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Original Contribution

Trends in Ranavirus Prevalence Among Plethodontid Salamanders in the Great Smoky Mountains National Park

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Abstract: Emerging pathogens are a potential contributor to global amphibian declines. Ranaviruses, which infect ectothermic vertebrates and are common in aquatic environments, have been implicated in die-offs of at least 72 amphibian species worldwide. Most studies on the subject have focused on pool-breeding amphibians, and infection trends in other amphibian species assemblages have been understudied. Our primary study objective was to evaluate hypotheses explaining ranavirus prevalence within a lungless salamander assemblage (Family Plethodontidae) in the Great Smoky Mountains National Park, USA. We sampled 566 total plethodontid salamanders representing 14 species at five sites over a 6-year period (2007–2012). We identified ranavirus-positive individuals in 11 of the 14 (78.6%) sampled species, with salamanders in the genus *Desmognathus* having greatest infection prevalence. Overall, we found the greatest support for site elevation and sampling year determining infection prevalence. We detected the greatest number of infections in 2007 with 82.5% of sampled individuals testing positive for ranavirus, which we attribute to record drought during this year. Infection prevalence remained relatively high in low-elevation sites in 2008 and 2009. Neither body condition nor aquatic dependence was a significant predictor of ranavirus prevalence. Overall, our results indicate that life history differences among species play a minor role determining ranavirus prevalence compared to the larger effects of site elevation and yearly fluctuations (likely due to environmental stressors) during sampling years.

Keywords: amphibians, lotic, pathogen, surveillance

INTRODUCTION

Global-scale population declines have been noted for many taxa, with amphibians displaying some of the highest rates of extinction (Collins and Storfer 2003; Stuart et al. 2004). Although primary drivers behind many species' declines include habitat destruction and alteration (e.g., Barrett and Guyer 2008) as well as global climate change (Kiesecker et al. 2001), emerging infectious diseases are also recognized as a major contributor (Mendelson et al. 2006; Blaustein et al. 2012). Throughout the last two decades,

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amphibian biologists have largely focused on the emergence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), which has played a primary role in dramatic declines of amphibian species with small geographic ranges in high-elevation environments of the Neotropics, Australia, and the western United States (Lips et al. 2006; Wake and Vredenburg 2009). A recently discovered salamander chytrid fungus (*B. salamandrivorans*) represents an emerging pathogen that may threaten salamander populations globally (Martel et al. 2013, 2014). In addition to these chytrid threats, there are other important amphibian diseases that have been understudied (Whitfield et al. 2013).

Ranavirus (Family Iridoviridae) represents another important pathogen that has been implicated in amphibian die-offs throughout the world (Miller et al. 2011), including North America (Petranka et al. 2007; Brunner et al. 2011; Hoverman et al. 2012), South America (Fox et al. 2006), Europe (Balseiro et al. 2009; Teacher et al. 2010), and Asia (Une et al. 2009, Geng et al. 2011). These double-stranded DNA viruses infect ectothermic vertebrates, including fish, amphibians, and reptiles associated with aquatic habitats (Gray et al. 2009a). Overall, pond-breeding anurans in the family Ranidae and species with rapidly developing larvae appear to be most susceptible to infection from ranavirus (Hoverman et al. 2011).

To date, most ranavirus surveillance research has included single survey or die-off events, with few long-term examinations of infection trends (Miller et al. 2011). In addition, the majority of surveillance efforts have focused on anurans (Miller et al. 2011), with only a few examining infection trends in salamander populations (e.g., Brunner et al. 2004, Gray et al. 2009b, Sousa et al. 2012; Rothermel et al. 2013). It is important to evaluate infection trends within plethodontid salamanders, as many of these species have different physiological requirements (i.e., lunglessness, varying levels of terrestriality) compared with pool-breeding amphibian species, which may have a large influence on infection prevalence. Second, plethodontid salamanders contribute significantly to ecological function in forested ecosystems. Specifically, plethodontid salamanders (1) are key components of detrital food webs in forest floor communities and top predators in primary and secondary stream ecosystems (Wyman 1998; Davic and Welsh 2004), (2) reside in high densities in many forested environments and provide an abundant food source for other predatory vertebrates (Petranka and Murray 2001; Davic and Welsh 2004), and (3) are indicators of environmental quality due to physiological adaptations, including moist, permeable skin, and species-specific temperature tolerances (Feder 1983; Wells 2007) that link them physiologically to changes in environmental conditions (Welsh and Droege 2001). Given that some plethodontid salamander species have experienced recent population declines (Corser 2001; Caruso and Lips 2013; Kroschel et al. 2014), it is crucial to evaluate if ranavirus may play a role in further declines of plethodontid salamanders (Gray and Miller 2013).

