Abstract

Title of Document:	ENVIRONMENTAL VARIABLES ASSOCIATED WITH POPULATION CHANGES OF PLETHODONTID SALAMANDERS IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK, USA
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I used a long term collection database to compare 72 current populations of six species and three hybrids of *Plethodon* salamanders in the Great Smoky Mountains National Park (GRSM). I analyzed population abundance and adjusted for detection probabilities, over time for each species, with respect to null models. I also examined biotic and abiotic factors as potential causes for changes in population abundance. Population response varied among species, *Plethodon glutinosus* and *P. teyahalee* declined while *P. jordani x metcalfi* and *P. ventralis* increased at a greater rate than what was expected by historic variation in abundance. Declines of GRSM salamanders most likely began in the late 1960's to early 1970's and were associated with cooler and moister habitats. I conclude that species' biology may explain the variation in population responses and propose future research to determine the cause.

ENVIRONMENTAL VARIABLES ASSOCIATED WITH POPULATION CHANGES OF PLETHODONTID SALAMANDERS IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK, USA

By

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2011

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Acknowledgements

This study was approved by the UMD Institutional Animal Care and Use Committee (R–09–09); research permits were issued from the Great Smoky Mountains National Park through the National Park Service.

I would like to express my gratitude to my advisor Dr. Karen Lips, for her immense support and guidance through my graduate degree. I would also like to thank the members of my committee, Dr. Daniel Gruner and Dr. James Dietz, for their assistance. I thank Dr. Jake Kerby for providing me the opportunity to learn DNA extraction and PCR techniques. Special thanks go to Dr. Richard Highton, whose incredible knowledge is a continued inspiration and the Smithsonian National Museum of Natural History: Addison Wynn, Roy McDiarmid, and Steve Gotte. I would also like to thank Paul Super for his assistance in getting around and living within the Great Smoky Mountains.

I am very appreciative to my loving parents, Frank and Maria, as well as my brother and sister, Matt and Jess, who have always been there for me and offered their love and support. I would like to thank Dot Lundberg for her support and enduring many stressful late nights of writing with me. I would like to thank the SIU and UMD herp labs: Sarah, Sandra, Brooke, Erin, Toren, Kyle, Cora, Stephanie, and Rebecca for their helpful suggestions and comments. I would also like to thank all of my volunteers for assistance in the field.

Table of Contents

List of Tables

List of Figures

Introduction

Plethodon salamanders were once immensely abundant throughout the United States. In the Great Smoky Mountains National Park (GRSM), densities of *Plethodon jordani* and *P. teyahalee* were estimated at 8,600 and 2,300 individuals/hectare respectively (Merchant 1972). In the Southern Appalachian region, salamander density was estimated at 5,961–9,935/ha (Hairston 1987), and salamander density in western North Carolina was estimated at 10,000/ha (Petranka et al. 1993). In a New Hampshire forest, Burton and Likens (1975a) found that *P. cinereus* occurred in such large densities that their estimated biomass exceeded the combined biomass of all small vertebrates.

Because of their naturally high abundance and density, *Plethodon* salamanders are important components of forest ecosystems (Davic and Welsh 2004). These salamanders are considered the dominant vertebrate predators of leaf litter arthropods (Hairston 1987) and function as keystone predators of leaf litter invertebrates (Davic 1983; Wyman 1998). Woodland salamanders also function as important prey species for birds and small mammals and form an important link in the food web. *Plethodon* salamanders are able to efficiently convert small prey (e.g., forest invertebrates) into available biomass for larger predators (e.g., small mammals; Burton and Likens 1975b).

In addition to their role in forest food webs, *Plethodon* salamanders are important to forest ecosystem function. Woodland salamanders are considered ecosystem engineers, because they are able to modify resource availability for other species by causing either physical or chemical changes in habitats (Jones et al. 1994). The mechanism by which salamanders alter their habitat is through the creation or use of underground burrows (Brooks 1946; Heatwole 1960), which may lead to translocation of

nutrients underground from the forest floor (for plants), deposition of excretory nutrients (for bacteria and fungi), and increased dispersion of gases through the soil (Davic and Welsh 2004). Through control of forest floor invertebrates, salamanders may have an indirect effect on leaf litter decomposition and nutrient recycling (Hairston 1987; Wyman 1998; Wyman 2003).

Woodland salamanders are excellent study organisms for monitoring abiotic and biotic components of forest ecosystems because fluctuations in their populations can be associated with changes in leaf litter, air and water pollution, invertebrates, pH, and moisture (Welsh and Droege 2001). Moreover, Welsh and Droege (2001), reported that salamander population trends can be detected more quickly and with fewer years of monitoring effort than with other vertebrates. Therefore, successive surveys of terrestrial salamanders over a long period of time can provide reliable estimates of population changes. These changes, therefore, will be important to assess the changes in the forest ecosystem.

Studies of *Plethodon* population abundance over time have shown opposing trends for different species, times, and geographic locations (Wyman 2003). From 1976 to 1990 at two different sites, Hairston and Wiley (1993) found no changes in abundance for *P. glutinosus* (*teyahalee*; Highton 1984) and *P. jordani*. Elsewhere, Highton (2005) described declines of both *P. jordani* and *P. teyahalee* over 40 years. Globally, at least 21 of 350 species within the family Plethodontidae have become more threatened in the last 20 years (Stuart et al. 2004). Declines of 13 of these species were attributed to habitat loss, while 8 were classified as enigmatic, declining despite residing in suitable habitat (Stuart et al. 2004). Currently, 22 species of

Plethodon salamanders have an IUCN listing as "Near Threatened" or higher, including *P. jordani* (IUCN 2010).

Synchronous, widespread declines occurred in 180 populations of 38 species of eastern U.S. *Plethodon* salamanders (Highton 2005; Figure 1); of these species only *P. welleri* is currently listed as "Endangered" (IUCN 2010). Reductions in abundance were first noticed in the late 1980's throughout these populations, which suggests that the same factor or factors affected these populations in a similar manner (Highton 2005). Species with populations that declined occurred at sites where other species did not decline (Highton 2005; Figure 1), which suggest that species' biology is an important factor in determining which species declined and which did not decline. Extensive habitat destruction was observed at 16 sites (22 populations; Highton 2005), but it did not explain the other 158 population declines documented by Highton (2005), including populations in protected areas, in which habitat loss, land–use change and overexploitation cannot explain these losses.

Plethodon salamanders face many different threats. Six identified main threats are likely the cause of most amphibian population declines: overexploitation, land use change, alien species, emerging infectious diseases, pollution (e.g., habitat acidification), and global climate change (Collins and Storfer 2003). Within *Plethodon*, the main threat to population abundance appears to be timber harvesting (Wyman 2003), although it has not caused any documented species extinctions (IUCN 2007); however, habitat acidification may be another important threat (Wyman 2003). Additionally, global climate change has been implicated in declines in Central American plethodontid

populations (Rovito et al. 2009) and *Plethodon* species are susceptible to emerging infectious diseases (Vazquez et al. 2009; Weinstein 2009)

Timber harvesting can create unsuitable habitat for terrestrial plethodontids (Ash 1988) by reducing leaf litter, shade soil moisture and increasing surface temperature (Bury 1983; Ash 1988). These conditions, therefore, create drier habitats that limit surface activity and may cause mortality due to increased physiological stress (Petranka et al. 1993). Additionally, Petranka et al. (1993, 1994) reported that *Plethodon* abundance may not recover until 50 – 70 years after harvesting because younger tree stands create less favorable (i.e., drier and warmer) microhabitats than mature stands. Although protected areas (e.g., National Parks) preserve salamander habitat from harvesting, changes in populations may be lagged effects of this past logging. Because *Plethodon* salamanders are lungless and rely on moist skin for cutaneous respiration, it is likely that all species are affected similarly to harvested forests (see Petranka et al. 1993); however, some evidence suggests that *P. teyahalee* may be less sensitive to drier forests, as a result of timber harvesting, than *P. jordani* (Ash 1997).

Acidic habitat can affect salamanders as well as their ecosystem. These acidic environments may create unsuitable habitat for terrestrial salamander populations (Wyman and Jancola 1992; Frisbie and Wyman 1995). Individuals raised in low pH (< 3.8) aquatic and terrestrial environments can experience direct mortality, increased deformity rates and reduced rates of growth and development (Rowe and Freda 2000; Wyman 2003). Additionally, salamanders living on acidic substrates (< 3.8) lose sodium, water, and overall body mass more readily than salamanders living on less acidic substrates (Frisbie and Wyman 1991). This disruption in sodium balance may create

unfavorable habitats to terrestrial salamander populations on acid soils or in areas receiving increased acid deposition (Wyman 2003).

Infectious diseases can cause amphibian morbidity and mortality. Chytridiomycosis is an emerging infectious disease of amphibians caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*; Longcore et al. 1999). *Bd* attacks the keratinized epidermis of adult amphibians and the keratinized mouthparts of tadpoles (Berger et al. 1998). Susceptible amphibian species with high intensity infections experience electrolyte depletion and osmotic imbalance, which leads to cardiac arrest and death (Voyles et al. 2007; 2009).

Disease occurrence varies among different hosts and different habitats and is determined by the interaction between three factors: the host, the pathogen and the environment (Wobeser 2006). Some amphibians can clear an infection or can persist without signs of disease (Daszak et al. 2004). In terrestrial salamanders, cutaneous antifungal bacteria (Harris et al. 2006) produce antifungal metabolites (Brucker et al. 2008), which can reduce the level of *Bd* infection (Harris et al. 2009a, b). *Bd* thrives under cooler temperatures (Piotrowski et al. 2004) and moist environments (Johnson et al. 2003) and can be cleared in warmer (Woodhams et al. 2003) and drier conditions (Johnson et al. 2003). Optimum growth of *Bd* occurs between 17 – 25°C (Piotrowski et al. 2004) and the critical thermal maximum of *Bd* is 30°C (Piotrowski et al. 2004). Consistent with *Bd*'s environmental tolerances, infection prevalence, in several empirical studies, increased during cooler months compared to warmer months (Woodhams et al. 2003; Berger et al. 2004; Kriger and Hero 2006; Weinstein 2009). Variation in species response to *Bd* has also been attributed to differences in

habitat, microhabitat (Lips et al. 2003; Rowley and Alford 2007; Brem and Lips 2008), thermoregulation (Richards–Zawacki 2010), amphibian immune response (Ramsey et al. 2010), skin peptides (Harris et al. 2006), and amphibian behavior (Longo et al. 2009), amphibian density (Briggs et al. 2010).

Arrival of *Bd* into a susceptible population is followed by amphibian mortality, increases in prevalence and intensity of disease, and population declines (Lips et al. 2006; Briggs et al. 2010). Following *Bd*'s arrival, amphibian communities experience a loss of regional and local scale diversity, in which many endemic species are lost (Crawford et al. 2009; Smith et al. 2009). Declines attributed to *Bd* have occurred in amphibian populations throughout the world (Berger et al. 1998; Lips et al. 2006; Rachowicz et al. 2006). Within the United States, confirmed cases of amphibian population declines attributed to *Bd* have occurred in California (Rachowicz et al. 2006) and Colorado (Muths et al. 2003) and mortality events associated with *Bd* have occurred in adult anurans in California, Colorado, North Dakota, North Carolina, and Wyoming (Green et al. 2002). Amphibian response to *Bd* throughout the US varies. Populations in the Western US experience epizootic outbreaks consistent with a naïve population, while Eastern US amphibians do not show the same patterns (Green et al. 2002).

Bd has caused salamander population declines worldwide (Berger et al. 1998; Collins and Storfer 2003; Lips et al. 2006) and is present on every continent in which amphibians occur (Berger and Speare 2000; Goka et al. 2009). In Peñalara National Park, Spain, *Salamandra salamandra* have experienced population declines (Martínez–Solano et al. 2003), in which heavy *Bd* infections were confirmed in several dead

individuals (Bosch and Martínez–Solano 2006). Although *Bd*–related declines and mortalities have rarely been reported for salamanders, *Bd* infections have been confirmed in many genera, including: *Ambystoma* (Davidson et al. 2003; Ouellet et al. 2005), *Notophthalmus* (Ouellet et al. 2005), *Necturus* (Speare and Berger 2000), *Siren* (Speare and Berger 2000), *Pseudotriton* (Speare and Berger 2000), *Desmognathus* (Grant et al. 2008), *Eurycea* (Grant et al. 2008), *Bolitoglossa* (Pasmans et al. 2004; Lips et al. 2006; Rovito et al. 2009; Crawford et al. 2010), and *Taricha* (Padgett–Flohr and Longcore 2007).

Within the genus *Plethodon*, reports of infected species include: *Plethodon neomexicanus* (Cummer et al. 2005), *P. cinereus* (www.spatialepidemiology.net/bd/), *P. glutinosus* (Chinnadurai et al. 2009), *P. yonahlossee* (Chinnadurai et al. 2009) and *P. metcalfi* (Vazquez et al. 2009). Although population declines attributed to *Bd* have occurred within *Plethodontidae* in Central America (Lips 1998; Lips and Donnelly 2005; Lips et al. 2004; Lips et al. 2006; Rovito et al. 2009; Crawford et al. 2010), there are no confirmed cases of *Plethodon* population declines directly caused by *Bd* in the US.

Ranavirus and has been implicated in amphibian declines in various species (e.g., Collins et al. 1988; Chinchar 2002; Ariel et al. 2009). In the US, ranaviruses have been associated with some amphibian mortalities (Green et al. 2002) especially in *Ambystoma* spp. (Green et al. 2002; Greer et al. 2009). Dead amphibians with ranaviruses have been found in New Hampshire, Colorado, North Dakota, Minnesota, Maine, Utah, Idaho, Tennessee, Massachusetts, Wyoming, and North Carolina (Green et al. 2002). Higher ranavirus prevalence estimates are associated with higher elevations (Gahl and Calhoun 2008) and its virulence is likely increased through

natural (e.g., metamorphosis) and anthropogenic stressors (e.g., nitrogenous compounds; Forson and Storfer 2006; Gray et al. 2007; Gray et al. 2009a). Within *Plethodon*, reports of susceptible species include: *P. jordani*, *P. metcalfi* and *P. jordani x metcalfi* (Gray et al. 2009b; M.J. Gray and N.M. Caruso *unpublished data*).

Climate change can impact protected amphibian populations (Carey et al. 2001; Carey and Alexander 2003; Rohr et al. 2008; Rodenhouse et al. 2009; Rohr and Raffel 2010). Warmer and drier climates are likely to negatively impact many amphibian populations (Blaustein et al. 2003; Pounds 2001; Rohr and Madison 2003). In the eastern US, predictive models predict warmer and wetter climates (Girvetz et al. 2009). These changes may be problematic for terrestrial lungless salamanders (i.e., plethodontid salamanders), which rely on moist, permeable skin to allow gas exchange (Whitford and Hutchison 1965). Because of their permeable skin, plethodontid salamanders are prone to dehydration, are restricted to cool, moist microhabitats, and have limited surface activity, even during favorable conditions (Feder 1983; Bernardo and Spotila 2006).

Amphibian species most at risk of global climate change include those that are already at their physiological limits of temperature and/or moisture (e.g., montane-adapted terrestrial plethodontids; Bernardo and Spotila 2006), those that depend on ephemeral wetlands, or those that cannot disperse (Reaser and Blaustein 2005). Based on the degree of metabolic depression associated with reduced temperature, current high elevation species of plethodontid salamanders with restricted ranges (e.g., *P. jordani*) are most likely distributed near the upper limits of their thermal tolerances (Bernardo and Spotila 2006; Bernardo et al. 2007). Global circulation

models predict that rising CO_2 levels will lead to climate changes in the northern hemisphere, including the GRSM (IPCC 2007), in which average temperatures will continue to increase (Girvetz et al. 2009). Because of these warming temperatures, lower elevation forest habitats may become unsuitable for southern Appalachian salamanders (Milanovich et al. 2010).

Changes in climate can also affect disease dynamics by shifting the host-pathogen relationship in favor of the pathogen (Wobeser 2006; Rohr et al. 2008). Decreases in temperature may reduce the host's ability to fight infection (Vazquez et al. 2009) while increased precipitation may create more suitable habitat for disease (Rohr et al. 2008; Weinstein 2009). Natural changes in climate, such as El Niño – Southern Oscillation (ENSO) events may create more favorable conditions for pathogen persistence and transmission (Weinstein 2009), in producing higher than average rainfall in the Eastern United States (NOAA 2010; NCDC 2010). Therefore, ENSO years may allow a pathogen that normally exists at low levels, which do not cause decreases in population abundance (Vredenburg et al. 2010), to increase transmission and cause declines.

Objectives

I analyzed the change in population abundance over 49 years for six species and three hybrids of *Plethodon* salamanders in the GRSM at 35 historic collecting sites. I used a collection dataset to determine historic population abundance and field surveys to determine current population abundance. My goal for this project was four–fold. (1) To determine if changes in abundance of *Plethodon* salamanders differed from expected

based on historic population abundance and adjust count indices based on detection probabilities; (2) to determine the relationship among changes in population abundance and species, sites, and elevations as well as determine the approximate timing of population declines; (3) to estimate the change in diversity within each site as a function of species presence/absence and abundance changes; (4) to search for associations between these population changes and potential threats (e.g., *Bd*'s predicted and actual occurrence, forest disturbance, current temperature, precipitation patterns, and changes in precipitation and temperature from 1961 – 2006). These analyses will indicate potential causes for declines in *Plethodon* salamander populations the GRSM.

If populations declined because of forest stand age or disturbance, I expected declines to have occurred in young forests, with stable or increasing populations in old growth forests. Furthermore, I would expect populations of *P. teyahalee* to remain stable because this species is more tolerant of timber harvesting (Ash 1997).

If acid deposition has caused *Plethodon* salamander declines in the GRSM then I expected an elevational pattern, with all high elevation, which is correlated with increased acid deposition in the GRSM (Robinson et al. 2008), population to declined, while all low elevation populations would remain stable or increase.

