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# First Report of Ranavirus and *Batrachochytrium dendrobatidis* in Green Salamanders (*Aneides aeneus*) from Virginia, USA

The Green Salamander (*Aneides aeneus*) is distributed from extreme southwest Pennsylvania, USA to northern Alabama and Mississippi with a disjunct population in southern North Carolina, northeastern Georgia, and northern South Carolina (Petranka 1998). Because of unique habitat requirements, Green Salamanders are thought to be at risk of range-wide declines and extirpations (Corser 2001). Green Salamanders primarily dwell in rock crevices within rock outcrops (Gordon 1952; Rossell et al. 2009). Like its western congeners, Green Salamanders also inhabit arboreal habitats (Petranka 1998; Waldron and Humphries 2005) as well as decaying logs and stumps on the forest floor (Fowler 1947).

Green Salamander populations, especially those in the disjunct Carolina populations, have experienced declines and extirpations (Snyder 1991; Corser 2001). From the 1970s to 1980s local extirpations occurred in once-abundant North Carolina populations (Snyder 1991), and since the 1980s reductions of up to 98% have occurred in remaining populations (Corser 2001). One of the most-studied Blue Ridge escarpment populations was located at Biscuit Rock (Highlands, North Carolina; Gordon 1952; Snyder 1991; Corser 2001), and in the past five years this population became extirpated without a definitive cause (Lori Williams, unpubl. data). Various hypotheses have been proposed for Green Salamander declines such as disease and loss of surrounding timber (Snyder 1991; Petranka 1998; Corser 2001). Diseases are one of the leading causes of worldwide amphibian declines (Stuart et al. 2004; Young et al. 2004; Sodhi et al. 2008). Currently the Green Salamander is listed as threatened or endangered in Indiana, Maryland, Mississippi, North Carolina, Ohio, and Pennsylvania (MDNR 2005; MMNS 2005; PGCPBFC 2005; IDNR 2006; NCWRC 2014; ODNR 2014) and as a species of greatest conservation need in all other inhabited states (GDNR 2005; SCDNR 2005; TWRA 2005; VDGIF 2005; WFFDADCNR 2005; WVDNR 2005; KDFWR 2013). However, the potential impact of diseases on Green Salamanders is unknown, especially in Virginia.

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Diseases caused by ranaviruses are responsible for amphibian die-offs throughout Europe and North America, including the southeastern United States (Green et al. 2002; Miller et al. 2011; Hoverman et al. 2012), and may contribute to population declines (Gray et al. 2009a). In the southern Appalachian Mountains, ranavirus infections have been reported in 18 species of plethodontid salamanders, but Green Salamanders have not been sampled (Miller et al. 2011; Hamed et al. 2013). Overall ranavirus prevalence in plethodontid salamanders has varied from 3–81% throughout the southern Appalachian Mountains (Gray et al. 2009b; Hamed et al. 2013) with salamanders from the genus *Desmognathus* having highest individual prevalence (Sutton et al. 2014). Only a single past study has sampled plethodontid salamanders for ranavirus within the Virginia portion of the Green Salamander's range (Wise Co.; Davidson and Chambers 2011a); overall ranavirus prevalence in this study was 33%, although Green Salamanders were not sampled.

Chytridiomycosis, the disease caused by *Batrachochytrium dendrobatidis* (*Bd*), has also been responsible for numerous amphibian declines and extirpations worldwide, especially in Central American anurans (Lips et al. 2006; Lötters et al. 2009). Infection rates of *Bd* vary throughout the southeastern U.S., with 17 species of plethodontid salamanders testing positive for *Bd* in past studies, and an additional 23 species not infected (Hughey et al. 2014). However, *Bd* is thought to have played a role in the declines of plethodontid salamanders in Central America and has been shown to be lethal to plethodontid salamanders in the western United States (Lips et al. 2006; Weinstein 2009; Cheng et al. 2011). Only a single prior study with a limited sampled size (N = 3) has surveyed Green Salamanders for *Bd*, with no positive results (Hill et al. 2011). Our goal was to determine ranavirus and *Bd* prevalence in Green Salamander populations from southwest Virginia.

