# NO SAFE SPACE: PREVALENCE AND DISTRIBUTION OF BATRACHOCHYTRIUM DENDROBATIDIS IN AMPHIBIANS IN A HIGHLY-PROTECTED LANDSCAPE

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Abstract.—Amphibian populations have experienced widespread and severe declines resulting from, in part, emerging pathogens. The Amphibian Chytrid Fungus, *Batrachochytrium dendrobatidis* (*Bd*), is a fungal pathogen implicated in the decline of numerous amphibian species. Human land use and elevation are factors that may affect the distribution of this pathogen and resultant chytrid-related declines. We assessed prevalence and pathogen load of *Bd* related to recreation intensity throughout the Adirondack State Park, New York, USA, a 2.4 million ha, protected wildland that experiences often intense hiking pressure. We collected DNA samples from amphibians during 90-min searches of natural cover objects at 43 public hiking trails. We found robust evidence that *Bd* is geographically widespread and prevalent in amphibians, particularly salamanders, at trailheads and along trails. Wood Frogs (*Lithobates sylvaticus*) and Eastern Newts (*Notophthalmus viridescens*) had the highest pathogen loads. Prevalence of *Bd* was not correlated with either elevation or recreation intensity, but was high in salamanders, which may serve as disease reservoirs and vectors in temperate forests and could additionally be adversely impacted by infection. Our results indicate amphibians are exposed to *Bd*, even in highly protected areas considered refugia, which has implications for conservation of amphibians and pathogen management in similar settings.

Key Words.--amphibian chytrid fungus; Adirondack Park; Bd; caudate; elevation; land use; recreation

#### INTRODUCTION

Amphibians are a diverse, well-distributed group of vertebrates that are suffering severe global declines (Stuart et al. 2004). Salamanders and anurans are important temperate forest and wetland predators, play a role in energy exchange and nutrient cycling and regulate invertebrate populations (Burton and Likens 1975; Davic and Welsh 2004; Capps et al. 2015). Approximately one third of the amphibian species worldwide are threatened (Gewin 2008), including nearly half of the species of salamander (International Union for Conservation of Nature [IUCN] 2017). Extinction rates for amphibians are estimated as high as 45,000 times greater than the background extinction rate (McCallum 2007). These declines are attributed to changes in land use, climate change, invasive species, and emerging diseases (Berger et al. 1998; Collins and Storfer 2003; Lips et al. 2006; McMenamin et al. 2008).

Chytridiomycosis is an amphibian fungal disease that has caused mass mortality events (Rachowicz et al. 2006) and is a threat to amphibian biodiversity (Berger et al. 1998). The causative agent of chytridiomycosis is the fungus, *Batrachochytrium dendrobatidis* (*Bd*; Amphibian Chytrid Fungus) that infects keratinized amphibian skin (Berger et al. 1998). *Bd* is known to infect > 350 species, including members from all orders of amphibians, and occurs on all continents with amphibian hosts (Fisher et al. 2009b; Churgin et al. 2013; Olson et al. 2013; Richards-Hrdlicka et al. 2013). Because of its broad host range and global distribution, *Bd* is an important biodiversity threat. *Bd* grows best at  $17-25^{\circ}$  C (Piotrowski et al. 2004) and can maintain infectivity in moist soil for up to 3 mo (Johnson and Speare 2005), making its persistence in the environment an additional risk factor.

Although research during the last decade has revealed much about the distribution and ecology of Bd, the mechanisms for local and regional dispersal of the pathogen are not well understood and may have complex interactions with the environment. Enigmatic amphibian declines, not related to habitat reduction or overexploitation, are positively correlated with elevation (Davidson et al. 2013), but it is not clear what drives this relationship. Sapsford et al. (2013) suggest that elevation represents an environmental gradient, likely related to temperature; however, Becker et al. (2012) propose that these declines are a result of disturbance to forest canopy, with low elevation, more disturbed sites having lower pathogen prevalence than high-elevation sites. The authors further suggest that disturbed habitat is typically warmer and drier than undisturbed habitat,

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**FIGURE 1.** Distribution and prevalence of *Batrachochytrium dendrobatidis* (*Bd*) throughout the Adirondack Park, New York, USA, 2015. Size of symbols indicate prevalence (proportion of *Bd* positive individuals 0-1.0) of *Bd* at a site (n = 43); elevation is shown in green (highest mountain peaks are white, valleys and water bodies are dark green), and the park boundary is shown in blue.

and that warmer, drier conditions may allow amphibians to clear *Bd* from their bodies (Becker et al. 2012). In evaluating changes in eight types of anthropogenic pressures, including extent of roads, built environments, and other disturbance measures, Venter et al. (2016) found 71% of the ecoregions in the world exhibit > 20% increases in the human footprint. Protected areas are thought to serve as refugia from development and disease pressures and are touted as solutions to amphibian conservation issues (Venter et al. 2016).

