OCCUPANCY MODELING AND DISEASE SUCCEPTIBILITY OF MONTANE SALAMANDER SPECIES IN WESTERN NORTH CAROLINA

A Thesis
by
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ABSTRACT

OCCUPANCY MODELING AND DISEASE SUCCEPTIBILITY IN MONTANE SALAMANDER SPECIES IN WESTERN NORTH CAROLINA

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Amphibians worldwide are in a state of decline. Factors linked to their declines include habitat destruction, chemical pollution, UV-B irradiation, exotic species, exploitation, climate change, and disease. Monitoring at-risk populations allows researchers to determine the extent to which populations are experiencing declines, provides data for future population comparisons and allows researchers to determine the causes of decline. I conducted surveys of terrestrial salamanders (Caudata, Plethodontidae) on Grandfather Mountain, a high elevation biosphere reserve in Avery Co, NC from 2010-2011 in six plethodontid taxa, including *Plethodon welleri,* a state listed species of special concern in North Carolina (listed as endangered by the IUCN). I estimated occupancy and detection probabilities, and determined how environmental variables influenced these probabilities. Moreover, in each species, I assessed abundance of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*. Estimates of occupancy and detection probabilities differed between 2010 and 2011 for most species. Habitat variables (PC's) comprised of tree assemblages best explained occupancy in all species except *P. montanus,* which was found at all sites for an occupancy estimate of 1, and *Desmognathus orestes*, whose top model suggested that occupancy was constant across sites. I sampled 308 plethodontid salamanders for the presence of *B. dendrobatidis.* Molecular analysis of skin swabs indicated no positive salamanders. Although I did not detect *B. dendrobatidis*, these data can be used to help assess changes in its distribution and prevalence, providing baseline information for future studies of *B. dendrobatidis*.

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This project and subsequent thesis would not have been possible without the help of multiple individuals. First and foremost I would like to thank my major advisor, Dr. Lynn Siefferman. Her patience, understanding, guidance, and willingness to work with a student wanting to pursue a project outside her area of interest were key to my success. I am very thankful for her invitation to join her lab and for the friendship that followed. Committee members Drs. Michael Gangloff and Wayne Van Devender were also instrumental to my success. I greatly appreciated Dr. Gangloffs insight, constant edits, and suggestions throughout the course of my project, and Dr. Van Devender's vast knowledge about herps. I am indebted to Jacob Fields for his constant support during the course of this project, both with fieldwork and emotionally. During 2010, Jacob helped with every survey, getting nothing more than a thank-you at the end. Without Jacob's encouragement and faith in our safety, our first night survey would have ended in the parking lot (and would have never led to a second) after our encounter with a large bear. I thank Grandfather Mountain for allowing me to do research on their property, supplying me with cover boards, and explaining to security why they needed to let me in after dark. Jesse Pope was key to this project in that he was willing to meet with me multiple times to discuss logistics, suggesting locations for transects, securing supplies, and allowing staff to occasionally assist in day surveys during working hours. For discussion on topics I had yet to grasp, I thank Mary Jane Carmichael. I am extremely thankful for the assistance of Pushpal Mukhopadhyay in my endeavor to understand occupancy modeling. At times he would receive an overwhelming

number of emails from me, none of which went unanswered. I would also like to thank Larissa Bailey and Jerome Pellet for help in understanding occupancy modeling. Lastly, I thank my as-of-yet unborn son for a boost in motivation, confidence, and happiness. Appalachian State University Office of Student Research and a Grant-In-Aid of Research from Sigma Xi supported my research.

DEDICATION

I would like to dedicate this thesis to Dr. Kristin B. Webb.

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FOREWORD

Chapters 2 and 3 of this thesis are being submitted for publication and are thus formatted to specific journal formats. Chapter 2 is being submitted to Biological Conservation and Chapter 3 is being submitted to the Journal of Herpetology.

CHAPTER 1

GENERAL INTRODUCTION

The earth is experiencing a biodiversity crisis: a sixth mass extinction event is currently taking place (Barnosky et al., 2011; Rands et al., 2010). While the previous five mass extinctions were caused by natural events, this event is being caused by human activity. We are altering habitats through urbanization and pollution, changing the world's climate, and introducing invasive species, all of which is contributing to a loss of biodiversity and ecosystem services (Pysek and Richardson, 2010).

Amphibians are the most threatened vertebrate taxon in the world (Wake and Vredenburg, 2008). Global amphibian declines were first noted in 1989 at the First World Congress of Herpetology, but reports of localized declines date back as far as the 1960's (Pechmann and Wilbur, 1994; Wake, 1991). It is not uncommon to see species loss in areas far removed from human influence and in relatively pristine environments (Carver et al., 2010; Waldman and Tocher, 1998). Threats to amphibians include habitat destruction, chemical pollution, UV-B irradiation, exotic species, exploitation, climate change, and disease, specifically chytridiomycosis (Beebee and Griffiths, 2005; McCallum, 2007).

The southern Appalachian Mountains are a hotspot for salamander diversity (Kiester, 1971; Lannoo et al., 2005), with the family Plethodontidae composing the bulk of the diversity (Milanovich et al., 2010). Because they are the most abundant vertebrate in eastern US forests and streams, it is important to understand their current distribution and what drives changes in their populations. Salamander distributions can be heavily influenced by environmental factors, such as temperature and moisture, so tracking trends in these variables should offer insight into patterns of distribution.

Abundance estimation has traditionally been employed to monitor salamander populations, and changes in abundance over time are presumed to reflect changes in population size or status (MacKenzie and Nichols, 2004). However, it is also important to take into account detection probabilities to make accurate assessments regarding these changes. To obtain accurate abundance estimates with detection probabilities, it is necessary to uniquely identify individuals throughout the course of the study. It is often the case that these studies must continue for multiple years over large spatial scales to compensate for model assumptions and model inaccuracies. An alternative approach to abundance estimation is occupancy modeling (MacKenzie et al., 2002). Occupancy modeling allows the researcher to assess changes in the proportion of area occupied by a species. This approach is often less expensive, does not require surveying over large spatial scales, and is usually conducted over shorter periods of time. At an “appropriate scale,” abundance and occupancy should be positively correlated, with occupancy increasing as abundance increases (Mackenzie and Nichols, 2004). Occupancy models use logistic regression to estimate the importance of covariates that are hypothesized to influence occupancy and detection probabilities, such as habitat variables (site-specific) and environmental variables (survey-specific) (MacKenzie et al., 2006).

In addition to occupancy modeling, amphibians can be sampled for disease. Chytridiomycosis, the disease caused by the fungus *Batrachochytrium dendrobatidis* (Bd), is thought to contribute to population declines. There are two life stages of Bd. The first stage consists of an immobile, reproductive body called the zoosporangium, and the second stage consists of a flagellated zoospore, which is released from the zoosporangium (Berger et al., 2005; Longcore, 1999; Weinstein, 2009). This two-stage process is dependent on aquatic environments, but the fungus can survive for up to 12 weeks in damp soils, with certain strains growing in soils with moisture levels as low as 10%.

Infection with Bd is restricted to the kertanized skin parts of the amphibian (Berger et al., 1998; Voyles, 2007). There are three hypotheses that address the ability of chytridiomycosis to cause mortality. The first hypothesis states that infection disrupts osmoregulation, the second states that the fungus releases toxins that affect internal organs, and the third states that mortality occurs as a combination of the two (Voyles, 2009). The skin of an amphibian is important for exchange of respiratory gasses, ions and water between it and the environment. Infection with chytridiomycosis in anurans is known to cause hyperkeratosis and hyperplasia (Berger et al., 1998), thus interfering with an amphibians ability to respire and obtain moisture. Experimental infection in frogs with the Bd pathogen by Voyles (2007) showed a reduction in blood plasma osmolality and electrolyte concentrations, demonstrating an osmotic imbalance in the skin (Vazquez et al., 2009; Voyles, 2007; Voyles, 2009).

Infection rate with chytridiomycosis in Southern Appalachian salamander assemblages has been understudied. Examining the presence of this disease, along with patterns of species occurrence, can provide important baseline information necessary to determine whether local salamander biodiversity is in decline.

CHAPTER 2

OCCUPANCY AND DETECTABILITY OF A SOUTHERN APPALACHIAN SALAMANDER ASSEMBLAGE

ABSTRACT

Amphibian declines are widespread and ongoing. Detecting population changes necessitates the need for intensive monitoring programs. Most traditional methods of estimating population abundance do not take into account detection probabilities. Because salamanders can be hard to detect, traditional methods yield unreliable estimates. Occupancy models, however, incorporate estimates of detection probability and can provide more reliable methods of assessing population parameters. In this study, I used occupancy models to examine southern Appalachian salamander assemblages on Grandfather Mountain, North Carolina using multiple environmental factors. I conducted night searches and cover board surveys for terrestrial salamanders in 2010, and cover board surveys in 2011, along an elevational gradient. I identified trees and measured precipitation and temperature. I found six species of plethodontid salamanders, including *Plethodon welleri*, a species considered globally at risk. Between 2010 and 2011, estimates of occupancy and detection probabilities differed for most species. Species distribution varied with tree assemblages, though some species were restricted to high elevation sites while others appeared restricted to lower elevation sites. Tree assemblage best explained occupancy in all species except *P. montanus*, which was found at all 25 sites, and *Desmognathus orestes,* whose occupancy was constant across sites. These data provide baseline estimates of occupancy and detectability of this

montane salamander assemblage and can be used to assess, in conjunction with additional years of data, future conservation needs.

INTRODUCTION

Amphibians, the most threatened group of vertebrate (Johnson et al., 2011), face multiple stressors including disease, habitat destruction, chemical pollution, UV-B irradiation, exotic species, exploitation, and climate change (Beebee and Griffiths, 2005; McCallum 2007, Ohmer and Bishop 2011). Monitoring amphibians has become an important component of conservation research and may provide managers with important information on population level trends, conservation priorities, and the extent of decline (Blaustein et al. 2002; Pellet and Schmidt 2005). The goal of most amphibian monitoring programs is to estimate population abundance, however the methods required to obtain these estimates can be expensive and time consuming. Also, estimates of abundance often assume perfect detection of a species within a season, even if that assumption is unrealistic and cannot be met. Only a handful of species that are present at a site will be detected perfectly by a research team (MacKenzie et al., 2006). When researchers fail to incorporate detection probability into population estimates, the data will likely be biased and potentially underestimated.

Occupancy modeling is an alternative approach to monitoring population abundance that takes into account imperfect species detection probabilities (Bailey et al., 2002; MacKenzie et al., 2002). Occupancy (ψ) is defined as the proportion of area occupied by a species and is determined by visiting survey sites numerous times within a season to compile a detection history. The target species is either detected with probability p, or not detected.

The goal of occupancy modeling is to determine the proportion of area occupied, and to derive these estimates based on detection probabilities <1. Assessing patterns within these detection histories allow estimation of occupancy and detection.

Salamanders (class: amphibia, order: caudata) are important to forest ecosystem functioning: they act as predators and prey, transfer energy throughout food webs, regulate decomposition rates by consuming soil invertebrates, and move nutrients, gasses, fungi and microorganisms from above to below ground (Davic and Welsh, 2004). Salamanders in the family Plethodontidae are terrestrial and primarily subterranean with only a small portion of their population above ground. Because of the difference between above and below ground populations, estimating abundance is difficult. Surface counts likely produce highly variable estimates (Bailey et al., 2004). Also, these estimates are likely poorly correlated with total population size (Bailey et al., 2004; Smith and Petranka, 2000). Occupancy modeling provides an excellent alternative to study population level dynamics of salamanders.

Both occupancy and detection probabilities can be influenced by site-specific covariates, which include elevation, and habitat variables, whereas detection can be influenced by survey-specific covariates, such as time, temperature, season, and precipitation (Bailey et al., 2004). It is important for researchers to consider the natural history of the target organism when formulating hypotheses about the factors that could influence parameter estimation in order to obtain appropriate estimates. For example, because Plethodontid salamanders are lungless with high moisture requirements, they may be more likely to be found above ground after precipitation events. Also, because salamanders are ectothermic, species may be more likely to be found above ground during warmer periods.