The primary objective of this study was to examine potential hypotheses explaining ranavirus prevalence in a southern Appalachian plethodontid salamander assemblage. Previous research suggests that ranavirus prevalence in amphibian assemblages may be determined by multiple factors, including elevation gradients (Gray et al. 2009b), individual variation in body condition (St-Amour et al. 2010), natural history differences (e.g., length of larval period; Hoverman et al. 2011), and natural variation among sampling years (Hoverman et al. 2012). Our study contributes to the findings of Rothermel et al. (2013) in that we used an information-theoretic approach to evaluate multiple hypotheses of ranavirus prevalence over a 6-year period (2007–2012) in a large assemblage (i.e., 14 species) of plethodontid salamanders.

Materials and Methods

Study Site Description

We conducted this study in the Great Smoky Mountains National Park (GSMNP), which is centrally located in the Blue Ridge physiographic province at the intersection between eastern Tennessee and western North Carolina, USA (Fig. 1). At nearly 210,875 ha, the GSMNP represents one of the largest national parks east of the Mississippi River in North America and was designated as an International Biosphere Reserve in 1973. The unique climate, elevation gradients, and forest communities of the GSMNP provide habitat for a wide variety of plant and animal species, including 43 amphibian species. Plethodontid salamanders are the most speciose and represent the greatest biomass of any amphibian family within the GSMNP (Tilley and Huheey 2001).

We surveyed five sites spanning an elevation range of 514–1,605 m (Fig. 1) over a 6-year period from 2007–2012. Three sites (Ash Hopper Branch [514 m], Chimney Tops Seep [823 m], and Indian Gap Seep [1,605 m]) were sampled once yearly from 2007 to 2012 and two sites (Cable