I searched for associations between declines and environmental variables (Lips et al. 2003; Gray et al. 2009a) and spatial relationships. If *Bd* were a potential cause for declines, declines should be associated with areas of higher *Bd* suitability [as predicted by Maxent models (Phillips et al. 2006)], I further expected that population declines would be spatially clustered in areas of high *Bd* suitability. Furthermore, I expected populations which inhabited cooler temperatures and higher precipitation, which may promote *Bd* or

ranavirus persistence, will have declined, while populations which inhabit warmer temperatures and lower precipitation will not have declined. Additionally, I expected to find these ranavirus or *Bd* within populations and species that have declined and at a lower prevalence in populations and species which have remained stable or increased.

If changes in temperature and precipitation were a potential direct cause for declines, I would expect that populations will have declined in areas that have become warmer and drier over the last 55 years. I expected populations to have remained stable or increased in areas that have become cooler and wetter since 1951. Additionally, I expected the timing of declines to be synchronous with extreme weather events (e.g., El Niño – Southern Oscillations; Pounds et al. 1999).

Methods

Study Area

The GRSM, which straddles the Tennessee–North Carolina border, encompasses 205,665 ha of contiguous forest, categorized into five major forest types: Cove Hardwood, Spruce–Fir, Northern Hardwood, Hemlock, and Oak–Pine (Jenkins 2007). Approximately 95% of the park is forested and 25% of this is old–growth forest (Davis 1993). Forest stands, especially mature stands, are critical habitat for *Plethodon* salamanders (Dupuis et al. 1995) because they provide extensive canopy coverage, which inhibits sunlight penetration (Phillips and Shure 1990) that can otherwise lead to higher temperatures and drier leaf litter (Ash 1995). Moreover, mature stands provide abundant coarse woody debris, which serves as moist retreat sites for terrestrial salamanders (Feder 1983). Elevations in the GRSM range from 267 – 2,025 meters with highly variable topography, which include gentle and steep slopes, level valleys, talus slopes, incised drainages and rocky summits (Jenkins 2007). The GRSM has a temperate climate; annual temperatures range from -2 to 31 ^{o}C in the low elevations (~265 meters), while at the highest elevations (2,025 meters) annual temperatures range from -18 to 19 ^{o}C (NPS 2010). Precipitation also varies with elevation; currently, the highest elevations receive an average of 2,160 mm of precipitation per year while the lower elevations average about 1,397 mm annually (NPS 2010). The GRSM is home to nearly 10% of the world's salamander species (Petranka 1998); at least 30 species (5 families) reside within the GRSM (Tilley and Huheey 2004). Because of the high salamander diversity the GRSM is

known as “The Salamander Capital of the World” (NPS 2010) and is an international temperate forest biodiversity refuge (NPS 2010).

Despite its protected status, GRSM salamander diversity may be threatened by factors such as historic timber harvesting, acid precipitation, emerging infectious diseases, and climate change. Although the GRSM is currently protected, historically, forest harvesting has occurred until 1939 (NPS 2010), while precipitation pH at the highest elevations (NPS 2010) is currently low enough to cause reduced growth or mortality of salamanders (Wyman 2003). *Bd* has been present in the southeastern US since 1978 (Daszak et al. 2005) and both *Bd* and ranavirus are present within the GRSM (Chatfield et al. 2009; Gray et al. 2009b; Todd-Thompson et al. 2009). Additionally, projections of future climate trends using species distribution models (SDMs) show range contractions for southern Appalachian salamanders as early as 2020 (Milanovich et al. 2010).

Site Selection

From the 1960 to 2001, Richard Highton collected over 17,000 plethodontid salamanders at over 300 sites throughout the GRSM. Detailed field notes were taken for every visit, including: geographic coordinates, date of collection, species, number of individuals encountered, and number of collectors. These collected specimens as well as the research notes are currently housed at the US National Museum of Natural History (USNM). These sites were sampled for over 50 years in the same manner, which allowed for unique opportunity to study multiple species throughout the GRSM for over a half century (Shaffer et al. 1997).

I selected 35 of Highton's collecting sites that occurred within the GRSM (Figure 2) and which had been sampled during multiple years. I sampled 2 plots within each site to the increase power for detecting changes in abundance (Smith and Petranka 2000). I selected sites that encompassed a wide geographic and elevation range (488 to 1,972 meters) to represent a variety of climate space and include the distribution of the 6 species of *Plethodon* salamanders within the GRSM. I collected sampled field sites during March, May through July and November of 2009 (Figure 2). I sampled seven low elevation sites (~500 m – 1,000 m), 19 mid elevation sites (~1,000 m – 1,500 m) and nine high elevation sites (~1,500 m – 2,000 m).

Study Species

Six species of *Plethodon* salamanders occur within the GRSM (Tilley and Huheey 2001). All six species are lungless, are completely terrestrial, and utilize natural cover objects as aboveground shelter on the forest floor (Wells 2007). One to three species occupied the 35 study sites for a total of 72 populations of *Plethodon* salamanders (Tables 2 and 3).

Susceptibility to *Bd* varies among species of terrestrial plethodontids. *Plethodon glutinosus* has been found to be infected with *Bd* in the wild (Chinnadurai et al. 2009; Table 3) while *Plethodon metcalfi* shows clinical signs of chytridiomycosis and mortality when infected with high doses of *Bd* in the lab (Vazquez et al. 2009). Susceptibility to *Bd* for the other species of GRSM salamanders is unknown, and no fully terrestrial plethodontid salamander has been found to be infected within the GRSM (Chatfield et al. 2009).

High elevation populations of terrestrial salamanders are most likely distributed near the upper limits of their thermal tolerances (Bernardo and Spotila 2006; Bernardo et al. 2007). High elevation species experience high rates of metabolic depression with increasing temperatures, which can limit their ability to disperse through lower valleys or adapt to warming conditions (Bernardo and Spotila 2006). *P. serratus, P. metcalfi, P. teyahalee*, and *P. jordani* inhabit all three elevational bands (Table 1), while *P. ventralis* is the only species that is not found at elevations over 1,000 m (Table 1) and *P. glutinosus* inhabits elevations below 1,500 m (Table 1). Species and populations inhabiting the warmer, low elevation habitats may not be able deal with the physiological stress associated with warming trends (Bernardo and Spotila 2006).

Field Methods

From 1960 – 2001, R. Highton and colleagues surveyed these 35 sites at least once and not more than seventeen times (Figure 3). Each site is characterized as an area marked by one geographic coordinate, in which Highton and colleagues searched natural cover objects (i.e., rocks and logs) for approximately one hour. All salamanders were captured by hand, identified and usually collected (R. Highton *personal communication*). During a site visit, Highton used topographic and quadrat maps to georeference the site location, which allowed him to return to the same site over many years.

I located using a combination of a handheld GPS Unit and topographic maps. I sampled each site two to four times and searched two different per visit. I surveyed each site multiple times to limit the chances that the number of salamanders encountered was not biased towards good or bad weather events but rather was a more accurate index of

abundance for that site during over time. I sampled each site during optimal conditions (e.g., early morning or during cooler, moister weather conditions) to maximize the chance of encountering salamanders, consistent with the historic sampling (R. Highton *personal communication*). Using red flagging, I marked off two 50 m x 3 m plots within each site. I combined relative abundance indices for the two plots for each site visit, which increases the power to detect population abundance changes in terrestrial plethodontids (Smith and Petranka 2000). Estimated home range sizes for *Plethodon glutinosus, P. jordani, P. metcalfi,* and *P. teyahalee* are less than 15 m^2 (Table 3; Merchant 1972; Nishikawa 1990; Marvin 1998), so I maintained at least 15 meters between plots within a site visit and between visits. To allow comparison among Highton's surveys and my surveys, I used the number of salamanders accounted for effort (the number of surveyors multiplied by the amount of time surveying) as abundance indices.

In each plot, I turned all natural cover objects and captured all salamanders by hand. Upon capture, I placed each salamander in a new plastic bag to prevent cross–contamination in case of infected individuals (Hyatt et al. 2007). I recorded the species identification, snout–to–vent length (SVL), tail–to–vent length (TVL), and mass for each individual. Additionally, I measured the length and width of each natural cover object that I searched under and indicated the type of cover (i.e., rock, log, surface, tree bark). To test for *Bd,* I used a skin swab, using a cotton–tipped plastic applicator (Medical Wire and Equipment, M113), I swabbed the ventral surface ten times and each limb five times for a total of 30 swabs per individual (Hyatt et al. 2007). I stored all swabs in vials (Fisher, 02–681–374) of 75% ethanol at

room temperature until quantitative PCR analysis, which I performed in January of 2010.

After processing, I released all salamanders at their point of capture and replaced the natural cover object to its original position to minimize habitat disturbance (Smith and Petranka 2000). I quantified the total amount of time searching for salamanders at a site. I did not include time spent handling and processing salamanders or measuring cover objects because these actions were not performed during historic surveys. Between captures, I used new powder–free latex gloves to prevent disease transmission.

Swab Analysis

I performed PCR analysis on all swabs at the University of South Dakota under the supervision of Dr. Jake Kerby. DNA from all swabs was extracted using a QIAGEN DNeasy tissue and blood purification kit (QIAGEN, 69581). DNA was amplified using a FAST PCR machine using the primers and probes described in Boyle et al. (2004). I pooled swabs into groups of three and ran those pooled swabs in triplicates to reduce the likelihood of obtaining a false negative (Hyatt et al. 2007). When the pooled swabs returned a positive result, we reran the three swabs individually in triplicates, which identified the positive sample and allowed for estimates of infection intensity. I estimated the prevalence of *Bd*, defined as the number infected out of the total sample, and calculated 95% Clopper–Pearson binomial confidence intervals using the *Hmisc* package (Harrell Jr. et al. 2010) in Program R (R Development Core Team 2009). Additionally, I estimated the intensity

of infection by multiplying the number of zoospores detected in each sample by 100 (the dilution of the final sample) to determine the level of infection. This value was used as the estimate of the number of *Bd* zoospores in each swab, or genomic equivalents (GE). Infection intensity is an important predictor of amphibian population responses to *Bd* (Vredenburg et al. 2010). High average infection intensity (> 10,000 GE) in a population is associated with individual mortality, population declines and extirpations (Vredenburg et al. 2010; V.T. Vredenburg *personal communication*).

Data Analysis

Detection Probabilities

I estimated detection probabilities for each species during survey decades to account for variation in amphibian detection and surface activity (Schmidt 2009). I used the software program Presence version 3.0 (Hines 2006) to determine detection probabilities for each species using presence/absence data for each survey visit and a multi-season model to estimate detection probabilities during each decade of survey. I used these detection probabilities to adjust count indices used in subsequent analyses (Schmidt 2009; Bailey et al 2004).

Patterns in Abundance and the Environment

I used R (R Development Core Team 2009) and ArcMap version 10.0 (ESRI, Redlands, California, USA) for all statistical analyses. I used a generalized linear mixed effects model (GLMM; lme4; Bates and Maechler 2010) to measure the

change in abundance over time and analyzed the relationship between abundance and changes in the environment (i.e., temperature and precipitation patterns) for each species.

I ran five models for each species (Table 7). First, I analyzed the relationship between population abundance and the year of survey (YEAR; Model 1) to determine if population abundance has changed. Next, I included environmental variables (average mean temperature, annual precipitation, change in annual temperature from 1951 – 2006, and change in annual precipitation from 1951 – 2006) as site covariates for the six taxa with more than one population. To evaluate *Bd* and ranavirus as a potential cause for declines I used annual mean temperature (AMT; Model 2) and annual precipitation (AMP; Model 3) from WorldClim (1 km resolution; Hijmans et al. 2005). These variables were chosen because they are important in determining patterns of *Bd* abundance using climatic envelope models (Puschendorf et al. 2009; Murray et al. 2011). These data were interpolated from a compilation of five different global weather stations (Hijmans et al. 2005). These variables demonstrate the magnitude of the annual temperature and precipitation at all sites, which allows comparison among sites based on their climate (e.g., warmer or cooler). Higher values for AMT or AMP denote areas that are currently warmer or wetter than the lower values.

To evaluate climate change as a potential cause for declines, I used two variables: the change in annual temperature (Δ Temp; Model 4) from 1951 – 2006 and change in annual precipitation (Δ Precip; Model 5) from 1951 – 2006 (Girvetz et al. 2009). These variables explain the direction (i.e., increase or decrease) and

magnitude that the annual temperature and precipitation over the 55 years of surveys at these sites. A positive value for Δ TEMP or Δ PRECIP indicates areas that have become warmer or wetter over the last 55 years respectively, while negative values for Δ TEMP or Δ PRECIP denotes areas that have become cooler or drier over the last 55 years respectively. I obtained estimates for of the climate change variables from Climate Wizard (4 km resolution; Girvetz et al. 2009), an online tool that provides an ensemble average of 16 global circulation models for future climate predictions, as well as provides a compilation of the last 50 years of climate trends. For the US, the past 55 years of climate were derived from 8,000 climate monitoring stations (Girvetz et al. 2009).

To extract climate data for each site, I used ArcGIS and Geospatial Modeling Environment (GME; Beyer 2010). Using ArcGIS, version 9.3.1 (ESRI, Redlands, California, USA), I imported my sites as a shapefile and imported the climate data as raster layers. I used the *insectpntrst* command in GME to extract the climate data for each site based on geographic location (Beyer 2010).

I modeled the relationship between the adjusted counts and the survey date and nested the site within the survey year as the random variable (Shaffer et al. 1997). Therefore I was able to evaluate each species as a composite of all populations, which allowed me to determine the overall change in relative abundance for that species, as well as compare each surveyed population at a given site. I standardized the effort by the number of person hours and used a Poisson distribution (e.g., Roulin and Bersier 2007) and fit each model using maximum likelihood. The fixed effects varied in units, therefore, I standardized (i.e., subtracted the mean and divided by the standard

deviation) the year of survey, AMT, AMP, Δ TEMP, and Δ PRECIP (Marquardt et al. 1980). All models were selected in the following manner. First, I ran each model with standardized year as the only fixed effect. Next, I ran each model with the standardized environmental site covariates (AMT, AMP, Δ TEMP, and Δ PRECIP) and selected the best fit models based on the lowest Akaike's information criterion with second order correction for small sample sizes (AICc; Sugiura 1978).

Determining Population Response

I used a conservative approach for classifying each population's change in abundance. I calculated the model coefficients and standard error for each population using the results from the overall GLMM (Qian et al. 2010) and assigned each population as "Decline", "Stable", or "Increasing" based on the coefficients (β_1) and standard error. If the upper and lower bounds of a population's standard error were negative, I considered this population to have declined. If the upper bound of a population's standard error was positive and lower bound was negative (i.e., the error bars crossed zero), I considered this population to be stable. If both of the upper and lower bounds of a population's standard error were positive, I considered this population to have increased. Additionally, I performed pairwise comparisons on all species to determine if changes in relative abundance varied among species. All pairwise comparisons were analyzed at the 95% confidence level. For each species, I graphed population changes over time from sites that had been sampled at least 8 times. I chose this number because 8 years of data is sufficient to detect changes in

abundance for *Plethodon* salamanders when surveying six sites (Smith and Petranka 2000), which was the average number of sites per species of my study.

Null Models

I created null models of changes in population abundance for each species through time based on the mean and variance of the historic relative abundance of each species from 1960 to 1979. I chose this time period because I wanted to know if my estimates for relative abundance change were within the normal fluctuations of relative abundance among historic sampling (Pounds et al. 1997) and during the "pre-decline" period reported by Highton (2005).

First, I estimated the mean and variance of adjusted relative abundance indices for all species with greater than one sampled populations between 1960 and 1979. Next, for each taxon, I generated 10,000 datasets of relative abundance during the total survey period (1960 – 2009). To generate these data, I used a quasi-Poisson distribution because the historic data were over dispersed (variance was greater than the mean; Ver Hoef and Boveng 2007). Next, I ran a generalized linear model (GLM), with a quasi-Poisson distribution (Ver Hoef and Boveng 2007) on each of run of the 10,000 datasets to determine the change in relative abundance over time. From these results, I determined if a population declined (i.e., standard error of β_1 were below 0), was stable (i.e., standard error of β_1 were above and below 0) or increased (standard error of β_1 were above 0; see Determining Population Response).

I performed a chi square test between the null model and my estimates, to determine if a species had changed more or less than expected, based on the null models.

Maxent Predictions for *Bd* in the GRSM

I used Maxent (version 3.3.0; Phillips et al. 2006) to model the suitability of *Bd* in the GRSM based on its presence in the United States and the climate. Maxent is a powerful presence-only SDM (Elith et al. 2006) that models the predicted environmental suitability (ES) based on the environmental conditions of the inputted presence localities for a given organism (Phillips et al. 2006). Maxent generates output ES maps for the target organism, in which higher values indicate areas that are predicted to be more suitable (Phillips et al. 2006). I obtained *Bd* localities from the Global *Bd*–mapping project (http://www.bd–maps.net/), an online resource providing information on *Bd*'s past and current range. Additionally, I used the locations of *Bd* occurrence in Appalachian plethodontids from previous research (Chatfield et al. 2009; Chinnadurai et al. 2009) and this study. I used 75 US locations with documented *Bd* presence to model predicted range. I used the 19 continuous bioclimatic variables (~5 km resolution; Hijmans et al. 2005) and followed the methods of Murray et al. (2009), in which all 19 variables were included in the full model and then selected those variables that contributed 90% of the information to the full model run. Next, I re–ran the trimmed model with the most important variables. I projected results of the trimmed model, using the same bioclimatic variables, onto the full range of the six GRSM Plethodon salamanders. This region

was obtained by clipping the trimmed bioclim variables by the shapefile of those species' distribution maps. Distribution maps were obtained from International Union for Conservation of Nature (IUCN 2011).

I used the area under the curve (AUC) of the receiver operator characteristic (ROC) to assess model accuracy (Fielding and Bell 1997). I used bootstrapping (N=100) on unique training and testing datasets (75%; 25% respectively) on the trimmed model to determine average and standard deviation of *Bd*'s suitability in the projected region.

