

Short Communication

High Occupancy of Stream Salamanders Despite High *Ranavirus* Prevalence in a Southern Appalachians Watershed

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Abstract: The interactive effects of environmental stressors and emerging infectious disease pose potential threats to stream salamander communities and their headwater stream ecosystems. To begin assessing these threats, we conducted occupancy surveys and pathogen screening of stream salamanders (Family Plethodontidae) in a protected southern Appalachians watershed in Georgia and North Carolina, USA. Of the 101 salamanders screened for both chytrid fungus (*Batrachochytrium dendrobatidis*) and *Ranavirus*, only two exhibited low-level chytrid infections. Prevalence of *Ranavirus* was much higher (30.4% among five species of *Desmognathus*). Despite the ubiquity of ranaviral infections, we found high probabilities of site occupancy (≥ 0.60) for all stream salamander species.

Keywords: *Desmognathus*, *Eurycea*, *Gyrinophilus*, occupancy, monitoring, disease surveillance

INTRODUCTION

Two emerging pathogens of amphibians—*Batrachochytrium dendrobatidis* (*Bd*) and *Ranavirus* (*Rv*)—have been detected at relatively protected sites in the southern Appalachian Mountains (Dodd 2004; Rothermel et al. 2008; Gray et al. 2009a), with unknown consequences for the host species and their headwater ecosystems. *Bd* is a specialized, pathogenic fungus in the Phylum Chytridi-

omycota that infects the keratinized tissues of amphibians (Berger et al. 1998; Longcore et al. 1999). Catastrophic die-offs of amphibians attributable to *Bd* have occurred in montane regions of Central America (Lips et al. 2003) and western North America (Daszak et al. 1999; Rachowicz et al. 2006; Vredenburg et al. 2010). In contrast, there have been very few cases of *Bd*-associated mortality in the eastern United States (e.g., Todd-Thompson et al. 2009; Bakkegard and Pessier 2010), despite high prevalence of *Bd* in ranids, hylids, and salamandrids (Longcore et al. 2007; Rothermel et al. 2008). Ranaviral disease has caused many more mass mortality events of amphibians in the eastern United States, mostly involving ranid frogs and ambystomatid salamanders (Green et al. 2002; Dodd 2004;

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Petranka et al. 2007; Gray et al. 2009b). Three of the six described species of *Rv* (Family Iridoviridae) can infect amphibians, and some genetically unique isolates may have enhanced virulence (Gray et al. 2009b). *Rv* is known to have a broad host range, but was only recently detected in plethodontid salamanders at stream sites in Great Smoky Mountains National Park (Gray et al. 2009a).

Although stream-breeding salamanders are sensitive to anthropogenic habitat disturbance and climate change (Willson and Dorcas 2003; Lowe 2012), intensive monitoring is required to assess the impacts of environmental stressors and emerging infectious disease. Toward that end, we completed an initial assessment of pathogen prevalence and site occupancy of stream salamanders in the Upper Tallulah River watershed (UTRW) in northeastern Georgia and southwestern North Carolina. Although this watershed was heavily disturbed by logging, mining, and other

activities in the early twentieth century (Wharton 1978), most of the UTRW has been protected as wilderness area (managed by the U.S. Forest Service) since 1984. At least nine species of plethodontids associated with streams and other aquatic habitats occur in the UTRW.

We employed an occupancy estimation approach to account for imperfect detection and generate species-specific estimates of the proportion of area occupied (MacKenzie et al. 2002). From 1 May to 23 August 2010, we surveyed 27 sites in the UTRW, including several first-order (i.e., headwater) streams, as well as larger second- and third-order streams (Fig. 1). Each site was surveyed one time, with three replicate plots per site serving as the repeated sample for estimating detection probabilities (MacKenzie et al. 2006). Observers completed two temporary removal passes of each 2×8 -m plot, turning rocks and other cover objects and capturing salamanders with