To determine the potential impact of ranaviruses and *Bd*, we sampled Green Salamanders from known historic locations as well as new locations with ideal habitat in southwestern Virginia (Dickenson, Scott, Washington, and Wise counties; Fig. 1; VDGIF 2014) and tested them for infection by these pathogens. Multiple observers searched rock crevices and surrounding trees and mid-story vegetation for Green Salamanders from May–August 2013. We also used burlap bands at three sites to intercept Green Salamanders climbing trees (Thigpen et al. 2010), but burlap proved to be ineffective due to repetitive damage from mammals, presumed to be Black Bears (*Ursus americanus*). Once located, we captured Green Salamanders by hand, while wearing nitrile gloves (Fisher Scientific, Pittsburg, Pennsylvania USA), and placed salamanders in individual 1.2-liter plastic bags, where they remained throughout processing and until released. We measured both snout–vent length (SVL) and total length (TL) using dial calipers to assess life stage (adult, subadult, juvenile; Waldron and Humphries 2005). To sample for ranavirus (N = 38), we followed procedures of Hamed et al. (2013) and used sterilized stainless steel forceps to collect a small tail section

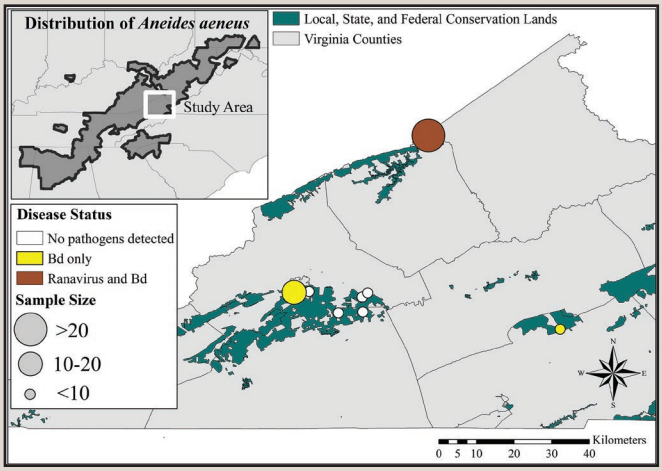


FIG. 1. Distribution of sampling sites and presence of ranavirus and *Batrachochytrium dendrobatidis* (Bd) for *Aneides aeneus* across four counties in southwest Virginia, USA. The diameter of the symbol for each sampling locality is proportional to the sample size at each site. Inset map indicates U.S. states and location of study area (white box) relative to the distribution of *A. aeneus* (dark gray).

from a natural break point. Each sample was placed in a sterile, 2-ml microcentrifuge tube with 99% reagent grade isopropyl alcohol. We did not sample for ranavirus if a salamander had tail damage, and we also excluded female salamanders guarding eggs. To sample for *Bd* infection (N = 41), we swabbed each Green Salamander with a Dryswab™ with wire shaft and rayon bud swab (MW&E, England). First, we swabbed each flank 5 times, then the ventral surface 10 times, and finally the bottom of each foot 5 times (Chatfield et al. 2012). Swab tips were placed in separate microcentrifuge tubes with 99% reagent grade isopropyl alcohol. We released all salamanders at their original location of capture. Bags and gloves were changed and forceps autoclaved after each use.

Genomic DNA was extracted from tail samples and swabs using Qiagen DNeasy Blood and Tissue Kits (Qiagen Inc., Valencia, California, USA) and then quantified utilizing a Qubit™ fluorometer (Life Technologies Corp., Carlsbad, California, USA). We used quantitative real-time PCR (qPCR) utilizing an ABI 7900HT PCR system (Life Technologies Corp.). For ranavirus testing, we used identical primers and protocol of Gray et al. (2012) and ran samples in duplicate. We utilized two positive (cultured virus and DNA from a confirmed positive animal) and two negative (water and DNA from a known negative animal) controls. We deemed samples to be positive if $C_T$ value ≤ 30, based on a 95% confidence interval that we derived from a standard curve originating from runs with known concentrations of virus (Caraguel et al. 2011). For *Bd* testing, we also used qPCR following the procedure and primers of Boyle et al. (2004) and ran samples in duplicate. We repeated analysis if $C_T$ values differed by more than 1 $C_T$ value. We used two positive (DNA from a *Bd* culture and DNA from a confirmed positive animal) and two negative (water and DNA from a known negative animal) controls. We considered samples to be positive for *Bd* infection if $C_T$ value ≤ 35, which was similarly based on a 95% confidence interval derived from a standard curve (Caraguel et al. 2011). All DNA extraction and PCR testing was conducted in the Center for Wildlife Health at the University of Tennessee. We established 95% confidence intervals (CIs) for prevalence using Lowry (2014) 2-sided CIs from a single proportion.

