

**Impacts of climate change, human land use, and mercury contamination on
Southern Appalachian Plethodontid salamanders**

**A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville**

**Michael Kevin Hamed
August 2014**

ACKNOWLEDGEMENTS

I would like to thank many people and institutions for their invaluable assistance, support, guidance, and encouragement. First, I would like to thank the chair of my dissertation committee, Dr. Matthew Gray, for his patience, guidance, and support during my doctoral journey. I greatly appreciate your willingness to attempt this undertaking from a non-traditional approach, which made the process even more difficult. Your inbox will feel lonely without my 3:00 AM emails. I would also like to thank the other members of my dissertation committee, Dr. David Buehler, Dr. Benjamin Fitzpatrick, and Dr. John Drake for the guidance and direction you provided. I must thank my two “unofficial” committee members, Dr. James and Della Organ, who provided an immensely value contribution to this project. I am more than honored that you entrusted your life’s work to me and I only hope you are proud of the results. Your friendship not only to me, but my family was amazing. I had hoped we all could sit on the cabin’s porch and watch red squirrels at your feeders while talking about the completed project. Della, you are missed.

As an educator I would be remised to not thank the many amazing teachers and professors that helped guide me to pursuing a career in biological sciences. Malcolm Sprinkle, Harriet Locke, Randy Lacy, Dr. Frank Bulow, and Dr. Michael “Mick” Harvey, sharing your passion for nature and learning was more important to me than any knowledge I gained; thank you! As a student and teacher, I incorporate much of what you taught me into my classroom and my life.

Completion of this degree and many other accomplishments in the past 20 years would not have been possible without the help of many true friends who provided support, assistance, and guidance including: Phil Gentry, Joel Keebler, Wallace Coffey, Jane Perry, Jason Hoverman,

Bill Sutton, Jason Matson, and Dexton Eller. My many colleagues also provided tremendous support including: Joel Rudy, Mary Snead, Sandy Davis, and Reva Russell. I owe a debt of gratitude to Josh Rudd for covering my classes during my one-year absence from Virginia Highlands Community College (VHCC). I am also very appreciative for the support and motivation of the administrators from VHCC for providing many resources to help me obtain this degree including: David Wilkin, Robert May, and Susan Fleming. Without The Virginia Community College System's (VCCS) Chancellor's Faculty Fellowship, I would not have been able to attend the University of Tennessee and I am very grateful for the support.

I wanted to thank the institutions and organizations that provided financial support for this project: the Virginia Department of Game and Inlands Fisheries, U.S. Fish and Wildlife Service, Tennessee Wildlife Resource Agency, VA Tech Prep Fund, VHCC professional growth and development funds, VCCS Paul Lee professional development grant, and the University of Tennessee College of Agricultural Sciences and Natural Resources Hazelwood scholarship.

Lastly, I must thank my family for without their encouragement and support I could not have completed such an enduring task. My parents, Mickey and Doris Hamed, tolerated a house full of every creature known throughout my childhood and I greatly appreciate their patience. They also instilled in me the only trait that allowed me to survive this process, being a hard worker. My sister, Alia, has always been my greatest supporter and I thank her for always being there for me. I know much of my passion for nature was created during my childhood as I would spend afternoons with my grandmother, Vesta Wilson, in her gardens as well as weekly walks down to the "lower 40" with my uncle, Ray Wilson; both were great influences and I appreciate the time they spent with me. Misty and I could not have survived this journey without much help and I am especially indebted to the time Sue King spent helping us. I have to thank my wife

of 18-years, Misty, for her support, tolerance, and patience through this process. I would not have been able to complete this degree without your loving encouragement. Lastly, my greatest accomplishment, not only during the six years of my dissertation but my life, was the arrival of my daughter, Maddie Grace. She changed my world and I cannot wait for the adventures we will now have time to undertake.

ABSTRACT

The Southern Appalachian Mountains are home to the greatest diversity of Plethodontid Salamanders in the world. The future for these salamanders is uncertain as globally amphibians have been experiencing significant population declines. We investigated the impact of habitat loss, chemical contaminants, and climate change on Plethodontid salamanders in northeastern Tennessee and southwestern Virginia. Four-toed salamanders (*Hemidactylium scutatum*) were negatively impacted by powerline right-of-way (ROW) mowing. Embryonic and larval survival significantly decreased in ROW after mowing and required up to 3 years of vegetation regrowth to reach post mowing survival. We also investigated mercury contamination in black-bellied salamanders (*Desmognathus quadramaculatus*) from Whitetop Mountain, Virginia. We found these salamanders to be contaminated with mercury levels equivalent to salamanders from point source pollution streams. Additionally, salamander from streams facing the prevailing wind direction had significantly greater levels of mercury contamination. To help land managers in planning long range habitat goals for the Mount Rogers National Recreation Area, we also developed salamander occupancy models based on parameters describing forest structure and composition. Elevation or other abiotic factors had a greater association to occupancy than many forest parameters. Lastly, to determine the influence of climate change on Mount Rogers National Recreation Area (MRNA) salamander distributions, we compared current distributions with those from the 1950s and 1990s and examined regional temperature changes since 1948. *Plethodon welleri*, a high elevation endemic, moved down slope since both the 1950s and 1990s. *Plethodon yonahlossee* expanded its range both up and down slope, but *P. cylindraceus* experienced range constrictions since the 1950s. No significant differences were observed for regional temperatures since 1948. We also modeled future salamander occupancy based on

current mean temperature preferences. Using the Canadian Centre for Climate Modeling and Analysis Coupled Global Climate Model (CGCM) and the Hadley Centre for Climate Prediction and Research (HAD) global circulation models with 2 different CO₂ [carbon dioxide] emission projections, we projected the extirpation of *P. welleri*, *D. organi*, and *Eurycea wilderae* from Whitetop Mountain by 2070. Our results provide land managers with data necessary to better prepare for the challenges to maintain Plethodontid salamander populations.

Table of Contents

INTRODUCTION	1
LITERATURE CITED	6
CHAPTER 1 : IMPACT OF POWERLINE RIGHT-OF WAY MOVING ON FOUR-TOED SALAMANDER NESTING AND LARVAL SURVIVAL	
ABSTRACT	13
INTRODUCTION.....	14
METHODS.....	19
RESULTS.....	27
DISCUSSION	35
CONCLUSIONS.....	45
LITERATURE CITED	48
APPENDIX	57
CHAPTER 2 : TEMPORIAL AND SPATIAL CHANGES OF MERCURY CONTAMINATION OF BLACK-BELLIED SALAMANDERS FROM WHITETOP MOUNTAIN, VIRGINIA.....	
ABSTRACT	78
INTRODUCTION.....	79
METHODS.....	83
RESULTS.....	87
DISCUSSION	89
CONCLUSIONS.....	96
LITERATURE CITED	98
APPENDIX	107
CHAPTER 3 : CHANGES IN SALAMANDER DISTRIBUTIONS ALONG AN ELEVATIONAL GRADIENT IN THE MOUNT ROGERS NATIONAL RECREATION AREA	
ABSTRACT	114
INTRODUCTION.....	115
METHODS.....	121
RESULTS.....	127
DISCUSSION	131
CONCLUSIONS.....	139
LITERATURE CITED	141

APPENDIX	151
CHAPTER 4 : VEGATATION AND BIOTIC HABITAT CHARACTERISTICS INFLUENCE PLETHODONTID SALAMANDER OCCUPANCY WITHIIN THE MOUNT ROGERS NATIONAL RECREATION AREA.....	186
ABSTRACT	187
INTRODUCTION.....	188
METHODS.....	192
RESULTS.....	199
DISCUSSION	204
CONCLUSIONS	213
LITERATURE CITED	215
APPENDIX	227
CHAPTER 5 : FUTURE CHANGES IN SALAMANDER DISTRIBUTIONS ALONG AN ELEVATION GRADIENT ON WHITETOP MOUNTAIN, VIRGINIA	254
ABSTRACT	255
INTRODUCTION.....	256
METHODS.....	261
RESULTS.....	267
DISCUSSION	273
CONCLUSIONS.....	282
LITERATURE CITED	284
APPENDIX	294
CONCLUSIONS.....	332
VITA	335

LIST OF TABLES

Table 1.1. Number of abandoned four-toed salamander nests per treatment (forest or right-of-way) each nesting year from the South Holston Weir Dam, Sullivan County, Tennessee (Bold values indicated significant differences).....	57
Table 1.2. Snout-vent-length of female four-toed salamander guarding nest per treatment for each nesting year per treatment (forest or right-of-way) each nesting year from the South Holston Weir Dam, Sullivan County, Tennessee.	58
Table 1.3. Microhabitat variables from nests with female salamanders present in each habitat (forest or right-of-way) during each nesting year from the South Holston Weir Dam, Sullivan County, Tennessee (Bold values indicated significant differences).	59
Table 1.4. Percent embryonic survival from nests with female four-toed salamander present from each habitat (forest or right-of-way) during each nesting season from the South Holston Weir Dam, Sullivan County, Tennessee (Bold values indicated significant differences).	60
Table 1.5. Snout-vent-length, total length, fecundity of returning female four-toed salamanders and the distance moved from past nest locations from the South Holston Weir Dam, Sullivan County, Tennessee.	61
Table 1.6. Tukey's post-hoc analysis with a Bonferroni adjustment results from an ANOVA for larval survival of four-toed salamanders from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.....	62
Table 1.7. Tukey's post-hoc analysis with a Bonferroni adjustment results for a repeated measures ANOVA of four-toed salamander larval survival from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee (2009-12).	63
Table 1.8. Results from an ANOVA and Tukey's post-hoc analysis with a Bonferroni adjustment for four-toed salamander larval length and mass from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee (2009-12). Results from post-hoc analysis are noted with an * for the <i>F</i> value.	64
Table 1.9. Tukey's post-hoc analysis with a Bonferroni adjustment results from an ANOVA comparing four-toed salamander mean days to metamorphosis from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee (2009-12).	65
Table 1.10. Results from Tukey's post-hoc analysis with a Bonferroni adjustment for a repeated measures ANOVA for larval survival of four-toed salamanders from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.	67
Table 1.11. Results from Mann-Whitney test with Bonferroni adjustment for a mean daily pool temperatures from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.....	68
Table 1.12. Results from Friedman test for mean daily pool temperatures at the same distance into the forest or right-of-way, but between years from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.	69

Table 1.13. Results from Mann-Whitney test with Bonferroni adjustment for a maximum daily pool temperatures from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.....	70
Table 1.14. Results from Mann-Whitney test with Bonferroni adjustment for a net primary production from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.....	71
Table 2.1. Comparison of THg in <i>D. quadramaculatus</i> tail tissue from Whitetop Mt., VA (2011-12) and other known amphibian tissue THg from published accounts.....	107
Table 2.2. Comparison of THg in <i>D. quadramaculatus</i> liver tissue from Whitetop Mt., VA (2011-12) and other known amphibian liver THg tissue accounts.	108
Table 3.1. Top models explaining occupancy probabilities for historic (1950s & 1990s) and modern (2008-11) sampling of salamanders within the Mount Rogers National Recreation Area, Virginia.	151
Table 3.2. Model-averaged parameter estimates, SEs, and 85% CIs for variables in AIC candidate models from occupancy probabilities form historic (1950s & 1990s) and modern (2008-11) surveys of salamander from the Mount Rogers National Recreation Area, Virginia.	154
Table 3.3. Mean and median changes (meters) in midpoint elevation since historic surveys (1950s & 1990s) for Plethodontid salamanders from Mount Rogers National Recreation Area, Virginia. Values in bold represent changes that were confirmed with occupancy modeling....	156
Table 3.4. Overall median range limit (meters) changes since historic survey periods (1950s & 1990s) for Plethodontid salamanders from Mount Rogers National Recreation Area, Virginia. Values in bold represent changes that were confirmed with occupancy modeling.	157
Table 3.5. Median range limit changes (meters) since each historic time period of <i>Plethodon welleri</i> from Mount Rogers National Recreation Area, Virginia (* = transect limit not sampled during that time period; ND=Not detected; ND90= Not detected in 1990s surveys).....	158
Table 3.6. Median range limit changes (meters) since each historic time period of <i>Plethodon cylindraceus</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during time period; ND=Not detected; ND2000= Not detected in 2000s surveys)	159
Table 3.7. Median range limit changes (meters) since each historic time period of <i>Plethodon yonahlossee</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during time period)	160
Table 3.8. Median range limit changes (meters) since each historic time period of <i>Plethodon cinereus</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during time period)	161
Table 3.9. Median range limit changes (meters) since each historic time period of <i>Plethodon montanus</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period)	162
Table 3.10. Median range limit changes (meters) since each historic time period of <i>Desmognathus organi</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period; ND=Not detected; ND50= Not detected in 1950s surveys)	163
Table 3.11. Median range limit changes (meters) since each historic time period of <i>Desmognathus orestes</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during time period)	164

Table 3.12. Median range limit changes (meters) since each historic time period of <i>Desmognathus fuscus</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period; ND=Not detected; ND2000= Not detected in 2000s surveys)	165
Table 3.13. Median range limit changes (meters) since each historic time period of <i>Plethodon richmondi</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period; ND=Not detected; ND90= Not detected in 1990s surveys) ..	166
Table 3.14. Median range limit changes (meters) since each historic time period of <i>Desmognathus quadramaculatus</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period; ND=Not detected).....	167
Table 3.15. Median range limit changes (meters) since each historic time period of <i>Desmognathus monticola</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during time period; ND=Not detected)	168
Table 3.16. Median range limit changes (meters) since each historic time period of <i>Eurycea wilderae</i> from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period; ND=Not detected; ND90= Not detected in 1990s surveys; ND2000= Not detected in 2000s surveys)	169
Table 3.17. Mean Climatic values for each 10-year period prior to last sampling year for historic and modern salamander surveys from the Tri-Cities Regional Airport (Sullivan Co., TN). Values in bold are significant.....	170
Table 3.18. Results from linear regression analysis of climatic variables in response to time from Tri-cities airport (Kingsport, TN) from 1948-2011.	171
Table 4.1. Model Terms and published accounts of habitat associations justifying their use in occupancy models for Plethodontid salamanders residing within the Mount Rogers National Recreation Area.	227
Table 4.2. Covariate descriptions used to model occupancy probabilities of Plethodontid salamanders within the Mount Rogers National Recreation Area (2008-11) and units of measurement.	228
Table 4.3. Covariates and covariate combinations used to model Plethodontid salamander occupancy probabilities within the Mount Rogers National Recreation Area, Virginia (2008-2011).	229
Table 4.4. Highest supported ($\Delta\text{QAIC}_c < 2$) models explaining Plethodontid salamanders occupancy within the Mount Rogers National Recreation Area, Virginia (2008-11).	232
Table 4.5. Model-averaged parameter estimates, standard errors, and 85% CIs for vegetation and abiotic variables explaining occupancy of Plethodontid salamanders within the Mount Rogers National Creation Area, VA form top QAIC _c candidate models (2008-11)	234
Table 5.1. Coupled Modeled Intercomparison Project phase 3 (CMIP3) model January and July mean monthly temperature predictions for 2030, 2050, and 2070 from the Whitetop Mountain, VA reference location.	294
Table 5.2. Mean temperature differences from the Coupled Modeled Intercomparison Project phase 3 (CMIP3) model reference location to each Whitetop Mountain, VA transect for January (2010-12) and July (2009-11) along Dells Branch (northern slope) and Whitetop Creek (southern slope).....	294
Table 5.3. Highest supported models describing salamander occupancy with mean January and July temperatures from Whitetop Mountain, VA from July 2009 – January 2012. Only models with $\Delta\text{AIC}_c < 2.0$ are listed.	296

Table 5.4. Model-averaged temperature, parameter estimates, standard errors, and 85% confidence intervals for variables explaining occupancy for Whitetop Mountain, VA salamanders from July 2009 – January 2012 in top QAIC _c candidate model.....	297
Table 5.5. Future occupancy probabilities for <i>Plethodon welleri</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.	298
Table 5.6. Future occupancy probabilities for <i>Plethodon welleri</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	299
Table 5.7. Future occupancy probabilities for <i>Desmognathus organi</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	300
Table 5.8. Future occupancy probabilities for <i>Desmognathus organi</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	301
Table 5.9. Future occupancy probabilities for <i>Eurycea wilderae</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	302
Table 5.10. Future occupancy probabilities for <i>Eurycea wilderae</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.	303
Table 5.11. Future occupancy probabilities for <i>Plethodon montanus</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	304
Table 5.12. Future occupancy probabilities for <i>Plethodon montanus</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	305
Table 5.13. Future occupancy probabilities for <i>Desmognathus orestes</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	306
Table 5.14. Future occupancy probabilities for <i>Desmognathus orestes</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	307
Table 5.15. Future occupancy probabilities for <i>Plethodon yonahlossee</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	308
Table 5.16. Future occupancy probabilities for <i>Plethodon yonahlossee</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	309
Table 5.17. Future occupancy probabilities for <i>Plethodon cinereus</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	310
Table 5.18. Future occupancy probabilities for <i>Plethodon cinereus</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	311

Table 5.19. Future occupancy probabilities for <i>Desmognathus quadramaculatus</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	312
Table 5.20. Future occupancy probabilities for <i>Desmognathus monticola</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	313
Table 5.21. Future occupancy probabilities for <i>Desmognathus quadramaculatus</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	314
Table 5.22. Future occupancy probabilities for <i>Desmognathus monticola</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	315
Table 5.23. Future occupancy probabilities for <i>Plethodon richmondi</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	316
Table 5.24. Future occupancy probabilities for <i>Plethodon richmondi</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	317
Table 5.25. Future occupancy probabilities for <i>Desmognathus fuscus</i> from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	318
Table 5.26. Future occupancy probabilities for <i>Desmognathus fuscus</i> from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.....	319

LIST OF FIGURES

Figure 1.1. Four-toed salamander mesocosm pool layout at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.	72
Figure 1.2. Mean percent four-toed salamander embryonic survival per treatment (forest or right-of-way) each year prior to and after ROW mowing at the South Holston Weir Dam, Sullivan County, Tennessee from 2007-12. Error bars represent SEM.	73
Figure 1.3. Four-toed salamander larval mean percent survival 9 meters into ROW with mesocosm pools at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12. Error bars represent +1 SD.	74
Figure 1.4. Four-toed salamander larval mean percent survival 6 meters into ROW with mesocosm pools at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12. Error bars represent +1 SD.	75
Figure 1.5. Relationship of mean days to metamorphosis and mean pool temperature for four-toed salamanders from mesocosm pools at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12. ($r_s = -0.589$; $P < 0.001$).	76
Figure 2.1. Relationship between liver and tail THg concentrations of <i>Desmognathus quadramaculatus</i> from Whitetop Mountain, Virginia collected 2011-2012 ($r_s = 0.83$, $P < 0.01$).	109
Figure 2.2. Mean total mercury of tail tissue of <i>Desmognathus quadramaculatus</i> from Big Branch (northern slope) and Whitetop Creek (southern slope) collected 2011-2. Error bars represent 95% CIs. Raw data are presented in the graph but analysis was conducted on $\log_{10}$ transformed data.	110
Figure 2.3. Mean total mercury of tail tissue of <i>Desmognathus quadramaculatus</i> from low elevations and high elevations on Big Branch, north slope of Whitetop Mountain, Virginia collected 2011-2. Error bars represent 95% CIs. Raw data are presented in the graph but analysis was conducted on $\log_{10}$ transformed data.	111
Figure 2.4. Mean percent change of total mercury in preserved tail tissue of <i>Desmognathus quadramaculatus</i> , collected from Big Branch north slope of Whitetop Mountain, from initial values stored in 65% ethanol. Error bars represent 95% CIs.	112
Figure 3.1. Relationship between elevation and occupancy probabilities of <i>Plethodon welleri</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	172
Figure 3.2. Relationship between elevation and occupancy probabilities of <i>Plethodon cylindraceus</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	173
Figure 3.3. Relationship between elevation and occupancy probabilities of <i>Plethodon yonahlossee</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	174
Figure 3.4. Relationship between elevation and occupancy probabilities of <i>Plethodon cinereus</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	175
Figure 3.5. Relationship between elevation and occupancy probabilities of <i>Plethodon montanus</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	176

Figure 3.6. Relationship between elevation and occupancy probabilities of <i>Desmognathus organi</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	177
Figure 3.7. Relationship between elevation and occupancy probabilities of <i>Desmognathus orestes</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	178
Figure 3.8. Relationship between elevation and occupancy probabilities of <i>Desmognathus fuscus</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	179
Figure 3.9. Relationship between elevation and occupancy probabilities of <i>Plethodon richmondi</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	180
Figure 3.10. Relationship between elevation and occupancy probabilities of <i>Desmognathus quadramaculatus</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	181
Figure 3.11. Relationship between elevation and occupancy probabilities of <i>Desmognathus monticola</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	182
Figure 3.12. Relationship between elevation and occupancy probabilities of <i>Eurycea wilderae</i> for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.	183
Figure 3.13. Relationship between mean temperature and year of collection (1948-2011) from Tri-cities airport, Kingsport, TN. [(January mean = $30.622 - 0.014x$; $r^2 = 0.007$; $P = 0.461$), (July mean = $28.792 - 0.002x$; $r^2 = 0.014$; $P = 0.741$), and (Annual mean = $13.516 - 0.000x$; $r^2 = 0.016$; $P = 0.983$)]	184
Figure 3.14. Relationship between mean precipitation and sample year (1948-2011) from Tri-cities airport, Kingsport, TN. [(July mean = $19.793 - 0.004x$; $r^2 = 0.016$; $P = 0.908$) (Annual mean = $284.186 - 0.09x$; $r^2 = 0.007$; $P = 0.446$)]	185
Figure 4.1. Sampling plots for measuring vegetation characteristics for Plethodontid salamanders from the Mount Rogers National Recreation Area, Virginia (2011). Overstory trees were quantified and diameter-breast-height was measured with in a 400 m ² plot. Saplings were quantified within a 100 m ² plot and seedlings were quantified within a 40 m ² plot. Circles indicate locations of ground cover and vertical structure measurements. Diagram is not to scale.	236
Figure 4.2. Occupancy probabilities of small bodied salamanders of the genus <i>Plethodon</i> in relation to elevation from the Mount Rogers National Recreation Area, Virginia (2008-2011).	237
Figure 4.3. Occupancy probabilities of <i>Plethodon welleri</i> and <i>Eurycea wilderae</i> in relation to slope from the Mount Rogers National Recreation Area, Virginia (2008-2011).	238
Figure 4.4. Occupancy probabilities of <i>Eurycea wilderae</i> , <i>Plethodon richmondi</i> and <i>P. welleri</i> in relation to yellow birch (> 11.43 cm DBH) relative frequency within 400 m ² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).	239
Figure 4.5. Occupancy probabilities of <i>Plethodon yonahlossee</i> in relation to yellow buckeye (> 11.43 cm DBH) relative frequency within 400 m ² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).	240

