BIODIVERSITY RESEARCH

Truly enigmatic declines in terrestrial salamander populations in Great Smoky Mountains National Park

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ABSTRACT

Aim Woodland salamanders (genus: *Plethodon*) declined synchronously across many protected areas in Eastern North America by the mid-1980s, but no cause was attributed to these declines. We hypothesized that the rapid, synchronous loss of several populations of many species was consistent with the invasive pathogen *Batrachochytrium dendrobatidis* (*Bd*), and we resurveyed historic sites to search for *Bd* in current populations.

Location Great Smoky Mountains National Park.

Methods We surveyed 35 sites 2–4 times between March and November 2009, quantified community composition and abundance of 72 populations of six species and three hybrids of *Plethodon* salamanders, and collected 665 skin swabs to detect *Bd*.

Results At 22 of the 35 sites, we were unable to find one or more species that were historically present. *Plethodon glutinosus* and *P. teyahalee* and their hybrids were less abundant than historically found, *P. jordani x metcalfi* and *P. ventralis* were more abundant and the remaining three species fluctuated but showed no net change. Yet, only one of the 665 salamanders was positive for *Bd*.

Main conclusions Declines were not associated with particular localities, but occurred in particular species. We conclude that over collecting, logging, and acid rain are unlikely to have caused these population declines, but we were unable to rule out disease or climate change as contributing factors. Population declines of *Plethodon* salamanders in the Park are substantial and have persisted for 30 years. Determining the cause and the extent of these declines is important for managing this area of global salamander biodiversity.

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INTRODUCTION

Understanding the causes for the 'enigmatic' population declines of amphibians has been – and remains – a major challenge for amphibian conservation. Identifying unusual or 'enigmatic' population declines of amphibians and the causes for those declines requires good population data, solid evidence of the presence of causative agents and knowledge of their mechanisms. Both sets of data are often limiting or non-existent for many species or areas, which prevents accurate estimates of biodiversity loss. This is important because over 40% of amphibian species are declining, as many as 113

species may be extinct worldwide and 23% are data deficient (Stuart *et al.*, 2004). The causes for amphibian declines are not uniformly distributed across the globe (Stuart *et al.*, 2004) and may involve multiple, synergistic factors. Dramatic declines have been reported from Central America (Lips *et al.*, 2006; Rovito *et al.*, 2009), California (Vredenburg *et al.*, 2010), Europe (Bosch *et al.*, 2001) and Australia (Berger *et al.*, 1998), but surprisingly, there are few reports of enigmatic losses of species from the well-studied eastern United States (Stuart *et al.*, 2004).

Extensive historic data allow for statistically robust estimates of species occurrence, population abundance and

A Journal of Conservation Biogeography

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population fluctuations (Pechmann *et al.*, 1991). These estimates can be improved by correcting for imperfect detection (Hyde & Simons, 2001; Schmidt, 2009; Bonardi *et al.*, 2011), comparing declines against natural population fluctuations (Pounds *et al.*, 1997) and comparing changes in community diversity (Smith *et al.*, 2009). However, long-term population data are often lacking (Pechmann *et al.*, 1991; Houlahan *et al.*, 2000) and rarely extend beyond a decade. Even when declines are well documented, it can be difficult to discriminate them from natural population fluctuations inherent in amphibian life histories (Alford & Richards, 1999; Welsh & Droege, 2000).

Likewise, it can be difficult to assign causation to a particular threat, either because of an inability to identify causative agents (Rovito *et al.*, 2009), insufficient evidence that causative agents were present at appropriate times (e.g. Daszak *et al.*, 2005), or uncertainty regarding mechanisms causing population declines (e.g. Pounds *et al.*, 2006). Museum collections and associated field data from large geographic areas and diverse species pools offer opportunities to assess changes in population status and evaluate potential causes (Rovito *et al.*, 2009; Lips, 2011). Several studies have correlated the timing of population declines with the presence of agents by examining museum specimens for pathogens (e.g. Cheng *et al.*, 2011), with historic land use patterns (Petranka *et al.*, 1993; Becker & Zamudio, 2011) or with regional weather data (e.g. Ron *et al.*, 2003; Burrowes *et al.*, 2004).