Our survey is the first known to document ranavirus and *Bd* infections in Green Salamanders (Miller et al. 2011; Hughey et al. 2014). We did not observe co-occurrence of pathogens in a single individual, but we did within the same site (Breaks Interstate Park; Fig. 1). We sampled a total of 42 Green Salamanders (21 adults, 15 subadults, and 6 juveniles), but due to tail damage, escape, or females guarding eggs we did not collect a tail sample or swab from every individual. We sampled 38 Green Salamanders for ranavirus, and salamanders averaged 41.22 ± 1.90 (mean ± SE) mm in SVL. Ranavirus prevalence was 8% (3/38), and no salamanders testing positive for ranavirus displayed external signs of infections (Table 1; Miller et al. 2011). Only one Green Salamander testing positive for ranavirus was an adult (50 mm SVL) while the remaining two salamanders were both subadults (40 mm SVL each). Green Salamanders infected with ranavirus were collected only from the Breaks Interstate Park (Dickenson Co.; Fig. 1), which had the greatest number of Green Salamanders encountered during the survey.

We swabbed 41 Green Salamanders for *Bd*, and salamanders averaged 42.70 ± 1.89 mm SVL. We detected *Bd* in 6 of 41 (15%) sampled Green Salamanders (Table 1). Two Green Salamanders infected with *Bd* were adults (52 and 57 mm SVL), whereas the other four were subadults (32–42 mm SVL). Only one salamander that tested positive for *Bd* displayed external signs of illness (e.g., emaciation). However, another Green Salamander was thin but not emaciated and did not test positive for either pathogen. Plethodontid salamanders from Mexico that were *Bd*-positive also lacked clinical external signs, thus suggesting positive salamanders do not always exhibit external signs of disease (Rooij et al. 2011). Green Salamanders infected with *Bd* were collected from Breaks Interstate Park (N = 4), Flag Rock (N = 1; Wise Co.), and Brumley Cove Camp (N = 1; Washington Co; Table 1; Fig. 1).

Due to the cryptic behavior of Green Salamanders and the presence of nesting females, we were only able to sample a single individual at six locations and are therefore cautious about inferring the potential disease status of these populations. However, two locations (Breaks Interstate Park, N = 22; Flag Rock, N = 13) provided a sufficient sample size to evaluate disease status. Only the Breaks Interstate Park had ranavirus-positive individuals, and this location was much lower in elevation (553 m) than Flag Rock (983 m), where no Green Salamanders tested positive for ranavirus. A similar trend was observed in the Great Smoky Mountains National Park where ranavirus prevalence was greater in salamander communities at lower elevation sites (Gray et al. 2009b; Sutton et al. 2014). However, our two sites were separated by almost 50 km and could have been influenced by other factors, including variation in temperature and moisture that have also been linked with ranavirus prevalence (Sutton et al. 2014). The Breaks Interstate Park also had the highest prevalence for *Bd*. A seep and subsequent stream were adjacent to three rock crevices at the Breaks Interstate Park. Both ranavirus and *Bd* are associated with streams and aquatic environments (Lips et al. 2006; Sutton et al. 2014).

Green Salamanders are not typically associated with aquatic habitats, but other hosts (i.e., those associated with aquatic habitats) may be transmitting one or both of these pathogens. For example, ranavirus has been demonstrated to be transmitted by an infected individual to an uninfected individual following a short contact time (Brunner et al. 2007). Also, *Bd* can be passed via water containing zoospores (Carey et al. 2006) and can survive for up to three months in river sands, thus suggesting moist rock ledges could harbor *Bd* (Johnson and Speare 2005). Brumley

TABLE 1. Ranavirus and *Batrachochytrium dendrobatidis* (*Bd*) prevalence in Green Salamanders (*Aneides aeneus*) from Southwest Virginia, USA, May–August 2013. I = total number of infected individuals; N = total number sampled.

