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Low Prevalence of the Amphibian Pathogen *Batrachochytrium dendrobatidis* in the Southern Appalachian Mountains

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Abstract.—**Global population and species-level amphibian declines are attributable to multiple environmental and biological factors including the disease chytridiomycosis caused by the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd). In North America, chytridiomycosis-mediated declines may be severe, but the occurrence of Bd is also patchy. The Southern Appalachian Mountains are a global hotspot for salamander diversity, yet relatively few surveys have focused on the prevalence of Bd in salamanders. From 2008 to 2013, we collected 668 swabs from 603 individual amphibians (some were captured and swabbed twice) of 43 species (seven Anura and 36 Caudata) from the Southeastern Piedmont and Southern Appalachian Mountains in western North Carolina and northeastern Tennessee. We used replicate PCR-assays and found that Bd was present but extremely uncommon (1.00%) in salamanders of the region and was not detected at all in the four anuran taxa sampled. We detected six Bd-positive salamanders, including five Spotted Salamanders (*Ambystoma maculatum*; 10% of individuals sampled) from Watauga County, North Carolina, and one Green Salamander (*Aneides aeneus*; 7.7% of individuals sampled) from Transylvania County, North Carolina. Collectively, our data suggest that Bd is very uncommon in this salamander hotspot. Thus, Bd may not be a cause of current and future declines in this region. These data serve as an important baseline for future studies of amphibian abundance, distribution, and assemblage structure in this region.**

Key Words.—*Ambystomatidae*; *Ambystoma maculatum*; *Aneides aeneus*; Chytrid; North Carolina; Plethodontidae

Introduction

Amphibians worldwide are in a state of rapid decline. Of the > 6,000 described species, 32.5% are considered at risk of extinction (Stuart et al. 2004; Beebee and Griffiths 2005; Smith et al. 2009). Currently, there are many plausible hypotheses to explain amphibian declines, including habitat destruction, chemical pollution, UV-B irradiation, introduced species, over-exploitation, and climate change (reviewed in Beebee and Griffiths 2005). Infectious disease is one factor that is currently threatening several amphibian species (Daszak et al. 2003; Skerratt et al. 2007).

Most notably, the disease chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis* (Bd; Phylum Chytridiomycota, Class Chytridiomycetes, Order Rhyzophydiales) and has been clearly linked to several well documented population declines (Berger et al. 1998; Daszak et al. 1999; Lips et al. 2003; Seimon et al. 2007; Murray et al. 2011).

In the last few decades, researchers have used epidemiological, pathological, and experimen-

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tal evidence to link chytridiomycosis to the decline of numerous amphibian taxa (Briggs et al. 2005; Lips et al. 2006; Voyles et al. 2009; Crawford et al. 2010). The degree to which infected animals exhibit symptoms of chytridiomycosis (e.g., loss of righting reflex, irregularly sloughing skin) is likely dependent upon a combination of environmental and biological factors including host species, host condition, and the abundance and distribution of Bd on the host (Bustamante et al. 2010; Searle et al. 2011). Bd is currently present in amphibian populations on six continents (Berger et al. 1998; Lips 1999; Garner et al. 2005; Goldberg et al. 2007; Rothermel et al. 2008; Yang et al. 2009) and occurs in a variety of amphibians in southeastern North America (Rothermel et al. 2008; Hossack et al. 2010; Chatfield et al. 2012; Souza et al. 2012). In the Southern Appalachian region, Bd prevalence across amphibian populations appears to be relatively low; however, surveys are sparse and further sampling efforts are needed (Keitzer et al. 2011; Caruso and Lips 2013; Rollins et al. 2013, Muletz et al. 2014).

The Appalachian Mountains are a region characterized by high salamander diversity and endemism (Kiester 1971; Petranka 1998; Green et al. 2013). In an ecological niche modeling study, Ron (2005) identified the Southern Appalachian region as a suitable area for Bd based on temperature and precipitation regimes. Chytridiomycosis outbreaks are primarily associated with cool temperatures, high moisture levels, and high elevations, and these conditions characterize many habitats in this region (Young et al. 2001; Drew et al. 2006). Also, few studies have directly targeted salamanders, and as a result the effects of chytridiomycosis and the distribution of Bd are not well-understood in salamander populations (but see Rothermel et al. 2008; Chinnadurai et al. 2009; Keitzer et al. 2011; Caruso and Lips 2013; Muletz et al. 2014). Moreover, recent studies suggest that salamanders in this region have undergone largely unexplained abundance, assemblage, and range shifts (Highton 2005; Caruso and Lips 2013; Moskwik 2014).

Here we present the results of a study examining the prevalence of Bd in amphibian populations in the Piedmont and Highland regions of the Southern Appalachian Mountains of North Carolina and Tennessee. Using a variety of survey techniques, we collected skin swabs of caudate and anuran species and used PCR to test for the presence of Bd. We expected that aquatic amphibian species and those at higher elevations were more likely to test positive for Bd (Kriger and Hero 2007).

Materials and Methods

Sample collection.—Using the sample collection protocol outlined in Kriger and Hero (2006), we swabbed 668 amphibians using sterile, individually wrapped, cotton-tipped swabs. We swabbed each animal 10 times making sure to cover the following body regions: dorsal sides and ventral surfaces, along legs, and between toes. All samples were transferred to a 1.5 mL microcentrifuge tube and were stored on ice and then at -20 °C.