Figure 4.6. Occupancy probabilities of <i>Plethodon yonahlossee</i> and <i>P. cinereus</i> in relation to striped maple (> 11.43 cm DBH) relative frequency within 400 m ² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).	241
Figure 4.7. Occupancy probabilities of <i>Plethodon yonahlossee</i> in relation to eastern hemlock (> 11.43 cm DBH) relative frequency within 400 m ² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).	242
Figure 4.8. Occupancy probabilities of large bodied salamanders of the genus <i>Plethodon</i> in relation to elevation from the Mount Rogers National Recreation Area, Virginia (2008-2011).	243
Figure 4.9. Occupancy probabilities of <i>Desmognathus organi</i> and <i>Plethodon cylindraceus</i> in relation to aspect from the Mount Rogers National Recreation Area, Virginia (2008-2011). ...	244
Figure 4.10. Occupancy probabilities of <i>Plethodon cinereus</i> in relation to cucumber magnolia (> 11.43 cm DBH) relative frequency within 400 m ² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).	245
Figure 4.11. Occupancy probabilities of <i>Plethodon cinereus</i> in relation to overstory richness from the Mount Rogers National Recreation Area, Virginia (2008-2011).	246
Figure 4.12. Occupancy probabilities of <i>Plethodon cinereus</i> and <i>P. richmondi</i> in relation to percent ground cover of moss from 4 x 1 m ² plots within the Mount Rogers National Recreation Area, Virginia (2008-2011).	247
Figure 4.13. Occupancy probabilities of <i>Plethodon cinereus</i> in relation to vegetation density < 1 m from the ground surface from the Mount Rogers National Recreation Area, Virginia (2008-2011).	248
Figure 4.14. Occupancy probabilities of <i>Plethodon richmondi</i> in relation to number of rock cover objects within 400 m ² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).	249
Figure 4.15. Occupancy probabilities of <i>Eurycea wilderae</i> and <i>Plethodon richmondi</i> in relation to sugar maple (> 11.43 cm DBH) relative frequency within 400 m ² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).	250
Figure 4.16. Occupancy probabilities of <i>Plethodon richmondi</i> in relation to American beech (> 11.43 cm DBH) relative frequency within 400 m ² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).	251
Figure 4.17. Occupancy probabilities of <i>Desmognathus fuscus</i> in relation to proportion of canopy cover from the Mount Rogers National Recreation Area, Virginia (2008-2011).	252
Figure 4.18. Occupancy probabilities of salamanders of the genus <i>Desmognathus</i> in relation to elevation from the Mount Rogers National Recreation Area, Virginia (2008-2011).	253
Figure 5.1. Future occupancy probabilities for <i>Plethodon welleri</i> by 2030 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.	320
Figure 5.2. Future occupancy probabilities for <i>Plethodon welleri</i> by 2050 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.	321

Figure 5.3. Future occupancy probabilities for <i>Plethodon welleri</i> by 2070 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.	322
Figure 5.4. Future occupancy probabilities for <i>Plethodon welleri</i> by 2030 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.	323
Figure 5.5. Future occupancy probabilities for <i>Plethodon welleri</i> by 2050 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.	324
Figure 5.6. Future occupancy probabilities for <i>Plethodon welleri</i> by 2070 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.	325
Figure 5.7. Future occupancy probabilities for <i>Desmognathus organi</i> by 2030 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.	326
Figure 5.8. Future occupancy probabilities for <i>Desmognathus organi</i> by 2050 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.	327
Figure 5.9. Future occupancy probabilities for <i>Desmognathus organi</i> by 2070 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.	328
Figure 5.10. Future occupancy probabilities for <i>Desmognathus organi</i> by 2030 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global	

Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.....	329
Figure 5.11. Future occupancy probabilities for <i>Desmognathus organi</i> by 2050 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.....	330
Figure 5.12. Future occupancy probabilities for <i>Desmognathus organi</i> by 2070 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.....	331

INTRODUCTION

Amphibian declines were first noted in the 1980s and numerous studies have continued to indicate significant declines (Wake 1991; Wake and Vredenburg 2008). The rates of declines are similar or even greater than other significant losses of biodiversity over the history of the planet. Some have suggested that we are in the midst of the sixth mass extinction (Wake and Vredenburg 2008). Currently, 33% of all amphibians are threatened with extinction and 43% are declining. Caudates especially are impacted as 47% of all salamanders are threatened with extinction (IUCN 2008). Within North America almost 20% of all southeastern salamanders are threatened (Young et al. 2004). Compared to birds or mammals, amphibians are 5 to 7 times more likely to be classified as critically endangered and 3 to 6 times more likely to be classified as endangered by the IUCN (Young et al. 2004). Habitat loss, climate change, over-exploitation, chemical contamination, diseases/pathogens, and synergistic factors have been linked to amphibian declines (Davidson et al. 2001; Lips et al. 2008; Gray et al. 2009; Relyea and Hoverman 2008; Semlitsch et al. 2009; Milanovich et al. 2010, and many others).

Amphibians have been considered a sentinel species and the “canary in the coal mine” as an environmental indicator (Carroll 1999). One factor that contributes to their environmental sensitivity is their permeable skin, which provides toxins an easy pathway of entry into the body of amphibians (Boone 2003). Most amphibians are reliant on aquatic environments early in their life history and their complex life cycle allows them to metamorphose onto land (Wells 2007). Thus, they are potentially exposed to toxins in both aquatic and terrestrial environments. Many species of anurans and some caudates (e.g. in the southeastern United States members of the family *Ambystomatidae*, *Hemidactylium scutatum*) must migrate to reach breeding sites, thus exposing them to additional anthropogenic mortality (Gibbs and Shriver 2005). For those

species that do not require standing water, they are still linked to moist environments and are susceptible to changing climate (Walls et al. 2013). Most amphibians have very limited dispersal abilities, which allowed for allopatric speciation of Southern Appalachian salamanders (Kozak and Wiens 2010), but recently has led to fragmented populations from anthropogenic habitat alteration (Bartoszek and Greenwald 2009). In addition to limited dispersion, Plethodontid salamanders can have home ranges of $< 10 \text{ m}^2$ thus making them extremely susceptible to habitat loss. When examining life history traits contributing to listing as an IUCN red list species, small distributions were the leading factor (Sodhi et al. 2008).

Hutchinson (1957) defined an individual's habitat requirements as its fundamental niche. Where the individual ultimately resides due to competition and other interactions was classified as its realized niche (Hutchinson 1957). Understanding an organism's fundamental and realized niche is critical for management decisions and conservation. Few Southern Appalachian salamanders have had their fundamental niche requirements identified and quantified (Welsh et al. 2006; Riedel et al. 2008; Dillard et al. 2008 & 2008b). Conservation efforts will require knowledge of these habitats for protection. Habitat loss is the most cited causes for amphibian declines as almost 90% of all IUCN listed amphibians are impacted by habitat loss (Young et al. 2004). Most studies on amphibian habitat loss have focused on timber harvesting and almost all studies have indicated a significant negative impact when the canopy is removed (Petranka 1993; deMaynadier and Hunter 1998; Reichenbach and Sattler 2007; Hoymack and Hass 2009; Semlitsch et al. 2009). Estimated losses of 14 million Plethodontid salamanders annually from western North Carolina were due to clear cutting (Petranka et al. 1993). Recovery times for salamanders from timber harvesting are estimated between 6 – 60 years (Petranka 1993; Ash 1997). However, enigmatic declines in pristine, protected areas have also been documented

(Stuart et al. 2004; Caruso and Lips 2012) suggesting other factors are involved in amphibian declines than habitat loss.

Chemical contamination has also been linked to amphibian declines and is second only to habitat loss as the leading factor cited for declines of IUCN red listed species (Young et al. 2004). Pesticides and herbicides are the leading cause of amphibian declines as glyphosates, atrazine, malathion, carbaryl, and many other have been shown to cause mortality or negatively impact amphibian populations (Bridges and Semlitsch 2000; Davidson et al. 2001; Hayes et al. 2002; Relyea 2004; Relyea and Hoverman 2008). Additionally, nitrogen has also negatively impacted amphibian populations either through direct runoff or as ions in acid rain (Wyman and Hawksley-Lescault 1987; Wyman and Jancola 1992; Moore and Wyman 2010). Heavy metals are another chemical contaminant that is impacting amphibians either through direct mortality or impaired neurological function (Bank et al. 2005; Ugarte et al. 2005; Bergeron et al. 2010; Burke et al. 2010; Rimmer et al. 2010). Mercury from both industrial point-sources and through atmospheric deposition, even in pristine areas such as the Florida Everglades, has been found to contaminate amphibians suggesting that globally amphibians could be impacted (Ugarte et al. 2005; Bergeron et al. 2010).

Climate change has negatively impacted amphibian populations in North and South American as future projections are indicating rapid population declines (Pounds et al. 2006; Milanovich et al. 2010). Amphibians have been negatively affected by changing climate through decreased leaf litter, greater competitive interactions, distribution shifts, increased effects of diseases and pathogens, and altered breeding phenology (Pounds et al. 2006; Whitfield et al. 2007; Kusano and Inoue 2008; Milanovich et al. 2010; Walls et al. 2013). Climate change is projected to negatively influence amphibians more than either birds or mammals in North

American due to their reliance on water and/or cool moist environments (Lawler et al. 2009). Many Southern Appalachian salamanders are projected to experience declines and even extirpations with warming environments. Plethodontid salamanders in the extreme southern portion of the Appalachian Mountains have been predicted to become extirpated as early as 2020 with climate models showing the greatest warming (Milanovich et al. 2010).

Conservation of amphibians will require a diverse approach targeting many anthropogenic factors that negatively impact amphibian populations. Amphibian declines (up to 48%) have often been deemed enigmatic as specific causes could not be related to declines (Stuart et al. 2004; Caruso and Lips 2013). Most likely these declines were due to synergistic effects of even minimal habitat loss, chemical contamination, diseases, and over exploitation. Therefore, we chose to investigate several different anthropogenic impacts (habitat loss/modification, chemical contamination, and climate change) to determine their potential impact on Southern Appalachian Plethodontid salamanders.

My dissertation is written in manuscript style and divided into 5 chapters each representing a possible threat to salamanders from anthropogenic sources. Initially, we focused on the impact of habitat loss/clearing on four-toed salamander (*Hemidactylium scutatum*) embryonic and larval survival within an electrical powerline right-of-way. We hypothesized that vegetation removal would negatively impact salamander nesting and larval survival, but the impact would decrease as time since mowing increased due to vegetation regrowth. Remaining chapters focused on Plethodontid salamanders within the Mount Rogers National Recreation Area (MRNRA). In Chapter 2, we determined the concentration of mercury in black-bellied salamanders (*Desmognathus quadramaculatus*) over a spatial and temporal scale from Whitetop Mountain. Since mercury typically enters ecosystems through atmospheric deposition, we

hypothesized streams higher in elevation and facing the prevailing winds would have salamanders with greater mercury contamination. In Chapter 3, we investigated salamander distribution changes of along elevational gradients in the MRNRA. We resurveyed 10 transects that were originally sampled in the 1950s and/or 1990s. Given the documented increase in global temperatures, we hypothesized that high elevation endemic salamanders would shift their distributions upslope to avoid warming temperatures and maintain their fundamental niche. In Chapter 4, we wanted to determine if forest characteristics could be associated with the occupancy of Plethodontid salamanders within the MRNRA. Since some salamanders have limited distributions on high elevations peaks, we hypothesized vegetation characteristics could be associated with salamander occupancy. These results will provide land managers with an initial strategy to manage habitat. Finally, in Chapter 5, we modeled occupancy of Plethodontid salamanders on both the north and south slope of Whitetop Mountain in relation to mean January and July temperatures at 30.5 m elevation intervals. Using global circulation models, we projected future temperatures at each elevation with 2 different emission scenarios. Salamander occupancy was then modeled based on future temperature projections for 2030, 2050, and 2070.

LITERATURE CITED

- Ash, A. N. 1997. Disappearance and return of Plethodontid salamanders to clear cut plots in the southern Blue Ridge Mountains. *Conservation Biology* 11:983-9.
- Bank, M.S., C. S. Loftin, and R. E. Jung. 2005. Mercury bioaccumulation in northern two-lined salamanders from streams in the Northeastern United States. *Ecotoxicology* 14:181-91.
- Bartoszek, J. and K. R. Greenwald. 2009. A population divided: railroad tracks as barriers to gene flow in isolated population of marbled salamanders (*Ambystoma opacum*). *Herpetological Conservation and Biology* 4:191-7.
- Bergeron, C. M., C. M. Bodinof, J. M. Unrine, and W. A. Hopkins. 2010. Mercury accumulation along a contamination gradient and nondestructive indices of bioaccumulation in amphibians. *Environmental Toxicology and Chemistry* 29:980-8.
- Boone, M. D. 2003. Effects of pesticides on amphibian populations. In *Amphibian conservation*, ed. R. D. Semlitsch, 152-67. Washington, DC: Smithsonian Books.
- Bridges, C. M. and R. D. Semlitsch 2000. Variation in pesticide tolerance of tadpoles among and within species of *Ranidae* and patterns of amphibian decline. *Conservation Biology* 14:1490-99.
- Burke, J. N., C. M. Bergeron, B. D. Todd, and W. A. Hopkins. 2010. Effects of mercury on behavior and performance of northern two-lined salamanders (*Eurycea bislineata*). *Environmental Pollution* 158:3546-51
- Carroll, D. M. 1999. *Swampwalker's journal. A wetlands year*. Boston: Houghton Mifflin.

- Caruso, N. M. and K. R. Lips. 2013. Truly enigmatic declines in terrestrial salamander populations in Great Smoky Mountains National Park. *Diversity and Distributions* 19:38-48.
- Davidson, C., H. B. Shaffer, and M. B. Jennings. 2001. Declines of the California redlegged frog: climate, UV-B, habitat, and pesticide hypotheses. *Ecological Applications* 11:464-79.
- deMaynadier, P. G., and M. L. Hunter. 1998. Effects of silviculture edges on the distribution and abundance of amphibians in Maine. *Conservation Biology* 12:340-52.
- Dillard, L. O., K. R. Russell, and W. M. Ford. 2008. Microhabitat models of occurrence for the threatened Cheat Mountain salamander, *Plethodon netting*. *Applied Herpetology* 5:201-24.
- Dillard, L. O., K. R. Russell, and W. M. Ford. 2008b. Site-level habitat models for the endemic, threatened Cheat Mountain salamander (*Plethodon netting*): the importance of geophysical and biotic attributes for predicting occurrence. *Biodiversity and Conservation* 17:1475-92.
- Gray, M. J., D. L. Miller, J. T. Hoverman. 2009. First report of *Ranavirus* infecting lungless salamanders. *Herpetological Review* 40:316-9.
- Gibbs, J. P. and W. G. Shriver. 2005. Can road mortality limit populations of pool-breeding amphibians? *Wetlands Ecology and Management* 13:281-9.
- Hayes, T.B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A.A. Stuart, and A. Vonk. 2002. Hermaphroditic, demasculinized frogs following exposure to the herbicide, atrazine, at ecologically relevant doses. *Proc. Nat. Acad. Sciences (USA)* 99: 5476-5480.
- Homyack, J. A. and C. A. Haas. 2009. Long-term effects of experimental forest

- harvesting on abundance and reproductive demography of terrestrial salamanders. *Biological Conservation* 142:110-21.
- Hutchinson, G. E. 1957. Concluding remarks. *Cold Springs Harbor Symposia on Quantitative Biology* 22:415-27.
- IUCN, Conservation International, and NatureServe. 2008. An Analysis of Amphibians on the 2008 IUCN Red List www.iucnredlist.org/amphibians. Downloaded on February 15, 2014.
- Kusano, T. and M. Inoue. 2008. Long-term trend toward earlier breeding of Japanese Amphibians. *Journal of Herpetology* 42:608-14.
- Lawler, J. J., S. L. Shafer, D. White, P. Kareiva, E. P. Maurer, A. R. Blaustein, and P. J. Barlein. 2009. Projected climate-induced faunal change in the Western Hemisphere. *Ecology* 90: 588-97.
- Lips, K. R., J. Diffendorfer, J. R. Mendelson, M. W. Sears. 2008. Riding the wave: reconciling the roles of disease and climate change in amphibian declines. *PLoS Biology* 6:441-54.
- Milanovich, J. R., W. E. Peterman, N. P. Nibbelink, J. C. Maerz. 2010. Project loss of a salamander diversity hotspot as a consequence of project global climate change.
- Moore, J. D. and R. L. Wyman. 2010. Eastern red-backed salamanders (*Plethodon cinereus*) in a highly acid forest soil. *American Midland Naturalist* 163:95-105.
- Petranka, J. W., M. E. Eldridge, and K. E. Haley. 1993. Effects of timber harvesting on low elevation populations of Southern Appalachian salamanders. *Conservation Biology* 7:363-70.
- Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N.

- Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, B. E. Young. 2006. Widespread amphibian extinctions from epidemic diseases driven by global warming. *Nature* 439:161-7.
- Reichenbach, N., and P. Sattler. 2007. The effects of timbering on *Plethodon hubrichti* over 12 years. *Journal of Herpetology* 41:622–629.
- Relyea, R. A. 2004. Growth and survival of five amphibian species exposed to combinations of pesticides. *Environmental Toxicology and Chemistry* 23:1737-42.
- Relyea, R. A., and J. T. Hoverman. 2008. Interactive effects of predators and a pesticide on aquatic communities. *Oikos* 117:1647-58.
- Riedel, B. L., K. R. Russell, W. M. Ford, K. P. O'Neill, H. W. Godwin. 2008. Habitat relationship of eastern red-backed salamanders (*Plethodon cinereus*) in Appalachian agroforestry and grazing systems. *Agriculture, Ecosystems and Environment* 12:229-36.
- Rimmer, C. C., E. K. Miller, K. P. McFarland, R. J. Taylor, S. D. Faccio. 2010. Mercury bioaccumulation and tropic transfer in the terrestrial food web of a montane forest. *Ecotoxicology* 19:697-709.
- Semlitsch, R. D., B. D. Todd, S. M. Blomquist, A. J. K. Calhoun, J. W. Gibbons, J. P. Gibbs, G. J. Grater, E. B. Harper, D. J. Hocking, M. L. Hunter Jr., D. A. Patrick, T. A. G. Rittenhouse, B. B. Rothermel. 2009. Effects of timber harvest on amphibian populations: understanding mechanisms from forest experiments. *BioScience* 59:853-62.
- Sodhi N.S., D. Bickford, A. C. Diesmos, T. M. Lee, L. P. Koh, B. B. Brook, C. H. Sekercioglu, C. J. A. Bradshaw. 2008. Measuring the Meltdown: Drivers of Global Amphibian Extinction and Decline. *PLoS ONE* 3(2): e1636. doi:10.1371/journal.pone.0001636.
- Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman,

- R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783-6.
- Ugarte, C. A., K. G. Rice, and M. A. Donnelly, 2005. Variation of total mercury concentrations in pig frogs (*Rana grylio*) across the Florida Everglades, USA. *Science of the Total Environment* 345:51-59.
- Wake, D. B. 1991. Declining amphibian populations. *Science* 253:860.
- Wake, D. B. and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Science* 105:11466-73.
- Walls, S. C., W. J. Barichivich, M. E. Brown, D. E. Scott, B. R. Hossack. 2013. Influence of drought on salamander occupancy of isolated wetlands on the Southeastern Coastal Plain of the United States. *Wetlands* 33:345-54.
- Wells, K. D. 2007. *The Ecology and Behavior of Amphibians*. Chicago, IL: The University of Chicago Press.
- Welsh, H. H., J. R. Dunk, W. J. Zielinski. 2006. Developing and applying habitat models using forest inventory data: an example using a terrestrial salamander. *The Journal of Wildlife Management* 70:671-81.
- Whitfield, S. M., K. E. Bell, T. Philippi, M. Sasa, F. Bolanos, G. Chaves, J. M. Savage, and M. A. Donnelly. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of National Academy of Science* 104:8352-6.
- Wyman, R. L. and D. S. Hawksley-Lescault. 1987. Soil acidity affects distribution, behavior, and physiology of the salamander *Plethodon cinereus*. 1987. *Ecology* 68:1819-27.

- Wyman, R. L. and J. Jancola. 1992. Degree and scale of terrestrial acidification and amphibian community structure. *Journal of Herpetology* 26:392-401.
- Young, B. E., S. N. Stuart, J. S. Chanson, N. A. Cox, and T. M. Boucher. 2004. *Disappearing Jewels: The Status of New World Amphibians*. NatureServe, Arlington, VA.

**CHAPTER 1: IMPACT OF POWERLINE RIGHT-OF WAY MOVING ON
FOUR-TOED SALAMANDER NESTING AND LARVAL SURVIVAL**

ABSTRACT

Over 2.8 million ha of powerline right-of-ways (ROW) are maintained each year in the United States, often with mechanical mowing. Limited data exist on the impact of this vegetation removal to amphibians especially Plethodontid salamanders. We investigated the impact of ROW mowing on the nesting and larval success of four-toed salamanders (*Hemidactylium scutatum*) throughout a 5-year mowing cycle. Nesting success was determined one year prior and 5 years after mowing by quantifying the number of embryos that survived to the larval stage. We established a 5-year mowing and annual mowing plot to determine larval recovery time after mowing. Within each plot, arrays of mesocosm pools were placed 9 m into both the ROW and into the forest at 3 m increments. Nesting success in the ROW was significantly less than in the forest 3 years after mowing. Almost 17% of female salamanders returning to forest nests had previously nested in the forest, but no female salamander that had previously nested in the ROW was found to return. Vegetation succession improved larval survival 6 and 9 m into the ROW as survival rates were significantly greater than from pools in the 5-year treatment than from pools in the annual mowing treatment 3 and 4 years post mowing, respectively. Larvae from pools 9 m into the annual treatment consistently produced smaller salamanders. There was a strong negative relationship between temperature and larval survival which suggested vegetation regrowth produced cooler temperatures which probably increased larval survival. Four-toed salamander reproductive efforts are negatively affected by mowing for at least 3 years after mowing.

INTRODUCTION

Amphibian populations worldwide have been declining for at least the past 25 years (Stuart et al. 2004; Wake and Vredenburg 2008). Habitat alteration, habitat loss, climate change, exotic species, and pathogens have been identified as contributors to this decline, with habitat loss and alteration as the leading global threat (Lannoo 2005; Gardner et al. 2007; Sodhi et al. 2008). In eastern North America, silvicultural practices have been linked to reductions in habitat suitability for many amphibian species (Knapp et al. 2003; Homyack and Haas 2009; Semlitsch et al. 2009). In particular, clear-cutting forests eliminates necessary shade and leaf litter for amphibians and has been shown to reduce terrestrial salamander abundance and diversity by increasing temperature and reducing moisture (Petranka et al. 1993). Of all forest-dwelling vertebrates, amphibians are most vulnerable to desiccation from forest removal due to their semi-permeable skin that lacks protective covering (Spight 1968).

Most of the research on the impacts of timber harvesting on amphibians has focused on anurans (frogs and toads) and salamanders in the family *Ambystomatidae*, but pond breeding Plethodontid salamanders have not been examined. The effects of habitat clearing on amphibians varied. Gray treefrogs (*Hyla versicolor*), wood frogs (*Rana sylvatica*), leopard frogs (*Rana pipiens*), and American toads (*Bufo americanus*) metamorphosed in less time, but were smaller in open areas such as forest clearcuts (Hocking and Semlitsch 2008; Skelly et al. 2002; Werner and Glennemeier 1999). Smaller amphibian metamorphs were less likely to reach adulthood than larger metamorphs (Rothermel and Semlitsch 2002). As the distance increased from the forest edge to the pool in which metamorphosing amphibians emerged, survival decreased (Rothermel 2004). Juvenile and adult amphibians experienced greater water loss in open areas due to increased temperatures, which contributed to increased mortality (Rothermel

and Luhring 2005; deMaynadier and Hunter 1998; Rothermel 2004). Microhabitat conditions (leaf litter and cover objects) needed for survival are significantly reduced in edge and open areas (deMaynadier and Hunter 1998). Thus, survival in forest openings was typically reduced (Semlitsch et al. 2009).

