Frick, M. G. 1999. *Siren lacertina* (greater siren). Parasitism. Herpetol. Rev. 30:162.
Goka, K., J. Yokoyama, Y. Une, T. Kuroki, K. Suzuki, M. Nakahara, A. Kobayashi, S. Inaba, T. Mizutani, and A. Hyatt. 2009. Amphibian chytridiomycosis in Japan: distribution, haplotypoes, and possible route of entry into Japan. Mol. Ecol. 18:4757–4774.
Graham, S. P., and E. Borda. 2010. First report of leech parasitism in the amphibian family Sirenidae. Comp. Parasitol. 77:105–107.
Hendricks, R. 2005. *Siren lacertina. In* M. J. Lannoo (ed.), Amphibian Declines: The Conservation Status of United States Species, pp. 912–914. University of California Press, Berkeley and Los Angeles, California.
Hyatt, A. D., D. G. Boyle, V. Olsen, D. B. Boyle, L. Berger, D. Obendorf, A. Dalton, K. Kriger, M. Hero, H. Hines, R. Phillott, R. Campbell, G. Marantelli, F. Gleason, and A. Colling. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis. Aquat. Org. 73:175–192.
Kriger, K. M., J. M. Hero, and K. J. Ashton. 2006. Cost efficiency in the detection of chytridiomycosis using PCR assay. Dis. Aquat. Org. 71:149–154.
Leja, W. T. 2005. *Siren intermedia. In* M. J. Lannoo (ed.), Amphibian Declines: The Conservation Status of United States Species, pp. 910–912. University of California Press, Berkeley and Los Angeles, California.
Longcore, J. R., J. E. Longcore, A. P. Pessier, and W. A. Halteman. 2007. Chytridiomycosis widespread in anurans of northeastern United States. J. Wild. Manage. 71:435–444.
McAllister, C. T., S. R. Goldberg, S. E. Trauth, C. R. Bursey, H. J. Holshuh, and B. G. Cochran. 1994. Helminths of the western lesser siren, *Siren intermedia nettingi* (Caudata, Sirenidae), from Arkansas. J. Helminthol. Soc. Washington 61:234–238.
Moler, P. E. 2005a. *Pseudobranchus axanthus. In* M. J. Lannoo (ed.), Amphibian Declines: The Conservation Status of United States Species, pp. 908–909. University of California Press, Berkeley and Los Angeles, California.
———. 2005b. *Pseudobranchus striatus. In* M. J. Lannoo (ed.), Amphibian Declines: The Conservation Status of United States Species, pp. 909–910. University of California Press, Berkeley and Los Angeles, California.
Ouellet, M., I. Mikaelian, B. D. Pauli, J. Rodrigue, and D. M. Green. 2005. Historical evidence of widespread chytrid infection in North American amphibian populations. Conserv. Biol. 19:1431–1440.
Pearl, C. A., E. L. Bull, D. E. Green, J. Bowerman, M. J. Adams, A. Hyatt, and W. H. Wente. 2007. Occurrence of the amphibian pathogen *Batrachochytrium dendrobatidis* in the Pacific Northwest. J. Herpetol. 41:145–149.
Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C. 592 pp.
Speare , R., and L. Berger. 2000. Global distribution of chytridiomycosis in amphibians. Available at: http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyglob.htm. 11 November 2000 (accessed 18 July 2010).
Vredenburg, V. T., R. A. Knapp, T. S. Tunstall, and C. J. Briggs. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. PNAS 107:9689–9694.
Wells, K. D. 2007. The Ecology and Behavior of Amphibians. The University of Chicago Press, Chicago. 1148 pp.

