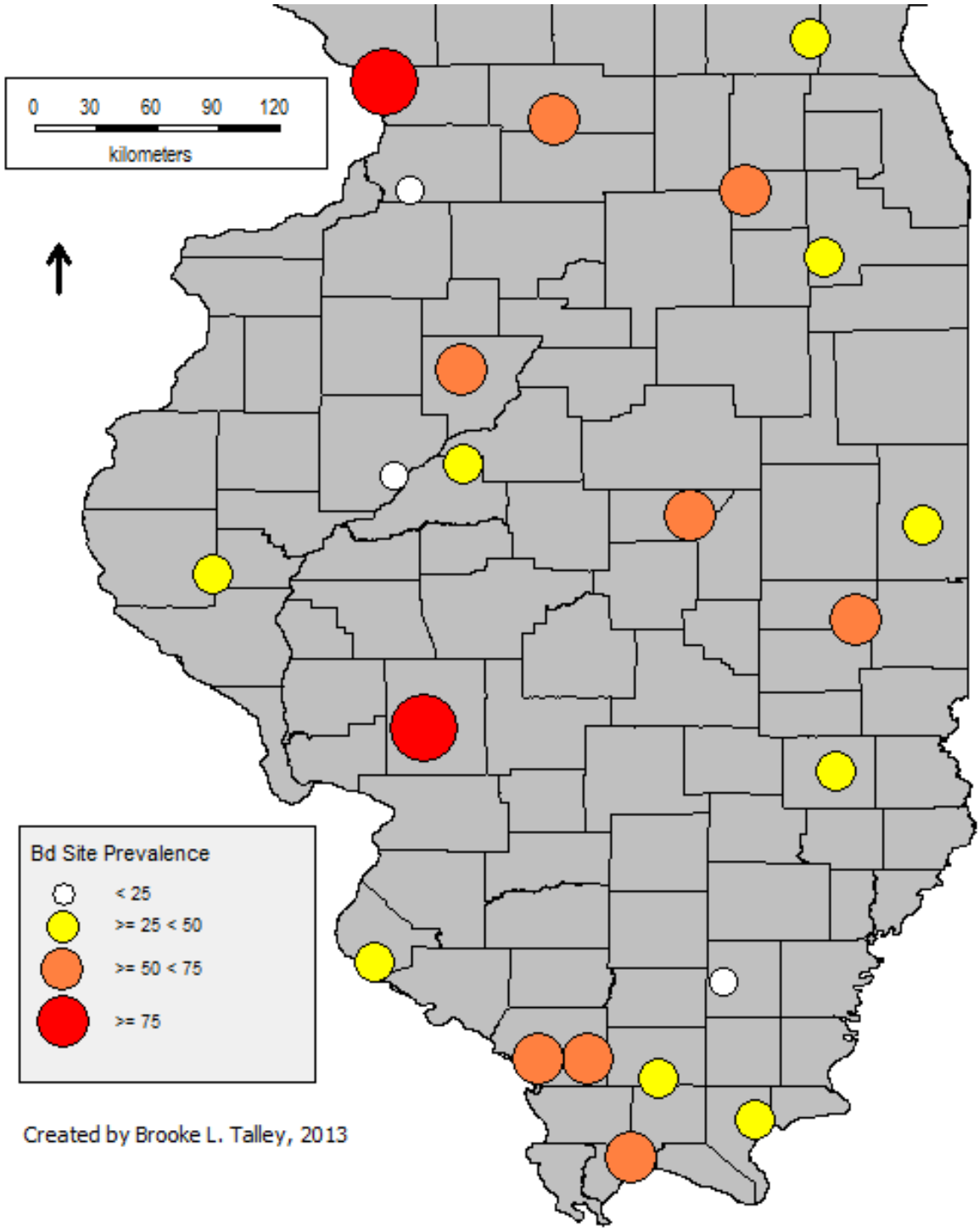


Figure A6.1 Site mean *Bd* prevalence.

