Ohio Biological Survey Notes 10: 13–21, 2021. © Ohio Biological Survey, Inc.

Infection Patterns in an Amphibian Fungal Pathogen in Ohio

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Abstract: The pathogenic fungus *Batrachochytrium dendrobatidis* (Bd) is a leading cause of mortality among amphibians worldwide. To provide a large-scale assessment of Bd in Ohio (USA), we analyzed aggregated data on Bd infection prevalence, microhabitat, and seasonality from across the state. Skin swabs (n = 2,200) were collected from 26 species of amphibians (and one hybrid form) across 22 counties in Ohio between 2006 and 2020. Of these, 20 species tested positive for Bd and at least one positive sample was found in 18 of 22 counties. Overall Bd prevalence among the tested samples was 17.7% (390/2,200). Frogs were infected at a significantly higher frequency (23.3%; 283/1,212) than salamanders (10.7%; 106/988). Amphibians sampled from aquatic microhabitats were infected at a significantly higher frequency (frogs: 24.9%; 237/953; salamanders: 15.0%; 105/706) than those sampled from terrestrial microhabitats (frogs: 17.4%; 45/259; salamanders: 0.4%; 1/282). Seasonal infection frequency differed among species, with some species showing seasonal infection patterns and others not. All skin swabs tested for another emerging fungal pathogen of amphibians (*Batrachochytrium salamandrivorans,* n = 186) tested negative. While these data suggest that Bd is widespread in Ohio and has strong taxonomic and microhabitat associations, they also underscore how much work remains to be done, with many species still undersampled (or not sampled at all) for this emerging pathogen.

Keywords: *Batrachochytrium dendrobatidis*, *Batrachochytrium salamandrivorans,* disease surveillance, frog, salamander, wildlife diseases.

Introduction

Amphibians are one of the most threatened groups of organisms on the planet, with nearly one-third of species thought to be at risk of extinction (Stuart et al., 2004). While the causes of amphibian declines are complex and often include multiple stressors (Blaustein and Kiesecker, 2002; Collins and Crump, 2009; Rohr and Palmer, 2013), an important driver of these losses is the emerging amphibian fungal pathogen *Batrachochytrium dendrobatidis* (hereafter Bd) (Berger et al. 1998; Scheele et al. 2017; Skerratt et al. 2007; Vredenburg et al. 2010). Overwhelming evidence indicates that this pathogen has played a major role in numerous extinctions and local extirpations, resulting in biodiversity loss (Collins and Crump, 2009; Skerratt et al., 2007; Vredenburg et al., 2010; Cheng et al., 2011). More recently, a newly discovered fungal pathogen, *Batrachochytrium salamandrivorans* (hereafter Bsal), has also been implicated in declines in some salamanders in Europe but has yet to be reported from the New World (Gray et al., 2015; Yap et al., 2015; Waddle et al., 2020).

Patterns of Bd infection, including taxonomic associations (O'Hanlon et al., 2018), environmental influences (Kärvemo et al., 2018), temporal (Watters et al., 2019) and geographic trends (Olson et al., 2013), and genetic divergence (Byrne et al., 2019), continue to be examined in detail by researchers. Global mapping efforts have been underway for some time (Olson et al., 2013; James et al., 2015), but patterns of Bd infection at regional spatial scales are still incomplete. Many studies that examine infection status of free-living individuals are based on information from one or a small number of sites. These studies, while important, are often hampered by small sample sizes. To effectively monitor wildlife pathogens and to rigorously investigate factors influencing infection patterns, large-scale studies with robust sample sizes are needed.

While there is often great interest in areas where wildlife disease outbreaks are occurring (epizootics; Lips et al., 2006), there is also a need for studies in areas where pathogens have long been established (enzootic stage; James et al., 2015; Scheele et al.,

2017). For example, Bd has been present in the midwestern United States since at least the late 19th century (Talley et al., 2015). Recent evidence suggests that partial population recovery from Bd in some taxa is possible even over relatively short time scales (Voyles et al., 2018). Therefore, intensive studies in areas where Bd has been present for long time periods are critically important to examine the potential mechanisms of recovery from declines and to assess continuing changes. Here, we present new data and aggregate scattered published and unpublished data on the distribution, prevalence, taxonomic associations, and seasonal and microhabitat infection patterns of Bd infection for amphibians in the state of Ohio (USA). These aggregated data allow us to overcome some of the limitations of small sample sizes of individual species to search for overall patterns. We also assess the occurrence of Bsal in Ohio.