We collected skin swabs from amphibian populations in western North Carolina, and northeastern Tennessee (Fig. 1) from 2008–2013 using a variety of surveying techniques. Except for the specific sampling methods below, LAW collected Bd samples opportunistically either personally or acquired them from other North Carolina amphibian researchers. DJM collected 306 terrestrial salamander samples (*Plethodon, Desmognathus*, *Eurycea* spp.) during cover-board surveys on Grandfather Mountain (Avery County, North Carolina) from July to October 2010 and 2011. Grandfather Mountain Biosphere Reserve is a protected, relatively pristine, high elevation (1,812 m) mountain in western North Carolina. Approximately one time per week, we conducted cover board surveys during daytime hours, and approximately one to two times per month, we conducted time-constrained night searches adjacent to each cover board plot. We used five, 100

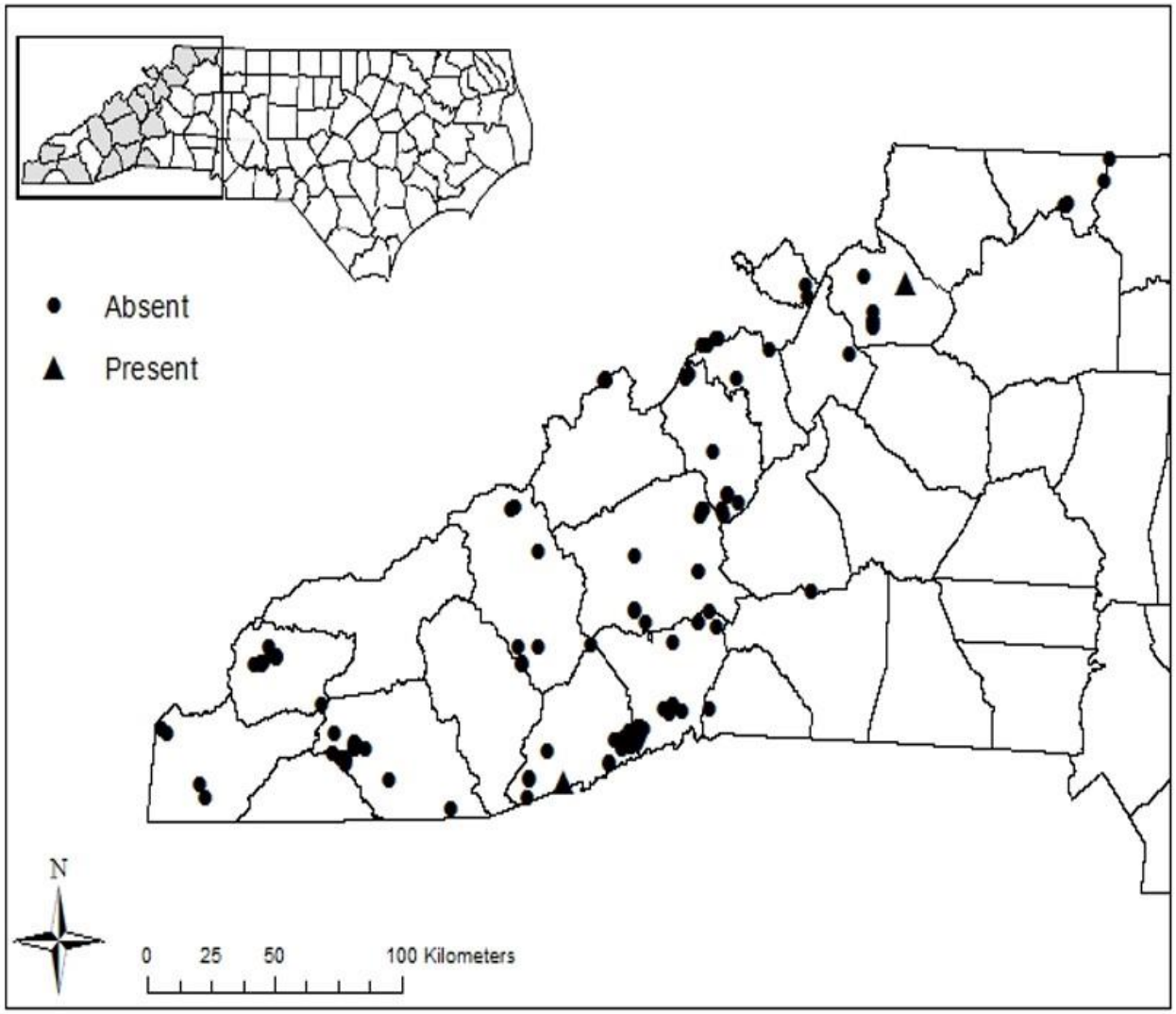

Figure 1. Map of sites where amphibians were swabbed for *Batrachochytrium dendrobatidis* (Bd) assays in western North Carolina and eastern Tennessee. Triangles denote sites where amphibians tested positive for Bd and ellipses are sites where Bd was not detected. Inset map shows area of detail in western North Carolina and eastern Tennessee.

m long transects on the south side of Grandfather Mountain along an elevation gradient (1533 m, 1445 m, 1356 m, 1311 m, and 1259 m). Each transect consisted of five coverboard (30 cm × 30 cm × 5 cm) plots equidistant along each 10 m × 10 m transect. There were nine boards within the boundaries of each plot for a total of 45 boards per transect (see Moffitt 2012). We conducted night surveys within 10 m × 2 m plots, with one plot placed adjacent to each cover board plot. We began surveys at dusk and continued until each plot had been searched for five minutes. When we encountered a salamander, we placed it into a clean, unused, sealable plastic bag. We identified salamanders to species, collected a skin swab, and individually marked each with Visible Implant Elastomer (VIE). Some animals (n = 66) were captured and swabbed two times during the season.