I determined the relationship between responses for each population (i.e., declined, stable, and increased; see Determining Population Response) and the average predicted suitability of *Bd*. I used a multinomial logistic regression (*mlogit*; Croissant 2010) with the population responses as the response variable and average predicted suitability as the explanatory variable.

Elevation and Forestry Practices Patterns in Abundance

To obtain site elevation data, I used USGS 1–arc second National Elevation Dataset (NED; Gesch et al. 2002). To obtain logging history data, I used a forest disturbance GIS polygon (Griggs 2009). This shapefile shows the extent of historic logging in the GRSM (Griggs 2009). Although the actual amount of logging cannot be derived, these data show relative amounts of logging from the most ("Heavy Cut") to the least ("Undisturbed"). I used the model coefficients and standard errors from the overall GLMM (see Determining Population Response) to determine population status and related the status to forestry practices and elevation. I approached these

analyses using R (R Core Development Team 2009) and a two–sided Kendall's Correlation Test (Hollander and Wolfe 1973).

Community Size Patterns

I compared pre-decline (1960 – 1979) to post-decline (2009) species richness (based on presence/absence of a species at a site) and determined the number of declined species (as determined by GLMMs; see Determining Population Response) at each site.

Temporal Patterns in Abundance

I used piecewise–linear relationships on generalized linear models to determine the approximate timing of population changes. First, I analyzed the linear relationship between the relative abundance of each species and the year of each survey using a generalized linear model (GLM) and fitted the data using a quasipoisson distribution, which fits the data to the Poisson distribution while accounting for overdispersion (Ver Hoef and Boveng 2007). The GLM for each species was then used to find "breakpoints" in the regression, which are periods in which the slope changes (e.g., the beginning of a decline in population abundance would be signified by a change from a positive slope to a negative slope). I approached this analysis using the *segmented* package in Program R (Muggeo 2003; Muggeo 2008). I used 10,000 iterations for each segmented model.

Spatial Patterns in Abundance

I used ArcMap to measure spatial clustering of population responses. First, I used the model coefficients and standard error for each population using the results from the overall GLMM (see Determining Population Response; Qian et al. 2010). Next, I gave each population a numeric value based on the model coefficients; "1" for declined, "2" for stable, and "3" for increased. Next, I imported these coded model coefficients into an ArcMap shapefile using the geographic coordinates for each site. I projected the shapefile using "NAD_1983_HARN_StatePlane_Tennessee". Similar to the GLMMs, I did not analyze the 3 taxa (*P. glutinosus x teyahalee, P. metcalfi,* and *P. jordani x teyahalee*) for which I sampled one population. I assigned the coded coefficients as the Input Field, and used inverse distance and Euclidean distance as the Conceptualization of Spatial Relationships and Distance Method respectively. I analyzed each species using the High/Low Clustering (Getis-Ord General G) in the Spatial Statistic tools, which measures concentrations of high (increased populations) or low (decreased populations) values. High Z scores indicate clustering of populations that increased while low Z scores indicate clusters of decreased populations (Getis and Ord 1992).

Results

Bd Surveys

I swabbed 665 plethodontid salamanders, which included four genera; 12 species (*Desmognathus imitator*, *D. ocoee, D. santeetlah, D. wrighti, Eurycea wilderae, Gyrinophilus porphyriticus danielsi*, *Plethodon glutinosus, P. jordani, P. metcalfi, P. serratus, P. teyahalee* and *P. ventralis*), and 2 hybrids (*P. jordani x metcalfi* and *P. jordani x teyahalee*). I swabbed 485 *Plethodon* species (Table 4) and none were found to be infected within the GRSM (95% C.I. = 0 – 0.79). I found one *D. santeetlah* with a low intensity of *Bd* infection (29 GE) near a small stream at a high elevation site (1504 meters) on July 10, 2009. Prevalence for all plethodontid salamanders in the GRSM was 0.15% (95% C.I. = 0.007 – 0.847).

Detection Probabilities

All GRSM *Plethodon* salamanders were detected imperfectly (i.e., $p < 1$) during at least one survey decade (Table 5). Therefore, for each species, I corrected the relative abundance indices by the formula $N = C/p$ (where N the true parameter value, C is a count index and p is a detection probability during the survey decade; Schmidt 2009). I used the adjusted count indices for all subsequent analyses.

Species Abundance Patterns

Changes in population abundance varied with species (Table 6); some species and populations declined, some fluctuated but remained stable, while others increased (Figures 4 – 11). *Plethodon glutinosus* ($z = -0.2977$; $p = 0.0087$), *P. teyahalee* ($z = -$

3.1655; $p < 0.0001$), *P. jordani x teyahalee* ($z = -0.3753$; $p = 0.0004$) and *P. glutinosus x teyahalee* ($z = -3.2135$; $p = 0.0281$) decreased in abundance overall (Table 7). *Plethodon glutinosus* declined at all 5 of the resurveyed populations (Table 6) and *P. teyahalee* declined at 13 sites, increased at one site, and remained stable at two of the 16 resurveyed populations (Table 6). *Plethodon jordani x teyahalee* (N = 1) and *P. glutinosus x teyahalee* (N = 1) declined at their resurveyed populations (Table 6).

Plethodon jordani ($z = -0.0287.$; $p = 0.8100$), *P. metcalfi* ($z = -0.1461$; $p = 0.4170$), and *P. serratus* ($z = 0.0409$; $p = 0.8640$) remained stable overall (Table 7) although individual populations showed mixed responses (Table 6). *Plethodon serratus* (N = 18) and *P. jordani* (N = 18) showed the most variation; both species had populations that declined, remained stable, and increased (Table 6). *Plethodon metcalfi* was stable at the one resurveyed population (Table 6).

Plethodon ventralis ($z = 0.3560$; $p < 0.0001$) and the hybrid *P. jordani x metcalfi* ($z = 0.6195$; $p < 0.0001$) increased in abundance overall (Table 7). *Plethodon ventralis* increased at all four of the resurveyed populations while *P. jordani x metcalfi* increased at 87% (N = 8) of the resurveyed populations and remained stable at 13% of the resurveyed populations (Table 6).

Null Models

For all species, null models predicted that some populations declined, some increased, while others remained stable (Figures 12 – 17). *Plethodon glutinosus* ($\chi^2 = 25.7975$; $df = 2$; $p < 0.0001$) and *P. teyahalee* ($\chi^2 = 50.1073$; $p < 0.0001$) declined at a

greater number of populations than expected (Figures 12 and 13). Population responses for *Plethodon jordani* ($\chi^2 = 1.9088$; df = 2; p = 0.385) and *P. serratus* ($\chi^2 = 0.9981 = 2$; p = 0.6071) were not significantly different than expected from the null models (Figures 14 and 15). *Plethodon jordani x metcalfi* ($\chi^2 = 26.5368$; df = 2; p < 0.0001) and *P. ventralis* ($\chi^2 = 20.1869$; df = 2; p < 0.0001) increased at a greater number of populations than expected (Figures 16 and 17).

Maxent Predictions for *Bd* in the GRSM

The trimmed model included seven Bioclim variables, which contributed ~94% information to the full model. The mean test AUC for the trimmed model was 0.993). BIO 2 (Mean Diurnal Temperature Range) contributed the most unique information to the trimmed model, while the most model training was lost when removing BIO 7 (Precipitation of Driest Quarter) from the trimmed model (Figures 18 and 19).

The model predicts that the western portion of the GRSM is more suitable for *Bd* than the eastern portion of the GRSM (Figures 21 and 22). Based on the 100 models, the maximum suitability was 0.90 (Figure 20), while average suitability in the GRSM of *Bd* was 0.51 (± 0.12 standard deviation; Figure 21). The median suitability was highest in the declined populations compared to the stable or increased populations (Figure 22). However, the likelihood of population responses (i.e., declined, stable, increased) were not significantly different ($\chi^2 = 0.8816$; df = 2; p = 0.6435) based on predicted average *Bd* suitability (Figure 22).

Elevation and Forestry Practices Patterns in Abundance

All three elevational bands included populations that exhibited all three responses (Figure 23). The mid (1000 – 1,500 m) and low (500 – 1000 m) elevation sites had the highest percentage of declined populations, while the low elevation sites also had the highest percentage of increased populations (Figure 23). There was no correlation between the percentage of declined species and elevation ($z = -0.9371$, $p = 0.3487$). For the two species that had the largest elevational range, *Plethodon jordani* (1130 m) and *P. serratus* (996 m), declined populations only occurred at elevations greater than 1,300 m.

Declined, stable and increased populations were found in forests of all ages (Figure 24) and there was no correlation between the population response (i.e., declined, stable, increased) and the type of disturbance at each site ($z = 0.4761$; $p = 0.634$).

Temperature and Precipitation Patterns in the GRSM

Populations in the lower elevations experienced the highest temperatures and the lowest precipitation (Hijmans et al. 2005; Table 8), which may be less favorable environment for *Bd* and salamanders than the cooler and moister higher elevations (Lips et al. 2003; Bernardo and Spotila 2006). Populations at the higher elevations experienced decreases in annual temperatures over the last 55 years (Girvetz et al. 2009; Table 8); these cooler temperatures may promote more suitable habitat for terrestrial salamanders and *Bd* (Lips et al. 2003; Bernardo and Spotila 2006) than the

lower and mid elevations, which have populations that have increased in temperature since 1961 (Girvetz 2009; Table 8).

Acid Precipitation History in the GRSM

Currently, the precipitation pH at low elevation (640 meters) ranges from 4.08 – 6.05 (NADP 2010), while the highest elevations experience rainfall pH range of 3.92 – 5.7, with an average of 4.62 (MACTEC 2010).

Diversity Patterns

Historic salamander community composition at the 35 sites ranged from one to three species (Table 2; Figure 25) and averaged 1.91 species per site. Current community composition ranged from zero to three species (Figure 25) and averaged 1.23 species per site. During my resurveys of those same populations, I encountered species for 41 of the 69 historic populations. However, at 3 sites, I encountered a species during my surveys that was not found during any historic visit; therefore, adding three new populations to the 69 historic populations. At 66% of sites (23 out of 35) had at least one declined species and 39% of populations (28 out of the 72) declined (Table 2).

Temporal Patterns in Abundance

Breakpoints, which indicate changes in the slope of population abundance over time (e.g., Lips et al. 2006) that fall within the temporal bounds of this study (i.e., 1960 – 2009), could be determined for one species (*Plethodon jordani;* Figure

26). *Plethodon jordani* began to change in 1970 (95% C.I. = 1965 – 1975; Figure 26). The pattern in abundance change for *Plethodon jordani* appears to be gradual over time (Figure 26).

Spatial Patterns in Abundance

Declined populations co-occurred with stable and increased populations for GRSM *Plethodon* salamanders. For all species, the 3 population responses ("declined", "stable" or "increased") did not show any significant clustering and were randomly distributed throughout the GRSM (Table 9). For a given species, populations that had declined or increased were not spatially clustered around other declined or increased populations of the same species.

Patterns in Abundance and the Environment

Based on AICc, adding the four environmental variables (AMT, AMP, Δ EMP and Δ PRECIP) to the separate species GLMMs, did not increase model fit ($p > 0.05$) for *Plethodon glutinosus*, *P. teyahalee*, *P. jordani x metcalfi,* and *P. ventralis* (Table 7). I did not have sufficient sample size to run GLMMs with environmental variables for the singlet populations of *P. glutinosus x teyahalee, P. jordani x teyahalee,* and *P. metcalfi*. *P. jordani* and *P. serratus* showed an increase in model fit compared to the Year model when adding one of the four environmental variables (Table 7).

The fit of the *P. jordani* model significantly increased when I added the average annual temperature (AMT; $\chi^2 = 7.1735$; df = 1; $p = 0.0074$) and average

annual precipitation (AMP; $\chi^2 = 7.6863$; df = 1; p = 0.0056; Table 7). Populations of *P. jordani* that decreased in relative abundance during the survey period, occupied cooler areas (z = 3.0830; p = 0.0021) and received a greater amount of annual precipitation (z = –3.1630; p = 0.0016; Table 7). Based on AICc, the AMP and AMT models performed better than Year model (Table 7).

Plethodon serratus also increased in model fit when I added AMP ($\chi^2 = 6.1449$; df = 1; p = 0.0132) and AMT ($\chi^2 = 4.4015$; df = 1; p = 0.0359; Table 7). Populations of *P. serratus* that have declined in relative population abundance since 1960 are cooler (z = 2.1920; p = 0.0284) and receive higher annual average precipitation (z = –2.6900; p = 0.0071; Table 7). Based on AICc, the AMP and AMT models performed better than Year model (Table 7).

Adding AMT to the *Plethodon ventralis* model increased the model performance based on AICc; however, this increase was not significant ($\chi^2 = 2.9104$; df = 1; p = 0.0880; Table 7). Although all populations for this species increased, populations of *P. ventralis* increased most in areas that are cooler (z = -2.0050; p = 0.0450; Table 7).

Discussion

Some *Plethodon* populations in the GRSM are declining since the 1960's and I found no consistent pattern in declines among species sites or populations. *Plethodon glutinosus* and *P. teyahalee* have declined at a rate greater than expected, while *P. jordani*, *P. metcalfi,* and *P. serratus* have remained stable overall, and *P. jordani x metcalfi* and *P. ventralis* have increased. I found no association among declining populations, elevation, forestry practices, *Bd* suitability, or spatial relationship, but I found evidence that declines began in the late 1960's to early 1970's. Furthermore, I concluded that salamanders in cooler and moister habitats may be more susceptible to the causative agent (e.g., *Bd*), while salamanders in warmer habitats are thriving.

Detection Probabilities and Null Models

Count indices can only be used as valid representations of population abundance when detection probability is constant (Schmidt 2009). During my study as well as throughout historic collections, terrestrial salamander detection probability varied over time and among the six species (Table 5). These findings are consistent with other studies, in which temporary subterranean emigration reduces and varies the proportion of salamanders available for capture on the surface among survey seasons (Bailey et al. 2004). Because of the difference in survey effort between my study and Highton's work as well as the decrease in salamander detection, detection probabilities were consistently lowest during "2000's" surveys (Table 5). I adjusted the relative abundance indices during my surveys as well as the historic collections to

reduce the likelihood of finding artificial variation in population abundance as well as account for differences (e.g., amount of effort) among surveys (Link and Nichols 1994; Shenk et al. 1998; Schmidt 2009). Future studies should use consistent sampling methods to reduce the variation in detection; however, because species and populations vary in detection (Schmidt 2009), future studies should also account for detection probabilities when using count data in addition to resurveying multiple plots within sites (Smith and Petranka 2000). Furthermore, count indices unadjusted for detection can lead to misinterpretation of data (Schmidt 2009). For example, if I used the unadjusted counts for *Plethodon glutinosus*, I would have determined that only two of the five populations declined, while the other three remained stable and I would have underestimated the extent to which this species had declined (Table 6).

Null models of population abundance are essential for understanding how the observed populations have changed with respect to natural fluctuations (Pounds et al. 1997) and can provide evidence if changes in abundance are a natural occurrence (Alford and Richards 1999). Although salamanders display natural variation in their populations due to the differences in surface and subterranean activity (Feder 1983; Bernardo and Spotila 2006) as well as detection (Bailey et al. 2004), terrestrial plethodontids exhibit one of the lowest amounts of variation in population abundance among amphibian families (Green 2003). My results indicated that the observed changes in abundance for *Plethodon glutinosus, P. teyahalee*, *P. jordani x metcalfi* and *P. ventralis* are beyond the normal variations for those populations.

These models examined, for each species, individual populations as well as the species as whole. Previous reports (Hairston and Wiley 1993) indicate no

declines in *P teyahalee* (*glutinosus*; Hairston 1992) and *P. jordani* at similar sites to my study from the 1970's to 1990's. However, the changes I observed during the course of this study highlight the importance of surveying multiple populations. For example, if I had chosen to survey the three sites in the GRSM at which *Plethodon teyahalee* had not declined in addition to any three other sites, I most likely would have reached the conclusion that this species had not declined beyond what is expected from natural variation. The power to determine if abundance has changed increases with the number of survey years and the number of surveyed populations (Smith and Petranka 2000); based on my results, I recommend that future studies should examine no less than six populations for each species of terrestrial plethodontids.

Maxent Predictions for *Bd* in the GRSM

Species distribution models (SDM) based on climatic envelope estimates can provide insight into the potential distribution of an organism (Phillips et al. 2006; Ron 2005; Lawler 2009; Lawler et al 2010; Milanovich et al. 2010) and have also been used to predict the impact of an infectious pathogen (*Bd*) on its amphibian hosts (Murray et al. 2011) and have shown very high probability for *Bd* to occur throughout the GRSM (Ron 2005). Similar to previous studies of *Bd*'s potential distribution using SDMs (Puschendorf et al. 2009; Murray et al. 2011), my results show that Mean Diurnal Range (BIO 2) and Precipitation during the Driest Quarter (BIO 7) are important predictors of *Bd*'s potential distribution in the GRSM, which is consistent with *Bd*'s biology (Johnson et al. 2003; Piotrowski et al. 2004). My results indicate

that suitability for *Bd* varies throughout the GRSM, and that the one locality where I found *Bd* had a slightly below average suitability of 0.429. Although I did not find *Bd* in other habitats, areas with higher predicted suitability (e.g., the western portion of the GRSM; Figure 21) than this locality may have a high probability for *Bd* occurrence because these habitats are generally cooler and receive more precipitation (Hijmans et al. 2005).

Results from Maxent modeling neither supported nor rejected the hypothesis that *Bd* is associated with declines in GRSM *Plethodon* salamanders. Although the median suitability was highest in declined populations, areas with the highest *Bd* suitability showed all three population responses, while areas with the lowest *Bd* suitability were associated with declined and increased populations (Figure 22). Murray et al. (2011), cautioned against using predicted suitability as an exact measure of disease risk, as other factors related to species' biology (e.g., amphibian immune response or skin peptides; Harris et al. 2006; Ramsey et al. 2010) may explain transmission and infection dynamics. Future studies should examine species-specific differences in microbial communities (Harris et al. 2006; Brucker et al. 2008a, b) and whether the effectiveness of these microbial communities are affected by temperature and moisture.