In addition to landscape factors, recreational land use may be a mechanism for landscape-scale disease transmission. Non-consumptive recreation can negatively impact local fauna (Liddle and Scoroe 1980; Steven et al. 2011; Burgin and Hardiman 2012). For example, Parent and Weatherhead (2000) noted that presence of human recreation altered the behavior of the Eastern Massasauga (*Sistrurus catenatus*). Although recreation-related disturbance can also impact species richness and distribution, the impact of recreation is not consistent between or within taxa (Francesco et al. 2006). Further, indirect transmission via deposition of contaminated soils via human activity could be an important mechanism in disease spread. Recreationists could be unknowingly transporting *Bd*-contaminated substrates between sites on equipment (e.g., boots, trekking poles, tires).

We studied the prevalence of Bd in terrestrial amphibians to examine the impact of recreation intensity on pathogen dynamics. To capture the potential relationship of recreation to Bd prevalence, we sampled amphibians for Bd throughout the 2.4 million ha park, which is a relatively understudied region (Becker et al. 2012). The forested, mountainous terrain of Adirondack Park provides an ideal site to examine the impact of both elevation and land use on Bd prevalence. This highly protected state park is the largest wildland in the contiguous United States and contains 85% of all wilderness in eastern North America (Scrafford 1990). The park consists of a mosaic of public and private land (approximately 50% of each type of ownership) and has strict laws that prohibit forest management on public land and govern development on private land. There is relatively little developed land; only 0.2% of the park is classified as industrial land and 7.2% as residential and commercial land (Adirondack Park Agency. 2014. Adirondack Park Land Use Classification Statistics. Available from https://www.apa.ny.gov/gis/stats/ colc201705.htm [Accessed 8 February 2018]); however, the park hosts an estimated 10 million visitors annually (Adirondack Council. 2017. State of the Park. Available from https://www.adirondackcouncil.org/page/ publications-20.html [Accessed 28 June 2018]) and visitation is not distributed equally across the park. The combination of high recreational traffic and minimal anthropogenic disturbance from development provides ideal conditions to examine the impact of recreation on pathogen prevalence.

We hypothesized that recreation, but not elevation, influences the impacts of Bd on amphibians. We predicted that recreation would have a positive relationship to pathogen prevalence and infection intensity and there would be no correlation between elevation and Bd pathogen dynamics. We expected Bd to be widely distributed throughout the Adirondack Park in New York, USA. An understanding of the pathogen dynamics of Bd across its host range and the mechanisms driving local pathogen prevalence and distribution would improve pathogen mitigation efforts and, ultimately, amphibian conservation and management.

#### MATERIALS AND METHODS

*Field methods.*—We surveyed public hiking trailheads in the Adirondack State Park for the presence of the amphibian chytrid fungus from 5 April to 21 June 2015 (Fig. 1). The Adirondack Park is dominated by temperate deciduous and conifer forest covers. Soils within the park tend to be acidic spodosols (https://

sdmdataaccess.sc.egov.usda.gov) and the climate of the region is typically cool and wet. The average spring and summer temperature (March-August) within the Adirondack Park is 12.2° C (SD = 1.3° C; http://prism. oregonstate.edu). All native amphibians (19 species; Gibbs et al. 2007) were expected at all surveyed sites and elevations. We used a 30-m digital elevation model (DEM) in ArcGIS (Esri, Redlands, California, USA) to designate survey sites into elevation classes of low (< 300 m), moderate (300–499 m), and high ( $\geq$  500 m). We classified recreation intensity, the number of hikers who signed into the trailhead registry during one year, into three classes: low (< 1,000), medium (1,000-4,999), and high ( $\geq$  5,000). We randomly selected five sites from each of the nine elevation-recreation treatment combinations from the Trail Registry Database, a set of public recreation trail heads managed by New York State Department of Environmental Conservation and compiled in 2013 (Abigail Larkin, pers. comm). The elevations ranged from 106-679 m (mean = 418.92; n = 43), and recreational intensity ranged from 48–25,821 hikers (mean = 3.815; n = 43). The low elevation-high recreation class contained only three possible survey sites.

We conducted 90-min amphibian surveys along the trail beginning at the trailhead registry, which included animal handling and processing time. We conducted surveys in the morning from 0600-1000 on dry days with no rain or wind. We searched natural cover objects (logs and rocks) and pools within 10 m of the trail for amphibians. We captured individual amphibians by hand or via dip net, and we changed gloves between handling of each individual. We collected DNA from each amphibian with sterile rayon swabs. We chose swabbing over other methods of detection because it provides the best DNA recovery and ensures minimal risk of sample contamination and effective logistics within the laboratory (Hyatt et al. 2007). We placed each captured amphibian in a separate, disposable plastic bag. We swabbed anurans five times across each femoral patch, 10 times across the ventral surface, and four times across each hind foot; we swabbed caudates 28 times across the ventral surface. We stored swabs in a cooler during sampling and transferred them to a -20° C freezer as soon as possible. We recorded elevation at the trailhead of each survey site. We cleaned equipment and boots of debris and decontaminated them between sites using a 10% bleach solution (Cashins et al. 2008).