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Low Prevalence of *Batrachochytrium dendrobatidis* Detected in Appalachian Salamanders from Warren County, Virginia, USA

B. GRATWICKE*
M. EVANS
Center for Species Survival, and Center for Animal Care Sciences Smithsonian Conservation Biology Institute, 3001 Connecticut Ave., NW, Washington, DC 20008, USA
E. H. CAMPBELL GRANT
NE Amphibian Research and Monitoring Initiative, USGS Patuxent Wildlife Research Center, 12100 Beech Forest Rd., Laurel, Maryland 20708, USA
J. GREATHOUSE
Oglebay's Good Zoo, 465 Lodge Drive, Wheeling, West Virginia 26003, USA
W. J. McSHEA
N. ROTZEL
R. C. FLEISCHER
Conservation Ecology Center, and Center for Conservation and Evolutionary Genetics, Smithsonian Conservation Biology Institute, 3001 Connecticut Ave., NW, Washington, DC 20008, USA

*Corresponding author e-mail: brian.gratwicke@gmail.com

Salamanders are one of the most important features of America's vertebrate fauna and the Appalachian region of the United States is a global salamander biodiversity hotspot (Young et al. 2004). Despite numerous studies of their biology at the species level, we have a poor understanding of overall threats to salamander biodiversity (Gratwicke 2008). We also have a poor understanding of the susceptibility of different salamander species to diseases, such as the fungus *Batrachochytrium dendrobatidis* (*Bd*). To date, there has been no systematic threat assessment of *Bd* on Appalachian salamanders, but several Appalachian salamander species are known to be susceptible to *Bd* in the wild including: *Ambystoma tigrinum* (Davidson et al. 2003); *Cryptobranchus alleganiensis* (Briggler et al. 2008); *Desmognathus conanti* (Timpe et al. 2008); *D. quadramaculatus* (Bartkus 2009); *D. fuscus* and *D. monticola* (Hossack et al. 2010); *Eurycea bislineata* (Grant et al. 2008); *E. cirrigera* (Byrne et al. 2008) and *Notophthalmus viridescens* (Bakkegard and Pessier 2010; Chatfield et al. 2009; Rothermel et al. 2008; Timpe et al. 2008; J. Ware and K. Duncan, unpubl.data; www.Bd-maps.net). *Bd* can also infect *D. monticola* and *Plethodon metcalfi* (Vazquez et al. 2009); *D. orestes* and *P. glutinosus* (Chinnadurai et al. 2009); and *P. cinereus* (Becker and Harris 2010; Becker et al. 2009) in laboratory settings. The effects of *Bd* on salamanders in both wild

TABLE 1. Salamanders encountered and swabbed to test for *Batrachochytrium dendrobatidis* (*Bd*) as part of the 2009 salamander bio-blitz on the Smithsonian Conservation Biology Institute in Warren County, Virginia, USA.

	Total Counted	Total Swabbed	*Bd* Positives	Number of Sites
Eurycea bislineata	13	13	0	4
Desmognathus fuscus	4	4	0	2
Desmognathus monticola	134	25	1	5
Gyrinophilus porphyriticus	3	3	0	1
Plethodon cinereus	225	142	0	25
Plethodon cylindraceus	21	19	0	7
Pseudotriton ruber	4	4	0	4
Total	404	211	0	34

and laboratory settings, however, are extremely variable depending on temperatures, salamander species, and skin flora (Becker and Harris 2010).

There has been no systematic effort to monitor *Bd* throughout Virginia, although the disease has been detected on amphibians in: 1) Virginia at Mountain Lake Biological Research Station and Upham Brook (Rothermel et al. 2008); 2) the Shenandoah National Park (Grant, unpubl. data); and 3) on A*cris crepitans*, *Lithobates catesbeianus*, *L. clamitans*, *L. spenocephalus*, and *N. viridescens* in Richmond and Charles Counties (J. Ware and K. Duncan, unpubl.data; www.Bd-maps.net). In order to investigate the status of *Bd* and its potential impact on wild salamanders, we surveyed the *Bd* status of salamanders on the 3200-acre Smithsonian Conservation Biology Institute, Front Royal site (SCBI-FR) in Warren County, Virginia (38.893279°N, 78.152381°W). SCBI-FR occupies a low point (elevation range ~300–600 m asl) on the western slope of the Blue Ridge Mountains of Virginia, with a topography characterized by a series of rolling hills and small valleys. Wooded areas are mainly covered by mature secondary Eastern mixed deciduous forest, with Tulip Poplar (*Liriodendron tulipifera*), Pignut Hickory (*Carya glabra*), Black Gum (*Nyssa sylvatica*), Mockernut Hickory (*C. alba*), Red Oak (*Quercus rubra*), and White Oak (*Q. alba*) being the major dominant canopy species.