Materials and Methods

Sample Collection. To collect samples to assess pathogen infection status, we hand-captured adult amphibians in the field, using new sterile gloves for each individual. In aquatic environments, we used dip nets. Fewer than ten larvae were captured; we pool these data with those from adults because they represent a very small fraction of the total. Captured individuals were swabbed either for 30 seconds or 25 times each, using a sterile swab. Skin swabs were collected between 2006 and 2020 in 22 counties in Ohio (USA) and stored in a freezer until they were processed in the laboratory (see below). Species identification was based on Pfingsten et al. (2013), but a small number of small *Plethodon* were determined to be hybrids between *Plethodon cinereus* and *P. electromorphus* based on coloration and morphological criteria in Lehtinen et al. (2016). We worked at 110 study sites across the state and, in general, all sites were sampled for amphibians only once. However, at Wooster Memorial Park (Wayne Co.), we repeatedly sampled amphibian populations from 2016 to 2020. This time series will be the subject of a separate manuscript, but the aggregate data are included here. For swabs from this site, each was assumed to be independent across years. In addition to these new data, our analysis also includes previously published data from Steiner and Lehtinen (2008), Krynak et al. (2012), Korfel and Hetherington (2014), and Sonn et al. (2019). Because each of the researchers was working independently of others at the time that skin swabs were collected in the field, sampling was haphazard.

Infection quantification using PCR. DNA was extracted from the swabs using Qiagen DNEasy Blood & Tissue kits. Isolated DNA was tested for Bd using either quantitative real-time PCR (n = 1,077), following a modified protocol based on Boyle et al. (2004) or conventional PCR (n = 1,124) following Annis et al. (2004). Because of differing protocols among researchers, some samples were run in triplicate while others were run in singlicate. For samples run in triplicate, they were deemed Bd positive if two or more PCR reactions for that sample were positive. All skin swab samples were run to assess Bd infection status; a smaller subset (all from Wooster Memorial Park in Wayne county) were also run for Bsal. Unfortunately, because different labs used different standards in their qPCR reactions, infection load numbers for positive samples cannot be meaningfully compared among researchers. For this reason, we present only infection frequency data.

Statistical Analysis. Our aggregate analyses pool infection data from many different species, allowing much larger sample sizes than would otherwise be available. However, due to uneven sampling among species, these pooled results should be interpreted cautiously. Multiple logistic regression was used to determine the influence of taxonomic order (frogs or salamanders), microhabitat type (terrestrial or aquatic), and season (spring, March–May; summer, June–August; or autumn, September–November) on Bd infection likelihood. Reference categories for these predictors were assigned: e.g., frogs, aquatic microhabitat, and spring, respectively. All predictor variables were coded as categorical and entered into the logistic regression model simultaneously. We also used χ^2 goodness-of-fit tests to test for differences in infection frequency across different seasons and microhabitats and between order, family, and species when sample sizes allowed. In addition to aggregated analyses, we also used χ^2 goodness-of-fit tests to assess seasonality and microhabitat influences for individual species that had at least n = 30 for each season or microhabitat. Cramér's V was used to assess the strength of association between variables. All statistical analyses were performed in SPSS version 27.0.

Results

A total of 2,200 skin swab samples from 26 species (and one hybrid form) in eight amphibian families were collected and processed from 22 Ohio counties. A total of 390 (17.7%) of these swabs tested positive for Bd, including swabs from 18 of the 22 counties sampled (Table 1). Twenty of 26 species tested positive for Bd in at least one individual (Tables 2, 3), however, all swabs tested for Bsal (n = 186) were negative.

As a group, frogs had a significantly higher frequency of infection (23.3%) than salamanders (10.7%; $\chi^2 = 60.893$, df = 1, n = 2,200, $p < 0.001$, Cramér's V = -0.165; Table 2). Within frogs, infection frequency was not equally distributed among families

(χ^2 = 41.494, df = 2, n = 1,234, p < 0.001, Cramér's V = 0.183), with Ranidae having the highest frequency of infection (31.0%) and Bufonidae the lowest (11.2%, Table 2). Similarly, within salamanders, infection frequency was also not equally distributed among families (χ^2 = 25.681, df = 4, n = 988, p < 0.001, Cramér's V = 0.161), with Cryptobranchidae having the highest frequency of infection (29.2%) and Plethodontidae the lowest (8.6%; Table 2).