*Laboratory analysis.*—We extracted DNA from swabs using Qiagen DNeasy blood and tissue kits (Qiagen, Venlo, Netherlands). We used quantitative PCR (qPCR) to assess pathogen presence and pathogen load. Using the ITS-1 Chytr3 and 5.8S Chytr primers developed by Boyle et al. (2004), we conducted SYBR Green quantitative PCR according to the protocols supplied by the manufacturer. The SYBR green assay was a less expensive but reliable alternative to TaqMan PCR, which allowed us to increase the number of sampled sites. We conducted quantitative PCR on a roto-gene qPCR (Qiagen, Venlo, Netherlands) machine using the default amplification setting for 50 cycles; reactions were prepared in triplicate. We set the high standard of 1,000 at 2 x 105 zoospores/mL and then diluted the remaining standards to a ratio of 1,000:100:10:1:0.1. We determined pathogen load by multiplying the output quantity by the dilution factor of each sample (5 µL of 1:10 dilution, with a volume of 40 µL). We re-ran incongruent samples twice in triplicate and discarded samples that did not show congruency following multiple runs. A negative control from a Green Frog (Lithobates clamitans) from another study was used in most runs.

Although we initially selected survey sites from a stratified list frame, we treated recreational intensity and elevation as continuous variables for the analyses. We used generalized linear models (family: binomial; link = logit) to analyze the effect of recreation and elevation on pathogen prevalence, using AIC to compare models (Burnham and Anderson 1998). We used the STATS package in base Program R (R Core Team 2017). We calculated AIC weights using R package MuMIn (Barton 2017).

#### RESULTS

We sampled 461 individuals of 11 amphibian species from 43 sites throughout the Adirondack Park (Table 1). Near public hiking trailheads, Bd is widely distributed and has high prevalence in the amphibian species surveyed (Fig. 1). Only one site was a nondetection for Bd (Appendix 1). The average prevalence across survey sites was 82% (SD = 27%, n = 43) and we documented Bd in 73% of individuals. Throughout Adirondack Park, we detected Bd in all species except Mink Frogs (Lithobates septentrionalis); the 10 species infected with Bd had a pathogen prevalence > 50%(Table 1). The mean pathogen load of Wood Frogs (Lithobates sylvaticus) was more than an order of magnitude higher than other species sampled (Table 1); however, no animals exhibited signs of illness from chytridiomycosis. The null model performed better than all other models, and as a result we did not identify a meaningful relationship between pathogen prevalence and either Julian day, elevation, or recreational intensity  $(\Delta AIC < 2, n = 43; Table 2)$ . Prevalence and pathogen load at low elevation sites were no greater than at high elevation sites in our study.

Service Norre	Common Norma			Mean	95% Confidence	Mean Pathogen	
Species Name	Common Name	Detections	n	Prevalence	Interval	Load	SD
Caudates							
Ambystoma maculatum	Spotted Salamander	3	4	0.75	0.30 - 0.95	93.58	260.72
Desmognathus spp.	Dusky spp.	47	65	0.72	0.60 - 0.82	657.83	1,905.6
Eurycea bislineata	Two-lined Salamander	23	25	0.92	0.75 - 0.98	58.79	134.77
Gyrinophilus porphyriticus	Spring Salamander	7	8	0.88	0.53 - 0.98	267.40	451.73
Notophthalmus viridescens (Adult)	Red-spotted Newt	11	11	1	0.74 - 1.00	48.52	62.85
Notophthalmus viridescens (Eft)	Red-spotted Newt	37	49	0.76	0.62 - 0.85	663.30	3,606.0
Plethodon cinereus	Red-backed Salamander	180	266	0.68	0.62 - 0.73	678.78	2,732.0
Anurans							
Lithobates catesbeiana	Bullfrog	2	2	1	0.34 - 1.00	1.18	0.00
Lithobates clamitans	Green Frog	7	9	0.78	0.45 - 0.94	20.50	18.31
Lithobates palustris	Pickerel Frog	4	8	0.50	0.22 - 0.78	96.76	67.65
Lithobates septentrionalis	Mink Frog	0	1	0	0.00 - 0.79	0.00	0.00
Lithobates sylvaticus	Wood Frog	8	9	0.89	0.57 - 0.98	7,050.24	14,314.3

**TABLE 1**. *Batrachochytrium dendrobatidis (Bd)* prevalence and pathogen load (+ SD) across amphibian species in Adirondack Park, New York, USA, 2015.

#### DISCUSSION

*Batrachochytrium dendrobatidis* is pervasive in the native amphibian population across Adirondack Park as we expected. The pathogen is widely distributed across the southern and eastern Adirondacks; in the northern region of the park the relationship is less clear, as there are relatively few public recreational trailheads. Our evidence that *Bd* is geographically widespread throughout the surveyed area suggests that the pathogen likely occurs broadly throughout publicly-accessible places across the entire park.