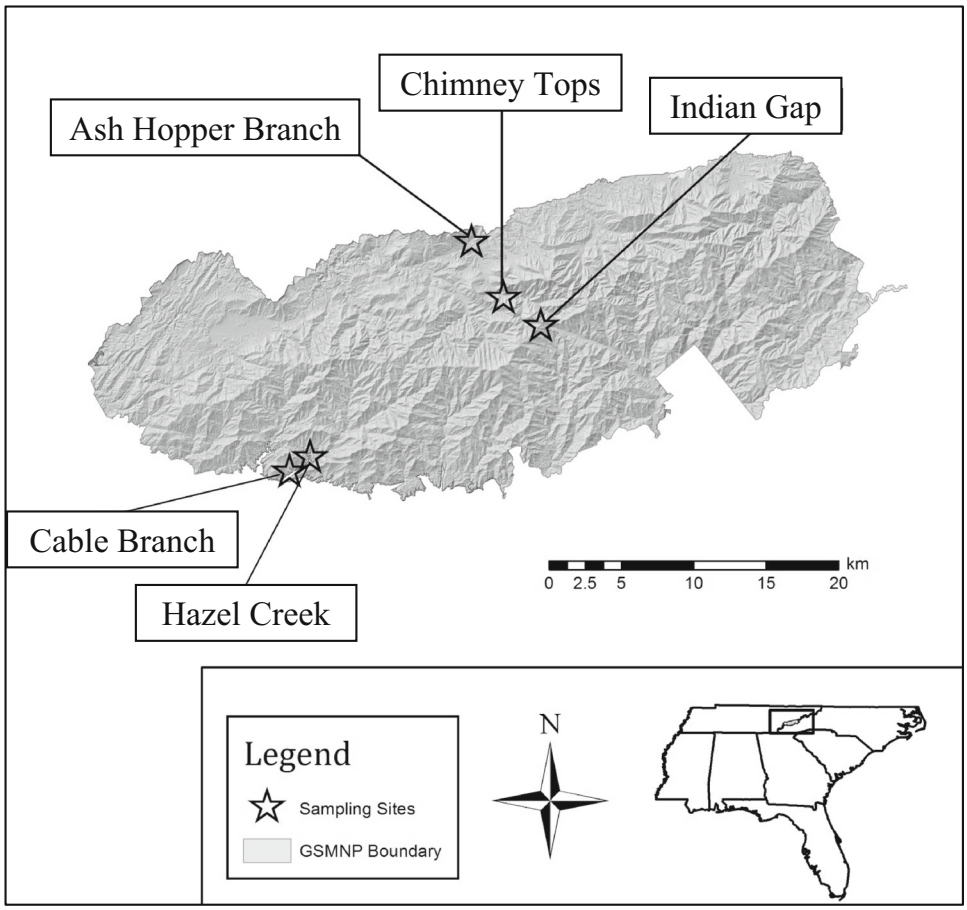

Figure 1. Location of salamander sampling sites to examine trends in ranavirus prevalence over a 6-year period (2007–2012) in the Great Smoky Mountains National Park, USA. Small *inset box* represents the location of the Great Smoky Mountains National Park in the southeastern United States of America

Branch [587 m] and Hazel Creek [537 m]) were sampled once in 2008. All sites were associated with lotic streams and seeps with a range of human access, including camping, nature instruction, and biological research. All sites were generally sampled during spring (e.g., mid-April).

Salamander Sampling

During sampling events, we surveyed salamanders opportunistically for a maximum of one hour or until we captured a maximum of 45 salamanders at each site. We generally sampled a maximum of 15 individuals per species and attempted to obtain samples from as many salamander species as possible at a given site. We captured salamanders by turning over cover objects (e.g., rocks, logs, leaf-packs) in both the aquatic and adjacent terrestrial environments. To prevent cross-contamination among captured individuals, all researchers wore disposable nitrile gloves during surveys. Captured salamanders were placed individually in plastic 2-L containers or plastic bags, and researchers that searched for salamanders did not process individuals. Captured salamanders were brought to an aseptic processing station, rinsed with distilled water, and placed in a clean, sealable plastic bag. We identified each salamander to species and measured snout-vent length (SVL; mm) and mass (g) with a dial caliper and Pesola® scale, respectively. We collected a small (<5 mm) tail clip from each salamander using sterilized stainless steel forceps and stored each tissue sample in an individual 2-mL microcentrifuge tube that contained 95% ethanol. We changed gloves after each salamander was processed and released salamanders near their original point of capture. We disinfected and rinsed containers, equipment, and footwear using 1% Nolvasan® (2% chlorhexidine diacetate AI; Fort Dodge Animal Health, Fort Dodge, IA, USA) to prevent the spread of pathogens among sites (Bryan et al. 2009).

Ranavirus Testing

All tissue samples were transported to the University of Tennessee Joe Johnson Animal Research and Teaching Unit for genomic DNA (gDNA) extraction and subsequent Polymerase Chain Reaction (PCR) testing. We extracted gDNA from individual tail tissue samples using a DNeasy

Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA), and concentrated the extracted DNA to 50 µL using a Savant DNA120 SpeedVac Concentrater (Thermo Fisher Scientific, Pittsburgh, PA, USA). We used quantitative real-time PCR (qPCR) by means of an ABI 7900HT Fast Real-Time PCR System (Life Technologies Corporation; Grand Island, NY, USA) to detect ranavirus in each tissue sample following the methods described in Picco et al. (2007) and Hoverman et al. (2010). We scored a sample as infected if the qPCR critical threshold value was less than 30 based on standardized optimization with known infected samples. For each qPCR analysis, we ran each sample in duplicate along with two positive controls (i.e., cultured viral DNA [FV3-like strain] and viral DNA from a ranavirus-positive amphibian) and two negative controls (i.e., DNA from a ranavirus-negative amphibian and a sample containing only molecular grade water). Inasmuch as qPCR on tail clips can result in false negatives (Gray et al. 2012), our estimates of infection prevalence may be lower than true prevalence.

