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A century of *Batrachochytrium dendrobatidis* in Illinois amphibians (1888–1989)

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ABSTRACT

The fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), causes the disease chytridiomycosis in amphibians and is responsible for the worst epizootics in vertebrate history. In some regions of the world (e.g., the Neotropics and Western United States), Bd has caused recent reductions in amphibian population abundance and species richness, while in other regions the impacts are less clear. Although Bd is present in the Midwestern United States, its history and impact in the region is not known. We used a qPCR assay to determine historic Bd prevalence in Illinois, testing 1028 specimens representing 10 anuran species, collected 1888–1989. We used two complementary sets of samples to first assess historic prevalence with the primary set, and used a secondary set to confirm Bd presence and examine older samples with a more sensitive technique. Prevalence varied among species; in the primary dataset of 1008 samples extracted with PrepMan Ultra collected 1892–1989, Bd was found in four species (11.1%, CI: 9.3–13.2%). *Rana* (*Lithobates*) *sphenocephala*, the southern leopard frog, had the highest prevalence (38.3%, CI 32.7–44.2%); prevalence among other infected species was <7%. Overall prevalence was <10% in most decades but >40% in the 1940s. In the secondary set of 50 samples extracted with Qiagen Blood and Tissue Kits (30 re-swabbed of the original 1008 and 20 additional older specimens), 17 of the 20 additional samples were Bd+ (85.0%, CI 64.0–94.8%) including the oldest Bd+ specimen, which was collected in 1888. We confirmed Bd presence by sequencing 42 Bd+ samples and found ⩾99% homology with Bd sequences in Genbank. By 1900, Bd was geographically widespread throughout Illinois—40 years earlier than the current oldest estimate in the U.S.—meaning that Illinois amphibians have been coexisting with Bd for at least 126 years. This long period of coexistence from our results raises new questions about the history of Bd in North America, possible coevolution between host and pathogen, and the potential role of Bd in historic population declines.

1. Introduction

Chytridiomycosis, a leading cause of amphibian population declines (Collins and Storfer, 2003), is an infectious disease of amphibians caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (Bd; Longcore et al., 1999). Where Bd has been introduced into naïve populations, mass die-offs have been followed by rapid population declines (Lips et al., 2006; Vredenburg et al., 2010) in spatiotemporal waves (Lips et al., 2008; Vredenburg et al., 2010). Population declines and extirpations have been described from areas like the Sierra Nevada of California (Vredenburg et al., 2010), the Cordillera Central of Central America (Cheng et al., 2011; Lips et al., 2006, 2008), and the Andes of South America (Catenazzi et al., 2011). There have been no reports of mass die-offs in the Eastern or Midwestern U.S. but there is evidence of some population declines in these areas (e.g., Caruso and Lips, 2013; Highton, 2005; Lannoo, 1998; Means and Travis, 2007). To date, none of these population declines have been attributed to Bd (Caruso and Lips, 2013; Muletz et al., 2014), and causes are considered "enigmatic."

The Midwestern U.S. harbors diverse communities of amphibians (Phillips et al., 1999), with evidence of historic population declines in some species (Lannoo, 2005). Enigmatic declines of

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Rana (*Lithobates*) *pipiens* were reported from across the upper Midwest in the 1960s (Collins and Crump, 2009; Nace, 1968) and continued into the 1970s (Hine et al., 1981; Koonz, 1992; Lannoo et al., 1994). Similarly, *Acris blanchardi* began declining in the 1950s, with losses peaking in the 1970s (Les, 1979; Vogt, 1981) and continuing today (Lannoo, 2005). The causes of these declines are unknown, but numerous hypotheses have been identified. The short lifespan of *A. blanchardi* may increase vulnerability to stochastic events (Burkett, 1984) and climate change (Hay, 1998). For *Rana* (*Lithobates*) *sphenocephala*, researchers have identified competition and predation (Orchard, 1992), pollutants (Koonz, 1992), and even succession of early-staged habitats (Orr et al., 1998) as causes of declines. Although Bd occurs in Midwestern populations of leopard frogs (Lannoo et al., 2011), chytridiomycosis has not been proposed as a cause of these historic declines.

