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LETTER

Conservation risk of *Batrachochytrium salamandrivorans* to endemic lungless salamanders

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Abstract

The emerging fungal pathogen, *Batrachochytrium salamandrivorans* (*Bsal*), is a significant conservation threat to salamander biodiversity in Europe, although its potential to affect North American species is poorly understood. We tested the susceptibility of two genera (*Eurycea* and *Pseudotriton*) and three populations of lungless salamanders (*Plethodontidae*) to *Bsal*. All species became infected with *Bsal* and two (*Pseudotriton ruber* and *Eurycea wilderae*) developed chytridiomycosis. We also documented that susceptibility of *E. wilderae* differed among populations. Regardless of susceptibility, all species reduced feeding when exposed to *Bsal* at the highest zoospore dose, and *P. ruber* and one population of *E. wilderae* used cover objects less. Our results indicate that *Bsal* invasion in eastern North America could have significant negative impacts on endemic lungless salamander populations. Future conservation efforts should include surveillance for *Bsal* in the wild and in captivity, and championing legislation that requires and subsidizes pathogen-free trade of amphibians.

KEYWORDS

amphibian, biodiversity, chytrid, conservation, disease, fungus, invasion, pathogen

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1 | INTRODUCTION

Emerging infectious diseases are a growing conservation concern, ranking among the top drivers of global species extinctions (Smith, Sax, & Lafferty, 2006). Leading among them are diseases caused by fungal pathogens (Fisher et al., 2012). For example, *Pseudogymnoascus destructans*, the causative agent of white nose syndrome (WNS), has resulted in precipitous declines of bats in North America since its introduction from Europe in 2006 (Turner, Reeder, & Coleman, 2011), with broad impacts on agricultural and wild systems (Boyles, Cryan, McCracken, & Kunz, 2011; Kunz, de Torrez, Bauer, Lobova, & Fleming, 2011). Despite significant impacts of WNS and other fungal pathogens on wildlife populations (e.g., *Ophidiomyces ophiodiicola*; Lorch et al., 2016), the fungi *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*) have caused the greatest disease-associated decline of biodiversity, influencing hundreds of species (Scheele et al., 2019). *Bd* and *Bsal* are pathogens that disrupt skin function and cause the disease chytridiomycosis in amphibians (Van Rooij, Martel, Haesebrouck, & Pasmans, 2015). Most declines caused by *Bd* chytridiomycosis have been associated with anuran species (Scheele et al., 2019); however, the recently discovered *Bsal* appears to be most pathogenic to salamanders (Martel et al., 2013, 2014).

Bsal was isolated in 2012 from a moribund European fire salamander (*Salamandra salamandra*) from a population in Bunderbos, the Netherlands, that had been declining since 2010 (Martel et al., 2013). Since its isolation, *Bsal* has been detected in captive and wild populations in six European countries (Fitzpatrick, Pasmans, Martel, & Cunningham, 2018; González et al., 2019; Sabino Pinto et al., 2015; Spitzen-van der Sluijs et al., 2016; Stegen et al., 2017). *Bsal*'s emergence has had negative impacts on susceptible host species. For example, Stegen et al. (2017) reported a >90% reduction in a Belgian *S. salamandra* population in as little as 6 months. Subclinical infections in wild populations and museum specimens of Asian salamander species, coupled with observations of high *Bsal* prevalence in salamanders imported to Europe from Asia, have led researchers to hypothesize that *Bsal* is endemic to the Asian continent and it was introduced to Europe through international trade (Laking, Ngo, Pasmans, Martel, & Nguyen, 2017; Martel et al., 2014; Nguyen, Nguyen, Ziegler, Pasmans, & Martel, 2017). Although *Bsal* has not been detected in North America, there is growing concern that it will enter similarly through unregulated trade (Grant et al., 2017; Gray et al., 2015). Preliminary risk analyses suggest that eastern North America is particularly vulnerable to *Bsal* invasion (Richgels, Russell, Adams, White, & Grant, 2016; Yap, Koo, Ambrose, Wake, & Vredenburg, 2015).

Initial host–pathogen challenge experiments suggest that newts (family Salamandridae) are very susceptible to *Bsal*,

with 14 of 15 species tested developing chytridiomycosis (Martel et al., 2014). The most diverse salamander family globally is the lungless salamanders (Plethodontidae; IUCN, 2019), and one of three species (*Hydromantes strinati*) previously tested developed *Bsal* chytridiomycosis (Martel et al., 2014). A global hotspot for biodiversity of lungless salamanders is eastern North America (Yap et al., 2015). Within this region, the most endemic tribe of lungless salamanders is the brook salamanders (Spelerpini; IUCN, 2019). Fifteen of 36 Spelerpini species (41.7%) are listed as vulnerable or endangered—most belonging to the *Eurycea* genus (IUCN 2019). A critical gap in understanding the conservation threat of *Bsal* to North America is estimating the susceptibility of possible host species (Gray et al., 2015).

The susceptibility of North American lungless salamander species to *Bsal* is unknown. Moreover, no studies have compared susceptibility among salamander populations. Thus, our objective was to estimate the susceptibility of Spelerpini species from two genera to *Bsal* infection and determine if susceptibility varied among populations for one species. Understanding host susceptibility to novel pathogens and if variation exists among populations is essential to plan and direct conservation efforts that target disease intervention and management (Woodhams et al., 2018).