The few reports of unusual or dramatic population declines from the eastern United States generated scepticism that declines occurred (e.g. Pechmann & Wilbur, 1994). A striking exception is the report of synchronous, widespread declines in 180 populations of 38 species of *Plethodon* salamanders by the 1980s (Highton, 2005). Highton (2005) attributed habitat loss to the disappearance of 22 populations at 16 sites but was unable to identify a cause in the remaining 158 population declines – including many within national parks and forests. The rapid and synchronous decline in multiple species of *Plethodon* across a large geographic region was suggestive of losses caused by *Batrachochytrium dendrobatidis* (*Bd*) in Central America (Lips *et al.*, 2008). Therefore, we designed a study to assess the status of 35 *Plethodon* communities in the Great Smoky Mountains National Park and to collect samples to determine the prevalence of *Bd*. We analysed changes in population abundance over 49 years for six species and three hybrids of *Plethodon* salamanders using data from Highton's field notes to determine historic (1960–2001) species richness and population abundance. We resampled these sites in the same manner, which allowed us to quantify changes in community composition and population abundance of multiple species for nearly half a century. Our objectives were as follows: (i) to determine whether community composition changed between historic and current surveys, (ii) to determine whether species or population abundances differed between historic and current surveys and (iii) to search for associations between declining populations and potential threats.

METHODS

Study area and species

The Great Smoky Mountains National Park encompasses 205,665 ha of contiguous forest, of which 95% is forested and 25% is old-growth forest (Davis, 1993). There are three primary habitats: boreal spruce-fir forests on the highest peaks, northern hardwood forests at mid-elevations and mesic hardwood forests at lower elevations and in protected coves and valleys. Soil pH ranges from 3.5 to 5.7, with more acidic soils at higher elevations (Jenkins, 2007). Annual rainfall varies from 140 cm at low elevations to over 200 cm on some high peaks (Jenkins, 2007).

At least 30 species of salamanders occur in the Park (Dodd, 2004), including six species of *Plethodon* (Table 1), three of which are endemic to the park and the immediate vicinity (*P. jordani*, *P. metcalfi* and *P. teyahalee*). We followed Highton's nomenclature based on his field notes when assigning populations to species (Table 1).

Field methods

Site selection

From 1960 to 2001, Highton (2005) repeatedly visited thousands of sites throughout the eastern United States. He collected over 17,000 plethodontid salamanders at 402 sites throughout the Park (Fig. 1) and deposited those animals and associated field notes at the U.S. National Museum of Natural History (USNM). Associated data included geographic coordinates, date of collection, species, number of individuals encountered and number of collectors. Surveyors turned over all natural cover objects (i.e. rocks and logs) for *c.* 1 h (Highton, 2005) and attempted to capture all salamanders encountered, noting the number of individuals that escaped or were not collected (R. Highton, pers. comm.).

We requested a database from the USNM of all *Plethodon* specimens collected by Highton from the Park. We selected resurvey sites that (i) had the greatest number of historic surveys for each of the six species, (ii) covered a broad elevational and geographic range and (iii) represented the major habitat types of the park (Fig. 1). For those 35 sites, the average number of historic surveys was 4.23 ± 3.4 (range = 1 –17). We sampled seven low-elevation (500–1000 m), 19 mid-elevation (1000–1500 m) and nine high-elevation (1500 –2000 m) sites during March, May–July and November of 2009, respectively. Most sites were > 100 m from the nearest stream. Undisturbed, old-growth forests constituted 29% of our study sites, intensively logged areas made up 9% of our sites, 26% of sites experienced moderate logging and the remaining 36% of sites were logged less intensively. Thirty-four percentage of our sites were comprised of high-elevation red oak forests, 31% were northern hardwood, 9% were spruce-fir and 11% were successional hardwood.

Table 1 Life history information for the six Smoky Mountain *Plethodon* species (Dodd, 2004; Kozak *et al.*, 2006)

Species	Phylogenetic complex	Size	Elevational range (m)	Geographic distribution	Maturation (years)	clutch size
P. glutinosus	*glutinosus*	Large	0–1500	Throughout E. United States	3–4	5–15
P. teyahalee	*glutinosus*	Large	0–1550	Southern Appalachians	5	–
P. jordani	*jordani*	Medium	200–1950	Smoky Mountains	3–6	–
P. metcalfi	*jordani*	Medium	> 750	Southern Appalachians	–	–
P. serratus	*cinereus*	Small	0–1690	Fragmented in Southeastern United States	2	5–7
P. ventralis	*welleri*	Small	< 600	Fragmented in Southeastern United States	–	2–5