In October 2011, DJM and MWP opportunistically captured 31 Yonahlossee Salamanders (*Plethodon yonahlossee*) during night surveys (Avery County, North Carolina). In March 2013, during a breeding-migration event along Meat Camp Creek (Watauga County, North Carolina), MWP captured 50 Spotted Salamanders. During the summers of 2011–2013, MWP collected 31 skin swabs from Eastern Hellbenders (*Cryptobranchus alleganiensis*) during snorkel surveys in the Watauga and French Broad River drainages (Watauga and Mitchell Counties, North Carolina and Carter County, Tennessee). We captured hellbenders by hand or dip nets and placed in clean mesh bags. We washed all mesh bags, nets, and the gear of researchers in a bleach solution and air-dried prior to re-use. We individually marked hellbenders with Passive Integrated Transponder (PIT) tags, but no animals were captured and swabbed multiple times.

PCR assays.—We extracted DNA from each sample using a Qiagen DNeasy kit. We used protocols outlined in the Qiagen Blood and Tissue Handbook for extractions. Extracted samples were stored at -20 °C prior to analysis. To assess presence/absence of Bd, we conducted PCR assays using Bd primers developed by Annis et al. (2004): Bd_1a (5'-CAGTGTGCCATATGTCACG-3') and Bd$_{2a}$ (5'-CATGGTTCATATCTGTCCAG-3'). Amplification mixtures included 1 μL of each primer, 6.25 μL of GoTaq Green Master Mix (Promega, Madison, Wisconsin, USA), 2.75 μL DI water, and 1 L of extracted DNA. We also included standard positive and negative controls for Bd. Positive Bd control samples were supplied by Joyce Longcore (1 μL extracted Bd DNA per 200 μL of sample mix). We also re-tested extracted DNA from animals that had previously tested positive for the presence of Bd to ensure consistency of the protocol.

We used a BioRad thermocycler (Hercules, California, USA) to perform amplification by initial denaturation 95 °C for 5 min, followed by 44 cycles of 93 °C for 45 s. Annealing temperature was set to 53.7 °C for 45 s and then the samples were incubated at 72 °C for 10 min before extension at 15 °C to complete the amplification. All samples were run on an agarose gel with ethidium bromide to help visualize the bands. We ran the PCR and electrophoresis in triplicate for each sample.

To ensure that we were able to detect the presence of Bd in an environmental sample, we swabbed fire-bellied toads (*Bombina* spp.) that were housed for sale in the pet trade. Four of seven samples produced positive Bd results and were then sent for quantitative analysis using qPCR to the Kerby Lab at the University of South Dakota to confirm results. The four samples ranged in the number of zoospores/μL from 0.005 zoospores/μL to 0.20 zoospores/μL. We calculated 95% confidence internals (CI) for our prevalence estimates. We used the number of positive individuals, the total number of individuals sampled, α = 0.05, and the Wilson score interval for the CI estimate (Agresti and Coull 1998) using the package PropCIs in R (R Core Development Team 2013).

Results

We tested 668 swabs taken from 603 individuals representing 43 amphibian species (seven anuran and 36 caudate taxa) from 17 counties in western North Carolina and eastern Tennessee (Appendix Table, Fig. 1). Bd was present but extremely uncommon (1.0%) in the salamanders and was not detected in the four anuran taxa sampled (Appendix Table). Of these, we collected 306 skins swabs from 241 plethodontid salamanders along five elevations on Grandfather Mountain. Of the 306 swabs, we sampled 175 Northern Gray-cheeked Salamander (*Plethodon montanus*; 50 were swabbed twice), 23 Eastern Red-backed Salamander (*P. cinereus*; four were swabbed twice), 19 Weller's Salamander (*P. welleri*; three were swabbed twice), 14 Blue Ridge Dusky Salamander (*Desmognathus orestes*; six were swabbed twice), five Northern Pigmy Salamander (*D. organi*; one was swabbed twice), four White-spotted Slimy Salamanders (*P. cylindraceus*; one was swabbed twice), and one Blue Ridge Two-lined Salamander (*Eurycea wilderae*). We detected Bd in five Spotted Salamander individuals (about 10% of individuals sampled from the Meat Camp Creek breeding population in Watauga County, North Carolina) and one Green Salamander (7.7% of those sampled from Transylvania County, North Carolina). We did not observe any symptoms of chytridiomycosis (loss of righting reflex, irregularly sloughing skin) in any infected or non-infected individuals.

Discussion

Bd was not present in the majority of amphibians sampled; of the 668 swabs taken from 603 animals, only five Spotted Salamanders (all from the same breeding population) and one Green Salamander tested positive for Bd, and none of these animals showed signs of chytridiomycosis. We are confident that we did not fail to detect Bd because of our rigorous methods, and the positive animals we tested in the pet trade confirmed that we were indeed able to detect Bd in animals with low densities of zoospores. It is possible that prevalence of Bd in salamander populations in the Southern Appalachian and Piedmont regions is so low that our sampling possibly failed to include infected individuals. Certainly, at many of our sampling locations, the number of individuals tested per species was quite low and thus we lack the power to suggest absence of Bd. Nonetheless, our study provides additional data to the limited number of previous studies that have systematically sampled terrestrial salamander assemblages in the Southern Appalachians (Rothermel et al. 2008; Keitzer et al. 2011; Caruso and Lips 2013; Muletz et al. 2014).