The history of Bd in the U.S. is not well understood, and becomes especially complicated considering Bd's global distribution (Olson et al., 2013) and evolutionary history (Rosenblum et al., 2013). Throughout its evolution, Bd has had a close association with amphibians (Farrer et al., 2013). The global pandemic lineage (Bd-GPL, Farrer et al., 2011) that is causing global declines (Fisher et al., 2009b; Schloegel et al., 2012) may date back at least 1000 years (Rosenblum et al., 2013) or may be a recently emerged clone (James et al., 2009; Morehouse et al., 2003; Morgan et al., 2007). The oldest record of Bd from North America is from a *Rana* (*Lithobates*) *catesbeiana* collected in California in 1928 (Huss et al., 2014). California amphibian populations have historic and current Bd infections with mixed effects on local populations (e.g., Briggs et al., 2010; Reeder et al., 2012; Vredenburg et al., 2013). In Brazil, stable Bd-amphibian host dynamics were reported over the last century (Rodriguez et al., 2014) yet in neighboring Peru, Bd's arrival caused amphibian community collapse and approximately 50% species loss in one of the most diverse amphibian landscapes on earth (Catenazzi et al., 2011). In the U.S., Bd may be native to parts of the country, could have arrived recently as a novel pathogen (e.g., Rodriguez et al., 2014; Vredenburg et al., 2013), or may be present as both native and invasive strains in the same location (e.g., Brazil, Rodriguez et al., 2014). The history of Bd among Midwestern U.S. amphibians needs to be resolved so that current potential impacts may be better understood.

The history of any pathogen is important to resolve because of the potential for evolutionary selection in the disease agent and host (Roy and Kirchner, 2000) and the need for different management options. Prolonged contact of hosts and pathogens should result in coevolution for resistance or tolerance among hosts (Roy and Kirchner, 2000). Field surveys revealed widespread prevalence and some high intensity Bd infections in Illinois amphibians (Talley unpublished data), so we hypothesized that the lack of observed mortality or ongoing declines might indicate adaptation of Illinois amphibians to Bd due to long-term infection and subsequent co-evolution. Alternately, Illinois amphibians may be infected with an invasive but less virulent strain of Bd than has been observed in global amphibian declines (e.g., Bd-GPL, Farrer et al., 2011). We surveyed museum specimens of Illinois amphibians to determine whether historic declines might have been caused by Bd. Our objectives were to determine the oldest evidence of Bd in Illinois, and to quantify prevalence among species and regions throughout this time period (1888–1989).

2. Methods

We sampled ten species (*A. blanchardi*, *Anaxyrus americanus*, *Anaxyrus fowleri*, *Hyla chrysoscelis*/*versicolor*, *Rana* (*Lithobates*) *blairi*, *R.* (*L.*) *catesbeiana*, *R.* (*L.*) *clamitans*, *R.* (*L.*) *pipiens*, *R.* (*L.*) *sphenocephala*, and *Pseudacris crucifer*) that have widespread distributions in Illinois (Phillips et al., 1999), are infected with Bd in Illinois today (Talley et al. unpublished data), and are relatively abundant in collections (Table A1). All these species have relatively stable populations in Illinois (NatureServe, 2013), and usually breed in lentic waterbodies between January and September (Phillips et al., 1999), but vary in longevity from one (*A. blanchardi*; Burkett, 1984) to ten years (*R.* (*L.*) *catesbeiana*; Casper and Hendricks, 2005).

For our primary dataset, we sampled 1008 museum specimens belonging to ten species collected between 1892 and 1989 (Table 1) across the entire state of Illinois (Fig. 1). We attempted to sample at least 30 individuals per species from as many decades as possible to capture historic geospatial and temporal patterns. Because of historic population declines reported in *A. blanchardi* and *Rana* (*Lithobates*) *sphenocephala*, we sampled all available specimens from those two species; some specimens were listed among museum records but were not available at time of sampling. We mapped specimen locations with Program R (hereafter R; version 2.15.3; R Core Team, 2013; package 'maps,' Brownrigg and Minka, 2013) to visualize collection localities.