2 | METHODS

2.1 | Susceptibility experiments

We used standardized host–pathogen challenges in a dose–response design to estimate the susceptibility of postmetamorphic *Eurycea wilderae*, *Eurycea lucifuga*, and *Pseudotriton ruber* to *Bsal* infection and chytridiomycosis (Gray et al., 2017). We challenged three distinct populations of *E. wilderae* obtained through wild collection or captive breeding of wild caught animals. Wild *E. wilderae* were captured in Nantahala National Forest (Macon and Clay counties, NC) and on private land adjacent to the Mount Rogers National Recreational Area (Smyth County, VA). Captive bred *E. wilderae* were originally collected in Rabun and Towns counties (GA), and bred by Indoor Ecosystems LLC (Whitehouse, OH). To minimize impacts on wild populations, *E. lucifuga* and *P. ruber* were also bred in captivity by Indoor Ecosystems. All breeding adults were wild caught, hence the salamanders used in our study were first-generation captive raised, which minimizes potential negative impacts of inbreeding depression on host immune response. Indoor Ecosystems also added fresh organic matter monthly to breeding terrariums to supplement skin microbiome (Harrison et al., 2017). The number of animals tested from each population and species varied by experiment due to availability (Table 1).

We performed each challenge experiment in environmental chambers at 15°C (Martel et al., 2014), which represents

TABLE 1 Sample size per treatment (n), percent infected and mortality observed during the experiment, and estimated zoospore exposure dose that resulted in 50% infection (ID-50) and 50% mortality (LD-50) for three Spelerpini species and three populations (Georgia, North Carolina, and Virginia) of *Eurycea wilderae* challenged with *Bsal* zoospores

Species	Treatment	n	Infected (%)	ID-50 zoospores (mL)	Mortality (%)	LD-50 zoospores (mL)
<i>Pseudotriton ruber</i>	Control	2	0	8,658	0	1,438,669
	5×10^3	5	40		0	
	5×10^4	5	80		0	
	5×10^5	5	100		0	
	5×10^6	5	100		100	
<i>Eurycea wilderae</i> (GA)	Control	5	0	54,488	0	651,779
	5×10^3	5	0		0	
	5×10^4	5	40		0	
	5×10^5	5	100		20	
	5×10^6	5	100		100	
<i>Eurycea wilderae</i> (NC)	Control	4	0	159,609	0	1,566,336
	5×10^4	4	0		0	
	5×10^5	4	100		0	
	5×10^6	4	100		100	
<i>Eurycea wilderae</i> (VA)	Control	6	0	5,762	0	7,412,661
	5×10^3	6	33.33		0	
	5×10^4	6	100		0	
	5×10^5	6	100		0	
	5×10^6	6	100		16.66	
<i>Eurycea lucifuga</i>	Control	2	0	105,417	0	No mortality
	5×10^3	5	0		0	
	5×10^4	5	60		0	
	5×10^5	5	80		0	
	5×10^6	5	80		0	

Note. *P. ruber*, *E. wilderae* (NC), and *E. lucifuga* were captive raised and tested 2–6 months postmetamorphosis (i.e., juveniles); *E. wilderae* (GA) and *E. wilderae* (VA) were captured from the wild and tested at >1 year postmetamorphosis (i.e., adults).

the optimal in vitro growth range of *Bsal* (Martel et al., 2013). This temperature also lies within the expected ambient temperatures where our species exist (Grant, Wiewel, & Rice, 2014; Voss, 1993). Environmental chambers were maintained at >90% humidity and set on a 12-hr light and 12-hr dark photoperiod. Each animal was housed terrestrially within a 1,940-cm² plastic container containing a moist paper towel and PVC cover object, which is similar to Martel et al. (2014). Every three days, we replaced each container, paper towel, and cover object with fresh materials to minimize accumulation of nitrogenous waste. Depending on oral gape size, we provided animals either bean beetles (*Callosobruchus maculatus*) or fruit flies (*Drosophila melanogaster*) at 2% of their body mass every 3 days. While replacing the housing materials every third day, we counted the number of invertebrates remaining and estimated the proportion eaten. Twice daily (AM/PM), we recorded whether salamanders were under the cover object and estimated percent use during the experiment.

We obtained *Bsal* isolated by Frank Pasman and An Martel (University of Ghent, Belgium) from a morbid wild fire salamander (*S. salamandra*), which is the same isolate used in Martel et al. (2014). *Bsal* cultures were maintained at Vanderbilt University under Biosafety Level 2 containment by LRS and LR. We grew cultures on TGhL plates and transported them to a biosecure facility at the University of Tennessee on the day prior to zoospore enumeration and animal exposure. We flooded *Bsal* culture plates with 7 mL of autoclaved dechlorinated water and filtered suspended *Bsal* zoospores and zoosporangia through a 20- μ m filter to remove zoosporangia. We enumerated zoospores using a hemocytometer and prepared four doses of 5×10^3 , 5×10^4 , 5×10^5 , and 5×10^6 *Bsal* zoospores/mL. We randomly assigned animals to each dose or to a control group. We placed exposed animals in 100-mL plastic cylindrical containers with 9 mL of autoclaved dechlorinated water and 1 mL of inoculum; control animals were treated identical except exposure occurred in 10 mL of autoclaved dechlorinated water. After 24-hr exposure, we

removed animals from their exposure containers and placed them in the previously described housing containers. We monitored animals daily and euthanized them at humane disease endpoints or at the end of the experiment. The euthanasia endpoints were loss of righting ability or unresponsiveness to prodding. The duration of experiments lasted between 42 and 79 days, which is sufficient time for *Bsal* chytridiomycosis to develop in susceptible species (Martel et al., 2014; Stegen et al., 2017). All procedures followed approved University of Tennessee Institutional Animal Care and Use Committee protocol #2395.