Statistical Analysis

We analyzed infection prevalence data using generalized linear mixed models (GLMMs) via the lme4 package in R v.3.02 (R Core Team 2013) to evaluate ranavirus infection trends throughout the 6-year study period. For all analyses, we created a binary dataset for the response variable, where 1 = detection and 0 = no detection of ranavirus DNA in a tail clip and modeled prevalence based on a binomial distribution and logit link function. We included sampling site elevation, salamander body condition index, and salamander aquatic dependency as fixed effects, and included site and sampling year as random effects in particular GLMMs. Across all analyses, we included site as a random effect in each model (except the year-only model) to test for the appropriate level of replication for fixed-effects at the site level. Overall, we completed three sets of analyses (explained in greater detail later), including (1) single-species analyses, (2) multi-species analyses across all sampling years (2007–2012), and (3) a multi-species analysis based on 2008 data only.

For respective analyses, we coded site elevation as low (1; 0–600 m), medium (2; >600–1,200 m), and high (3; >1,200 m). To estimate an index of body condition, we used the standardized residuals produced by regressing SVL by mass for all individuals within a given species where positive and negative residuals represented high and low body conditions, respectively (Schulte-Hostedde et al. 2005). We coded aquatic dependency on a scale of 1–3 as follows: (1) species that reside primarily in stream or streamside habitats (i.e., Black-bellied Salamander [*Desmognathus quadramaculatus*], Santeetlah Salamander [*D. santeetlah*], Seal Salamander [*D. monticola*] and Spotted Dusky Salamander [*D. conanti*]; (2) semi-terrestrial, streamside salamander species (i.e., Blue Ridge Spring Salamander [*Gyrinophilus porphyriticus danielsi*], Blue Ridge Two-lined Salamander [*Eurycea wilderae*], Imitator Salamander [*D. imitator*], and Ocoee Salamander [*D. ocoee*]); and (3) primarily terrestrial species (i.e., Red-cheeked Salamander [*Plethodon jordani*] and Pygmy Salamander [*D. wrighti*]). We consulted Petranka (1998) and Lannoo (2005) to determine categories of aquatic dependency for each species.

For single-species evaluations, we tested species with ≥10 occurrences of ranavirus, which included *D. quadramaculatus*, *D. imitator*, *D. santeetlah*, *D. conanti*, and *E. wilderae* (Table 1). We chose species with a minimum of 10 positive cases of ranavirus because it provided an adequate sample size to evaluate factors determining ranavirus prevalence among individual species. We used an information-theoretic approach (e.g., Burnham and Anderson 2002) to evaluate hypotheses explaining ranavirus prevalence. We evaluated five models (i.e., site; year; site and year; site and body condition; and site, year, and body condition) using Akaike's Information Criterion adjusted for small sample sizes (AIC_c) for each salamander species. We ranked candidate models based on AIC_c weights and model-averaged parameter estimates and unconditional variances for model terms with a $\Delta AIC_c < 2.0$ compared with the top-ranked model as recommended in Burnham and Anderson (2002). We also reported variance estimates for the random effects of site and year term to evaluate potential contributions for explaining prevalence.

For the second analysis, we combined infection history from all species with a minimum of 15 captures throughout the study period (2007–2012), which included ten species (*D. conanti*, *D. imitator*, *D. monticola*, *D. ocoee*, *D. quadramaculatus*, *D. santeetlah*, *D. wrighti*, *E. wilderae*, *G. porphyriticus danielsi*, and *P. jordani*; total $n = 539$). We chose species with a minimum of 15 captures as this provided a large enough sample size to calculate body condition scores within a given species and to permit analysis of species with and without infections. We evaluated nine total models, including singular models containing only site and year terms, along with all other additive

Table 1. Species and Number of Plethodontid Salamanders Detected During Terrestrial and Aquatic Stream Surveys in the Great Smoky Mountains National Park, 2007–2012

Scientific name	Common name	Sites detected	Number infected	Number sampled	Percent infected
Desmognathus conanti	Spotted Dusky Salamander	AH*, CB*, CT*, HC*	22	73	30.1
Desmognathus imitator	Imitator Salamander	CT*, IG*	16	78	20.5
Desmognathus marmoratus	Shovel-nosed Salamander	HC*	1	1	100
Desmognathus monticola	Seal Salamander	AH*, CB*, HC*, IG	7	17	41.2
Desmognathus ocoee	Ocoee Salamander	CT*, IG*	4	31	12.9
Desmognathus quadramaculatus	Black-bellied Salamander	AH*, CT*, IG*	16	73	21.9
Desmognathus santeetlah	Santeetlah Dusky Salamander	AH, CT*, IG*	13	83	15.7
Desmognathus wrighti	Pygmy Salamander	CT*, IG	1	25	4
Eurycea guttolineata	Three-lined Salamander	CB	0	1	0
Eurycea wilderae	Blue Ridge Two-lined Salamander	AH*, CT*, CB, HC*, IG*	16	86	18.6
Gyrinophilus porphyriticus danielsi	Blue Ridge Spring Salamander	AH, CT*, IG	1	25	4
Plethodon jordani	Red-cheeked Salamander	CT*, IG*	6	67	9.0
Plethodon serratus	Southern Red-backed Salamander	CT	0	3	0
Plethodon ventralis	Southern Zigzag Salamander	AH	0	3	0
Total			103	566	NA