We collected skin swab samples from museum specimens housed at Southern Illinois University Carbondale (SIUC), University of Illinois Museum of Natural History (UIMNH), and the Illinois Natural History Survey (INHS). We followed methods described in Cheng et al. (2011) for sample collection and analysis. Specifically, we handled each specimen with new gloves to reduce possible cross-contamination of Bd DNA. Before sampling for Bd DNA, we rinsed each specimen with 80% EtOH to remove floating Bd DNA or pieces of skin on the specimen that may have originated from others stored in the same jar. We used rayon-tipped Medical Wire swabs (MW113) to collect genetic material by swabbing the ventral surface of each specimen 25 times (five strokes per rear foot, inner thigh, and on the abdomen). Samples were stored in 80% EtOH until DNA extraction.

We extracted DNA from the skin swabs using Prepman Ultra (hereafter Prepman) and used quantitative Polymerase Chain Reaction (qPCR) protocols developed by Boyle et al. (2004) and refined for museum specimens by Cheng et al. (2011). We ran negative controls (water, TE Buffer) and positive Bd standards (100, 10, 1.0, and 0.1 zoospore genomic equivalents) with each qPCR reaction (see example plot Fig. A1). None of the negative controls amplified during qPCR. We tested all samples once, and if they showed exponential amplification before cycle 50 we ran them two additional times. We coded samples as positive if two of three runs showed exponential amplification before cycle 50 (Cheng et al., 2011), which are approximately 0.01 zoospore equivalents (Boyle et al., 2004).

We re-swabbed 12 *A. blanchardi* and 18 *R.* (*L.*) *sphenocephala* specimens (1892–1957) from our original sample to verify historic positives under the strict conditions of an ancient DNA laboratory (as in Muletz et al., 2014), and swabbed 20 additional specimens (9 *A. blanchardi*, 9 *R.* (*L.*) *pipiens*, and 2 *R.* (*L.*) *sphenocephala*; 1888–1948) to extend the timeline as far back as possible. For these 50 swabs, we extracted DNA in an ancient DNA lab at the Smithsonian Center for Conservation and Evolutionary Genetics, and used the Qiagen Blood and Tissue Kit for Tissue Extraction (hereafter Qiagen) with an overnight incubation in lysis buffer and a final elution volume of 80 µl. All other methods were the same as outlined above, including triplicate qPCR runs. To confirm Bd identification, we sequenced 42 of the Bd+ samples using the ITS and 5.8S primers (Boyle et al., 2004). We cleaned amplified PCR products with ExoSAP-IT (United States Biochemical), and sequenced the cleaned amplicons using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.). The sequenced products were column filtered, dried down, rehydrated with 10 µl of HPLC purified formamide, and then analyzed on an Applied Biosystems 3130xl DNA

Table 1
Results from qPCR for historical Bd prevalence by decade for ten anuran species; no specimens were examined in the 1910s.