Maintaining powerline, pipelines, or railroad right-of-ways (ROW) through forested areas removes trees and creates a narrow linear corridor of disturbed edge habitat thus modifying the habitat in a manner similar to a small clearcut. Over 2.8 million ha of powerline right-of-ways (ROW) in the United States must be maintained each year. Mechanical mowing is the most common method of vegetation control, out ranking chemical treatment 2.7:1 (Sulak and Kielbaso 2000). Utility companies prefer chemical application, but negative public opinion has reduced its use. New regulations established in September 2013 by the Federal Energy Regulatory Commission require that all electric utility providers maintain ROWs from vegetation and recommended clearing beyond the minimal requirements. If vegetation within a ROW causes a disruption to electrical service, the utility would receive a considerable fine (USFERC 2013). Therefore, many utilities are now clearing an even greater amount of vegetation for ROWs.

Numerous studies have examined the effects of ROWs on birds (Robinson et. al 1995; Askins et al. 2012;), reptiles (Soper and Schoeberl 1997; Wester and Kolb 1997), and mammals (Johnson et. al 1979; Gossen and Marsh 1997; Rieucan et al. 2007; Clarke and White 2008), but limited data exist on the impacts of ROWs on amphibian populations. In Pennsylvania, red-backed (*Plethodon cinereus*), spotted (*Ambystoma maculatum*), and Jefferson salamanders (*Ambystoma jeffersonianum*) used ROWs in the wire zone (area directly under the lines) and in the border zone (area between wires and forest that is cleared and maintained, Yahner et al.

2001). Although a difference in the number of salamanders in the ROW compared to the adjacent forest was detected, survival rate or occupancy duration was not examined (Yahner et al. 2001). Use of ROWs by amphibians could be increasing as wetlands are being constructed therein for mitigation (T. Biebighauser, USDA Forest Service, personal communication). Amphibians are often drawn to ROWs to reproduce with unknown consequences.

In addition to the loss of forested habitat, powerline ROWs create edge habitat, which has been shown to act as barriers to movement, increase predation, and serve as population sinks for many species of wildlife (Noss 1991; Willyard et al 2004). Amphibians are affected by edge habitat by decreased microhabitat conditions (e.g. increased soil temperature and decreased leaf litter depth; deMaynadier and Hunter 1998). Edge effects could also reach deep into the forest, greatly expanding the width of the potential ROW impact. Edge effects on Plethodontid salamanders from roads extended greater than 50 m into forest areas from each side of the road (Semlitsch et al. 2008). Spotted salamanders (*Ambystoma maculatum*) avoided grassland edges which served as a barrier to juvenile dispersal (Rittenhouse and Semlitsch 2006). Additionally, amphibian predators such as snakes are more abundant in edge habitat than in the forest which contributed to the decreased survival of juveniles Ambystomatid salamanders (Yahner et al. 2001; Rothermel and Semlitsch 2002).

In the eastern United States a salamander in the family *Plethodontidae*, the four-toed salamander (*Hemidactylium scutatum*), nests in small pools and shallow streams with limited flow in flooded forest. During the non-breeding season, the species resides in the upland forest (Petranka 1998). The salamander can be found from Nova Scotia to Florida (Petranka 1998), but is often found in isolated populations due to forest fragmentation. Because of a reliance on forested habitat, the four-toed salamander could potentially suffer negative effects from ROW

maintenance. A four-toed salamander population in Sullivan County, Tennessee at the Tennessee Valley Authority's South Holston Weir Dam resides in an area dissected by three separate electric transmission lines which carry electricity generated from the South Holston Dam (Hamed and Gentry 2003). One transmission line passes through a four-toed salamander nesting area, thus providing an opportunity to investigate the potential effects of ROW maintenance on four-toed salamander survival and recruitment.

Potential negative effects of ROWs on four-toed salamanders could have major implications as it has a limited distribution throughout its range. In the 30 eastern states it inhabits, 9 states list this species as being rare (Chalmers 1998). In Tennessee, the four-toed salamander is found mainly in the central and eastern portions of the state, with four disjunct locations in the northeastern portion of the state (Redmond and Scott 1996; Herman 2009). This sparse distribution has resulted in the four-toed salamander being classified as "in need of management" in Tennessee (TWRA 2000). Four-toed salamanders are nesting in ROWs other than this site, as four-toed salamanders have been documented to nest in ROWs in Kentucky (Personal conversation Biebighauser), Wisconsin (Willyard et al 2004), and potentially other areas. The impact to the nesting success of these animals is not known.

Four-toed salamander nests are usually found within moss clumps of the genera *Thuidium*, *Mnium*, *Sphagnum*, *Climacium*, *Atrichum*, *Aulatcomnium*, *Cirriphyllum*, *Entodon*, *Eurhynchium*, *Hypnum*, *Leptodictyum*, *Leucobryum*, or *Plagiothecium* above the waterline around pools and sluggish streams (Gilbert 1941; Wood 1955; Chalmers and Loftin 2006; Wahl et al. 2008). Four-toed salamanders are adapted to short hydroperiods and have one of the shortest aquatic larval periods (38–60 days; 21 days in laboratory conditions) of a Plethodontid salamander (Blanchard 1923; Wood 1955; Berger-Bishop 1996; Harris and Ludwig 2004).

Temperature influences nesting success, as greater nest temperatures on south facing pond slopes have been shown to decrease embryonic survival (Wahl et al. 2008). Vegetation clearing could increase nest temperatures thereby decreasing embryonic survival. Additionally, high incidence of solar radiation in ROWs could increase temperature in water bodies where four-toed salamander larvae develop and decrease survival and recruitment rates. Development rates of amphibian larvae are positively correlated with ambient temperature, thus greater rates also could lead to faster development and metamorphosis at smaller body size (Hocking and Semlitsch 2008, Schiesari 2006). Body size at metamorphosis has been shown to be positively related with adult fitness (Semlitsch et al. 1988). Therefore, impacts from ROW mowing could carry into the adult population.

A standard practice to maintain ROWs is mechanical mowing every 5 years, which occurs at the South Holston Dam breeding site. The effects of mowing would likely decrease as vegetation proceeds through succession. To date, no studies have investigated the effects of mowing ROWs on breeding amphibian populations. Natural and manmade depressions exist in the South Houston Dam ROW and are being used for breeding by 4-toed salamanders. Given the amount of land dedicated to ROWs in the United States, it is important to measure the impacts of this land use practice on amphibians. Additionally, given the rarity of four-toed salamanders, improved management could benefit the species not only at our study site, but in other areas with ROWs. We examined the effects of ROW mowing on four-toed salamander nesting and larval survival. Nesting success was compared before mowing and each season after mowing for 5 years. Additionally, we used mesocosm pools to examine the potential impact of ROW mowing on larval success and development between mowing cycles.

METHODS

Study area

Our study area was located in eastern Sullivan County, Tennessee on the Tennessee Valley Authority (TVA) South Holston Dam property. Four-toed salamanders nested within an area bordered by Holston View Dam Road to the north, South Holston River to the west, and the TVA property boundary to the south and east. Two roads (TVA Road S and Henson Hill Rd.) bisected the sites and are perpendicular to ROW. The study site was centered on the county record for the four-toed salamander (Hamed and Gentry 2003). Prior to the completion of the South Holston Dam in 1950, the entire area was in the floodplain and typically flooded at least every 5 years. Three electric powerlines dissect the TVA property, but only one dissects the study area. This powerline has a maintained ROW with a mean width of 40 m.

Four-toed salamanders nested in the ROW and the forested area adjacent to the ROW. The floodplain forest consisted mainly of boxelder (*Acer negundo*), sycamore (*Plantus occidentalis*), white oak (*Quercus alba*), sweetgum (*Liquidambar stryaciflua*), and paw paw (*Asimina triloba*) trees. Most trees within the forested area are less than 60 years old as the dam was completed in 1950. Vegetation in the ROW varied depending on time since last mowing, but was typically composed of blackberries (*Rubus fruticosus*), iron weed (*Vernonia altissima*), poison ivy (*Toxicodendron radicans*), common milkweed (*Asclepias syriaca*), and sweetgum (*Liquidambar stryaciflua*). The forested area and the ROW had separate ephemeral streams flowing through them and into the South Holston River.

Nesting success

Four-toed salamanders are known to nest between February – May in the Southern Appalachian Region (Petranka 1998). In previous work that we have performed, four-toed

salamanders typically began egg laying in mid-March at the study site (Hamed, unpubl. data). Thus, we began to search for nests in March from 2007-12. We searched clumps of moss in known four-toed salamander nesting locations as well as undocumented areas with potential nesting habitat in both the ROW and forest. Once a nest was located, we placed a metal wire, with a plastic flag and labeled with an identification number in the moss behind the nest to mark its location. We noted the presence or absence of the female salamander at each nest. Checking nesting habitat for eggs and female presence has not deterred females from future nesting or cause females to abandon their nest (Harris et al. 1995). We captured female salamanders in the nest by hand and measured their snout-vent length (SVL) to the nearest 0.1 mm with dial calipers from the tip of their snout to the posterior portion of the cloacal opening. We also measured the total length (TL) from the tip of the snout to the posterior tip of the tail with dial calipers to the nearest 0.1 mm. We compared both SVL and TL between salamanders nesting in the ROW and forest. To determine potential predation attempts, we noted females with tail loss or damage and compared rates of damage between forest and ROW nests. A female salamander could have nested in either forested or ROW areas in one nesting attempt and then chosen a different location in subsequent years as vegetation changed. To allow for future identification, we took a digital photograph of each female salamander's ventral surface with a Nikon 5400 Coolpix camera. Four-toed salamanders have unique ventral spot patterns that allow for identification (Bishop 1941; Harris and Gill 1980; Harris et al. 1995). We placed the female in the lid of a petri dish and then placed the base portion in the lid above the salamander. We applied slight pressure to the petri dish base which pressed the ventral surface against the larger lid and rotated the petri dish was so that a picture of the ventral surface could be obtained. This method has been successfully used to photograph ventral surfaces in other four-toed salamander studies

(Harris and Ludwig 2004). To determine nest site fidelity, we compared photographs from each nesting year using at least 20 different observers per nesting seasons. Observers consisted of general biology students who volunteered to assist with the project. Each semester from 2010 – 2012, a different group of student volunteers compared images. Observers were assigned a small group of female salamander photographs and then compared all other photographs from previous nesting years to their salamanders. Once individual salamanders were identified, nesting locations were compared each year to determine if site fidelity exists.

If a female salamander was in the process of laying eggs when discovered, we revisited the nest 48 hours later and subsequently until completion of egg laying. We counted eggs by gently sifting through the moss. We revisited nests without a female present and if the female salamander was still not present we classified the nest as abandoned. Four-toed salamanders are known to have joint nest with multiple females depositing eggs in the same nest (Blanchard 1934) and we classified nests as being joint if the number of eggs was ≥ 40 . The maximum number of eggs a female will lay is 39 (Blanchard 1934; Gilbert 1941; Breitenbach 1982; Harris 2008) and previous work in our study area indicated singled nests have less than 40 eggs (Hamed, unpubl. data). To determine the number of eggs that survive to the larval stage, we installed a 3.79-L plastic bucket just below each nest and filled with water from the pool below the nest. When eggs hatched larvae attempted to fall down into the pool but were be captured by the buckets. The number of larvae captured in the bucket represented the embryonic survival for the nest. We compared the percentage of embryonic survival from ROW and forest nests to determine the impact of ROW mowing on 4-toed nesting success. In 2007, one year prior to ROW mowing, we randomly chose 11 nests in the forest and 10 nests in ROW utilizing a table of random numbers. We installed buckets on the randomly chosen nests and determined

embryonic survival as part of a pilot study on embryonic survival. In all future years we sampled every nest in both ROW and forested areas by installing buckets.

Nests microhabitat comparisons

We first measured microhabitat in March 2008 (first year post-mowing) and continued through the conclusion of the 2012 nesting season (April 2012). We measured various nest characteristics including distance above the water, pool depth, and nest aspect. We compared nest characteristics among forest and ROW nests and related characteristics to survival to determine which characteristics were related to four-toed salamander nesting success. We measured distance above the water to the center of the egg mass (nest) to the nearest 0.1 cm using a metric measuring tape. We measured pool depth below the nest at the deepest location using a metal meter stick (0.1 cm). To determine nest aspect we placed a compass at the edge of the nest slope, in which the larvae would fall once they hatch, and recording the slope aspect to the nearest 5°.

Impact of ROW mowing on larval survival and fitness

We used aquatic mesocosms to determine the effects of ROW management on four-toed salamander larval development and survival. We placed mesocosms in two ROW treatment areas: annual mowing and 5-year mowing. Treatment areas were 15 x 15 m in the ROW and an equal size in the adjacent forest (Figure 1.1). The entire ROW was mowed in August 2007 with a tractor and rotary cutter. All vegetation 5 cm above the ground was cut and debris was mulched. This area served as our 5-year mowing treatment and was not mowed until the conclusion of the project (August 2012), which was also the end of the current mowing cycle. We established an annual mowing treatment in the ROW which was mowed each August (2008-

11) to the same height as the initial mowing treatment in August 2007. Both treatments were in the same ROW and were separated by an unmowed buffer of 20 m.

Mesocosms were 200-L plastic wading pools (General Foam Plastic, Norfolk, VA) covered with charcoal colored, fiberglass window screen (1.5 mm mesh) tops. We attached screen covers to weighted plastic tubing which encircled the outer pool edge. We established a 7 x 4 array (row x column) of mesocosm pools for each mowing treatment in May of each year, with rows of pools positioned different distances from the forest edge (Figure 1.1). For each treatment, we placed one row of 4 pools at the forest edge, with three subsequent rows of 4 pools set every 3 m (distance between pool centers) into the forest and ROW out to 9 m. Thus, each array consisted of 28 pools (Figure 1.1). We filled pools with 189 L of tap water obtained directly from the Bristol, Tennessee water plant and left in pools for 4 days to dechlorinate. Then we added 100 g of leaf litter (obtained from the study site) and 2 g of Purina Rabbit Chow® to the pools to provide nutrients (Relyea et al. 2005). We inoculated pools 3 days later with 1 L of water from a local vernal pool (~400 m from pool arrays) to provide zooplankton for developing four-toed salamander larvae. Care was taken not to introduce predators into mesocosms with zooplankton.

We pooled four-toed salamander larvae that were collected in plastic buckets below nests to determine embryonic survival and randomly divided larvae into groups of 10 within 24 hours of hatching. Each mesocosm pool received a group of 10 larvae. This density was representative of densities found in naturally occurring four-toed pools in the study area (Hamed, unpubl. data). Before larvae were separated into groups, we randomly selected and euthanized 13-15 larvae with MS-222. We measured their SVL and TL using dial calipers to the nearest 0.1 mm and measured mass using an Ohaus (Parsippany, NJ) Adventure Pro digital scale to the

nearest 0.001 g. To determine if differences existed between hatching size each year, initial values for each year were compared. We pooled individuals as measuring each larva before placing them into mesocosm pools would have caused additional stress and injuries, potential affecting the results of the mesocosm experiment. Once we placed larvae into pools, we placed fiberglass screen covers over the pools and secured them with stakes, which created constant contact of the screen to the pool edge and eliminated the possibility of larvae or metamorphs escaping from the pools.

Each week we searched pools by hand to locate all surviving larvae. We captured larvae by using a small dip net (20 x 12 cm) with 2 mm mesh and placed larvae into a separate container to ensure they were only counted once. After a complete investigation, we recorded the number of surviving larvae and returned all larvae to the pool. We continued to count the number of surviving larvae until all larvae metamorphosed or died. This ensured that true larval survival was recorded and any escaped larvae were not considered as a mortality event.

We checked pools twice daily (morning and late evening) as time to metamorphosis approached. Metamorphosed larvae were typically found along the pool edge just above the water line. Once larvae reach metamorphosis we measured their SVL, TL, and mass with the same methods used to measure adults. We collected any larvae that drown during metamorphosis and measured their SVL and TL. Mass was not measured because of additional water added due to absorption. We deemed individuals that drown as survivors because they reached metamorphosis and most likely could have exited a natural pool. We recorded the date each salamander emerged from the water as its metamorphosis date and calculated time to metamorphosis as time of initial placement in the pool (1-day post hatching) until metamorphosis date. We compared time to metamorphosis between treatments and years since

mowing. We also compared larval survivor rates, size at metamorphosis, and time to metamorphosis among treatments, time since mowing, and distance into the forest and ROW.

We measured pool temperatures and productivity to determine the potential impact on larval survivorship, size at metamorphosis, and days to metamorphosis. We placed a HOBO pendant data logger (Onset Corporation; Bourne, MA) in each pool to measure water temperature every 10 minutes. We compared both mean and mean maximum pool temperatures. Since pool temperatures continued to increase from May – July and larvae completed metamorphosis much quicker in some pools; comparing pool temperatures from the beginning to the end of each season would have provided unrealistic views of what larvae actually experienced. Therefore, we no longer recorded pools temperatures 48 hours after the last larvae metamorphosed, thus mean and maximum temperatures reported reflected temperatures actually experienced by the larvae. We compared pool temperatures between treatments and distance into the ROW and forest. To determine net primary productivity (NPP), we measured dissolved oxygen (DO; 0.01 mg/L) every hour from each pool for 24 hours. We used a YSI model number 55 DO meter (Yellow Springs Instruments, Yellow Springs OH) to determine DO levels in 2009 & 2010 and a YSI model number 50B DO meter (Yellow Springs Instruments, Yellow Springs OH) in 2011 & 2012. Since different meters were used, comparisons between years was not attempted, but comparison within the same year between treatments and distances in the ROW were made. Changes in DO levels have been used to determine total net productivity by comparing changes in DO levels from sunrise to sunset (Earl and Semlitsch 2012; Wetzel and Likens 2000). We used the peak DO measurement instead of the measurement immediately before sunset. Other studies (Earl and Semlitsch 2012; Williams et al. 2008) found no difference in DO levels prior to sunset and maximum values, but in our pools different levels of shading,

due to location and time since mowing, created differences in maximum and minimum DO. Therefore, we chose to use the highest level recorded, which was typically between 17:00 – 19:00 hours. We measured dissolved oxygen 14 days after larvae were introduced into pools and only if no precipitation had occurred, cloud cover was minimal during day light hours, and wind velocity was minimal to prevent ripping of water. If these conditions were violated, sampling occurred the next day in which conditions would be met. As long as those conditions were obtained, we assumed a diffusion coefficient (k) of 0.05 as established for pools (Wetzel and Likens 2000). We used the following formula (Earl and Semlitsch 2012) to establish NPP:

$$NPP = (O_{2(\text{highest})} - O_{2(\text{lowest})}) - (k (O_{2(\text{average})} - O_{2(\text{average saturation})})).$$

Average oxygen saturation was correlated with air temperature (°C), which we obtained with DO readings, and based on published values from Wetzel and Likens (2000). We compared net primary production between treatments, pool location, and time since mowing.

Statistical analyses

We used a chi-square goodness-of-fit test to determine if joint or guarded nests were associated with forest or ROW areas. Additionally, we used a chi-square test to determine if female salamanders experienced tail damage or loss more frequently in forest than ROW nests. Before comparisons between nesting areas or mesocosm treatments were made, we tested the assumptions for analysis of variance (ANOVA). We used a Shapiro-Wilk's test to assess data for normality (IBM 2011). We $\log_{10}$ transformed data or utilized non-parametric analyses for data which were not distributed normally. We employed a one-way ANOVA to determine if size of nesting females, numbers of eggs per nest, nest height above water, water depth, and percent egg survival were greater in the forest than in the ROW. We conducted a Pearson's correlation to determine the relationships for each microhabitat variable (distance above the nest, pool depth,

and aspect) as well as female SVL and embryonic survival. We compared percent survival of larvae, mean SVL, mean TL, mean mass of larvae, and mean days to metamorphosis from mesocosm pools between treatments using a one-way ANOVA and a Tukey's post-hoc analysis. We also made comparisons with dependent variables between distances into the ROW or forest but within the same treatment (e.g. annual or 5-year mow) and at the same distance into the ROW or forest but between treatments. We used a repeat measures ANOVA to compare dependent variables between treatment years. To determine the potential interaction of ROW treatment and distance on the dependent variables from above, we used a two-way ANOVA. We compared mean, maximum temperature, mean temperature, and mean net primary productivity with pools at the same distance into ROW or forest between treatments with a Mann-Whitney test (IBM 2011). We compared temperature and productivity between distances but within the same treatment with a Kruskal-Wallis test with post-hoc, pair-wise comparisons utilizing a Mann-Whitney test with Bonferroni corrections (IBM 2011). Pool temperatures were compared between treatment years with a Friedman's test. Lastly, we used a Spearman's rank-order correlation to determine if there is a correlation between mean temperature, mean maximum temperature, and NPP of mesocosm pools and the mean days to metamorphosis or percent survival.

RESULTS

Nesting success and microhabitat comparisons

We located 381 four-toed salamander nests from 2007 – 2012 and nests per season ranged from 82 (2007) to 47 (2010). Prior to mowing, no association was detected between nest location and abandonment ($\chi^2 = 2.86$, $df = 1$, $P = 0.09$; Table 1.1). However, after mowing,

female salamanders abandoned ROW nests significantly more than expected and the trend continued for 2 years (2010) after mowing ($\chi^2 = 6.70 - 13.97$, $df = 1$, $P = 0.001 - 0.01$; Table 1.1). Abandonment rates in ROW nests did not differ from expected 3 and 4 years (2011-12) after mowing ($\chi^2 = 0.13 - 3.77$, $df = 1$, $P = 0.07 - 0.72$; Table 1.1). Within each treatment, abandoned nest had significantly lower survival than guarded nests. The mean survival rate for guarded nests was 62.36% (95% CI, 56.21 – 68.51) in the forest, but was significantly reduced to 21.61 % (95% CI, 9.02 – 34.19; $F_{(1,168)} = 29.37$, $P < 0.001$) for abandoned nests. A similar trend existed in the ROW as 38.35% (95% CI, 30.07 – 46.64) of embryos in guarded nests survived while only 7.78% (95% CI, 0.05 – 15.5; $F_{(1,132)} = 19.81$, $P < 0.001$) survived in abandoned nests. The presence of mowing was unrelated to the rate of joint nesting as joint nests were as associated with forested areas (8.3 – 35.0 %) or ROW (4.5 – 35.9%) areas across all treatment years ($\chi^2 = 0.01 - 3.41$, $df = 1$, P - values: 0.132 – 0.946).

No difference in female SVLs was detected from nests in forested areas and ROW at any treatment period (all P -values > 0.135 ; Table 1.2). However, in the ROW mean percent survival was weakly, positively correlated with female SVL ($r = 0.250$, $P = 0.021$). Because few female salamanders exhibited signs of recent predation attempts (tail loss) each year, all values were combined and compared across the experiment. We did not detect a relationship between tail damage and ROW treatment ($\chi^2 = 0.971$, $df = 1$, $P = 0.325$).

Forest nests were significantly (73.63%) higher than ROW nests 2-years post-mowing (PM) as mean height above water was 8.98 cm (95% CI, 6.86 – 11.75) in forested areas and 5.89 cm (4.18 – 6.27) in ROW areas ($F_{1,42} = 10.77$, $P = 0.002$). Forested nests were also significantly (69.36%) higher 4-years PM. Mean height of forest nests was 8.18 cm (6.79 – 9.85), but in ROW areas mean nest height was 4.83 cm (3.77 – 6.20; $F_{1,55} = 12.40$, $P = 0.001$; Table 1.3).