| Location | *Bd* I / N | Prevalence (95% CI) | Ranavirus I / N | Prevalence (95% CI) |
|---|---|---|---|---|
| Breaks Interstate Park (37.2947°N; 82.4505°W) | 4 / 22 | 0.18 (0.07–0.39) | 3 / 22 | 0.14 (0.05–0.33) |
| Flag Rock (36.9194°N; 82.6268°W) | 1 / 13 | 0.08 (0.01–0.33) | 0 / 14 | 0 (0–0.22) |
| Brumley Cove Camp (36.8315°N; 81.9901°W) | 1 / 1 | 1.00 (0.21–1.00) | — | — |
| Clear Creek Gorge (36.9217°N; 82.5908°W) | 0 / 1 | 0 (0–0.79) | 0 / 1 | 0 (0–0.79) |
| Guest River Gorge (36.9183°N; 82.4505°W) | 0 / 1 | 0 (0–0.79) | — | — |
| Jaybird Branch (36.9081°N; 82.4638°W) | 0 / 1 | 0 (0–0.79) | 0 / 1 | 0 (0–0.79) |
| Kitchen Rock (36.8703°N; 82.5208°W) | 0 / 1 | 0 (0–0.79) | — | — |
| Little Stony Gorge (36.8726°N; 82.4623°W) | 0 / 1 | 0 (0–0.79) | — | — |
| Total | 6 / 41 | 0.15 (0.07–0.28) | 3 / 38 | 0.08 (0.03–0.27) |

Cove Camp had a *Bd*-positive individual and a large stream (Brumley Creek) that drains a reservoir (Hidden Valley Lake) is within a few meters of the rock face. We observed *Desmognathus ochrophaeus* (Alleghany Mountain Dusky Salamander) on the Brumley Cove Camp rock faces. In southwest Virginia, two sister species, *D. ochrophaeus* and *D. orestes* (Blue Ridge Dusky Salamander), have tested positive for ranavirus (Davidson and Chambers 2011a; Hamed et al. 2013) and the former also has tested positive for *Bd* (Davidson and Chambers 2011b). Thus, it is possible that salamanders of the genus *Desmognathus* could be moving both ranavirus and *Bd* out of aquatic systems to rock crevices. Flag Rock had no flowing or standing water, and only a single salamander tested positive for *Bd*, with no ranavirus infections.

The only observation of negative pathogen impacts was the emaciated Green Salamander that tested positive for *Bd* infection. We did not collect tissue samples for histological examination, but the animal was also lethargic. Anurans with *Bd* infections are often emaciated, with symptoms persisting for 2–3 months after clearing *Bd* infections (Parker et al. 2002). However, emaciation does not always imply a *Bd* infection, as anurans with parasite infections often appear emaciated (Young et al. 2012). We are cautious in assigning the condition of this salamander to *Bd* without histological evidence, but our results warrant further sampling, including histological examination and surveys for visibly infected or dead Green Salamanders.

Our finding of both ranavirus and *Bd* in Green Salamanders for the first time suggests that both pathogens could impact Green Salamander populations. Due to the conservation status of Green Salamanders throughout their range, our findings support implementation of range-wide monitoring. These results also confirm that Green Salamanders can be infected by either or both pathogens, suggesting that past declines could have been influenced by pathogens. Ranavirus prevalence has been shown to increase in drought years (Gray et al. 2009b; Sutton et al. 2014), and thus future long-term monitoring is warranted at yearly intervals to detect possible infection trends, especially on dry rock outcrops. Lastly, on rock faces where other amphibians are utilizing the same rock ledges or those in close proximity to known Green Salamander crevices, a broader sampling effort would determine if other amphibians are potentially spreading ranavirus and/or *Bd* from aquatic environments to rock faces inhabited by Green Salamanders.

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## LITERATURE CITED

BOYLE, D. G., D. B. BOYLE, V. OLSEN, J. A. T. MORGAN, A. D. HYATT. 2004. Rapid quantitative detection of chtridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis. Aquat. Org. 60:141–148.

BRUNNER, J. L, D. M. SCHOCK, J. P. COLLINS. 2007. Transmission dynamics of the amphibian ranavirus *Ambystoma tigrinum* virus. Dis. Aquat. Org. 77:87–95.

CARAGUEL, C. G. B, H. STRYHN, N. GAGNE, I. R. DOHOO, K. L. HAMMELL. 2011. Selection of a cutoff value for real-time polymerase chain

reaction results to fit a diagnostic purpose: analytical and epidemiologic approaches. J. Vet. Diagn. Invest. 23:2–15.

Carey, C., J. E. Bruzgul, L. J. Livo, M. L. Walling, K. A. Kuehl, B. F. Dixon, A. P. Pessier, R. A. Alford, and K. B. Rogers. 2006. Experimental exposure of boreal toads (*Bufo boreas*) to a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). EcoHealth 3:5–21.

Chatfield, M. W. H., P. Moler, C. L. Richards-Zawacki. 2012. The amphibian chytrid fungus, *Batrachochytrium dendrobatis,* in fully aquatic salamanders from southeastern North America. PLoS ONE 7:e44821.