Species	1890s (Positive/total) (Prevalence %) (95% CI)	1900s	1920s	1930s	1940s	1950s	1960s	1970s	1980s	Total
Acris blanchardi	0/7 0 0–35.4	0/2 0 0–65.8	2/16 12.5 3.5–36.0	0/30 0 0–11.4	3/42 7.1 2.5–19.0	1/117 0.9 0–4.7	0/52 0 0–6.9	0/23 0 0–14.3	0/1 0 0–94.9	6/290 2.1 1.0–4.4
Anaxyrus americanus	–	–	–	0/9 0 0–29.9	0/3 0 0–56.1	0/3 0 0–56.1	0/47 0 0–7.6	0/12 0 0–24.2	0/23 0 0–14.3	0/97 0 0–3.8
Anaxyrus fowleri	–	–	–	0/34 0 0–10.1	–	0/8 0 0–32.4	0/29 0 0–11.7	0/24 0 0–13.8	0/5 0 0–43.4	0/100 0 0–3.7
Hyla chrysoscelis/versicolor	–	–	–	–	–	0/18 0 0–17.6	0/19 0 0–16.8	–	2/17 11.8 3.3–34.3	2/54 3.7 1.0–12.5
Rana (*Lithobates*) *blairi*	–	–	–	–	–	0/2 0 0–65.8	–	–	0/12 0 0–24.2	0/14 0 0–21.5
Rana (*Lithobates*) *catesbeiana*	–	–	–	–	0/9 0 0–29.9	0/11 0 0–25.9	0/32 0 0–10.7	0/1 0 0–94.9	0/5 0 0–43.4	0/58 0 0–6.2
Rana (*Lithobates*) *clamitans*	–	–	–	0/2 0 0–65.8	0/1 0 0–94.9	0/26 0 0–12.9	0/41 0 0–8.6	0/1 0 0–94.9	0/15 0 0–20.4	0/86 0 0–4.1
Rana (*Lithobates*) *pipiens*	–	–	–	–	–	0/8 0 0–32.4	1/3 33.3 1.7–79.2	0/2 0 0–65.8	0/2 0 0–65.8	1/15 6.7 0.3–29.8
Rana (*Lithobates*) *sphenocephala*	0/1 0 0–94.9	1/2 50 2.6–97.4	1/2 50 2.6–97.4	0/12 0 0–24.2	56/84 66.7 56.1–75.8	37/66 56.1 44.1–67.4	3/73 4.1 1.4–11.4	3/12 25.0 8.9–53.2	2/17 11.8 3.3–34.3	103/269 38.3 32.7–44.2
Pseudacris crucifer	–	–	–	0/13 0 0–22.8	0/3 0 0–56.1	0/6 0 0–39.0	0/3 0 0–56.1	–	–	0/25 0 0–13.3
Total	0/8 0 0–32.4	1/4 25 1.3–69.9	3/18 16.7 5.8–39.2	0/100 0 0–3.7	59/142 41.5 33.8–49.8	38/265 14.3 10.6–19.1	4/299 1.3 0.5–3.4	3/75 4.0 1.4–11.1	4/97 4.1 1.6–10.1	112/1,008 11.1 9.3–13.2