Dash denotes missing information.
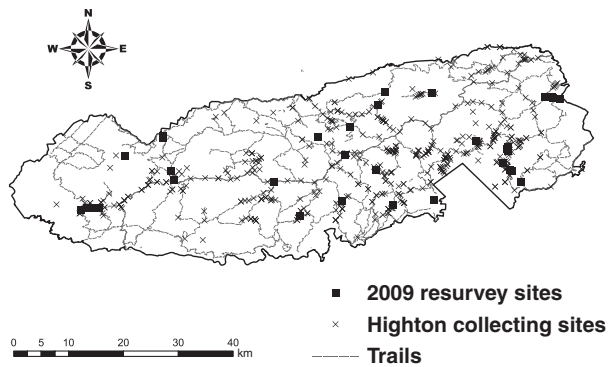
Figure 1 Map of the Great Smoky Mountain National Park study sites (boxes) and Highton's original collecting sites (x's). Lines are trails.

The remaining 15% of sites were acid cove, chestnut-oak or oak hickory forests.

Field sampling

We recorded GPS coordinates, elevation, weather conditions, number of surveyors, number of salamanders and the amount of time searching (excluding processing time) at each location. We surveyed two 50 m × 3 m plots at each site to include representative habitats and resurveyed each site 2–4 times to minimize effects of climate variation on salamander surface activity (Smith & Petranka, 2000; Bailey *et al.*, 2004).

Within each plot, we turned all natural cover objects and captured all salamanders by hand using a new powder-free latex glove. We placed each salamander in a new plastic bag to facilitate swabbing (Hyatt *et al.*, 2007). We released all salamanders at their point of capture and replaced natural cover objects (Smith & Petranka, 2000).

We calculated catch per unit effort (CPUE) as the number of animals captured per person during the search time for the two plots combined. We used program Presence version 3.0 (Hines, 2006) to estimate detection probabilities based on the presence/absence (Bailey *et al.*, 2004) of those populations that were surveyed at least once each decade. Data

from all populations sampled from all decades were pooled together to estimate detection probabilities in a multi-season occupancy model where season is equivalent to decade. We corrected CPUE estimates for detection by dividing the raw counts by the species-specific decadal detection probabilities (Schmidt, 2009) and used these values for all subsequent analyses.

Laboratory analyses

We tested 665 skin swabs collected from 14 taxa [*Desmognathus imitator* (37), *D. ocoee* (50), *D. santeetlah* (three), *D. wrighti* (63), *Eurycea wilderae* (24), *Gyrinophilus porphyriticus danielsi* (three), *Plethodon glutinosus* (seven), *P. jordani x metcalfi* (110), *P. jordani x teyahalee* (29), *P. jordani* (228), *P. metcalfi* (28), *P. serratus* (19), *P. teyahalee* (eight) and *P. ventralis* (56)] to determine *Bd* infection using standard techniques (Hyatt *et al.*, 2007) in the laboratory of Jake Kerby at South Dakota State University. We used Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) for DNA extraction and used PCR primers developed by Boyle *et al.* (2004). In each 96-well reaction plate, we included a negative control and standards in triplicate (i.e. 100 zoospores, 10 zoospores, 1 zoospores and 0.1 zoospores). We estimated the intensity of infection or genomic equivalents (GE) of *Bd* zoospores in each swab, by multiplying the number of zoospores detected in each positive sample by 100 (the dilution of the final sample). Sample plates were run on the ABI 7300 PCR machine for 50 amplification cycles.

Data analysis

Change in population abundance

We used R (R Development Core Team, 2011) and ArcMap version 10.0 (ESRI, 2011) for all statistical analyses. We ran generalized linear mixed effects model (GLMM; *lme4* package; Bates & Maechler, 2010) to measure changes in abundance over time for each species. We modelled the relationship between counts corrected for detection and the standardized survey year (model: YEAR); we included populations nested within survey year as a random variable

(Shaffer *et al.*, 1997) and fit the model using maximum likelihood. Additionally, we fit the model with a Poisson error distribution (e.g. Roulin & Bersier, 2007); as correcting for detection produced estimates with fractional amounts, we rounded those estimates to integers to fit the Poisson regression.

We used a conservative approach for classifying change in abundance. We calculated the model coefficients and standard error for each population using the results from the YEAR model for each species (Qian *et al.*, 2010) and determined whether each population was 'Declining', 'Stable', or 'Increasing' based on the coefficients and standard error (Pechmann & Wilbur, 1994). If the upper and lower bounds of a population's standard error were negative, we considered the population to have declined. A population was stable if the upper bound of a population's standard error was positive and lower bound was negative (i.e. the error bars crossed zero). If both the upper and lower bounds of a population's standard error were positive, then this population increased.