Plethodon welleri was a focal species in this study. *Plethodon welleri* are found primarily in moist, deciduous, spruce-fir forests (Petranka, 1998). Most populations occur on mountaintops and may be isolated relics of a once widespread distribution. They are listed as a species of special concern in North Carolina, with a single population located on Grandfather Mountain, Avery Co. NC. Since 1977 (Cooper et al., 1977), authorities have recognized this species as special concern and in 1990 the status of 'special concern' became official. The species is also listed as endangered by the IUCN Red list (IUCN, 2004).

Here, I present the results of a two-year study in which I assessed site-occupancy and detection probabilities of a plethodontid salamander assemblage in a pristine, southern Appalachian Mountain ecosystem. I modeled occupancy and detection probabilities using survey method (cover board surveys versus night surveys), rainfall and temperature, tree assemblage, and elevation as covariates in my models.

METHODS

Surveys

In the summers of 2010 and 2011, I conducted surveys on Grandfather Mountain, Avery Co., North Carolina to establish plethodontid salamander detection histories. I conducted cover board surveys approximately one time per week in 2010 and 2011, and conducted night surveys approximately 1-2x times per month during 2010.

Five, 100 m long transects were placed along an elevation gradient on the south face of Grandfather Mountain in April, 2010. The highest elevation transect was placed at 1533 m and the remaining transects were placed at 1445 m, 1356 m, 1311 m and 1259 m. Locations were selected to avoid high public traffic areas or steep and difficult terrain. At each location, I used a Garmin 60CSx handheld Global Positioning System (GPS) to measure latitude, longitude and elevation, which gave a reading within 6-9 m of accuracy depending on density of vegetation, weather and atmospheric pressure. I placed five 10 m x 10 m equally-spaced plots along each transect for use as cover board plots. Cover boards were made of untreated, weathered spruce lumber (30 x 30 x 5 cm). I placed nine boards within the boundaries of each 10 x 10 m plot for a total of 45 boards per transect.

I conducted night surveys as a means to increase sample size while the newly placed cover boards weathered. Night survey plots were located adjacent to cover board plots and measured 10 x 2 m. I conducted night searches approximately 1-2 x per month in 2010. Surveys began at dusk and continued until all plots had been searched for five minutes each. In some cases, the number of salamanders obtained during a night survey was high which required that surveys be conducted on two consecutive nights. Salamanders were continuously collected and processed following the completion of the survey.

When checking cover boards, each board was lifted quickly to reduce the number of escapees. Salamanders found during either survey were placed into a clean, 20 x 25 cm Ziplock® bag inverted over the hand. Salamanders were identified to species based on morphology. After processing, salamanders was placed next to the cover board, or placed in the center of the night survey plot. If salamanders became dried during handling, I poured a small amount of water into the Ziplock® before release to rehydrate the animal. Ziplock® bags were discarded after each use and hands were sanitized prior to switching plots with 65% ethyl alcohol.

Tree Surveys

I conducted tree surveys on September 24, 2010 at all 25 plots. All trees that contributed to the canopy and found within the plots were identified to species along with the subcanopy species. I used principal component analysis (PCA) to transform multiple correlated variables into a set of uncorrelated principal components that described the tree assemblage within survey plots. Based on initial extraction, I excluded tree species with loading factors <0.4. Using the remaining tree species, I extracted three principal components that accounted for a total of 66.2% of the total variance of the 11 variables. Principal component 1 (PC1), explained 34.9% of the variance, principal component 2 (PC2) accounted for 15.8% of the remaining variance, and principal component 3 (PC3) accounted for 15.5% of the remaining variance. Sites with high principal component 1 scores were characterized by high numbers of *Fagus grandifolia*, *Acer saccharum, Hamamelis virginiana*, *Quercus prinus*, and *Q. rubra*, and low numbers of *Picea rubens*, *Betula alleghaniensis*, and *Sorbus americana* (Table 1). Sites with high principal component 2 scores were characterized by high numbers of *B. alleghaniensis* and *S. americana*, and low numbers of *Tsuga canadensis* and *Fraxinus americana* (Table 1). Sites with high principal component 3 scores were characterized by low numbers of *Acer rubrum*, and *Q. prinus* (Table 1).

Modeling ψ and p

I used occupancy models to estimate detection and occupancy probabilities for plethodontid salamanders found within the 25 cover board plots and night survey plots. There were multiple covariates that were hypothesized to influence both detection and occupancy probabilities (Table 2). Site-specific covariates characterized habitat qualities that did not change throughout the survey season. Site-specific covariates included elevation, and a survey of tree assemblages, which yielded three habitat variables: PC1, PC2, and PC3. Survey-specific covariates characterized dynamic environmental variables at time of sampling. I included the following survey-specific covariates in my analysis: average temperature of the day (temp), the amount of rainfall the day before (rain), and the survey method used (method; for 2010 analysis only). Temperature and rainfall data were obtained from National Oceanic and Atmospheric Administration (NOAA). These covariates were hypothesized to influence detection probabilities, which can vary between sampling occasions.

Detection histories were analyzed for each species by fitting models to the data that included covariates that were hypothesized to influence detection and occupancy probabilities. Included in the suite of models were the global model, which consisted of additive effects of all covariates, and the probability constant model in which no covariate influenced probabilities. The best model was selected using Akaike's Information Criterion with a correction for small sample size (AICc) to rank models (the model with the lowest AICc value) (Burnham & Anderson 2002). A comparison between the 'best' ranked model and subsequent models can be done by using the ΔAICc value, which uses the difference between AICc values for each subsequent model and the 'best' ranked model. Models with ΔAICc values ranging from 0-2 have substantial support; ΔAICc values of 4-7 have much less support; models with ΔAICc values greater than 10 are not supported (Burnham & Anderson 2002). I used model averaging when multiple models fell within 2 ΔAICc units of the top-ranked model to account for model uncertainty and to obtain a weighted average of those models.

RESULTS

Surveys conducted during 2010 and 2011 yielded six terrestrial salamander species, but not all species were found at all elevations (Table 3). Occupancy and detection probabilities varied and were often functions of the site and survey-specific covariates.

Plethodon welleri

In 2010, *Plethodon welleri* was found at five of 25 sites for a naïve occupancy estimate of 0.20. The top-ranked model suggested that occupancy was a function of tree assemblage (PC1), and detection probability was a function of temperature (Table 4). *Plethodon welleri* were more common in habitats with low numbers of *F. grandifolia*, *A. saccharum*, *H. virginiana*, and *Q. rubra*, and detection probabilities increased with increasing temperature (Figure 1). Based on the $\psi(.)p(.)$ model, occupancy was estimated at $\psi=0.200\pm0.080$ and detection was estimated at $p=0.186\pm0.032$. Based on the beta estimates, the odds of detecting *P. welleri* increased by 8.9% for every degree increase in temperature (Table 5).

There was one model that ranked within 2 ΔAICc units of the top model in 2010. While the tree assemblage (PC1) remained as a covariate of occupancy, detection probabilities became constant across surveys. Because multiple models were plausible, I used model-averaging to obtain estimates of occupancy and detection probabilities.

Estimates of detection probabilities ranged from *p*=0.126±0.042 to *p*=0.226±0.057 and model-averaging provided an occupancy estimate of ψ=0.091±0.067.

In 2011, *P. welleri* again had a naïve occupancy estimate of 0.20. The top-ranked model suggested that occupancy was a function of tree assemblage (PC1), and detection probability was constant across surveys (Table 6). Based on the ψ(.)p(.) model, occupancy was estimated at ψ=0.201, and detection probability was estimated at *p*=0.236. Two models ranked within 2 ΔAICc units from the top model. Tree assemblage (PC1) remained as a covariate of occupancy in both models but detection became a function of temperature in the next highest model, and rain in the third model. Model-averaged estimates of detection probabilities ranged from *p*=0.196±0.047 to *p*=0.303±0.091 and occupancy was estimated at ψ=0.090±0.067.

Plethodon cinereus

In 2010, *Plethodon cinereus* was found at 14 of 25 sites for a naïve occupancy estimate of 0.56. The top-ranked model suggested that occupancy was best explained by tree assemblage (PC1), and detection probability varied by survey method (Table 7). *Plethodon cinereus* were more common in habitats with higher amounts of *F. grandifolia*, *A. saccharum*, *H. virginiana*, and *Q. rubra*, and detection increased during day surveying methods (Figure 2). Based on the ψ(.)p(.) model, occupancy was estimated at ψ=0.599±0.108 and detection was estimated at *p*=0.087±0.015. Based on beta estimates, the odds of detecting this species during a day survey increased by 54.4% (Table 8).

There were four models that ranked within 2 ΔAICc units of the top model of *P. cinereus* in 2010. While tree assemblage (PC1) remained as a covariate of occupancy in all four models, detection probabilities varied. The next top-ranking model held detection constant across surveys. The models that followed included detection as a function of rainfall, additive effects of temperature and method used, and additive effects of rainfall and method used. I used model-averaging to estimate detection and occupancy probabilities. Estimates of model-averaged detection probabilities ranged from p=0.059±0.020 to p=0.101±0.018, while occupancy was estimated at ψ=0.887±0.121.

In 2011, *P. cinereus* was found at 12 of 25 sites for a naïve occupancy estimate of 0.48. The top-ranked model suggested that occupancy remained a function of tree assemblage (PC1) and detection probability was a function of temperature (Table 9) with detection probability increasing with decreasing temperature (Figure 3). Based on the model ψ(.)p(.), occupancy was estimated at ψ=0.488±0.102 and detection was estimated at p=0.171±0.024. Based on the beta estimates, the odds of detecting *P. cinereus* decreases by 7% for every degree increase in temperature (Table 10).

There was only one model that ranked within 2 ΔAICc units of the top model of *P. cinereus* in 2011. Tree assemblage (PC1) remained as a covariate to describe occupancy, and detection probabilities became constant across surveys. Estimates of model-averaged detection probabilities ranged from p=0.134±0.029 to p=0.245±0.055 with an occupancy estimate of ψ=0.783±0.162.

Plethodon cylindraceus

In 2010, *Plethodon cylindraceus* was found at five of 25 sites for a naïve occupancy estimate of 0.20. The top-ranked model suggested that occupancy was a function of tree assemblage (PC1) and that detection probability was constant across all sites (Table 11).

Plethodon cylindraceus were more commonly found in habitats with higher amounts of *F. grandifolia*, *A. saccharum*, *H. virginiana*, and *Q. rubra*. Based on the model ψ(.)p(.), occupancy was estimated at ψ=0.381±0.230 and detection probability was estimated at *p*=0.024±0.016. There were two models within 2 ΔAICc units of the top model. Tree assemblage (PC1) remained the best covariate for occupancy, but detection probability was modeled as a function of temperature, and additive effects of temperature and method. Model-averaged estimates of detection probabilities ranged from *p*=0.018±0.010 to *p*=0.054±0.028 and occupancy was estimated at ψ=0.205±0.182.

In 2011, *P. cylindraceus* was found at three out of 25 sites for a naïve occupancy estimate of 0.12. Because captures were low, it was not possible to estimate occupancy or detection probability with reliable results.

Plethodon montanus

In 2010, *P. montanus* was found at 25 out of 25 sites for an occupancy estimate of ψ=1.00. Because this species occupied every site, I estimated detection probabilities only and fixed ψ at 1. The top-ranked model suggested that detection probability was best explained as a function of the additive effects of rainfall, temperature, and method used (Table 12). Detection of *P. montanus* increased with increasing rainfall, increasing temperatures, and during night surveys. Based on the model ψ(1)p(.), detection probability was estimated at *p*=0.284±0.16. Based on the beta estimates, the odds of detecting this species increases by 18.6% for every degree increase in temperature after adjusting for method and rainfall; the odds of detection decreased by 5% for every unit increase in rainfall after adjusting for temperature and method; the odds of detection during a night survey were 2.5 x higher than during a day survey after adjusting for temperature and rainfall (Table 13).

There was one model within 2 ΔAICc units of the top model of *P. montanus* in 2010. This model contained additive effects of rainfall and temperature as covariates of detection probability. Model-averaged detection probability estimates ranged from *p*=0.178±0.021 to *p*=0.552±0.042.