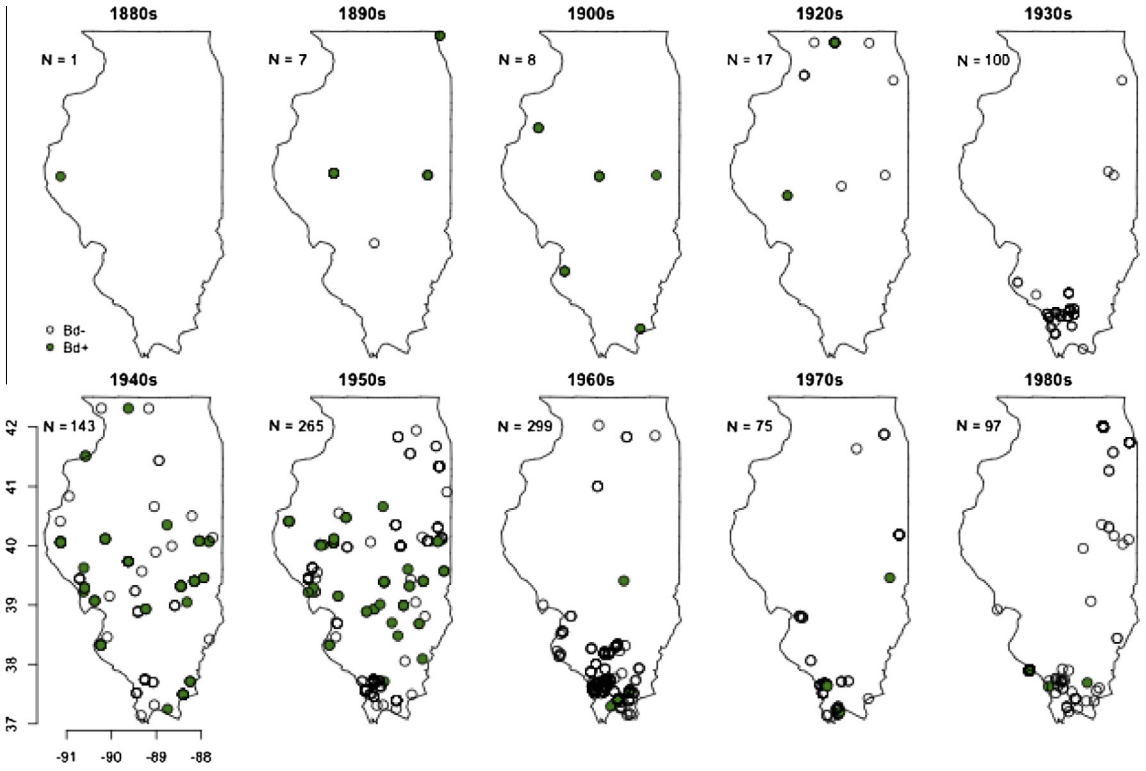

Fig. 1. Sampling effort and results of qPCR analyses (Prepman and Qiagen extractions) by decade show widespread Bd infection but unequal sampling effort among regions.

Analyzer. Individual sequences were assembled and edited in Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI USA).

We used R for all statistical analyses (version 2.15.3; R Core Team, 2013). We determined 95% confidence intervals (CIs) for prevalence estimates by decade and species, using alpha = 0.05 and the Wilson score interval for binomial proportion interval estimates (R package 'Hmisc,' Harrell, 2012; Agresti and Coull, 1998). To examine geographic distribution of historic Bd infection, we grouped specimen locations into southern, central, and northern Illinois regions that correspond to USDA hardiness zones, related to seasonal temperatures (USDA Plant Hardiness Zone Map, 2012; Fig. B1).

We did not include the second set of 50 samples in this analysis because differences in the sensitivity of the two Bd DNA extraction methods affected detection (Cheng et al., 2011). Instead, we did the following analyses for all qPCR samples from the primary dataset, and for a subset of samples omitting *R.* (*L.*) *sphenocephala.* We created a logistic regression model with species as a fixed effect, followed by Pearson's chi-square test for significance values (R package 'stats,' R Core Team, 2013). We then used generalized linear hypothesis testing (function 'glht') for multiple comparisons of means (function 'mcp'), employing Tukey's HSD test (R package 'multcomp,' Hothorn et al., 2008) to compare Bd prevalence among species.

3. Results

We found that 112 of 1008 specimens were Bd+ (prevalence = 11.1%, CI: 9.3–13.2%) using Prepman extractions; including samples that only amplified 1 of 3 times ($n = 22$) raised overall prevalence to 13.3% (CI: 11.3–15.5%), but those samples were not used in the remaining analyses to minimize potential false positive results. Prevalence estimates from Qiagen extractions were higher (17/20; 85.0%, CI: 64.0–94.8%) than those from Prepman extractions, so we did not combine these datasets. We found that Bd was widespread in Illinois by 1900 and that the oldest Bd+ specimen was collected in 1888. The oldest Bd+ specimens for each region were from 1900 in Southern Illinois (Hardin Co.), from 1888 in Central Illinois (Adams Co.), and from 1892 in Northern Illinois (Champaign Co.; Fig. 1).

The prevalence of Bd varied among species ($\chi^2 = 262.34$, $df = 9$, $p < 0.01$). Among all species, Bd prevalence was highest during the 1940s (40.8%, CI: 33.1–49.1%), with the next greatest prevalence in the 1950s (14.3%, CI: 10.6–19.1%, Fig. 2a). *Rana* (*Lithobates*) *sphenocephala* had the highest overall Bd prevalence among species (38.3%, CI 32.7–44.2%; Fig. 2b), followed by *R.* (*L.*) *pipiens* (6.7%, CI: 0.3–29.8%), *H. chrysoscelis* (3.7%, CI: 1.0–12.5%), and *A. blanchardi* (2.1%, CI 1.0–4.4%; Fig. 2c); we did not detect Bd in the remaining six species (Table 1).