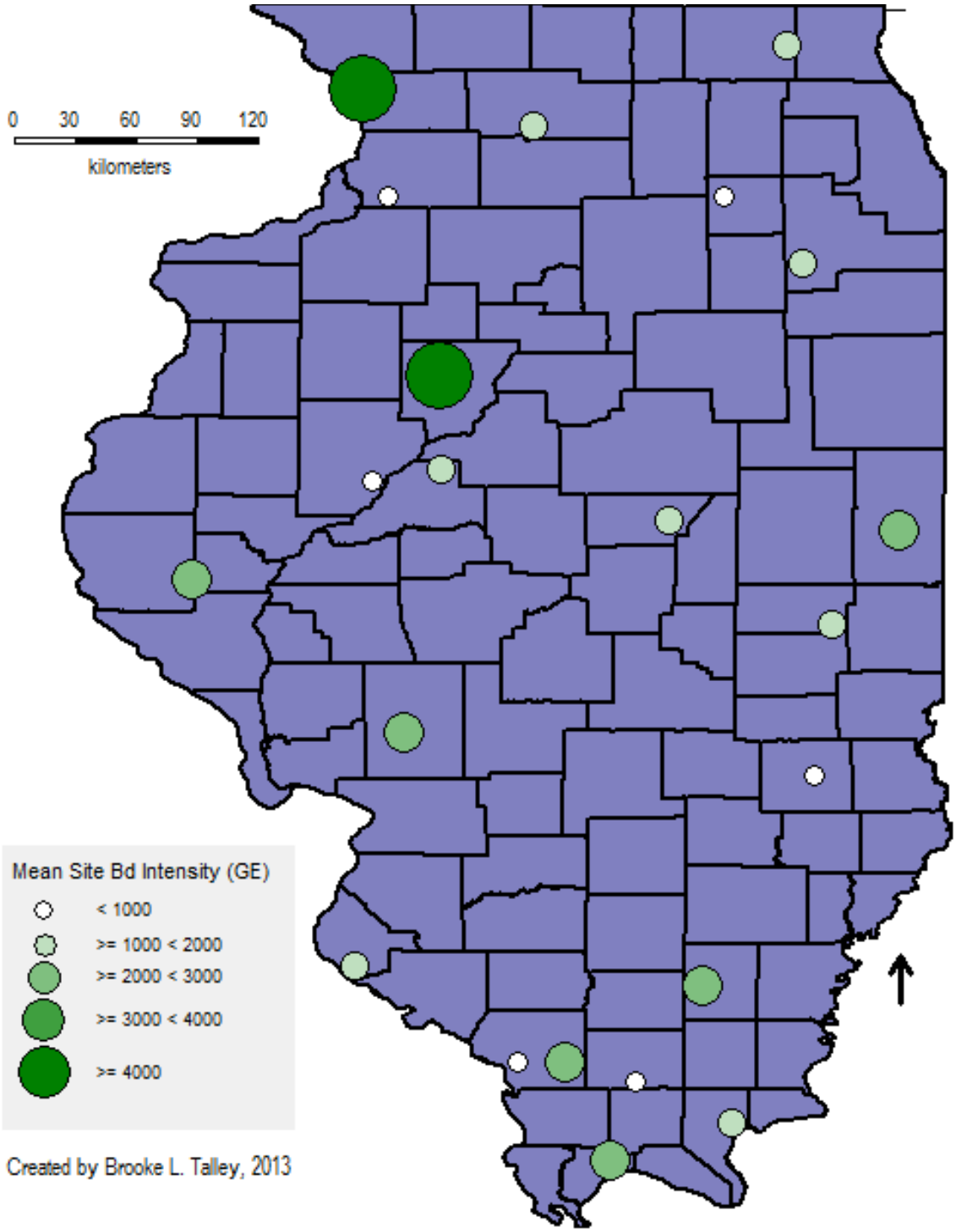


Figure A6.2 Site mean *Bd* intensity.

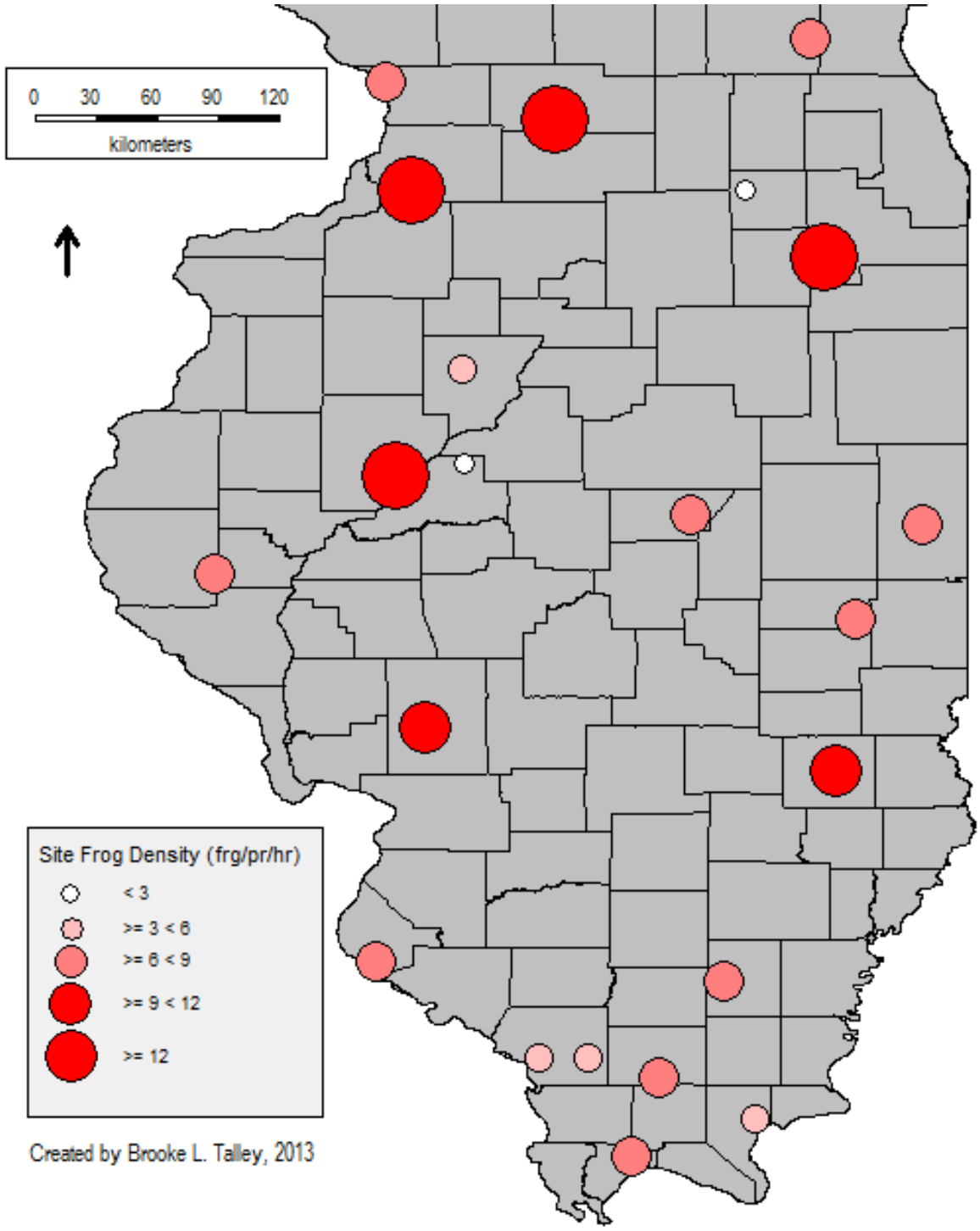


Figure A6.3 Frog density among sample sites, calculated as frogs captured per person per hour.