We tested whether these declines were within the natural range of declines (e.g. Pechmann *et al.*, 1991; Pounds *et al.*, 1997; Alford & Richards, 1999). For each species, we estimated the mean and variance of relative abundance of the corrected counts from the period 1960–1979 and simulated abundance estimates for the entire study period (1960–2009), assuming a quasi-Poisson error distribution to account for overdispersion (Ver Hoef & Boveng, 2007). We resampled this simulated data set 10,000 times and assigned each of those 10,000 estimates to one of three population states (i.e. stable, declining, increasing) using a generalized linear model (GLM), which fit a linear relationship between abundance and time for each species. We used the coefficient and standard error for all of the populations within each taxon to determine overall population response. This generated a distribution of abundances expected under normal population fluctuations to which we compared the fit of our observed responses with a chi-squared test.

We compared Highton's original estimates of percentage decline to our estimates in three ways to determine the impact our more conservative analysis had on assigning population response. We compared our uncorrected estimates to Highton's uncorrected estimates using a Wilcoxon sign-rank test (i.e. the analysis used in Highton, 2005), we compared our uncorrected estimates using mixed models, and finally, we used both mixed models and estimates corrected for detection (Schmidt, 2009).

Change in community composition

We compared changes in community composition at all 35 sites between pre-decline (1960–1979) and post-decline (2009) periods using the *rich* package (Rossi, 2011). We used a bootstrap analysis (10,000 randomizations) to estimate mean species richness for both pre- and post-decline periods (*rich* function) and used a randomization test (*c2m* function;

10,000 randomizations) to compare the two bootstrapped average richness values.

We compared beta diversity between pre-decline and post-decline communities within the Park (Smith *et al.*, 2009). We created a null prediction for declines among species by assigning each species at each site a fixed and equal probability of decline, defined as the number of species declining at each site divided by the number of species at each site (see Smith *et al.*, 2009). We simulated this null prediction 10,000 times and calculated, for each species, the average number of declined populations over those simulations. We compared the average number of declines estimated by our simulated null prediction to the actual number of declines derived from examining the slope coefficients from the YEAR model with a permutation test (*exactRankTests* package; Hothorn & Hornik, 2011).

Spatial, taxonomic and threat-specific patterns

We evaluated the possibility that collecting contributed to population declines. We compared the population responses generated from the YEAR model to the total number of salamanders removed at each population, expecting a negative effect of collection size on population size. We fit the data with a GLM with quasi-Poisson error distribution (Ver Hoef & Boveng, 2007).

We searched for spatial patterns in population response among all populations. If certain areas of the park were more affected, then wind patterns (Davidson *et al.*, 2002) or anthropogenic land use (Johnson *et al.*, 2011) may explain these declines. We analysed the spatial relationship of population response for the combined data set using spatial autocorrelation in the spatial statistic tools in ArcMap.

We determined whether changes in population abundance were associated with elevation, current average annual temperature and precipitation, environmental suitability for *Bd*, historic forestry practices and change in annual temperature and precipitation. If declines were correlated with elevation, this might indicate a cause that shows elevational bias (e.g. acidic conditions; Jenkins, 2007). We hypothesized that if *Bd* were involved in declines, then declining populations were more likely to occupy cool and moist habitats (Kilpatrick *et al.*, 2010) or those habitats that were most suitable for *Bd*. If forestry practices were a potential cause for these declines, then we expected them to occur in areas that had been most intensively logged. Lastly, we expected that if changes in temperature and precipitation caused population declines, then declines would likely be associated with habitats that have become warmer and drier since 1951.

We determined elevation of a site using a 1-arc second National Elevation Dataset (Gesch *et al.*, 2002). We determined the average annual temperature (AMT; 1950–2000) and average annual precipitation (AMP; 1950–2000) for each site using data from WorldClim (1-km resolution; Hijmans

et al., 2005). We used a forest disturbance GIS polygon (National Park Service, Great Smoky Mountains National Park, 2007) to determine the magnitude of logging at each site during the last phase of timber harvesting (1935–1939). We quantified the amount of logging using existing categories to indicate most ('heavy cut') to the least ('undisturbed'). We ascertained the change in annual temperature (ΔTemp) and the change in annual precipitation (ΔPrecip) from 1951 to 2006 from the Climate Wizard database (Girvetz et al., 2009).