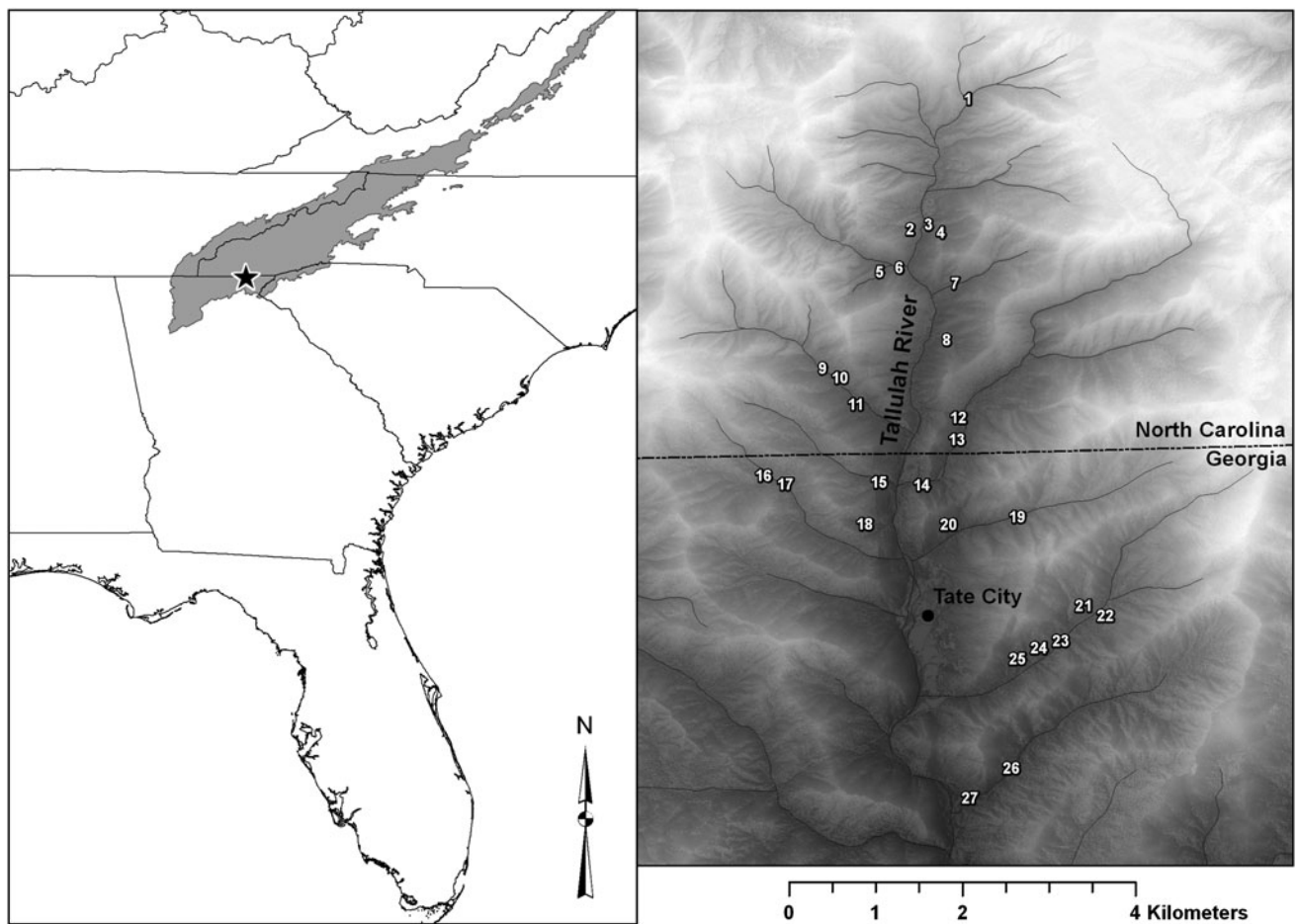


Figure 1. Location of the Upper Tallulah River watershed within the Appalachian Highlands region in the southeastern U.S. (black star; left panel) and approximate locations of 27 sites (right) surveyed for stream salamanders in May–August 2010. The watershed encompasses areas within Clay County, North Carolina (13 sites), Towns County, Georgia (seven sites), and Rabun County, Georgia (seven sites). Multiple sites on the same stream were separated by ≥ 100 m and all sites were ≥ 15 m from trail crossings and ≥ 50 m upstream of any roads.