We also repeatedly sampled Grandfather Mountain plethodontid salamanders during coverboard surveys along transects on an elevation gradient (1259–1533 m) to determine whether timing or elevation influenced the prevalence of Bd. However, because no Grandfather Mountain plethodontids were positive, we were unable to address this question. This low incidence of Bd among plethodontid salamanders corroborates the data reviewed by Muletz et al. (2014) demonstrating only 15 Bd positive individuals of 1,230 sampled in the eastern US.

A review of the literature (Rollins et al. 2013) indicates that 10 anuran and 10 caudate species have tested positive for Bd from Southern Appalachian states (Virginia, West Virginia, North Carolina, South Carolina, Tennessee, Kentucky, Georgia, Alabama), including anurans from the genera *Acris*, *Anaxyrus*, *Lithobates*, and *Pseudacris*, and caudates from the genera *Desmognathus*, *Eurycea*, *Notophthalmus*, *Plethodon*, and *Pseudotriton*. Our study adds two additional caudate species to the list: Spotted Salamanders and Green Salamanders. Of the five localities in the Southern Appalachian states that have reported Bd-related mortality events, all are pond breeding species Eastern Newt (*Notophthalmus viridescens*), chorus frogs (*Pseudacris* spp.), and true frogs (*Lithobates* spp.) that were sampled within

or near mountainous areas (reviewed in Rollins et al. 2013).

Because Bd is a largely aquatic fungus and because aquatic environments typically experience lower thermal extremes relative to terrestrial environments, Bd will more readily infect aquatic or semi-aquatic amphibians (Weldon et al. 2004; Kriger and Hero 2007; Chatfield et al. 2012). Indeed, the impact of Bd on semi-aquatic frogs is well-documented, and fully aquatic frogs and salamanders may be serving as important vectors of Bd (Garner et al. 2005; Chatfield et al. 2012). Although they apparently are less likely to carry Bd than anurans (Rothermel et al. 2008; Timpe et al. 2008), we detected Bd only in salamanders. Our data suggesting low prevalence of Bd support six other studies that have sampled headwater- or seep-associated amphibians in the US where Bd has been detected in only 3% of 1,322 individuals from 21 species (reviewed in Hossack et al. 2010). Five of six Bd-positive animals came from the same breeding pond, and Bd could occur across the landscape in pathogen hotspots. Future research should investigate how local microclimate, topography, hydrology, or anthropogenic land use attributes influence the distribution of Bd in our study sites, and whether outbreaks have a centralized origin.

In contrast to Spotted Salamanders, Green Salamanders are terrestrial breeders and inhabit crevices in relatively mesic to xeric rock outcroppings in the Southern Appalachian Mountains at elevations from 500–1300 m (Petranka 1998; Corser 2001). Two disjunct populations occur in western North Carolina, and the species is listed as endangered by the North Carolina Wildlife Resources Commission (NCWRC 2005). Long-term monitoring of seven Green Salamander populations in western North Carolina revealed a 98% decline in relative abundance between 1971 and 1990 (Corser 2001). Habitat loss and changing climate were believed to be the primary stressors to this population, but Bd has been heretofore detected in three of nine individuals tested in this species (Lori Williams, unpubl. data). Thus, our data suggest that future research should focus on testing the hypothesis that Bd could be a factor in the declines of Green Salamander populations.

Unlike many of the other salamanders sampled, Eastern Hellbenders are entirely aquatic and occupy large, permanently-flowing streams (Petranka 1998). Bd has been detected in numerous fully-aquatic salamander species in the genera *Amphiuma*, *Necturus*, *Pseudobranchus*, and *Siren* sampled in Florida, Mississippi, and Louisiana (Chatfield et al. 2012). Moreover, because Bd has been reported from hellbender populations in Arkansas, Missouri, Indiana, North Carolina, New York, Ohio, Pennsylvania, Virginia (Briggler et al. 2008; Bodinof et al. 2011; Burgmeier et al. 2011; Williams and Groves 2014; Bales et al. 2015), we expected some hellbenders to be Bd-positive. Many of our Eastern Hellbender sampling sites were located in the headwaters of the Watauga Drainage and although a previous study detected Bd in hellbenders of the upper Watauga drainage (Williams and Groves 2014), it is still unknown what role land use, habitat quality, and stream order might play in limiting Bd exposure in this high-quality watershed (Pugh 2013).

To date, amphibians occurring in the Southern Appalachian Highlands of North Carolina and Tennessee have been sporadically sampled for the presence of Bd. Moreover, these samples have come from a handful of protected and relatively pristine ecosystems (reviewed in Muletz et al. 2014). By sampling amphibian populations across a much broader spatial and taxonomic scale, our study provides evidence that Bd incidence is either extremely uncommon in the region or exhibits dramatic seasonal shifts in detectability. Although Bd was not detected from the majority of sampled taxa or locations, these data can be used to help assess changes in its distribution and prevalence, providing baseline information for future studies on Bd. Understanding the distribution and spread of Bd is an important factor for conservation, and disease incidence should be monitored closely to ensure protection

of sensitive amphibian populations and taxa.

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Literature Cited

Agresti A, and B.A. Coull. 1998. Approximate is better than "exact" for interval estimation of binomial proportions. American Statistician 52:119–126.