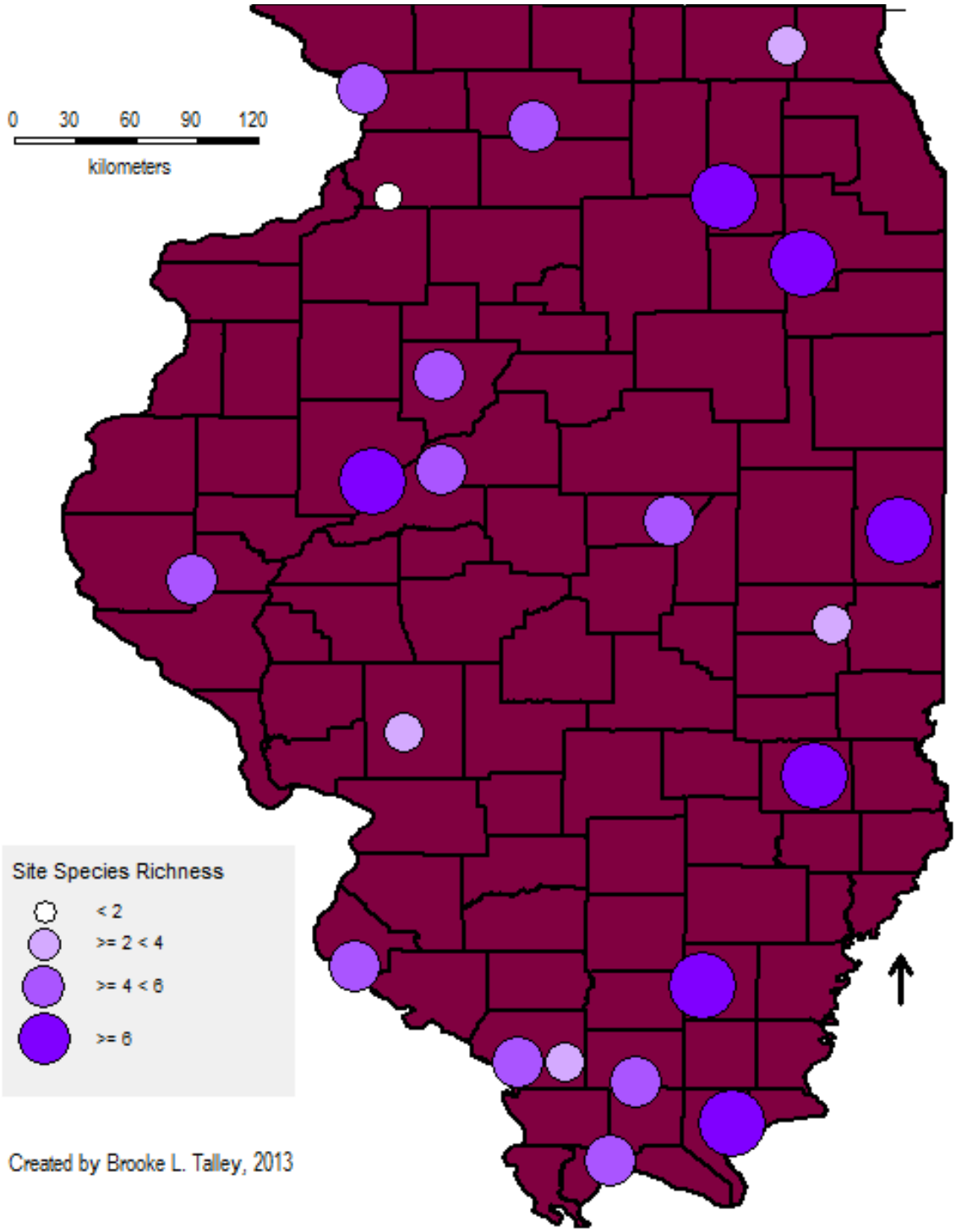


Figure A6.4 Anuran species richness among sample sites at time of sampling.

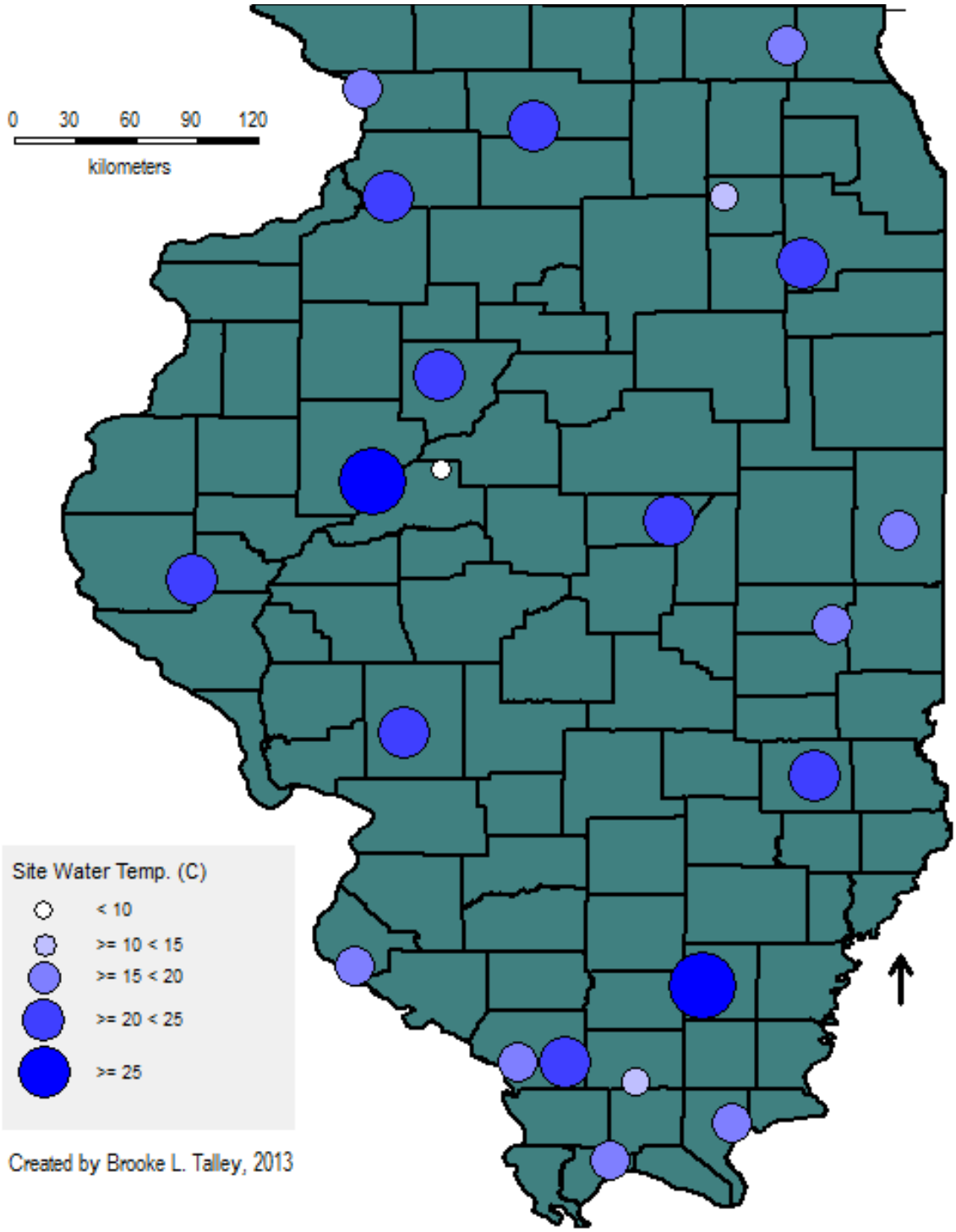


Figure A6.5 Water temperature among sample sites at time of sampling.

APPENDIX 7
Wetland-level analyses of current *Bd* distribution among widespread anurans.