Table 1. Naïve Occupancy (i.e., Proportion of Sites) and Estimated Site Occupancy (Ψ) and Detection Probability (p) for Stream Salamanders in the Upper Tallulah River Watershed in Georgia and North Carolina.

Species	Prop. of sites occupied	$\Psi \pm SE$	$p \pm SE$
Black-bellied salamander (<i>Desmognathus quadramaculatus</i>)	1.00	–	–
Seal salamander (<i>D. monticola</i>)	0.96	0.9767 \pm 0.0379	0.7584 \pm 0.0517
Ocoee salamander (<i>D. ocoee</i>)	0.89	0.9611 \pm 0.1194 ^a	0.5780 \pm 0.0686
Dwarf black-bellied salamander (<i>D. folkertsi</i>)	0.70	0.7478 \pm 0.0972	0.6109 \pm 0.0746
Shovel-nosed salamander (<i>D. marmoratus</i>)	0.59	0.5987 \pm 0.1245 ^a	0.7836 \pm 0.0627
Carolina spring salamander (<i>Gyrinophilus porphyriticus dunni</i>)	0.70	0.9007 \pm 0.1557	0.3975 \pm 0.0858
Blue Ridge two-lined salamander (<i>Eurycea wilderae</i>)	0.85	0.8958 \pm 0.0761	0.6340 \pm 0.0661

Estimates shown here are from the model with constant occupancy and constant detection, which was the highest ranked model for every species; see Table A1 of the online Appendix for a comparison of results from all candidate models, including their associated Akaike's information criteria and Akaike weights.

^aStandard error was corrected for overdispersion because $\hat{c} > 1.0$ (Donovan and Hines 2007).

dipnets (see online Appendix for details). We used Program PRESENCE (version 4.2) to estimate site occupancy (Ψ) and detection probability (p) over one season for species captured at more than one site (see online Appendix for detailed statistical methods).

Every captured salamander was visually examined to confirm species identification and detect gross signs of disease. To determine prevalence of *Bd* and *Rv*, we collected samples from 92 postmetamorphic *Desmognathus* spp. (15–20 haphazardly chosen individuals of each species, from 20 sites), eight larval Carolina spring salamanders (*Gyrinophilus porphyriticus dunni*; 5 sites) and one adult Blue Ridge two-lined salamander (*Eurycea wilderae*). A skin swab (of ventral surfaces and hind feet), toe-clip (one toe from the right hind foot), and tail-clip from each individual were preserved in separate vials containing 70% ethanol. Skin-swab samples were tested for *Bd* using polymerase chain reaction (PCR)-based assays (Annis et al. 2004). Because we expected low levels of *Bd* infection in desmognathine salamanders, we tested toe-clips from the same individuals using real-time PCR (Boyle et al. 2004). The tail-clip samples were screened for *Rv* using conventional PCR (Mao et al. 1996, 1997; see online Appendix for details).

The two most common species detected were black-bellied salamanders (*Desmognathus quadramaculatus*) and seal salamanders (*D. monticola*; Table 1). Most captures of Blue Ridge two-lined salamanders and all but one capture of Carolina spring salamanders were larvae. Because black-bellied salamanders were captured at every site, there was logically no way to improve the occupancy estimate by accounting for detection. Occupancy estimates (Ψ) for the remaining stream-associated species were high, ranging from 0.5987 to 0.9767 (Table 1). Additional species

encountered at fewer than three sites included two adult seepage salamanders (*D. aeneus*; sites 3 and 19), three larval three-lined salamanders (*Eurycea guttolineata*; site 7), one juvenile red-spotted newt (*Notophthalmus viridescens viridescens*; site 3), and several postmetamorphic American bullfrogs (*Lithobates catesbeianus*; sites 15, 19, and 21).