We determined whether any parts of the study area were within the environmental niche of Bd, using Maxent (version 3.3.0; Phillips et al., 2006) to model environmental suitability based on 75 Bd+ locations from the United States (http://www.bd-maps.net/; Chatfield et al., 2009; Chinnadurai et al., 2009). We followed the methods of Murray et al. (2011) and used all 19 bioclimatic variables (c. 5-km resolution; Hijmans et al., 2005) to generate the initial model. We selected the top-ranking variables that contributed c. 90% of information to the full model, which reduced over-fitting the model (Parolo et al., 2008). We retained the seven informative variables and used bootstrapping (n = 100) with unique sets of training and testing data (75/25% respectively) to allow uncertainty in our predictions. We used the average of the 100 bootstrapped models as the average environmental suitability. We used the conservative, 'lowest presence threshold' (LPT) approach to designate the threshold for Bd's average environmental suitability, which identifies suitable pixels if they are at least as suitable as where Bd was present (Pearson et al., 2007).

We used GLMMs to determine which environmental variable best explained the variation in changes in population abundance for Plethodon salamanders. For all models, we standardized all continuous explanatory variables, rounded corrected counts to integers, nested populations within survey year as a random variable (Shaffer et al., 1997) and fit models using maximum likelihood and a Poisson error distribution (e.g. Roulin & Bersier, 2007). We selected the best models based on lowest Akaike's information criterion with second-order correction for small sample sizes (AIC$_c$; Sugiura, 1978) and determined AIC$_c$ weights for top models (AICcmodavg package; Mazerolle, 2012). We report models with AIC$_c$ differences less than or equal to two units (AIC$_i$−AIC$_{min}$ = Δ_i ≤ 2).

RESULTS

Detection

Detection varied among decades and among species, and no species was detected perfectly in all decades (Table 2). Detection probabilities for P. jordani and P. jordani x metcalfi were relatively constant across all decades, but detection probabilities for P. glutinosus, P. teyahalee, P. serratus and P. ventralis decreased over time.

Table 2 Decadal detection probabilities for the six taxa with ≥ 1 population

Taxon	P (1960s)	P (1970s)	P (1980s)	P (1990s)	P (2000s)
Plethodon glutinosus	0.8303	1.0000	1.0000	1.0000	0.3077
P. teyahalee	0.8786	0.5521	0.6176	*	0.0722
P. jordani	0.9643	1.0000	1.0000	1.0000	0.9425
P. jordani x metcalfi	0.8750	1.0000	–	1.0000	1.0000
P. serratus	0.6030	0.7599	0.3000	0.5000	0.2195
P. ventralis	0.8000	0.7698	*	1.0000	0.3969

Asterisk denotes decades when no salamanders were found, and dash denotes decades when no surveys were conducted.

Community composition

At no multispecies site did all species disappear, but at 28 (39%) sites, we were unable to find one or more species that was present historically. As a result, historic community size was larger (range: 1–3 species, μ = 1.51) than current size (range: 0–3, μ = 0.99; randomization test: P = 0.01). This was supported by the analysis showing a significant decline in regional beta diversity (P = 0.016), indicating the selective loss of species across communities.

Population abundance

We combined all populations of all species and analysed the change in abundance using GLMMs and found a significant decline in abundance (z = −2.326; P = 0.020). Twenty-eight (39%) of the 72 populations declined, and 20 of those populations were P. glutinosus, P. teyahalee and their hybrids. The remaining eight declining populations were P. jordani (five) and P. serratus (three). No population of P. metcalfi, P. ventralis and P. jordani x metcalfi declined (Fig. 2).

We determined whether changes in species abundance differed from expected by comparing their response to null models. These models showed that Plethodon glutinosus (χ^2 = 25.798; d.f. = 2; P < 0.0001) and P. teyahalee (χ^2 = 50.107; d.f. = 2; P < 0.0001) declined more than expected. Populations of P. jordani x metcalfi (χ^2 = 26.537; d.f. = 2; P < 0.0001) and P. ventralis (χ^2 = 20.187; d.f. = 2; P < 0.0001) increased more than expected. Plethodon jordani and P. serratus were no different from expected (P > 0.35).