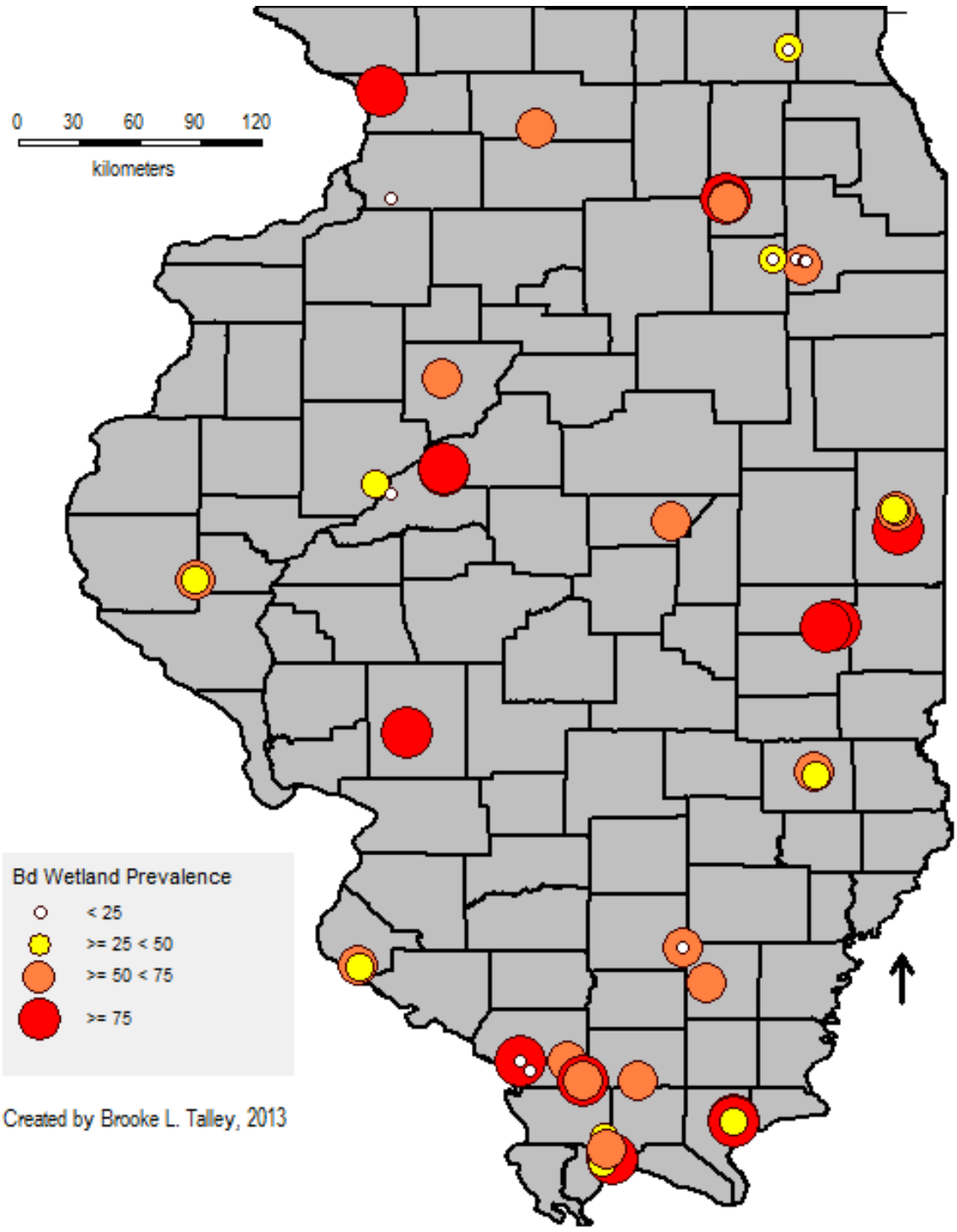


Figure A7.1 Wetland mean *Bd* prevalence.

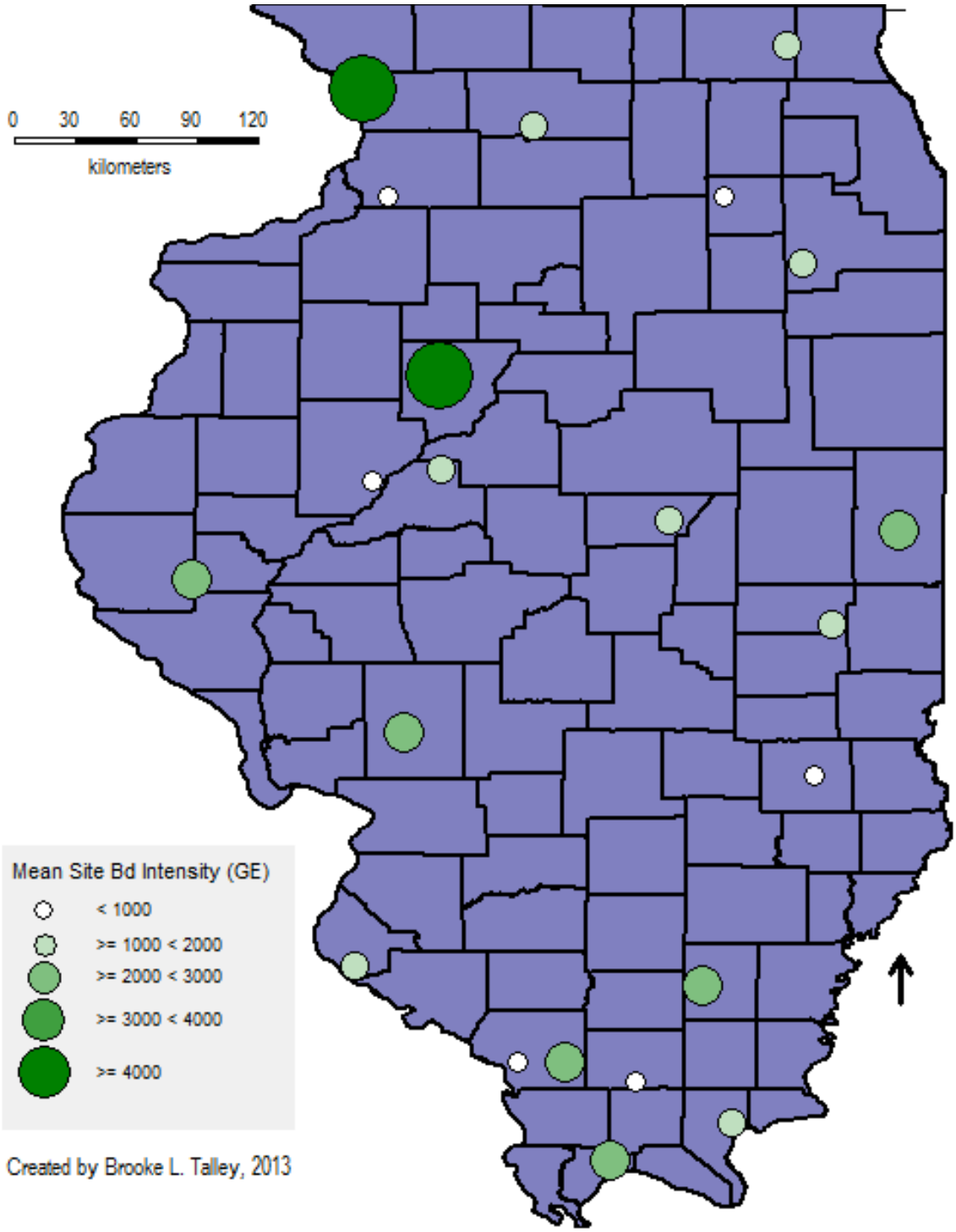


Figure A7.2 Wetland mean *Bd* intensity.