Annis, S.L., F.P. Dastoor, H. Ziel, P. Daszak, and J.E. Longcore. 2004. A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. Journal of Wildlife Disease 40:420–428.

Bales, E.K. O.J. Hyman, A.H. Loudon, R.N. Harris, G. Lipps, E. Chapman, K. Roblee, J.D. Kleopfer and K.A. Terrell. 2015. Pathogenic chytrid fungus *Batrachochytrium dendrobatidis*, but not *B. salamandrivorans* detected in Eastern Hellbenders. PloS one 0116405. doi: 10.1371/journal.pone.0116405

Beebee, T.J.C., and R.A. Griffiths. 2005. The amphibian decline crisis: a watershed for conservation biology? Biological Conservation 125:271–285.

Berger, L., R. Speare, P. Daszak, D.E. Green, A.A. Cunningham, C.L. Goggin, and H. Parkes. 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 95:9031–9036.

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

Briggler, J.T., K.A. Larson, and K.J. Irwin. 2008. Presence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) on Hellbenders (*Cryptobranchus alleganiensis*) in the Ozark Highlands. Herpetological Review 39:443–444.

Briggs, C.J., V.T. Vredenburg, R.A. Knapp, and L.J. Rachowicz. 2005. Investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. Ecology 86:3149–3159.

Burgmeier, N.D., S.D. Unger, J.L. Meyer, T.M. Sutton, and R.N. Williams. 2011. Health and habitat quality assessment for the Eatern Hellbender (*Cryptobranchus alleganiensis*) in Indiana, USA. Journal of Wildlife Diseaese. 47:836–848.

Bustamante, H.M., L.J. Livo, and C. Carey. 2010. Effects of temperature and hydric environment on survival of the Panamanian Golden Frog infected with a pathogenic chytrid fungus. Integrative Zoology 5:143–153.

Caruso, N.M., and K.R. Lips. 2013. Truly enigmatic declines in terrestrial salamander popuations in Great Smoky Mountains National Park. Biodiversity Research 2012:1–11.

Chatfield, M.W.H., P. Moler, and C.L. Richards-Zawacki. 2012. The amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, in fully aquatic salamanders from southeastern North America. PloS one 7:e44821. doi: 10.1371/journal.pone.0044821

Chinnadurai, S.K., D. Cooper, D.S. Dombrowski, M.F. Poore, and M.G. Levy. 2009. Experimental infection of native North Carolina salamanders with *Batrachochytrium dendrobatidis*. Journal of Wildlife Diseases 45:631–636.

Corser, J.D. 2001. Decline of disjunct Green Salamander (*Aneides aeneus*) populations in the southern Appalachians. Biological Conservation 97:119–126.

Crawford, A.J., K.R. Lips, and E. Bermingham. 2010. Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. Proceedings of the National Academy of Sciences 107:13777–13782.

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

Daszak, P., A.A. Cunningham and A.D. Hyatt. 2003. Infectious disease and amphibian population declines. Diversity and Distributions 9:141–150.

Drew, A., E.J. Allen, and L.J.S. Allen. 2006. Analysis of climatic and geographic factors affecting the presence of chytridiomycosis in Australia. Diseases of Aquatic Organisms 68:245–250.

Garner, T.W.J., S. Walker, J. Bosch, A.D. Hyatt, A.A. Cunningham, and M.C. Fisher. 2005. Chytrid fungus in Europe. Emerging Infectious Diseases 11:1639–1641.

Goldberg, T.L., A.M. Readel, and M.H. Lee. 2007. Chytrid fungus in frogs from an equatorial African montane forest in Western Uganda. Journal of Wildlife Diseases 48:521–524.

Green, D.M., L.A. Weir, G.S. Casper, and M. J. Lannoo. 2013. North American Amphibians: Distribution and Diversity. University of California Press, Berkeley, California.

Highton, R. 2005. Declines in populations of eastern North American woodland salamanders (*Plethodon*). Pp. 34-46 In: Amphibian Declines: The Conservation Status of U.S. Species. Lannoo, M.J. (Ed.). University of California Press, Berkeley, USA.

Hossack, B.R., M.J. Adams, E.H.C. Grant, C.A. Pearl, J.B. Bettaso, W.J. Barichhivich, W.H. Lowe, K. True, J.L. Ware, and P.S. Corn. 2010. Low prevalence of chytrid fungus (*Batrachochytrium dendrobatidis*) in amphibians of U.S. headwater streams. Journal of Herpetology 44:253–260.

Keitzer, S.C. , R. Goforth, A.P. Pessier, and A.J. Johnson. 2011. Survey for the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* in southwestern North Carolina salamander populations. Journal of Wildlife Diseases 47:455–458.

Kiester, A.R. 1971. Species density of North American amphibians and reptiles. Systematic Zoology 20: 127–137.

Kriger, K.M., and J.M. Hero. 2006. Survivorship in wild frogs infected with chytridiomycosis. Ecohealth 3:171–177.

Kriger, K.M., and J.M. Hero. 2007. The chytrid fungus *Batrachochytrium dendrobatidis* is nonrandomly distributed across amphibian breeding habitats. Diversity and Distributions 13:781-788.

Lips, K.R. 1999. Mass mortality and population declines of anurans at an upland site in western Panama. Conservation Biology 13:117–125.

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 103:3165–3170.

Lips, K. R., D.E. Green, and R. Papendick. 2003. Chytridiomycosis in wild frogs from southern Costa Rica. Journal of Herpetology 37:215–218.