Species Patterns in Abundance

I used a conservative approach when determining population responses for all species (see Determining Population Response). Using this approach, only populations that showed a distinct decline pattern (i.e., upper and lower standard error of the slope estimate were below zero) were considered declined populations, which

may have led to an underestimation of declines. However, this approach provided confidence in

Changes in population abundance varied among species (Table 6). *P. glutinosus* and *P. teyahalee*, declined while *P. jordani, P. metcalfi* and *P. serratus* remained stable and *P. jordani x metcalfi* and *P. ventralis* increased (Tables 7 and 8). These results may indicate that these species vary in their susceptibility to the agent for declines.

The observed variation in population response among species does not support the idea that historic park forestry practices caused the observed changes. If these changes were the cause of immature forest stands (Petranka et al. 1993) then I expected *Plethodon teyahalee*, which tolerates drier habitats (Ash 1997), would have remained stable despite the negative impacts of immature forest stands on salamander habitat (Ash 1997; Petranka et al. 1993, 1994).

Susceptibility to chytridiomycosis and ranavirus varies among amphibians (Berger et al. 2004; Gray et al. 2009a; Vazquez et al. 2009; Crawford et al. 2010). Within GRSM terrestrial plethodontids, *P.metcalfi* and *P. glutinosus* are susceptible to chytridiomycosis in the laboratory, while infected *Plethodon glutinosus* have been recovered from the wild (Chinnadurai et al. 2009; Vazquez et al. 2009). With respect to ranavirus, species-specific susceptibilities from laboratory tests are not known although *P. jordani*, *P. metcalfi, P. jordani x metcalfi* have been found to be infected in the wild (Gray et al. 2009b; M.J.G and N.M.C unpublished data). Based on the observed variation in population responses alone, it is unlikely that ranavirus or *Bd* has caused the observed declines as *Plethodon jordani x metcalfi* has not shown

reductions in population abundance. If either of these disease-causing agents has contributed to population declines in the GRSM, then I would expect that interactions between *Bd* and other factors, such as natural (e.g., host density; Gray et al. 2009a; Briggs et al. 2011) and anthropogenic stressors (e.g., pesticides; Gray et al. 2009a; Carey et al. 1999) or differences in host immunity (Ramsey et al. 2010) or skin microbes (Harris et al. 2006) would explain the variation in response in these populations because of the variation among populations within each species (Table 6).

Future research is needed to understand the actual variation in species ability to cope with the stress associated with a changing climate (Bernardo and Spotila 2006; Bernardo et al. 2007) beyond SDMs and environmental suitability (Lawler 2009; Lawler et al. 2010; Milanovich et al. 2010). To determine which species are most at risk, studies should measure the stress (e.g., metabolic depression; Bernardo and Spotila 2006) associated with warming temperatures. Specifically, to determine climate's role in the declines of GRSM salamanders, future studies should determine if *Plethodon glutinosus* and *P. teyahalee* are more sensitive to changes in temperature and precipitation than *P. ventralis* and *P. jordani x metcalfi* given that these species co-occur but have opposite changes in population abundance.

Elevation and Forestry Practices Patterns in Abundance

Population declines occurred at all elevations and no elevation was more or less affected than others (Figure 23). This pattern is most likely a consequence of the elevational range of these species rather than from the environmental effects

associated with elevation changes. The only species that increased at the high elevations (> 1,500 m) was *Plethodon jordani x metcalfi* and 36% of the resurveyed high elevation populations consisted of this hybrid (Table 1). Because declines were not limited to specific elevations, these data may suggest a biological/evolutionary link (e.g., sensitivity to changes in climate or presence of microbial symbionts) for declines rather than an environmental cause because not all species declined in the same habitat. Therefore, species which are closely related to *Plethodon glutinosus* or *P. teyahalee* (e.g., *P. aureolus*; Kozak et al. 2006) may be similarly sensitive or susceptible.

If acidic precipitation has caused the observed declines, I expected that higher elevation populations would have been the most negatively impacted given that the precipitation pH decreases with increasing elevation (Robinson et al. 2008). However, elevation patterns may not explain the actual variation in acidic habitats in the GRSM. Future studies should examine the exact range of acidic conditions experienced by GRSM salamanders to determine if they experience pH levels low enough to cause mortality or impact growth (Rowe and Freda 2000). If habitat acidification is not a cause for the declines of GRSM salamanders then I expect the soil pH to be above 3.8.

Population responses were random with respect to their spatial relationship (Table 9) and declined populations co-occurred with stable and increased populations. These data are consistent with the earlier finding of Highton (2005) for Eastern US *Plethodon* species. These results indicate that the cause for these declines is species-specific and is not limited to specific areas, which may further suggest a biological or

evolutionary cause for declines rather than environmental or habitat acidification. Biological factors such as density (Briggs et al. 2010), skin peptides (Harris et al. 2006), ecology (Lips et al. 2003), phylogenic clumping (Corey and Waite 2008), or variation in susceptibility to disease (Smith et al. 2009; Crawford et al. 2010) may explain these patterns.

Forestry practices cannot explain patterns in GRSM *Plethodon* declines as declined, increased, and stable populations occurred in all levels of historic logging in similar proportions (Figure 24). Furthermore, the lowest proportion of declined populations occurred in the "heavy cut" sites which were expected to have the highest percentage of declined populations if forestry practices were the cause of declines. Given that the last timber harvest in the GRSM took place in 1939 (NPS 2010), it is unlikely that past timber harvesting has affected present-day salamanders in the GRSM.

Diversity Patterns

Although all six species were present during current surveys, my results suggest that the GRSM has lost some of its historic diversity as 23 sites have had at least one species decline (Table 2). Moreover, the majority of the declined populations (20 out of 28) were the large-bodied salamanders, *P. glutinosus* and *P. teyahalee* and their hybrids. These results may indicate that the declines of GRSM *Plethodon* salamanders are non-random and may further suggest that the cause of declines is linked to these species' biology. Furthermore, these results may suggest a

loss of a functional role as these large salamanders generally eat larger prey items that may be too large for other *Plethodon* species (Adams and Rolf 2003).

Temporal Patterns in Abundance

Plethodon jordani declines most likely began in the late 1960's or early 1970's. The timing of declines may indicate a climate-link to the observed changes. During the early and mid-1970's, the GRSM experienced a strong ENSO event (1972; NOAA 2010). This ENSO corresponded to periods of cool, moist conditions (Ropelewski and Halpert, 1986), in which monthly precipitation remained well-above average throughout the year (NCDC 2010). Terrestrial salamanders are expected to thrive in cool, moist conditions (Grover 1998); however, these conditions may also promote pathogen (e.g., *Bd*) persistence in the soil (Johnson and Speare 2005), and increase transmission in dense salamander populations (Briggs et al. 2010). If moist conditions increase pathogen intensity and prevalence and dry conditions decrease it (see Weinstein 2009) then further strong ENSO years may be associated with increases in *Bd* or ranavirus transmission and further decreases in salamander abundance. If these declines are caused by a climate-disease link then I expect high pathogen presence will be found in museum specimens collected at the beginning of decline declines (late 1960's to early 1970's) as well as during periods of prolonged and above average rainfall in the GRSM (1972 – 1975; NCDC 2010).

Environmental Patterns

Precipitation and temperature patterns were important in explaining the variation in relative abundance changes among salamander populations (Table 7), which are consistent with both *Bd* and ranavirus biology (Chinchar 2002; Piotrowski et al. 2004). Forest soils that receive a greater amount of precipitation and are cooler may create more favorable conditions for *Bd* and ranavirus to persist and infect amphibians (Chinchar 2002; Piotrowski et al. 2004). During periods of high or continuous precipitation pathogen prevalence may increase. High soil moisture can allow for pathogen survival in the soil (Johnson and Speare 2005; Brunner et al. 2007), which can increase the likelihood of infections. Wet conditions can also promote increases in salamander activities and densities (Grover 1998), which lead to increases in contact with infected individuals (Briggs et al. 2010). Dry conditions, on the other hand, have increased survivorship of infected terrestrial salamanders in empirical studies (Weinstein 2009). Because surveys for *Bd* and ranavirus in the GRSM during 2009 (this study; M.J.G. and N.M.C unpublished data) and 2006 – 2007 (Chatfield et al. 2009) were made during months that received lower than average rainfall (NCDC 2010), prevalence estimates for terrestrial plethodontids in this region may be underestimated.

This study provides further evidence of declines in *Plethodon* salamanders (Highton 2005). Consistent with Highton (2005), this study shows widespread declines with no clear spatial pattern in population response. However, Highton (2005) reported declines in 88% of Eastern US *Plethodon* populations, while this study shows that 39% GRSM *Plethodon* populations have declined. This difference

may be as a result of the difference in spatial scale or because of the differences in species surveyed.

Building upon the reported declines by Highton (2005), I analyzed patterns in population responses to determine potential causes for declines. This study provides evidence that historic logging in the GRSM was not the cause for population declines in *Plethodon* salamanders while acid precipitation, disease, and climate change may still be potential causes. Because of the complexity in these changes in population abundance (e.g., variation in species' response and variation in species within a site) it is likely declines have been caused by multiple factors (e.g., disease epizootic caused by fluctuations in the climate).

Conservation Implications

This study highlights the need for future studies to examine multiple populations when considering if a species has declined. Using a power analysis, Smith and Petranka (2000) show that the number of plots or sites needed to detect real changes in abundance varies with the number of survey years. Future studies should conduct similar analyses to determine if the number of sites is sufficient to detect changes in abundance. For similar studies, I recommend at least six sites per species. Furthermore, future studies should search multiple plots within each of these sites, which have also shown to increase power to detect changes in abundance (Smith and Petranka 2000).

Currently, both *Bd* and ranavirus are present in few locations within the GRSM and at a low prevalence in terrestrial salamander populations (Chatfield et al. 2009; Gray et al. 2009b; this study; N.M.C. and M.J.G. unpublished data); however, abiotic or biotic factors (e.g., increased rainfall or increased amphibian density) may favor pathogen transmission (Weinstein 2009; Briggs et al. 2011). Therefore, it is important to reduce the spread of these pathogens into naïve areas. I recommend installing boot-wash stations at GRSM trailheads and educating the public (e.g., trail signs or posting information on websites) about these pathogens to prevent anthropogenic spread.

More data are needed in order to determine the role of *Bd* in the declines of GRSM *Plethodon* salamanders. First, future studies are needed to determine the arrival of *Bd* in the GRSM by examining histology of museum specimens. Additionally, studies should examine museum specimens for an increase in *Bd*

prevalence following its initial arrival. These data may suggest an epizootic event, which has been indicative of *Bd* as a potential cause (Lips et al. 2006). Future studies should also examine susceptibility of current populations of *Plethodon* salamanders as well as for the presence of cutaneous bacteria or *Bd*-inhibiting metabolites (Harris et al. 2006; Brucker 2008a, b), which may suggest a co-evolutionary history between these salamanders and *Bd*. Presence of pathogen-defenses (e.g., bacteria; Harris et al. 2006) in population or species that have not declined (e.g., *Plethodon jordani x metcalfi*) and absence of these defenses in populations that have declined (e.g., *P. glutinosus*) may suggest *Bd* as a potential cause for declines.

Further research is also needed to determine the role of climate change in the declines of GRSM *Plethodon* salamanders. First, studies are needed to determine the relationship between the soil conditions (i.e., temperature and moisture) experienced by terrestrial salamanders and air temperature and precipitation. Additionally, studies should examine the effects of changes in air temperature and precipitation on the overall fitness of salamanders (e.g., metabolic stress; Bernardo and Spotila 2006) and to determine which populations are at risk based on the magnitude of temperature and precipitation changes. If variation in species response, as determined by these studies, suggest climate change as a potential cause (e.g., *Plethodon glutinosus* and *P. teyahalee* are more sensitive than other species), then future monitoring is needed for these populations and potentially relocating the high risk populations to habitats that are more suitable for terrestrial salamanders.

This study documents two species of concern, *Plethodon glutinosus* and *P. teyahalee*. Although these species are still present in the GRSM, they have declined

throughout their resurveyed range and show decline patterns in other studies (Highton 2005). Future studies should examine these species, as well as other closely related *glutinosu*s group species (Kozak et al. 2006) throughout their range to determine where they have declined. Currently, both *Plethodon glutinosus* and *P. teyahalee* are listed as "least concern" and have populations that are considered "stable" (IUCN 2010). Because of the population abundance trends for both of these species throughout their range (Highton 2005; this study); I recommend reevaluation of the IUCN red list category and criteria for both *Plethodon glutinosus* and *P. teyahalee*.

Tables

Table 1: Number of populations surveyed for each species. Low elevations are between 500 – 1,000 m, Mid elevations are between 1,000 – 1,500 m and High elevations are above 1,500 m.

Species	Total Populations	Low Elevation Populations	Mid Elevation Populations	High Elevation Populations
P. glutinosus	5	4	1	0
P. glutinosus x teyahalee	1	1	0	0
P. teyahalee	16	2	12	2
P. jordani x teyahalee	1	0	1	0
P. jordani	18	1	13	4
P. metcalfi	1	0	0	1
P. jordani x metcalfi	8	0	4	4
P. serratus	18	5	13	0
P. ventralis	4	4	0	0
Total	72	17	44	11

Table 2: Number of sites based on the number of historic species present. Low elevations are between 500 – 1,000 m, Mid elevations are between 1,000 – 1,500 m and High elevations are above 1,500 m. The number of declined species is based off of GLMM results. "–" indicates that no sites with three species historically were sampled at high elevations

	Historic Number of Species	Number of Sites	Sites with ≥ 1 Declined Species
Low Elevation	1	2	2
	2	1	1
	3	4	4
Mid Elevation	1	2	0
	2	11	7
	3	6	6
High Elevation	1	7	2
	2	2	1
	3	–	–

Table 3: Biological information for GRSM *Plethodon* salamanders. For Bd/Ranavirus, the "+" denotes that a species has been found infected in the field with the respected pathogen, the "-"denotes a species that has not been found to be infected with the respected pathogen, and "0" denotes species that have not been surveyed for that pathogen (Rothermel et al. 2008; Gray et al. 2009b; Chinnadurai et al. 2009; this study; M.J. Gray and N.M. Caruso *unpublished data*.

Species	Groups (Kozak et al. 2006)	Elevational Range	Home Range	Distribution	Bd/Rana virus
P. glutinosus	*glutinosus* group	Up to 1,500 m (Petranka 1998)	4 – 14 m^2 (Merchant 1972; Marvin 1998)	Throughout the Eastern US (Petranka, 1998).	+/0
P. teyahalee	*glutinosus* group	Up to 1,550 m (Petranka 1998)	6.5 – 14.3 m^2 (Nishikawa 1990)	Blue Ridge Province of southeastern TN, southwestern NC, northwestern SC and northeastern GA (Highton 1987)	–/–
P. jordani	*glutinosus* group	213 – 1951 m (Grobman 1944)	1.7 – 11.4 m^2 (Merchant 1972)	TN–NC border; disjunct population in extreme northeastern GA (Petranka 1998)	–/+

Table 3: continued

P. metcalfi	*glutinosus* group	Above 750 m (Highton and Peabody 2000).	1.87 – 5.04 m^2 (Nishikawa, 1990).	Southern Blue Ridge Mountains in NC, SC, and GA (Highton and Peabody 2000)	–/+
P. serratus	*cinereus* group	Up to 1,686 m (Huheey and Stupka 1967)	Unknown	Four disjunct isolates: southeastern MO and western IL; northwestern GA, eastern AL, eastern TN, and western NC; central LA; southeastern OK and western AR (Petranka, 1998)	–/–
P. ventralis	*welleri* group	Up to 579 m (King 1939; Highton 1972)	Unknown	Scattered from northern MS to southeastern VA, including populations in northern AL and GA, eastern TN, and western NC (Petranka 1998)	–/0

Table 4: Prevalence and intensity of *Bd* infection for all species of *Plethodon* salamanders sampled during my 2009 surveys. The number of total animals swabbed, number of infected individuals, prevalence estimates, 95% Clopper-Pearson binomial confidence intervals, and the average intensity are summarized.

Species	Number Swabbed	Number Infected	Prevalence (%)	95% CI
P. glutinosus	7	0	0	0.00–35.43
P. teyahalee	8	0	0	0.00–32.44
P. jordani x teyahalee	29	0	0	0.00–11.70
P. jordani	228	0	0	0.00–1.66
P. metcalfi	28	0	0	0.00–12.06
P. jordani x metcalfi	110	0	0	0.00–3.37
P. serratus	19	0	0	0.00–16.82
P. ventralis	56	0	0	0.00–6.42
Total	485	0	0	0.00–0.79

Table 5: Detection probabilities for each species. Detection probabilities (p[ ]) were estimated for each decade. "–" denotes decades in detection probabilities could not be estimated.

Species	p [1960's]	p [1970's]	p [1980's]	p [1990's]	p [2000's]
P. glutinosus	0.8303	1.0000	1.0000	1.0000	0.3077
P. glutinosus x teyahalee	–	1.0000	1.0000	–	0.0000
P. teyahalee	0.8786	0.5521	0.6176	–	0.0722
P. jordani x teyahalee	1.0000	1.0000	1.0000	–	1.0000
P. jordani	0.9643	1.0000	1.0000	1.0000	0.9425
P. metcalfi	–	1.0000	1.0000	–	1.0000
P. jordani x metcalfi	0.8750	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

Table 6: Number of declined, stable and increased populations for each species. Pairwise comparisons with unlike letters show differences among species ($p < 0.05$). Numbers in parentheses show percentages of total populations.