We tested 101 salamanders from 20 sites in 11 major stream drainages for both *Bd* and *Rv* (Table A2). Although *Bd* was not detected in any swab samples using conventional PCR, an adult Ocoee salamander (site 17) and a juvenile black-bellied salamander (site 23) were positive by qPCR on the hind toe; average Ct values were 34 and 37, respectively, suggesting low infection intensity. Thus, *Bd* prevalence was 2.2% among *Desmognathus* spp. ($n = 92$; 95% CI: 0.3–7.6%). *Rv* was detected in five species of *Desmognathus*, with an overall prevalence of 30.4% ($n = 92$; 95% CI: 21.3–40.9%; Fig. 2).

None of the 1,288 salamanders captured during our surveys exhibited morbidity or gross lesions, but on a return visit to site 14 in September 2010, we found an adult black-bellied salamander that was lethargic and sitting in an exposed position. It subsequently died after two days in captivity. Tissues were PCR-positive for *Rv* and histopathological examination of the liver revealed vacuolar degeneration of cells, increased numbers of melanomacrophage centers, and rare intracellular structures consistent with viral inclusion bodies.

Most previous surveys in the southern Appalachians have failed to detect *Bd* in stream salamanders (e.g., Chinnadurai et al. 2009; Hossack et al. 2010; Conor Keitzer et al. 2011). The prevalence of *Bd* infection among *Desmognathus* spp. in the UTRW was only 2.2%, similar to the low prevalence reported for Ocoee salamanders in south-

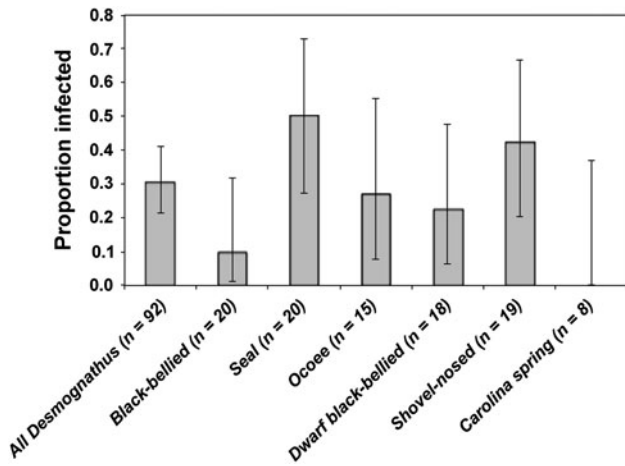


Figure 2. *Ranavirus* prevalence (\pm 95% CI) in six species of stream salamanders (and in the five species of *Desmognathus* combined) in the Upper Tallulah River watershed, based on PCR assays of tail-clips collected in May–June 2010. Given limited resources for PCR testing, we prioritized screening of postmetamorphic *Desmognathus* spp.; additional sampling would be needed to obtain reliable estimates of prevalence in Carolina spring salamanders and Blue Ridge two-lined salamanders (not shown; the one individual we tested was *Rv*-negative).

western North Carolina (5.6%; Kiemiec-Tyburczy et al. 2012) and seal salamanders and northern dusky salamanders (*D. fuscus*) in northern Virginia (3.4%; Gratwicke et al. 2011) and in Virginia and Maryland (4.8%; Hossack et al. 2010). The low prevalence of *Bd* in these field studies and the low infection intensity found in this study are consistent with laboratory experiments showing resistance to infection in *Desmognathus* spp. (Chinnadurai et al. 2009; Vazquez et al. 2009). Cutaneous bacteria prevent morbidity associated with *Bd* infection in red-backed salamanders (*Plethodon cinereus*; Becker and Harris, 2010), but it is unknown whether desmognathine salamanders possess similar skin defenses. It should be noted that higher *Bd* prevalence has been observed in desmognathine salamanders at sites in Maryland (13.4%; Grant et al. 2008) and southwestern Virginia (17.9%; Davidson and Chambers 2011). Our sampling was conducted in May–June, when *Bd* prevalence tends to be high in pond-breeding species in our study area (Rothermel et al. 2008), so the low prevalence in stream salamanders is not attributable to timing of sampling.