However, the year immediately after mowing ROW nests were significantly (39.05%) higher than forest nests ($F_{1,51} = 4.32$, $P = 0.04$). Nests in ROWs had a mean height of 8.19 cm (7.20 – 9.33) above water, but mean height for forests nests was 5.89 (4.12 – 8.42) cm above water (Table 1.3). Differences in mean height above water were not significant for 1-year or 3-years PM ($P = 0.587$; $P = 0.538$ respectively). Mean water depth was significantly deeper (125.32%) in forested areas than ROW areas immediately after mowing ($F_{1,52} = 23.57$, $P < 0.001$). Breeding pools in forested areas averaged 10.59 cm deep (7.97 – 14.13), but pools in ROWs only averaged 4.70 cm (3.82 – 5.78) deep. Water depths between treatments were not significant for other years (P -values: 0.151 – 0.67). Median aspects for nesting locations did not differ between forest or ROW nests for any treatment year (P -values: 0.072 – 0.79; Table 1.3). Mean distance above water, pool depth, or aspect were not significantly correlated with mean percent embryonic survival (r : -0.154 – 0.097, P -values: 0.265 – 0.854).

Embryonic survival in nests with female four-toed salamanders present was significantly greater in forested areas compared to ROWs in the year of mowing and for two subsequent years PM. Survival immediately following mowing was 73.14% (± 7.84 ; SEM) in forested nests, but only 42.63% (± 7.52) in ROW nests. Both 1-year and 2-years PM, survival was significantly ($F_{1,42} = 5.76$, $P = 0.02$; $F_{1,47} = 4.87$, $P = 0.03$; respectively) greater in forested areas (62.70% $\pm$ 6.53; 63.99% $\pm$ 7.23; respectively) than ROWs (34.20% $\pm$ 11.67; 28.16% $\pm$ 10.76; respectively). However, no significant differences were observed the year prior to mowing or 3- and 4-years PM (Table 1.4; Figure 1.2).

Female salamanders re-nesting differed between treatments. Overall 10% of females nesting from 2010-2 had nested previously. All returning salamanders had previously nested in and returned to the forested area. No salamanders initially identified from the ROW were

recaptured. The percent of nesting females returning did increase each year from 12.0% (2010) to 17.14% (2012). All but one female returned the year immediately after her previous nesting attempt. The lone female salamander that skipped a year nested in 2009 and returned in 2011. Females not only returned to the forest again, but 61.5% of returning females nested in the exact same location as their previous nesting attempt. The other (30.8%) females moved within 10 m of their original attempt (Table 1.5). One female moved 26 m between nesting attempts and this was the lone female that skipped a nesting season. A female displaying tail damage in her initial nesting year returned the next 2 years with a regrown tail and produced full clutches (38) eggs (Table 1.5).

Impact of ROW mowing on larval survival

Larval percent survival varied among treatments and years since mowing. One-year PM larvae from pools in the annual mowing treatments (AT) showed significant differences in mean percentage survival with distance into the ROW ($F_{6,21} = 6.73$, $P < 0.001$). Mean percent survival was also significantly different in the 5-year mowing treatment (FT) with distance into the ROW both 1- and 2- years PM ($F_{6,21} = 4.82$, $P = 0.003$; $F_{6,21} = 3.23$, $P = 0.021$; respectively). A post-hoc analysis indicated that distance into the ROW significantly affected larval survival as ROW pools had lower mean percent survival. In the AT, mean percent survival was significantly less from pools 9 m (7.50%) and 6 m (7.50%) into the ROW compared to pools at the edge (62.50%), 3 m into the forest (55.00%), and 6 m (57.50%) into the forest (Table 1.6). A similar trend was noted in the FT as larvae from pools 9 m (15%) and 6 m (10%) into the ROW had significantly less mean survivorship than larvae from pools 6 m into the forest (80%; Table 1.6). Two-years PM in the FT, mean percent survival in pools 3 m into the ROW (22.50%) was significantly less than pools 6 m (80%) into the forest (Table 1.6). Mean percent survival was only significantly

different between treatments at 9 m into the ROW and from 4-years PM (80.0 % FT; 27.50% AT; Table 1.6). A 2-way ANOVA indicated no evidence of interaction between treatment and distance on larval survival (P -values: 0.171 – 0.609).

A repeated measures ANOVA indicated years since mowing did significantly affect mean larval percent survival in the AT, 6 m into the ROW ($F_{3,9} = 9.39$, $P = 0.004$) and in the FT at 9 m ($F_{3,9} = 5.27$, $P = 0.011$), 6 m ($F_{3,9} = 6.85$), and 3 m into the ROW ($F_{3,9} = 15.82$, $P = 0.001$). A post-hoc analysis with a Bonferroni adjustment indicated survival significantly increased in FT pools, 9 m into the ROW from 1-year (15%) and 2-years (27.5%) PM to 4-years PM (80%; Table 1.7; Figure 1.3). Mean percent survival from pools 6 m into ROW increased from 1-year PM (10%) to 3- (70%) and 4-years PM (52.5%; Table 1.7; Figure 1.4). A similar trend was observed 3 m into the ROW as mean percent survival significantly increased from 1-year PM (35.00%) to 3-years PM (65%; Table 1.7). Also at 3 m into the ROW, mean percent survival significantly increased from 2-years PM (22.5%) to 3- and 4-years PM (65%; Table 1.7). Within the AT the only significant increase was 6 m into the ROW from 1-year PM (7.5%) to both 2- (65%) and 3-years (55%) PM (Table 1.7).

Larval starting size did not differ among years ($F_{3,50}$: 1.51-2.18, P -values: 0.092 – 0.196). Minimal differences in SVL for four-toed salamander larvae raised in microcosm pools were detected and were inconsistent with changing ROW conditions (Table 1.8). No significant differences were detected in mean SVL from larvae from pools at different distances within the same treatment and years (P -values: 0.06 – 0.854) or as time since mowing increased (P -values: 0.157 – 0.817). There was no interaction detected between distance and treatment for larval SVL (P -values: 0.294 – 0.636).

Time since mowing and distance into the ROW significantly affected larval TL. Four-toed salamander larvae from FT pools, 9 m into the ROW were significantly larger 2-years PM (25.52 mm) than larvae from AT pools at the same distance (23.06 mm; Table 1.8). Larvae from FT pools, 6 m into the ROW were significantly larger (25.92 mm) than larvae from AT pools at the same distance (24.41 mm; Table 1.8), 2-years PM. Total length also varied within treatments and years. Mean TL was significantly different from AT pools at different distances for both 2010 ($F_{6,21} = 2.69$, $P = 0.043$) and 2012 ($F_{6,20} = 3.30$, $P = 0.02$). A Tukey post-hoc analysis indicated mean larval TL from pools 9 m into the ROW (23.06 mm) was significantly shorter than from pools 6 m into the ROW (25.57 mm) 2-years PM (Table 1.8). A similar trend continued 4-years PM, as mean larval TL from pools 9 m into the ROW, was shorter (22.32) than from pools 3 m into the ROW (25.16) and 3 m into the forest (25.38; Table 1.8). However, TL did not significantly differ with time since mowing as a repeat measures ANOVA indicated no differences (P -values: 0.158 – 0.787). A 2-way ANOVA did not indicate interactions between treatment and distance into the ROW on larval TL (P -values: 0.106 – 0.992).

Mean mass at metamorphosis was significantly greater for larvae from the FT (0.11 g) than AT (0.08 g) in pools 3 m into the ROW, 1-year PM (Table 1.8). Four-years PM, larvae from the FT (0.08 g) were significantly heavier than those from AT (0.06) 9 m into the ROW (Table 1.8). However, no significant differences were detected in mean mass with time since mowing (P -values: 0.222 – 0.658) or as distance varied into the ROW and forest (P -values: 0.077 – 0.621). An interaction between treatment and distance and mean mass at metamorphosis was not detected (P -values: 0.09 – 0.756).

Mean days to metamorphosis did not differ significantly between treatments at the same distances into the ROW and forest during each year PM (P -values: 0.071 – 0.891). However,

significant differences were detected within each treatment as distances increased into the ROW and forest for 2-years PM in both the AT ($F_{6,21} = 3.18, P = 0.022$) and FT ($F_{6,21} = 17.47, P < 0.001$); 3-years PM in the AT ($F_{6,19} = 10.08, P < 0.001$) and FT ($F_{6,19} = 4.87, P = 0.004$); 4 years PM in the AT ($F_{6,20} = 5.43, P = 0.002$) and FT ($F_{6,21} = 3.68, P = 0.012$). A post-hoc analysis indicated that mean days required to reach metamorphosis was significant quicker in pools 9 m into the ROW than those at the edge and into the forest for both treatments 2-4 years PM (Table 1.9). A repeated measures ANOVA indicated mean number of days to metamorphosis changed with time since mowing only for pools 6 and 9 m into the forest in both treatments, but no significant differences were detected within either ROW treatment (Figure 1.10). There was no interaction detected between distance and treatment on mean days to metamorphosis (P -values: 0.536-0.990).

Comparisons of mean pool temperatures between treatments and at different distances into the ROW indicated significant differences 3- and 4-years PM. Pools 9 m into the ROW were significantly warmer in the AT ($Mdn = 24.45\text{ }^{\circ}\text{C}$) than the FT ($Mdn = 22.12\text{ }^{\circ}\text{C}; U = 0.00, z = -2.309, P = 0.029$) 3-years and 4-years PM (AT, $Mdn = 23.36\text{ }^{\circ}\text{C}$; FT, $Mdn = 20.52\text{ }^{\circ}\text{C}; U = 0.00, z = -2.309, P = 0.029$). Mean pool temperatures also differed significantly between distances into the forest and ROW with each treatment and year PM. One-year PM mean pool temperatures differed significantly only within the AT ($\chi^2(6) = 15.64, P = 0.016$). However, significant differences were indicated between distances in both AT and FT, 2-years ($\chi^2(6) = 18.42, P = 0.005$; $\chi^2(6) = 16.97, P = 0.009$, respectively), 3-years ($\chi^2(6) = 21.52, P = 0.001$; $\chi^2(6) = 20.59, P = 0.002$, respectively), and 4-years ($\chi^2(6) = 16.40, P = 0.012$; $\chi^2(6) = 16.13, P = 0.013$, respectively) PM. Within the AT mean, mean pool temperatures 9 m into the ROW ($Mdn = 23.36 - 25.47\text{ }^{\circ}\text{C}$) were significantly greater each year than pools at the edge ($19.32 - 20.47\text{ }^{\circ}\text{C}$;

Table 1.11). Three-years PM, AT pools 6 m into the ROW (21.23 °C) were also significantly warmer than those at the edge (19.35 °C, Table 1.11). Within the FT, mean temperatures from pools 9 m into the ROW were significantly warmer 2 and 3-years PM (23.81 & 22.17 °C) than pools at the edge (20.23 °C & 19.19 °C; Table 1.11). Four-years PM, FT pools 9 m into the ROW (20.50 °C) were significantly warmer than pools 3 m into the forest (19.17 °C; Table 1.11). Mean pool temperature differed significantly within the AT and at the same distances into the ROW, between treatment years, but no significant differences were detected within the FT (Table 1.12). These results suggest pools in the AT are affected by seasonal temperature fluctuation more than pools in the FT. Mean pool temperatures were moderately, negatively related to mean days to metamorphosis ($r_s = -0.589$; $P < 0.001$; Figure 1.5) and weakly related to mean percent survival ($r_s = -0.207$; $P = 0.006$), which suggested larvae develop quicker in warmer pools, but experience an increase in mortality.

Mean maximum pool temperatures within the same treatment were significantly greater 9 m into the ROW for both treatments than the edge up to 3-years PM (Table 1.13). Mean maximum pool temperatures 9 m into the ROW from the AT ($Mdn = 39.16$ °C) were significantly warmer than pools from the FT ($Mdn = 34.90$ °C; $U = 0.00$, $z = -2.323$, $P = 0.029$) 3-years PM. The trend continued 4-years PM as AT pools ($Mdn = 36.73$ °C) were significantly warmer than pools from the FT ($Mdn = 30.21$ °C; $U = 0.00$, $z = -2.309$, $P = 0.029$) 9 m into the ROW. Mean maximum pool temperatures within each treatment at each distance were compared between each treatment year. Pools 6 m into the ROW, within the AT, differed significantly from time since mowing ($\chi^2(3) = 10.34$, $P = 0.042$). However, no other comparisons were significant. One-year PM ($Mdn = 31.52$ °C), mean maximum pool temperatures were significantly warmer than 4-years PM ($Mdn = 28.66$ °C; $P = 0.024$). There was a moderate

negative relationship between the mean number of days to metamorphosis and mean maximum pool temperature ($r_s = -0.566$; $P < 0.001$). However, a negligible relationship between mean maximum pool temperature and mean survival was indicated ($r_s = -0.148$; $P = 0.019$). This also suggested warmer pools decrease days to metamorphosis but increase mortality.

Net primary production (NPP) differed significantly at the same distances into the ROW between treatments. Four-years PM, NPP was significantly greater in pools 9 m into the ROW from the AT ($Mdn = 2.58$) than from the FT ($Mdn = 0.48$; $U = 0.00$, $z = -2.309$, $P = 0.029$). Within the same treatment years NPP differed significantly with distance into the ROW and forest. Net primary production was significantly greater both 1 and 2-years PM from ROW pools than pools at the edge and/or forest in both treatments (Table 1.14). However, 4-years PM only pools in the AT differed significantly in NPP as pools 9 m into the ROW had greater NPP than forest pools (Table 1.13). No comparisons were made between years as the use of different DO meters would have added the potential of error. However, as NPP increased, the number of days to metamorphosis decreased ($r_s = -0.141$; $P = 0.041$) as did the percent larval survival ($r_s = -0.213$; $P = 0.001$), but both relationships were negligible.

DISCUSSION

Right-of-way mowing negatively impacted four-toed salamander reproductive effort by decreasing nesting success and increasing larval mortality. Nest abandonment occurred more after mowing and continued until ROW vegetation had grown for 2 additional years (3 years total). Abandonment has been shown to decrease four-toed embryonic survival (Harris et al. 1995; Harris and Gill 1980) and we observed a similar trend as abandoned nests were significantly less successful than those with a female present in both treatments. Greater success

of guarded nests might have been the results of a decrease in pathogens and fungi on eggs (Banning et al. 2008). Female four-toed salamanders have been observed to weave through their eggs daily, rubbing their ventral and dorsal surfaces across all eggs (Herman 2009). Anti-microbial properties of bacteria on the skin of four-toed salamanders inhibit the growth of *Batrachochytrium dendrobatidis* and potentially other fungal species (Harris et al. 2006; Banning et al. 2008). Four-toed salamander eggs infected with fungi had greater survival when anti-*Mariannaea* bacteria from female four-toed salamander's skin were present on the egg (Banning et al. 2008). Another Plethodontid salamander, *P. cinereus*, also had higher embryo survival with the female present in laboratory conditions without predators, which suggests benefits other than physical defense (Highton and Savage 1961). Predation did not seem to be the cause of abandonment as no significant differences in the rates of tail damage or loss were detected between ROW and forest nests. We acknowledge that abandoned nests could have been the results of successful predation attempts and tail damage would not have been observed. Additionally, no female four-toed salamanders, initially nesting in the ROW, were found returning to nest in previous years as opposed to those nesting in the forest, which did have repeat nesting. This suggests the missing females could have been predated. Since Plethodontid salamanders rely on cutaneous respiration, warmer and drier condition in the ROW could have exceeded their physiological limits for respiration and facilitated abandonments (Petranka 1998; Bernardo and Spotila 2006). Timber clearcuts have greater light penetration and temperatures which have caused amphibians to experience greater water loss (deMaynadier and Hunter 1998; Rothermel and Semlitsch 2002). Loss of canopy within the ROW, due to mowing, could have created a similar effect. If environmental conditions in ROW nests became extreme, female

salamanders could have abandoned their nest for self preservation which then lead to greater embryonic mortality.

In addition to abandonment, nesting success was also negatively impacted by ROW mowing when females remained to guard nests. Embryonic survival was greater in forest nests than ROW nests during the mowing year and for 2 additional years even with the female present. However, no significant differences were detected prior to mowing or 3- and 4-years PM. Most likely the additional vegetation shaded ROW nests and created cooler temperatures. Four-toed salamander nest temperatures have been negatively correlated with embryo survival in other areas (Wahl et al. 2008).

Differences in embryonic survival between ROW and forest nests did not appear to be related to female condition. Female salamanders guarding nests were not significantly larger in either treatment. However, embryonic survival was positively related to female SVL in ROW nests. Larger Plethodontid salamanders would have a smaller surface area to volume ratio than smaller salamanders, thus decreasing water loss (Semlitsch 1981). Therefore, large female salamanders would have a slight advantage over smaller salamanders as they would have potentially been able to stay with their eggs longer, with less risk of desiccation or decreased gas exchange. Four-toed salamander embryonic success has been linked to the duration of female presence in the nest (Harris and Gill 1980; Harris et al. 1995). Given the progressive warming of temperatures as the nesting season progresses (March – May), larger females might have been able to tolerate warmer temperatures slightly longer than smaller females, which could have improved embryonic survival.

The potential negative impact to female four-toed salamanders nesting in the ROW could be even greater as no female was recaptured in later nesting seasons. Even though rates of

recapture were low (<17.14%) from forest nests, females were still recaptured in future nesting years. Harris and Ludwig (2004) found the probability of females skipping 3 consecutive years of nesting to be 0.016, which suggests if female four-toed salamanders survived nesting in the ROW, they should have returned within 4-years PM. In other studies, female four-toed salamanders that did return the next year were those that had been supplemented with a high amount of food. This also suggests that nesting in the ROW could require a large amount of resources to prepare females for their next breeding attempt, thus requiring several years before renesting (Harris and Ludwig 2004). Additionally, female salamanders with major tail damage were observed returning to nest the next season while producing typical clutch sizes and having regrown their tail. Traditionally, tail loss in female Plethodontid salamanders was thought to greatly impact ova production the following year as the tail is an area of high lipid storage (Petranka 1998). *Plethodon cinereus* (Yurewicz and Wilbur 2004) and *Batrachoseps attenuatus* (Maiorana 1977) have shown decreased or lack ova production in the year following tail loss. Ova reduction after tail loss has also been observed in female lizards that experience tail autonomy (Dial and Fitzpatrick 1981). However, some female four-toed salamanders at our study site appear to be able to recover from tail loss within one summer and return the next nesting season, producing full clutches of eggs. Of the female salamanders returning to their nesting area several returned to the exact location of previous nests. Four-toed salamanders nest fidelity had not been previously observed. Females have been observed returning to the same pond to nest over several breeding seasons (Harris and Gill 1980), but never repetitively using the exact same nesting site.

Differences in nest microhabitat conditions were detected between treatments, although we did not detect a correlation between microhabitat variables (aspect, height above nest, and

pool depth) and embryonic survival for nests with female salamanders present. We also did not detect differences in aspect between treatments. Water depth below nests was only significantly different during the nesting season immediately after mowing, but was not correlated to embryonic success during the entire study including the season after mowing. Nesting areas, including ditches, were filled with precipitation and its subsequent drainage. Without a continual supply of water these areas were susceptible to evaporation. With the absence of a vegetative layer overhead, ROW nests probably experienced greater rates of evaporation and thus shallower pools one-year after mowing. Female four-toed salamander have been shown to prefer nesting sites above areas of deeper water (Chalmers and Loftin 2006), but embryonic survival was not correlated with water depth (Wahl et al. 2008). Nests in forested and ROW areas varied between heights above water as each location was significantly higher than the other during one nesting year. However, our results indicated nest height did not impact embryonic survival.

Right-of-way mowing continued to negatively impact four-toed salamander recruitment as larvae suffered greater mortality in ROW pools. One-year PM significantly fewer larvae survived in AT and FT pools from the ROW than the forest. The trend continued 2-years PM as pools 9 and 6 m into the ROW from the FT had significantly less larvae survival. However, vegetation succession most likely produced conditions around pools 3 m into the ROW, which were conducive to larval survival as forested pools. No differences were detected 3- or 4-years PM between pool location and survival from AT pools, which was due to decreased survival from forest pools, but survival from pools 9 m into the ROW were still low while survival improved from ROW pools in the FT. After 3 years, no overall differences between treatments were observed, but differences within treatments between distances were noted.

Larval survival significantly increased in the FT as time since mowing increased. Recovery time varied with distance into the ROW as pools 3 and 6 m into the ROW had significantly greater survival 3- and 4-years PM than 1 year PM. An extra year of vegetation growth was needed for pools 9 m into the ROW, which had significantly greater survival 4-years PM than 1-year PM. This trend was not observed in the AT which suggests vegetation succession could be responsible for the increased survival. The benefit of vegetation regrowth was also apparent as larval survival was greater in pools 9 m into the ROW from the FT than from pools in the AT after 4 years of vegetation regrowth. However, no significant differences were detected in any other comparisons between treatments at the same distances, which suggested that ROW pools closer to the edge in the AT have conditions conducive to survival similar to pools in the FT and required less time to recover after mowing than pools 9 m into the ROW. Increased larval survival was not observed in ROW pools from the AT except for pools 6 m into the ROW which appeared to suffer increased mortality 1-year PM compared to 2- and 3-years PM. However, differences were probably not due to changing AT conditions, but to the unusual mortality from 1-year PM as pools 3 m into the ROW did not improve. Therefore, pools 3 and 6 m into the ROW recovered after 3 years, but pools 9 m into the ROW required 4 years. Improved survival was mostly likely due to increased vegetative cover, which produced slightly cooler temperatures. Mean and maximum pool temperatures 9 m into the ROW were cooler in the FT compared to the AT after 3 and 4 years of vegetation regrowth. Mean and maximum pool temperatures were weakly related to mean percent survival as warmer temperatures decreased survival. In laboratory conditions other Plethodontid larvae (*Eurycea lucifuga*) had higher mortality rates as water temperatures increased, which suggests that four-toed salamanders could

have also experienced greater mortality due to warmer water temperatures (Ringia and Lips 2007).

For larvae that survived to metamorphosis, increased size at metamorphosis has been shown to increase juvenile survivorship and adult fitness for other amphibians (Semlitsch et al. 1988; Scott et al. 2007), thus four-toed larvae metamorphosing at a large size might have a better chance to reach adulthood. Only a single comparison between SVLs from FT and AT was significant, suggesting mowing treatment had minimal impact on larvae SVL. However, larval TLs were impacted by distance into the ROW and time since mowing. In the AT larvae were consistently shorter from pools 9 m into the ROW than those just 6 m into the ROW and even into the forest during some treatment years. Larvae 9 and 6 m into the ROW from the FT were also longer than those from the AT after only 2-years PM and the trend continued 4-years PM. However, when compared to forest and edge locations, no differences were detected from FT pools each year PM. Larvae also had greater mass from pools in the FT than those in the AT, 9 m into the ROW and after 4 years of regrowth. Similar mass differences occurred 3 m into the ROW, but only after 1 year of regrowth. The closer pools from the FT were to the forest edge, the less time PM, mass and TL exceed larvae in the AT, and by 4-years PM, even pools 9 m away from the edge were producing larvae significantly large than pools from the annual mow treatment. However, no differences were observed between distance into the forest or ROW, but as vegetation success occurred larvae from the FT were large than those from the AT. In AT pools, 9 m into the ROW, four-toed larvae consistently metamorphosed smaller (mass and TL) than those from edge or a forest pool, which suggests conditions were not ideal; mostly like due to temperature. The Wilbur-Collins model predicted that amphibians will metamorphosis smaller, but quicker in unfavorable conditions such as high temperatures (Wilber and Collins

1973). Amphibians have been shown to metamorphosis earlier and at smaller sizes in warmer conditions (Hocking and Semlitsch 2008). However, pond breeding Plethodontid salamanders have not been examined, but stream breeding Plethodontid salamanders (*Desmognathus ochrophaeus* and *Eurycea lucifua*) did metamorphosis significantly larger in cooler temperatures (Beachy 1995; Ringia and Lips 2007). The warmest pool for both maximum and mean temperatures, 9 m into ROW from AT, had consistently smaller metamorphs, which was similar to gray treefrogs in open clear-cuts (Hocking and Semlitsch 2008). Larvae were significantly larger than those from the AT after 2 years of regrowth for TL and 4 years for mass 9 m into the ROW and within the FT. This suggests a benefit from vegetation succession as potential negative impacts on the four-toed salamander population were partially reduced after 2 years. Smaller metamorphs, for other amphibian species experienced reduced survival from desiccation as they tried to disperse back to forest from clearcuts, which implies that four-toed salamander metamorphs could continue to experience greater mortality before they reach the forest (Rothermel and Semlitsch 2002). The few larvae that survived from AT pools most likely will experience reduced survivorship later in life thus negatively impacting the population.