Prevalence of *Bd* in the Adirondack Park is greater for many species than reported in other studies in the northeastern U.S. (Becker et al. 2012; Lenker et al. 2014; Richards-Hrdlicka et al. 2013). High pathogen prevalence and wide distribution may indicate high environmental suitability for Bd in the Adirondack Park. Although Bd was widespread, no clinical signs of chytridiomycosis were documented in any amphibians, suggesting that Bd may exist at subclinical levels. Amphibians in the region may be able to cope with documented pathogen loads without incidence of disease. We did not note any Bd-related morbidity or mortality; however, such signs can be difficult to detect in caudates because of their secretive life histories (i.e., many species are largely fossorial, nocturnal, or cryptic).

Our data suggest that caudates, particularly Eastern Newts, may serve as a reservoir for *Bd* and perform as a vector in pathogen transmission. Juvenile (eft) and adult stage newts were common and widespread throughout the Adirondack Park and exhibited high pathogen prevalence (mean 0.76 and 1.00, respectively) in our study. Efts may migrate or move extensive distances over one or more years and are capable of traveling up to 100 m per day (Roe and Grayson 2008), which predisposes the eft to be a carrier of *Bd*. Lenker et al. (2014) found adult newts in central New York pools were one of three species driving prevalence of *Bd*, which supports our assertion that juvenile and adult newts may serve as a host for the pathogen.

The relatively high prevalence (means 0.72–0.92) of Bd occurrence in Two-lined Salamanders (Eurycea bislineata). Spring Salamanders (Gyrinophilus porphyriticus), and Dusky Salamanders (Desmognathus spp.) may be associated with their biphasic life histories. Each of these species is associated with forested streams and seeps and has a relatively small home range (Petranka 1998; Gibbs et al. 2007). Aquatic habitats embedded in a forested matrix potentially provide favorable microhabitats for the pathogen and could increase the risk for Bd exposure in these species (but see Kriger and Hero 2007; Greenberg et al. 2017). Our work demonstrates that terrestrial salamanders can carry high pathogen loads on par with amphibians in semipermanent and permanent wetlands.

The pathogen dynamics of Bd across the full range of potential hosts has not been adequately explored. Much of the current research centers on the ecology and impacts of Bd on anurans, while few studies have assessed the prevalence of Bd in caudates (Cheng et al. 2011; Richards-Hrdlicka et al. 2013; Muletz et al. 2014; Windstam and Olori 2014), and none have examined population impacts of chytridiomycosis on caudates.

**TABLE 2.** Akaike information criterion (AIC) values for generalized linear models assessing the relationship of *Batrachochytrium dendrobatidis* (*Bd*) pathogen prevalence to recreation intensity (Rec), elevation (Elev), and Julian day (Day), in Adirondack Park, New York, USA.

Model	K	AIC	ΔΑΙC	W <sub>i</sub>	-2loglikelihood
Null	1	37.41	0	0.339	35.408
Rec	2	38.64	1.23	0.183	34.643
Day	2	39.38	1.98	0.126	35.385
Elev	2	39.43	2.03	0.123	35.435
Day +Rec	3	40.58	3.17	0.069	34.581
Rec + Elev	3	40.71	3.30	0.065	34.709
Day + Elev	3	41.40	3.99	0.046	35.396
$\text{Rec} + \text{Elev} + \text{Rec}^*\text{Elev}$	4	42.25	4.84	0.011	34.250
Day + Rec + Elev	4	42.63	5.23	0.025	34.634
Day + Rec + Elev + Rec*Elev	5	44.21	6.80	0.011	34.207

Many northeastern terrestrial salamanders are abundant and long-lived (Petranka 1998), and some species of ephemeral wetland-breeding salamanders move up to 0.6 km from natal pools (Calhoun and deMaynadier 2004). Thus, caudates could serve as an important *Bd* reservoir, potentially allowing the pathogen to persist even after anuran hosts have become locally extirpated.

Environmental, land use, and temporal variables were not correlated with *Bd* prevalence. In our study area, average temperature exceeded the tolerance range of *Bd* only along the periphery of the park (http://prism. oregonstate.edu), in particular the Champlain Valley in the eastern region of the park. In the warmer, lower valleys and slopes of that region, amphibians may be able to better clear the pathogen than in cooler interior sites, although microclimate certainly influences that ability. That elevation did not affect pathogen prevalence across hiking trails in Adirondack Park lends support to the assertion of Becker et al. (2012) that elevation may serve as a proxy for disturbance regimes rather than environmental gradients.

Although Becker and Zamudio (2011) found that disease is driven by habitat destruction, which occurs more widely in lowlands, the Adirondack Park has minimal large-scale disturbance and we would not expect to see the same relationship. Our sites are on forested, public land that cannot be developed, and while parcel-based development does occur on private land, at a landscape scale the Adirondack region did not experience increases in the human footprint from 1993-2009 (Venter et al. 2016). Pristine habitats are generally at higher risk for Bd-related declines because of higher host species richness and more optimal fungal microclimates than in disturbed forest or open canopy sites (Becker and Zamudio 2011; Beyer et al. 2015), suggesting the Adirondack region is optimal for Bd. The impact of land use on pathogen distribution, prevalence, and pathogen load could be further investigated by

pairing high-resolution remote sensing data with field surveys.