Many populations declined even after correcting for detection. If we used uncorrected data and Wilcoxon signed-rank analysis, 65% of these populations were in decline (W = 3173.5, P = 0.020). If we analysed our data with mixed models but without correcting for detection, then 46% of populations declined. Using the most conservative analysis, 39% of populations declined (Table 3).
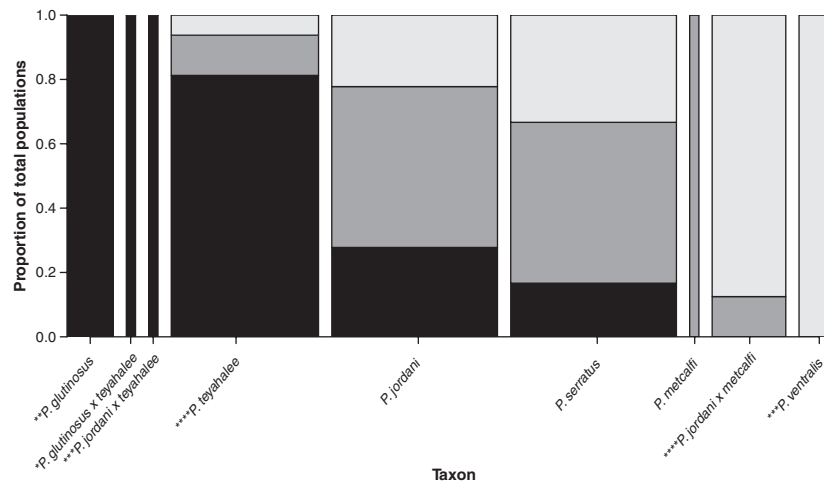
Figure 2 Taxa-specific population responses. Percentage of populations declining (black), stable (grey) or increasing (light grey). Column width represents the total number of populations surveyed (data in Table 3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

Table 3 Estimates of the number (and percentage) of declining populations (n) for each species using (i) Highton's Wilcoxon signed-rank test on the number of salamanders/person/hour in each population; (ii) mixed models not correcting for detection; and (iii) mixed models correcting for detection

| Taxon	No. pops.	Highton method	Uncorrected	Corrected
P. glutinosus	5	5 (100)	5 (100)	5 (100)
P. glutinosus x teyahalee	1	1 (100)	1 (100)	1 (100)
P. teyahalee	16	15 (94)	14 (88)	13 (81)
P. jordani × teyahalee	1	1 (100)	1 (100)	1 (100)
P. jordani	18	11 (61)	5 (28)	5 (28)
P. metcalfi	1	0	0	0
P. jordani x metcalfi	8	0	0	0
P. serratus	18	8 (44)	5 (28)	3 (17)
P. ventralis	4	0	2 (50)	0
Total	72	65%	46%	39%

Spatial, taxonomic and threat-specific correlation

Highton collected 1–578 salamanders from each of our 35 sites (average = 49.47 individuals species^{-1} site^{-1}; 3562 total). We did not find any significant relationship between the number of individuals collected and population response ($F_{1,70} = 1.955$; $P = 0.166$).

We found no spatial clustering of population response for all combined populations ($Z = -1.738$; $P = 0.082$). Declined populations co-occurred with populations classified as either stable or increased.

One of the 665 swabbed salamanders was *Bd* positive (*Desmognathus santeetlah*). It had a low intensity of infection (29 GE) and occurred at a site where the resident *Plethodon metcalfi* showed no decline.

Most of the Park was suitable for *Bd*, although suitability declined from west to east (Fig. 3a, b). The final environmental
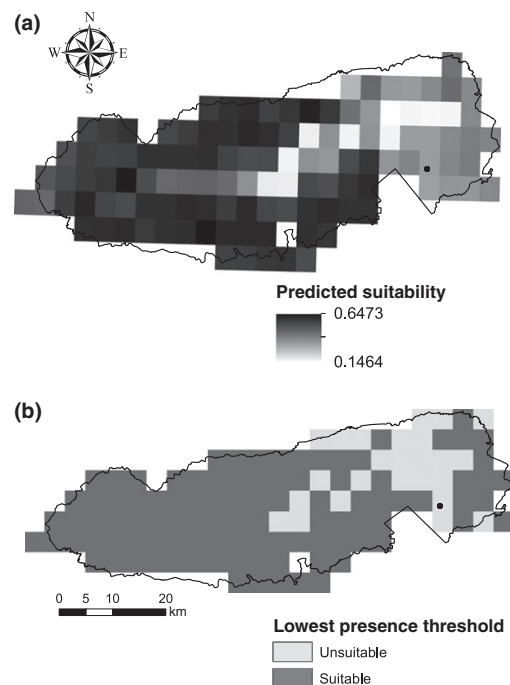
Figure 3 Results of Maxent modelling of (a) average environmental suitability for *Bd*, *Bd*-positive locality (dot) and (b) suitability based on lowest presence threshold (Pearson *et al.*, 2007).