On 9 May 2009, a salamander bio-blitz event was held for 40 volunteer participants recruited from various local natural history and herpetology groups. Teams surveyed a total of seven stream sites that were selected by dividing each stream on the property into 500-m segments and then selecting stream segments for survey using a random number table. At each survey site, two 25-m transects were sequentially established along one stream bank and all cover objects 1 m either side of the transect line were searched. An additional 27 terrestrial transects were established by dividing the wooded portion of the property (excluding animal enclosures) into 1-km^2 blocks and using a random number generator to select 50 blocks; the transect was placed in the center of each block. At each site, two parallel 25-m transects were established 10-m apart from each other, running in a randomly assigned cardinal direction. All cover objects within 1 m of either side of the transect line were searched. Amphibians encountered in either survey were captured and identified to species level, and fresh powder-free nitrile gloves were used for each animal handled. The first 10 individuals of each species at each site were swabbed for *Bd* by rubbing a cotton-tipped swab 15 times in both directions along the salamanders' ventral surfaces and five times on each foot. Exact GPS coordinates for each specimen were recorded.

Swabs were stored dry at room temperature for four weeks before analysis. Anywhere from one to three swabs were incubated together in an oscillating thermal incubator at 56°C and 30 rpm in 400 µl of lysis buffer and 30 µl proteinase K (Qiagen) for 24 h. After incubation, DNA was extracted from the lysate solutions using a Qiagen Biosprint 96 DNA Blood Kit according to the instructions. Testing for the presence of *Bd* was performed using Qiagen's QuantiTect SYBR Green PCR Kit, using the primers ITS1-3Chytr and 5.8sChytr, developed by (Boyle et al. 2004). Positive and negative controls were included in both the extraction and the realtime-PCR reactions. A melt-curve analysis was used to ensure only *Bd* DNA was amplified. No contamination was present in the DNA or subsequent analysis of these swabs.

Seven salamander species (404 individuals) were encountered in the survey transects, and 211of these individuals were swabbed (Table 1). Only a single *D. monticola* swab tested positive for *Bd* (georeference 38.895383°N, 78.151369°W). Other amphibian species encountered on the property that day during unconstrained searches, but were not swabbed include: *Ambystoma maculatum; Anaxyrus americanus; Anaxyrus fowleri*; *Hyla versicolor*; *Lithobates catesbeianu;*, *Lithobates palustri;*, *Lithobates clamitans*; *Lithobates sylvaticus*; and *Pseudacris crucifer.*

We used a binomial distribution calculator (http://faculty.vassar.edu/lowry/binomialX.html) to calculate the maximum prevalence rate using a 95% confidence limit with our sample of 1 infected swab (success) from 211 salamanders (trials). Assuming 100% detectability on all animals swabbed, we estimate that overall prevalence rates in the combined aquatic and terrestrial salamander assemblage are lower than 2.1%. We did not have sufficient sample sizes to conduct species-specific prevalence rates.

Bd is present in salamanders at very low prevalence rates in Warren County, Virginia. Other studies testing for *Bd* on salamanders in the Appalachians found very low occurrences (Bartkus 2009). The actual level of threat that *Bd* poses to wild Appalachian salamander populations remains an unresolved question. *Bd* is very temperature dependent and varies seasonally, therefore, spring was selected as a time of year when temperatures are optimal for *Bd* (Longcore et al. 2007). The observed prevalence levels of less than 2.1% on the 3200-acre SCBI property at a time of year optimal for detecting the pathogen means that it is unlikely that *Bd* is significantly affecting the salamander populations at that site. Furthermore, our observations in Virginia are consistent with observations elsewhere in the Appalachian region, where others have found that although *Bd* appears to be widely distributed, it is found at low prevalence rates in aquatic salamanders (Bartkus 2009; Byrne et al. 2008; Hossack et al. 2010), but is uncommon in terrestrial salamanders (e.g., Chatfield et al 2009).