We did not detect differences in time to metamorphosis between treatments each year but, within treatments significant differences were observed between distances into the ROW and forest. Larvae in pools 9 and/or 6 m into the ROW, 2-4 years PM, metamorphosed quicker than pools at the edge, 3 m into the ROW, or into the forest in both AT and FT, even with vegetation regrowth. Temperature was most likely the cause of differences in the days to metamorphosis as it was negatively related. Gray treefrogs have been shown to metamorphose quicker from pools in open canopies, presumably due to warmer temperatures (Hocking and Semlitsch 2008). Other Plethodontid salamanders larvae (*Desmognathus ochrophaeus* and

Eurycea lucifuga) have also decreased days to metamorphosis as water temperatures increased (Beachy 1995; Ringia and Lips 2007). Anurans (e.g. *Scaphiopus* spp.) rapidly increased metamorphosis when pools began to dry or became excessively warm as predicted by the Wilbur-Collins model (Newman 1992; Wilbur and Collins 1973). We observed minimal evaporation in either ROW or forest mesocosm pools and changes in time to metamorphosis were not related to water depth suggesting temperature was the driving force. We acknowledge that natural ROW pools could dry quicker than forested pools, potentially initiating early metamorphosis in natural conditions, but drying was not observed in mesocosm pools. Since four-toed salamander larvae are able to metamorphose quicker in ROW pools, they might be able to survive pool drying. Comparisons of time to metamorphosis from pools at the same distances into the ROW or forest varied inconsistently with years. Differences were detected within the forest and annual treatments with the quickest metamorphosis consistently occurring 2-years PM, which also had the warmest pool temperatures of the project. This also suggested differences were related to temperatures between years and not the effects of treatments.

Pool temperatures apparently influenced survival, size, and days to metamorphosis as temperatures differed by pool location and time since mowing. In the AT and FT, pools 9 m into the ROW were significantly warmer each year than pools at the edge or forest. When mean and mean maximum pool temperatures were compared between treatments within the same year and distance, pools 9 m into the ROW from the FT were cooler than pools from the AT, 3- and 4-years PM, which implied at least 3 year of regrowth was needed before ROW conditions were significantly different between treatments. Mean pool temperatures within the same treatments but between years did vary, but only in the annual treatment. All pools within the AT ROW fluctuated from year to year with 2-years PM being the warmest year. No differences were

detected within the FT, which suggested that additional vegetation growth helped to buffer seasonal temperature variations and created a more consistent breeding environment year to year. Mean maximum pool temperatures were warmer 9 m into either mowing treatment compared to the edge, forest, or 3 m into the ROW within the same year. Even with vegetative growth within the FT, ROW pools were still significantly warmer than edge and forest locations. Mean maximum temperatures were negatively related to survival and days to metamorphosis, but the relationship was less than that of mean pool temperatures.

Net primary production was significantly greater from pools 9 m into the ROW from the AT compared to the FT. This also suggests that additional plant growth blocked enough sunlight to decrease NPP in the FT to forest levels. In every year except year 4, ROW pools in both treatments had significantly greater NPP than edge or forest pools within the same treatment. However, NPP was weakly related to time to metamorphosis and survival, which suggests that productivity might not be as critical for four-toed larvae as other amphibians. Additionally, the most consistently productive pools (9 m in ROW from AT) produced the smallest larvae and had the lowest survival. O’Laughlin and Harris (2000) found no association between food levels and development rates for four-toed salamander larvae. Since four-toed salamander larvae have the shortest larval period of any Plethodontid salamander, they begin the process of metamorphosis as soon as they hatch (< 3 days after hatching; Berger-Bishop and Harris 1996; O’Laughlin and Harris 2000). Therefore, increasing or decreasing food does not trigger a plasticity response to change metamorphosis timing. Plethodontid salamander larval periods have varied with food availability. *Desmognathus ocoee* had shorter larval periods with an abundance of prey items (Bernardo and Agosta 2003), but *Desmognathus ochrophaeus* has been shown to not alter time to metamorphosis with different feeding regimes (Beachy 1995). Four-toed salamanders are

forest residents (Petranka 1998) and unlike some anuran species (*Rana sylvatica*, *R. pipiens*, and *Hyla veriscolor*), which have greater breeding success in edge and/or open habitats (Skelly et al. 2002; Schiesari 2006; Hocking and Semlitsch 2008), four-toed salamanders are most likely adapted to breeding in pools with low productivity due to closed forest canopies. Pond productivity does not seem to influence four-toed salamander larval success or time to metamorphosis and should not be a critical factor for management decisions.

The period of time needed for recovery of salamander populations in cleared and edge habitat is variable. Recovery time of terrestrial Plethodontid salamanders from timber harvest has varied from 2-60 years (Pough et al 1987; Clawson et al 1997). Stream dwelling Plethodontid salamanders are projected to need 40 years to recover (Crawford and Semlitsch 2008). Right-of-way mowing is not as extensive of a habitat disturbance as a large clearcut and four-toed salamanders are spending the majority of the year in areas that are still forested, only using the ROW for nesting. However, four-toed salamander recovery was at least 3 years and for salamanders nesting on pools farther into the ROW recovery time might even be 4 years. After 4 years of vegetation regrowth, larval survival was greater from pools 9 m into the ROW than those from the same distance in the AT and differences were detected after 2 years of regrowth from forest or edge pools.

CONCLUSIONS

Right-of-way mowing negatively impacted four-toed salamanders at the South Holston Weir Dam. Female salamanders abandoned nests significantly more in the ROW, which led to greater embryonic loss. Even when female salamanders remained with their eggs, nesting success was significantly lower in the ROW for 3 nesting seasons after mowing which only left 2

nesting seasons before the ROW was mowed again on the current management cycle. The fate of female salamanders nesting in the ROW was not determined, but none were observed nesting again for 3 years after they were first located unlike females nesting in the forest of which ~17% were found nesting again. Four-toed salamander larvae were also negatively impacted by conditions created from ROW mowing. Larvae residing in ROW pools 9 m from the edge had significantly less survival than those closer to the edge and within the forest for 2-years PM. However, after 4 years of vegetation growth, even pools 9 m into the ROW had greater larval survival than pools in the annual mowing treatment and no difference from forest pools. Larvae from pools 9 m into the ROW in the AT metamorphosed quicker, but smaller than larvae from pools at the edge or in the forest. Reduced size at metamorphosis could negatively impact the salamander later as other amphibians have been shown to have reduced survival and delayed maturity if they metamorphosis smaller. Quicker time to metamorphosis could benefit four-toed salamanders larvae if ROW pools dry quicker than forest pool, but we did not observe evaporation differences in ROW pools. Differences in larval survival, time to metamorphosis and size appear to be related to water temperature and not net primary productivity as has been observed for other amphibians. Temperature was related to survival and time to metamorphosis suggests that observed difference were mostly like due to changes in water temperature. The impact of ROW on four-toed nesting could vary throughout it range and latitudes with varying climates.

Acknowledgments

This research was completed with funds provided by the Tennessee Wildlife Resource Agency through a State Wildlife Grant from the U.S. Fish and Wildlife Service. A special thanks to P. Gentry, B. Parks, G. Poe, S. Bobo, L. Huffman, and E. Studholme, for field assistance.

Additionally, we are grateful to the Tennessee Valley Authority staff for project assistance, yearly mowing, and access to our study sites. We are grateful to the City of Bristol Tennessee, water treatment facility for providing water needed to fill mesocosm pools. We appreciate the assistance of Appalachian Well Drilling and East High Volunteer Fire Department for assistance in moving water to fill mesocosm pools. All sampling was approved by the Tennessee Wildlife Resource Agency (Scientific Collection Permit #1986) and followed UT Institutional Animal Care and Use Committee protocol #2130-0412.

LITERATURE CITED

- Askins, R. A., C. M. Folsom-O'Keefe, and M. C. Hardy. 2012. Effects of vegetation, corridor width and regional land use on early successional birds on powerline corridors. PLoS ONE 7: e31520.
- Banning, J. L., A. L. Weddle, G. W. Wahl, M. A. Simon, A. Lauer, R. L. Walters, R. N. Harris. 2008. Antifungal skin bacteria, embryonic survival, and communal nesting in four-toed salamanders, *Hemidactylium scutatum*. Oecologia 156:423-9.
- Beachy, C. K. Effects of larval growth on metamorphosis in a stream-dwelling salamanders (*Desmognathus ochrophaeus*). Journal of Herpetology 29:375-82.
- Berger-Bishop, L. E. and R. N. Harris. 1996. A study of the caudal allometry in the salamander *Hemidactylium scutatum* (Caudata: Plethodontidae) Herpetologica 52:515-25.
- Bernardo, J. and S. Agosta. 2003. Determinants of clinal variation in life history of mountain dusky salamanders (*Desmognathus ocoee*): prey abundance and ecological limits on foraging time restrict opportunities for larval growth. *Proceedings of the Zoological Society of London* 259:411-421.
- Bernardo, J. and J. R. Spotila. 2006. Physiological constraints on organismal response to global warming: mechanistic insights from clinally varying populations and implications for assessing endangerment. Biology Letters 2:135-9.
- Bishop, S. C. 1941. Salamanders of New York. New York State Museum Bulletin 324:1-365.
- Blanchard, F. N. 1923. The life history of the four-toed salamander. The American Naturalist 57:262-8.

- Blanchard, F. N. 1934. The relation of the female four-toed salamander to her nest. *Copeia* 1934:137-8.
- Breitenbach, G. L. 1982. The frequency of communal nesting and solitary brooding in the salamander, *Hemidactylium scutatum*. *Journal of Herpetology* 16:341-6.
- Chalmers, R. J. 1998. Wetland and nest scale habitat use by the four-toed salamander (*Hemidactylium scutatum*) in Maine, and a comparison of survey methods [thesis]. [Orono, Maine]: The University of Maine. p109.
- Chalmers, R. J. and C. S. Loftin. 2006. Wetland and microhabitat use by nesting four-toed salamanders in Maine. *Journal of Herpetology* 40:478-85.
- Clarke, D. J. and J. G. White. 2008. Recolonisation of powerline corridor vegetation by small mammals: timing and the influence of vegetation management. *Landscape and Urban Planning* 87:108-16.
- Clawson, R. G., B. G. Lockaby, and R. H. Jones. 1997. Amphibian responses to helicopter harvesting in forested floodplains of low order, blackwater streams. *Forest Ecology and Management* 90:225-35.
- Crawford, J. A. and R. D. Semlitsch. 2008. Post-disturbance effects of even-aged timber harvest on stream salamander in southern Appalachian forests. *Animal Conservation* 2008:1-8.
- deMaynadier, P. G., and M. L. Hunter. 1998. Effects of silviculture edges on the distribution and abundance of amphibians in Maine. *Conservation Biology* 12:340-52.
- Dial, B. E. and L. C. Fitzpatrick. 1981. The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia* 51:310-7.

- Earl, J. E. and R. D. Semlitsch. 2012. Reciprocal subsidies in ponds: does leaf litter input increase frog biomass export? *Oecologia* 170:1077-87.
- Gardner T.A., J. Barlow, C. A. Peres. 2007. Paradox, presumption, and pitfalls in conservation biology: The importance of habitat change for amphibians and reptiles. *Biological Conservation* 138: 166-79.
- Gilbert, P. W. 1941. Eggs and nests of *Hemidactylium scutatum* in the Ithaca region. *Coepia* 1941:47.
- Goosem, M. and H. Marsh. 1997. Fragmentation of a small mammal community by a powerline corridor through tropical rainforest. *Wildlife Research* 24:613-29.
- Hamed, M. K. and P. D. Gentry. 2003. Geographic Distribution. *Hemidactylium scutatum*. *Herpetological Review* 34:160-1.
- Harris, R. N. 2008. Body condition and order of arrival affect cooperative nesting behavior in four-toed salamanders *Hemidactylium scutatum*. *Animal Behaviour* 75:229-33.
- Harris, R. N. and D. E. Gill. 1980. Communal nesting, brooding behavior, and embryonic survival of the four-toed salamander *Hemidactylium scutatum*. *Herpetologica* 36:141-4.
- Harris, R. N. and W. W. Hames, I. T. Knight, C. A. Carreno, and T. J. Vess. 1995. An experimental analysis of joint nesting in the salamander *Hemidactylium scutatum* (Caudata: Plethodontidae): the effects of population density. *Animal Behavior* 50:1309-16.
- Harris, R. N., T. Y. James, A. Lauer, M. A. Simon, and A. Patel. 2006. Amphibian pathogen *Batrachochytrium dendrobatidis* is inhibited by the cutaneous bacteria of amphibian species. *EcoHealth* 3:53-6.

- Harris, R. N. and P. M. Ludwig. 2004. Resource level and reproductive frequency in female four-toed salamanders, *Hemidactylium scutatum*. *Ecology* 85:1585-90.
- Herman, T. A. 2009. Range-wide phylogeography of the four-toed salamander (*Hemidactylium scutatum*): Out of Appalachia and into the glacial aftermath [thesis]. [Bowling Green (OH)]: Bowling Green State University. 64 p.
- Highton, R and T. Savage. 1961. Functions of the brooding behavior in the female Red-backed Salamander, *Plethodon cinereus*. *Evolution* 14:351-60.
- Hocking, D. J. and R. D. Semlitsch. 2008. Effects of experimental clearcut logging on gray treefrog tadpole (*Hyla veriscolor*) performance. *Journal of Herpetology* 42:689-98.
- Homyack, J. A. and C. A. Haas. 2009. Long-term effects of experimental forest harvesting on abundance and reproductive demography of terrestrial salamanders. *Biological Conservation* 142:110-21.
- IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.
- Johnson, W. C., R. K. Schreiber, and R. L. Burgess. 1979. Diversity of small mammals in a powerline right-of-way and adjacent forest in East Tennessee. *The American Midland Naturalist* 101:231-5.
- Knapp, S. M., C. A. Hass, D. N. Harpole, R. L. Kirkpatrick. 2003. Initial effects of clearcutting and alternative silvicultural practices on terrestrial salamander abundance. *Conservation Biology* 17:752-62.
- Lannoo, M. J. 2005, ed. *Amphibian declines: The conservation status of United States species*. Berkley: University of California Press.

- Maiorana, V. C. 1977. Tail autotomy, functional conflicts and their resolution by a salamander. *Nature* 265:533-5.
- Newman, R. A. 1992. Adaptive plasticity in amphibian metamorphosis. *BioScience* 42:671-8.
- Noss, R. F. 1991. Landscape connectivity: different functions at different scales. In: Hudson, W. E. editor. *Landscape linkages and biodiversity*. Washington, DC: Island Press. P27-39.
- O’Laughlin, B. E. and R. N. Harris. 2000. Models of metamorphic timing: an experimental evaluation with the pond-dwelling salamander *Hemidactylium scutatum* (Caudata: Plethodontidae). *Oecologia* 124:343-50.
- Petranka, J. W. 1998. *Salamanders of the United States and Canada*. Washington, DC: Smithsonian Institution Press. 587 p.
- Petranka, J. W., M. E. Eldridge, and K. E. Haley. 1993. Effects of timber harvesting on low elevation populations of Southern Appalachian salamanders. *Conservation Biology* 7:363-70.
- Pough, F. H., E. M. Smith, D. H. Rhodes, and A. Collazo. 1987. The abundance of salamanders in forest stands with different histories of disturbance. *Forest Ecology and Management* 20:1-9.
- Relyea, R. A., N. M. Schoeppner, J. T. Hoverman. 2005. Pesticides and amphibians the importance of community context. *Ecological Applications* 15:1125-34.
- Rieucou, G. W. L. Vickery, G. J. Doucet, and B. Laquerre. 2007. An innovative use of white-tailed deer (*Odocoileus virginianus*) foraging behavior in impact studies. *Canadian Journal of Zoology* 85:839-46.

- Ringia, A. M. and K. R. Lipps. 2007. Oviposition, early development and growth of the cave salamanders, *Eurycea lucifuga*: surface and subterranean influences on a troglomorphic species. *Herpetologica* 63:258-68.
- Redmond, W. H. and A. F. Scott. 1996. Atlas of Amphibians in Tennessee. Misc. Publ. No. 12, The Center for Field Biology, Austin Peay State University, Clarksville, Tennessee 94 pp. [Hard copy and internet versions, the latter of which includes a link to information on amphibians in Tennessee having appeared since 1996], <http://www.apsu.edu/amatlas>, accessed 2 February 2014).
- Rittenhouse, T. A. G. and R. D. Semlitsch. 2006. Grasslands as movement barriers for forest associated salamanders: migration behaviors of adult and juvenile at a distinct habitat edge. *Biological Conservation* 131:14-22.
- Robinson, S. K., F. R. Thompson, T. M. Donovan, D. R. Whitehead, J. Faaborg. 1995. Regional forest fragmentation and the nesting success of migratory birds. *Science* 267:1987-90.
- Rothermel, B.B. 2004. Migratory success of juveniles: A potential constraint on connectivity for pond-breeding amphibians. *Ecological Applications* 14:1535-46.
- Rothermel, B. B. and T. M. Luhring. 2005. Burrow availability and desiccation risk of mole salamanders (*Ambystoma talpoideum*) in harvested versus unharvested forest stands. *Journal of Herpetology* 49:619-26.
- Rothermel, B. B. and R. D. Semlitsch. 2002. An experimental investigation of landscape resistance of forest versus old-field habitats to emerging juvenile amphibians. *Conservation Biology* 16:1324-32.

- Schiesari, L. 2006. Pond canopy cover: a resource gradient for anuran larvae. *Freshwater Biology* 51:412-23.
- Scott, D. E., E. D. Casey, M. F. Donovan, and T. K. Lynch. 2007. Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival. *Oecologia* 153:521-32.
- Semlitsch, R. D. 1981. Terrestrial activity and summer home range of the mole salamander (*Ambystoma talpoideum*). *Canadian Journal of Zoology* 59:315-22.
- Semlitsch, R.D., T.J. Ryan, K. Hamed, M. Chatfield, B. Drehman, N. Pekarek, M. Spath, and A. Watland. 2007. Salamander abundance along road edges and within abandoned logging roads in Appalachian forests. *Conservation Biology* 21:159-167.
- Semlitsch, R. D., D. E. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology* 69:184-92.
- Semlitsch, R. D., B. D. Todd, S. M. Blomquist, A. J. K. Calhoun, J. W. Gibbons, J. P. Gibbs, G. J. Grater, E. B. Harper, D. J. Hocking, M. L. Hunter Jr., D. A. Patrick, T. A. G. Rittenhouse, B. B. Rothermel. 2009. Effects of timber harvest on amphibian populations: understanding mechanisms from forest experiments. *BioScience* 59:853-62.
- Skelly, D. K., L. K. Friedenburd, and J. M. Kiesecker. 2002. Forest canopy and the performance of larval amphibians. *Ecology* 83:983-92.
- Sodhi, N. S., D. Bickford, A. C. Diesmos, T. M. Lee, L. P. Koh, B. W. Brook, C. H. Sekercioglu, C. J. A. Bradshaw. 2008. Measuring the meltdown: drivers of global amphibian extinction and decline. *Plosone* 3:1-8.
- Soper, R. and K. Schoeberl. 1997. Mitigation of impacts to timber rattlesnakes (*Crotalus horridus horridus*) during replacement of a natural gas transmission line in Southeastern New York. In: Williams, J.R., J. W. Goodrich-Manney, J. R. Wisniewski, and J.

- Wisniewski, editors. Proceedings of the sixth international symposium on environmental concerns in right-of-way management; 1997 February 24-26; New Orleans, Louisiana. Elsevier. p. 327-331.
- Spight, T. M. 1968. The water economy of salamanders: Evaporative water loss. *Physiological Zoology* 41:195-203.
- Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783-6.
- Sulak, J. A. and J. J. Kielbaso. 2000. Vegetation management along transmission utility lines in the United States and Canada. *Journal of Arboriculture* 26:198-205.
- Tennessee Wildlife Resource Agency. 2000. Wildlife in need of management. Tennessee Wildlife Resource Agency Proclamation No.00-14, Nashville.
- U. S. Federal Energy Regulatory Commission. 2013. Docket Number: RM-12-16000; Order 785. Accessed 15 February 2013. Available from <https://www.ferc.gov/whats-new/comm-meet/2013/091913/E-4.pdf>.
- Wahl, G. W., R. N. Harris, and T. Nelms. 2008. Nest site selection and embryonic survival in four-toed salamanders, *Hemidactylium scutatum* (Caudata: Plethodontidae). *Herpetologica* 64: 12-19.
- Wake, D. B. and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Science* 105: 11466-73
- Werner, E. E. and K. S. Glennemeier. 1999. Influence of forest canopy cover on the breeding pond distributions of several amphibian species. *Copeia* 1999:1-12.

- Wester, E.E. and J. W. Kolb. 1997. Conservation of the gopher tortoise and associate species during construction of the Florida gas transmission company phase III expansion project. In: Williams, J.R., J. W. Goodrich-Manney, J. R. Wisniewski, and J. Wisniewski, editors. Proceedings of the sixth international symposium on environmental concerns in right-of-way management; 1997 February 24-26; New Orleans, Louisiana. Elsevier. p. 333-40.
- Wetzel, R. G. and G. E. Likens. 2000. *Limnological Analyses*. New York, NY: Springer. 429 p.
- Wilbur, H. M. and J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. *Science* 182:1305-14.
- Williams, B. K., T. A. G. Rittenhouse, R. D. Semlitsch. 2008. Leaf litter input medians tadpole performance across forest canopy treatments. *Oecologia* 155:377-84.
- Willyard, C. J., S. M. Tikalsky, and P. A. Mullins. 2004. Ecological effects of fragmentation related to transmission line right-of-way: a review of the state of the science. Resource Strategies, Inc., Madison, Wisconsin. 63p.
- Wood, J. T. 1955. The nesting of four-toed salamander, *Hemidactylium scutatum* (Schlegel), in Virginia. *The American Midland Naturalist* 53:381-9.
- Yahner, R. H., W. C. Bramble, and W. R. Byrnes. 2001. Effects of vegetation maintenance of an electric transmission right-of-way on reptile and amphibian populations. *Journal of Arboriculture* 27:24-9.
- Yurewicz, K. L. and H. M. Wilbur. 2004. Resource availability and costs of reproduction in the salamander *Plethodon cinereus*. *Copeia* 2004:28-36.