suitability model included seven bioclimatic variables, which contributed ~94% of the information to the full model, and the mean test AUC for the final average model was 0.993. The one *Bd+* salamander occupied a site with a suitability index of 0.429 (Fig. 3a), which was used to determine the LPT for the park (Fig. 3b). Mean diurnal temperature range contributed the most useful information to the final model, while the most model training was lost when removing precipitation of driest quarter from the final average model.

When all candidate factors were included in the model to evaluate potential causes, the top GLMM covariate was AMP ($AIC_c = 2692.5$; AIC_c weight = 0.49), while elevation ($AIC_c = 2693.7$; $\Delta_i = 1.2$; AIC_c weight = 0.27) and AMT ($AIC_c = 2694.0$; $\Delta_i = 1.5$; AIC_c weight = 0.23) also explained some variation in population responses. Populations that declined were associated with areas that received more precipitation ($z = -3.453$; $P = 0.001$), higher elevations ($z = -3.023$; $P = 0.003$) and cooler temperatures ($z = 3.066$; $P = 0.002$). The remaining four covariates (ΔTemp, ΔPrecip, *Bd* suitability and timber harvesting) were > 9 AIC_c units from the top model and did not contribute to the overall AIC_c weight.

DISCUSSION

Overview

Populations of plethodontid salamanders declined throughout the Park, including four of the nine taxa and 39% of all populations. Declines were species specific and affected the largest-bodied species, causing a loss of regional diversity within the Park. We found no association between the full population database and forestry practices, environmental suitability for *Bd* or the change in temperature or precipitation. However, we found that annual average precipitation, elevation and annual average temperature did explain variation in population response of *Plethodon* salamanders. The lack of any spatial pattern of decline is inconsistent with site-specific threats such as logging (Petranka *et al.*, 1993), acid deposition (Wyman & Jancola, 1992) or physiological stress caused by climate change (e.g. Bernardo & Spotila, 2006). We were unable to identify an association of declines with *Bd* environmental suitability, although geographically widespread losses of certain species are consistent with emerging infectious diseases (Crawford *et al.*, 2010; Cheng *et al.*, 2011).

Evaluating causes

We found no evidence that scientific collection contributed to these declines. Many animals were collected, but relatively few from any particular site. Interestingly, populations where the largest collections were made were those with the highest abundances, and those populations continue to have the highest abundances today. This suggests that some inherent aspect of the site or the population determines population size and may be important in contributing to management plans.

We found mixed support for climate change as a cause for these declines. No declines were associated with changes in annual temperature and precipitation, and the species with the most restricted geographic and elevational ranges (*P. jordani* and *P. metcalfi*) did not show declines overall. Our findings lend empirical support to conclusions made by Milanovich *et al.* (2010) who projected that *P. jordani* – which

has a small range and is restricted to the higher elevations – was expected to decline by at least 90% by 2020 and that *P. serratus* would decline by at least 50%. They analysed the *P. glutinosus* group across its entire range and predicted it would decline by 10%. Had they analysed the other endemic species of the park (*P. teyahalee* and *P. metcalfi*) they would likely have found similarly high probability of decline, as they share similar risk factors to *P. jordani*. Next steps should include quantifying population responses at the northern end of the Appalachians, as well as experimental studies to determine species and population-specific responses to changes in climate variables.