In 2011, *P. montanus* was again found at all sites for an occupancy estimate of ψ=1.00. The top-ranked model contained the additive effects of rain and temperature as covariates of detection probability (Table 14). Detection increased as rainfall and temperature increased (Figures 4 and 5). Based on the model ψ(1)p(.), detection was estimated at *p*=0.189±0.017. Based on the beta estimates, the odds of detection increased by 6.7% for every degree increase in temperature after adjusting for rainfall, and the odds of detection also increased by 1% for every mm increase in rainfall after adjusting for temperature (Table 15).

There was one model within 2 ΔAICc units of the top model for *P. montanus* in 2011. The next model contained temperature as a function of detection probability. Model-averaged detection probability estimates ranged from *p*=0.127±0.027 to *p*=0.235±0.032.

Desmognathus orestes

In 2010, *D. orestes* was found at nine of 25 sites for a naïve occupancy estimate of ψ=0.36. The top-ranked model demonstrated that occupancy was constant across sites and detection probability was a function of temperature and method used (Table 16). Detection probability increased during night surveys and as temperature increased (Figure 6). Based on the model $\psi(.)p(.)$, occupancy was estimated at $\psi=0.371\pm0.099$ and detection was estimated at $p=0.111\pm0.020$. Based on the beta estimates, the odds of detecting this species increased by 22.5% for every degree increase in temperature after adjusting for method, and the odds of detection during a night survey were 7.8 x greater than detection during a day survey after adjusting for temperature (Table 17). Because the next most likely model was greater than 2 ΔAICc units from the top model, I did not model average my estimates.

In 2011, *D. orestes* was found at eight of 25 sites for a naïve occupancy estimate of $\psi=0.32$. The top-ranked model suggested that occupancy was constant across sites and detection probability was influenced by rain (Table 18). Detection of *D. orestes* increased as rainfall increased (Figure 7). Based on the model $\psi(.)p(.)$, occupancy was estimated at $\psi=0.367\pm0.112$ and detection was estimated at $p=0.089\pm0.024$. Based on the beta estimates, the odds of detection increased by 2.3% for every mm increase in rainfall (Table 19).

There were two models that ranked within 2 ΔAICc units of the top model for *Desmognathus orestes* in 2011. Occupancy in this model became a function of tree assemblage (PC1) and detection probability remained a function of rainfall. Model-averaged estimates of detection probabilities ranged from $p=0.076\pm0.023$ to $p=0.289\pm0.132$ with an occupancy estimate of $\psi=0.357\pm0.113$.

Desmognathus wrighti

In 2010, *D. wright* was found at eight of 25 sites for a naïve occupancy estimate of $\psi=0.32$. The top-ranked model suggested that occupancy was best explained as a function of tree assemblage (PC1), and detection probability was a function of temperature and method used (Table 20). *Desmognathus wrighti* were more common in habitats with fewer numbers of *F. grandifolia*, *A. saccharum*, *H. virginiana*, and *Q. rubra*, and detection increased during night surveys and as temperature increased (Figure 8). Based on the model $\psi(.)p(.)$, occupancy was estimated at $\psi=0.348\pm0.103$ and detection probability was estimated at $p=0.080\pm0.019$. Based on the beta estimates, the odds of detecting this species increased by 27.3% for every degree increase in temperature after adjusting for method, and the odds of detecting this species during a night survey were 2.66 x greater than detection during a day survey after adjusting for temperature (Table 21).

There was one model within 2 ΔAICc units of the top model for *D. wrighti* in 2010. Occupancy in this model became constant across sites and detection probability remained a function of temperature and method used. Model-averaged detection probability estimates ranged from $p=0.008\pm0.005$ to $p=0.213\pm0.067$ and occupancy was estimated at $\psi=0.304\pm0.113$.

In 2011, *D. wrighti* was found at six of 25 sites for a naïve occupancy estimate of 0.24. The top-ranked model demonstrated that occupancy was a function of tree assemblage (PC1), and detection probability was constant across sampling occasions (Table 22). Based on the model $\psi(.)p(.)$, occupancy was estimated at $\psi=0.398\pm0.193$ and detection was estimated at $p=0.041\pm0.022$.

There were multiple models within 2 ΔAICc units of the top model for *D. wrighti* in 2011. The next model was an occupancy and detection probability constant model. The model that followed suggested that occupancy was constant, but detection became a function of temperature. Occupancy in the third model was best explained by tree assemblabe (PC1), and detection remained a function of temperature. Finally, occupancy in the fourth model was a function of elevation while detection was constant across surveys. Model-averaged detection probabilities ranged from p=0.028±0.017 to p=0.045±0.020 and occupancy was estimated at ψ=0.433±0.235.

DISCUSSION

I modeled salamander occupancy and detection probabilities as functions of rainfall the day before, average temperature, survey method used, elevation, and three habitat covariates based on tree assemblages, PC1, PC2, and PC3. My results indicate that salamander occupancy was not associated with elevation, PC2 or PC3. However, tree assemblage (PC1) best explained occupancy in all species except *P. montanus* and *D. orestes*. *Plethodon montanus* was found at all sites for an occupancy estimate of 1, and no measured habitat variables explained occupancy in *D. orestes*.

None of my top models included elevation as a covariate for any salamander species. This was perplexing because I predicted that elevation would influence occupancy, particularly in *P. welleri* as I only found this species at the highest elevation (1533 m). It is possible that my sampling design across an elevation gradient was too narrow to provide adequate inference about its effect on species distribution. Rather than elevation, PC1 of tree assemblage was the most important factor influencing occupancy; *P. welleri* was generally found in areas with high PC1 or in forests with more *Picea rubens, Betula alleghaniensis*, and *Sorbus americana*. Similarly, Cooper (1977) reported that this species inhabits forests in which *T. canadensis, P. rubens, B. alleghaniensis,* and *Rhododendron maximum* are commonly occurring species. By using a linear regression to examine the relationship between elevation and PC1 I found that there was a significant correlation between the two variables (R^2=0.53, df=4, p<0.001). At the lowest elevation sites, PC1 scores were higher and generally decreased with increasing elevation. Therefore, tree assemblage changed with elevation, such that there are more deciduous forests at lower elevations. However, because the relationship was <1, I opted to use both covariates in my models.

In 2010 and 2011, multiple species (*P. cinereus, P. welleri, P. montanus, D. orestes,* and *D. wrighti*) had top-ranked models containing either rain or temperature or a combination of both as a covariate of detection probability; *P. welleri, P. montanus* and *D. orestes* were more commonly found during warmer and/or wetter surveys. Other studies also demonstrate that precipitation and temperature influence detection of amphibians (Haan et al. 2007; Roloff et al. 2011). Salamanders are dependent on moisture (Spight, 1968; Spotila and Berman, 1976; Grover, 2000), especially the plethodontids as their skin acts as their primary respiratory organ, which must remain moist to effectively facilitate gas exchange. This dependence restricts their activity to periods of high moisture or humidity (Spotila 1972; Grover 2000). Similarly, temperature influences salamander movement (Hendrickson 1954; Maiorana 1976) with changing temperatures altering surface activity (Hyde and Simons, 2001) and salamanders showing preference for cooler microhabitats (Heatwole, 1962; Haan 2007). Moisture and temperature have been shown to be important factors in amphibian breeding activity (Beebee, 1995; Blaustein et al., 2001) and capture rates (deMaynadier and Hunter, 1998). Thus I predicted that temperature and moisture would be influential factors in the detection of salamanders.

However, relationships with temperature and moisture varied across species. *Plethodon welleri, P. montanus, D. orestes,* and *D. wrighti* were all positively associated with these covariates with an increase in temperature and/or moisture increasing detection probabilities. However, I observed the opposite relationship for *P. cinereus* in 2011. When estimates of detection were plotted against a range of temperatures, this species was more likely to be found at the lower end of the spectrum. Because salamanders are ectotherms, they behaviorally regulate their body temperatures by selecting the appropriate microhabitat (Spotila, 1972). Also, *P. cinereus* has a much lower ‘preferred temperature’ as compared to other salamanders in the family Plethodontidae. This preferred temperature refers to the thermal range in which a species exhibits its normal activity range. Of the 13 different species of plethodontid salamanders this study compared, *P. cinereus* had the second lowest preferred temperature range.

In 2010, I used night surveys to supplement newly placed cover boards. Night surveys were more likely to detect *P. montanus, D. orestes,* and *D. wright*, which is intuitive because these animals are nocturnal. However, *P. cinereus* was more likely to be detected during daytime cover board surveys. *Plethodon cinereus* are highly territorial and will aggressively defend cover objects with a food supply from both conspecific and heterospecific salamanders (Mathis, 1989; Lang and Jaeger 2000; Moore 2001; Riedel et al. 2012). It is possible that increased detectability under cover boards may indicate a behavioral response to a sufficient food supply.

Because some models resulted in detectability or occupancy being constant across time or space, it is possible that I failed to measure other important covariates. Other covariates I could have considered include aspect (Haan et al. 2007), distance to nearest riparian zone (Birx-Raybuck 2009), prior disturbance history (Bailey et al. 2004), distance to nearest road, or potentially incorporating body condition or other measurements into my models. While other factors may have been influential, the basic covariates I chose to incorporate were based on simple ecological or biological relationships and can provide useful information for salamander occupancy modeling studies. These data can also help researchers focus their often limited resources on appropriate survey methods and under which environmental conditions it would be best to survey to increase detection of plethodontid salamanders.

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TABLES AND FIGURES

TABLES

Table 1. Principal component (PC) loading factors for tree species.

Tree Species	PC1	PC2	PC3
American Beech (*Fagus grandifolia*)	0.866	0.001	0.393
Sugar Maple (*Acer saccharum*)	0.787	0.243	0.450
Red Maple (*Acer rubrum*)	0.232	0.007	-0.786
Witch Hazel (*Hamamelis virginiana*)	0.885	0.221	0.286
Chestnut Oak (*Quercus prinus*)	0.585	0.081	-0.512
Northern Red Oak (*Quercus rubra*)	0.841	0.066	-0.205
Red Spruce (*Picea rubens*)	-0.431	0.200	0.294
Yellow Birch (*Betula alleghaniensis*)	-0.441	0.459	0.022
Mountain Ash (*Sorbus americana*)	-0.441	0.531	0.352
Eastern Hemlock (*Tsuga canadensis*)	-0.057	-0.780	0.214
White Ash (*Fraxinus americana*)	0.033	-0.693	0.296

Table 2. Covariates used to model salamander occurrence (ψ) and detection probability (*p*) used in analysis of capture history on Grandfather Mountain, Avery Co. NC.

Covariate	Use	Value	Description
Elevation	ψ	1259 –1533	Elevation (m)
PC1	ψ	-0.043 – 4.207	PC1 of tree community
PC2	ψ	-0.009 – 3.110	PC2 of tree community
PC3	ψ	-0.018 – 2.407	PC3 of tree community
Rain	*p*	0.00 – 8.40	The average rainfall (mm) previous 24 hours
Temp	*p*	0.00 - 21.94	The average temperature (°C)
Method	*p*	0, 1	Method used: day versus night

Table 3. Number of Plethodontid salamander species encountered during the 2010 and 2011 field seasons and the elevation in which they were found.