Although we could not statistically examine temporal and geographical patterns in our data because of non-independence, visual inspection indicated Bd prevalence variation in space and time but not like that of a historic epizootic wave (Fig. 1). The greatest Bd prevalence in *R.* (*L.*) *sphenocephala* was in the 1940s (66.7%, CI: 56.1–75.8) and 1950s (56.1%, CI: 44.1–67.4), followed by the 1970s (25.0%, CI: 8.9–53.2; Fig. 2b); prevalence was <12% in all other decades (Fig. 2b; Table 1). Infection prevalence in *R.* (*L.*) *sphenocephala* was lowest in the south (18.1%, CI: 12.6–25.4), at intermediate levels in the north (55.6%, CI: 37.3–72.4) and highest in central Illinois (60.0%, CI: 50.4–68.9). Only 9 of 742 specimens from the other 9 species were Bd+ (1.2%, CI: 0.6–2.3%; Table 1), with no differences among species ($\chi^2 = 14.44$, $df = 8$, $p = 0.07$). Prevalence was lower in these other species and ranged from 0.0% (CI: 0.0–5.7) in the 1970s to a high of 5.2% (CI: 1.8–14.1) in the 1940s (Fig. 2a).

The two extraction methods varied in sensitivity. We resampled 30 specimens that were previously tested from Prepman extractions (18 Bd+ and 12 Bd−) and extracted these new skin swabs with Qiagen extractions; we confirmed all 18 Bd+ and an

Table 2
Results of qPCR by decade from Qiagen extractions and DNA sequencing for Bd confirmation of 30 Prepman samples, and further exploration of additional museum samples.

Species	Bd confirmation (Positive/total) (Prevalence %) (95% CI)					Oldest Bd sample identification (Positive/total) (Prevalence %) (95% CI)				
	1890s	1900s	1920s	1940s	1950s	1880s	1890s	1900s	1920s	1940s
Acris blanchardi	3/7	2/2	2/3	–	–	–	0/2	7/7	–	–
	42.9	100	66.7				0	100		
	15.8–75.0	34.2–100	20.8–98.3				0–65.8	64.6–100		
Rana (*Lithobates*) *pipiens*	–	–	–	–	–	–	4/4	1/1	2/3	1/1
							100	100	66.7	100
							51.0–100	5.1–100	20.8–98.3	5.1–100
Rana (*Lithobates*) *sphenocephala*	1/1	2/2	1/1	8/8	6/6	1/1	1/1	–	–	–
	100	100	100	100	100	100	100			
	5.1–100	34.2–100	5.1–100	67.6–100	61.0–100	5.1–100	5.1–100			
Total	4/8	4/4	3/4	8/8	6/6	1/1	5/7	8/8	2/3	1/1
	50	100	75	100	100	100	71.4	100	66.7	100
	21.5–78.5	51.0–100	30.1–98.7	67.6–100	61.0–100	5.1–100	35.9–91.8	67.6–100	20.8–98.3	5.1–100

additional 7 that were previously classified Bd− to be Bd+. For an additional 20 samples (collected 1888–1948) extracted with Qiagen, prevalence estimates (17/20; 85.0%, CI 64.0–94.8%) were higher than those from Prepman extractions (Table 2). We sequenced all Qiagen Bd+ samples ($n = 42$), producing 29 high quality consensus sequences (5.8S and ITS primers) that were $\geqslant$99% match to Bd sequences in GenBank (Table C1). We obtained high quality Bd sequences (>80% quality) from the earliest samples (1888–1900s, $n = 18$) indicating the ability to obtain short DNA sequences (<200 bp) of fungal pathogen DNA from century old formalin-fixed museum specimens.

4. Discussion

Our results show that Bd infected amphibians in North America by 1888. This individual, a *R.* (*L.*) *sphenocephala* from Central Illinois, is also the oldest reported global record for Bd, closely followed by the 1894 Bd positive *Hypsiboas pulchellus* from Atlantic Coastal Forest in Brazil (Rodriguez et al., 2014). Such old Bd infections with stable infection presence over a century suggest this pathogen has likely been influencing amphibian population dynamics for longer than previously suspected. We do not know whether current populations previously declined from Bd, whether declines are ongoing, or whether lack of die-offs represent host-pathogen coevolution (Roy and Kirchner, 2000).