We swabbed animals every 6 days using the standardized protocol for *B. dendrobatidis* (Boyle, Boyle, Olsen, Morgan, & Hyatt, 2004). We extracted genomic DNA from all collected swabs using Qiagen DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany). To detect and quantify *Bsal* DNA present on each swab sample, we performed quantitative PCR (qPCR) similar to Blooi et al. (2013). All qPCR testing was performed on an Applied Biosystems Quantstudio 6 Flex qPCR instrument (Thermo Fisher Scientific Inc. Headquarters in Waltham, MA). We ran all swab samples in duplicate and considered an animal pos-

itive if both replicates reached cycle threshold prior to 50 amplification cycles. We used a standard curve created from a dilution series of synthetic *Bsal* DNA (gBlock) to estimate the number of *Bsal* zoospore copies/ μ L. We confirmed *Bsal* chytridiomycosis in all individuals that died or were euthanized at humane endpoints by examining histological cross-sections of the skin stained with hematoxylin and eosin. Representative images showing histological signs of *Bsal* chytridiomycosis (e.g., thalli invading the epidermis; Van Rooij et al., 2015) for each species or population are provided.

2.2 | Statistical analyses

As indices of susceptibility, we estimated mortality rates and median infectious (ID-50) and lethal dose (LD-50) levels for each species and *Eurycea* population. Specifically, we used Kaplan–Meier survival analyses and Cox-proportional hazard models from the “survival” package in R studio (version 3.5.3) to test for and estimate differences in mortality rates among species and populations exposed at the same zoospore dose and among doses within a species or population (Goel,

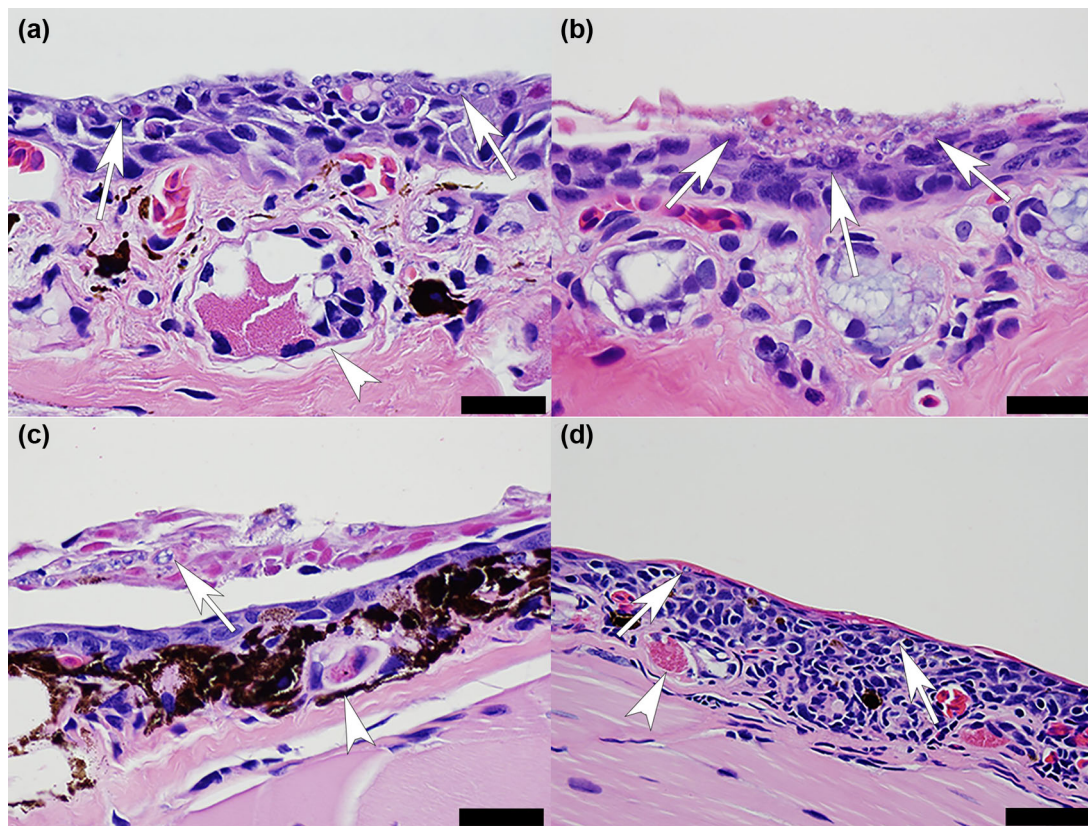


FIGURE 1 Hematoxylin and eosin stained skin sections of Spelerpini experimentally exposed to 5×10^6 *Batrachochytrium salamandrivorans* (*Bsal*) zoospores. (a) Skin from the foot of *Pseudotriton ruber*. (b) Skin from the caudal body of *Eurycea wilderae* (Georgia). (c) Skin from the dorsal pelvic area of *E. wilderae* (North Carolina). (d) Skin from the tail of *E. wilderae* (Virginia). In all sections, *Bsal* thalli (arrows) are invading the epidermis, which is pathognomonic for chytridiomycosis (Martel et al., 2013; Van Rooij et al., 2015). Within the dermis, granular glands (arrowheads) are often present. Bar = 20 μ m