NCWRC (North Carolina Wildlife Resourcs Commission). 2005. North Carolina Wildlife Action Plan. North Carolina Wildlife Resourcs Commission, Raleigh, North Carolina.

Moffitt, D.J. 2012. Occupancy modeling and disease susceptibility of montane salamander species in western North Carolina. M.Sc. Thesis, Appalachian State University, Boone, North Carolina, USA. 107 p.

Moskwik, M. 2014. Recent elevational range expansions in plethodontid salamanders (Amphibia: Plethodontidae) in the southern Appalachian Mountains. Journal of Biogeography 41:1957–1966.

Muletz, C., N.M. Caruso, R.C. Fleischer, R.W. McDiarmid, K.R. Lips. 2014. Unexpected rarity of the pathogen *Batrachochytrium dendrobatidis* in Appalachian *Plethodon* salamanders: 1957–2011. PloS one 9(8):e103728. doi: 10.1371/journal.pone.0103728

Murray, K.A., R.W.R. Retallick, R. Puschendorf, L.F. Skerratt, D. Rosauer, H.I. McCallum, L. Berger, R. Speare, and J. VanDerWal. 2011. Assessing spatial patterns of disease risk to biodiversity: Implications for the management of the amphibian pathogen *Batrachochytrium dendrobatidis*. Journal of Applied Ecology 48:163–173.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C., USA.

R Core Development Team. 2013. R: A Language and Environment for Statistical Computing. Foundation for Statistical Computing, Vienna, Austria.

Rollins, A.W., J.E. Copeland, H. Barker, and D. Satterfield. 2013. The distribution of *Batrachochytrium dendrobatidis* across the southern Appalachian states, USA. Mycosphere 4:250–254.

Ron, S.R. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. Biotropica 37:209–221.

Rothermel, B.B., S.C. Walls, J.C. Mitchell, C.K. Dodd, L.K. Irwin, D.E. Green, V.M. Vazquez, J.W. Petranka, and D.J. Stevenson. 2008. Widespread occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the southeastern USA. Diseases of Aquatic Organisms 82:3–18.

Searle, C.L., S.S. Gervasi, J. Hua, J.I. Hammond, R.A. Relyea, D.H. Olson, and A.R. Blaustein. 2011. Differential host susceptibility to *Batrachochytrium dendrobatidis*, an emerging amphibian pathogen. Conservation Biology 25:965–974.

Seimon, T.A., A. Seimon, P. Daszak, S.R.P. Halloy, L.M. Schloegel, C.A. Aguilar, P. Sowell, A.D. Hyatt, B.E. Konecky, and J. Simmons. 2007. Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. Global Change Biology 13:288–299.

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

Smith, K.G., K.R. Lips, and J.M. Chase. 2009. Selecting for extinction: nonrandom disease-

associated extinction homogenizes amphibian biotas. Ecology Letters 12:1069–1078.

Souza, M.J., M.J. Gray, P. Colclough, and D.L. Miller. 2012. Prevalence of infection by *Batrachochytrium dendrobatidis* and Ranavirus in Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in eastern Tennessee. Journal of Wildlife Disease 48:560–566.

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

Timpe, E.K., S.P. Graham, R.W. Gagliardo, R.L. Hill, and M.G. Levy. 2008. Occurrence of the fungal pathogen *Batrachochytrium dendrobatidis* in Georgia's amphibian population. Herpetological Review 39:447–449.

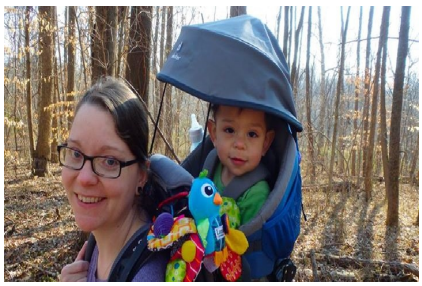
Voyles, J., S. Young, L. Berger, C. Campbell, W.F. Voyles, A. Dinudom, D. Cook, R. Webb, R.A. Alford, L.F. Skerratt, and R. Speare. 2009. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. Science 326:582–585.

Weldon, C., L.H. Du Preez, A.D. Hyatt, R. Muller, and R. Speare. 2004. Origin of the amphibian chytrid fungus. Emerging Infectious Diseases 10:2100.

Williams, L.A and J.D. Groves. 2014. Prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* in Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) in western North Carolina, USA. Herpetological Conservation and Biology 9:454–467.

Yang, H., R. Speare, R. Webb, S. Park, T. Kim, K.C. Lasater, S. Shin, S. Son, J. Park, et al. 2009. First detection of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in free-ranging populations of amphibians on mainland Asia: survey in South Korea. Diseases of Aquatic Organisms 86:9–13.

Young, B.E., K.R. Lips, J.K. Reaser, R. Ibáñez, A.W. Salas, J.R. Cedeño, L.A. Coloma, S. Ron, E.L.A. Marca, J.R. Meyer, et al. 2001. Population declines and priorities for amphibian conservation in Latin America. Conservation Biology 15:1213–1223.



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(Photographed by Jon Groves).

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Table A1. Caudate and anuran species in the Piedmont and Highland regions of the Southern Appalachian Mountains of North Carolina and Tennessee that were swabbed for the presence of *Batrachochytrium dendrobatidis*, their primary habitat (T- terrestrial, A- aquatic, S- semi-aquatic), county, number of swabbed animals (positive animals in parentheses), and prevalence binomial confidence intervals (reported as a percent).