We examined the effect of recreation on pathogen prevalence and, contrary to our hypothesis, found no evidence suggesting level of hiking intensity as measured impacts Bd prevalence across the landscape. Even the least-visited site, which had fewer than 50 visitors in a year (540 times fewer hikers than the most-visited site), had a prevalence rate of 0.60 (Appendix 1). Although our data are limited to publicly accessible sites, it is possible that Bd has been present in the region for some time. Batrachochytrium dendrobatidis globally infects introduced populations of the American Bullfrog, Lithobates catesbeiana (Garner et al. 2006). Bullfrogs are found throughout Adirondack Park and are a potential region-wide host for *Bd*. Alternately, it is possible that even very low levels of human activity on the landscape may spread Bd. We suspected hikers could pick up and redistribute contaminated substrate on equipment during their travels, but our work suggests recreation may not contribute appreciably to disease dynamics in this landscape. Other forms of recreation (i.e., fishing, all-terrain vehicle use, mountain biking) may have more significant effects on pathogen dynamics if they move quantities of sediment or zoospores across the landscape. Although human visitation can negatively impact fauna within protected areas (Sarmento and Berger 2017), it is unclear how recreation influences disease dynamics. We recommend careful study of controlled variables, such as number of users, to better mitigate and monitor Bd and other amphibian pathogens.

We documented no clinical infection or mortality in any amphibians, but our study was limited to public recreation sites and the incidence of chytridiomycosis throughout the Adirondack Park is unknown. Future studies should assess the impact of *Bd* on local amphibian populations, especially for caudates. Whereas we documented high prevalence and pathogen load in Wood Frogs, Crespi et al. (2015) found that *Bd* infections of Wood Frogs were rare and of low severity. This apparent contradiction warrants further investigation.

Given the widespread occurrence of Bd and other threats to amphibians, measures could be taken to safeguard populations if chytridiomycosis or novel diseases are identified in the future. First, genotyping Bd strains occurring within the region could provide insight on pathogen virulence (Berger et al. 2005; Fisher et al 2009a). Additionally, testing for the local strain(s) would enable more precise estimates in pathogen loads, because of variation among strains in genomic copies. Second, if amphibian species or populations at risk for disease are identified, managers could bolster populations to provide a buffer preventing local extinction (Scheele et al. 2014). For example, the Adirondack Mountains are the southern range limit of Mink Frogs, which may be at risk from climate change impacts (Popescu and Gibbs 2009); additionally, several salamanders and frogs are species of concern in the state (New York Natural Heritage Program. 2018. List of Endangered, Threatened and Special Concern Fish & Wildlife Species of New York State. Available from https://www.dec.ny.gov/animals/7494.html. [Accessed 13 February 2018]). Last and perhaps most germane in our study area, monitoring and surveillance can provide important information regarding pathogen distribution, incidence of disease, and detection of emerging diseases such as Batrachochytrium salamandrivorans, which particularly threatens caudates (Martel et al. 2013).

Although there are many challenges to a proactive management strategy, reactive management of Bd in other regions has proven largely ineffective. Decision analysis could prove to be an important tool to help resource managers and policymakers enact meaningful and effective management to protect amphibian diversity (Grant et al. 2017). It is clear from our research that even highly protected landscapes are at risk for Bd establishment, and surveillance should be considered as an important tool in understanding disease dynamics and distribution. Gray et al. (2017) cover key considerations in designing surveillance programs and provide an overview on potential intervention strategies to prevent entry, transmission, or decrease host stress. Strategies include habitat management to decrease host density, disruption of disease transmission pathways, or mitigation of environmental stressors by providing microsites for basking and pathogen-clearing (Becker et al. 2012). We recommend continuous monitoring of terrestrial amphibian populations for pathogens and advocate planning for future conservation needs of all native fauna.

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#### LITERATURE CITED

- Barton, K. 2017. MuMIn: Multi-Model Inference. R package version 1.40.0. https://CRAN.R-project.org/ package=MuMIn.
- Becker, C.G., and K.R. Zamudio. 2011. Tropical amphibian populations experience higher disease risk in natural habitats. Proceedings of the National Academy of Sciences of the United States of America 108:9893–9898.
- Becker, C.G., D. Rodriguez, A.V. Longo, A.L. Talaba, and K.R. Zamudio. 2012. Disease risk in temperate amphibian populations is higher at closed–canopy sites. PLoS ONE 7, 1-7. https://doi.org/10.1371/ journal.pone.0048205.
- Berger, L., G. Marantelli, L.F. Skerratt, and R. Speare. 2005. Virulence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* varies with the strain. Diseases of Aquatic Organisms 68:47–50.
- Berger, L., R. Speare, P. Daszak, D.E. Green, A.A. Cunningham, C.L. Goggin, R. Slocombe, M.A. Ragan, A.D. Hyatt, K.R. McDonald, et al. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences of the United States of America 95:9031–9036.
- Beyer, S.E., C.A. Phillips, and R.L. Schooley. 2015. Canopy cover and drought influence the landscape epidemiology of an amphibian chytrid fungus. Ecosphere 6:1–18.
- Boyle, D.G., D.B. Boyle, V. Olsen, J.A.T. Morgan, and A.D. Hyatt. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*)

in amphibian samples using real-time Taqman PCR assay. Diseases of Aquatic Organisms 60:141–148.