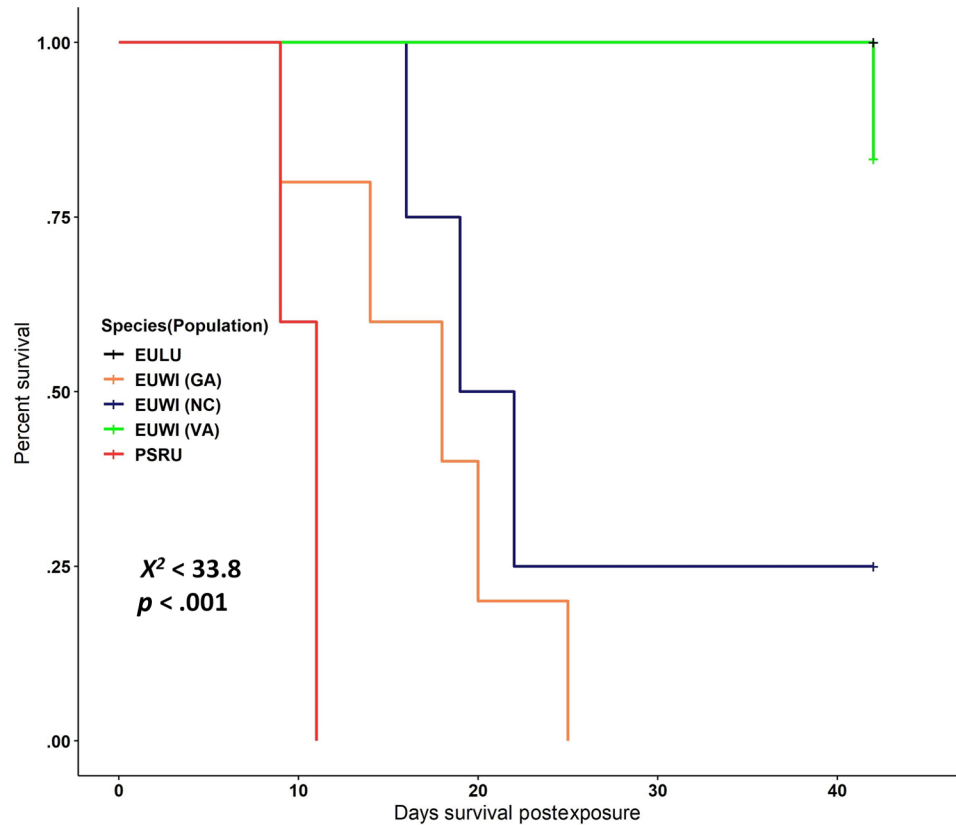


FIGURE 2 Kaplan–Meier survival curves for *Eurycea lucifuga* (black; 100% survival—not visible), *E. wilderae* from Virginia (green), *E. wilderae* from North Carolina (blue), *E. wilderae* from Georgia (orange), and *Pseudotriton ruber* (red) exposed to 5×10^6 zoospores. For the analysis, survival for *E. lucifuga* and *E. wilderae* (VA) was censored at the end of the experiment (denoted by cross marks). Rates of mortality differed among species/populations using a log-rank significance test ($n = 4\text{--}6$ salamanders per group)

Khanna, & Kishore, 2010). Survival comparisons made among species and populations were censored at the shortest experimental duration of 42 days. We estimated ID-50 and LD-50s for each species and population using the MASS package in R (Venables & Ripley, 2002), which fits a generalized linear model with a binomial (probit) link function to the proportion of animals that became infected and died, respectively. In our study, the ID- and LD-50s were the estimated exposure dose of *Bsal* zoospores per mL where 50% of the salamanders became infected and died, respectively.

We compared the *Bsal* loads (DNA copies/ μL) from swabs collected at necropsy between animals that survived and those that died due to chytridiomycosis via Wilcoxon signed-rank tests (Conover, 1999). For each species and population, we used Kruskal–Wallis tests to evaluate differences among zoospore doses in median percent of food items (invertebrates) consumed and percent cover object use during the experiment (Conover, 1999). If the Kruskal–Wallis test was significant, we used pairwise Wilcoxon signed rank tests to determine if differences existed between levels of each factor (Conover, 1999). Nonparametric tests were performed because data were not normally distributed; we evaluated all tests at $\alpha = 0.05$.