Cheng, T. L., S. M. Rovito, D. B. Wake, and V. T. Vrendenburg. 2011. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. Proc. Nat. Acad. Sci. 108:9502–9507.

Corser, J. D. 2001. Decline of disjunct green salamander (*Aneides aeneus*) populations in the southern Appalachians. Biol. Conserv. 97:119–126.

Davidson, S. R. A. and D. L. Chambers. 2011a. Ranavirus prevalence in amphibian populations of Wise County, Virginia, USA. Herpetol. Rev. 42:540–542.

———, and ———. 2011b. Occurrence of *Batrachochytrium dendrobatidis* in Amphibians of Wise County, Virginia, USA. Herpetol. Rev. 42:214–216.

Fowler, J. A. 1947. Record for *Aneides aeneus* in Virginia. Copeia 1947:144.

Georgia Department of Natural Resources [GDNR]. 2005. A comprehensive wildlife strategy for Georgia. Georgia Department of Natural Resources Wildlife Resources Division, Social Circle, Georgia.

Gordon, R. E. 1952. A contribution to the life history and ecology of the plethodontid salamander *Aneides aeneus* (Cope and Packard). Am. Midl. Nat. 47:666–701.

Gray, M. J., D. L. Miller, and J. T. Hoverman 2009a. Ecology and pathology of amphibian ranaviruses. Dis. Aquat. Org. 87:243–266.

———, ———, and ———. 2009b. First report of ranavirus infecting lungless salamanders. Herpetol. Rev 40:316–319.

Gray, M. J., D. L. Miller, and J. T. Hoverman. 2012. Reliability of nonlethal surveillance methods for detecting ranavirus infection. Dis. Aquat. Org. 99:1–6.

Green, D. E., K. A. Converse, and A. K. Schrader. 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. Ann. New York Acad. Sci. 969:323–339.

Hamed, M. K., M. J. Gray, D. L. Miller. 2013 First report of ranavirus in plethodontid salamanders from the Mount Rogers National Recreation Area, Virginia, USA. Herpetol. Rev. 44:455–457.

Hill, R. L., M. G. Levy, E. K. Timpe, J. B. Kaylock. 2011. Additional reports of amphibian chytrid fungus *Batrachochytrium dendrobatidis* from Georgia, USA. Herpetol. Rev. 42:376–379.

Hoverman, J. T., M. J. Gray, D. L. Miller, and N. A. Haislip. 2012. Widespread occurrence of ranavirus in pond-breeding amphibian populations. EcoHealth 9:36–48.

Hughey, M. C., M. H Becker, J. B. Wake, M. C. Swartwout, L. K. Belden. 2014. *Batrachochytrium dendrobatidis* in Virginia amphibians: within and among site variation in infection. Herpetol. Rev. 45:428–438.

Indiana Department of Natural Resources [IDNR]. 2006. Indiana Comprehensive Wildlife Strategy. Department of Natural Resources Division of Fish and Wildlife, Indianapolis, Indiana.

Johnson, M. L., and R. Speare. 2005. Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. Dis Aquat. Org. 65:181–186.

Kentucky Department of Fish and Wildlife Resources [KDFWR]. 2013. Kentucky's Comprehensive Wildlife Conservation Strategy. Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky.

Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Coey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc. Nat. Acad. Sci. 103:3165–3170.

Lötters, S., J. Kielgast, J. Bielby, S. Schmidtlein, J. Bosch, M. Veith, S. F. Walker, M. C. Fisher, and D. Rödder. 2009. The link between rapid enigmatic amphibian decline and the globally emerging chytrid fungus. EcoHealth 6:358–372.

Lowry, R. 2014. VassarStats: Website for Statistical Computation. http://http://vassarstats.net.

Maryland Department of Natural Resources [MDNR]. 2005. Maryland Wildlife Diversity Conservation Plan. Department of Natural Resources Wildlife and Heritage Services, Annapolis, Maryland.

Miller, D., M. Gray, A. Storfer. 2011. Ecopathology of ranaviruses infecting amphibians. Viruses 3:2351–2373.

Mississippi Museum of Natural Science [MMNS]. 2005. Mississippi's Comprehensive Wildlife Conservation Strategy. Mississippi Department of Wildlife, Fisheries and Parks, Mississippi Museum of Natural Science, Jackson, Mississippi.

North Carolina Wildlife Resources Commission [NCWRC]. 2014. Protected Wildlife Species of North Carolina. North Carolina Wildlife Resources Commission, Raleigh, North Carolina.