Species	Pairwise Comparisons	Total Populations	Decline	Stable	Increasing
P. glutinosus	AB	5	5 (100%)	0	0
P. glutinosus x teyahalee	A	1	1 (100%)	0	0
P. teyahalee	BCG	16	13 (81%)	2 (12%)	1 (6%)
P. jordani x teyahalee	ABD	1	1 (100%)	0	0
P. jordani	AE	18	5 (28%)	9 (50%)	4 (22%)
P. metcalfi	EF	1	0	1 (100%)	0
P. jordani x metcalfi	BC	8	0	1 (13%)	7 (87%)
P. serratus	ABCDEFG	18	3 (17%)	10 (55%)	5 (28%)
P. ventralis	BCF	4	0	0	4 (100%)
Total		72	28 (39%)	23 (32%)	21 (29%)

Table 7: AICc scores and GLMM estimates for each model. Bolded numbers indicate significant variable for each model ($p < 0.05$). Models are ranked in descending order based on AICc score. K indicates the number of parameters in each model, while ωAICc is the model weight.

Species	Model	K	AICc	ωAICc	Estimate	Standard Error	Z value	p value
P. glutinosus	Year	5	211.5	0.47	-0.2977	0.1135	-2.6240	**0.0087**
	Year + Δ TEMP	6	213.1	0.15	-0.0896	0.1100	-0.8150	0.4153
	Year + AMT	6	214.1	0.13	-0.0220	0.0741	-0.2970	0.7662
	Year + AMP	6	214.1	0.13	0.0228	0.0885	0.2580	0.7963
	Year + Δ PRECIP	6	214.2	0.12	-0.0022	0.1494	-0.0150	0.9883
P. glutinosus x teyahalee	Year	5	84.2	1.00	-3.2135	1.4634	-2.1960	**0.0281**
P. teyahalee	Year	5	200.6	0.31	-3.1655	0.7546	-4.1950	**< 0.0001**
	Year + AMP	6	200.8	0.27	-0.3822	0.2649	-1.4430	0.1490
	Year + AMT	6	201.3	0.21	0.3143	0.2572	1.2220	0.2217
	Year + Δ TEMP	6	202.5	0.12	-0.2534	0.3177	-0.7980	0.4252
	Year + Δ PRECIP	6	202.8	0.10	0.0330	0.2789	0.1180	0.9026

Table 7: continued

P. jordani x teyahalee	Year	5	59.0	1.00	-0.3753	0.1061	-3.5370	**0.0004**
	Year + AMP	6	820.4	0.53	-0.2740	0.0866	-3.1630	**0.0016**
	Year + AMT	6	820.9	0.41	0.2670	0.0866	3.0830	**0.0021**
P. jordani	Year	5	825.8	0.03	0.0287	0.1194	0.2410	0.8100
	Year + Δ TEMP	6	828.1	0.01	0.0163	0.1038	0.1570	0.8760
	Year + Δ PRECIP	6	828.1	0.01	-0.0021	0.1086	-0.0190	0.9850

Table 7: continued

P. metcalfi	Year	5	66.7	1.00	-0.1491	0.1836	-0.8120	0.4170
P. jordani x metcalfi	Year	5	204.0	0.46	0.5484	0.1079	5.0820	**< 0.0001**
	Year + Δ PRECIP	6	205.8	0.19	-0.0745	0.0725	0.1028	0.3040
	Year + Δ TEMP	6	206.6	0.12	-0.0356	0.0691	-0.5160	0.6060
	Year + AMP	6	206.7	0.12	-0.0237	0.0869	-0.2720	0.7850
	Year + AMT	6	206.8	0.11	0.0166	0.0875	0.1900	0.8500
P. serratus	Year + AMP	6	462.3	0.60	-0.6023	0.2239	-2.6900	**0.0071**
	Year + AMT	6	464.1	0.25	0.5055	0.2306	2.1920	**0.0284**
	Year	5	466.3	0.08	0.0409	0.2394	0.1710	0.8640
	Year + Δ TEMP	6	467.8	0.04	0.2849	0.3031	0.9400	0.3470
	Year + Δ PRECIP	6	468.4	0.03	-0.0906	0.2614	-0.3470	0.7290

Table 7: continued

	Year + AMT	6	612.6	0.35	-0.4247	0.2118	-2.0050	**0.0450**
	Year	5	612.8	0.34	0.3560	0.0551	6.4650	**< 0.0001**
P. ventralis	Year + Δ TEMP	6	614.8	0.12	0.1571	0.1270	1.2370	0.2160
	Year + AMP	6	615.2	0.10	0.1020	0.1305	0.7820	0.4344
	Year + Δ PRECIP	6	615.3	0.09	0.0860	0.1734	0.4960	0.6200
P. metcalfi	Year	5	66.7	1.00	-0.1491	0.1836	-0.8120	0.4170

Table 8: Temperature and precipitation ranges among the three elevational groups. Temperature range and annual precipitation were derived from Hijmans et al. (2005). Annual temperature change and annual precipitation change reflect the magnitude of change from 1951 – 2006 (Girvetz et al. 2009).

	Temperature Range (^{o}C)	**Annual Precipitation Range (mm)**	**Annual Temperature Change (^{o}C)**	**Annual Precipitation Change (%/year)**
Low Elevations	-5 to 29	1,323 to 1,511	-0.067 to 0.023	-0.067 to 0.104
Mid Elevations	-7 to 26	1,485 to 1,845	-0.077 to 0.034	-0.148 to 0.151
High Elevations	-8 to 24	1,770 to 1,946	-0.079 to -0.007	-0.138 to 0.274

Table 9: Results of High/Low clustering analysis (Getis-Ord General G). Only species with at least 2 surveyed populations were analyzed. Species without variation in population responses could not be analyzed.

Species	Observed General G	Expected General G	Z score	p value
P. glutinosus	–	–	–	–
P. teyahalee	0.058	0.0667	-1.6504	0.7207
P. jordani	0.0001	0.0002	-0.6525	0.5141
P. jordani x metcalfi	0.1414	0.1429	-0.3575	0.117
P. serratus	0.0543	0.0588	-1.5674	0.0989
P. ventralis	–	–	–	–

Figures

Figure 1: Map showing the widespread declines in *Plethodon* salamander populations. At each resurveyed sites; these declines occurred during the 1980s (Highton 2005). "Decline" indicates populations where the 1990s average abundance was lower than the average abundance from 1960s – 1980s. "Non-Decline" indicates populations where the 1990s average abundance was greater than or equal to the 1960s – 1980s average abundance. This map was created using the data in Highton (2005).

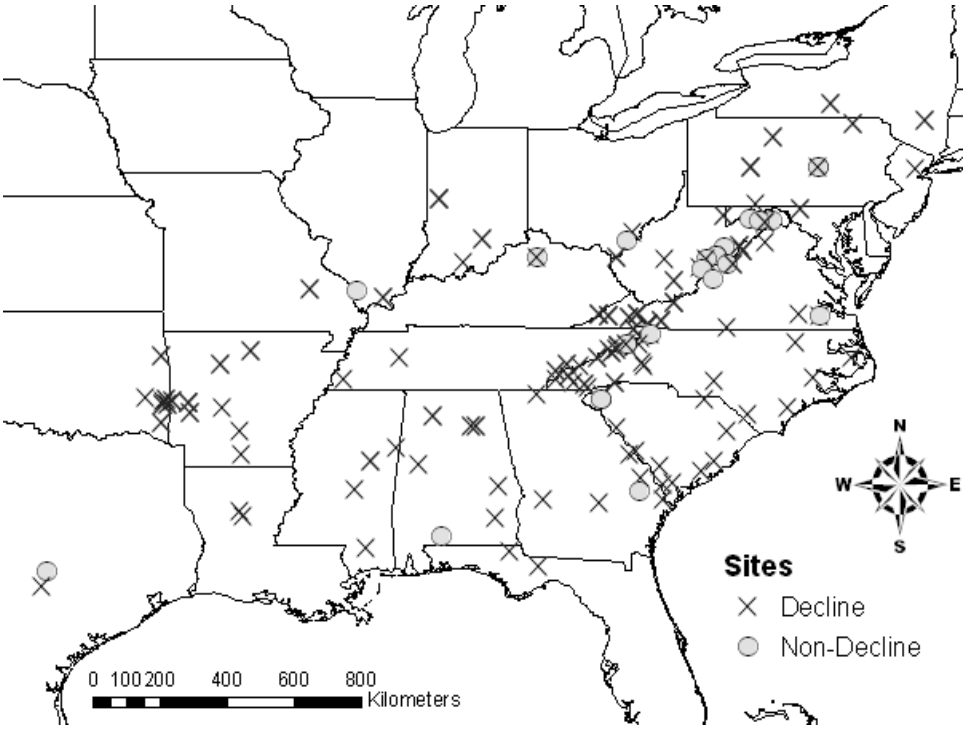


Figure 2: Map showing the location of the resurveyed sites in the GRSM with respect to elevation.

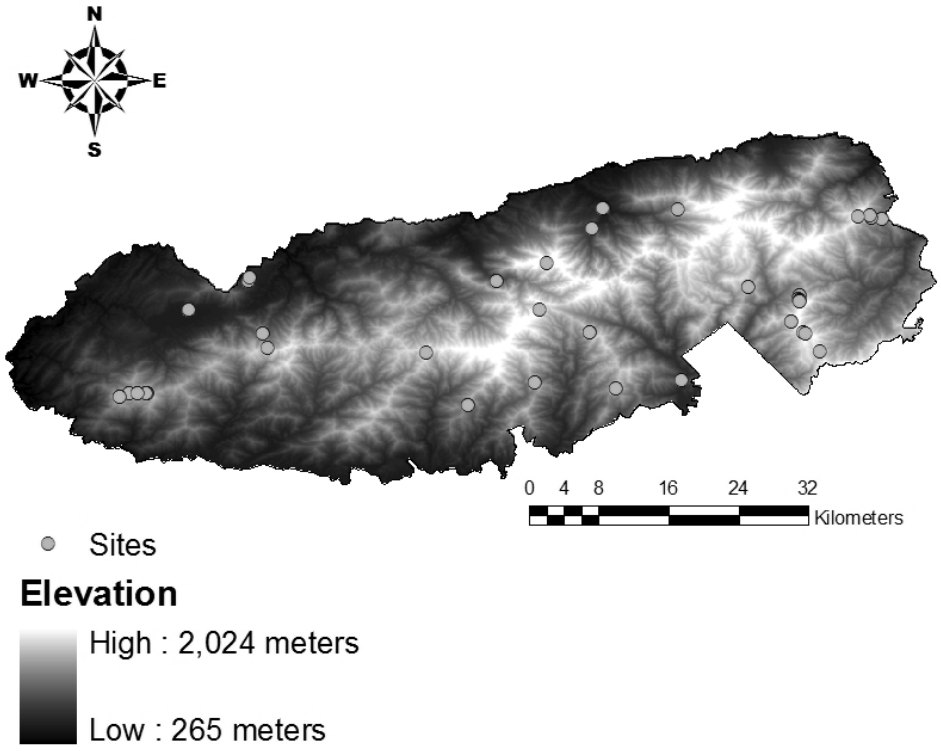


Figure 3: Frequency graph showing the distribution in historic sampling. Numbers along the x-axis show the number of visits at a particular site.

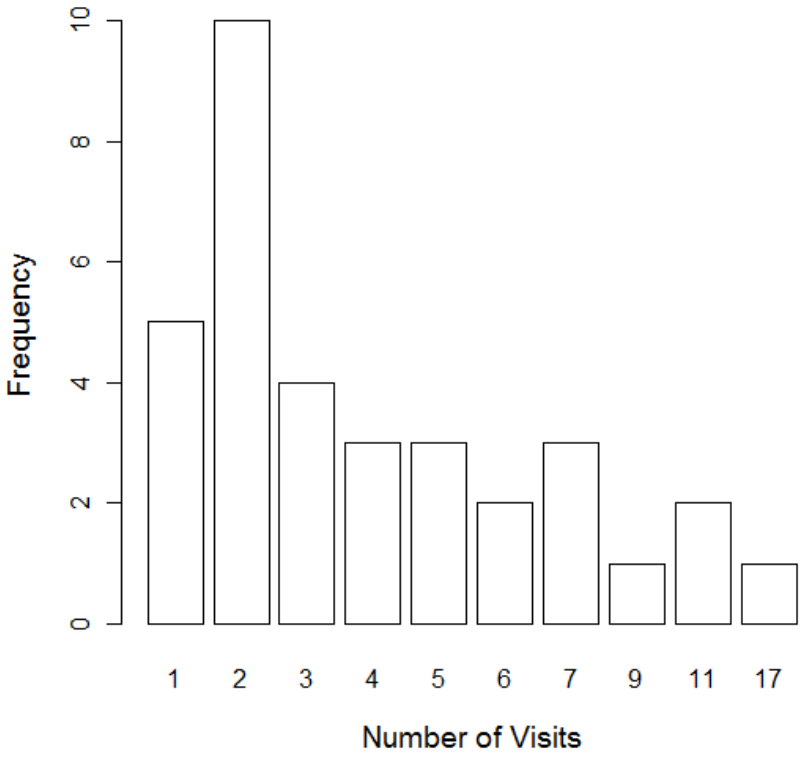


Figure 4: Relative population abundance over time at selected sites for *Plethodon glutinosus*. Filled-in circles represent one sampling occasion. Numbers in the gray-shaded area represent the site's elevation. The solid line represents the linear relationship between the adjusted abundance index and the survey year.

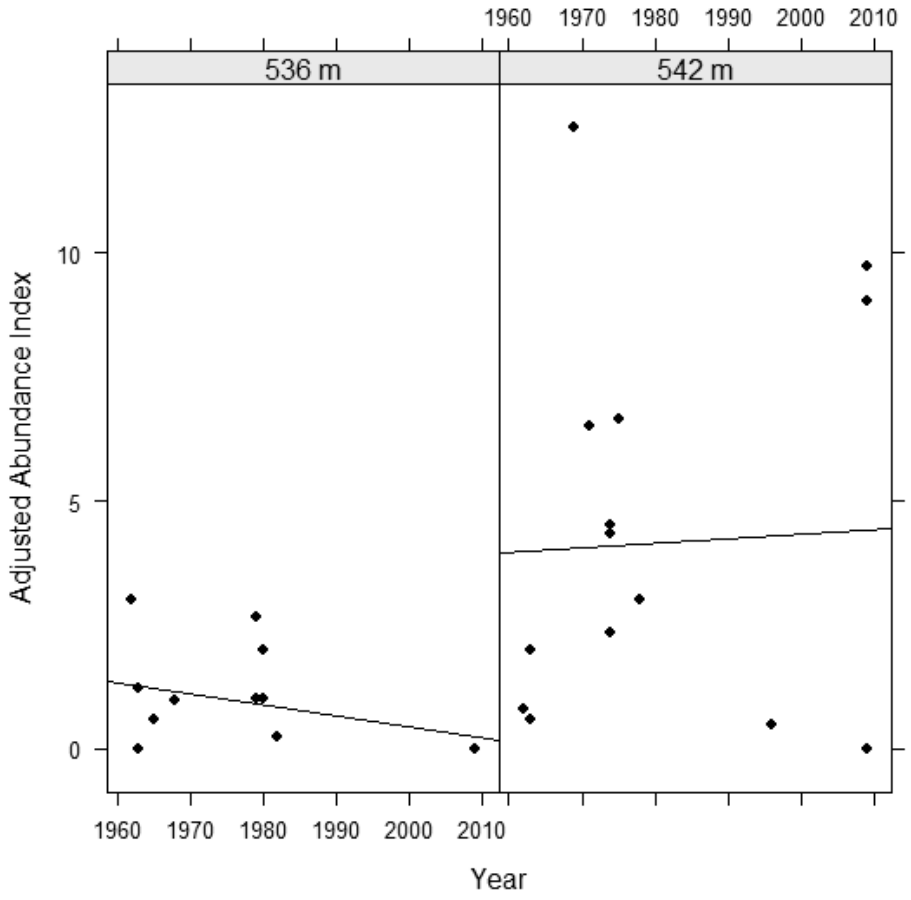


Figure 5: Relative population abundance over time at selected sites for *Plethodon jordani x metcalfi*. Filled-in circles represent one sampling occasion. Numbers in the gray-shaded area represent the site's elevation. The solid line represents the linear relationship between the adjusted abundance index and the survey year.

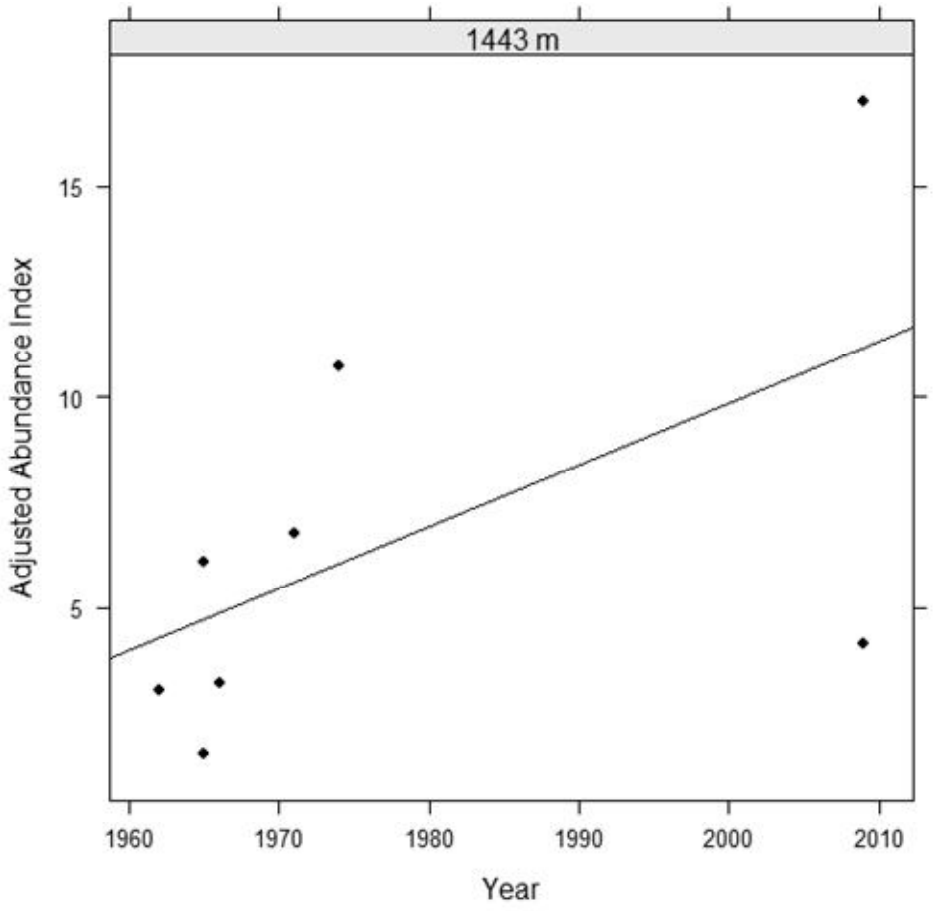


Figure 6: Relative population abundance over time at selected sites for *Plethodon jordani x teyahalee*. Filled-in circles represent one sampling occasion. Numbers in the gray-shaded area represent the site's elevation. The solid line represents the linear relationship between the adjusted abundance index and the survey year.