3 | RESULTS

All Spelerpini species that we tested became infected with *Bsal* at relatively low zoospore doses (Table 1). The lowest ID-50 estimate was for *P. ruber* (8,658 zoospores) followed by *E. wilderae* (averaged across three populations; 73,286 zoospores) and *E. lucifuga* (105,417 zoospores; Table 1). Of the three *E. wilderae* populations, the Virginia population had the lowest ID-50 estimate, whereas the North Carolina population had the greatest ID-50 estimate (Table 1). We detected *Bsal* chytridiomycosis (determined by histopathology) in all species (Figure 1) except *E. lucifuga*. Survival was dose-dependent (Figure S1). Tolerance to *Bsal* infection differed among species, with *E. wilderae* (Georgia population) having the lowest LD-50 estimate (651,779 zoospores), whereas all *E. lucifuga* survived infections (Table 1). At the greatest zoospore dose (5×10^6), mortality rates differed among species and *E. wilderae* populations (Figure 2). Median survival duration for *P. ruber* and *E. wilderae* from Georgia and North Carolina were 11, 18, and 20.5 days, respectively. *Bsal* loads on final skin swabs taken at necropsy were greater on animals that died compared to those that survived (Figure 3, Table S1), providing additional

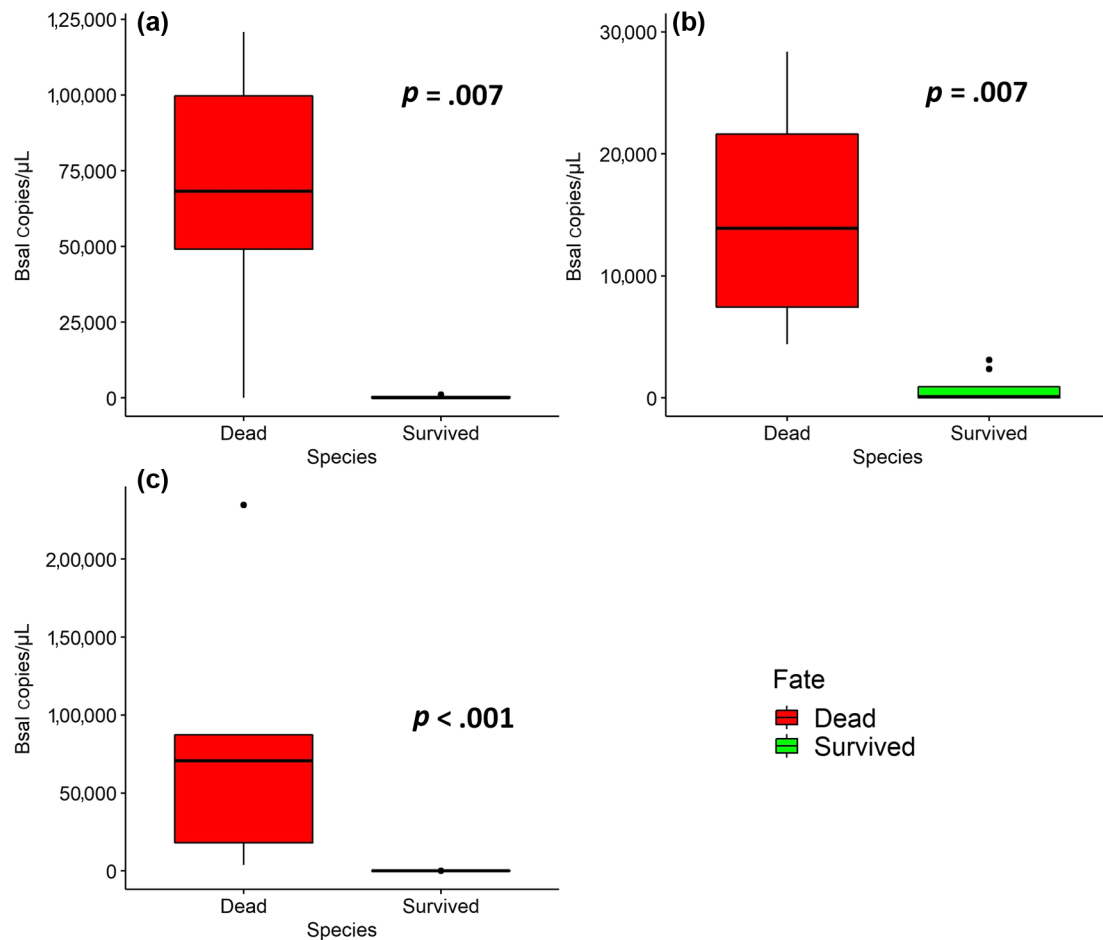


FIGURE 3 Final *Bsal* zoospore loads (copy/ μL) estimated from standardized skin swabs at necropsy for *Eurycea wilderae* from Georgia (a) and North Carolina (b) and *Pseudotriton ruber* (c). Wilcoxon p -values are given for testing differences in *Bsal* loads between animals that either died or survived during the experiment. The bottom line extending from each boxplot is the $1 - 1.5 \times$ interquartile range (IQR), whereas the line extending from the upper portion of each box is the $3 + 1.5 \times$ IQR. The lower and upper portion of each box is the first and third quartile, the midline is the median, and points extending beyond the boxplot are outliers

evidence that *Bsal* chytridiomycosis caused the observed mortality.

We detected statistically significant differences in behavior associated with *Bsal* infection. Across species and populations, salamanders consumed 11–69% less invertebrates in the highest zoospore dose compared to control animals (Figure 4, Table S2). *Pseudotriton ruber* and *E. wilderae* (North Carolina) also spent 2–4.5 \times less time under cover objects compared to controls (Figure S2, Table S3).