In contrast to Rodriguez et al. (2014), whose results showed stable prevalence over the entire sample period (1890–2000), we identified an increase in Bd prevalence from 1940 to 1949. This increase, followed by a decrease back to 1930s levels may indicate an epizootic, although additional sampling is needed to confirm this supposition. The peak in historic Bd prevalence around the 1940s seems to driven by *R.* (*L.*) *sphenocephala*. Alternatively, this may be a result of uneven sampling effort among species and years or other factors influencing disease dynamics (Phillips et al., 2012), such as changes in pathogen strain virulence and evolution (Fisher et al., 2009a,b), changes in host species susceptibility (Searle et al., 2011), or changes in the environment (Kriger et al., 2007). We found no evidence of an epizootic wave moving through naïve populations (as per Lips et al., 2008; Vredenburg et al., 2010). This observation suggests that Bd may be native to Illinois or that the introduction of Bd predates the earliest collection year of this study. Additionally, the role of multiple Bd strains in Illinois should be further examined on historic and current samples because historic strain prevalence may be unrelated to current conditions. Prevalence varied geographically, and this could be due to regional variation in seasonal temperatures that affect Bd growth and survival or other environmental factors (Kriger et al., 2007).

Historic Bd prevalence varied among species, which may reflect differences in historic population responses to Bd infection. Periods of historic Bd infection in both *R.* (*L.*) *pipiens* and *A. blanchardi* may have been associated with reports of population declines from the 1970s (Collins and Crump, 2009; Les, 1979; Nace, 1968; Vogt, 1981). We were unable to obtain sufficient samples of *R.* (*L.*) *pipiens* for decades after the 1950s, which may indicate reduced abundance, as was described for *A. blanchardi* in Illinois (Reeder et al., 2005). For *A. blanchardi*, we identified low historic Bd prevalence (Fig. 2c); present day *A. blanchardi* populations have high Bd prevalence (Talley unpublished data) and recent population declines (Lannoo, 2005). However, we cannot compare historic with current prevalence because the technique we used in this study typically underestimates prevalence (see Cheng et al., 2011). In contrast to *A. blanchardi*, *R.* (*L.*) *sphenocephala* has not shown any evidence of declines (Lannoo, 2005), despite a long history of infection and current high prevalence (Talley unpublished data). Future investigations into potential mechanisms of tolerance in *R.* (*L.*)

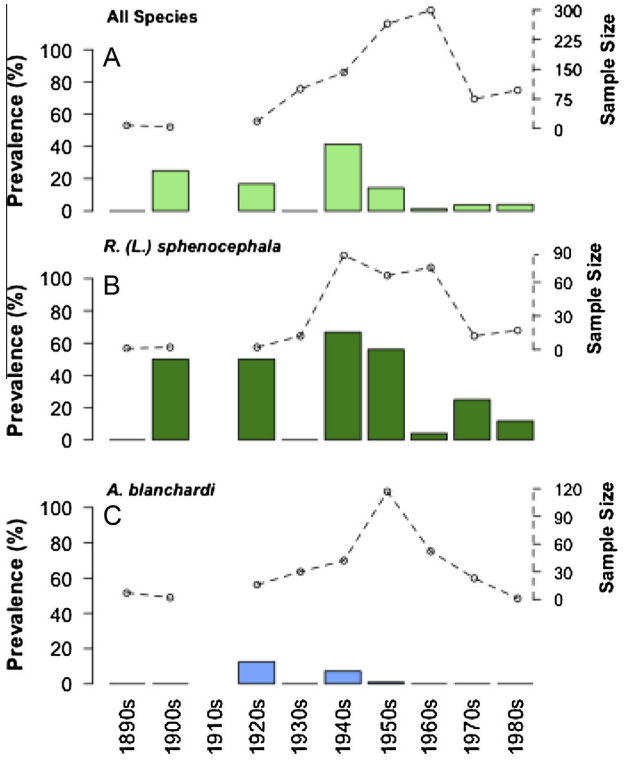

Fig. 2. Considering all sampled species, the greatest historic Bd prevalence was in the 1940s, a result that stems primarily from *R.* (*L.*) *sphenocephala*. Here we show Bd prevalence of Prepman samples by decade for (A) all sampled species (n = 1008) and for the two species with greatest sample sizes, (B) *R.* (*L.*) *sphenocephala* (n = 269), and (C) *A. blanchardi* (n = 290).

sphenocephala are needed, as are mark-recapture studies to generate demographic information needed to assess population status.