Species	2010	2011	Elevation
Plethodon cinereus	49	64	1259, 1311, 1356
Plethodon cylindraceus	10	3	1259, 1311
Plethodon montanus	401	137	1259, 1311, 1356, 1445, 1533
Plethodon welleri	40	50	1533
Desmognathus orestes	63	20	1259, 1311, 1356, 1445
Desmognathus wrighti	21	14	1311, 1445, 1533

Table 4. Model selection and parameter estimation of site occupancy for *Plethodon welleri* in 2010. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAIC	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(PC1)p(temp)	0.000	0.209	0.047	0.059
ψ(PC1)p(.)	0.040	0.205	0.047	0.059
ψ(PC1)p(rain)	2.170	0.071	0.047	0.059
ψ(PC1)p(rain, temp)	2.670	0.055	0.047	0.059
ψ(PC1)p(method)	2.830	0.051	0.047	0.059
ψ(PC1)p(temp, method)	3.020	0.046	0.047	0.059
ψ(PC2)p(temp)	3.330	0.039	0.138	0.086
ψ(PC2)p(.)	3.380	0.039	0.138	0.086
ψ(elevation)p(temp)	3.920	0.029	0.199	0.080
ψ(.)p(temp)	3.950	0.029	0.200	0.080
ψ(elevation)p(.)	3.960	0.029	0.199	0.080
ψ(.)p(.)	4.250	0.025	0.200	0.080
ψ(PC1)p(rain, method)	5.240	0.015	0.047	0.059
ψ(PC2)p(rain)	5.500	0.013	0.138	0.086
ψ(PC2)p(rain, temp)	6.010	0.010	0.138	0.086
ψ(elevation)p(rain)	6.090	0.010	0.199	0.080
ψ(PC3)p(temp)	6.100	0.010	0.191	0.081
ψ(.)p(rain)	6.120	0.010	0.200	0.080
ψ(PC3)p(.)	6.140	0.010	0.191	0.081
ψ(PC2)p(method)	6.160	0.010	0.138	0.086
ψ(elevation, PC3)p(.)	6.210	0.009	0.188	0.082
ψ(.)p(rain, temp)	6.320	0.009	0.200	0.080
ψ(PC2)p(temp, method)	6.350	0.009	0.138	0.086
ψ(elevation)p(rain, temp)	6.590	0.008	0.199	0.080
ψ(.)p(temp, method)	6.670	0.007	0.200	0.080
ψ(elevation)p(method)	6.750	0.007	0.199	0.080
ψ(.)p(method)	6.780	0.007	0.200	0.080
ψ(elevation)p(temp, method)	6.940	0.007	0.199	0.080
ψ(PC1, PC2, PC3, elevation)p(.)	7.890	0.004	0.036	0.107
ψ(PC3)p(rain)	8.270	0.003	0.191	0.815
ψ(PC2)p(rain, method)	8.580	0.003	0.138	0.086
ψ(PC3)p(rain, temp)	8.770	0.003	0.191	0.081
ψ(.)p(rain, method)	8.890	0.002	0.200	0.080

ψ(PC3)p(method)	8.930	0.002	0.191	0.081
ψ(PC3)p(temp, method)	9.120	0.002	0.191	0.081
ψ(elevation)p(rain, method)	9.160	0.002	0.199	0.080
ψ(PC3)p(rain, method)	11.340	0.000	0.191	0.081
ψ(elevation, PC1, PC2, PC3)p(rain, temp, method)	17.720	0.000	0.036	0.017
Model averaged			*0.091*	*0.067*

Table 5. Beta estimates based on the top-ranked model ψ(PC1)*p*(rain, temp) for *Plethodon welleri* in 2010. SE represents the standard error followed by the lower confidence interval (LCI) and the upper confidence interval (UCI).

Parameter	Covariate	Beta	SE	LCI	UCI
p	Intercept	-2.947	1.015	-4.936	-0.958
p	Temp	0.085	0.056	-0.024	0.194
ψ	Intercept	-3.906	2.123	-8.067	0.256
ψ	PC1	-3.019	1.320	-5.607	-0.431

Table 6. Model selection and parameter estimation of site occupancy for *Plethodon welleri* in 2011. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAICc	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(PC1)p(.)	0.000	0.263	0.047	0.059
ψ(PC1)p(temp)	0.360	0.219	0.047	0.059
ψ(PC1)p(rain)	1.660	0.115	0.047	0.059
ψ(PC1)p(rain, temp)	2.820	0.064	0.047	0.059
ψ(PC2)p(.)	3.330	0.050	0.138	0.086
ψ(PC2)p(temp)	3.700	0.041	0.138	0.086
ψ(elevation)p(.)	3.920	0.037	0.199	0.080
ψ(.)p(.)	4.210	0.032	0.201	0.080
ψ(elevation)p(temp)	4.280	0.031	0.199	0.080
ψ(.)p(temp)	4.310	0.030	0.201	0.080
ψ(PC2)p(rain)	5.000	0.022	0.138	0.086
ψ(elevation)p(rain)	5.580	0.016	0.199	0.080
ψ(.)p(rain)	5.610	0.016	0.201	0.080
ψ(PC3)p(.)	6.100	0.012	0.191	0.082
ψ(PC2)p(rain, temp)	6.150	0.012	0.138	0.086
ψ(PC3)p(temp)	6.460	0.010	0.191	0.081
ψ(.)p(temp, rain)	6.460	0.010	0.200	0.080
ψ(elevation)p(rain, temp)	6.730	0.009	0.199	0.080
ψ(PC3)p(rain)	7.760	0.005	0.191	0.082
ψ(PC3)p(rain, temp)	8.910	0.003	0.191	0.082
ψ(elevation, PC1, PC2, PC3)p(rain, temp)	12.980	0.000	0.036	0.108
Model averaged			*0.090*	*0.067*

Table 7. Model selection and parameter estimation of site occupancy for *Plethodon cinereus* in 2010. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAIC	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(PC1)p(method)	0.000	0.274	0.888	0.121
ψ(PC1)p(.)	0.130	0.257	0.888	0.121
ψ(PC1)p(rain)	1.770	0.113	0.888	0.121
ψ(PC1)p(temp, method)	1.930	0.104	0.888	0.121
ψ(PC1)p(rain, method)	1.960	0.103	0.888	0.121
ψ(PC1)p(temp)	2.040	0.099	0.888	0.121
ψ(PC1)p(rain, temp)	3.690	0.043	0.888	0.121
ψ(PC2)p(method)	11.810	0.001	0.761	0.160
ψ (PC2)p(.)	11.920	0.001	0.762	0.160
ψ(elevation)p(method)	13.430	0.000	0.592	0.107
ψ(elevation)p(.)	13.550	0.000	0.592	0.107
ψ(PC2)p(rain)	13.570	0.000	0.761	0.160
ψ(PC2)p(temp, method)	13.750	0.000	0.761	0.161
ψ(PC2)p(rain, method)	13.770	0.000	0.761	0.161
ψ(elevation, PC1, PC2, PC3) p(rain, temp, method)	13.800	0.000	0.922	0.285
ψ(PC2)p(temp)	13.830	0.000	0.761	0.160
ψ(.)p(method)	14.090	0.000	0.599	0.108
ψ(.)p(.)	14.460	0.000	0.599	0.108
ψ(PC3)p(method)	14.730	0.000	0.604	0.112
ψ(PC3)p(.)	14.850	0.000	0.605	0.112
ψ(elevation)p(rain)	15.200	0.000	0.592	0.107
ψ(elevation)p(temp, method)	15.370	0.000	0.592	0.107
ψ(elevation)p(rain, method)	15.390	0.000	0.592	0.107
ψ(elevation)p(temp)	15.460	0.000	0.592	0.107
ψ(PC2)p(temp, rain)	15.490	0.000	0.761	0.160
ψ(.)p(temp, method)	15.720	0.000	0.599	0.108
ψ(.)p(rain, method)	15.750	0.000	0.599	0.108
ψ(.)p(rain)	15.850	0.000	0.599	0.108
ψ(.)p(temp)	16.120	0.000	0.599	0.108
ψ(PC3)p(rain)	16.500	0.000	0.605	0.112
ψ(PC3)p(temp, method)	16.670	0.000	0.604	0.112

ψ(PC3)p(rain, method)	16.690	0.000	0.604	0.112
ψ(PC3)p(temp)	16.760	0.000	0.605	0.112
ψ(.)p(rain, temp, method)	17.110	0.000	0.598	0.108
ψ(elevation)p(rain, temp)	17.120	0.000	0.592	0.107
ψ(.)p(rain, temp)	17.470	0.000	0.599	0.108
ψ(PC3)p(rain, temp)	18.420	0.000	0.604	0.112
Model averaged			*0.887*	*0.122*

Table 8. Beta estimates based on the top-ranked model ψ(PC1)*p*(method) for *Plethodon cinereus* in 2010. SE represents the standard error followed by the lower confidence interval (LCI) and the upper confidence interval (UCI).

Parameter	Covariate	Beta	SE	LCI	UCI
p	Intercept	-1.388	0.587	-2.539	-0.237
p	Method	-0.785	0.493	-1.751	0.182
ψ	Intercept	2.072	1.217	-0.312	4.457
ψ	PC1	5.608	2.665	0.384	10.832

Table 9. Model selection and parameter estimation of site occupancy for *Plethodon cinereus* in 2011. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAIC	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(PC1)p(temp)	0.000	0.512	0.784	0.162
ψ(PC1)p(.)	1.120	0.292	0.784	0.162
ψ(PC1)p(rain, temp)	3.090	0.109	0.784	0.162
ψ(PC1)p(rain)	3.640	0.083	0.784	0.162
ψ(elevation)p(temp)	13.570	0.001	0.486	0.102
ψ(elevation, PC1, PC2, PC3)	14.050	0.000	0.674	0.361
ψ(.)p(temp)	14.280	0.000	0.487	0.102
ψ(PC2)p(temp)	14.290	0.000	0.495	0.109
ψ(elevation)p(.)	14.690	0.000	0.487	0.102
ψ(PC2)p(.)	15.400	0.000	0.495	0.109
ψ(.)p(.)	15.650	0.000	0.488	0.102
ψ(PC3)p(temp)	15.730	0.000	0.488	0.104
ψ(elevation)p(rain, temp)	16.660	0.000	0.486	0.102
ψ(PC3)p(.)	16.850	0.000	0.488	0.105
ψ(.)p(rain, temp)	17.060	0.000	0.487	0.102
ψ(elevation)p(rain)	17.210	0.000	0.486	0.102
ψ(PC2)p(rain, temp)	17.380	0.000	0.495	0.109
ψ(.)p(rain)	17.910	0.000	0.488	0.102
ψ(PC2)p(rain)	17.920	0.000	0.495	0.109
ψ(PC3)p(rain, temp)	18.820	0.000	0.488	0.104
ψ(PC3)p(rain)	19.370	0.000	0.488	0.105
Model averaged			*0.783*	*0.161*

Table 10. Beta estimates based on the top-ranked model ψ(PC1)*p*(rain) for *Plethodon cinereus* in 2011. SE represents the standard error followed by the lower confidence interval (LCI) and the upper confidence interval (UCI).

Parameter	Covariate	Beta	SE	LCI	UCI
p	Intercept	-0.471	0.557	-1.562	0.620
p	Rain	-0.073	0.036	-0.144	-0.001
ψ	Intercept	1.289	0.955	-0.582	3.160
ψ	PC1	5.608	2.549	0.611	10.605

Table 11. Model selection and parameter estimation of site occupancy for *Plethodon cylindraceus* in 2010. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAICc	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(PC1)p(.)	0.000	0.350	0.200	0.183
ψ(PC1)p(temp)	0.270	0.305	0.199	0.181
ψ(PC1)p(temp, method)	1.920	0.134	0.198	0.179
ψ(PC1)p(rain)	2.860	0.084	0.200	0.183
ψ(PC1)p(rain, temp)	3.390	0.064	0.199	0.181
ψ(PC1)p(rain, method)	4.660	0.034	0.199	0.181
ψ(.)p(.)	8.970	0.004	0.381	0.230
ψ(.)p(temp)	9.010	0.004	0.378	0.228
ψ(elevation)p(.)	9.900	0.002	0.339	0.211
ψ(elevation)p(temp)	10.200	0.002	0.337	0.208
ψ(.)p(method)	10.240	0.002	0.379	0.228
ψ(.)p(temp, method)	10.390	0.002	0.376	0.225
ψ(elevation)p(method)	11.420	0.001	0.337	0.209
ψ(PC3)p(.)	11.500	0.001	0.377	0.226
ψ(PC2)p(.)	11.570	0.001	0.381	0.230
ψ(.)p(rain)	11.570	0.001	0.381	0.230
ψ(PC3)p(method)	11.800	0.001	0.374	0.224
ψ(.)p(rain, temp)	11.830	0.001	0.378	0.228
ψ(elevation)p(temp, method)	11.870	0.001	0.335	0.206
ψ(PC2)p(method)	12.380	0.001	1.000	0.000
ψ(elevation)p(rain)	12.760	0.001	0.339	0.211
ψ(.)p(rain, method)	13.090	0.001	0.379	0.228
ψ(elevation)p(rain, temp)	13.310	0.000	0.337	0.208
ψ(PC2)p(temp, method)	13.470	0.000	0.371	0.222
ψ(PC2)p(temp, method)	14.080	0.000	1.000	0.000
ψ(PC3)p(rain)	14.350	0.000	0.377	0.226
ψ(PC2)p(rain)	14.420	0.000	0.381	0.230
ψ(elevation)p(rain, method)	14.570	0.000	0.337	0.209
ψ(PC3)p(rain, temp)	14.910	0.000	0.374	0.224
ψ(PC2)p(rain, temp)	15.500	0.000	1.000	0.000
ψ(PC3)p(rain, method)	16.170	0.000	0.374	0.224
ψ(PC2)p(rain, method)	16.240	0.000	0.379	0.228

ψ(elevation, PC1, PC2, PC3)p(rain, temp, method)	18.370	0.000	0.130	0.183
Model averaged			*0.205*	*0.182*

Table 12. Model selection and parameter estimation of site occupancy for *Plethodon montanus* in 2010. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAICc	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(1)p(rain, temp, method)	0.000	0.559	1.000	0.000
ψ(1)p(temp, method)	0.475	0.441	1.000	0.000
ψ(1)p(temp, rain)	21.014	0.000	1.000	0.000
ψ(1)p(temp)	23.651	0.000	1.000	0.000
ψ(1)p(method)	49.919	0.000	1.000	0.000
ψ(1)p(rain, method)	49.924	0.000	1.000	0.000
ψ(1)p(.)	75.311	0.000	1.000	0.000
ψ(1)p(rain)	75.477	0.000	1.000	0.000

Table 13. Beta estimates based on the top-ranked model $\psi(1)p$(temp, rain, method) for *Plethodon montanus* in 2010. SE represents the standard error followed by the lower confidence interval (LCI) and the upper confidence interval (UCI).