At the species level, Bd infection frequency was also not equally distributed among frog species (χ^2 = 91.411, df = 10, n = 1,234, p < 0.001, Cramér's V = 0.272; Table 3). *Lithobates catesbeianus* (60.3%) and *L. clamitans* (30.3%) had particularly high infection frequencies. In one frog species (*Hyla versicolor*), no infection was detected. Bd infection frequency was also not equally distributed among salamander species (χ^2 = 60.058, df = 15, n = 991, p < 0.001, Cramér's V = 0.246; Table 3). *Ambystoma jeffersonianum* (34.4%) and *Cryptobranchus alleganiensis* (29.2%) had particularly high infection frequency. In four salamander species (two *Ambystoma* and two *Plethodon*), no infection was detected (Table 3).

Microhabitat use significantly influenced infection status. Amphibians in aquatic microhabitats were infected with Bd at significantly higher frequency than those in terrestrial microhabitats (χ^2 = 64.460, df = 1, n = 2,200, p < 0.001, Cramér's V = 0.170). This also held true when frogs and salamanders were analyzed separately (frogs: χ^2 = 14.307, df = 1, n = 1,234, p < 0.001, Cramér's V = 0.108; salamanders: χ^2 = 45.124, df = 1, n = 991, p < 0.001, Cramér's V = 0.213; Table 4). Notably, only two species of frogs sampled in a terrestrial microhabitat were Bd positive (*P. crucifer*: 1/2; *A. americanus*: 12/115). Similarly, of the 282 salamander samples taken from terrestrial microhabitats, only one was positive (*E. bislineata*: 1/60). Blanchard's cricket frog was the only frog with a large enough sample size in both aquatic and terrestrial habitats to permit a within-species test. This species did not have significantly different infection frequencies between habitats (11/50 [22.0%] in terrestrial habitats and 74/393 [18.8%] in aquatic habitats; χ^2 = 0.288, df = 1, n = 443, p = 0.592). Of salamanders with large enough sample sizes for a within-species test, infection was significantly more frequent in aquatic habitats in both species: the infection frequency in *E. bislineata* was 1/60 (1.7%) in terrestrial habitats and 47/315 (14.9%) in aquatic habitats (χ^2 = 6.678, df = 1, n =423, p = 0.01, Cramér's V = 0.126), and in *P. cinereus*, infection occurred in 0/115 (0.0%) of individuals in terrestrial habitats and 10/53 (18.9%) in aquatic habitats (χ^2 = 19.341, df = 1, n = 178, p < 0.001, Cramér's V = 0.330).

Bd infection frequency in all amphibians was not equally distributed between seasons (χ^2 = 9.181, df = 2, n = 1,662, p = 0.010, Cramér's V = 0.074). For all amphibian

County	Infection		
	Total	Positive	% Bd Positive
Auglaize	43	6	14.0
Belmont	15	1	6.7
Clinton	155	24	15.5
Columbiana	1	0	0.0
Cuyahoga	149	45	30.2
Delaware	210	72	34.3
Franklin	381	75	19.7
Geauga	115	10	8.7
Greene	62	7	11.3
Hardin	66	0	0.0
Harrison	6	6	100.0
Jefferson	23	7	30.4
Lake	43	18	41.9
Marion	7	1	14.3
Medina	77	4	5.2
Monroe	6	0	0.0
Preble	19	3	15.8
Stark	27	0	0.0
Summit	40	5	12.5
Union	10	9	90.0
Warren	158	43	27.2
Wayne	587	54	9.2
Total	**2,200**	**390**	**17.7**

Table 1. Bd infection frequency across 22 Ohio counties in frogs and salamanders (n = 2,200, all species pooled).

Family		Infection		
		Total	Positive	% Bd Positive
Frogs	Bufonidae	125	14	11.2
	Hylidae	510	90	17.6
	Ranidae	577	179	31.0
	Total	**1,212**	**283**	**23.3**
Salamanders	Ambystomatidae	123	20	16.3
	Cryptobranchidae	24	7	29.2
	Plethodontidae	802	69	8.6
	Proteidae	8	2	25.0
	Salamandridae	32	8	25.0
	Total	**988**	**106**	**10.7**

Table 2. Summary of frog and salamander Bd infection frequency in Ohio by family (n = 2,200). Infection was significantly higher in frogs than salamanders and not equally distributed across families.

Table 3. Summary of Bd infection frequency in Ohio by species (n = 2,200). *Plethodon* hybrids were *P. cinereus* x *P. electromorphus*.