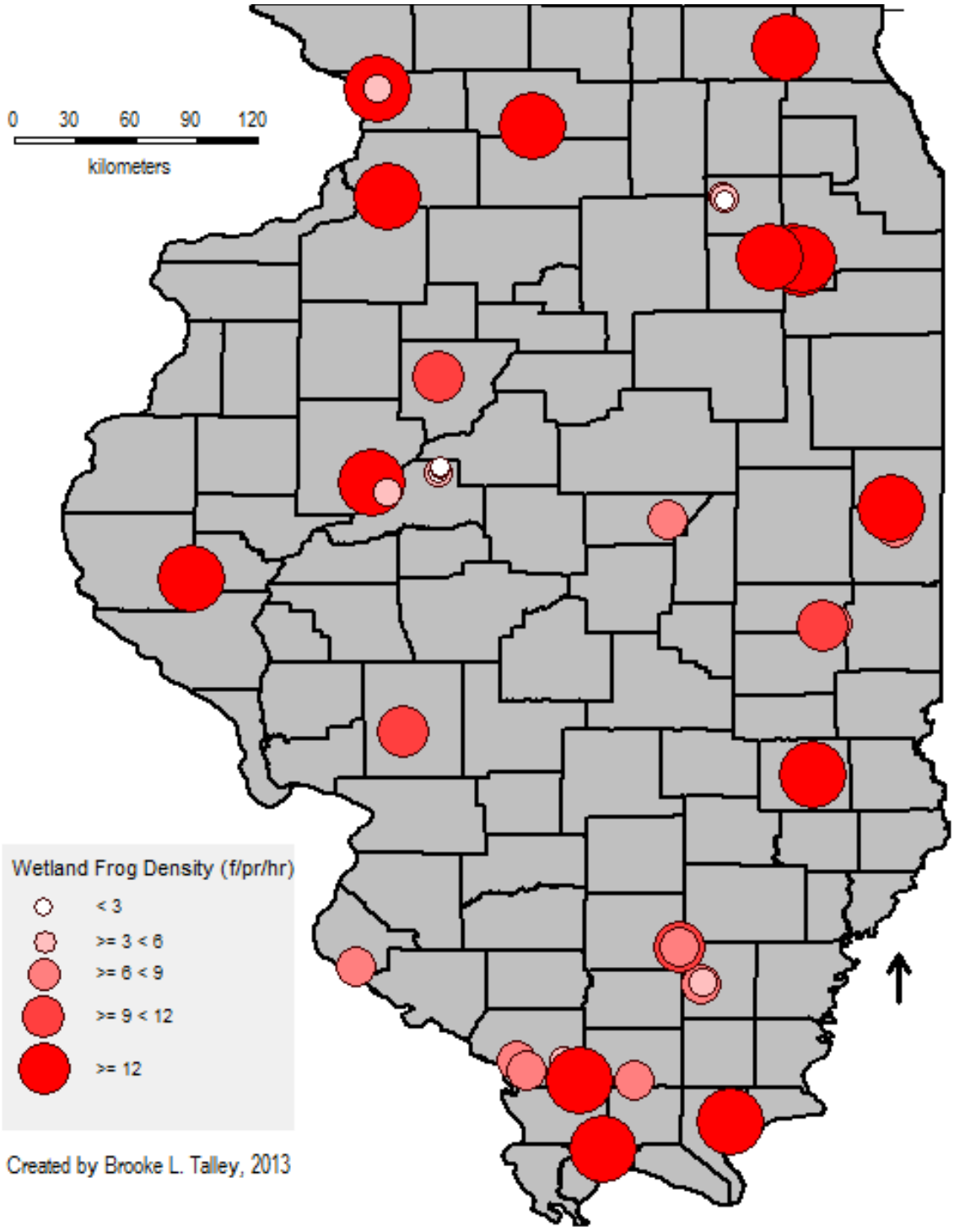


Figure A7.3 Frog density among sample wetlands, calculated as frogs captured per person per hour.