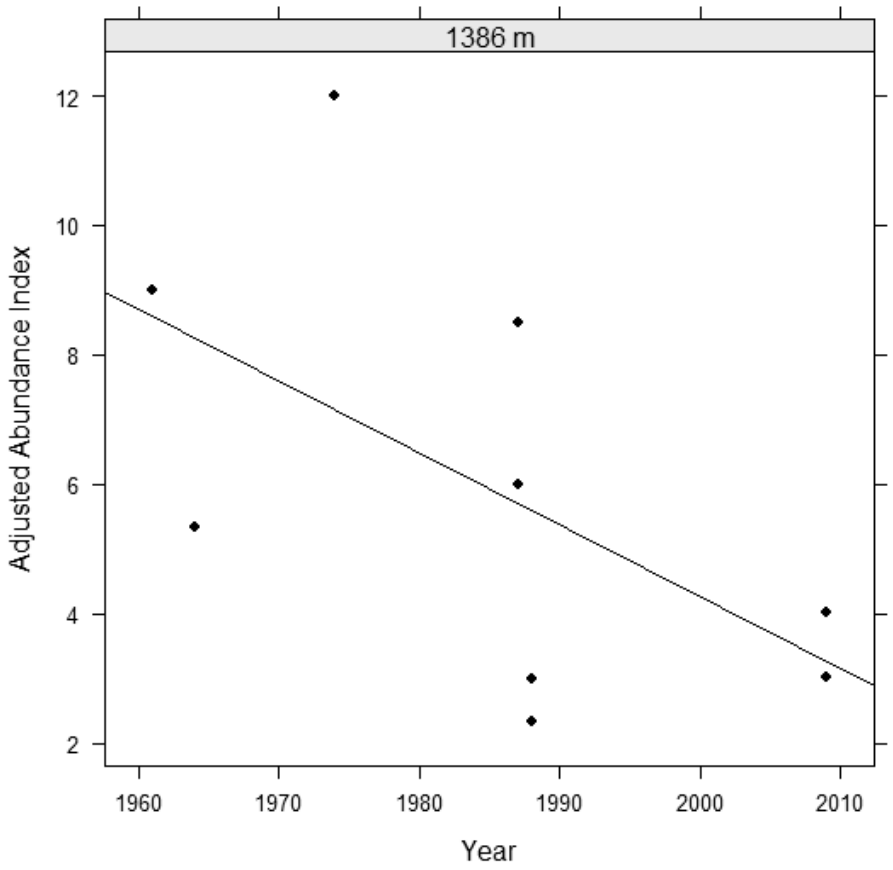


Figure 7: Relative population abundance over time at selected sites for *Plethodon jordani*. Filled-in circles represent one sampling occasion. Numbers in the gray-shaded area represent the site's elevation. The solid line represents the linear relationship between the adjusted abundance index and the survey year.

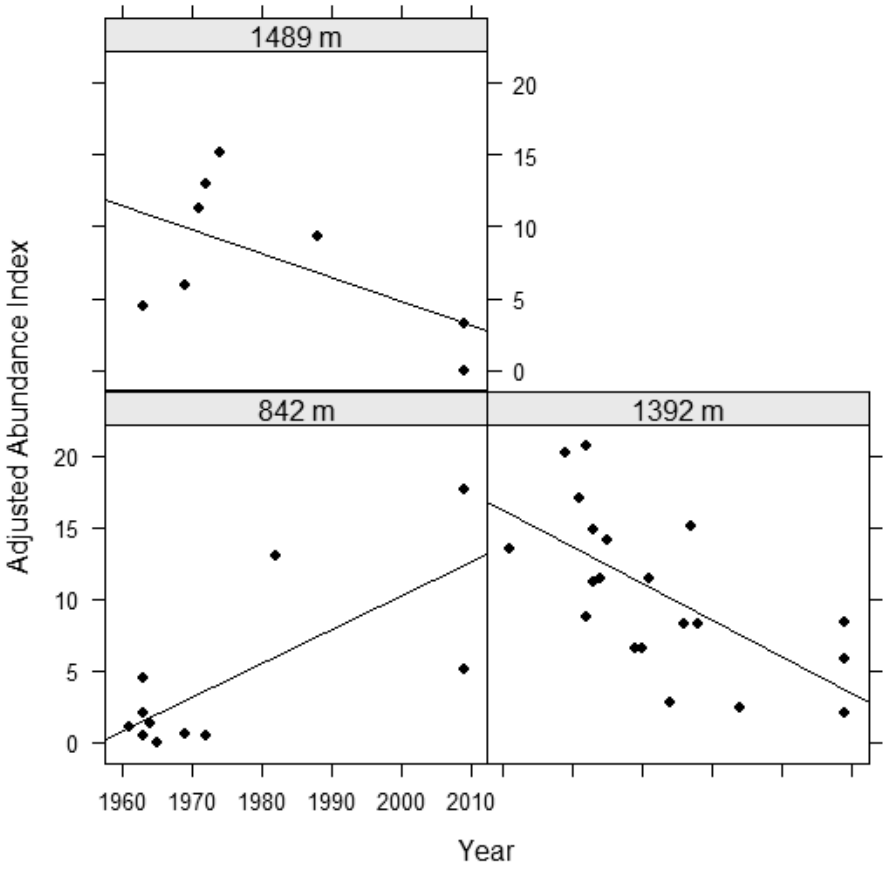


Figure 8: Relative population abundance over time at selected sites for *Plethodon metcalfi.* Filled-in circles represent one sampling occasion. Numbers in the gray-shaded area represent the site's elevation. The solid line represents the linear relationship between the adjusted abundance index and the survey year.

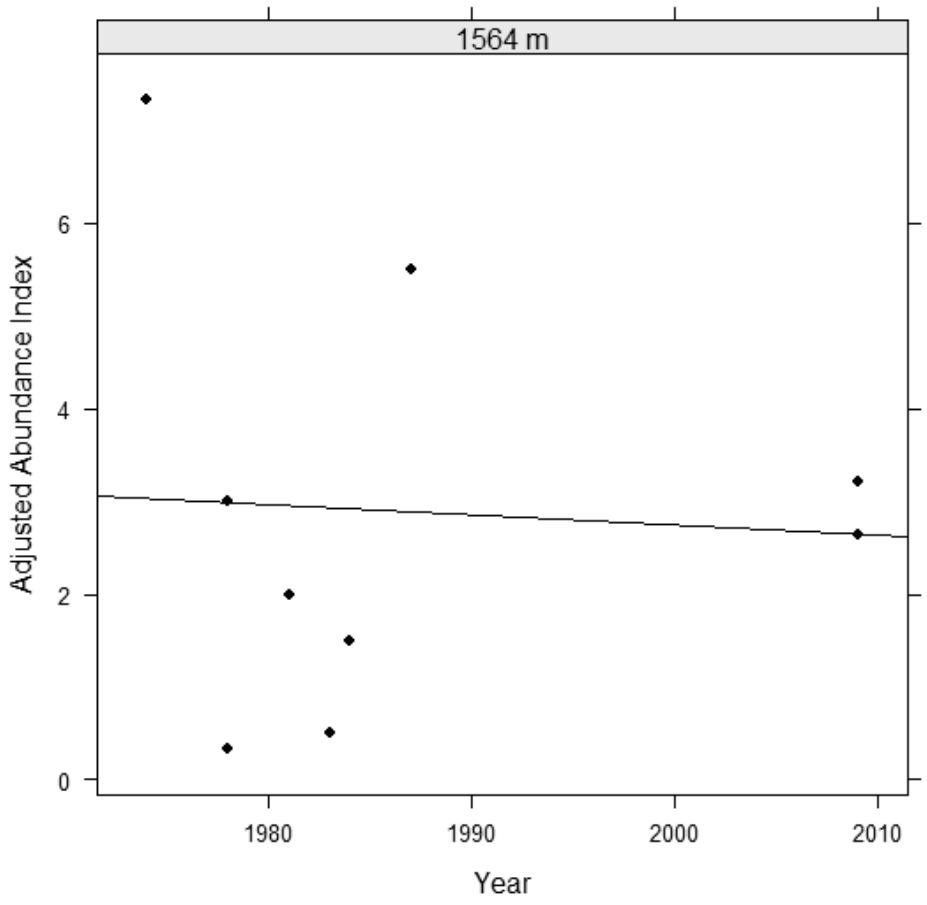


Figure 9: Relative population abundance over time at selected sites for *Plethodon serratus*. Filled-in circles represent one sampling occasion. Numbers in the gray-shaded area represent the site's elevation. The solid line represents the linear relationship between the adjusted abundance index and the

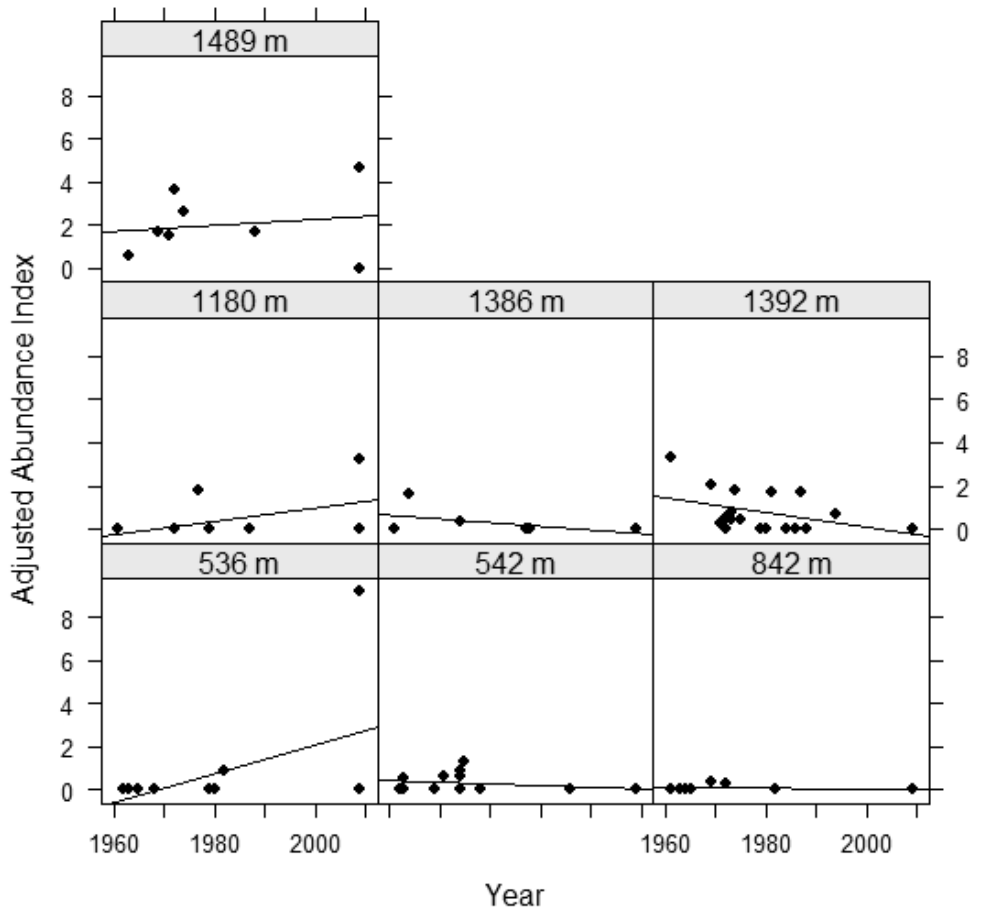


Figure 10: Relative population abundance over time at selected sites for *Plethodon teyahalee.* Filled-in circles represent one sampling occasion. Numbers in the gray-shaded area represent the site's elevation. The solid line represents the linear relationship between the adjusted abundance index and the survey year.

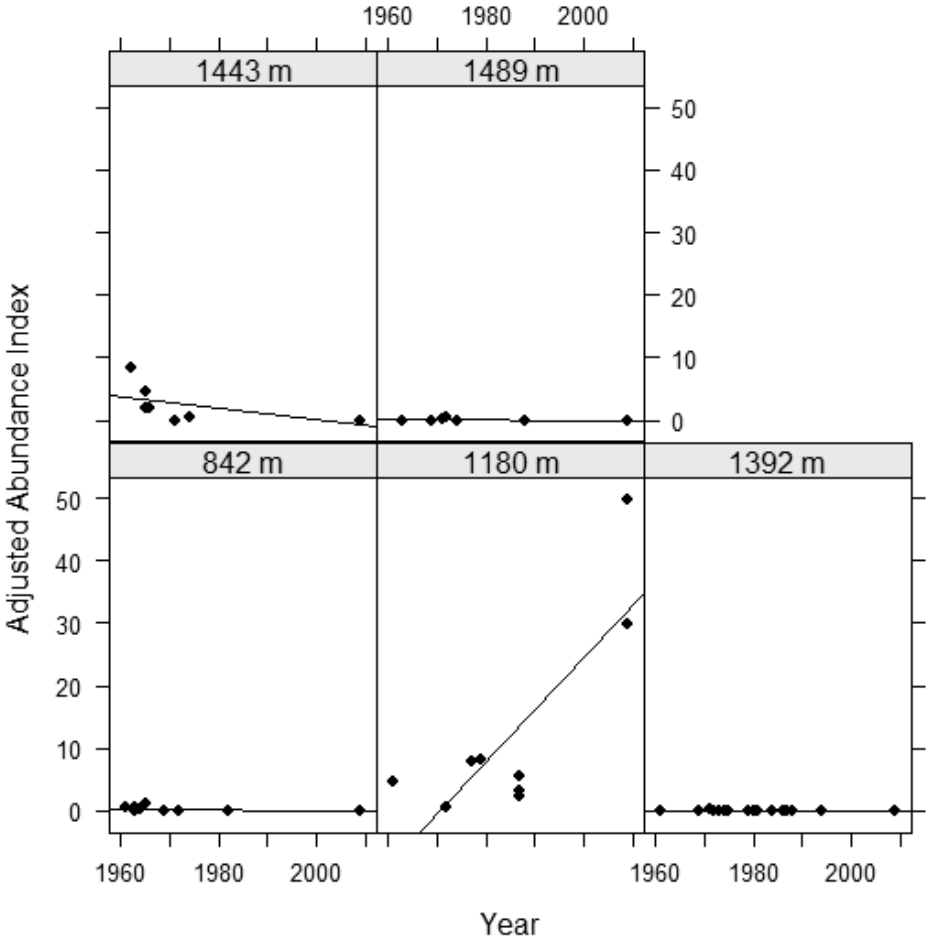


Figure 11: Relative population abundance over time at selected sites for *Plethodon ventralis*. Filled-in circles represent one sampling occasion. Numbers in the gray-shaded area represent the site's elevation. The solid line represents the linear relationship between the adjusted abundance index and the survey year.