Taxon	Habitat	County	N	Prevalence
Order Anura				
Family Bufonidae				
Anaxyus americanus (American Toad)	S	Cherokee	1	(0-79)
Anaxyus americanus (American Toad)	S	Graham	1	(0-79)
Anaxyus americanus (American Toad)	S	Haywood	2	(0-66)
Anaxyus americanus (American Toad)	S	Transylvania	2	(0-66)
Family Hylidae				
Hyla chrysoscelis(Cope's Treefrog)	S	Graham	1	(0-79)
Pseudacris brachyphona(Mountain Chorus Frog)	S	Cherokee	9	(0-30)
Pseudacris crucifer(Spring Peeper)	S	Cherokee	2	(0-66)
Pseudacris crucifer(Spring Peeper)	S	Graham	1	(0-79)
Pseudacris crucifer(Spring Peeper)	S	Henderson	1	(0-79)
Pseudacris crucifer(Spring Peeper)	S	Transylvania	2	(0-66)
Family Ranidae				
Lithobates catesbeianus(American Bullfrog)	A	Henderson	1	(0-79)
Lithobates catesbeianus(American Bullfrog)	A	Transylvania	3	(0-56)
Lithobates clamitans(Green Frog)	A	Haywood	1	(0-79)
Lithobates clamitans(Green Frog)	A	Transylvania	1	(0-79)
Lithobates sylvaticus(Wood Frog)	S	Buncombe	1	(0-79)
Lithobates sylvaticus(Wood Frog)	S	Transylvania	1	(0-79)
Order Caudata				
Family Abystomatidae				
Ambystoma maculatum(Spotted Salamander)	S	Buncombe	4	(0-49)
Ambystoma maculatum(Spotted Salamander)	S	Graham	4	(0-49)
Ambystoma maculatum(Spotted Salamander)	S	Haywood	1	(0-79)
Ambystoma maculatum(Spotted Salamander)	S	Macon	1	(0-79)
Ambystoma maculatm(Spotted Salamander)	S	Watauga	50(5)	(4-21)
Ambystoma opacum(Marbled Salamander)	S	Cherokee	1	(0-79)
Ambystoma talpoideum(Mole Salamander)	S	Buncombe	1	(0-79)
Family Cryptobranchidae				
Cryptobranchus alleganiensis alleganiensis (Eastern Hellbender)	A	Carter (TN)	16	(0-19)
Cryptobranchus alleganiensis alleganiensis (Eastern Hellbender)	A	Mithell	2	(0-66)
Cryptobranchus alleganiensis alleganiensis (Eastern Hellbender)	A	Watauga	13	(0-23)
Family Plethodontidae				
Aneides aeneus(Green Salamander)	T	Henderson	21	(0-16)
Aneides aeneus(Green Salamander)	T	Jackson	1	(0-79)
Aneides aeneus(Green Salamander)	T	Macon	1	(0-79)
Aneides aeneus(Green Salamander)	T	Transylvania	1	(0-79)

Continued in Table A2 on next page.

Table A2. continued from Table A1).

Taxon	Habitat	County	N	Prevalence
Desmognathus aeneus (Seepage Salamander)	T	Clay	1	(0-79)
Desmognathus aeneus (Seepage Salamander)	T	Graham	2	(0-66)
Desmognathus aeneus (Seepage Salamander)	T	Macon	1	(0-79)
Desmognathus carolinensis (Carolina Dusky Salamander)	S	Buncombe	3	(0-56)
Desmognathus carolinensis (Carolina Dusky Salamander)	S	Haywood	2	(0-66)
Desmognathus carolinensis (Carolina Dusky Salamander)	S	Henderson	1	(0-79)
Desmognathus carolinensis (Carolina Dusky Salamander)	S	Yancey	1	(0-79)
Desmognathus fuscus (Northern Dusky Salamander)	S	Macon	1	(0-79)
Desmognathus fuscus (Northern Dusky Salamander)	S	McDowell	2	(0-66)
Desmognathus fuscus (Northern Dusky Salamander)	S	Yancey	4	(0-49)
Desmognathus monticola (Seal Salamander)	A	Graham	5	(0-43)
Desmognathus monticola (Seal Salamander)	A	Haywood	3	(0-56)
Desmognathus monticola (Seal Salamander)	A	Macon	2	(0-66)
Desmognathus ocoee(Ocoee Salamander)	S	Haywood	1	(0–79)
Desmognathus ocoee(Ocoee Salamander)	S	Macon	6	(0–39)
Desmognathus orestes (Blue Ridge Dusky Salamander)	S	Avery	14	(0–22)
Desmognathus organi (Northern Pigmy Salamander)	T	Buncombe	6	(0–39)
Desmognathus organi (Northern Pigmy Salamander)	T	Yancey	3	(0–56)
Desmognathus quadramaculatus (Black-bellied Salamander)	A	Haywood	1	(0–79)
Desmognathus quadramaculatus (Black-bellied Salamander)	A	Macon	2	(0–66)
Desmognathus quadramaculatus (Black-bellied Salamander)	A	Polk	1	(0–79)
Desmognathus quadramaculatus (Black-bellied Salamander)	A	Transylvania	1	(0–79)
Desmognathus quadramaculatus (Black-bellied Salamander)	A	Watauga	1	(0–79)
Desmognathus santeetlah (Santeetlah Dusky Salamander)	S	Graham	4	(0–49)
Desmognathus organi (Northern Pigmy Salamander)	T	Avery	5	(0–44)
Desmognathus organi (Northern Pigmy Salamander)	T	Jackson/Haywood	3	(0–56)
Eurycea guttolineata (Three-lined Salamander)	S	Graham	2	(0–66)
Eurycea junaluska (Junaluska Salamander)	S	Graham	6	(0–39)
Eurycea longicauda (Longtail Salamander)	S	Haywood	1	(0–79)
Eurycea longicauda (Longtail Salamander)	S	Macon	1	(0–79)
Eurycea longicauda (Longtail Salamander)	S	Watauga	1	(0–79)
Eurycea wilderae (Blue Ridge Two-lined Salamander)	S	Avery	1	(0–79)
Eurycea wilderae (Blue Ridge Two-lined Salamander)	S	Buncombe	1	(0–79)
Eurycea wilderae (Blue Ridge Two-lined Salamander)	S	Graham	2	(0–66)
Eurycea wilderae (Blue Ridge Two-lined Salamander)	S	Haywood	1	(0–79)
Eurycea wilderae (Blue Ridge Two-lined Salamander)	S	Henderson	1	(0–79)
Eurycea wilderae (Blue Ridge Two-lined Salamander)	S	Macon	2	(0–66)
Eurycea wilderae (Blue Ridge Two-lined Salamander)	S	Transylvania	2	(0–66)
Eurycea wilderae (Blue Ridge Two-lined Salamander)	S	Yancey	2	(0–66)
Hemidactylium scutatum (Four-toed Salamander)	S	Buncombe	2	(0–66)
Notophthalmus viridescens (Red-spotted Newt)	A	Graham	3	(0–56)
Notophthalmus viridescens (Red-spotted Newt)	A	Henderson	1	(0–79)