4 | DISCUSSION

Our results provide the first evidence that *Bsal* infection and chytridiomycosis can occur in multiple lungless (Plethodontidae) salamander species and are the first cases documented for *Eurycea* and *Pseudotriton* (Spelerpini). These findings represent a significant conservation concern given that all

36 Spelerpini species are endemic to eastern North America (IUCN, 2019), and 41.7% are listed by the IUCN as vulnerable to extinction or endangered (IUCN 2019). Within the geographic range of Spelerpini, the Appalachian Mountains contain the greatest species richness (6–10 species; Figure 5) and might represent the region at greatest conservation risk to *Bsal* invasion. This region also has high environmental suitability for *Bsal* (Richgels et al., 2016; Yap et al., 2015), hence natural resource professionals should consider focusing surveillance efforts and developing strategies in this area for disease intervention in the event that *Bsal* is introduced (Hopkins et al., 2018).

Tolerance to *Bsal* infection differed among species. All Spelerpini species we tested became infected with *Bsal* and most developed chytridiomycosis; *E. lucifuga* was the only species that did not develop chytridiomycosis. Species variation in tolerance to *Bsal* infection has been reported (Martel et al., 2014; Stegen et al., 2017). The mechanisms

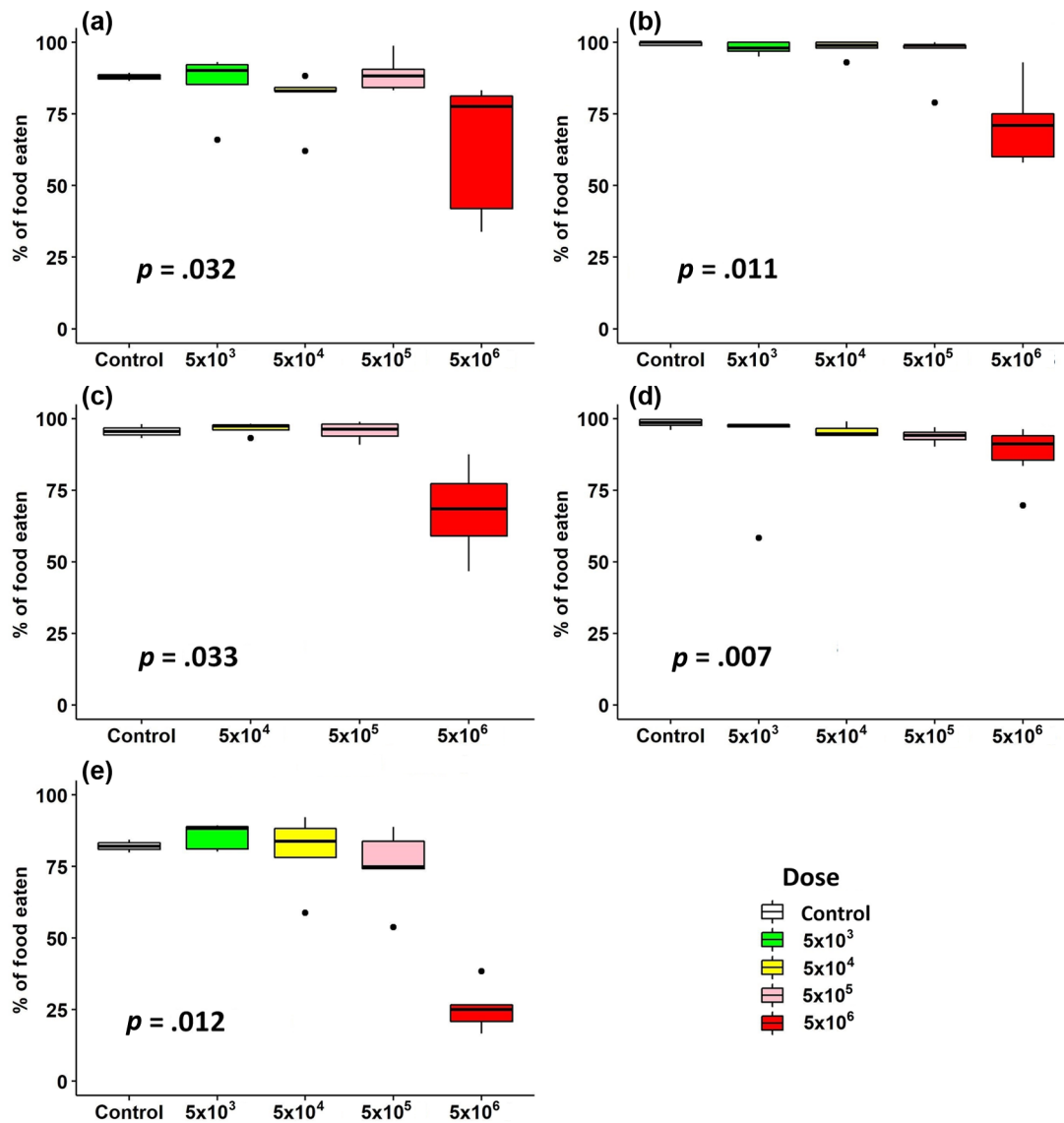


FIGURE 4 Average percent food consumed among zoospore doses for *Eurycea lucifuga* (a), *E. wilderae* from Georgia (b), North Carolina (c) or Virginia (d) and *Pseudotriton ruber* (e). Kruskal–Wallis p -values are provided for testing differences in food consumed among doses for each species. The bottom line extending from each boxplot is the $1 - 1.5 \times$ interquartile range (IQR), whereas the line extending from the upper portion of each box is the $3 + 1.5 \times$ IQR. The lower and upper portion of each box is the first and third quartile, the midline is the median, and points extending beyond the boxplot are outliers

connoting infection tolerance are poorly understood for *Bsal* (Van Rooij et al., 2015) but might include an interaction of innate and adaptive immune defenses, similar to host responses to *Bd* infection (Woodhams et al., 2018).