Site abbreviations are as follows: *AH* Ash Hopper Branch, *CB* Cable Branch, *CT* Chimney Tops, *HC* Hazel Creek, *IG* Indian Gap. Species common and scientific names follow those designated in Crother (2012).

* Indicate sites where a positive case of ranavirus was confirmed for a given species.

combinations of site, sampling year, body condition, and aquatic dependency. As with the single-species comparisons, we used AIC_c weights to evaluate the relative fit of each model and calculated model-averaged parameter estimates and unconditional variances where appropriate.

Lastly, we tested infection data from sites sampled in 2008 only, which included Ash Hopper Branch, Chimney Tops, Indian Gap, Hazel Creek, and Cable Branch for species with $\geq$15 captures throughout the 6-year study period. This additional analysis permitted us to test elevation as a fixed effect. We tested three models, including site and elevation, site and body condition, and site and species aquatic dependency. The low sample size (five total sites in 2008 only) prevented us from testing singular fixed effects of elevation, body condition, and species terrestriality.

Results

Over the course of the study, we collected demographic and ranavirus infection data from 566 plethodontid salamanders representing 14 species (Table 1). *Desmognathus quadramaculatus*, *D. conanti*, *E. wilderae*, *D. santeetlah*, *D. imitator*, and *P. jordani* represented 81.2% of the total salamander captures during the study (Table 1). Besides the shovel-nosed salamander (*D. marmoratus*), which was limited to one capture, *D. monticola* had the greatest infection prevalence (41.2%). For species with greater than 20 captures, *D. conanti* and *D. quadramaculatus* had the greatest infection prevalence at 30.1% and 21.9%, respectively, whereas both *D. wrighti* and *G. porphyriticus danielsi* had the lowest infection prevalence at 4.0% (Table 1). Across genera, ranavirus prevalence was the greatest among *Desmognathus* (21.0%) and *Eurycea* (18.4%) and the lowest among *Plethodon* (8.2%) and *Gyrinophilus* (4.0%) genera.

For species with $\geq$10 positive cases of ranavirus, we observed the highest support for a model containing only the year term for *D. conanti* (ω_i = 0.86), *D. santeetlah* (ω_i = 0.80), and *E. wilderae* (ω_i = 0.82; Table 2). Variance estimates of the random year term for *D. conanti* and *D. santeetlah* were 11.0 $\pm$ 3.3 and 8.6 $\pm$ 2.9, respectively, which suggests a large effect of sampling year on infection prevalence. The variance estimate of the random year term for *E. wilderae* was also supported, but with a relatively high standard error (2.0 $\pm$ 1.5). Infection prevalence for *E. wilderae* and *D. santeetlah* was the greatest in 2007 (66.7% and 84.7%, respectively) and remained low throughout the remainder of the study (0–10.0% and 0–6.7%, respectively). Infection prevalence for *D. conanti* was the

Table 2. The highest Supported (ΔAIC <2.0) Predictive Models Describing Ranavirus Prevalence in a Plethodontid Salamander Assemblage in the Great Smoky Mountains National Park (2007–2012)

Species	Model	−2 LL[a]	K[b]	AIC_c[c]	ΔAIC_c[d]	ω_i[e]
Desmognathus conanti[f]	Year	37.50	2	43.21	0.00	0.86
Desmognathus imitator[f]	Site + Year	44.80	3	54.23	0.00	0.75
Desmognathus quadramaculatus[f]	Year	63.90	2	68.99	0.00	0.58
	Site + Year	62.40	3	70.80	1.81	0.24
Desmognathus santeetlah[f]	Year	39.80	2	45.00	0.00	0.80
Eurycea wilderae[f]	Year	55.70	2	60.79	0.00	0.82
All species 2007–2012[g]	Site + Year	284.88	3	302.51	0.00	0.57
	Site + Year + BC	293.10	4	304.18	1.66	0.25
All species 2008 only[g]	Site + Elev	67.50	3	97.50	0.00	0.98

Please see the statistical methods section for a description of model construction.