Species		Infection		
		Total	Positive	% Bd Positive
Frogs	*Acris blanchardi*	441	85	19.3
	Anaxyrus americanus	125	14	11.2
	Hyla chrysoscelis	15	4	26.7
	Hyla versicolor	25	0	0.0
	Lithobates catesbeianus	58	35	60.3
	Lithobates clamitans	409	124	30.3
	Lithobates palustris	11	3	27.3
	Lithobates pipiens	14	1	7.1
	Lithobates sylvaticus	85	16	18.8
	Pseudacris crucifer	28	1	3.6
	Pseudacris triseriata	1	0	0.0
	Total	**1,212**	**283**	**23.3**
Salamanders	*Ambystoma jeffersonianum*	32	11	34.4
	Ambystoma maculatum	56	9	16.1
	Ambystoma opacum	5	0	0.0
	Ambystoma texanum	30	0	0.0
	Cryptobranchus alleganiensis	24	7	29.2
	Desmognathus fuscus	56	5	8.9
	Desmognathus ochrophaeus	22	3	13.6
	Eurycea bislineata	420	47	11.2
	Eurycea longicauda	13	1	7.7
	Necturus maculosus	8	2	25.0
	Notophthalmus viridescens	32	8	25.0
	Plethodon cinereus	177	10	5.6
	Plethodon electromorphus	34	0	0.0
	Plethodon glutinosus	46	0	0.0
	Plethodon spp. hybrid	17	0	0.0
	Pseudotriton ruber	16	3	18.8
	Total	**988**	**106**	**10.7**

species, there was a slightly higher infection frequency during the spring than in the summer or autumn, but patterns differed between frogs and salamanders (Table 5). Infection frequency was distributed relatively equally among seasons in frogs, with the highest frequency in the spring (27.0%), followed by lower (but still high) infection frequencies of 21.2% in the summer and 22.3% in the autumn. Only two species of frogs had large enough sample sizes in all three seasons for statistical analysis. Of these, *Acris blanchardi* did not have significantly different Bd infection frequency among seasons ($\chi^2 = 5.566$, df = 2, n = 443, p = 0.062, Cramér's V = 0.112; Table 6), but *L. clamitans* did ($\chi^2 = 8.569$, df = 2, n = 409, p = 0.014, Cramér's V = 0.131; Table 6). Overall salamander infection frequency was lower; it was also highest in spring (14.0%) and intermediate in the summer (11.9%), but markedly lower in the autumn (7.5%, Table 5). Only one salamander species had large enough sample sizes in all three seasons for statistical analysis. *Eurycea bislineata* did not have significantly different Bd infection frequency among seasons ($\chi^2 = 2.338$, df = 2, n = 423, p = 0.311, Cramér's V = 0.074; Table 7).

Logistic regression of the infection data for all species pooled identified several important predictors of infection status (Omnibus χ^2= 138.377, df = 4, n = 2,200, p < 0.001; Table 8), explaining between 6.0% (Cox and Snell r^2) and 10.0% (Nagelkerke r^2) of the variance in infection prevalence. The strongest predictor of infection status was microhabitat. Individuals sampled from aquatic microhabitats were more than four times as likely to be infected with Bd compared to individuals sampled from terrestrial microhabitats (Table 8). Taxonomic differences also contributed significantly to the model, with frogs more than twice as likely to be infected with Bd compared to salamanders. The season in which an individual was sampled was also a significant predictor of Bd infection status (Table 8), with summer-sampled individuals 1.5 times less likely to be infected than spring-sampled individuals. Spring and fall were not different from one another (Table 8).

Microhabitat		Infection		
		Total	Positive	% Bd Positive
Frogs	Aquatic	953	237	24.9
	Terrestrial	259	45	17.4
	Total	**1,212**	**282**	**23.3**
Salamanders	Aquatic	706	105	15.0
	Terrestrial	282	1	0.4
	Total	**988**	**106**	**10.7**

Table 4. Aggregate infection frequency of frogs and salamanders in aquatic and terrestrial microhabitats (n = 2,200).

Discussion

Attempts to synthesize what we know about Bd and its effects on its hosts have been crucial to identify information gaps, catalyze further research, and enable conservation efforts (James et al., 2015; Lips, 2016). Our relatively large sample sizes from aggregated data allow for firmer conclusions than is often the case about the distribution and frequency of Bd infections, as well as identifying key factors influencing infection patterns. Our results indicate that Bd occurs in most sampled species (20 of 26) and is widely distributed across Ohio; infected amphibians were found in 18 of 22 sampled counties, but counties without Bd detection often had small sample sizes and further sampling would likely detect it (Table 1). Bd is also known to occur in other Ohio counties not directly assessed in this paper (e.g., Butler, Clermont, and Hamilton counties; Rumschlag and Boone, 2020) and probably occurs statewide.