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Literature cited

Bakkegard, K., and A. Pessier. 2010. *Batrachochytrium dendrobatidis* in adult *Notophthalmus viridescens* in North-Central Alabama, USA. Herpetol. Rev. 41:45–47.

Bartkus, C. J. 2009. The occurrence of *Batrachochytrium dendrobatidis* in salamander populations of West Virginia. MSc thesis, Marshall University, Huntington. http://ow.ly/3elhs

Becker, M., and R. Harris. 2010. Cutaneous bacteria of the redback salamander prevent morbidity associated with a lethal disease. PLoS One 5:e10957.

Becker, M. H., R. M. Brucker, C. R. Schwantes, R. N. Harris, and K. P. C. Minbiole. 2009. The bacterially-produced metabolite violacein is associated with survival in amphibians infected with a lethal disease. Appl. Environ. Microbiol. doi:10.1128/AEM.01294–09.

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. Dis. Aquat. Org. 60:141–148.

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. Herpetol. Rev. 39:443–444.

Byrne, M., E. Davie, and J. Gibbons. 2008. *Batrachochytrium dendrobatidis* occurrence in *Eurycea cirrigera*. Southeast. Nat. 7:551–555.

Chatfield, M. W. H., B. B. Rothermal, C. S. Brooks, and J. B. Kay. 2009. Detection of *Batrachochytrium dendrobatidis* in amphibians from the Great Smoky Mountains of North Carolina and Tennessee. USA. Herpetol. Rev. 40:176–179.

Chinnadurai, S., D. Cooper, D. Dombrowski, M. Poore, and M. Levy. 2009. Experimental infection of native North Carolina salamanders with *Batrachochytrium dendrobatidis*. J. Wildl. Dis. 45:631.

Davidson, E. W., M. Parris, J. P. Collins, J. E. Longcore, A. P. Pessier, and J. Brunner. 2003. Pathogenicity and transmission of chytridiomycosis in tiger salamanders (*Ambystoma tigrinum*). Copeia 2003:601–607.

Grant, E. H. C., L. L. Bailey, J. Ware, and K. Duncan. 2008. Prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* in stream and wetland amphibians in Maryland, USA. Appl. Herpetol. 5:233–241.

Gratwicke, B. 2008. Proceedings of the Appalachian Salamander Conservation Workshop. p. 38. UCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN, Hosted by Smithsonian's National Zoological Park, facilitated by the IUCN/SSC Conservation Breeding Specialist Group. http://ow.ly/3el3B

Hossack, B., M. Adams, E. Campbell Grant, C. Pearl, J. Bettaso, W. Barichivich, W. Lowe, K. True, J. Ware, and P. Corn. 2010. Low prevalence of chytrid fungus (*Batrachochytrium dendrobatidis*) in amphibians of US headwater streams. J. Herpetol. 44:253–260.

Longcore, J., J. Longcore, A. Pessier, and W. Halteman. 2007. Chytridiomycosis widespread in anurans of northeastern United States. J. Wildl. Manage. 71:435–444.

Rothermel, B., S. Walls, J. Mitchell, C. Dodd, L. Irwin, D. Green, J. Petranka, and D. Stephenson. 2008. Widespread occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the southeastern USA. Dis. Aquat. Org. 82:3–18.

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 populations. Herpetol. Rev. 39:447–449.

Vazquez, V., B. Rothermel, and A. Pessier. 2009. Experimental infection of North American plethodontid salamanders with the fungus *Batrachochytrium dendrobatidis*. Dis. Aquat. Org. 84:1–7.

Young, B., S. Stuart, J. Chanson, N. Cox, and T. Boucher. 2004. Disappearing jewels—The status of New World amphibians. NatureServe, Arlington, Virginia. http://www.natureserve.org/publications/disappearingjewels.jsp