Year sampling year, *Site* sampling site, *Elev* survey site elevation, *BC* index of salamander body condition.

[a] −2 Log-likelihood derived from Generalized Linear Mixed Model output.

[b] Number of model parameters including random and fixed intercepts.

[c] Akaike's Information Criterion adjusted for small sample sizes.

[d] Relative difference between candidate models and model with the highest support.

[e] Relative model weight; higher values indicate higher support.

[f] Individual species with ≥10 cases of ranavirus.

[g] All 10 salamander species with ≥15 captures throughout the study period (see Table 1).

greatest in 2007 (92.9%) and 2009 (50.0%). Infection prevalence for *D. imitator* was best explained by a single model (ω_i = 0.75) that contained both sampling year and site elevation model terms (Table 2). Prevalence was greater for *D. imitator* at the mid-elevation site (26.8%) compared to the high-elevation site (4.5%), with the greatest prevalence in 2007 (84.6%). Prevalence for *D. quadramaculatus* was best explained by a model containing only sampling year (ω_i = 0.58) and a model containing site and sampling year (ω_i = 0.24; Table 2). Prevalence for *D. quadramaculatus* was the greatest in 2007 (87.5%) at the lowest elevation site (27.5%) throughout the study period compared with the mid-(9.1%) and high-(10.0%) elevation sites. Variance estimates for the random year term in the highest supported model for both *D. imitator* and *D. quadramaculatus* were 22.2 ± 4.7 and 3.7 ± 1.9, respectively, indicating a large effect of sampling year on prevalence.

For salamander species with ≥15 captures, we found the highest support for two models, including the Site + Year (ω_i = 0.57) and Site + Year + BC (ω_i = 0.25) models (Table 2). In the highest supported model, the variance estimate for the year term (4.28 ± 2.07) provided relatively good support for explaining prevalence. In 2007, infection prevalence was greatest (95.2%) at the low-elevation (i.e., Sugarlands) site (Fig. 2a). In 2008 and 2009, prevalence remained the greatest (38.5% and 29.2%, respectively) at the low-elevation site compared with the mid (Chimney Tops; 0% and 6.7%, respectively) and high-elevation (Indian Gap; 3.0% and 6.9%, respectively) sites (Fig. 2a). All sites had comparatively low infection prevalence in 2010 and 2012 (Fig. 2a); however, we documented a slight increase in prevalence during 2011 for all three sites (Fig. 2a). We also observed support for a model (ω_i = 0.25) containing site, year, and body condition model terms (Table 2). However, the body condition term was not well supported (β = –0.28 ± 0.21; CI −0.69, 0.13), suggesting that site and year model terms were responsible for relatively high support of the second model.

In the 2008 analysis, we found highest support (ω_i = 0.98) for the model containing the elevation and site terms (Table 2). We observed an effect of elevation (β = –2.41 ± 0.72; CI −3.8, −1.0) on ranavirus prevalence, with greater prevalence in low-elevation sites (43.8%) compared to the mid (0%) and high (3.0%) elevation sites.

We did not find statistical support for a relationship between body condition and infection prevalence for the single-species and multi-species comparisons (Table 2). However, we observed a negative trend between infection prevalence and body condition (Fig. 2b). Specifically, mean body condition (1.4 ± 0.1) was the greatest in 2010, which was one of the years where mean infection prevalence was the lowest (2.1 ± 1.2%) for salamander species with ≥15 captures (Fig. 2b).