The difficulty in unraveling historic Bd effects is compounded by not only the known underestimation of incidence among museum specimens, but also by not knowing what the environmental conditions were like when the animals were collected (Cheng et al., 2011). In present day samples, Bd infection outcomes are determined by interactions of the host (e.g., Reeder et al., 2012), agent (e.g., Schloegel et al., 2012), and environmental (e.g., Piotrowski et al., 2004) characteristics. We propose that the same types of controlling factors determined the outcomes of historic Bd infections. For example, the increase in prevalence in the 1940s may represent a shift in environmental conditions, a change Bd virulence, and/or alteration of the amphibian response to infection that favored infection.

We suggest that the Midwestern U.S. should be examined as a potential point of origin for Bd because of the widespread distribution of Bd since the 1890s and the steady presence of Bd in museum specimens. North America has been over-looked as a potential geographic origin of Bd because of epizootics in the Western U.S. (e.g., California, Vredenburg et al., 2010; Muths et al., 2003) that typically indicate the arrival of a novel pathogen into naïve populations. Additionally, the most well studied North American Bd lineages belong to a recently derived Bd-GPL (Farrer et al., 2011) clade, Bd-GPL-1 (Schloegel et al., 2012). However, Midwestern U.S. samples from wild populations are typically missing from phylogenetic analyses (e.g., James et al., 2009; Rosenblum et al., 2013). Supporting the possibility of an older lineage, the only Midwestern wild sample from Schloegel et al. (2012), a *R.* (*L.*) *pipiens* from Ohio, grouped with a lesser-derived Bd-GPL clade (Farrer et al., 2011), Bd-GPL-2. The paucity of Bd isolates from Midwestern U.S. amphibians that have been genetically profiled leaves open the possibility that there is a basal strain in the Midwestern U.S.

5. Summary and conclusions

The taxonomic variation among host species for historic Bd prevalence reminds us that caution is needed when interpreting negative Bd results from historic samples, especially when sample sizes are small. If we had not sampled *R.* (*L.*) *sphenocephala* the historic Bd prevalence in Illinois would have been much lower than indicated here. These results should be considered for future retrospective surveys, particularly in the Midwestern U.S., since other species yielded few Bd positive specimens. The role that *R.* (*L.*) *sphenocephala* played in the historic distribution of Bd in Illinois, and its current role in disease dynamics also needs further investigation.

We demonstrate that Bd was present in Illinois over 120 years ago. Interestingly, we did not find a pattern of Bd invasion as seen in areas where Bd epizootics were followed by amphibian population collapse, but instead describe a pattern of relatively constant Bd prevalence over the 100 year sample period, except for the 1940s. We describe a twofold increase in prevalence in the 1940s followed by a decline in prevalence back to pre-increase levels, suggesting that infection dynamics have changed over the last century. Other studies have either shown a steady host pathogen dynamic with relatively unchanged Bd prevalence over a similar time period (Rodriguez et al., 2014) or have described the invasion of Bd into naïve populations quickly followed by an increase in prevalence from zero to 100% before mass die-offs occur (Vredenburg et al., 2010).

Understanding historical distributions of Bd is crucial for unraveling the complex story of the origin and evolution of this deadly pathogen. We contribute to the growing evidence pool that Bd has been present in some regions of the world for much longer than originally realized. Museum collections afford a great opportunity to explore the history of Bd (Lips, 2011), allowing us to establish infection baselines for some amphibian populations. Amphibians are declining globally (Fisher et al., 2009b), so understanding historical pathogen prevalence is important in planning contemporary management strategies.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2014.12.007.

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