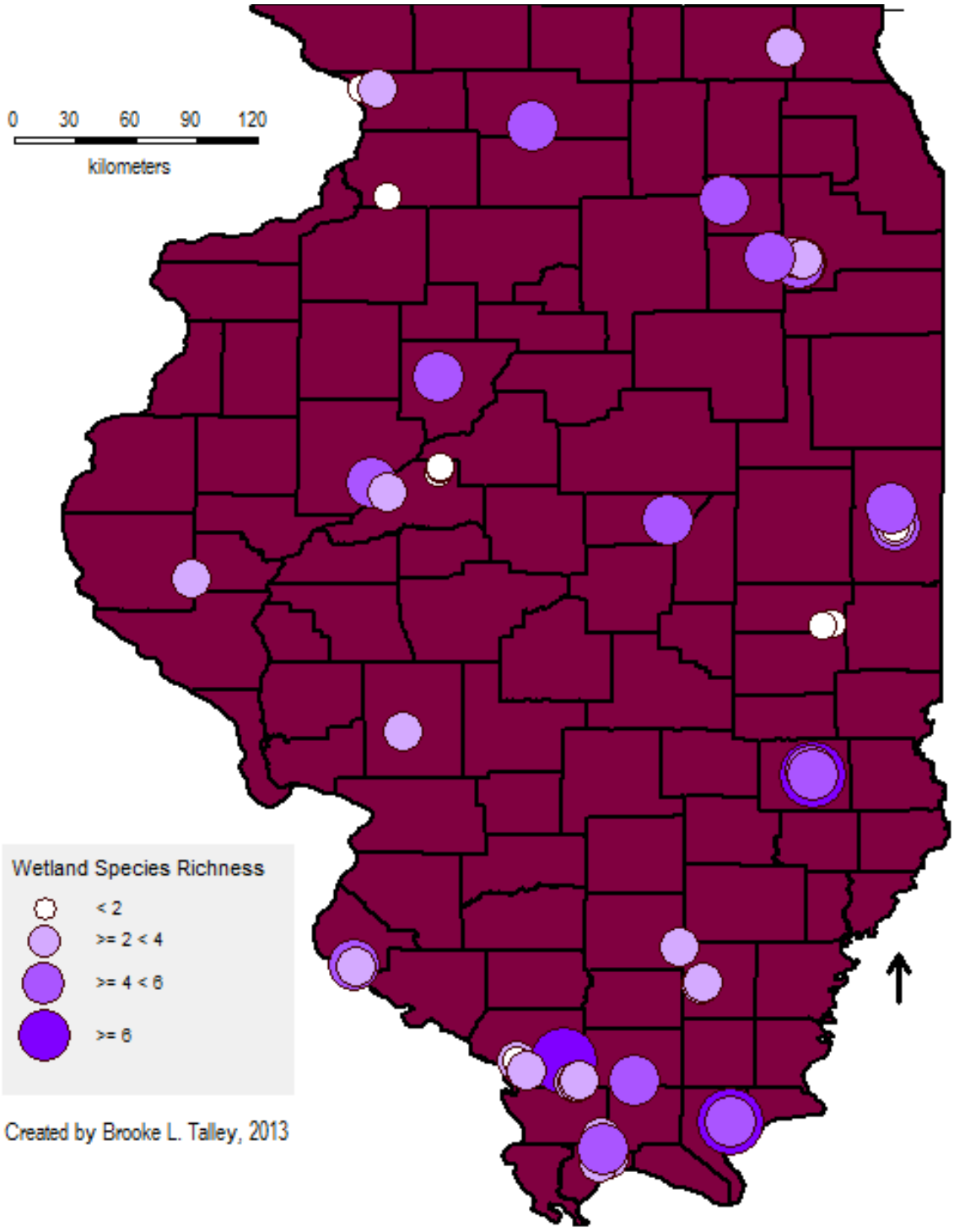


Figure A7.4 Anuran species richness among sample wetlands at time of sampling.

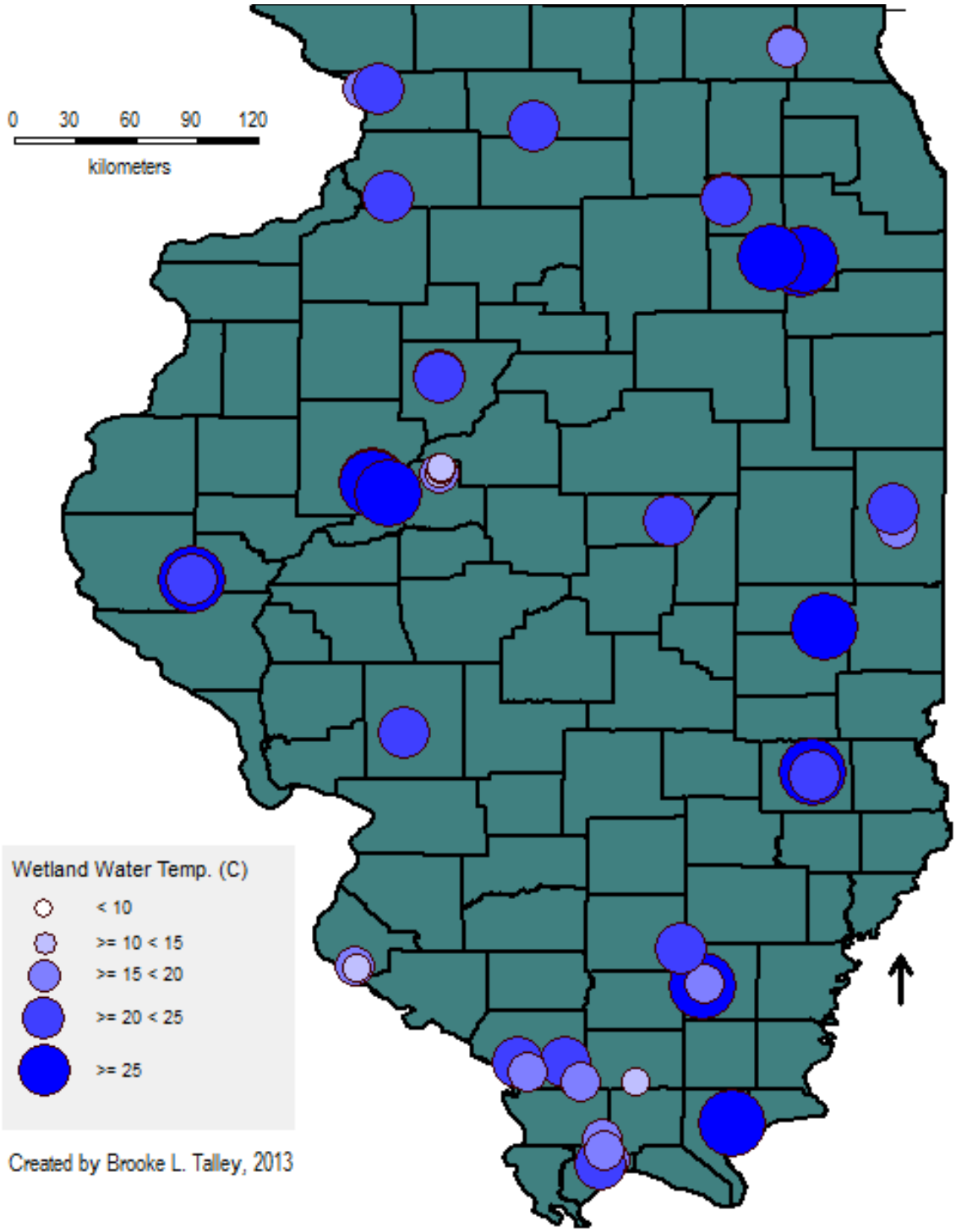


Figure A7.5 Water temperature among sample wetlands at time of sampling.