Parameter	Covariate	Beta	SE	LCI	UCI
p	Intercept	-5.032	0.618	-6.244	-3.821
p	Temp	0.171	0.029	0.113	0.229
p	Rain	-0.049	0.027	-0.103	0.005
p	Method	0.925	0.188	0.556	1.293
ψ	Intercept	0.000	0.000	0.000	0.000

Table 14. Model selection and parameter estimation of site occupancy for *Plethodon montanus* in 2011. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAICc	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(1)p(temp, rain)	0.000	0.413	1.000	0.000
ψ(1)p(temp)	0.146	0.384	1.000	0.000
ψ(1)p(.)	2.359	0.127	1.000	0.000
ψ(1)p(rain)	3.369	0.077	1.000	0.000

Table 15. Beta estimates based on the top-ranked model ψ(1)*p*(rain, temp) for *Plethodon montanus* in 2011. SE represents the standard error followed by the lower confidence interval (LCI) and the upper confidence interval (UCI).

Parameter	Covariate	Beta	SE	LCI	UCI
p	Intercept	-2.579	0.479	-3.518	-1.639
p	Rain	0.066	0.028	0.011	0.121
p	Temp	0.009	0.006	-0.001	0.021
ψ	Intercept	0.000	0.000	0.000	0.000

Table 16. Model selection and parameter estimation of site occupancy for *Desmognathus orestes* in 2010. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAICc	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(.)p(temp, method)	0.000	0.419	0.367	0.098
ψ(PC1)p(temp, method)}	2.490	0.120	0.362	0.100
ψ(elevation)p(temp, method)}	2.630	0.113	0.366	0.099
ψ(PC2)p(temp, method)}	2.960	0.095	0.366	0.099
ψ(PC3)p(temp, method)}	3.140	0.087	0.367	0.098
ψ(.)p(method)}	4.230	0.051	0.368	0.098
ψ(.)p(rain, method)}	5.370	0.029	0.368	0.098
ψ(PC1)p(method)}	6.420	0.017	0.362	0.100
ψ(elevation)p(method)}	6.550	0.016	0.367	0.099
ψ(PC2)p(method)}	6.880	0.013	0.367	0.099
ψ(PC3)p(method)}	7.070	0.012	0.368	0.098
ψ(PC1)p(rain, method)}	7.870	0.008	0.362	0.100
ψ(elevation)p(rain, method)}	8.000	0.008	0.367	0.099
ψ(PC2)p(rain, method)}	8.330	0.007	0.367	0.099
ψ(PC3)p(rain, method)}	8.510	0.006	0.368	0.098
ψ(elevation, PC1, PC2, PC3)p(rain, temp, method)}	17.540	0.000	0.357	0.101
ψ(.)p(temp)}	24.970	0.000	0.370	0.099
ψ(.)p(temp, rain)}	25.460	0.000	0.370	0.099
ψ(PC1)p(temp)}	27.160	0.000	0.364	0.101
ψ(elevation)p(temp)}	27.290	0.000	0.369	0.099
ψ(PC2)p(temp)}	27.620	0.000	0.369	0.099
ψ(PC3)p(temp)}	27.810	0.000	0.371	0.099
ψ(PC1)p(rain, temp)}	27.960	0.000	0.364	0.101
ψ(elevation)p(rain, temp)}	28.090	0.000	0.369	0.099
ψ(PC2)p(temp, rain)}	28.420	0.000	0.369	0.099
ψ(PC3)p(rain, temp)}	28.600	0.000	0.370	0.099
ψ(.)p(.)}	29.290	0.000	0.371	0.099
ψ(.)p(rain)}	30.420	0.000	0.371	0.099
ψ(PC1)p(.)}	31.220	0.000	0.365	0.101
ψ(elevation)p(.)}	31.350	0.000	0.369	0.100

ψ(PC2)p(.)}	31.680	0.000	0.370	0.100
ψ(PC3)p(.)}	31.860	0.000	0.371	0.100
ψ(PC1)p(rain)	32.610	0.000	0.365	0.101
ψ(elevation)p(rain)	32.750	0.000	0.369	0.100
ψ(PC2)p(rain)	33.070	0.000	0.370	0.100
ψ(PC3)p(rain)	33.260	0.000	0.370	0.099
Model averaged			*0.366*	*0.099*

Table 17. Beta estimates based on the top-ranked model ψ(.)*p*(method, temp) for *Desmognathus orestes* in 2010. SE represents the standard error followed by the lower confidence interval (LCI) and the upper confidence interval (UCI).

Parameter	Covariate	Beta	SE	LCI	UCI
p	Intercept	-8.750	2.026	-12.722	-4.778
p	Temp	0.203	0.094	0.018	0.387
p	Method	2.172	0.430	1.329	3.013
ψ	Intercept	-0.543	0.422	-1.371	0.284

Table 18. Model selection and parameter estimation of site occupancy for *Desmognathus orestes* in 2011. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAICc	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(.)p(rain)	0.000	0.257	0.363	0.110
ψ(PC1)p(rain)	1.050	0.152	0.338	0.119
ψ(PC2)p(rain)	2.240	0.084	0.361	0.112
ψ(PC3)p(rain)	2.570	0.071	0.362	0.111
ψ(.)p(.)	2.630	0.069	0.367	0.112
ψ(elevation)p(rain)	2.770	0.064	0.363	0.110
ψ(.)p(rain, temp)	2.850	0.062	0.363	0.110
ψ(PC1)p(.)	3.410	0.047	0.342	0.121
ψ(PC1)p(rain, temp)	4.210	0.031	0.338	0.119
ψ(PC2)p(.)	4.610	0.026	0.365	0.114
ψ(PC3)p(.)	4.930	0.022	0.366	0.113
ψ(.)p(temp)	5.060	0.020	0.367	0.112
ψ(elevation)p(.)	5.130	0.020	0.367	0.112
ψ(PC2)p(rain, temp)	5.400	0.017	0.361	0.112
ψ(PC3)p(rain, temp)	5.720	0.015	0.362	0.111
ψ(elevation)p(rain, temp)	5.920	0.013	0.363	0.110
ψ(PC1)p(temp)	6.100	0.012	0.342	0.21
ψ(PC2)p(temp)	7.300	0.007	0.365	0.114
ψ(PC3)p(temp)	7.620	0.006	0.366	0.113
ψ(elevation)p(temp)	7.830	0.005	0.367	0.112
ψ(elevation, PC1, PC2, PC3)p(rain, temp)	9.630	0.002	0.083	0.124
Model averaged			*0.357*	*0.113*

Table 19. Beta estimates based on the top-ranked model ψ(.)*p*(rain) for *Desmognathus orestes* in 2011. SE represents the standard error followed by the lower confidence interval (LCI) and the upper confidence interval (UCI).

Parameter	Covariate	Beta	SE	LCI	UCI
p	Intercept	-2.550	0.324	-3.185	-1.916
p	Rain	0.023	0.009	0.005	0.041
ψ	Intercept	-0.562	0.475	-1.494	0.370

Table 20. Model selection and parameter estimation of site occupancy for *Desmognathus wrighti* in 2010. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAICc	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(PC1)p(temp, method)	0.000	0.339	0.257	0.125
ψ(.)p(temp, method)	1.150	0.190	0.345	0.102
ψ(elevation)p(temp, method)	2.720	0.087	0.342	0.102
ψ(PC3)p(temp, method)	3.280	0.066	0.338	0.105
ψ(PC1)p(temp)	3.760	0.052	0.259	0.126
ψ(PC1)p(method)	4.240	0.041	0.258	0.125
ψ(PC2)p(temp, method)	4.310	0.039	0.345	0.102
ψ(PC1)p(rain, temp)	5.130	0.026	0.258	0.125
ψ(.)p(temp)	5.210	0.025	0.347	0.102
ψ(.)p(method)	5.700	0.020	0.347	0.103
ψ(.)p(rain, temp)	6.290	0.015	0.347	0.103
ψ(elevation)p(temp)	6.490	0.013	0.344	0.103
ψ(elevation)p(method)	6.970	0.010	0.344	0.103
ψ(PC3)p(temp)	7.040	0.010	0.339	0.106
ψ(.)p(rain, method)	7.520	0.008	0.346	0.103
ψ(PC3)p(method)	7.520	0.008	0.339	0.106
ψ(elevation)p(rain, temp)	7.860	0.007	0.344	0.103
ψ(PC1)p(.)	7.890	0.007	0.260	0.126
ψ(PC2)p(temp)	8.070	0.006	0.347	0.103
ψ(PC3)p(rain, temp)	8.420	0.005	0.339	0.106
ψ(PC2)p(method)	8.550	0.005	0.347	0.103
ψ(elevation)p(rain, method)	9.090	0.004	0.343	0.103
ψ(PC2)p(rain, temp)	9.440	0.003	0.347	0.103
ψ(.)p(.)	9.610	0.003	0.348	0.103
ψ(PC3)p(rain, method)	9.650	0.003	0.339	0.106
ψ(PC1)p(rain)	9.760	0.003	0.259	0.126
ψ(elevation)p(.)	10.620	0.002	0.345	0.103
ψ(PC2)p(rain, method)	10.670	0.002	0.346	0.103
ψ(PC3)p(.)	11.170	0.001	0.340	0.106
ψ(.)p(rain)	11.220	0.001	0.348	0.103
ψ(PC2)p(.)	12.200	0.001	0.348	0.103

ψ(elevation)p(rain)	12.490	0.001	0.345	0.103
ψ(PC3)p(rain)	13.040	0.001	0.340	0.106
ψ(PC2)p(rain)	14.070	0.000	0.348	0.103
ψ(elevation, PC1, PC2, PC3)p(rain, temp, method)	14.830	0.000	0.205	0.186
Model averaged			*0.304*	*0.113*

Table 21. Beta estimates based on the top-ranked model ψ(PC1)*p*(temp, method) for *Desmognathus wrighti* in 2010. SE represents the standard error followed by the lower confidence interval (LCI) and the upper confidence interval (UCI).

Parameter	Covariate	Beta	SE	LCI	UCI
p	Intercept	-8.562	2.480	-13.423	-3.701
p	Temp	0.242	0.116	0.013	0.470
p	Method	1.297	0.486	0.344	2.249
ψ	Intercept	-1.777	1.188	-4.104	0.550
ψ	PC1	-1.060	0.653	-2.340	0.221

Table 22. Model selection and parameter estimation of site occupancy for *Desmognathus wrighti* in 2011. ΔAIC is the difference between the top ranked model (lowest AIC) and subsequent models, *w* is the Akaike weight, $\hat{\Psi}$ is the actual estimate of occupancy and the SE($\hat{\Psi}$) is the standard error of the estimate.