- Burgin, S., and N. Hardiman. 2012. Is the evolving sport of mountain biking compatible with fauna conservation in national parks? Australian Zoology 36:201–208.
- Burnham, K.B., and D.R. Anderson. 1998. Model Selection and Multimodel Inference. 2<sup>nd</sup> Edition. Springer, Fort Collins, Colorado, USA.
- Burton, T.M., and G.E. Likens. 1975. Energy flow and nutrient cycling in salamander populations in the Hubbard Brook Experimental Forest, New Hampshire. Ecology 56:1068–1080.
- Calhoun, A.J.K., and P.G. deMaynadier. 2004. Forestry habitat management guidelines for vernal pool wildlife. MCA Technical Paper No. 6. Metropolitan Conservation Alliance, Wildlife Conservation Society, Bronx, New York, USA. 32 p.
- Capps, K.A., K.A. Berven, and S.D. Tiegs. 2015. Modelling nutrient transport and transformation by pool-breeding amphibians in forested landscapes using a 21-year dataset. Freshwater Biology 60:500– 511.
- Cashins, S.D., L.F. Skerratt, and R.A. Alford. 2008. Sodium hypochlorite denatures the DNA of the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. Disease of Aquatic Organisms 80:63– 67.
- Cheng, T.L., S.M. Rovito, D.B. Wake, and 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 of the United States of America 108:9502–9507.
- Churgin, S.M., B.L. Raphael, J.B. Pramuk, J.G. Trupkiewicz, and G. West. 2013. *Batrachochytrium dendrobatidis* in aquatic caecilians (*Typhlonectes natans*): a series of cases from two institutions. Journal of Zoo and Wildlife Medicine 44:1002–1009.
- Collins, J.P., and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. Diversity and Distributions 9:89–98.
- Davic, R.D., and H.H. Welsh. 2004. On the ecological roles of salamanders. Annual Review of Ecology, Evolution, and Systematics 35:405–434.
- Davidson, C., C. Williamson, K. Vincent, S. Simonich, K. Yip, J.M. Hero, and K. Kriger. 2013. Anuran population declines occur on an elevational gradient in the Western Hemisphere. Herpetological Conservation and Biology 8:503–518.
- Fisher, M.C., J. Bosch, Z. Yin, D. A. Stead, J. Walker, L. Selway, A.J.P. Brown, L.A. Walker, N.A.R. Gow, J.E. Stajich, et al. 2009a. Proteomic and phenotypic profiling of the amphibian pathogen

*Batrachochytrium dendrobatidis* shows that genotype is linked to virulence. Molecular Ecology 18:415–429.

- Fisher, M.C., T.W.J. Garner, and S.F. Walker. 2009b. Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. Annual Review of Microbiology 63:291–310.
- Francesco, G.F., R. Sacchi, S. Scali, A. Gentilli, F. De Bernardi, and P. Galeotti. 2006. Vertebrates respond differently to human disturbance: implications for the use of a focal species approach. Acta Oecologica 31:109–118.
- Garner, T.W.J., M.W. Perkins, P. Govindarajulu, D. Seglie, S. Walker, A.A. Cunningham, and M.C. Fisher. 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American Bullfrog, *Rana catesbeiana*. Biology Letters 2:455–459.
- Gewin, V. 2008. Riders of a modern-day ark. PLoS Biology 6(1): e24. http://doi:10.1371/journal. pbio.0060024.
- Gibbs, J.P., A.R. Breisch, P.K. Ducey, G. Johnson, J.L. Behler, and R.C. Bothner. 2007. The Amphibians and Reptiles of New York State. Oxford University Press, Inc., New York, New York, USA.
- Gray, M.J., A.L.J. Duffus, K.H. Haman, R.N. Harris, M.C. Allender, T.A. Thompson, M.R. Christman, A. Sacerdote-Velat, L.A. Sprague, J.M. Williams, et al. 2017. Pathogen surveillance in herpetofaunal populations: guidance on study design, sample collection, biosecurity, and intervention strategies. Herpetological Review 48:334–351.
- Grant, E.H.C., E. Muths, R.A. Katz, S. Canessa, M.J. Adams, J.R. Ballard, L. Berger, C.J. Briggs, J.T. Coleman, M.J. Gray, et al. 2017. Using decision analysis to support proactive management of emerging infectious wildlife diseases. Frontiers in Ecology and the Environment 15:214–221.
- Greenberg, D. A., W.J. Palen, and A.Ø. Mooers. 2017. Amphibian species traits, evolutionary history and environment predict *Batrachochytrium dendrobatidis* infection patterns, but not extinction risk. Evolutionary Applications 10:1130–1145.
- Hyatt, A.D., D.G. Boyle, V. Olsen, D.B. Boyle, L. Berger, D. Obendorf, A. Dalton, K. Kriger, M. Hero, H. Hines, et al. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 73:175–192.
- International Union for Conservation of Nature (IUCN) 2017. IUCN Red List of Threatened Species. http://www.iucnredlist.org.
- Johnson, M.L., and R. Speare. 2005. Possible modes of dissemination of the amphibian chytrid

*Batrachochytrium dendrobatidis* in the environment. Diseases of Aquatic Organisms 65:181–186.