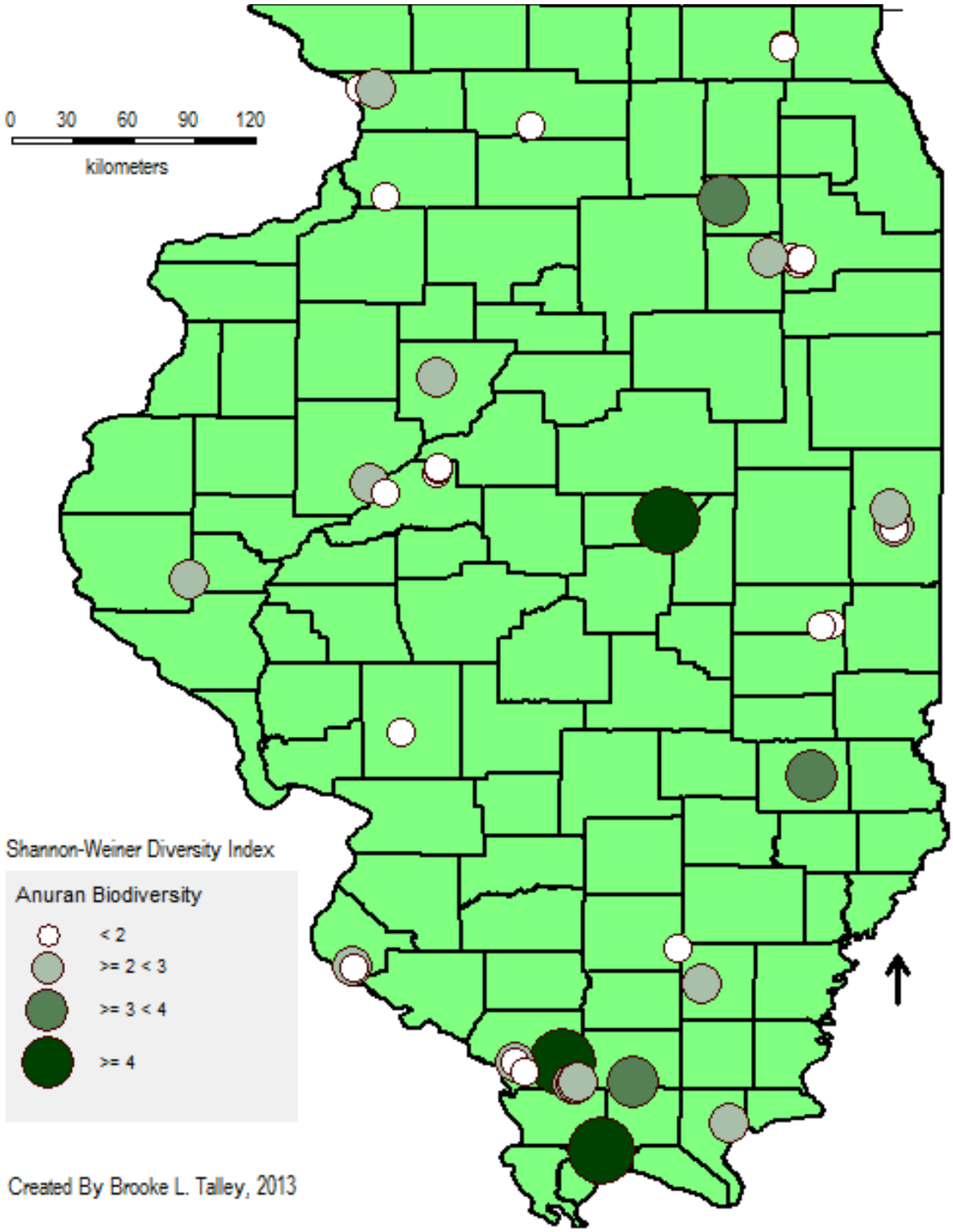


Figure A7.6 Shannon-Weiner diversity index among sample wetlands at time of sampling.

APPENDIX 8
Historic occurrence of *Bd* among widespread anuran species.

Histologic Examination, Museum Specimens:

We performed histological analyses on museum specimens from three museums: Southern Illinois University Carbondale (SIUC), University of Illinois Museum of Natural History (UIMNH), and Illinois Natural History Survey (INHS). We analyzed 454 anuran specimens from SIUC, 211 from UIMNH, and 144 from INHS. Skin samples were collected from specimens collected from 1950 – 1989. We collected more skin samples than we examined histologically because we discovered this technique yielded high false-negative rates, which led us to employ molecular techniques (see below) instead of examining every microscope slide. We found zero *Bd*-positive samples during visual inspection of 1,618 slides (2 slides per 809 specimens), but a number of samples had suspicious morphological structures that will get further examination from outside experts. We took photos of any suspect-positive samples, although BLT has yet to identify any structures consistent with *Bd* zoospores.

The geographic locations of all anurans we used in our historic survey using histological examination are displayed below (Figure A8.1).