Model	ΔAICc	*w*	$\hat{\Psi}$	SE($\hat{\Psi}$)
ψ(PC1)p(.)	0.000	0.180	0.471	0.328
ψ(.)p(.)	0.060	0.175	0.398	0.193
ψ(.)p(temp)	1.170	0.100	0.396	0.192
ψ(PC1)p(temp)	1.390	0.090	0.469	0.348
ψ(elevation)p(.)	1.850	0.071	0.382	0.182
ψ(.)p(rain)	2.380	0.055	0.398	0.193
ψ(PC1)p(rain)	2.590	0.049	0.470	0.332
ψ(PC2)p(.)	2.650	0.048	0.378	0.193
ψ(PC3)p(.)	2.660	0.048	0.398	0.193
ψ(elevation)p(temp)	3.220	0.036	0.380	0.180
ψ(.)p(rain, temp)	3.960	0.025	0.396	0.192
ψ(PC2)p(temp)	4.020	0.024	0.396	0.192
ψ(elevation)p(rain)	4.430	0.020	0.382	0.181
ψ(PC1)p(rain, temp)	4.480	0.019	0.469	0.348
ψ(PC3)p(temp)	4.600	0.018	0.999	0.016
ψ(PC2)p(rain)	5.230	0.013	0.398	0.193
ψ(PC3)p(rain)	5.240	0.013	0.398	0.193
ψ(elevation)p(rain, temp)	6.310	0.008	0.380	0.180
ψ(PC2)p(rain, temp)	7.110	0.005	0.396	0.192
ψ(PC3)p(rain, temp)	7.790	0.004	1.000	0.002
ψ(elevation, PC1, PC2, PC3)p(rain, temp)	15.240	0.000	0.091	0.240
Model averaged			*0.433*	*0.235*

FIGURES

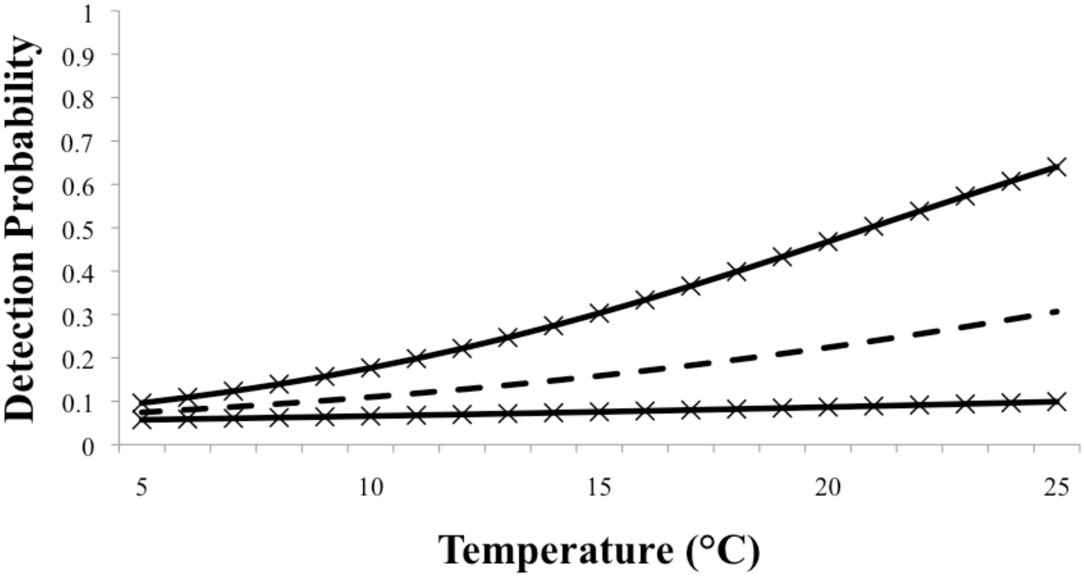

Figure 1. The effect of temperature on estimated detection probabilities based on the top-ranked model, ψ(PC1)p(temp) (*p* +/- 1 SE), for *Plethodon welleri* in 2010.

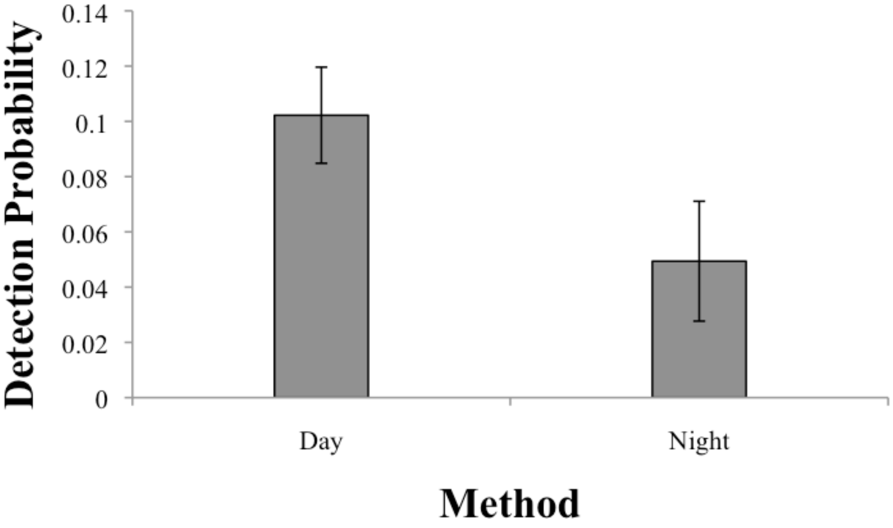

Figure 2. Effect of method on estimated detection probabilities based on the top-ranked model, ψ(PC1)p(method) (p +/- 1 SE), for *Plethodon cinereus* in 2010.

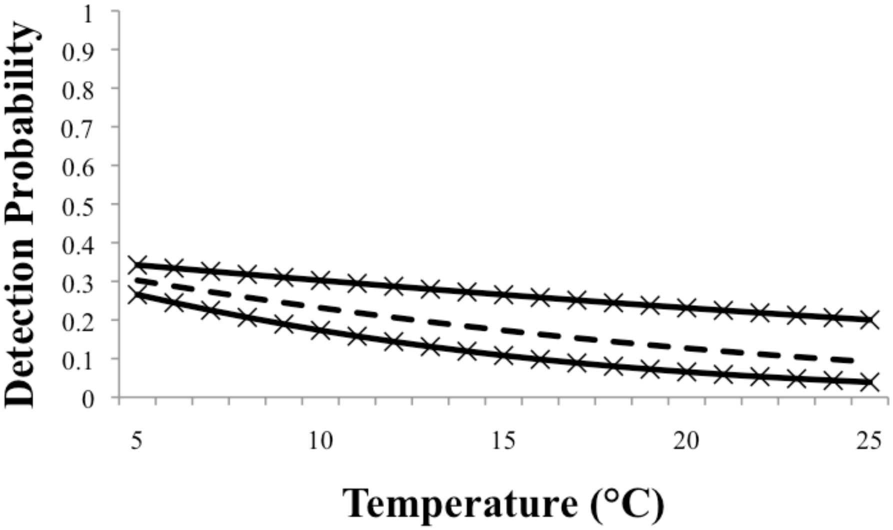

Figure 3. Effect of temperature on estimated detection probabilities based on the top-ranked model ψ(PC1)*p*(temp) (*p* +/- 1 SE), for *Plethodon cinereus* in 2011.

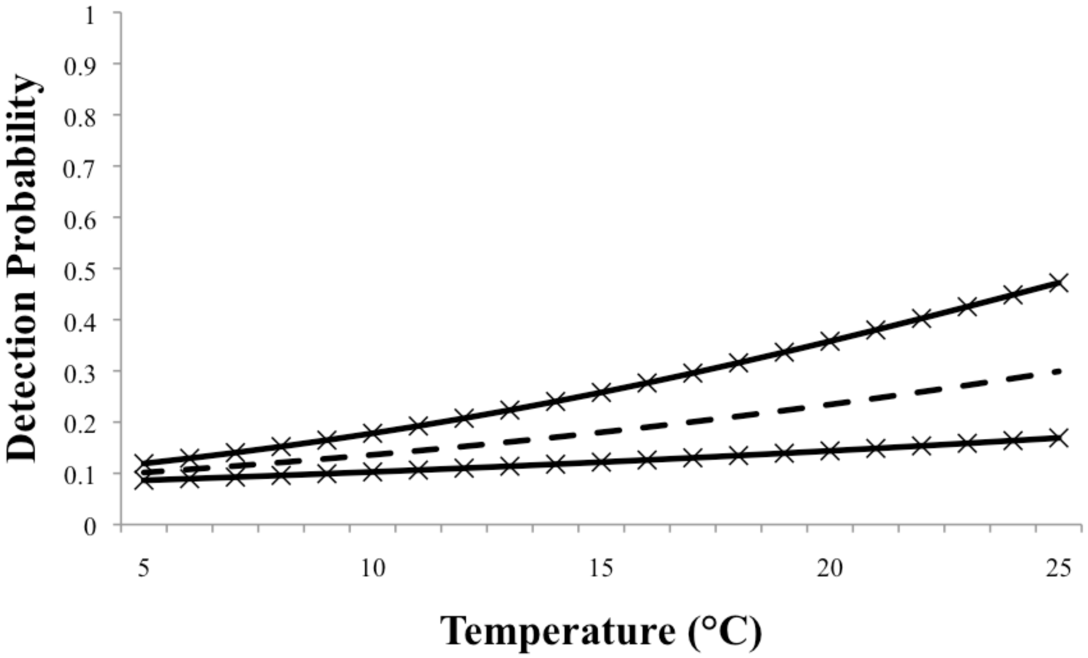

Figure 4. Effect of temperature after adjusting for rainfall on estimated detection probabilities based on the top-ranked model, $\psi(1)p(\text{temp, rain})$ (p +/- 1 SE), for *Plethodon montanus* in 2011.

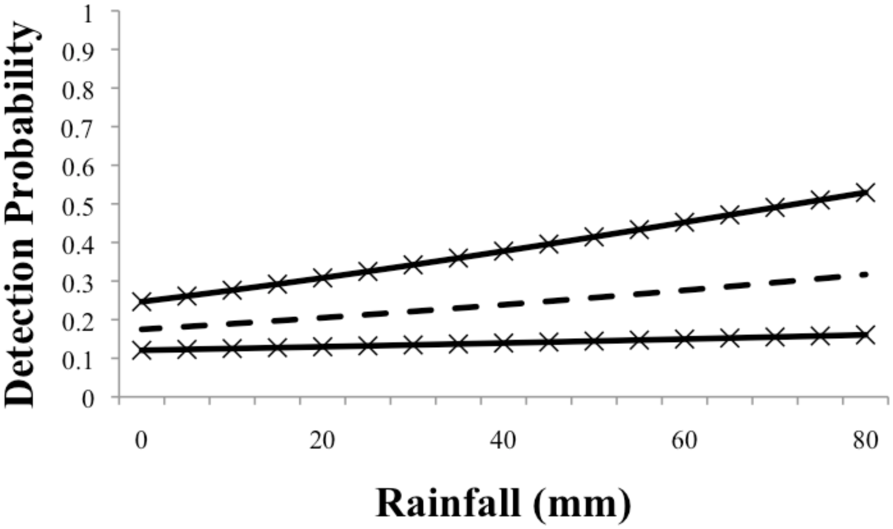

Figure 5. The effect of rainfall after adjusting for temperature on estimated detection probabilities based on the top-ranked model, ψ(1)p(temp, rain) (*p* +/- 1 SE), for *Plethodon montanus* in 2011.