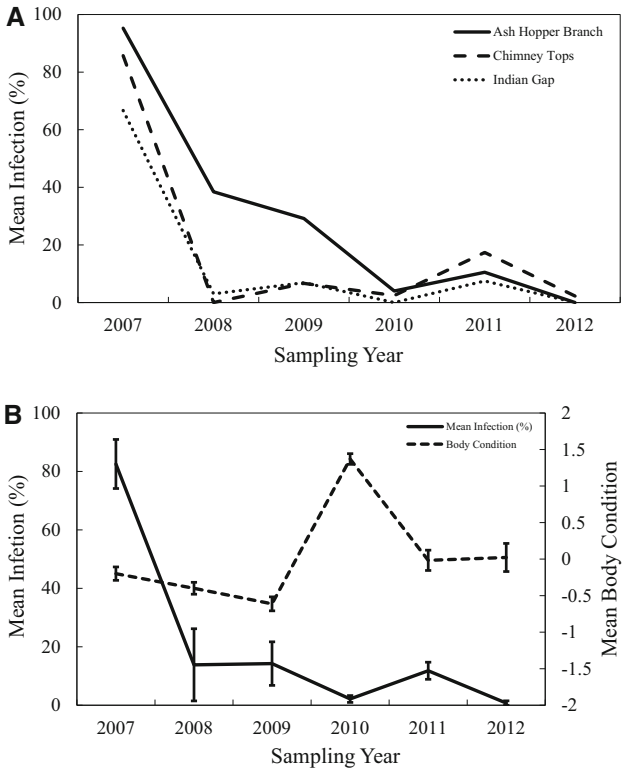

Figure 2. **a** Percent ranavirus prevalence in plethodontid salamanders at three sites (Ash Hopper Branch, Chimney Tops, and Indian Gap) in the Great Smoky Mountains National Park, USA (2007–2012). **b** Relationship between mean percent infection and body condition (±SE) from three sites (Ash Hopper Branch, Chimney Tops, and Indian Gap) in the Great Smoky Mountains National Park (2007–2012). The *solid line* represents percent infection and the *dotted line* represents average body condition. Salamander species in both figures had ≥15 captures (see Table 1)

Discussion

Our results illustrate that patterns of ranavirus infection in plethodontid salamander assemblages vary widely among years, and ecological and environmental factors may play potential roles in these patterns. We identified that yearly fluctuations, along with site differences (i.e., elevation), were important explanatory variables of ranavirus prevalence. We hypothesize that environmental conditions may have been responsible for the relatively high infection prevalence observed in 2007 (Gray et al. 2009b). Seasonal and annual variation in ranavirus prevalence is common (Hoverman et al. 2012), and it is likely that prevalence is impacted by abiotic and biotic factors. Environmental stressors such as drought and temperature extremes have been suggested as possible drivers of wildlife disease outbreaks (Acevedo-Whitehouse and Duffus 2009). In the GSMNP, rainfall amounts were 23.26 in (59.10 mm) below average in 2007 (www.ncdc.noaa.gov), making it one of the driest years over the previous 50 years. Drought may have reduced the availability of aquatic habitats, thereby increasing contact rates among individuals and ranavirus transmission, which has been shown to occur in a density-dependent manner (Greer et al. 2008). The role of ranavirus in amphibian communities during drought years needs greater investigation because climate change is projected to cause some geographic regions to become drier over the next century (Kundzewicz et al. 2008).

We detected a trend of salamander species having greater infection prevalence at low-elevation sites, which may have been due to greater virion concentration as a result of viral shedding upstream with accumulation downstream. A downstream gradient of increased concentration of pathogens has been documented elsewhere (Whitman et al. 1995; Byappanahalli et al. 2003). Inasmuch as infected amphibians are known to shed ranavirus virions and persistence in water may be greater than 1 month (Langdon 1989; Nazir et al. 2012), it is reasonable to hypothesize that the chance for contact with ranavirus in lotic environments would increase at lower elevations in a watershed as virions travel downstream (Gray et al. 2009b). In addition, two higher elevation sites (Chimney Tops and Indian Gap) with relatively lower occurrences of infection were primarily seep habitats with limited connection to lotic environments.

We found that salamanders in the genus *Desmognathus* and *Eurycea* tended toward greater ranavirus prevalence compared to salamanders in the genus *Plethodon* and *Gyrinophilus*. Salamanders in the genus *Desmognathus* and *Eurycea* are primarily aquatic and inhabit a variety of stream environments (Petranka 1998; Tilley and Huheey 2001). In addition, species in these genera typically have an aquatic larval stage that can last for a period from 6 months to three years (Petranka 1998). Ranaviruses often infect ectothermic vertebrates associated with aquatic environments, which is probably due to efficient transmission in water (Gray et al. 2009a; Hoverman et al. 2010). Indeed, species strongly associated with aquatic habitats, such as *D. monticola*, *D. conanti*, *D. quadramaculatus*, and *E. wilderae* had greater infection prevalence compared with more terrestrial species. Rothermel et al. (2013) found higher infection prevalence in *Desmognathus* salamanders compared to other salamander genera. Thus, terrestrial plethodontids (i.e., genus *Plethodon*) may be at lower risk of ranavirus infection compared to stream-dwelling species.