We provide the first evidence of variation in *Bsal* infection tolerance among populations for one species (*E. wilderae*). The Georgia population of *E. wilderae* had the lowest infection tolerance (i.e., lowest LD-50 estimate) but they were raised in captivity. Considering that microbiome composition can change in captivity (Kueneman et al., 2016) and might be a source of *Bsal* resistance, comparison with the Georgia population could be confounded. Individuals from North Carolina and Virginia populations were collected from

the wild and captivity was minimized prior to experiments (<2 weeks). Total mortality and mortality rate were greater and the LD-50 estimate lower in the North Carolina population compared to the Virginia population. Interestingly, the ID-50 estimate for the Virginia population was lower than North Carolina, suggesting greater tolerance of the Virginia population to *Bsal* infection (Wilber, Knapp, Toothman, & Briggs, 2017). Variation in infection tolerance to amphibian pathogens among populations has been reported and could be related to differences in genetic diversity or local conditions that affected innate and adaptive immune defenses (Pearman & Garner, 2005; Savage & Zamudio, 2011). More research is needed to understand the mechanisms

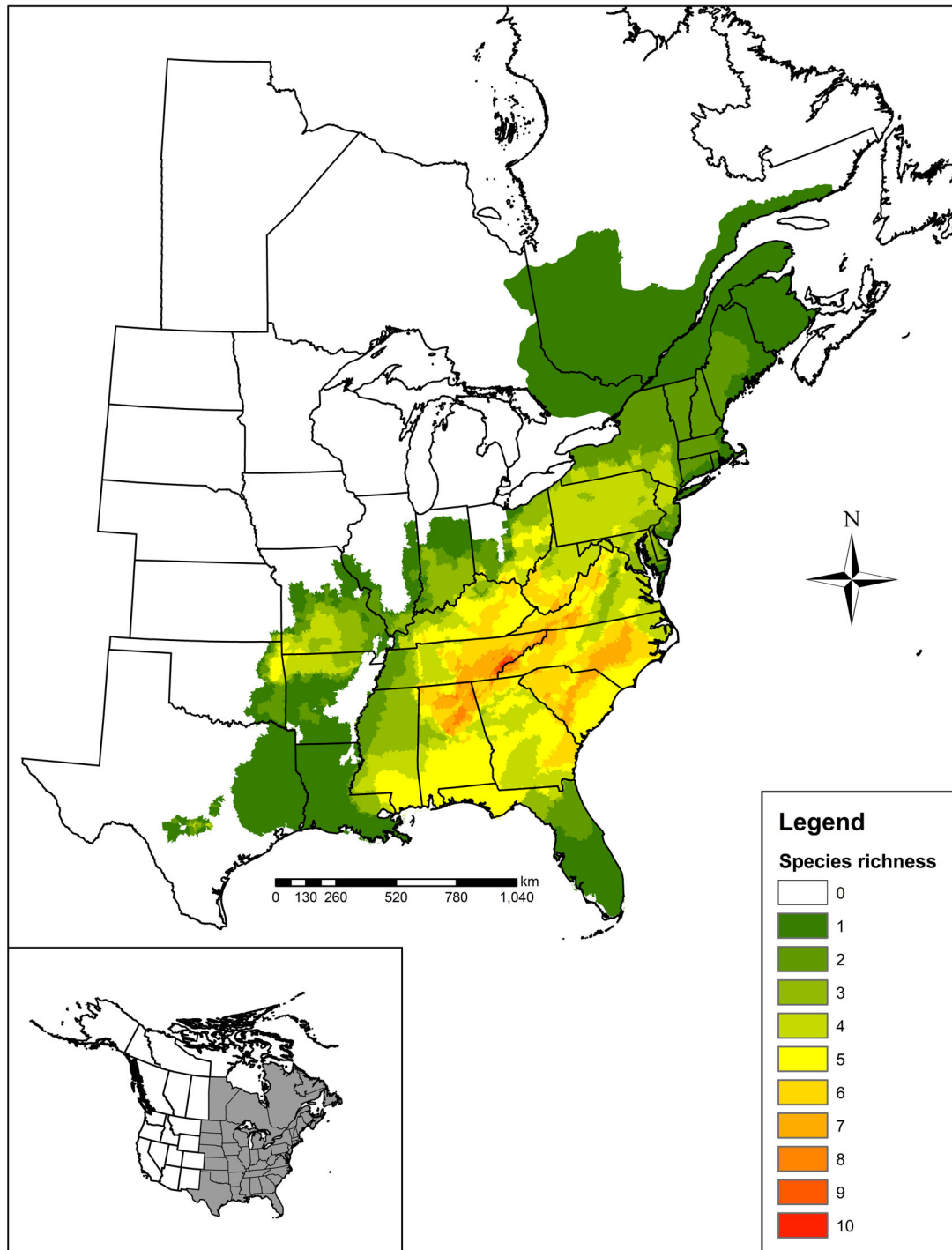


FIGURE 5 Distribution of Spelerpini salamander species in North America. Green shades represent relatively low species richness, whereas yellow, orange and red shades represent greater species richness. We used spatial distribution data available from the Gap Analysis Project (<https://www.usgs.gov/core-science-systems/science-analytics-and-synthesis/gap>) and the International Union for the Conservation of Nature (<https://www.iucn.org/>) to create the final richness raster layer at a spatial resolution of 300 meters. U.S. states at greatest threat of Spelerpini loss due to *Bsal* invasion include Alabama, Georgia, Kentucky, Pennsylvania, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia

driving population-level variation in tolerance to *Bsal* infection, which has not been observed in *S. salamandra* in Europe (Schmidt, Bozzuto, Lotters, & Steinfartz, 2017; Stegen et al., 2017). This finding suggests that mapping *Bsal* invasion risk

and planning disease intervention may be more complex than focusing on species-level susceptibility or environmental suitability of *Bsal* (Beukema et al., 2018; Richgels et al., 2016).

We found that individuals that died from *Bsal* chytridiomycosis had greater pathogen loads on their skin at necropsy than those that were infected and survived. These results suggest there may be a threshold to *Bsal* infection that results in clinical chytridiomycosis, as proposed for *Bd* (Vredenburg, Knapp, Tunstall, & Briggs, 2010). Across species, the average *Bsal* genomics equivalents (GE) on swabs from animals that died ranged from 15,125 to 82,902 copies/ μ L, which is greater than the proposed 10,000 GE lethal threshold for *Bd* (Vredenburg et al., 2010). *Bsal* chytridiomycosis is characterized by necrotic ulcerations in the epidermis (Figure 1), which might affect skin respiration and osmoregulation or facilitate secondary bacterial infections (Bletz et al., 2018; Van Rooij et al., 2015).

Despite these findings, estimates of LD-50 levels for our species were greater than reported for European newt species (Martel et al., 2014). Although Martel et al. (2014) did not estimate LD-50 levels, 100% mortality occurred in highly susceptible species when exposed to 5,000 zoospores. Thus, Spelerpini species may be more tolerant to *Bsal* infection than some Salamandridae species, such as the fire salamander and eastern newt (*Notophthalmus viridescens*; Longo, Fleischer, & Lips, 2019; Martel et al., 2014), which have been referred to as hypersusceptible *Bsal* hosts. Indeed, Longo et al. (2019) reported 50% mortality of eastern newts—a North American Salamandridae species—exposed to 10,000 *Bsal* zoospores. Given the higher tolerance of the Spelerpini species we tested to *Bsal*, it is possible they could serve as reservoirs and facilitate spillover of *Bsal* to eastern newts where species distributions and habitat use overlap. Similarly, there could be epidemiological feedbacks where highly susceptible eastern newts amplify *Bsal* zoospores in the environment via high rates of shedding to concentrations about the LD-50 levels of Spelerpini species. Future research should investigate the role of amphibian community composition on epidemiological outcomes.

We also report the first evidence of behavioral changes associated with exposure to *Bsal*. Across all species, salamanders at the highest zoospore dose consumed 11–69% less food compared to control animals. Additionally, *P. ruber* and one population of *E. wilderae* reduced cover object use, potentially resulting in greater opportunities for exposure-related mortality in the wild. Changes in feeding behavior and activity have been reported for other amphibian pathogens (Venuesky, Parris, & Storer, 2009), and highlight potential fitness consequences on hosts of being subclinically infected with *Bsal*. Most of the individuals in our experiments experienced mortality at the highest zoospore dose, hence changes in behavior would not have impacted their fitness. Of the individuals that survived, remained infected, and had altered behavior (e.g., fed less), our experiments were too short to identify if fitness consequences of subclinical infections occurred (e.g.,

reduced reproduction), which will be an important area for future research.

Collectively, these results emphasize that conservation organizations in North America need to develop a better understanding of the susceptibility of lungless salamander species to *Bsal*. Given that many species in Plethodontidae inhabit complex habitats such as streams, trees, rocky outcrops, and caves, employment of management strategies that effectively contain or eradicate *Bsal* could be challenging. As such, preventing the introduction of *Bsal* to North America should remain a top conservation policy priority (Grant et al., 2017). Banning the trade of amphibian species that are capable of being infected with *Bsal* can reduce the likelihood of introduction into naïve areas; however, conservation planners and legislators also should consider policies and programs that require and subsidize pathogen-free trade of amphibians (Baláž et al., 2017). Additionally, conservation planners in North America should begin educational programs to inform the public about *Bsal*, and provide recommendations to tourists traveling from Europe or Asia (where *Bsal* is present) to North America on decontaminating footwear and recreational gear (Gray et al., 2017), which is believed to be how white-nose syndrome was introduced. International trade of infected amphibians and contaminated fomites attached to international travelers are two logical anthropogenic pathways for *Bsal* introduction to North America. However, other anthropogenic and natural pathways are possible, such as importation of contaminated materials (e.g., soil and water) and zoospores adhering to migratory birds (Stegen et al., 2017), and should be considered when assessing *Bsal* invasion risk and conservation policy options.

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