APPENDIX

Table 1.1. Number of abandoned four-toed salamander nests per treatment (forest or right-of-way) each nesting year from the South Holston Weir Dam, Sullivan County, Tennessee (Bold values indicated significant differences).

Nesting Year	Time Since Mowing (Years)	Forest Abandoned/ Total Nests	ROW Abandoned/ Total Nests	χ^2 - value (P)
2007	Prior	8/43	14/40	2.86 (0.091)
2008	Mowing Year	7/21	34/42	13.97 (0.001)
2009	1	6/44	10/23	7.40 (0.007)
2010	2	3/28	9/21	6.70 (0.010)
2011	3	9/37	6/21	0.13 (0.723)
2012	4	3/38	6/23	3.77 (0.070)

Table 1.2. Snout-vent-length of female four-toed salamander guarding nest per treatment for each nesting year per treatment (forest or right-of-way) each nesting year from the South Holston Weir Dam, Sullivan County, Tennessee.

Nesting Year	Time Since Mowing (Years)	Forest Mean SVL SD	ROW Mean SVL SD	<i>F</i>	<i>P</i>
2007	Prior	36.09 (± 2.79)	35.49 (± 2.28)	(1, 57) 0.795	0.376
2008	Mowing Year	36.54 (± 1.56)	36.35 (± 2.53)	(1, 41) 0.059	0.809
2009	1	36.83 (± 2.33)	37.66 (±1.81)	(1, 46) 1.254	0.269
2010	2	38.49 (± 2.36)	37.85 (± 1.86)	(1, 34) 0.635	0.431
2011	3	35.81 (± 2.47)	36.88 (± 2.33)	(1, 36) 1.648	0.207
2012	4	37.28 (± 3.22)	35.86 (± 2.07)	(1, 45) 2.319	0.135

Table 1.3. Microhabitat variables from nests with female salamanders present in each habitat (forest or right-of-way) during each nesting year from the South Holston Weir Dam, Sullivan County, Tennessee (Bold values indicated significant differences).

Time Since Mowing	Aspect		Distance Above Water (cm)		Water Depth (cm)	
	Forest Median (SD)	ROW Median (SD)	Forest Mean (SD)	ROW Mean (SD)	Forest Mean (SD)	ROW (SD)
Prior to Mowing	---	---	---	---	---	---
1	1.89 (± 1.18)	1.04 (± 0.41)	4.76 (± 1.82)	4.38 (± 1.68)	7.12 (± 1.87)	6.27 (± 1.46)
2	1.89 (± 1.01)	1.41 (± 0.98)	8.98 (± 1.92)	5.12 (± 1.52)	4.41 (± 2.23)	3.86 (± 2.07)
3	1.80 (± 1.02)	1.76 (± 1.25)	4.89 (± 1.88)	4.42 (± 1.67)	8.00 (± 1.92)	6.53 (± 1.52)
4	1.90 (± 0.96)	1.49 (± 1.16)	8.18 (± 1.72)	4.83 (± 1.13)	7.20 (± 1.64)	5.66 (± 2.13)

Table 1.4. Percent embryonic survival from nests with female four-toed salamander present from each habitat (forest or right-of-way) during each nesting season from the South Holston Weir Dam, Sullivan County, Tennessee (Bold values indicated significant differences).

Nesting Year	Time Since Mowing (Years)	Forest		ROW		<i>F</i>	<i>P</i>
		% Survival (SEM)	95% CI	% Survival (SEM)	95% CI		
2007	Prior	55.52 (± 10.02)	32.86 – 78.19	37.62 (± 13.77)	5.86 – 69.37	(1, 17) 1.14	0.30
2008	Mowing Year	73.14 (± 7.84)	56.05 – 90.23	42.63 (± 7.52)	27.28 – 57.98	(1, 42) 5.76	0.02
2009	1	62.70 (± 6.53)	49.44 – 75.96	34.20 (± 11.67)	8.79 – 59.60	(1, 47) 4.87	0.03
2010	2	63.99 (± 7.23)	49.03 – 78.95	28.16 (± 10.76)	4.18 – 52.15	(1, 33) 7.68	0.01
2011	3	57.45 (± 8.09)	40.82 – 74.09	36.83 (± 12.11)	10.85 – 62.81	(1, 40) 2.14	0.15
2012	4	62.63 (± 6.65)	49.08 – 76.19	42.56 (± 9.48)	22.22 – 62.90	(1, 45) 2.95	0.09

Table 1.5. Snout-vent-length, total length, fecundity of returning female four-toed salamanders and the distance moved from past nest locations from the South Holston Weir Dam, Sullivan County, Tennessee.

Female	Nesting Year	SVL	TL	Number of Eggs	Location Change (m) From Original Nest Site
1	2009	37.9	52.2	31	
	2010	41.9	96	59	3.0
2	2009	36.8	82	32	
	2010	37.6	44.9	45	0.0
3	2009	36.4	79.7	34	
	2010	36.4	86.4	34	0.0
4	2009	38.1	76.1	27	
	2011	38.3	85.4	42	26.0
5	2010	39.4	49.4	32	
	2011	41.0	81.8	38	0.0
	2012	41.0	88.6	71	0.0
6	2010	38.9	86.5	38	
	2011	39.0	86.5	32	3.4
7	2010	37.8	82.8	91	
	2011	39.5	85.8	36	0.0
8	2011	33.2	75.4	42	
	2012	36.4	81.1	26	0.0
9	2011	38.2	85.4	38	
	2012	39.6	88.8	25	0.0
10	2011	38.1	74.9	38	
	2012	38.1	85.8	82	6.5
11	2011	38.4	84.9	39	
	2012	39.5	82.4	24	0.0
12	2011	38.9	89.5	36	
	2012	39.6	88.8	25	7.3

Table 1.6. Tukey's post-hoc analysis with a Bonferroni adjustment results from an ANOVA for larval survival of four-toed salamanders from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.

Distance (m)	Treatment	Year(s) since mowing	Mean % Survival	95% CI	<i>P</i>
9 ROW	AT	1	7.50	-7.3 – 22.73	0.007
6 ROW			7.50	-7.3 – 22.73	
Edge			62.5	16.8 – 108.2	
3 Forest			55.0	27.44 – 82.56	
6 Forest			57.5	37.45 – 77.52	
9 ROW	FT	1	15.0	-22.88 – 52.88	0.005
6 ROW		1	10.0	-2.99 – 22.99	0.002
6 Forest		1	80.0	45.63 – 114.37	
3 ROW	FT	2	22.5	14.54 – 30.46	0.03
6 Forest		2	80.0	67.01 – 92.99	
9 ROW	FT	4	80.0	67.01 – 92.99	0.006
	AT	4	27.5	-10.10 – 65.10	

Table 1.7. Tukey's post-hoc analysis with a Bonferroni adjustment results for a repeated measures ANOVA of four-toed salamander larval survival from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee (2009-12).

Distance (m)	Treatment	Year(s) since mowing	Mean % Survival	95% CI	<i>P</i>
9 ROW	FT	1	15.0	-22.88 – 52.88	0.016
		2	27.5	-18.2 – 73.20	0.018
		4	80.0	67.01 – 92.00	
6 ROW	FT	1	10.0	-2.99 – 22.99	
		3	70.0	44.02 – 95.99	0.004
		4	52.5	37.27 – 67.44	0.003
3 ROW	FT	1	35.0	7.44 – 62.56	
		3	65.0	49.09 – 80.91	0.046
3 ROW	FT	2	22.5	14.54 – 30.46	
		3	65.0	49.09 – 80.91	0.003
		4	65.0	49.09 – 80.91	0.003
6 ROW	AT	1	7.5	-7.5 – 22.74	
		2	65.0	55.81 – 74.19	0.003
		3	55.0	39.09 – 70.91	0.01

Table 1.8. Results from an ANOVA and Tukey's post-hoc analysis with a Bonferroni adjustment for four-toed salamander larval length and mass from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee (2009-12). Results from post-hoc analysis are noted with an * for the *F* value.

Length	Distance (m)	Treatment	Year(s) Since Mowing or Mass (g)	Mean Length (mm)	95% CI	<i>F</i>	<i>P</i>
SVL	6 ROW	FT	3	15.25	14.50 – 15.51	11.19	0.016
		AT		14.63	14.09 – 15.71		
TL	9 ROW	FT	2	25.52	24.90 – 26.13	8.58	0.001
		AT		23.06	22.42 – 23.69		
TL	6 ROW	FT	2	25.92	24.87 – 26.97	13.54	0.01
		AT		24.41	23.63 – 25.19		
TL	9 ROW	AT	2	23.06	22.42 – 23.69	*	0.034
	6 ROW			25.57	24.08 – 26.35		
TL	9 ROW	AT	4	22.32	19.63 – 25.0	*	0.003
	3 ROW			25.16	24.01 – 26.31		
	3 Forest			25.38	23.01 – 27.75		
Mass	3 ROW	FT	1	0.11	0.07 – 0.14	7.93	0.03
		AT		0.08	0.07 – 0.09		
Mass	9 ROW	FT	4	0.08	0.07 – 0.09	8.98	0.024
		AT		0.06	0.04 – 0.08		

Table 1.9. Tukey's post-hoc analysis with a Bonferroni adjustment results from an ANOVA comparing four-toed salamander mean days to metamorphosis from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee (2009-12).

Distance (m)	Treatment	Year(s) since mowing	Mean Days to Metamorphosis	95% CI	<i>P</i>
3 ROW 9 ROW	AT	2	35.57 27.5	25.58 – 45.56 23.36 – 29.89	0.037
6 ROW 3 ROW Edge 3 Forest 6 Forest 9 Forest	FT	2	28.52 33.21 37.88 35.57 35.01 35.09	26.48 – 30.56 29.44 – 36.98 32.35 – 43.40 33.75 – 37.79 33.29 – 36.73 33.74 – 36.43	0.019 < 0.001 < 0.001 0.001 0.001
9 ROW 3 ROW Edge 3 Forest 6 Forest 9 Forest	FT	2	27.88 33.21 37.88 35.57 35.01 35.09	27.48 – 28.27 29.44 – 36.98 32.35 – 43.40 33.75 – 37.79 33.29 – 36.73 33.74 – 36.43	0.006 < 0.001 < 0.001 < 0.001 < 0.001 < 0.001
6 ROW Edge 3 Forest 6 Forest 9 Forest	AT	3	32.29 38.02 39.71 39.61 39.53	30.0 – 34.58 32.27 – 43.77 36.82 – 42.60 37.58 – 41.63 36.53 – 42.54	0.036 0.004 0.005 0.006

Table 1.9. Continued

Distance (m)	Treatment	Year(s) since mowing	Mean Days to Metamorphosis	95% CI	<i>P</i>
9 ROW	FT	3	33.26	25.75 – 40.77	0.013
3 Forest			40.75	38.93 – 42.57	
6 Forest			40.94	34.30 – 47.57	
6 ROW	FT	3	34.55	32.19 – 36.19	0.03
3 Forest			40.75	38.93 – 42.57	
6 Forest			40.94	34.30 – 47.57	
9 ROW	AT	4	32.58	26.67 – 38.50	0.006
Edge			41.69	39.53 – 43.85	
3 Forest			40.40	33.40 – 47.19	
6 Forest			41.53	37.34 – 45.72	
9 Forest			43.16	38.26 – 48.05	
9 ROW	FT	4	35.46	30.77 – 40.15	0.023
Edge			44.12	39.39 – 48.86	
3 Forest			43.46	40.54 – 46.38	
9 Forest			44.50	35.66 – 53.34	

Table 1.10. Results from Tukey's post-hoc analysis with a Bonferroni adjustment for a repeated measures ANOVA for larval survival of four-toed salamanders from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.

Distance (m)	Treatment	Year(s) since mowing	Mean Days to Metamorphosis	95% CI	<i>P</i>
9 Forest	AT	2	34.79	31.99 – 37.60	0.004
		3	39.71	36.82 – 42.60	
6 Forest	AT	2	33.66	28.74 – 38.58	0.003
		4	41.53	37.34 – 45.72	
3 Forest	FT	2	35.57	33.75 – 37.39	0.002
		3	40.75	38.93 – 42.57	
		4	43.46	40.54 – 46.38	0.044
6 Forest	FT	2	35.01	33.29 – 36.73	0.003
		1	43.43	41.62 – 45.23	
		4	42.56	38.01 – 47.11	0.044

Table 1.11. Results from Mann-Whitney test with Bonferroni adjustment for a mean daily pool temperatures from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.

Treatment	Year(s) since mowing	Distance (m); (Median Mean Daily Temperature C°)	Distance (m); (Median Mean Daily Temperature C°)	<i>P</i>
AT	1	9 ROW (25.47)	Edge (19.67)	0.047
	2	9 ROW (24.53)	Edge (20.47)	0.031
	3	9 ROW (24.45)	Edge (19.35)	0.001
	4	9 ROW (23.36)	Edge (19.32)	0.038
AT	3	6 ROW (21.23)	Edge (19.35)	0.029
FT	2	9 ROW (23.81)	Edge (20.23)	0.013
	3	9 ROW (22.17)	Edge (19.19)	0.003
FT	4	9 ROW (20.50)	3 Forest (19.17)	0.047

Table 1.12. Results from Friedman test for mean daily pool temperatures at the same distance into the forest or right-of-way, but between years from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.

Distance (m)	Treatment	Year(s) since mowing	Median Production	<i>P</i>
9 ROW	AT	1	25.47	0.027
		4	23.36	
6 ROW	AT	1	22.46	0.006
		4	20.55	
3 ROW	AT	2	20.33	0.027
		4	19.40	

Table 1.13. Results from Mann-Whitney test with Bonferroni adjustment for a maximum daily pool temperatures from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.

Treatment	Year(s) since mowing	Distance (m); (Median Maximum Temperature C°)	Distance (m); (Median Maximum Temperature C°)	<i>P</i>
AT	1	9 ROW (39.39)	Edge (24.74)	0.04
	2	9 ROW (37.27)	Edge (26.24)	0.02
	3	9 ROW (39.16)	Edge (25.95)	0.002
AT	4	9 ROW (36.73)	3 ROW (26.00)	0.032
			6 Forest (25.32)	0.027
FT	3	9 ROW (34.90)	Edge (25.46)	0.004
FT	3	6 ROW (31.07)	Edge (25.46)	0.047

Table 1.14. Results from Mann-Whitney test with Bonferroni adjustment for a net primary production from mesocosm pools at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.

Treatment	Year(s) since mowing	Distance (m); (Median Net Primary Production)	Distance (m); (Median Net Primary Production)	<i>P</i>
AT	1	6 ROW (2.52)	Edge (0.09)	0.023
FT	1	3 ROW (2.26)	6 Forest (0.04) 9 Forest (0.02)	0.03 0.03
AT	2	9 ROW (4.49)	Edge (-0.56) 3 Forest (-0.54)	0.014 0.02
FT	2	9 ROW (5.24)	3 Forest (-0.66)	0.036
AT	4	9 ROW (5.24)	6 Forest (-0.18) 9 Forest (-0.17)	0.023 0.023

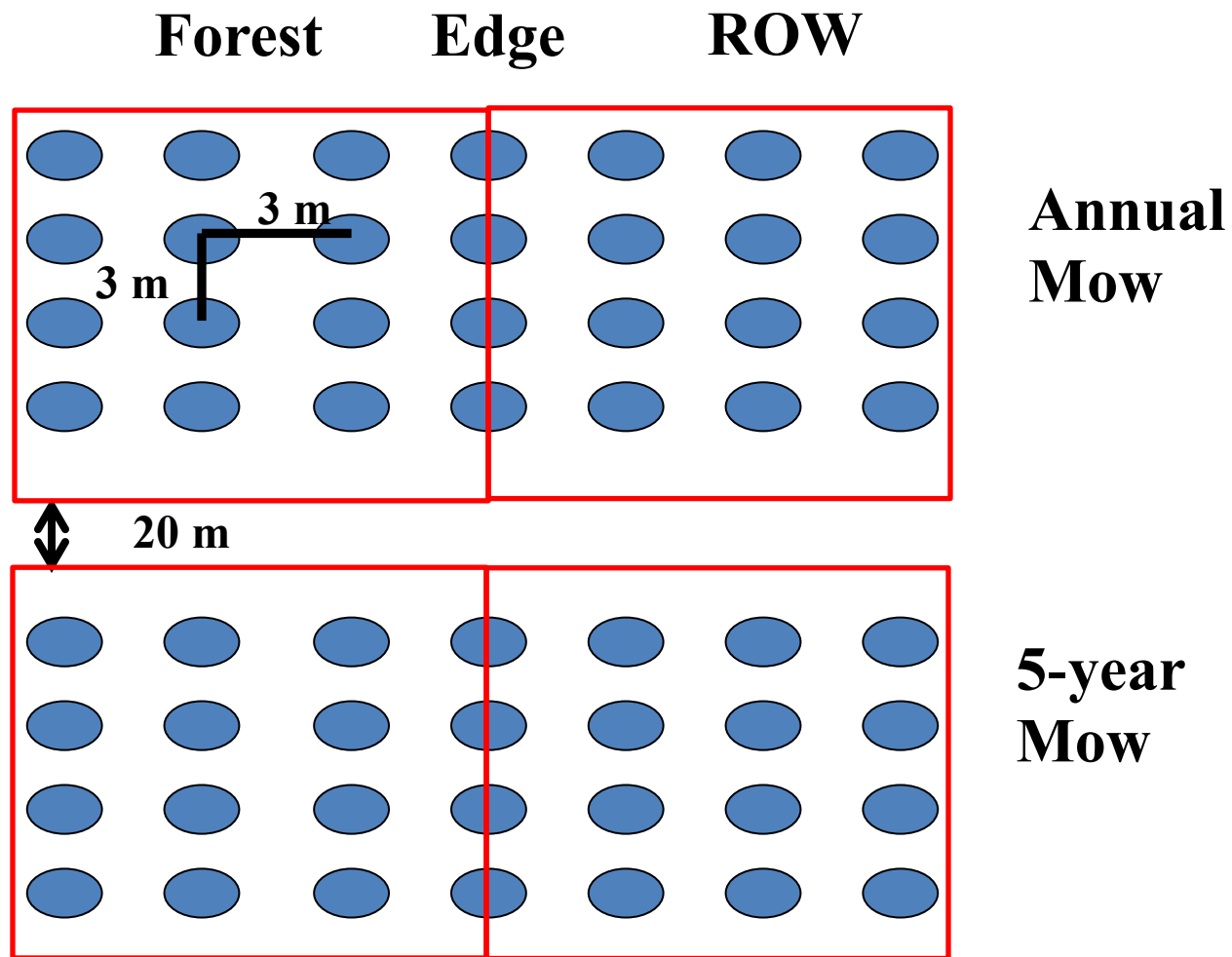


Figure 1.1. Four-toed salamander mesocosm pool layout at 3 m increments into the electric transmission line right-of-way and forest at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12.

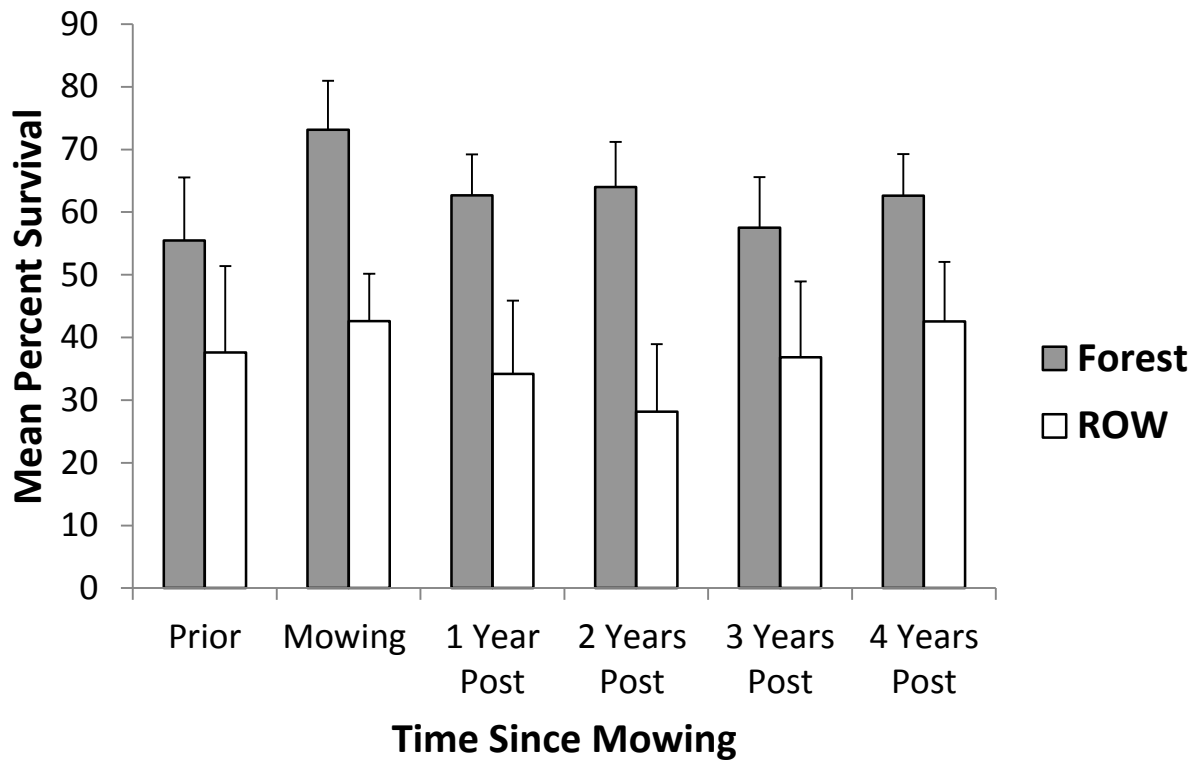


Figure 1.2. Mean percent four-toed salamander embryonic survival per treatment (forest or right-of-way) each year prior to and after ROW mowing at the South Holston Weir Dam, Sullivan County, Tennessee from 2007-12. Error bars represent SEM.