- Kriger, K.M., and J.M. Hero. 2007. The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. Diversity and Distributions 13:781–788.
- Lenker, M.A., A.E. Savage, C.G. Becker, D. Rodriguez, and K.R. Zamudio. 2014. *Batrachochytrium dendrobatidis* infection dynamics vary seasonally in upstate New York, USA. Diseases of Aquatic Organisms 111:51–60.
- Liddle, M.J., and H.R.A. Scoroe. 1980. The effects of recreation on freshwater plants and animals: a review. Biological Conservation 17:183–206.
- Lips, K.R., F. Brem, R. Brenes, J.D. Reeve, R.A. Alford, J. Voyles, C. Carey, L. Livo, A.P. Pessier, and J.P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences of the United States of America 103:3165– 3170.
- Martel, A., A. Spitzen-van der Sluijs, M. Blooi, W. Bert, R. Ducatelle, M.C. Fisher, A. Woeltjes, W. Bosman, K. Chiers, F. Bossuyt, et al. 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. Proceedings of the National Academy of Sciences of the United States of America 110:15325–15329.
- McCallum, M.L. 2007. Amphibian decline or extinction? Current declines dwarf background extinction rate. Journal of Herpetology 41:483–491.
- McMenamin, S.K., E.A. Hadly, and C.K. Wright. 2008. Climatic change and wetland desiccation cause amphibian decline in Yellowstone National Park. Proceedings of the National Academy of Sciences of the United States of America 105:16988–16993.
- Muletz, C., N.M. Caruso, R.C. Fleischer, R.W. McDiarmid, and K.R. Lips. 2014. Unexpected rarity of the pathogen *Batrachochytrium dendrobatidis* in Appalachian *Plethodon* salamanders: 1957–2011. PLoS ONE 9, 1–7. https://doi.org/10.1371/journal. pone.0103728.
- Olson, D.H., D.M. Aanensen, K.L. Ronnenberg, C.I. Powell, S.F. Walker, J. Bielby, T.W.J. Garner, G. Weaver, and M.C. Fisher. 2013. Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. PLoS ONE 8, 1-13. https://doi.org/10.1371/journal.pone.0056802.
- Parent, C., and J. Weatherhead. 2000. Behavioral and life history responses of Eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*) to human disturbance. Oecologia 125:170–178.
- Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institute Press, Washington, D.C., USA.

- Piotrowski, J.S., S.L. Annis, and J.E. Longcore. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycological Society of America 96:9–15.
- Popescu, V.D., and J.P. Gibbs. 2009. Interactions between climate, beaver activity, and pond occupancy by the cold-adapted Mink Frog in New York State, USA. Biological Conservation 142:2059–2068.
- R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project. org.
- Rachowicz, L.J., R.A. Knapp, J.A.T. Morgan, M.J. Stice, V.T. Vendenburg, J.M. Parker, and C.J. Briggs. 2006. Emerging infectious disease as a proximate cause of amphibian mass mortality. Ecology 87:1671–1683.
- Richards-Hrdlicka, K.L., J.L. Richardson, and L. Mohabir. 2013. First survey for the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in Connecticut (USA) finds widespread prevalence. Diseases of Aquatic Organisms 102:169–180.
- Roe, A.W., and K.L. Grayson. 2008. Terrestrial movements and habitat use of juvenile and emigrating adult Eastern Red-Spotted Newts, *Notophthalmus viridescens*. Journal of Herpetology 42:22–30.
- Sapsford, S.J., R.A. Alford, and L. Schwarzkopf. 2013. Elevation, temperature, and aquatic connectivity all influence the infection dynamics of the amphibian chytrid fungus in adult frogs. PloS ONE 8:e82425. https://doi.org/10.1371/journal.pone.0082425.
- Sarmento, W.M., and J. Berger. 2017. Human visitation limits the utility of protected areas as ecological baselines. Biological Conservation 212:316–326.
- Scrafford, C.W. 1990. The Adirondack Park State Land master plan origins and current status. The Adirondack Park in the Twenty-First Century, technical reports, vol. 1, Commission on the Adirondacks in the Twenty-First Century, State of New York, Albany, New York, USA.
- Scheele, B.C., D. A. Hunter, L.F. Grogan, L. Berger, J.E. Kolby, M.S. McFadden, G. Marantelli, L.F. Skerratt, and D.A. Driscoll. 2014. Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. Conservation Biology 28:1195–1205.
- Steven, R., C. Pickering, and J.G. Castley. 2011. A review of the impacts of nature based recreation on birds. Journal of Environmental Management 92:2287–2294.
- Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, S.L. Ana, D.L. Fischman, R.W. Waller, S. Science, N. Series, and M. Planum. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.
- Venter, O., E.W. Sanderson, A. Magrach, J.R. Allan, J. Beher, K.R. Jones, M. Fekete, M.A. Levy, H.P.