We found a correlation between higher rainfall, higher elevations, cooler temperatures and declining populations, which is consistent with declines caused by *Bd* (Lips *et al.*, 2006). Various types of circumstantial evidence support *Bd* as a cause for these declines. First, our Maxent model indicated that most areas of the Park are generally suitable for *Bd*. Second, *Bd* is present within the Park, although at low prevalence and where populations have not declined. However, where *Bd* is enzootic, prevalence and intensity are generally low, including Central American populations of plethodontid salamanders that declined following invasion by *Bd* (Rovito *et al.*, 2009; Cheng *et al.*, 2011). Surveys in eastern United States have found moderate prevalence of *Bd* in pond-breeding amphibians (Chatfield *et al.*, 2009), but low prevalence in stream and terrestrial salamanders (Campbell Grant *et al.*, 2008; Chatfield *et al.*, 2009). Third, *Bd* has been present in the area for at least 40 years. Ouellet *et al.* (2005) reported *Bd* from northern Virginia in the 1970s, and Daszak *et al.* (2005) found *Bd*-positive frogs collected from South Carolina in 1978–1981 – although they concluded that long-term hydrological patterns contributed to population declines at that site. Taken with this circumstantial evidence, our results lead us to hypothesize that *Bd* invaded the Appalachian region in the 1960s or 1970s causing widespread population declines in the most susceptible species and that populations have not recovered because *Bd* is now enzootic. We attribute low current day prevalence in wild populations to one or more mechanisms that would allow salamanders to avoid or minimize infections, such as genetic (Savage & Zamudio, 2011), microbial (Harris *et al.*, 2006) or ecological traits (e.g. Lips *et al.*, 2003). We have begun surveys of museum specimens to search for *Bd* from these sites and species that would provide evidence in support of our hypothesis.

The role of ecology

Ecological differences among species may explain variation in population response. We found that larger-bodied salamanders declined, which is consistent with reports of increased risk of decline in larger-bodied amphibians (Lips *et al.*, 2003; Cooper *et al.*, 2007). In Mexico and Guatemala, Rovito *et al.* (2009) showed widespread declines in several large-bodied species of terrestrial *Pseudoeurycea*. Typically,

larger animals have longer generation times and lower population density; both these factors may impede their ability to recover from a reduction in population size (Cardillo *et al.*, 2005; Hero *et al.*, 2005).

Plethodon glutinosus and *P. teyahalee* are sister taxa (Kozak *et al.*, 2006), which may indicate a phylogenetic-based susceptibility (Corey & Waite, 2008; Crawford *et al.*, 2010). Sister taxa often share many ecological, habitat or behavioural similarities. In contrast, *P. cinereus* rarely die when exposed to *Bd* in the laboratory and are protected by cutaneous microbial flora (Harris *et al.*, 2006). It is not known whether these microbes also occur on members of the *glutinosus* group or whether the sticky secretions of the *glutinosus* group are related to declines.

Detecting population declines

The eastern United States is home to the debate whether amphibian declines are 'unusual' or are merely 'natural population fluctuations' (Pechmann & Wilbur, 1994; Travis, 1994). Numerous long-term ecological studies (e.g. Hairston & Wiley, 1993; Daszak *et al.*, 2005) have documented population fluctuations, but extirpations and mysterious die-offs may have been overlooked or not reported (e.g. Hunsaker & Potter, 1960; Houlahan *et al.*, 2000). Recently, Corser (2001), Highton (2005) and Means & Travis (2007) have reported enigmatic population declines during the mid-1970s to 1980s in three genera of salamanders in three regions of the eastern United States. In no case have populations recovered or a cause been definitively identified. The cryptic nature of *Plethodon* species may have limited recognition of those declines, and their fossorial habits make it unlikely that anybody would notice a die-off if it did occur, making it difficult to claim a species is completely absent. The combination of broad geographic and elevational ranges of these species minimizes the chance that a species will go extinct, as surviving populations can act as source populations. Researchers often attribute these low abundances to short-term weather conditions (Daszak *et al.*, 2005) or historic collecting. This is widely appreciated as the shifting baseline problem (Pauly, 1995), which can obscure continual but slight declines (e.g. Whitfield *et al.*, 2007) unless compared with long-term data. We suggest that other 'enigmatic' declines in the east are likely to be identified when historic collections and data are re-evaluated (e.g. Houlahan *et al.*, 2000), especially where museum collections are available for corroboration.

CONCLUSIONS

The southern Appalachians are one of the world's hotspots for salamander biodiversity, and one of the best-studied faunas (e.g. Dunn, 1926; Tilley, 1973; Hairston, 1981). We found statistically robust evidence of historic widespread population declines in multiple species of salamanders throughout the study area. Until recently, these losses were undetected and have yet to receive the attention they deserve.

Extensive collections and detailed field notes provided the historic data necessary to conduct a systematic, large-scale resurvey needed to discriminate geographic, taxonomic and threat-specific patterns of decline. Despite this, we were unable to identify a definitive cause, although we were unable to rule out disease or climate change. These results are likely to stimulate discussions regarding shifting baselines, use of historic data sets and potential causes for declines. We hope these discussions convince others of the 'enigmatic' loss of amphibian biodiversity in the Appalachians.

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BIOSKETCHES

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Editor: David Green.