Continued in Table A3 on next page.

Table A3. continued from Table A2).

Taxon	Habitat	County	N	Prevalence
Plethodon amplus (Blue Ridge Gray-cheeked Salamander)	T	Buncombe	1	(0–79)
Plethodon amplus (Blue Ridge Gray-cheeked Salamander)	T	Henderson	1	(0–79)
Plethodon cinereus (Eastern Red-backed Salamander)	T	Avery	23	(0–14)
Plethodon cinereus (Eastern Red-backed Salamander)	T	Alleghany	2	(0–66)
Plethodon cinereus (Eastern Red-backed Salamander)	T	Mitchell	1	(0–79)
Plethodon cinereus (Eastern Red-backed Salamander)	T	Yancey	1	(0–79)
Plethodon cylindraceus (White-spotted Slimy Salamander)	T	Avery	4	(0–49)
Plethodon cylindraceus (White-spotted Slimy Salamander)	T	Alleghany	1	(0–79)
Plethodon cylindraceus (White-spotted Slimy Salamander)	T	Buncombe	1	(0–79)
Plethodon cylindraceus (White-spotted Slimy Salamander)	T	Mitchell	1	(0–79)
Plethodon glutinosus (Northern Slimy Salamander)	T	Graham	2	(0–66)
Plethodon longicrus (Crevice Salamander)	T	Henderson	2	(0–66)
Plethodon metcalfi (Southern Gray-cheeked Salamander)	T	Haywood	1	(0–79)
Plethodon metcalfi (Southern Gray-cheeked Salamander)	T	Henderson	4	(0–49)
Plethodon metcalfi (Southern Gray-cheeked Salamander)	T	Jackson/Haywood	1	(0–79)
Plethodon metcalfi (Southern Gray-cheeked Salamander)	T	Macon	1	(0–79)
Plethodon metcalfi (Southern Gray-cheeked Salamander)	T	Transylvania	8	(0–32)
Plethodon montanus (Northern Gray-cheeked Salamander)	T	Avery	175	(0–2)
Plethodon montanus (Northern Gray-cheeked Salamander)	T	Madison	1	(0–79)
Plethodon montanus (Northern Gray-cheeked Salamander)	T	Mitchell	1	(0–79)
Plethodon montanus (Northern Gray-cheeked Salamander)	T	Yancey	3	(0–56)
Plethodon serratus (Southern Red-backed Salamander)	T	Haywood	3	(0-–56)
Plethodon shermani (Red-legged Salamander)	T	Macon	13	(0–14)
Plethodon teyahalee (Southern Appalachian Salamander)	T	Macon	1	(0–79)
Plethodon ventralis (Southern Zigzag Salamander)	T	Buncombe	4	(0–49)
Plethodon wehrlei (Wehrle's Salamander)	T	Alleghany	6	(0–39)
Plethodon wehrlei (Wehrle's Salamander)	T	Surry	3	(0–56)
Plethodon welleri (Weller's Salamander)	T	Avery	19	(0–17)
Plethodon wehrlei (Wehrle's Salamander)	T	Madison	6	(0–39)
Plethodon wehrlei (Wehrle's Salamander)	T	Mitchell	8	(0–32)
Plethodon yonahlossee (Yonahlossee Salamander)	T	Avery	31	(0–11)
Plethodon yonahlossee (Yonahlossee Salamander)	T	Buncombe	2	(0–66)
Pseudotriton ruber(Red Salamander)	S	Buncombe	1	(0–79)
Pseudotriton ruber (Red Salamander)	S	Graham	4	(0–49)
Pseudotriton ruber (Red Salamander)	S	Haywood	1	(0–79)
Pseudotriton ruber (Red Salamander)	S	McDowell	1	(0–79)
Pseudotriton ruber (Red Salamander)	S	Transylvania	1	(0–79)