Season		Infection		
		Total	Positive	% Bd Positive
Frogs	Spring	397	107	27.0
	Summer	491	104	21.2
	Autumn	323	72	22.3
	Total	**1,211**	**283**	**23.4**
Salamanders	Spring	308	43	14.0
	Summer	311	37	11.9
	Autumn	373	28	7.5
	Total	**988**	**106**	**10.7**

Table 5. Aggregated seasonal Bd infection data for frogs and salamanders from Ohio (n = 2,200).

Species	Spring		Summer		Autumn	
	Positive/ Total	% Bd Positive	Positive/ Total	% Bd Positive	Positive/ Total	% Bd Positive
Acris blanchardi	**31/117**	**26.5**	**34/199**	**17.1**	**20/125**	**16.0**
Anaxyrus americanus	8/78	10.3	6/40	15.0	0/7	0.0
Hyla chrysoscelis	4/12	33.3	0/0	0.0	0/0	0.0
Hyla versicolor	0/1	0.0	0/23	0.0	0/1	0.0
Lithobates catesbeianus	19/26	73.1	6/19	31.6	10/13	76.9
Lithobates clamitans*	**27/67**	**40.3**	**57/174**	**32.8**	**40/168**	**23.8**
Lithobates palustris	3/5	60	0/6	0.0	0/0	0.0
Lithobates pipiens	1/2	50.0	0/11	0.0	0/1	0.0
Lithobates sylvaticus	14/65	21.5	1/15	6.7	1/5	20.0
Pseudacris crucifer	0/25	0.0	0/1	0.0	1/2	50.0
Pseudacris triseriata	0/0	0.0	0/0	0.0	0/1	0.0
Total	**107/398**	**26.9**	**104/491**	**21.2**	**72/323**	**22.3**

Table 6. Bd infection frequency in frogs by species among seasons. % Bd Positive indicates the percentage of swabs from that species during that season that tested positive for Bd. Bolded species had large enough sample sizes in all seasons (n > 30) to permit statistical analysis. Asterisks indicate species with statistically significant differences in Bd infection among seasons.

Species	Spring		Summer		Autumn	
	Positive/ Total	% Bd Positive	Positive/ Total	% Bd Positive	Positive/ Total	% Bd Positive
Ambystoma jeffersonianum	11/31	35.5	0/1	0.0	0/0	0.0
Ambystoma maculatum	9/53	17.0	0/3	0.0	0/0	0.0
Ambystoma opacum	0/0	0.0	0/5	0.0	0/0	0.0
Ambystoma texanum	0/30	0.0	0/0	0.0	0/0	0.0
Cryptobranchus alleganiensis	0/0	0.0	4/21	19.0	3/3	100.0
Desmognathus fuscus	2/12	16.7	3/34	8.8	0/10	0.0
Desmognathus ochrophaeus	0/1	0.0	3/21	14.3	0/0	0.0
Eurycea bislineata	**4/65**	**6.2**	**20/150**	**13.3**	**24/208**	**11.5**
Eurycea longicauda	0/1	0.0	0/11	0.0	1/1	100.0
Necturus maculosus	0/0	0.0	2/8	25.0	0/0	0.0
Notophthalmus viridescens	8/20	40.0	0/9	0.0	0/2	0.0
Plethodon cinereus	8/67	11.9	2/16	12.5	0/95	0.0
Plethodon electromorphus	0/17	0.0	0/1	0.0	0/16	0.0
Plethodon glutinosus	0/6	0.0	0/17	0.0	0/12	0.0
Plethodon spp. hybrid	0/4	0.0	0/0	0.0	0/13	0.0
Pseudotriton ruber	0/0	0.0	3/14	21.4	0/2	0.0
Total	**42/307**	**13.7**	**37/311**	**11.9**	**28/373**	**7.5**

Table 7. Bd infection frequency in salamanders by species across seasons. % Bd Positive indicates the percentage of swabs from that species during that season that tested positive for Bd. Bolded species had large enough sample sizes (n > 30) in all seasons to permit statistical analysis. No species varied significantly in Bd infection among seasons.