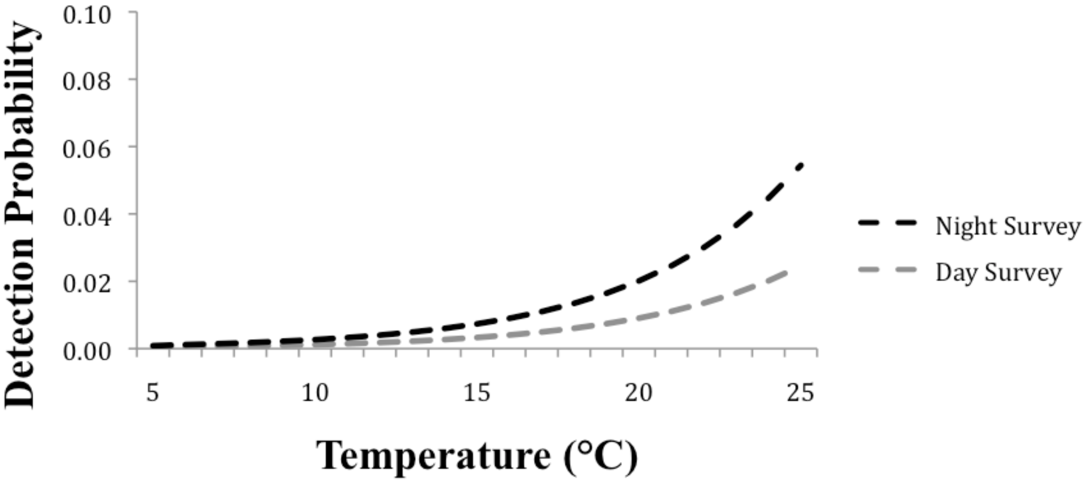

Figure 6. The effect of temperature and method on estimated detection probabilities based on the top-ranked model, ψ(.)p(temp, method), for *Desmognathus orestes* in 2010.

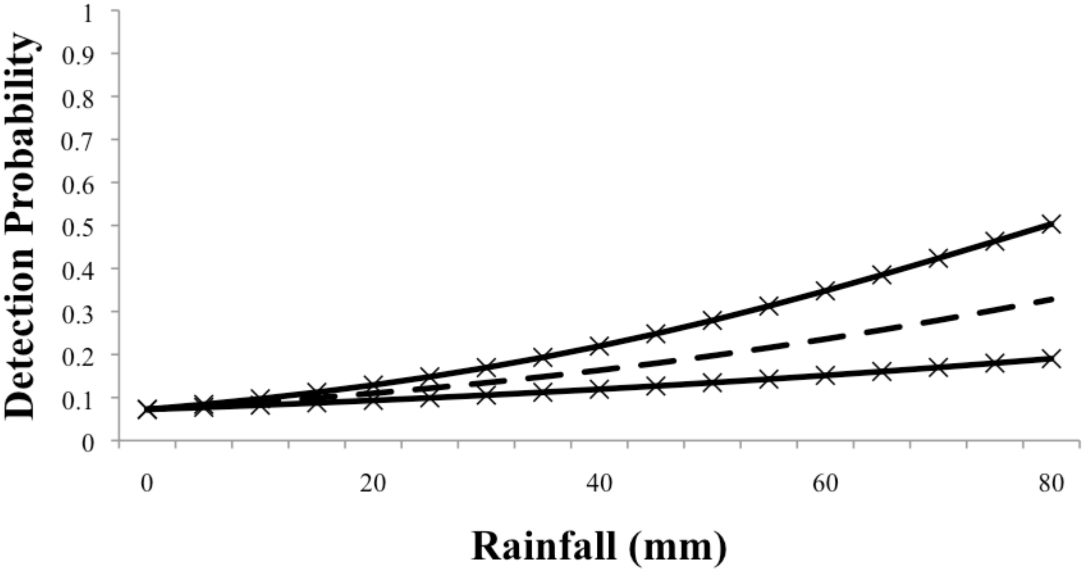

Figure 7. The effect of rainfall on estimated detection probabilities based on the top-ranked model, ψ(.)p(rain) (*p* +/- 1 SE), for *Desmognathus orestes* in 2011.

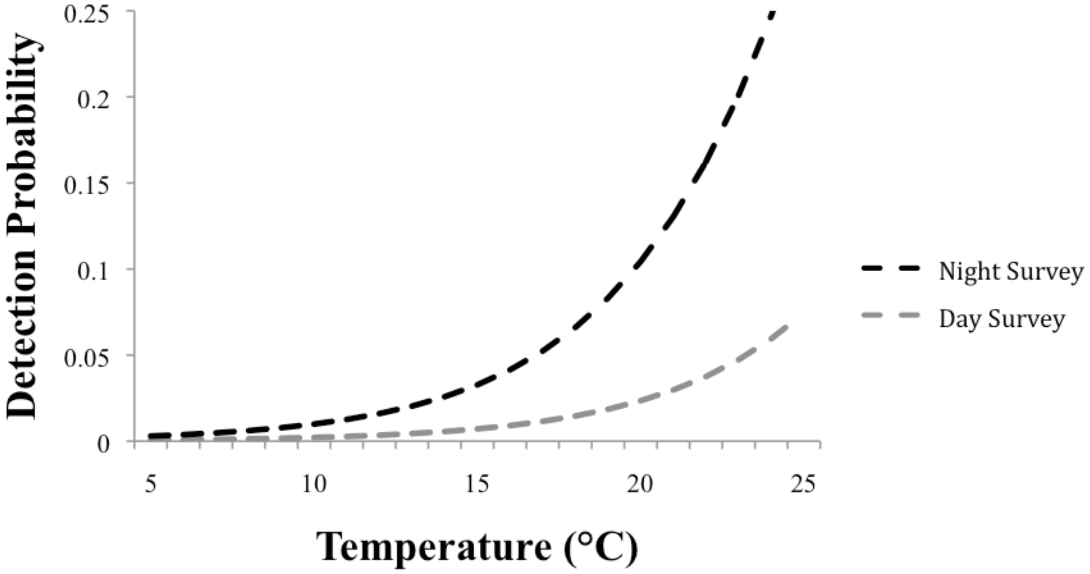

Figure 8. The effect of temperature and method on estimated detection probabilities based on the top-ranked model, ψ(.)p(temp, method), for *Desmognathus wrighti* in 2010.

CHAPTER 3

ASSESSING THE THREAT OF *BATRACHOCHYTRIUM DENDROBATIDIS* IN SOUTHERN APPALACHIAN SALAMANDERS

ABSTRACT

Amphibian decline is caused by multiple factors, including the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (Bd). The southern Appalachian Mountains are a global hotspot for salamander diversity. Because of its high diversity, it is important to assess whether Bd is present in this region. From July 1st to October 23rd, 2010 I sampled 308 salamanders of the family Plethodontidae along an elevation gradient on Grandfather Mountain, North Carolina. Sampled species included *Desmognathus orestes, D. wrighti, Plethodon cinereus, P. cylindraceus, P. montanus,* and the endangered species *P. welleri*. I used mark-recapture techniques to track salamanders through time to assess changes in fungal infection rates. Using PCR on extracted DNA, I found 0 Bd positive samples. Although I did not detect Bd, these data can be used to help assess changes in its distribution and prevalence, providing baseline information for future studies on Bd.

INTRODUCTION

Amphibians worldwide are in a state of rapid decline. Approximately 32.5% of the > 6,000 described species are considered at risk of extinction (Stuart et al., 2004; Beebee and Griffiths, 2005; Smith et al., 2009). At times, declines have been documented in habitats far removed from human influence and in relatively pristine environmental conditions

(Waldman and Tocher, 1998; Carver et al., 2010). Infectious disease is one factor that is currently threatening several species of amphibian (Daszak et al., 2003; Skerratt et al., 2007). The disease chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis* (Bd) [Phylum Chytridiomycota, Class Chytridiomycetes, Order Rhyzophydiales] and was first described by Longcore et al. (1999). Of all known pathogens to infect amphibians, Bd is the most clearly linked to declines and extinctions (Berger et al., 1998; Daszak et al., 1999; Murray et al., 2011).

Bd is the first chytrid reported to parasitize vertebrates where it infects the keratinized layers of the epidermis in post-metamorphic amphibians and the oral disk of tadpoles (Berger et al., 1998; Voyles, 2007). In infected amphibians, Bd can cause a thickening of the outer layer of skin (Berger et al., 1999). Cutaneous infection in amphibians poses a unique problem because amphibian skin is physiologically active, regulating respiratory gasses, ions and water between the skin and the environment. Bd can infect a broad number of species and can survive for weeks without a host (Johnson and Speare, 2005; Weinstein, 2009). Bd has an optimal growth temperature of 23°C (Berger et al., 2004), however in the lab Bd grows *in vitro* at temperatures between 6°C and 28°C, though growth is significantly impaired at temperatures just 1°C warmer (Longcore et al., 1999). Experimentally infected *Mixophyes fasciolatus* have shown that the pathogenicity of Bd decreases at temperatures above 23°C (Berger et al., 2004). The effect of temperature on Bd growth is important as temperatures can fluctuate drastically within a season and with elevation. A study of wild-caught frogs in Australia found that infection rates vary significantly between seasons with higher Bd prevalence during the winter and early spring (Kriger and Hero, 2006).

Bd currently infects amphibians on every continent except Antarctica (Berger et al., 1998; Lips, 1999; Garner et al., 2005; Goldberg et al., 2007; Rothermel et al., 2008; Yang et al., 2009). Within the United States, Bd has been found in the Pacific Northwest and the Northeastern and Southeastern U.S. (Rothermel et al., 2008). Forest-associated amphibian species that live in or near streams are more likely to be affected by Bd relative to their terrestrial counterparts (Hossack et al., 2005), but few studies have investigated the distribution of Bd in less aquatic amphibians (Grant et al., 2008). Also, few studies have directly targeted salamanders. Moreover, the effects of chytridiomycosis and its distribution of Bd are not well understood in salamander populations.

Salamander species richness in the Southern Appalachian Mountains is greater than anywhere else in the United States (Kiester, 1971; Lannoo et al., 2005), but many species are imperiled (Highton, 2005; Vazquez, 2009). Chytridiomycosis outbreaks are primarily associated with cool temperatures, high moisture levels, and high elevations (Young et al., 2001; Drew et al., 2006). In an ecological niche modeling study, Ron (2005) demonstrated that the Southern Appalachian region should be favorable for Bd.

Grandfather Mountain Biosphere Reserve (GFMBR) is a protected, relatively pristine, high elevation (1,812 m) mountain located in Western North Carolina. Ongoing studies on GFMBR provided an opportunity to examine the prevalence of Bd in a highly diverse ecosystem with potentially favorable conditions for Bd. Furthermore, the pristine condition of GFMBR reduces the likelihood that salamander declines, past or present, will occur due to habitat loss or alteration, introduced species, or species exploitation. Finally, GFMBR also provides an opportunity to sample at multiple elevations. Within the boundaries of Grandfather Mountain State Park, there is a high diversity of salamander species, including the endemic species *Plethodon welleri*. This species occurs at the highest elevations and is listed as 'special concern' in the state of North Carolina by the N.C. Wildlife Resources Commission and is also listed as endangered by the IUCN (IUCN 2004).

METHODS

Surveys

From July 1st, 2010 to October 23rd, 2010, I conducted cover board surveys approximately one time per week, time constrained night searches within each survey plot approximately 1-2x per month. I placed five, 100 m long transects along an elevation gradient on the south side of Grandfather Mountain in April, 2010. Transects were located at five elevations (1533 m, 1445 m, 1356 m, 1311 m and 1259 m). I placed five cover board plots equidistant along each 10 m x 10 m transect. I used untreated spruce lumber for use as cover boards, and each board measured 30 cm x 30 cm x 5 cm. I placed nine boards within the boundaries of each plot for a total of 45 boards per transect. I conducted night surveys as a means to increase sample size while the newly placed cover boards weathered. Night survey plots measured 10 m x 2 m, with one plot placed adjacent to each cover board plot. I began surveys at dusk and continued until each plot had been searched for five minutes. When I captured a salamander, I placed it into a clean, unused 20 cm x 25 cm sealable plastic Ziplock® bag. I identified salamanders to species based on morphology and individually marked each with Visible Implant Elastomer (VIE), and swabbed each to obtain a DNA sample.

Bd Swabbing

I swabbed 308 plethodontid salamanders for the presence of Bd beginning July 1st, 2010 and followed the protocol of Kriger and Hero (2006) using sterile, individually wrapped cotton-tipped swabs (Medical Wire and Equipment). Each sample was placed into a 1.5 mL microcentrifuge tube and placed on ice. Upon return from the field, I placed the samples into a -20° C freezer.