We observed that infection prevalence was relatively lower for *G. porphyriticus danielsi* (4.0% prevalence), which is also a species commonly encountered in streamside environments. Rothermel et al. (2013) also observed low ranavirus prevalence in this species, suggesting that some plethodontid species may be resistant to infection via ranavirus. Further research is necessary to evaluate whether ranavirus infection in plethodontid salamanders occurs on a phylogenetic basis, given that phylogenetic links have been established for infection and mortality among salamanders due to both *B. dendrobatidis* and *B. salamandrivorans* (Rovito et al. 2009; Martel et al. 2014).

We were unable to document a statistically significant relationship between body condition and ranavirus prevalence in plethodontid salamanders. St-Amour et al. (2010) identified a positive relationship between fluctuating asymmetry (a relative measure of stress and organismal condition) and ranavirus prevalence in Green Frogs (*Lithobates clamitans*). In addition, Davis et al. (2009) found that the prevalence of Rickettsial bacteria was greater in Eastern Red-backed Salamanders (*Plethodon cinereus*) with relatively greater body condition; however, they found that infection prevalence tended to be greater in male salamanders, which they attributed to gender-specific behavioral differences. Although we detected a negative trend between body condition and infection prevalence (specifically in 2010), the lack of a consistent trend across all years suggests that body condition may not serve as a good proxy for predicting infection prevalence. In addition, low-grade infections due to ranavirus may not result in an immediate reduction in body condition.

Although we detected ranavirus in 11 out of 14 (78.6%) plethodontid salamander species, population-level effects are unknown. Ranavirus susceptibility experiments for *E. wilderae* and four-toed salamander (*Hemidactylium scutatum*) larvae via water bath exposure resulted in 40% and 50% mortality, respectively (Gray and Miller, unpublished data). Hoverman et al. (2011) and Haislip et al. (2011) conducted laboratory experiments with over 20 species of amphibians and reported a strong correlation between infection prevalence and case mortality, which suggests that infection prevalence may be used to infer relative mortality rates (Gray and Miller 2013). If this is accurate, ranaviruses likely played a minor role in population dynamics of GSMNP plethodontid salamanders in most years during our study except for 2007 and 2008 when infection prevalence was the greatest (range 45–96%). As seen with the amphibian chytrid fungus (e.g., Whiles et al. 2006), emergence of a pathogen during a few years may significantly affect amphibian community structure and aquatic ecosystem function. As we have not witnessed amphibian morality which can be attributed to ranavirus, it remains unknown to what degree ranavirus is contributing to population fluctuations in the GSMNP.

Long-term studies are necessary to evaluate infection trends and identify potential drivers of infection in amphibian assemblages (Gray and Miller 2013). For example, Petranka et al. (2007) reported that ranavirus outbreaks caused amphibian die-offs over a 6-year period in at least 40% of the wetlands in a restored wetland complex—during several years, little to no recruitment was observed for two amphibian species (Petranka et al. 2003). In addition, Teacher et al. (2010) reported ranavirus as the primary cause of Common Frog (*Rana temporaria*) declines in the United Kingdom over a 28-year period. We encourage additional studies that track ranavirus prevalence in amphibian communities over multiple years. Properly designed surveillance studies should take a replicated site approach and attempt to account for influences of temporal, seasonal, ecological, and biological variables on disease prevalence. Although our study was able to account for temporal (year), ecological (elevation), and biological (body condition and aquatic dependency) effects on ranavirus prevalence, our lack of site and seasonal replication limited the inference of our study. Long-term, replicated studies can be useful in determining if ranavirus is emerging and to inform disease intervention strategies. Our results indicate that ranavirus is present in the GSMNP and that prevalence can vary greatly by year. In addition, life history differences and body condition appear to play a minimal role in ranavirus prevalence when viewed in the larger context of elevation gradients and prevailing environmental conditions in a given sampling year.

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