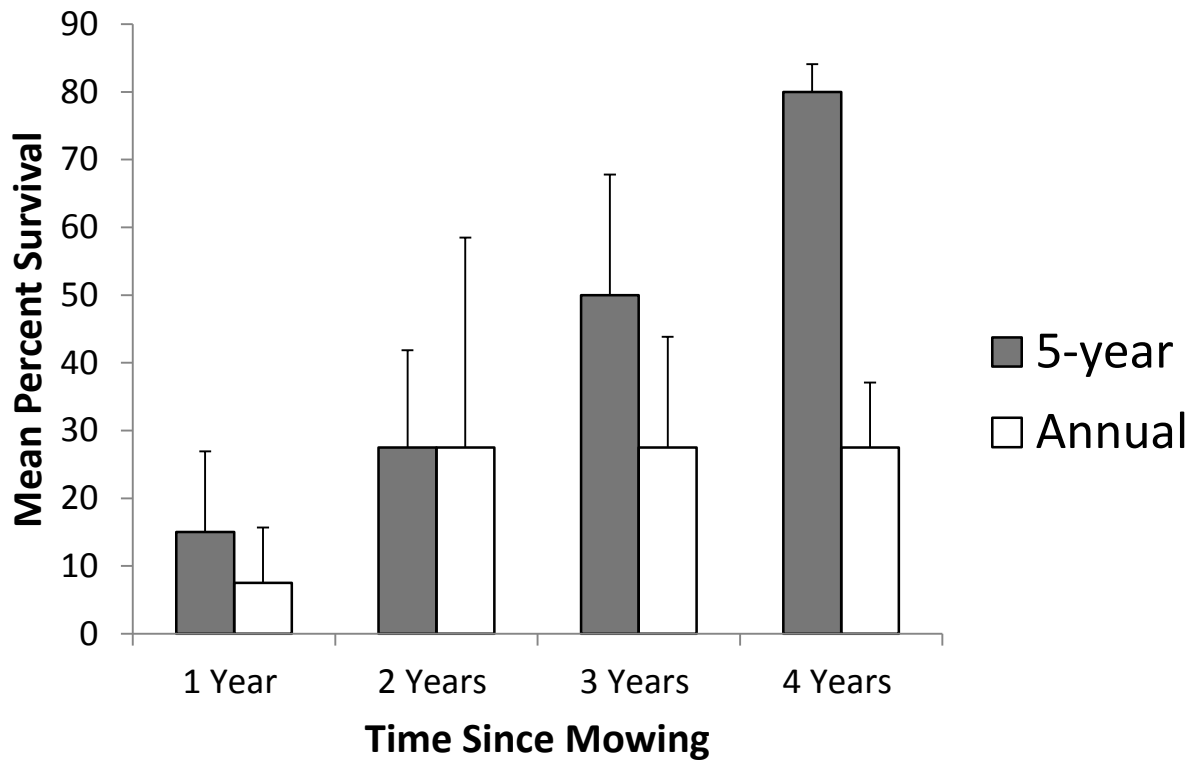


Figure 1.3. Four-toed salamander larval mean percent survival 9 meters into ROW with mesocosm pools at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12. Error bars represent +1 SD.

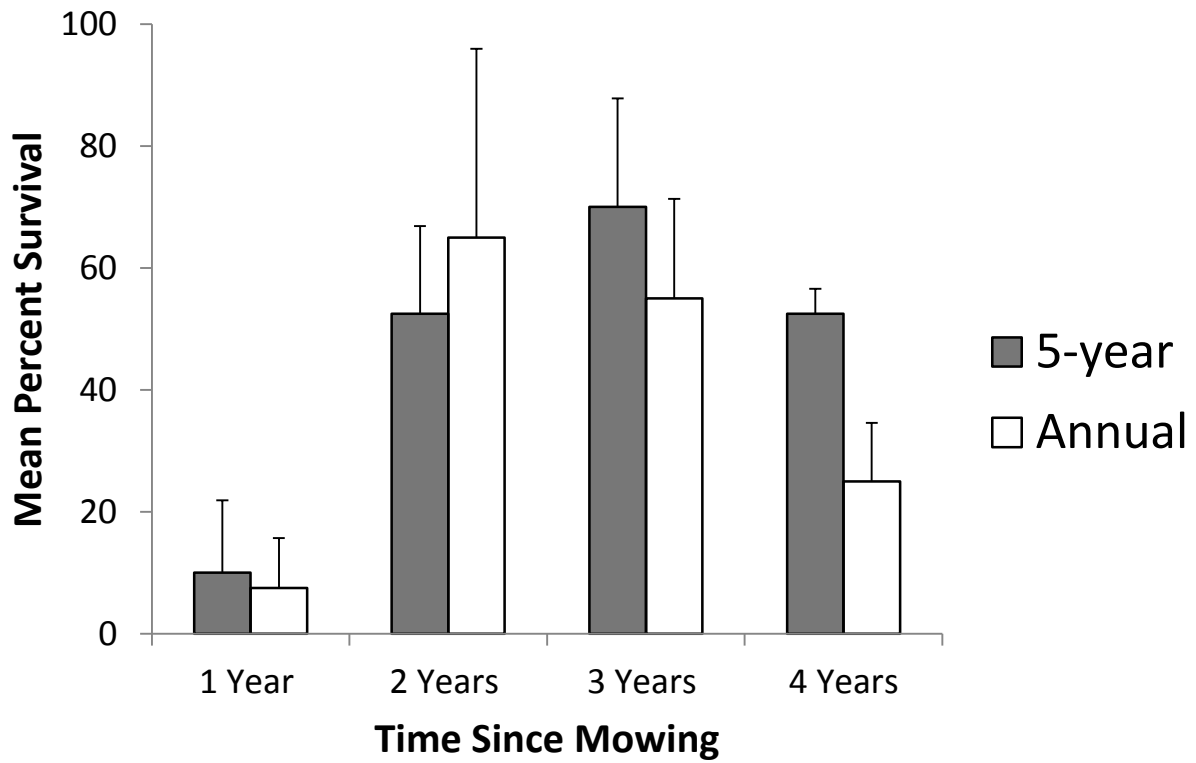


Figure 1.4. Four-toed salamander larval mean percent survival 6 meters into ROW with mesocosm pools at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12. Error bars represent +1 SD.

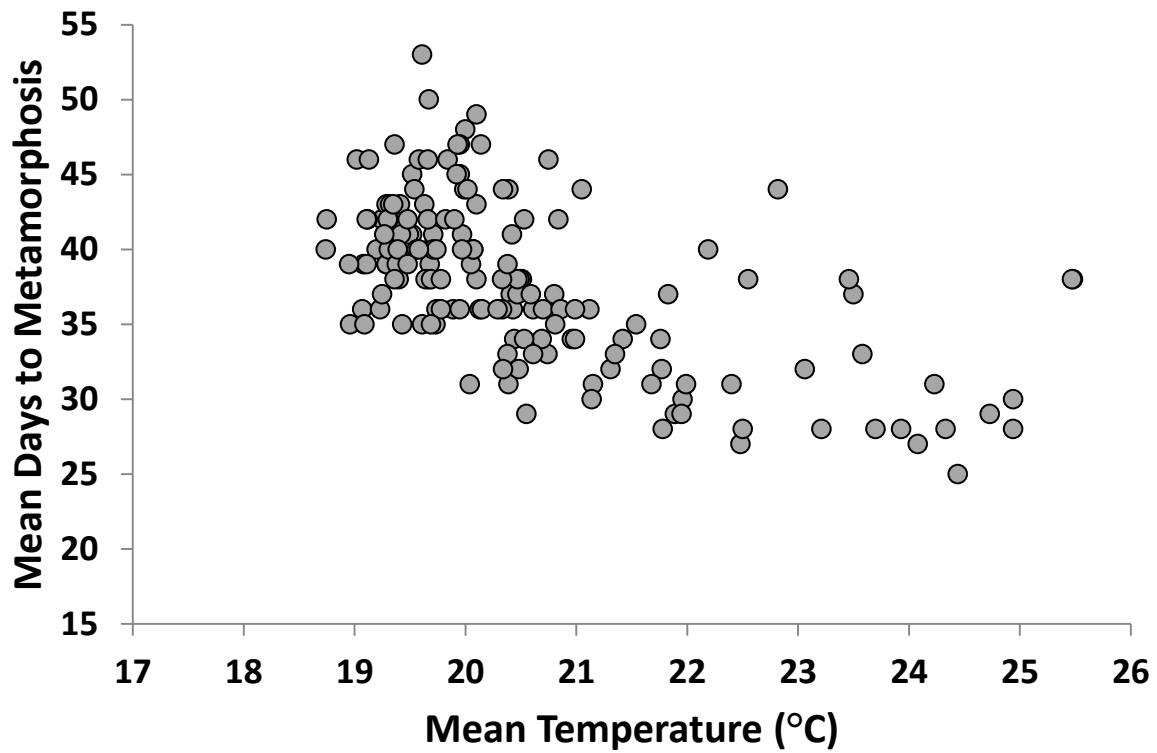


Figure 1.5. Relationship of mean days to metamorphosis and mean pool temperature for four-toed salamanders from mesocosm pools at the South Holston Weir Dam, Sullivan County, Tennessee from 2009-12. ($r_s = -0.589$; $P < 0.001$).

**CHAPTER 2: TEMPORIAL AND SPATIAL CHANGES OF MERCURY
CONTAMINATION OF BLACK-BELLIED SALAMANDERS FROM
WHITETOP MOUNTAIN, VIRGINIA**

ABSTRACT

Mercury (Hg) is a known neurotoxin that can enter ecosystems through atmospheric deposition and point sources from anthropogenic activities. Contamination levels and resulting negative effects have been established in numerous taxa of wildlife. Recently, Hg contamination in amphibians has been investigated, but data are lacking on Southern Appalachian salamanders. We investigated Hg concentrations in black-bellied salamanders (*Desmognathus quadramaculatus*) on Whitetop Mountain, VA. Both tail and liver tissue THg levels in *D. quadramaculatus* from Whitetop Mountain are comparable and even greater than THg levels in other amphibians from contaminated environments with health advisories. Liver THg was more than double levels documented for pig frogs in the Everglades National Park, for which a human consumption warning has been issued. Total mercury levels in 100% of liver and 56% of tail tissue sampled exceed the Environmental Protection Agency's piscivorous predator warning. Salamanders from northern aspect streams had THg levels 2.51 times greater than those from southern slopes and THg levels in *D. quadramaculatus* from higher elevations (> 1128 m) were 1.87 times greater than lower elevations (945 – 1097 m). We established nonlethal sampling methods by determining a correlation between total Hg (THg) levels in liver and tail tissue ($r_s = 0.834$). This method allowed tail tissue to be used for analysis. We also demonstrated that preserved salamander tissue could be used to provide historic levels of Hg contamination as we detected no significant change in THg levels of preserved black-bellied salamander tail tissue for 160 days after an initial 11% increase. Comparing current THg levels of *D. quadramaculatus* from museum specimens collected from Whitetop Mountain between 1957-9 were 4.5 – 15.3 times greater than current levels which indicated a reduction in contamination.

INTRODUCTION

Amphibian populations have been declining for the past 25 years and recently rates of decline have been increasing (Stuart et al. 2004; Wake and Vredenburg 2008). Atmospheric deposition of aerosolized chemicals and subsequent environmental contamination has been linked to the amphibian declines (Corn 2000). Mercury (Hg) is thought to negatively impact amphibian populations as it is easily deposited in the environment. Reduced reproductive success, neurological function, and hormone production in many wildlife species have been attributed to increased Hg levels (Driscoll et al. 2007). Mercury and other anthropogenic factors potentially act synergistically to negatively affect amphibians (Stebbins and Cohen 1995; Sparling et al. 2000). However, limited data exists on the impact of mercury on amphibians, especially Southern Appalachian salamanders (Houlahan et al. 2000).

Non-point source Hg enters ecosystems predominately through atmospheric deposition (Driscoll et al. 2007). Once on the ground and in the water, bacteria and chemical reactions convert Hg to its biologically active form of methylmercury (MeHg). Mercury bioaccumulates as it moves up food chains, especially in montane forest with shallow soils and in aquatic ecosystems (Driscoll et al. 2007; Rimmer et al. 2010). Long-lived predators at the apex of food chains often have greater Hg levels than short lived omnivores (Stemberger and Chen 1998) with mammalian and avian apex predators having Hg levels 1,000,000 times greater than that of bacteria initially fixing Hg.

Each year over 6600 metric tons of Hg enters the atmosphere with more than 50% originating from coal-fired power plants. Mercury emissions were the greatest in the late 1970s and have since decreased (Driscoll et al. 2007). Atmospheric Hg is deposited through wet precipitation events (rain and snow) or dry deposition through clouds and fogs (Driscoll et al.

2007). However, only areas with extended periods of cloud cover are impacted by dry deposition. Forested mountains are able to intercept greater amounts of mercury due to the increased surface area of the canopy and coniferous forest, such as red spruce (*Picea rubens*), intercept more Hg than deciduous forest due to more persistent needle surface area (Grigal 2002). High elevation peaks in the Southern Appalachians provide an opportunity to investigate the potential of Hg atmospheric deposition as most are forested, have summits dominated by red spruce forest, and have frequent cloud cover (Pyle and Schafale 1988).

Mercury levels in fish and piscivorous birds have been studied for many years but only recently have amphibians been examined. The few anurans that have been investigated displayed bioaccumulation of Hg (Bank et al. 2007). Pig frogs (*Lithobates grylio*) in the Everglades, FL should not be consumed for food as THg levels exceed federal limits (Ugarte et al. 2005) and bullfrogs (*Lithobates catesbianus*) in Texas have elevated THg levels (Chumchal et al. 2011). In headwater streams many salamanders occupy a similar niche as piscivorous birds or predatory fish (Petranka 1998). Adult and larvae *Eurycea bislineata* (northern two-lined salamander) have significantly greater levels of Hg than brook trout (*Salvelinus fontinalis*) from the same stream (Bank et al. 2005). *Plethodon cinereus* (red-backed salamander), *Eurycea bislineata*, and *Anaxyrus americanus* (American toad) inhabiting both aquatic and terrestrial environments contaminated from an industrial point source of Hg, contained up 22 times greater Hg levels than salamanders in non-contaminated areas (Bergeron et al. 2010). Larvae of *E. bislineata* had elevated Hg levels in high elevation forest and adults showed even greater levels of mercury than larvae of *E. bislineata* in lower elevation lakes (Bank et al. 2005).

Elevated mercury levels could have great impacts on salamander populations as other vertebrate taxa have displayed negative population effects. Sublethal concentrations of Hg

affected fish behavior and decreased productivity (Wiener and Spry 1996). Leatherback sea turtles (*Dermochelys coriacea*) showed lower reproductive success as Hg levels increased (mean liver THg = 17 ng/g wet weight; Perrault et al. 2011). White ibises (*Eudocimus albus*) had reduced egg production, increased male-male pairing, and decreased male-female pairing when exposed to Hg (THg blood > 1600 ng/g and feathers > 14,130 ng/g; Frederick and Jayasena 2011). Mallards (*Anas platyrhynchos*) produced fewer eggs and experienced increased embryo mortality when fed a diet contaminated with Hg (500 ng/g; Boening 2000). Female Atlantic bottlenose dolphins (*Tursiops truncatus*) have been shown to pass Hg to offspring which increased calf mortality (mean blood THg = 570 ng/g; Woshner et al. 2008).

The few studies to investigate Hg impacts on amphibian populations indicate potential negative population effects. Responsiveness and speed to capture prey were decreased in northern two-lined salamanders with elevated Hg levels (Burke et al. 2010). American toad tadpoles from females exposed to Hg swam significantly slower than those from controls. Additionally, toad larvae consuming a high Hg diet were smaller than those fed a control diet as well as those from females exposed to Hg (Bergeron et al. 2011). If these trends are consistent with other amphibians, Southern Appalachian salamanders could be experiencing similar effects due to Hg exposure.

Tracking the change of Hg concentrations in many species of wildlife has been useful to determine changes in atmospheric deposition. Museum specimens have served as a reservoir of data for environmental contamination, diseases, and distributions and have been useful to determine historic changes in Hg contamination (Suarez and Tsutsui 2004). Mercury levels from feathers of Swedish bird museum specimens collected from 1850 – 1950 were compared to feathers collected in the 1960s showed an increase in Hg levels (Berg et al. 1966). Feathers from

museum collections have also been utilized to determine Hg concentration changes in wading birds from the Everglades (Fredrick et al. 2004). Preserved museum fish have been used as a historical reference for comparison of current mercury levels in numerous studies (Hill et al. 2010; Martins et al. 2006; Kelly et al. 1975; Gibbs et al. 1974; Barber and Cross 1972). The potential for Hg tissue levels to change while in preservation fluids has caused concern for the accuracy of these methods (Gibbs et al. 1974). After one year of preservation, Hg was not detected in preservation fluids for Walleyes (*Sander vitreum*) fixed in 10% formalin and stored in 70% ethylalcohol (Kelly et al. 1975). Mercury levels in preserved longear sunfish (*Lepomis megalotis*) increased 18% over the first 40 days but remained stable for the remainder of preservation time (Hill et al. 2010). If preserved salamander tissues also maintain Hg levels in preservative, temporal comparisons could be made.

Our first object was to determine if Hg concentrations of *D. quadramaculatus* in tail tissue were correlated with liver concentrations, which would allow nonlethal sampling for the remainder of the project. This correlation has been established for *Eurycea bislineata* and *Plethodon cinereus* (Bergeron et al. 2010), but the authors recommended establishing this correlation prior to nonlethal sampling for other salamander species. The second objective was to determine if spatial variation in Hg accumulation occurs with elevation and aspect. My next objective was to determine the potential of preserved salamander tissue to determine past Hg concentrations, which would be investigated by preserving *D. quadramaculatus* tissue and measuring mercury concentrations every 40 days to determine the percent and rate of change. Finally, we compared current Hg concentrations with those from specimens collected from 1957-9.

METHODS

Study area and species

The Mount Rogers National Recreation Area (MRNA) is located in southwestern Virginia and lies within Grayson, Smyth, and Washington counties. Over 48,000 ha comprise the MRNA and within the area are the two highest peaks in Virginia: Mt. Rogers (1,746 m) and Whitetop Mountain (1,684 m). The summit (>1,554 m) of Whitetop Mountain is predominately red spruce (*Picea rubens*), while northern hardwood forest extends below the summit to approximately 1,158 m (Fisher et al. 2002). Whitetop Mountain is drained on the south side by Byars and Whitetop Creek. The north aspect is drained by Dells, Big, and Pennington Branch. We chose Whitetop Creek (south facing) and Big Branch (north facing) as my study sites. Whitetop Creek has intermittent flow at 1554 m and becomes permanent at 1524 m while Big Branch is intermittent at 1280 m and becomes permanent 1260 m. There were no streams on the north slope with flowing water as high as those on the south slope.

Whitetop Mountain annually receives 100-130 cm of precipitation. The mountain is covered with clouds 30-35% of the year (Baumgardner et al. 2003), providing up to an additional 50 cm of moisture each year (Thornton et al. 1994). An Environmental Protection Agency (EPA) Mountain Cloud Chemistry Program station detected the mean pH of cloud water to be 3.49 with levels as low as 2.59 (Vong et al. 1991). Cloud water analysis indicated fossil fuels were the main contributor to low pH levels (USEPA 1989). Mercury is released during the combustion of fossil fuels and could also be deposited on Whitetop Mountain.

Black-bellied salamanders (*Desmognathus quadramaculatus*) are common in Southern Appalachian headwater streams where they are usually apex predators (Peterman et al. 2008). Their complex life cycle potentially exposes them to Hg in their aquatic larval phase and also in

the terrestrial environment as an adult. *Desmognathus quadramaculatus* feeds predominately (82%) on aquatic invertebrates (Davic 1991) during a 24 – 26 month aquatic larval stage on Whitetop Mountain (Organ 1961). After metamorphosing, the diet of *D. quadramaculatus* shifts to terrestrial insects, large aquatic invertebrates such as crayfish, and other salamanders including smaller conspecific salamanders (Camp and Lee 1996; Bishop 1924). On rainy nights *D. quadramaculatus* will feed farther than 1 m from the stream bank consuming terrestrial prey items (Peterman et al. 2008). As an adult, many of their invertebrate prey species feed on leaves in the canopy or those that have recently fallen, which are the primary interceptor of atmospheric Hg from cloud water (Grigal 2002). Other salamanders that are preyed upon by *D. quadramaculatus* have also eaten the same invertebrates which create the potential for bioaccumulation of Hg as *D. quadramaculatus* has a maximum life span up to 13 years (Bruce 1988; Bruce et al. 2002).

Tissue collection

We collected black-bellied salamanders from Big Branch (945 – 1280 m) in September 2011 and Whitetop Creek (1158 – 1524 m) in June/July 2012. Sampling occurred every 30.5 m of elevation and sampling locations matched collections made by Organ (1961). We located salamanders by turning rocks and logs within and along the stream edge (n = 50 from Whitetop Creek and n = 50 from Big Branch). We used dial calipers to measure the snout-vent length (SVL) and total length (TL) to the nearest 0.1 mm of each salamander and used an Ohaus HP-320 scale measured the mass of each salamander to the nearest 0.1 g.

We used a sterile scalpel to remove a small portion of the tail tip from each salamander and sealed each wound with vetbondTM. Individual salamanders displaying signs of recent tail regeneration were excluded from all portions of the study. We placed tail samples individually

in a glass vial prepared in the laboratory and in accordance to the University of Maine Sawyer Environmental Chemistry Laboratory cleaning procedure of washing in lab detergent (LiquidnoxTM) followed by five rinses in 2% aqua regia solution. A rinse with deionized water occurred after detergent washing and each aqua regia rinse. We stored collection vials with tissue samples were stored in a cooler during field collection and immediately placed in a freezer upon return to the laboratory.

Nonlethal Sampling

To determine the effectiveness of non-lethal sampling, we compared total mercury levels of liver and tail tissue. Additionally, the ratio between total mercury in liver and tail tissue can determine if contamination is ongoing or the results of past exposure (Sorensen 1991). A liver THg / muscle THg > 1 would suggest ongoing exposure while ratios < 1 would indicate a past exposure event with less ongoing contamination in a pulse manner (Jagoe et al. 1998; Sorensen 1991). We selected a sub-sample of 30 salamanders across all elevations along Big Branch and euthanized salamanders with an overdose of buffered MS-222 (FinquelTM), placed them on ice, and transported salamanders in a cooler. We returned salamanders to the laboratory where we removed a tail tip and approximately half of the liver, placed tissue samples in a glass vial, and then froze the samples.

Historic museum samples

We evaluated the effects of long term preservation levels on salamander tissue by preserving the remainder of each salamander previously used for liver samples (n = 30). We followed the fixation and preservation procedure of the University of Michigan Museum of Zoology (Schneider personal communication) and it was the same procedure used by Organ (personal communication) for his collection. We initially fixed salamanders in 10% buffered

formalin for 7 days and then rinsed in tap water for 2 days. We then placed specimens in 65% ethanol and stored salamanders from the same elevation in the same glass specimen jar. We removed a tail sample every 40 days after placement in alcohol for a duration of 160 days. We placed all tail samples glass vials and immediately frozen.

Salamanders collected from 1957-9 by Organ (1961) are housed in the University of Michigan Museum of Zoology collection, Ann Arbor, Michigan. Each individual specimen has an identification tag with a specimen number that corresponds to the collection location and elevation in Organ's field notes. In March 2010 and July 2012, we visited the museum and located 50 salamanders from both Whitetop Creek and Big Branch across all elevations sampled for a total of 100 salamanders. My collection times of June/July and September and locations were the same as Organs. We collected a tail sample from each salamander, placed it in a glass vial, and froze the sample.

Total mercury analysis

All mercury analysis was conducted by the University of Maine Sawyer Environmental Chemistry Laboratory. Samples were freeze dried for 48 hours to remove moisture (Labconco LYPH-LOCK 4.5 Freeze Dry System; Kansas City, KS) and then weighed. Total mercury analysis procedure followed EPA method 7473 (USEPA 1998) and was conducted with Direct Mercury Analyzer (DMA-80; Milestone Inc., Monroe, CT) by combustion-amalgamation-cold vapor atomic absorption spectrophotometry. This procedure has been successful for numerous salamander and amphibian Hg studies (Bergeron et al. 2010; Bergeron et al. 2010b, Bergeron et al. 2007). Each group of 12 to 15 samples included a sample replicate and at least one standard reference material (DM-1 and DM-2 dogfish muscle cell [National Research Council of Canada, Ottawa, ON]). Mercury detection limits ranged from 1.00 to 100.29 ng/g and samples with THg

concentrations below detection limits were excluded from the study. Mean percent difference from replicate samples was $3.87 \pm 0.34\%$ ($n = 42$; mean $\pm$ standard error of the mean). Mean percent recoveries of THg for standard reference material DM cell 1 and cell 2 were $98.44 \pm 0.44\%$ ($n = 42$) and $97.39 \pm 0.67\%$ ($n = 17$), respectively. Our THg values were calculated as dry weight but salamander muscle tissue has been shown to be 73-75% moisture (Burke et al. 2010). Therefore an equal comparison to wet weight (ww) studies would be 25% of our concentrations.