While Bd infection prevalence was generally low to moderate, infection was most frequent in true frogs (Ranidae) and in salamanders with aquatic life stages. These more aquatic amphibians are presumably exposed to Bd zoospores more frequently than terrestrial amphibians, resulting in overall levels of infection over four times higher in amphibians sampled from aquatic microhabitats than from terrestrial ones. Bd zoospores must remain moist to cause infection and disease (Berger et al., 2005; Johnson and Speare, 2005; Van Rooij et al., 2012), and in other regions, similar associations of Bd with aquatic microhabitats have been identified (Lips et al., 2006; Kriger and Hero, 2007; Greenberg et al., 2017). Infection frequency of salamanders was generally low across Ohio when compared to frogs (9.3% of 805 sampled individuals). However, salamander infection frequency in Ohio was much higher than in Tennessee and Virginia, which showed minimal infection (0.7%) in *Plethodon* salamanders (Muletz et al., 2011). Our results were more similar to those reported by Jongsma et al. (2019) from Canada (maximum of 12.9% in *Plethodon cinereus*).

Overall seasonal infection frequency patterns in frogs and salamanders showed similar peaks of infection in the spring, though fall infection frequency dropped more strongly in salamanders than in frogs (Table 5). However, our aggregated analyses may oversimplify the situation; each species may or may not conform to these general patterns, and species with large sample sizes may bias the results toward patterns they exhibit. For example, when looking at seasonal Bd infection patterns in the green frog (*L. clamitans*), we found significant differences among seasons with lower frequency in the summer and autumn compared to spring (see also Korfel and Hetherington 2014). Other studies have reported similar decreased Bd infection frequency during the warmer or drier months of the year (Berger et al., 2004; Kriger and Hero, 2007; Padgett-Flohr, 2008; Lannoo et al.,

	B	S.E.	Wald	df	P-value	Odds
Microhabitat	**1.498**	**.217**	**47.649**	**1**	**< 0.001**	**4.471**
Season			**13.161**	**2**	**0.001**	
Autumn	.034	.146	0.053	1	0.818	1.034
Summer	.454	.147	9.588	1	0.002	1.575
Order	**0.824**	**.124**	**44.085**	**1**	**< 0.001**	**2.294**
Constant	-3.529	.241	213.527	1	**< 0.001**	.029

Table 8. Multiple logistic regression results of variables predicting likelihood of Bd infection, all species pooled (Omnibus Chi-square = 138.377, df = 4, n = 2,200, p < 0.001). Significant predictors are in bold. Salamanders, spring, and aquatic microhabitat were used as reference categories. Odds indicates the odds of infection in the reference category compared to the category being assessed (n = 2,200).

2011; Chatfield et al., 2012; Watters et al., 2019), possibly due to clearance of infections at higher temperatures that are less optimal for Bd growth (Longcore et al., 1999; Weinstein, 2009). Similarly, Blanchard's cricket frog (*Acris blanchardi*) tended to have higher infection in spring than in other seasons (as documented in Sonn et al., 2019; Sonn et al., 2020) even though this pattern did not quite reach statistical significance in our analysis. However, the northern two-lined salamander (*Eurycea bislineata*) showed no statistically significant difference in infection frequency among seasons in Ohio, with large samples from all seasons (Tables 6 and 7). Thus, while some species may clear Bd infections under warmer conditions, others seem to maintain their infections throughout the active season.

North America is a hotspot for amphibian biodiversity, especially for salamanders; it accounts for almost half of the world's recognized species (Yap et al., 2015). Studies that monitor amphibian populations and their pathogens play an essential role in providing baseline infection data that document infection patterns. Aggregating data among researchers provides stronger baseline information and allows for a greater understanding of the interactions between amphibians and their pathogens. Despite the large amount of aggregated data presented here, however, only a few species have robust samples (Tables 6 and 7). Many species in Ohio are still sparsely sampled or have yet to be assessed for Bd infection at all. This underscores the need for ongoing disease surveillance to help mitigate current and future threats to wildlife populations from emerging diseases.

Acknowledgments

RML, EMB, NCN and JNG-L thank the Biology Department and the Copeland Fund at the College of Wooster for support. GJL's work was supported by the State Wildlife Grants Program, administered jointly by the U.S. Fish and Wildlife Service and the Ohio Division of Wildlife with funds provided by the Ohio Biodiversity Conservation Partnership between Ohio State University and the Ohio Division of Wildlife. CLR-Z was supported by NSF grant #1649443 and thanks Dan Wetzel and Aimee Danley for lab support.

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