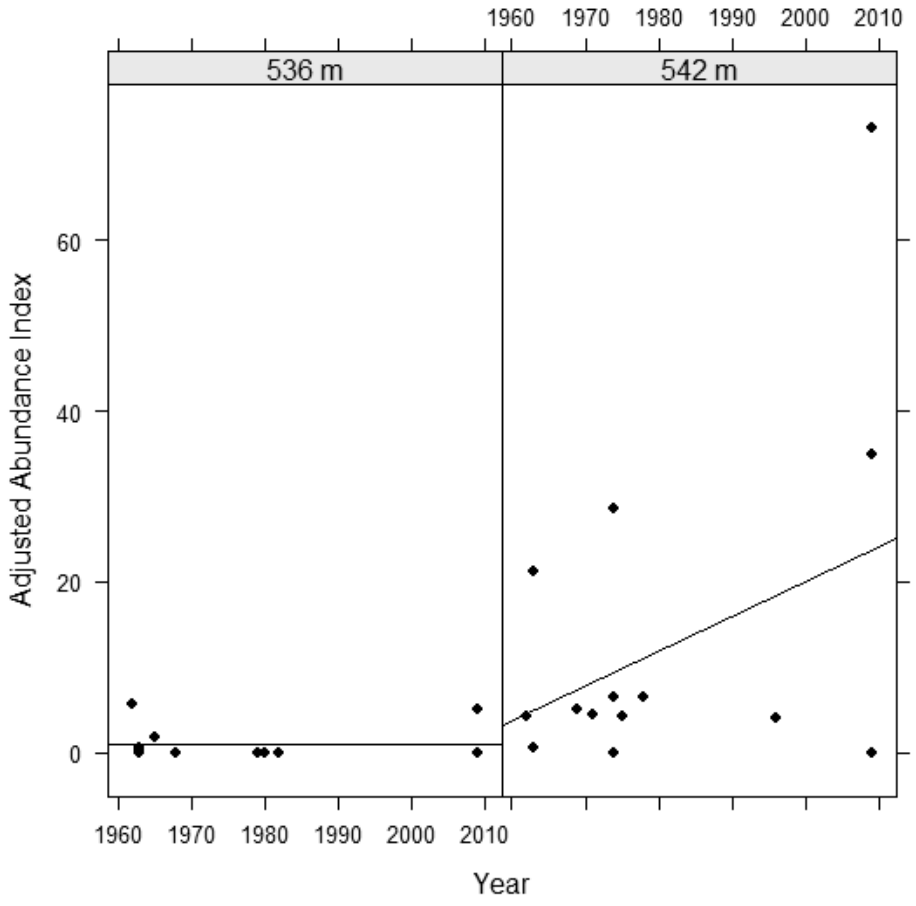


Figure 12: Observed and expected population responses for *Plethodon glutinosus*. The χ^2 statistic and p value are displayed at the top of the graph

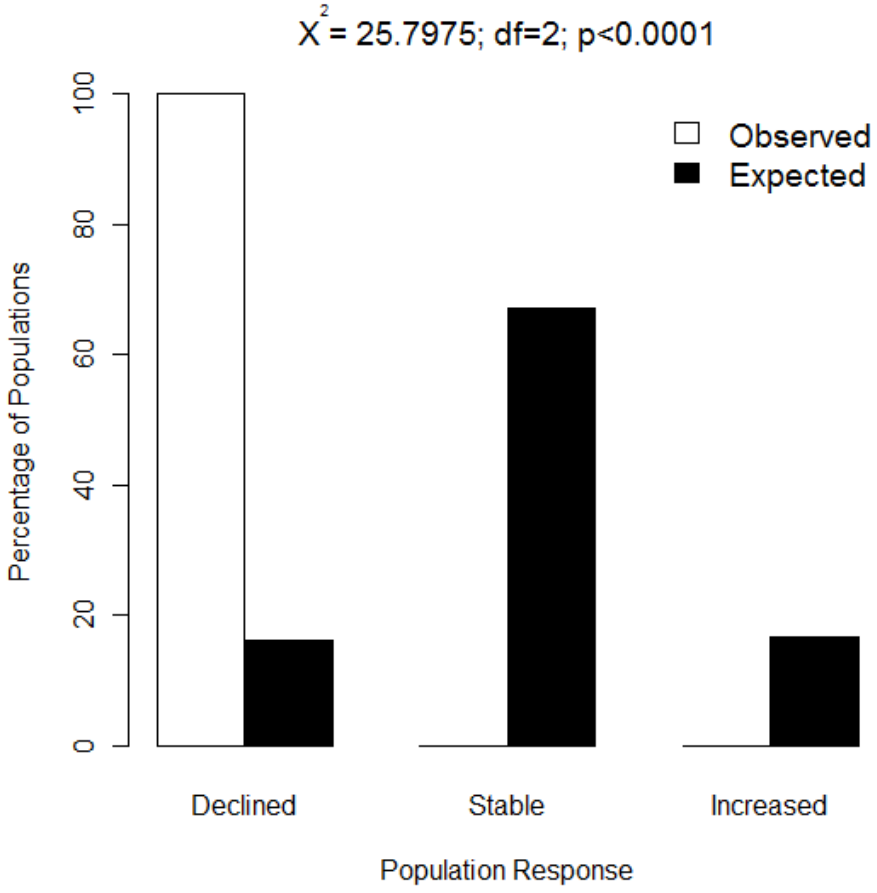


Figure 13: Observed and expected population responses for *Plethodon teyahalee*. The χ^2 statistic and p value are displayed at the top of the graph.

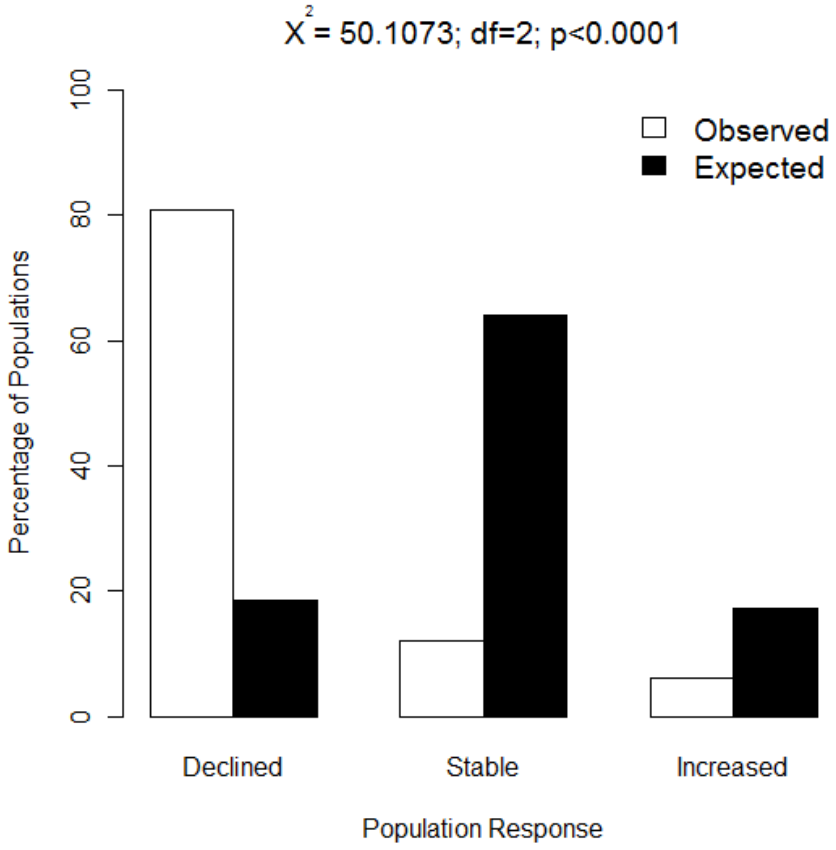


Figure 14: Observed and expected population responses for *Plethodon jordani*. The χ^2 statistic and p value are displayed at the top of the graph.

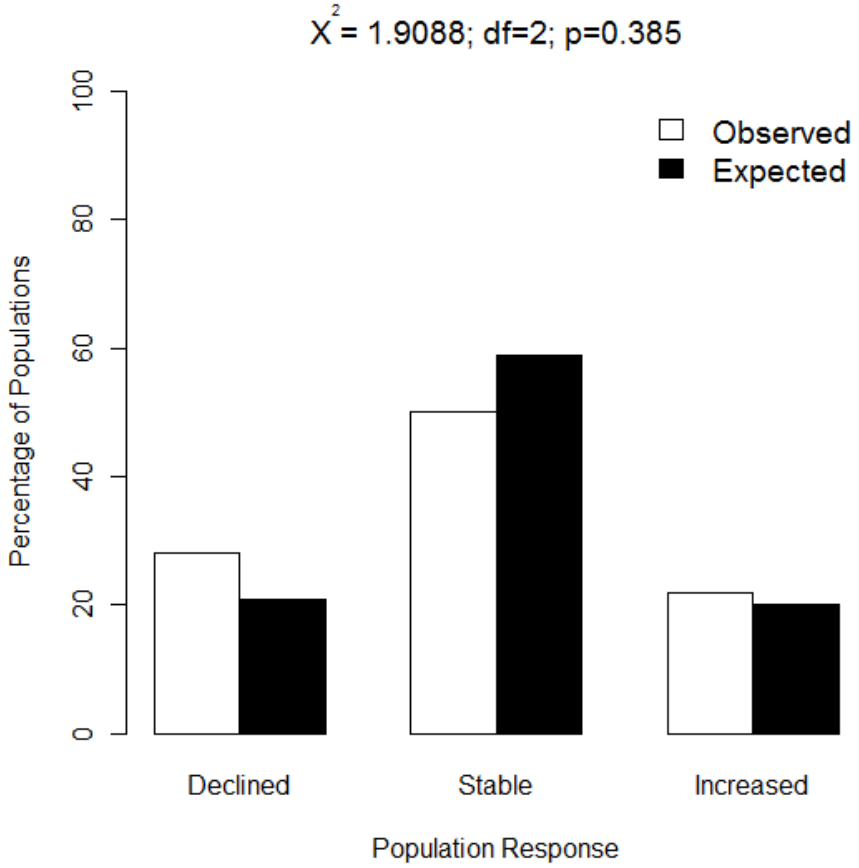


Figure 15: Observed and expected population responses for *Plethodon serratus*. The χ^2 statistic and p value are displayed at the top of the

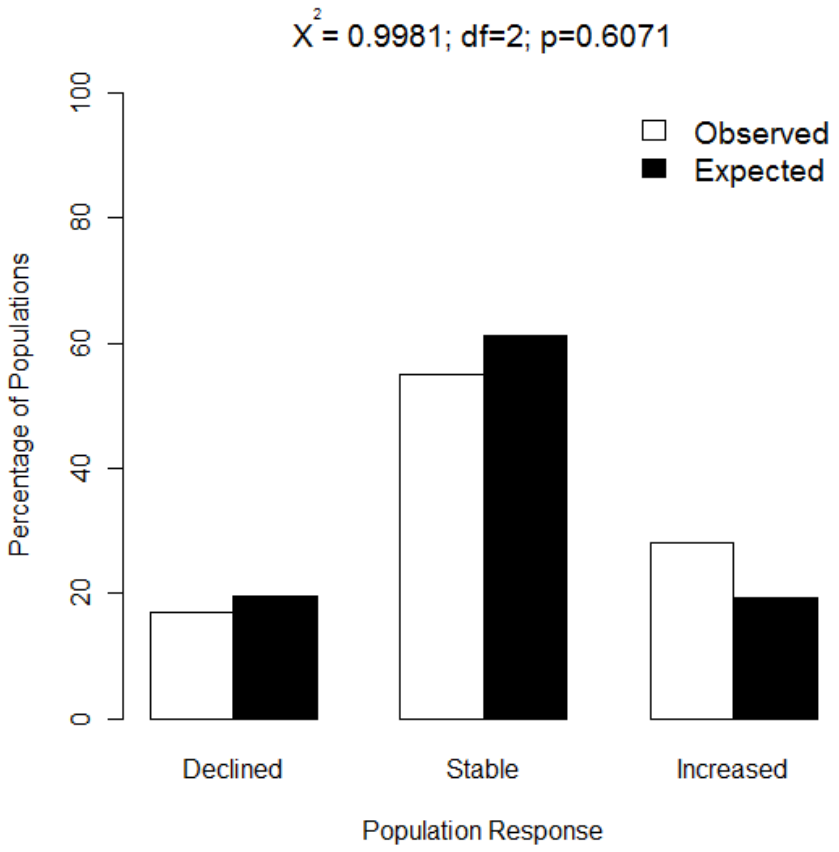


Figure 16: Observed and expected population responses for *Plethodon jordani x metcalfi*. The χ^2 statistic and p value are displayed at the top of the graph.

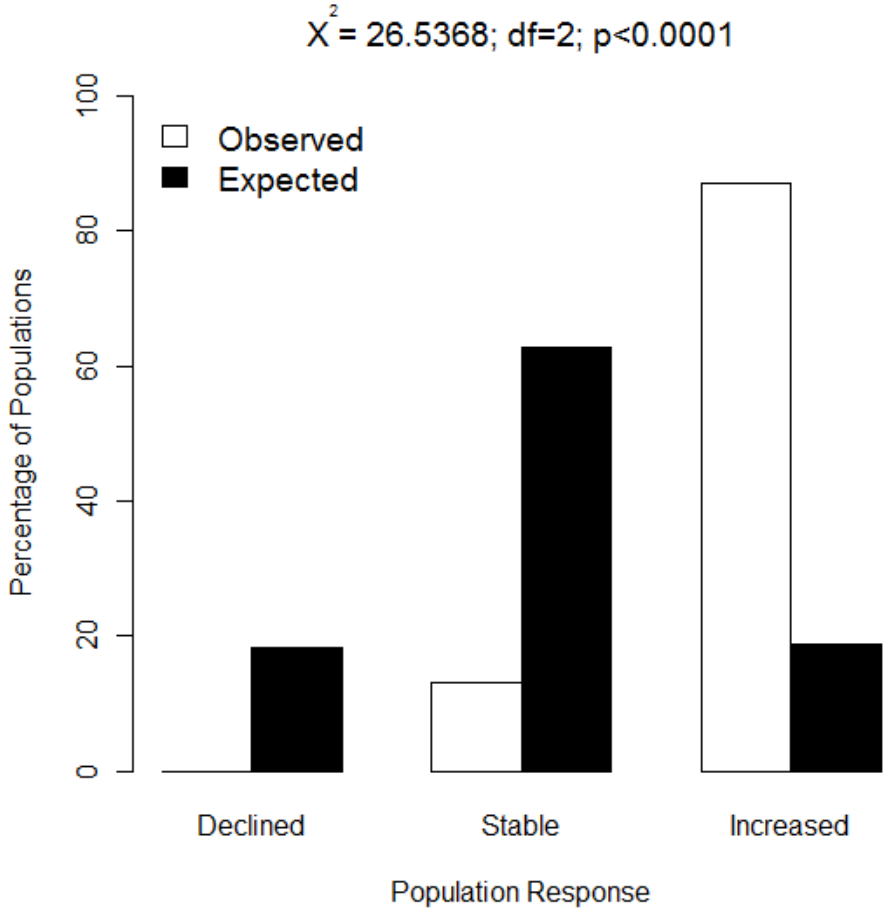


Figure 17: Figure 15: Observed and expected population responses for *Plethodon ventralis.* The χ^2 statistic and p value are displayed at the top of the graph.

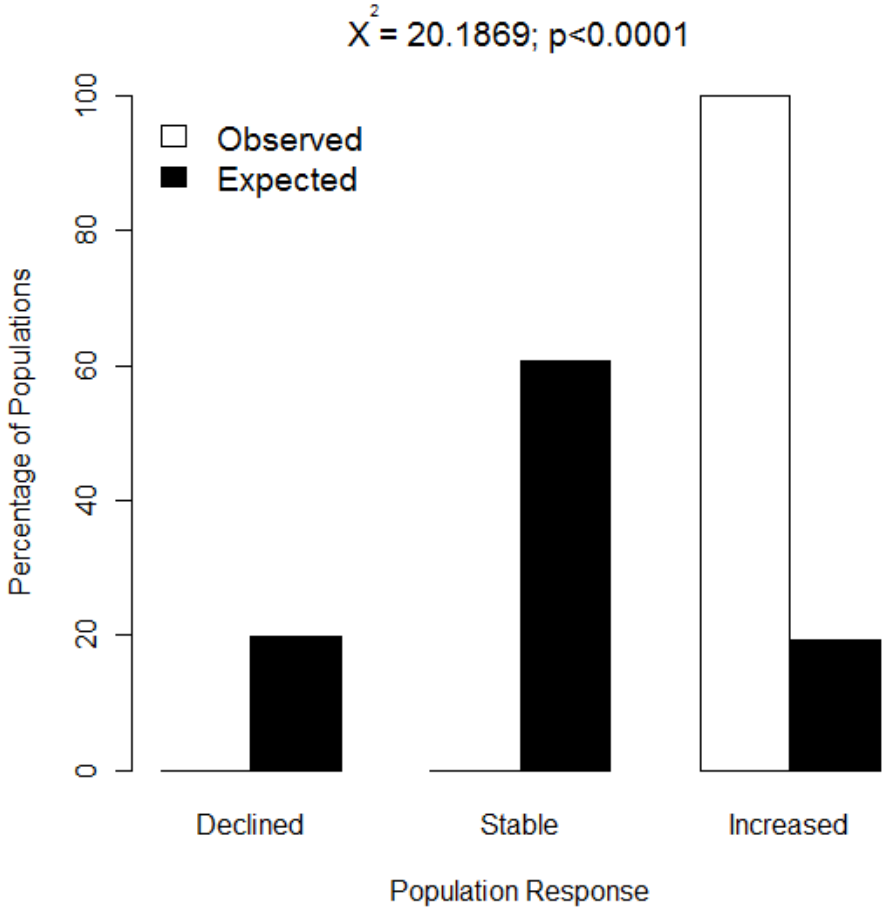


Figure 18: Variable contributions to training gain for the trimmed model of *Bd* in the GRSM. "Only variable" indicates the training gain when a single variable is run in isolation; "without variable" denotes the effect of removing a single variable from the full model (jack-knife). Values are means from 100 replicates

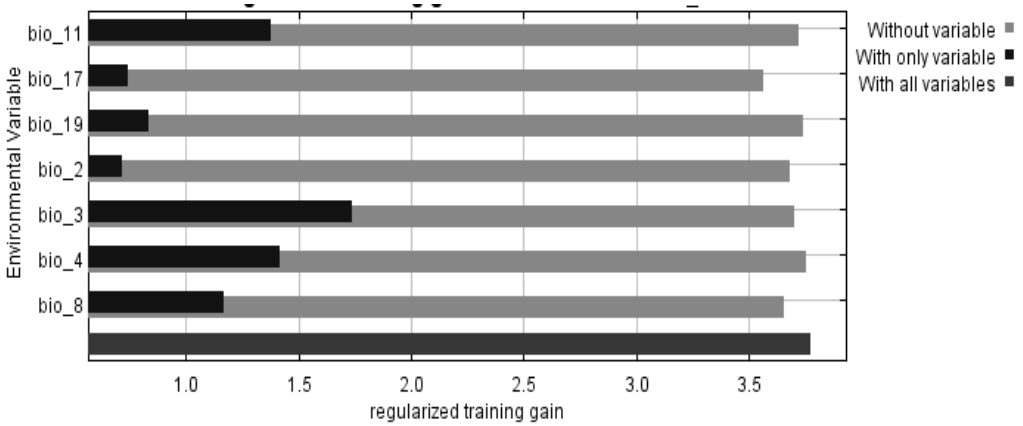


Figure 19: Variable contributions to area under the curve (AUC) for the trimmed model for *Bd* in the GRSM. "Only variable" indicates the AUC when a single variable is run in isolation; "without variable" denotes the effect of removing a single variable from the full model AUC (jack-knife). Values are means from 100 replicates

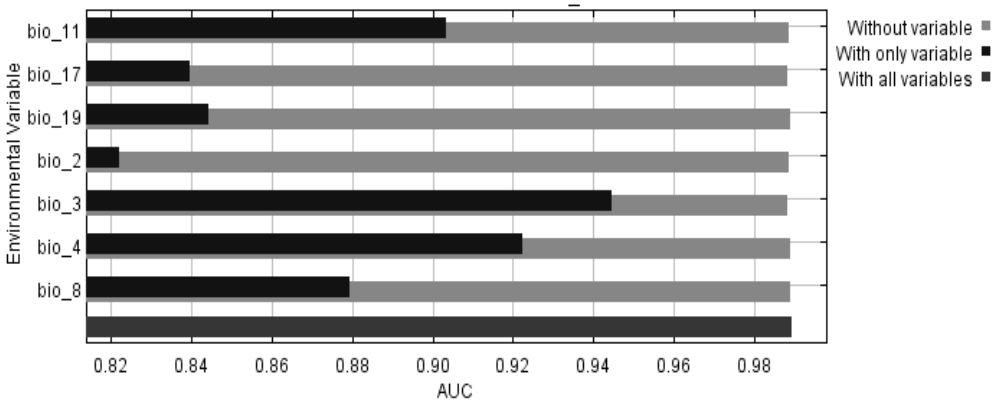


Figure 20: Predicted maximum suitability of *Bd* in the GRSM. Higher values denote areas that are more suitability for *Bd* occurrence. Maximum suitability was determined by the model with the highest suitability for the GRSM. This Figure shows that very few areas in the GRSM reach the maximum suitability level (0.9 – 1), while the maximum model predicts some areas to have low suitability (0.33 – 0.4).