Detection of *Rv* in five species and 10 of 11 stream drainages with only minimal sampling suggests this pathogen is ubiquitous even within this relatively protected watershed. Furthermore, our study may have underestimated

prevalence, given false-negative rates of \sim 20% for PCR testing of tail-clips (Gray et al. 2012). The only other studies of *Rv* in aquatic salamanders have found higher prevalence at some sites in eastern Tennessee (Gray et al. 2009a; Souza et al. 2012). We do not know if *Rv* was recently introduced or is endemic to our study area, though our observations are more consistent with pathogen and host species having co-existed long enough to allow for evolution of reduced virulence, enhanced immunity, or both. All but one of the *Rv*-positive salamanders had sub-clinical infections. In contrast, during 2008–2010, we detected *Rv* in red-spotted newts and observed recurring morbidity and mortality consistent with ranaviral disease in larval ranids inhabiting ponds in the Tallulah River valley (unpubl. data). As noted earlier, we observed postmetamorphic American bullfrogs and red-spotted newts using streamside habitats in our study area; both species likely serve as reservoirs for *Rv* and *Bd* (Daszak et al. 2004; Garner et al. 2006; Hoverman et al. 2012) and could potentially facilitate spread of pathogens from pond to stream habitats (Grant et al. 2008).

Streams in the UTRW still appear to support robust populations of all species of salamanders known to occur in this area historically, based on specimens deposited in the Georgia Museum of Natural History, Athens, Georgia, in 1961–1968. Despite high prevalence of *Rv* (Fig. 2), naïve occupancy was \geq 0.59 for all seven stream-associated species (Table 1). Three-lined salamanders and seepage salamanders typically do not breed in or inhabit fast-flowing streams (Petranka 1998), which explains why they were rarely detected at our sites.

Genetic sequencing of *Rv* from our study area is needed to determine if it is similar to *Frog virus 3* (FV3) or a novel isolate. Infection with an FV3-like strain (rather than *Ambystoma tigrinum* virus) would be consistent with sequencing results for isolates from salamanders in Great Smoky Mountains National Park, including plethodontids (Gray et al. 2009a) and ambystomatid larvae collected during a localized die-off in 2009 (Waltzek, University of Florida, pers. comm.; Todd-Thompson 2010). Available data suggest a high rate of sublethal *Rv* infections in stream salamander communities, but controlled experimental studies are needed to clarify fundamental questions about routes of transmission and other aspects of host-pathogen dynamics. Once the UTRW strain has been isolated and sequenced, there are many avenues for future research, including reciprocal exposure studies (e.g., Schock et al. 2009; Hoverman et al. 2011) to explore localized adaptation

of hosts and pathogen and the susceptibility of different species to this presumably endemic strain versus novel strains.

Because the UTRW is largely protected from habitat destruction, any future declines of stream salamanders could signal broad environmental changes. Based on their modeling of future suitable climatic habitat for 41 species of plethodontid salamanders, Milanovich et al. (2010) predicted imminent declines of salamander species richness along the southeastern edge of the Appalachians. Such declines could be precipitous if rising temperatures or other environmental stressors were to alter salamander immune responses, making them more susceptible to *Ranaviruses* or other pathogens (Raffel et al. 2006). Thus, we see a great need for development of a broader salamander monitoring program in the southern Appalachians and for integrating disease surveillance into population monitoring.

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