Ohio Department of Natural Resources [ODNR]. 2014. Ohio's Endangered Species. Ohio Department of Natural Resources Division of Wildlife Publication 5356: Columbus, Ohio.

Parker, J. M., I. Mikaelian, N. Hahn, and H. E. Diggs. 2002. Clinical diagnosis and treatment of epidermal chytridiomycosis in African clawed frogs (*Xenopus tropicalis*). Comp. Med. 52:256–268.

Pennsylvania Game Commission and Pennsylvania Boat and Fish Commission [PGCPBFC]. 2005. Pennsylvania's State and Wildlife Commission, Harrisburg, Pennsylvania.

Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institute Press, Washington, DC. 587 pp.

Rooji, P. A., A. Martel, J. Nerz, S. Voitel, F. V. Immerseel, F. Haesebrouck, and Pasmans. 2011. Detection of *Batrachochytrium dendrobatidis* in Mexican bolitoglossine salamanders using an optimal sampling protocol. EcoHealth 8:237–243.

Rossell, C. R., J. Hicks, L. A. Williams, and S. C. Patch. 2009. Attributes of rock crevices selected by green salamanders, *Aneides aeneus*, on the Blue Ridge escarpment. Herpetol. Rev. 40:151–153.

Snyder, D. H. 1991. The green salamander (*Aneides aeneus*) in Tennessee and Kentucky, with comments on the Carolinas' Blue Ridge populations. J. Tennessee Acad. Sci. 66:165–169.

Sodhi, N. S., D. Bickford, A. C. Diesmos, T. M. Lee, L. P. Koh, B. W. Brook, C. H. Sekercioglu, and C. J. A. Bradshaw. 2008. Measuring the meltdown: drivers of global amphibian extinction and decline. PLoS ONE 3:e1636.

South Carolina Department of Natural Resources [SCDNR]. 2005. Comprehensive Wildlife Conservation Strategy. South Carolina Department of Natural Resources, Columbia, South Carolina.

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.

Sutton, W. B., M. J. Gray, J. T. Hoverman, R. G. Secrist, P. E. Super, R. H. Hardman, J. L. Tucker, and D. L. Miller. 2014. Trends in ranavirus prevalence among plethodontid salamanders in the Great Smoky Mountains National Park. EcoHealth doi: 10.1007/s10393-014-0994-z.

Tennessee Wildlife Resource Agency [TWRA]. 2005. Tennessee's Comprehensive Wildlife Conservation Strategy. TWRA Wildlife Technical Report 05-08: Nashville, Tennessee.

Thigpen, T. F., W. J. Humphries, and J. C. Marez. 2010. Effectiveness of using burlap bands to sample arboreal green salamander populations in the Blue Ridge Mountains of Georgia and North Carolina. Herpetol. Rev. 41:159–162.

Virginia Department of Game and Inland Fisheries [VDGIF]. 2014. Virginia Fish and Wildlife Information Service Database, Virginia Department of Game and Inland Fisheries, Richmond, Virginia. Available at http://www.apsu.edu/reptatlas/, accessed 10 April 2015.

———. 2005. Virginia's Comprehensive Wildlife Strategy. Virginia Department of Game and Inland Fisheries, Richmond, Virginia.

Waldron, J. L., and W. J. Humphries. 2005. Arboreal habitat use by the green salamander, *Aneides aeneus*, in South Carolina. J. Herpetol. 39:486–492.

Weinstein, S. B. 2009. An aquatic disease on a terrestrial salamander: individual and population level effects of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, on *Batrachoseps attenuatus* (Plethodontidae). Copeia 2009:653–660.

West Virginia Division of Natural Resources [WVDNR]. 2005. West Virginia Wildlife Action Plan. West Virginia Division of Natural Resources Wildlife Resources Section, Charlestown, West Virginia.

Wildlife and Freshwater Fisheries Division, Alabama Department of Conservation and Natural Resources [WFFDADCNR]. 2005. Conserving Alabama's wildlife: a comprehensive strategies. Alabama Department of Conservation and Natural Resources, Montgomery, Alabama.

Young, B. E., S. N. Stuart, J. S. Chanson, N. A. Cox, and T. M. Boucher. 2004. Disappearing Jewels: The Status of New World Amphibians. NatureServe, Arlington, Virginia.

Young, S., L. F. Skerratt, D. Mendez, R Speare, L. Berger, and M. Steele. 2012. Using community surveillance data to differentiate between emerging and endemic amphibian diseases. Dis. Aquat. Org. 98:1–10.