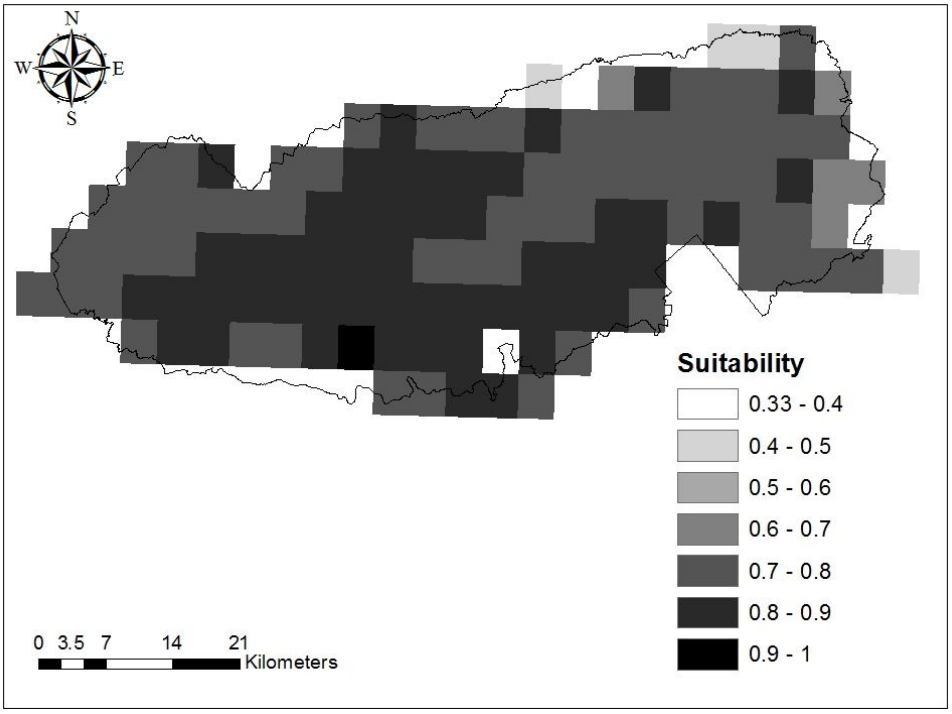


Figure 21: Predicted average suitability of *Bd* in the GRSM. Higher values denote areas that are more suitability for *Bd* occurrence. Average is based on all 100 models. This Figure shows that the western portion of the GRSM has higher suitability for *Bd* than the eastern half of the GRSM.

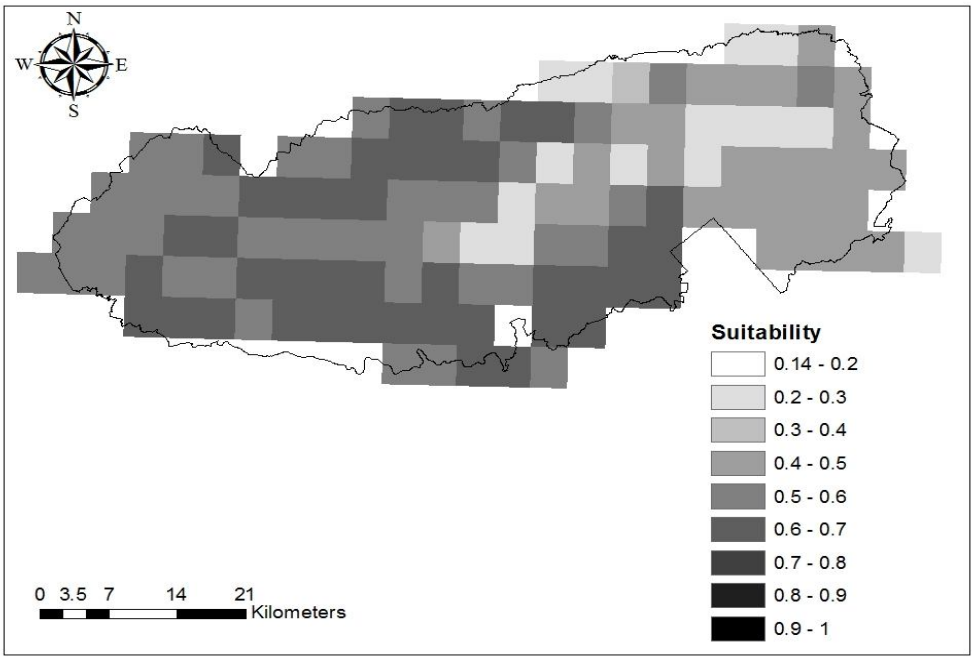


Figure 22: Average suitability of each population response. The filled in circles represent the median suitability for each population response. This Figure shows that the average suitability does not vary with population response for GRSM *Plethodon* salamanders.

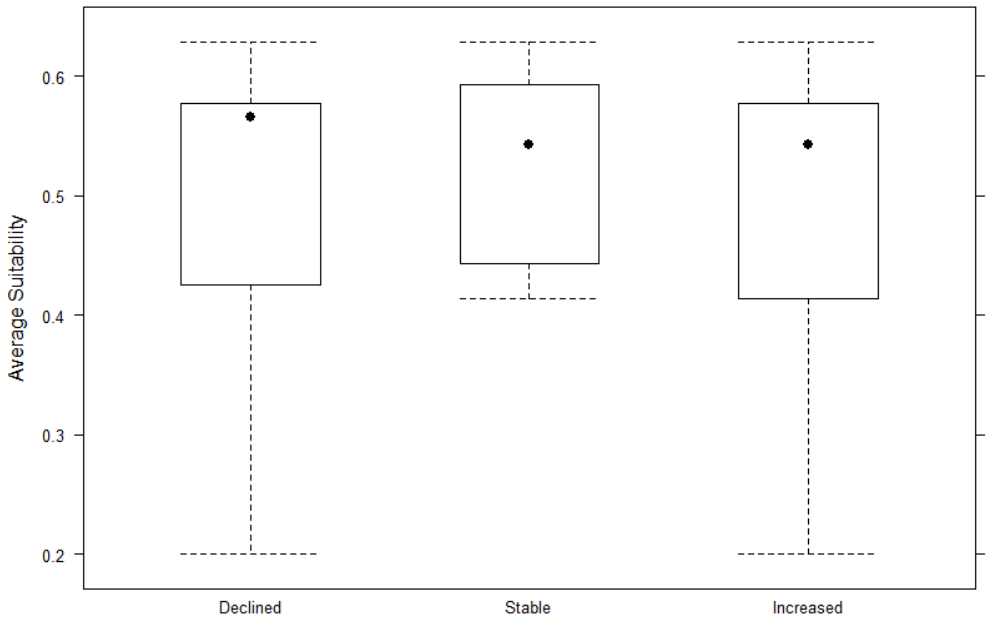


Figure 23: Proportion of population response based elevational bands. For population responses, "1" denotes declined populations, "2" is stable populations and "3" is increased populations, while the numbers on the right denote the proportion of the specific population response in each of the elevational bands (Low: 500 – 1,000 m; Mid: 1,000 – 1,500 m; High: > 1,500 m)

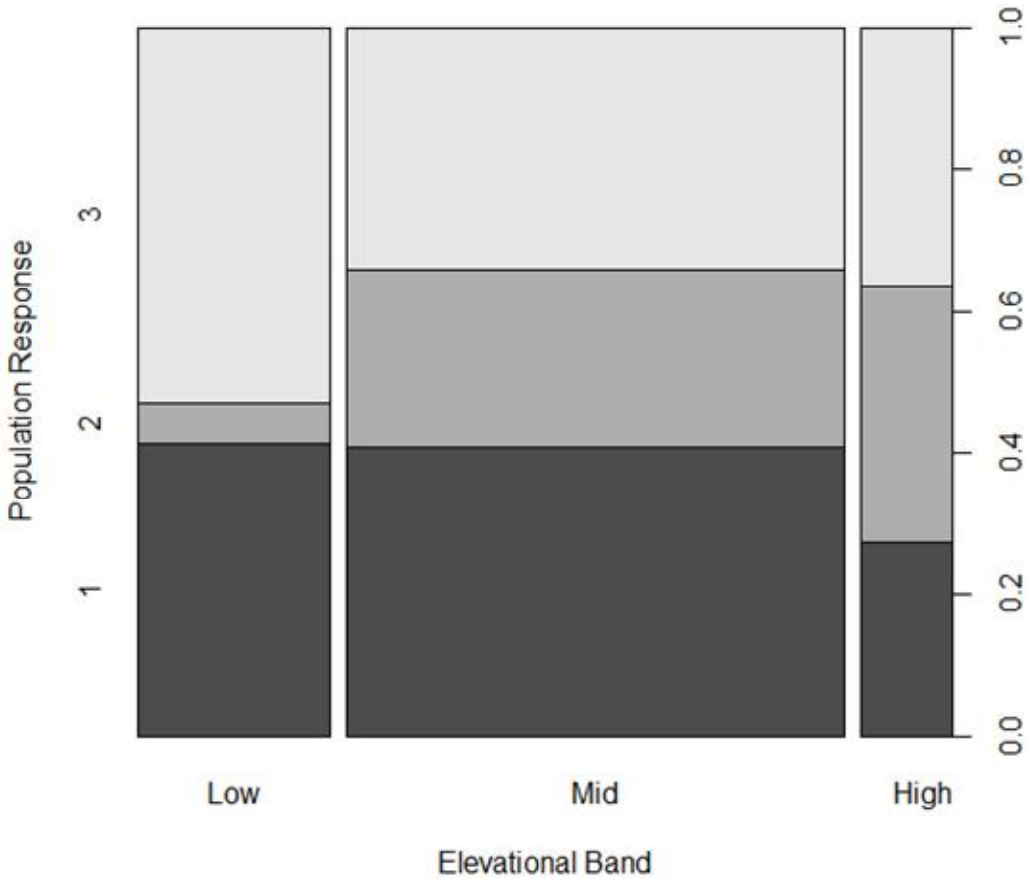


Figure 24: Proportion of population response based on type of historic logging (NPS 2007). For population responses, "1" denotes declined populations, "2" is stable populations and "3" are increased populations, while the numbers on the right denote the proportion of the specific population response in each of the logging categories.

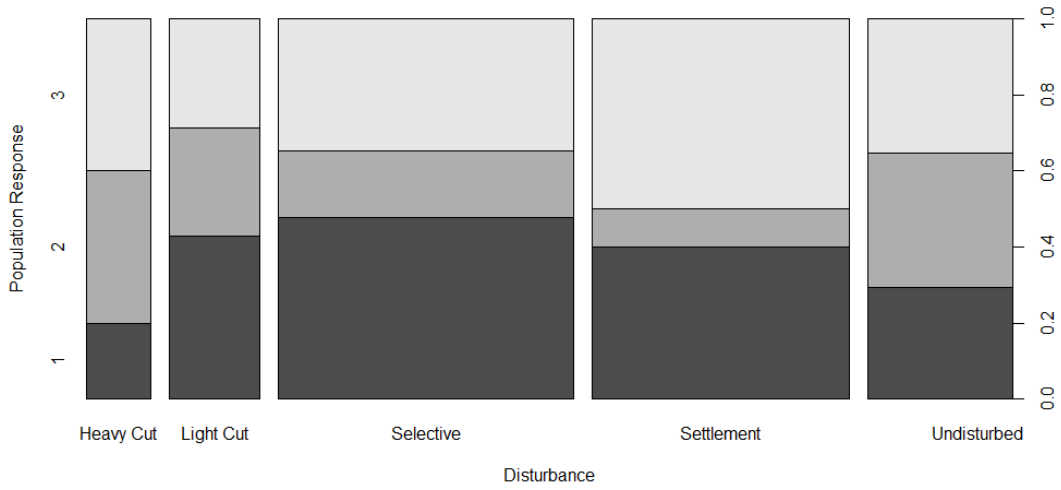


Figure 25: Number of populations found during historic (1960 – 1979) and current (2009) surveys based on community size.

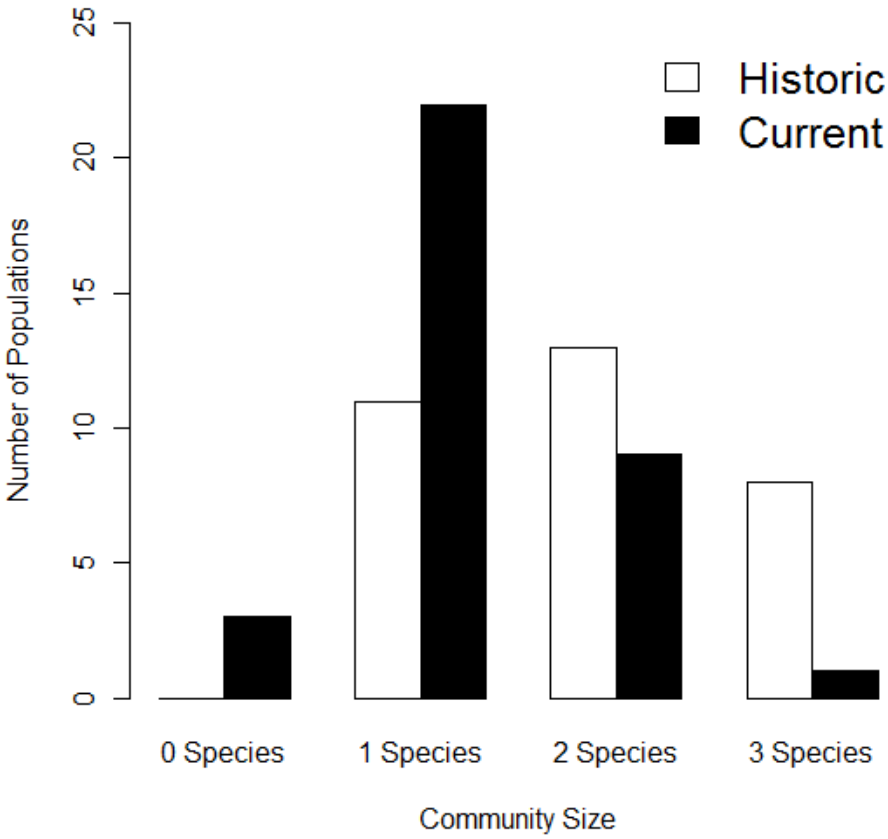
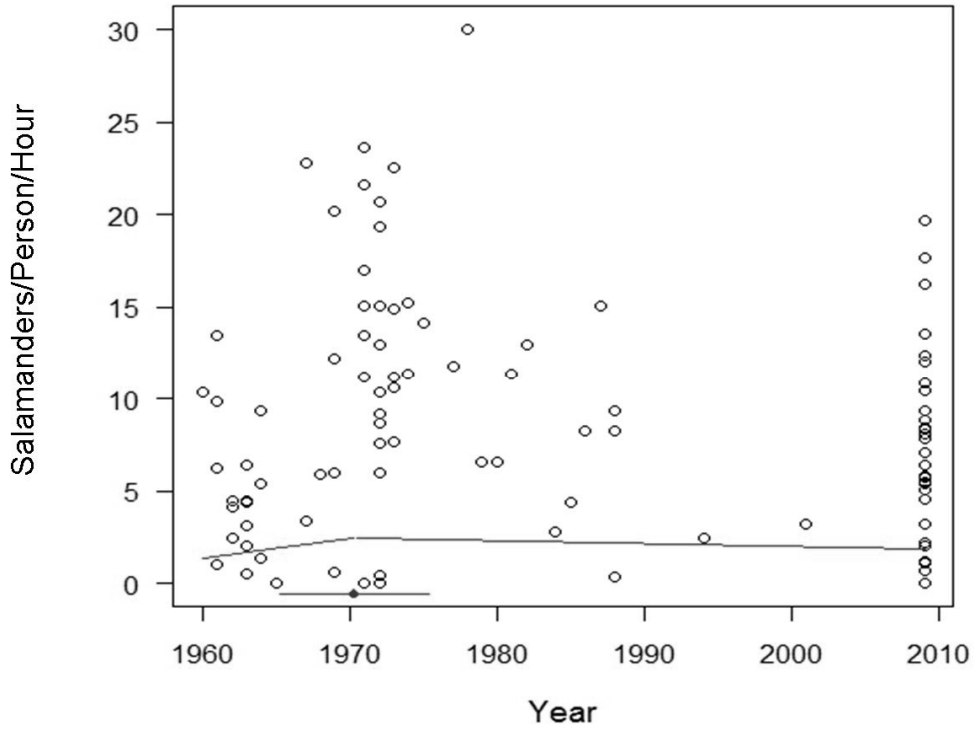


Figure 26: Graph showing the adjusted relative abundance for *Plethodon jordani* from 1962 – 2009. Solid lines indicate the piecewise linear regressions. The solid point with error bars represents the estimated point of change and 95% confidence intervals. This Figure shows that declines for this species most likely began during the late 1960's to early 1970's.



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