DNA analysis

I extracted DNA from the swabs using a Qiagen® DNeasy kit following the "Animal Tissues: Spin-Column" protocol guidelines from the Qiagen® Blood and Tissue Handbook. The extracted samples were stored at -20° C. To determine whether Bd was present in the samples, I conducted amplification and PCR analysis of the extracted DNA using the Bd primers Bd1a (5-CAGTGTGC-CATATGTCACG-3) and Bd2a (5-CATGGT-TCATATCTGTCCAG-3) from Annis et al. (2004).

Amplification reactions consisted of 1 µL of each primer, 6.25 µL of GoTaq® Green Master Mix (Promega®), 2.75 µL water, and 1 µL of DNA from each extracted sample. A thermocycler was used to perform amplification by initial denaturation at 95°C for five min, followed by 44 cycles of 93°C for 45 sec, an annealing temperature of 53.7°C for 45 sec, and 72°C for 1 min. This was followed by a 10 min hold at 72°C and a final extension of 15°C to complete amplification. I used a Bd positive DNA sample provided by Joyce Loncore from University of Maine for the positive control and water for use as a negative control. I then used gel electrophoresis in agarose gel to separate DNA fragments and positive reactions produced a 300 bp band. I ran all samples 3x.

To ensure that I was able to detect the presence of Bd in an environmental sample, I swabbed Fire-bellied toads (*Bombina* spp.) that were housed for sale in the pet trade. Four of seven samples produced positive Bd results and were then sent for quantitative analysis using qPCR to the Kerby Lab at the University of South Dakota to confirm results. The four samples ranged in the number of zoospores/µL from 0.005 zoospores/µL to 0.20 zoospores/µL.

RESULTS

Of the 307 salamanders I swabbed, I did not detect Bd on any of them. Of the 307 salamanders, I sampled 27 *Plethodon cinereus*, five *P. cylindraceus*, 225 *P. montanus*, 22 *P. welleri*, 21 *Desmognathus orestes*, six *D. wrighti,* and one *Eurycea wilderae* (Table 1). Of the *P. cinereus*, four of the 27 were recaptures, one of the five *P. cylindraceus* was a recapture, 50 of the 225 *P. montanus* were recaptures, three of the 22 *P. welleri* were recaptures, seven of the 21 *D. orestes* were recaptures, and one of the six *D. wrighti* was recaptured.

Not all species were found at every elevation (Table 2); *Plethodon cinereus* was found at the lowest three elevations, *P. cylindraceus* was found at the lowest two elevations, *P. montanus* was found at all elevations, *P. welleri* was found only at the highest elevation, *D. orestes* was found at all but the highest elevations, *D. wrighti* was found sporadically at the second, fourth and highest elevations, and *E. wildarea* was found at the lowest elevation.

DISCUSSION

Bd was not present in sampled *P. cinereus, P. cylindraceus, P. montanus, P. welleri, D. orested, D. wrighti* or *E. wilderae*. Because I did not find any positive animals, it is not possible to know if these salamanders are resistant or remain unexposed. My data corroborate those of Rothermel et al. (2008) who sampled amphibians in multiple locations in the Southeastern US (including NC) and found no plethodontid salamander positive for Bd, and those of Caruso & Lips (2012) who found one positive plethodontid (*Desmognathus santeetlah*) in 665 swabbed salamanders of the Great Smoky Mountains National Park. I am confident that I did not fail to detect Bd due to my rigorous methods, and the environmental samples I ran confirmed that I was able to detect Bd at low levels. My study is unique in that a limited number of previous studies have sampled terrestrial salamanders or systematically sampled any population of amphibian while resampling the same population throughout a single sampling season.

While I did not detect Bd in GFMBR plethodontid salamander assemblages, it is still possible that Bd exists in the environment. Other amphibians in North Carolina that have tested positive for Bd include *Notophthalmus viridescens*, *Rana catesbiana* tadpoles and *R. sphenocephala* (Rothermel et al., 2008). Also, other researchers have found Bd positive plethodontids in historical museum specimens, indicating that these salamanders are susceptible to Bd infection (Table 3; Weinstein, 2009; Richards-Hrdlicka, 2012). Within the plethodontids, salamanders in the genera *Batrachosepts, Bolitoglossa, Desmognathus, Eurycea, Oedipina,* and *Pseudoeurycea* have had positive Bd samples.

It has also been proposed that amphibian populations of the Southern Appalachians are in post-chytrid decline (Caruos and Lips 2012). Highton (2005) noted widespread declines in the Eastern United States during the 1980's (Caruso and Lips, 2012). During that time, he found that 180 populations of 38 species of salamanders in the genus *Plethodon* were in decline. While he used habitat loss to explain the disappearance of 22 populations in 16 sites, he was unable to account of the remaining 158 population declines. Curiously, many populations were located in national parks and forests. This type of widespread loss is indicative of infection with Bd infections. Caruso and Lips (2012) hypothesize that Bd was causing declines in the Appalachian region during the 1960's and 1970's and that populations have still not recovered because Bd remains at low levels within populations. This would suggest that these species have evolved a defense mechanism within one or two generations that allows them to fight off infection. Although this is not a likely scenario, the only way to test this hypothesis is to examine historical specimens.

A more likely explanation for the lack of Bd in my survey is that some species of amphibian have antimicrobial skin secretions that can inhibit the growth of bacteria, protozoa, viruses and fungi, including Bd (Rollins-Smith et al., 2005; Harris et al., 2006). Some types of bacteria that occupy amphibian skin produce antibiotics that restrict the growth of pathogens. Antibiotic secretions are most common in amphibian species that brood their eggs, inhibiting the growth of fungi with their bodily secretions. Secretions produced by *Plethodon cinereus, P. ventralis* and *Hemidactylium scutatum* inhibit Bd growth in the lab (Austin, 2000; Harris et al., 2006). These skin secretions may help avoid infection caused by injuries (Fredricks and Dankert, 2000) and protect the eggs of salamanders that brood their young (Lauer et al., 2007).

In salamander species that exhibit parental care, abandoned clutches often die of fungal infections. Of the salamander species that I sampled, *P. cinereus, P. cylindraceus, P.*

welleri, D. orestes, D. wrighti are known to brood their young (Petranka, 1998). *Plethodon montanus* has never been observed exhibiting parental care but it is hypothesized that this behavior does take place in this species. Whether *Eurycea wildarea* exhibits parental care is unknown. While laboratory studies indicate that *P. cinereus* has the ability to protect itself from infection with Bd, a single historical museum specimen from a sample of five tested positive for the fungus (Richards-Hrdlicka, 2012). None of the other species in my sample are known to have tested positive for Bd.

By sampling populations of Plethodontid salamanders, I provide evidence that overall, Bd is either rare or nonexistent in GFMBR. Although Bd was not detected in this location, these data can be used to help assess changes in its distribution and prevalence, providing baseline information for future studies on Bd. Understanding the distribution and spread of the fungus is an important factor for conservation and needs to be monitored closely.

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TABLES AND FIGURES

TABLES

Table 1. Plethodontid salamander species that were swabbed for the presence of Bd, number of each that were swabbed, and number of recaptures.

Species	Number Swabbed	Recaptures
Plethodon cinereus	20	4
Plethodon cylindraceus	5	0
Plethodon montanus	200	50
Plethodon welleri	17	3
Desmognathus orestes	21	6
Desmognathus wrighti	6	1
Eurycea wilderae	1	0

Table 2. Plehtodontid salamander species that were swabbed for the presence of Bd, number of swabbed animals per season, and elevation.

Species	Summer (June-Aug)	Fall (Sept-Nov)	Elevation (m)
Plethodon cinereus	20	7	1259, 1311, 1356
Plethodon cylindraceus	5	0	1259, 1311
Plethodon montanus	200	25	1259, 1311, 1356, 1445, 1533
Plethodon welleri	17	4	1533
Desmognathus orestes	21	0	1259, 1311, 1356, 1445
Desmognathus wrighti	6	0	1311, 1445, 1533
Eurycea wilderae	1	0	1259

Table 3. Plethodontid salamander species that have tested positive for Bd. When information is available, table includes salamander life history, sample size, number of positives, geographic location, sample type (wild caught (w), captive (c) or historical samples (h)), and reference paper.

Species	Life history	Sample Size	# Positive	Geography	Sample type	Reference
Oedipina grandis	Terrestrial		1	Central America	W	Lips et al., 2003
Bolitoglossa dofleini	Terrestrial	6	6	Central America	W	Pasmans et al., 2004
Plethodon neomexicanus	Terrestrial		1	New Mexico	W	Cummer et al., 2005
Bolitoglossa colonnea	Terrestrial			Central America	W	Lips et al., 2006
Bolitoglossa schizodactyla	Terrestrial		2	Central America	W	Lips et al., 2006
O. collaris	Terrestrial		2	Central America	W	Lips et al., 2006
O. cf. parvipes	Terrestrial		1	Central America	W	Lips et al., 2006
E. bislineata	Semi aquatic	14 9 4	1 1 (1968) 4 (2006)	 Connecticut Connecticut	W H H	Grant et al., 2008 Richards-Hrdlicka, 2012
E. cirrigera	Semi aquatic	50	21	Alabama	W W	Byrne et al., 2008 Lannoo et al., 2011
Desmognathus conanti	Aquatic	86	2	Georgia	W W	Timpe et al., 2008 Lannoo et al., 2011
D. fuscus	Semi aquatic	67 12 4 26 10 3 3	9 1 4 (1968) 4 (1969) 2 (2003) 3 (2005) 2 (2007)	 Maryland, Virginia Connecticut Connecticut Connecticut Connecticut Connecticut	W W H H H H H	Grant et al., 2008 Hossack et al., 2010 Richards-Hrdlicka, 2012
D. santeetlah	Aquatic	3	1		W	Caruso and Lips, 2012
E. longicauda	Semi aquatic				W	Lannoo et al., 2011
E. neotenes	Aquatic	16	1	Texas	W	Gaertner et al., 2009
E. pterophila	Aquatic	20	1	Texas	W	Gaertner et al., 2009

E. nana	Aquatic	17	1	Texas	W	Gaertner et al., 2009
E. sosorum	Aquatic	1	7	Texas	W	Gaertner et al., 2009
E. tonkawae	Aquatic	14	6	Texas	W	Gaertner et al., 2009
Batrachoseps attenuatus	Terrestrial	549 12 3	37 12 (1973) 3 (1974)	California	W H	Weinstein, 2009
Batrachoseps gavilanensis	Terrestrial	3	3 (1993)	California	H	Weinstein, 2009
Batrachoseps nigriventris	Terrestrial	3	3 (1993)	California	H	Weinstein, 2009
Batrachoseps relictus	Semi aquatic	3	1 (1995)	California	H	Weinstein, 2009
Batrachoseps wrightorum	Terrestrial	1	1 (1994)	Oregon	H	Weinstein, 2009
D. monticola	Semi aquatic	50	2	Maryland, Virginia	W	Hossack et al., 2010
Bolitoglossa rufescens	Terrestrial	2 7	2 6	Mexico	W C	Van Rooji et al., 2011
Pseudoeurycea cephalica	Terrestrial	4 3	4 2	Mexico	W C	Van Rooji et al., 2011
Pseudoeurycea firscheini	Terrestrial	1	1	Mexico	W	Van Rooji et al., 2011
Pseudoeurycea leprosa	Terrestrial	2 3	2 3	Mexico	W C	Van Rooji et al., 2011
Bolitoglossa platydactyla	Terrestrial	2	2	Mexico	C	Van Rooji et al., 2011
Pseudoeurycea belli	Terrestrial	4	2	Mexico	C	Van Rooji et al., 2011
Pseudoeurycea longicauda	Terrestrial	4	1	Mexico	C	Van Rooji et al., 2011
Plethodon cinereus	Terrestrial	5	1 (1968)	Connecticut	H	Richards-Hrdlicka, 2012
Plethodon glutinosus	Terrestrial	39	1	North Carolina	W	Chinnadurai et al., 2009
Plethodon yonahlossee	Terrestrial	40	1	North Carolina	W	Chinnadurai et al., 2009
Pseudotriton ruber	Semi aquatic				W	Lannoo et al., 2011

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