Possingham, W.F. Laurance, et al. 2016. Sixteen years of change in the global terrestrial human footprint and implications for biodiversity conservation. Nature Communications 7: 12558–12569.

Windstam, S.T., and J.C. Olori. 2014. Proportion of hosts carrying *Batrachochytrium dendrobatidis*, causal agent of amphibian chytridiomycosis, in Oswego County, NY in 2012. Northeastern Naturalist 21:25–35.



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## Robinson et al.—Prevalence and distribution of *Bd* in temperate amphibians.

Site Name	Site Code	Visitors	Elevation (m)	Positive	n	Prevalence	95% Confidence Interval
Bear Mountain	BEAR	3,413	458	15	15	1.00	0.08-1.00
Beaver Brook Tract	BEAV	1,003	292	8	8	1.00	0.68-1.00
Bennett Lake Trail	BENN	910	284	15	19	0.79	0.57-0.91
Black Mt.	BLAC	4,164	443	15	16	0.94	0.72-0.99
Bloody Pond	BLOO	313	287	8	8	1.00	0.68-1.00
Blue Hill Trail	BLUE	728	282	9	16	0.56	0.33-0.77
Buck Mt	BUCK	2,238	364	1	10	0.10	0.02-0.40
Pilot Knob Trail	BUMT	9,487	106	12	13	0.92	0.67-0.99
Clay Meadow	CLAY	3,760	120	8	8	1.00	0.68-1.00
Clockmill Corners	CLOC	168	557	10	10	1.00	0.72-1.00
Cod Pond	CODD	164	422	9	9	1.00	0.70-1.00
Crow Mt	CROW	2,228	679	8	8	1.00	0.68-1.00
Dacy Clearing	DACY	8,453	417	8	12	0.67	0.39-0.86
Elk Lake	ELKK	325	619	9	9	1.00	0.70-1.00
Giant Mountain	GIAN	7,274	387	4	8	0.50	0.22-0.78
Goose Pond	GOOS	1,047	329	8	8	1.00	0.68-1.00
Gore Mt.	GORE	645	319	7	8	0.88	0.53-0.98
Hadley Mt.	HADL	9,899	353	6	18	0.33	0.16-0.56
Hurrel Vly Trail	HURR	153	548	14	18	0.78	0.55-0.91
Indian Pass	INDI	7,469	663	8	8	1.00	0.68-1.00
Jay Mountain	JAYY	864	454	11	11	1.00	0.74-1.00
Jockeybush Lake Trail	JOCK	1,110	539	11	11	1.00	0.74-1.00
John's Brook Trail	JOHN	14,144	469	8	8	1.00	0.68-1.00
Kane Mt.	KANE	4,893	492	0	13	0.00	0.00-0.23
King's Flow Trail	KING	6,131	528	11	11	1.00	0.74-1.00
Lampson Falls	LAMP	5,698	247	6	6	1.00	0.61-1.00
Moose Mt. Pond	MOOS	417	279	5	8	0.63	0.31-0.86
Mount Severance	MTSE	3,265	262	7	8	0.88	0.53-0.98
Nine Corners	NINE	5,490	490	12	12	1.00	0.76-1.00
Panther Mt.	PAMT	7,154	527	9	25	0.36	0.20-0.55
Panther Pond	PANT	232	455	9	9	1.00	0.70-1.00
Pharaoh Lake	PHAR	2,170	286	11	11	1.00	0.74-1.00
Pillsbury Mt.	PILL	1,674	658	11	11	1.00	0.74-1.00
Prospect Mt.	PROS	6,082	182	12	15	0.80	0.55-0.93
Raymond Brook	RAYY	279	323	6	6	1.00	0.61-1.00
Rondaxe Fire Tower	ROND	25,821	599	12	15	0.80	0.55-0.93
Shelving Rock Trail	SHEL	2,882	150	10	14	0.71	0.45-0.88
Snowy Mt.	SNOW	3,304	551	2	8	0.25	0.07-0.59
Tubmill Marsh Trail	TUBM	702	287	10	10	1.00	0.72-1.00
Twin Lakes	TWIN	48	512	6	10	0.60	0.31-0.83
Upper Works Trail	UPPE	5,726	549	7	8	0.88	0.53-0.98
Wakely Mt.	WAKE	1,193	648	8	8	1.00	0.68-1.00
Wilson Pond	WILS	943	564	5	8	0.63	0 31-0 86

APPENDIX	<ol> <li>Pathogen</li> </ol>	prevalence at	t recreational	trailheads	throughout	the Adirond	lack Park	. New	York.	USA.	in 201	5.
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