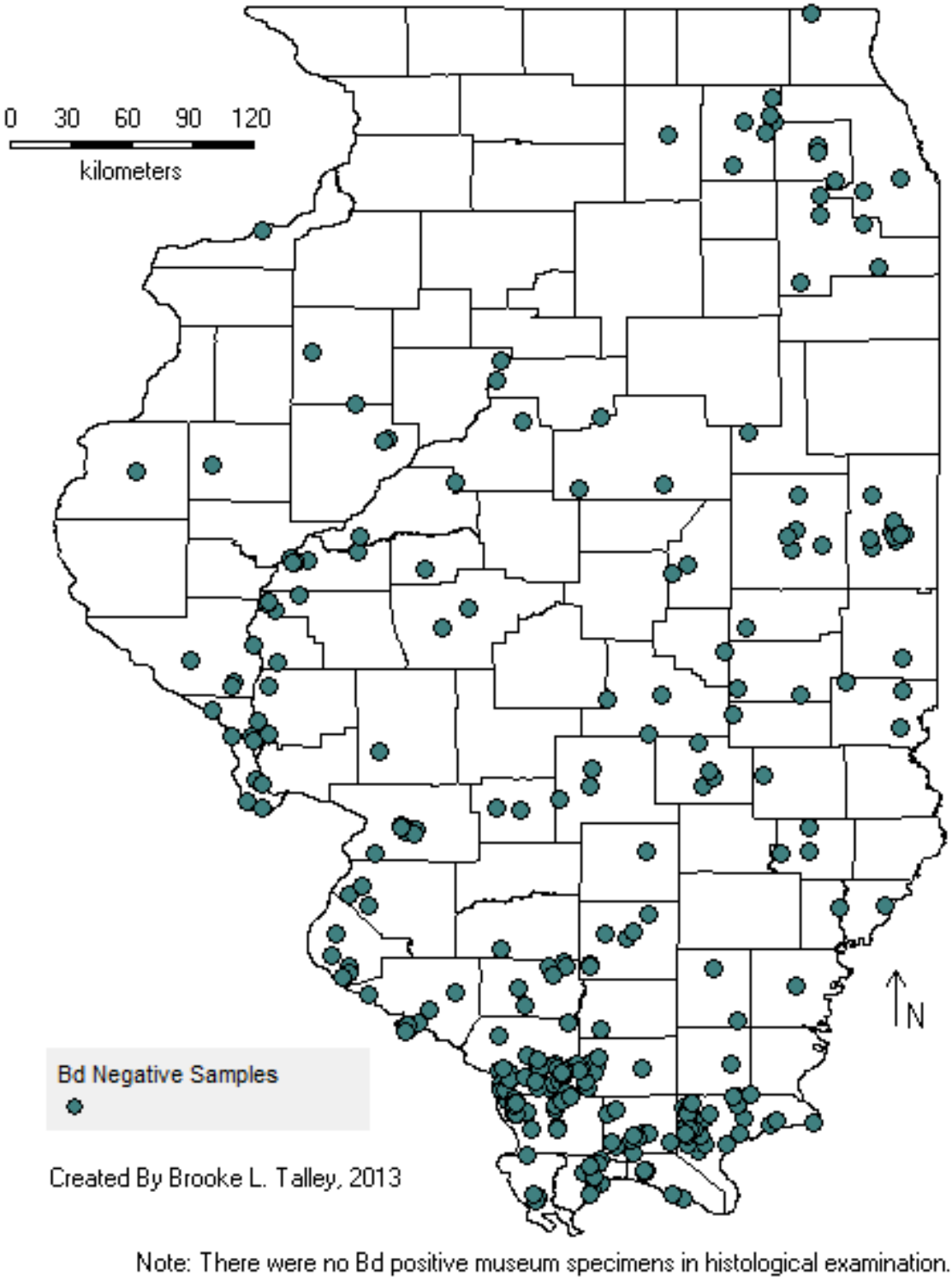


Figure A8.1 Geographic locations of all anurans we used in the histological examination of museum specimens.

Genetic Analyses, Museum Specimens:

We analyzed 682 museum specimens from SIUC, 120 from UIMNH, and 210 from INHS. We performed genetic analyses on museum specimens from the same museums in Illinois. Some of the same specimens were examined using both techniques, but most of the molecular analyses were performed on a different set of specimens. Initial testing revealed several *Bd*-positive samples that predated kknown records from the Midwest. This prompted us to test even older museum specimens (before 1950). Specimens were collected from 1892 – 1989.

We used the protocol developed by Cheng et al. (2011) to sample museum specimens, and test them for *Bd* zoospores using qPCR. Because this is a relatively new technique, we are conducting DNA sequencing on a subset of positive amples to verify that these positives are truly batrachochytrium dendrobatidis. We ran all positive samples in triplicate to help reduce the false-positive rate (Table A8.1).

Table A8.1 Number of *Bd* positive samples from molecular analyses. All samples were processed in triplicate; numbers indicate whether a sample amplified only once (singlicate), twice (duplicate), or all three times (triplicate).

Collection	Singlicate Run	Duplicate Run	Triplicate Run	Total Sampled
INHS	13	14	86	210
SIUC	5	1	9	682
UIMNH	6	0	0	120

When we examined *Bd* prevalence across decades, we found low prevalence levels from more recent samples (post-1960s) and high prevalence pre-1960s (Table A8.2). *Bd* was present throughout multiple decades, and remains present today (See Appendix 2). We will continue to explore these data and assess whether this prevalence trend follows that of an epizootic wave before the 1960s.

Table A8.2 *Bd* prevalence by decade, based upon samples that amplified two or three times during molecular processing, suggests temporally well-established disease agent in Illinois.

Sample Timeline	Positive	Negative	Total	Prevalence (%)	Prev. 95% CI
pre-1960s	100	242	342	29.2	24.7 – 34.3
1960s	4	289	293	1.4	0.5 – 3.4
1970s	2	70	72	2.8	0.8 – 9.6
1980s	4	91	95	4.2	1.6 – 10.3

As with the *Bd* survey among current anurans in Illinois (see Appendix 2), we detected *Bd* prevalence varied among species with some species having high prevalence (e.g., *L. sphenocephalus*) while others are very low (e.g., *L. catesbeianus*). We were most surprised to find that *A. crepitans* had such low prevalence values since current *Bd* levels are high for that species. We will continue to explore these data to determine how the temporal and geographical relationships among species could help explain variation among *Bd* prevalence levels.

Table A8.3 *Bd* prevalence by species, based upon samples that amplified two or three times during molecular processing.

Species	Positive	Negative	Total	Prevalence (%)	Prev. 95% CI
Acris crepitans	5	285	290	1.7	0.7 – 4.0
Anaxyrus americanus	0	97	97	0	0 – 3.8
Anaxyrus fowleri	0	100	100	0	0 – 3.7
Hyla chrysoscelis/versicolor	2	52	54	3.7	1.0 – 12.5
Lithobates blairi	0	14	14	0	0 – 21.5
Lithobates catesbeianus	0	58	58	0	0 – 6.2
Lithobates clamitans	0	89	89	0	0 – 4.1
Lithobates pipiens	1	14	15	6.7	0.3 – 29.8
Lithobates sphenocephalus	102	168	270	37.8	32.2 – 43.7
Pseudacris crucifer	0	25	25	0	0 – 13.3

APPENDIX 9
Some Important *Batrachochytrium dendrobatidis* literature, often used by BLT

Adams, M.J., N.D. Chelgren, D. Reinitz, R.A. Cole, L.J. Rachowicz, S. Galvan, B. McCreary, C.A. Pearl, L.L. Bailey, J. Bettaso, E.L. Bull, and M. Leu. 2010. Using occupancy models to understand the distribution of an amphibian pathogen, *Batrachochyrium dendrobatidis*. Ecological Applications 20(1):289-302.

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Altizer, S., D. Harvell, and E. Friedle. 2003. Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology and Evolution 18:589–596.

Becker, C.G., and K.R. Zamudio. 2011. Tropical amphibian populations experience higher disease risk in natural habitats. PNAS 108(24):9893–9898.

Berger, L., R. Speare, P. Daszak, D.E. Green, A.A. Cuningham, C.L. Goggin, R. Slocombe, M.A. Ragan, A.D. Hyatt, K.R. McDonald, H.B. Hines, K.R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proceedings of the National Academy of Sciences 95:9031–9036.

Berger, L., R. Speare, H.B. Hines, G. Marantelli, A.D. Hyatt, K.R. McDonald, L.F. Skerratt, V. Olsen, J.M. Clarke, G. Gillespie, M. Mahony, N. Sheppardii, C. Williams, and M.J. Tyler. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Australian Veterinary Journal 82:434–439.

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

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

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and M.C. Fisher. 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, Rana catesbeiana. Biology Letters 2(3):455–459.

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Kriger, K.M., and J.-M. Hero. 2006. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. Journal of Zoology 271: 352–359.

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Laurance, W.F., K.R. McDonald, and R. Speare. 1996. Epidemic disease and catastrophic decline of Australian rain forest frogs. Conservation Biology 10:406-413.

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