Statistical analyses

We used a Spearman's Rank Order to establish relationships between liver and tail muscle THg concentrations as $\log_{10}$ transformed data were not normally distributed. To account for mercury bioaccumulation as individuals aged, we calculated THg as ng/g per millimeter of SVL. We used an analysis of variance (ANOVA) to compare THg concentrations between stream aspects, stream elevations, days in preservative, and collection time periods. We $\log_{10}$ transformed data that was not normally distributed to meet assumptions necessary for ANOVA analysis. Due to a small sample size of salamanders from each elevation, samples were pooled into two classes from high elevations (1128 - 1280 m) and low elevations (945 – 1097 m) along Big Branch. However, all but one transect along Whitetop Creek was above 1097 m not allowing for elevational comparisons. We conducted all analyses with SPSS v.20.0 (IBM 2011) with an α value of 0.05.

RESULTS

Four salamanders sampled for non-lethal collection had tail, liver, or both tissue samples with THg levels below detection limit (1 ng/g) and were not included in the analysis; therefore 26 salamanders were used for analysis of non-lethal collection. There was a strong, positive correlation between liver and tail muscle THg levels ($r_s = 0.83$, $P < 0.01$) (Figure 2.1). These

results allowed only tail samples to be collected for the remainder of the project thus establishing a means of nonlethal sampling. Mean liver THg / tail muscle THg ratio was 8.025 ± 0.770 (SE) and values ranged from 3.51 – 16.93 for all salamanders sampled along Big Branch.

Sixteen (n= 8 Whitetop Creek; n= 8 Big Branch) tail samples had THg levels below detection limits and were not included for aspect and elevation comparisons resulting in 84 total samples. For all *D. quadramaculatus* sampled, mean detectable total mercury levels in liver tissue was 2,783.6 ng/g (246.6 – 16,545.6; n=26) and 133.8 ng/g (8.5 – 1,258.3; n=84) in tail muscle tissue across both sites and all elevations.

Total mercury concentrations varied by both aspect and elevation. Mean tail muscle THg (ng/g per mm of svl) from black-bellied salamanders in northern-facing Big Branch was 2.51 times greater than salamanders in southern-facing Whitetop Creek ($F_{1,82} = 39.03$, $P < 0.001$; Figure 2.2). Black-bellied salamanders from higher elevations (1128-1280 m) in Big Branch had THg tail muscle concentrations that were 1.87 times greater than those from lower elevations (945 – 1097 m; Figure 2.3) ($F_{1,40} = 5.039$, $P = 0.03$).

Effects of Preservation & Historical Comparisons

Only 26 salamanders sampled were used to examine the effects of preservation as four salamanders had THg levels below detection limits and three of the four also did not have an adequate amount of tail tissue to use for five samples. Over the entire 160 days the mean percent change in THg was 9.38% (± 1.44) with a maximum of 11.20% (Figure 2.4). The greatest change of THg levels in *Desmognathus quadramaculatus* tail tissue due to preservation occurred during the first 40 days (7.51% increases). There were no significant differences between percent change from 40, 80, 120, and 160 days ($F_{3,86} = 0.283$, $P = 0.837$).

Historic mercury levels were calculated based on a maximum of 11.2% reduction obtained from examining the increase of Hg levels due to preservation process obtained in the previous portion of this study. Therefore, THg levels obtained from museum specimens were decreased by 11.2% to compensate for the change in preservation. Total Hg levels from 1957-59 Big Branch samples were 4.59 times greater than those collected in 2011 ($F_{1,87} = 171.96$, $P < 0.001$) while THg levels from Whitetop Creek were 15.25 times greater than those from 2012 ($F_{1,93} = 359.19$, $P < 0.001$)

DISCUSSION

The greatest liver and tail muscle THg levels from *D. quadramaculatus* on Whitetop Mountain were 16,545 and 1,258 ng/g respectively. Limited studies have investigated mercury in salamanders, but when compared to these studies our results are comparable to amphibians in contaminated environments (Tables 2.1 & 2.2). In a point source, contaminated stream *Plethodon cinereus* and *Eurycea bislineata* had tail tissue THg levels of approximately 90-600 ng/g and 1,000-6,000 ng/g respectively (Bergeron et al. 2010). We had several *D. quadramaculatus* with THg tail tissue levels exceeding 1,000 ng/g indicating levels equal to or greater than salamanders from a point source. Pig frogs in the Everglades had liver THg of 269 - 544.4 ng/g (ww) and have a warning against human consumption (Table 2.2; Ugarte et al. 2005). On Whitetop Mt., *D. quadramaculatus* had liver THg levels which greatly exceed levels found in pig frogs. Liver THg levels in black-bellied salamanders on Whitetop Mountain ranged from 17 – 4,136 ng/g when they were converted to wet weight. Caddo Lake, Texas has fish with some of the highest THg levels in the state (Chumchal et al. 2011). Bullfrogs from Caddo Lake had mean liver THg levels of 1,228 ng/g (dw, dry weight) and American alligators, an apex predator,

had liver THg levels of 2,263 ng/g (dw) while the mean liver THg from *D. quadramaculatus* on Whitetop Mountain was 2,784 ng/g (dw) which is twice bullfrogs and slighter greater than American alligator THg levels from one of the worst mercury contamination sites in Texas (Table 2.2; Chumchal et al. 2011). Black-bellied salamanders from Whitetop Mountain have liver and tail muscle THg levels that are comparable and greater to areas of known point source and atmospheric contamination with levels requiring consumptions advisories.

In addition to protecting humans from Hg contamination, a MeHg criterion (77 ng/g) for the protection of avian and mammalian piscivorous predators has been established (USEPA 1997). We did not quantify MeHg, but research has documented MeHg levels in salamanders. Methylmercury composed 73-97% of THg from *Eurycea bislineata* larvae in Acadia National Park, Maine (Banks et al. 2005) and 56.8% of THg from contaminated sites in Virginia (Bergeron et al. 2010). Adult *E. bislineata* and *P. cinereus* from the same contaminated Virginia stream had 61% and 46% of their THg as MeHg (Bergeron et al. 2010). By conservatively applying the lowest value documented of 46% THg as MeHg, all Whitetop salamanders sampled would exceed the wildlife protection criterion for liver tissue and 56% would exceed the standard for tail tissue. Black-bellied salamanders have been known to be preyed upon shrews, snakes, and other salamanders (Petranka 1998). Bioaccumulation of Hg may also be a problem for salamander predators.

Ratios of liver THg to tail THg indicated that Hg contamination is ongoing in *D. quadramaculatus* on Whitetop Mt. Our mean ratio (8.03) is greater than those from other hepatofaunal species in contaminated areas. American alligators in the Everglades, which has a history of Hg contamination, had a mean ratio of 7.15 (Jagoe et al. 1998). Additionally, alligators from a Savannah River Site with Hg contaminated fish had a mean ratio of 4.21. Pig

frogs from the Everglades had a mean liver THg to leg muscle ratio of THg 2.84 and were recommended to not be harvested or consumed due to Hg levels (Ugarte et al. 2005). Based on our liver to muscle tissue ratios, black-bellied salamanders from Whitetop Mt. are being exposed to Hg at a rate that is similar and greater than other areas of known Hg contamination.

Total mercury levels in *D. quadramaculatus* were 2.51 times greater from the northern slope than the southern slope which suggested spatial deposition differences. Mercury typically enters ecosystems through atmospheric deposition (Driscoll et al. 2007). Given the degree of acid deposition from precipitation and cloud deposition on Whitetop Mountain from fossil fuels, atmospheric deposition could be a potential source of mercury entering this high elevation ecosystem (Vong et al. 1991). From 1986-1988 the EPA maintained a data collection center, including prevailing wind direction, on Whitetop Mountain as part of the Mountain Cloud Chemistry Program (USEPA 1989). During this study, the prevailing wind direction was the northwest as 50.9% of all wind currents originated from this direction. Southeast, southwest, and northeast composed 26.4%, 15.9%, and 6.8% of wind currents respectively. Streams from the slope facing the prevailing wind had black-bellied salamanders with the highest THg. Prevailing winds have also been shown to increase many pollutants that return to the ground through atmospheric deposition through precipitation and cloud vapor. On Whitetop Mt., lead levels in American Beech (*Fagus gradifolia*) trees were significantly greater on northwestern slopes which was similar to THg levels in black-bellied salamanders (Fisher et al. 2002). Mercury deposition increases with prevailing wind direction in the Adirondacks, NY (Yu et al. 2013), Wisconsin (Kolker et al. 2010), Alberta, Canada (Sanei et al. 2010), and China (Kim et al. 2012). The best model describing THg level in osprey (*Pandion haliaetus*) eggs and feathers from western Canada included proximity to western coast and prevailing winds (Guigueno et al.

2012). Amphibian contamination from pesticides deposited through atmospheric deposition has been shown to be greatly influenced by prevailing wind direction. Californian anuran populations suffered declines in eastern mountain regions downwind from intense pesticide use compared to western mountainous regions (Sparling et al. 2000; Davidson et al. 2001; Davidson et al. 2002). *Desmognathus quadramaculatus* in areas of the Great Smoky Mountains National Park receiving prevalent winds displayed elevated pesticide levels (Freake and Lindquist 2008). We infer that prevailing winds intercepting Whitetop Mountain could be increasing THg concentrations in *D. quadramaculatus*.

Mercury deposition has also been documented to be greater at higher elevations as these areas often receive greater precipitation and are exposed to greater cloud cover. Deposition increased with higher elevations in the Adirondacks especially with the amount intercepted by coniferous trees increasing 3 times from 800 to 1600 meters in elevation (Yu et al. 2013). Tropical birds at higher elevation cloud forest have THg blood concentrations 2-20 times greater than lower elevation rainforest (Townsend et al. 2013). This pattern was repeated on Whitetop Mountain in American beech leaf levels deposited through atmospheric deposition (Fisher et al. 2002). On Whitetop Mountain black-bellied salamanders at high elevations on northern facing slopes had THg levels that were almost twice as great as those from lower elevations. Results from black-bellied salamanders suggest that elevation could be contributing to Hg deposition on Whitetop Mountain.

Numerous nonlethal sampling methods have been developed due to positive correlations between different types of body tissue in many species of herpetofauna and fish. Our results provide additional evidence that nonlethal sampling can be used for Hg sampling in salamanders. Tail muscle THg was highly correlated with liver THg. Bergeron et al. (2010) established

nonlethal techniques with *E. bislineata*, *P. cinereus*, and *A. americanus* with r-values ranging from 0.916 – 0.994 for correlations between tail muscle and whole body analysis. A positive correlation ($r=0.88$) between toe tissue and blood samples of American toads provided justification for nonlethal sampling (Todd et al. 2012). Nonlethal sampling has been used successfully for other taxa as well. Nails and blood from freshwater turtles in a Hg contaminated stream were positive related (r_s 0.69-0.75; Hopkins et al. 2013). A positive correlation was identified in largemouth bass (*Micropterus salmoides*) between muscle tissue and scales ($r^2 = 0.89$; Ryba et al. 2008). Bergeron et al. (2010) expressed caution of assuming tissue correlations in other salamander species and locations without first establishing a correlation. Our association (0.83) varied slightly from Bergeron et al. (2010), but still indicated a significant positive relationship, confirming a relationship should be established prior to nonlethal sampling for each species. Given the similarities between our results and those of other studies, we conclude that tail tissue for black-bellied salamanders provides an accurate method to monitor THg concentrations.

There is limited evidence for negative impacts of Hg contamination on amphibians especially salamanders. However, *Eurycea bislineata* from contaminated streams required more time to move 1 meter and made fewer strikes at prey items in a laboratory setting (Burke et al. 2010). *Eurycea bislineata*, making few prey strikes, had mean whole body THg levels of 4,519 ng which was greater than our mean tail tissue samples but considerably less than mean THg liver levels (Burke et al. 2010). Southern leopard frogs (*Lithobates sphenoccephalus*) experienced longer times for tail absorption and greater larval mortality with body THg of ~250 ng/g, which were lower than THg levels in our study (Urine et al. 2004). Marbled salamander (*Ambystoma opacum*) larvae experienced 50% mortality with THg levels of 103 ng/g (Sparling et al. 2000).

American toad tadpoles fed Hg and with THg whole body level of ~1,000 ng/g were significantly smaller than animals from a reference population and tadpoles from females exposed to Hg were also smaller (Bergeron et al. 2011). Black-bellied salamanders from Whitetop Mountain had both tail and liver THg that were comparable to or exceeded amphibians from other studies in which significant developmental impacts or mortality were observed suggesting that there could be negative effects in black-bellied salamanders due to Hg.

This study was the first known use of preserved salamander tissue to establish historic mercury tissue concentrations. The use of museum specimens to measure historical changes in mercury contamination has been successful with bird feathers which require no preservation (Frederick et al. 2004; Berg et al. 1966). However, fish and amphibians require fixation and storage in a preservative. There has been much debate on the reliability of preserved fish tissue to provide accurate historical Hg levels. Gibbs et al. (1974) cautioned against using preserved fish tissue as a measure of historic Hg until changes in preservation were better understood. Hill et al. (2010) determined Hg concentration increased 18% during the first 40 days in preservative and remained somewhat constant for the remainder of 160 days indicating that preservation had minimal effects after 40 days. Additionally, no detectable levels of Hg were found in ethanol that had been used to preserve museum specimens of fish indicating that leaching of Hg from specimens was not affecting concentrations (Miller et al. 1972). The greatest potential change to Hg concentrations in preserved tissue would be due to changes in the sample weight through dehydration (Evans et al. 1972; Hill et al. 2010). Both salamander and fish tissue are estimated to be 75% water and storage in alcohol is suspected to dehydrate the sample (Evans et al. 1972; Kelly et al. 1975). Even with dehydration, we conclude the use of preserved black-bellied salamander tissue does provide an accurate estimate of past Hg contamination as historic values

can be corrected for initial changes caused by preservation. Our mean rate of change was 9.38% which was less than the 18% rate of change observed in long eared sunfish over the same time period (Hill et al. 2010). Fish tissue samples experienced the greatest change within the first 40-50 days of preservation and black-bellied salamander tissue experienced similar patterns (Hill et al. 2010; Kelly et al. 1975). Therefore, it appears the greatest change will occur initially after preservation and calculated concentrations can be adjusted.

We were able to demonstrate that THg concentrations in *D. quadramaculatus* from Whitetop Mountain have decreased significantly since 1958-9. Salamanders from Whitetop Creek had the greatest THg decline as levels were 15.25 times greater in 1958 and concentrations from Big Branch were 4.59 times greater in 1959. Regulatory programs implemented in the 1970s mandated a reduction in Hg emissions (Krabbenhoft et al. 2005). Subsequently Hg reductions have been documented in both air emissions and fish tissue especially in the northeastern United States. Deposition rates declined by 4 times from the 1960s to 1980s (Engstrom and Swain 1997) and air emissions dropped by almost 50% from the mid-1990s to 2002 (Chalmers et al. 2011). Fish populations from throughout the US showed declining THg concentrations at 6 times greater rates than those increasing from 1969-1987 (Chalmers et al. 2011). Walleye (*Sander bitreus*) and lake trout (*Salvelinus namaycush*) in the Great Lakes have shown THg reductions from 1980s to 2000s with rates of decrease as high as 120 ng/g per decade (Bhavsar et al. 2010). Our results from Whitetop Mountain are consistent with those from other aquatic ecosystems where historic comparisons have been made. Even though significant reductions have occurred in black-bellied salamanders from Whitetop Mountain, current Hg concentrations remain high with large individuals falling within established limits for the protection of avian and mammalian piscivorous predator warnings.

CONCLUSIONS

Our results provide additional evidence that salamanders inhabiting protected habitats great distances from point sources of Hg can be contaminated with THg tissue levels comparable to areas of industrial pollution (Fitzgerald et al. 1998). We demonstrated for the first time that black-bellied salamanders, serving as apex predators, have elevated Hg tissue levels. Liver and tail muscle tissue THg levels were comparable and some exceeded other herpetofauna from known contaminated populations (Chumchal et al. 2011; Bergeron et al. 2010; Ugarte 2005). Black-bellied salamanders exceeded the EPA piscivorous predator limit which suggests mercury could be moving to higher trophic levels through salamanders. Prevailing winds and cloud deposition could be increasing THg concentrations in salamanders as those from northern slopes and higher elevations had significantly higher THg levels. Additionally, nonlethal sampling techniques of using tail tissue were established eliminating the need for sacrificing animals for future research. Total mercury levels in preserved salamander tissue appear to stabilize after 40 days in preservative with a maximum increase of ~11%. By applying our findings to historic samples from Whitetop Mountain, mercury contamination in black-bellied salamanders has decreased significantly in the past 50 years. We acknowledge the need for additional investigations to determine if THg levels in preserved tissue will remain stable for time periods greater than 160 days, but based on fish tissue we conclude levels will remain somewhat constant and provide a comparison for historical change. Our results recognize another anthropogenic factor that could impact Southern Appalachian salamanders. Additional research should be conducted to determine what impacts Hg contamination could have on *D. quadramaculatus* populations, but if effects are similar to other salamanders, negative neurological and reproductive effects could be observed.

Acknowledgments

This research was completed with funds provided by the Virginia Department of Game and Inland Fisheries through a State Wildlife Grant from the U.S. Fish and Wildlife Service and additional funding from the Virginia Community College System Paul Lee Professional Development Grant. We thank J. and D. Organ for providing sampling locations, field notes of 1950s sampling, and guidance. A. and T. Horton provided field assistance. A special thanks to G. Schinder and the staff of the Univ. of Michigan Museum of Zoology for assistance in obtaining museum specimens. We also thank C. Devoy and M. Langerklint, Univ. of Maine Sawyer Envir. Chemistry Laboratory for help with analytical analysis. Additionally, we are grateful to the MRNA staff for project assistance and to many private land owners for access to our study sites. All sampling was approved by the Virginia Department of Game and Inland Fisheries (Scientific Collection Permit #41396) and followed UT Institutional Animal Care and Use Committee protocol #2084-0412.

LITERATURE CITED

- Bank, M. S., J. Crocker, B. Connery, and A. Amirbahman. 2007. Mercury bioaccumulation in green frog (*Rana clamitans*) and bullfrog (*Rana catesbiana*) tadpoles from Acadia National Park, Maine, USA. *Environmental Toxicology and Chemistry* 26:118-25.
- Bank, M.S., C. S. Loftin, and R. E. Jung. 2005. Mercury bioaccumulation in northern two-lined salamanders from streams in the Northeastern United States. *Ecotoxicology* 14:181-91.
- Barber, R. T. and F. A. Cross. 1972. Mercury concentrations in recent and ninety-year old benthopelagic fish. *Science* 178:636-9.
- Baumgardner, R. E., S. S. Isil, T. F. Lavery, C. M. Rogers, and V. A. Mohnen. 2003. Estimates of cloud water deposition at Mountain Acid deposition program sites in the Appalachian Mountains. *Journal of the Air and Waste Management Association* 53:291-308.
- Berg, W., A. Johnels, B. Sjostrand, and T. Westermark. 1966. Mercury content in feathers of Swedish birds from the past 100 years. *Oikos* 17:71-83.
- Bergeron, C. M., W. A. Hopkins, C. M. Bodinof, S. A. Budischak, H. Wada, J. M. Unrine. 2011. Counterbalancing effects of maternal mercury exposure during different stages of early ontogeny in American toads. *Science of the Total Environment* 409: 4746-4752.
- Bergeron, C. M., C. M. Bodinof, J. M. Unrine, and W. A. Hopkins. 2010. Mercury accumulation along a contamination gradient and nondestructive indices of bioaccumulation in amphibians. *Environmental Toxicology and Chemistry* 29:980-8.
- Bergeron, C. M., C. M. Bodinof, J. M. Unrine, and W. A. Hopkins. 2010b.

- Bioaccumulation and maternal transfer of mercury and selenium in amphibians.
Environmental Toxicology and Chemistry 29:989-97.
- Bergeron, C. M., J. F. Husak, J. M. Unrine, C. S. Romanek, and W. A. Hopkins. 2007.
Influence of feeding ecology on blood mercury concentrations in four species of turtles.
Environmental Toxicology and Chemistry 26:1733-41.
- Bhavsar, S. P., S. B. Gewurtz, D. J. McGoldrick, M. J. Keir, and S. M. Backus. 2010. Changes in
mercury levels in Great Lakes fish between 1970s and 2007. Environmental Science and
Technology 44:3273-3279.
- Bishop, S. C. 1924. Notes on salamanders. New York State Museum Bulletin 253:87-96.
- Boening, D. W. 2000. Ecological effects, transport, and fate of mercury: a general
review. Chemosphere 40:1335-51.
- Bruce, R. C., J. Castenet, and H. Francillon-Vieillot. 2002. Skeletochronological analysis
of variation in age structure, body size, and life history in three species of desmognathine
salamanders. Herpetologica 58:181-93.
- Bruce, R. C. 1988. Life history variation in the salamander *Desmognathus quadramaculatus*.
Herpetologica 44:218-227.
- Burke, J. N., C. M. Bergeron, B. D. Todd, and W. A. Hopkins. 2010. Effects of mercury
on behavior and performance of northern two-lined salamanders (*Eurycea bislineata*).
Environmental Pollution 158:3546-51.
- Camp, C. D. and T. P. Lee. 1996. Intraspecific spacing and interaction within a population of
Desmognathus quadramaculatus. Copeia 1996:78-84.

- Chalmers, A. T., D. M. Argue, D. A. Gay, M. E. Brigham, C. J. Schmitt, D. L. Lorenz. 2011. Mercury trends in fish from rivers and lakes in the United States, 1969-2005. *Environmental Monitoring Assessment* 175:175-191.
- Chumchal, M. M., T. R. Rainwater, S. C. Osborne, A. P. Roberts, M. T. Abel, G. P. Cobb, P. N. Smith, F. C. Bailey. 2011. Mercury speciation and biomagnification in the food web of Caddo Lake, Texas and Louisiana, USA, A subtropical freshwater ecosystem. *Environmental Toxicology and Chemistry* 30:1153-1162.
- Corn, P. S. 2000. Amphibian declines: review of some current hypotheses. In: Sparling DW, Bishop CA, Linder G, editors. *Ecotoxicology of amphibians and reptiles*. Pensacola FL: Society of Environmental Toxicology and Chemistry. p 663-696.
- Davic, R. D. 1991. Ontogenetic shift in diet of *Desmognathus quadramaculatus*. *Journal of Herpetology* 25:108-111.
- Davidson, C., H. B. Shaffer, and M. R. Jennings. 2002. Spatial tests of pesticide drift, habitat destruction, UV-B, and climate change hypotheses for California amphibian declines. *Conservation Biology* 16:1588-1601.
- Davidson, C., H. B. Shaffer, and M. R. Jennings. 2001. Declines of the California red-legged frog: climate, UV-B, habitat, and pesticides hypothesis. *Ecological Applications* 11:464-479.
- Driscoll, C. T., H. Young-Ji, C. Y. Chen, D. C. Evers, K. F. Lambert, T. M. Holsen, N. C. Kamman, and R. K. Munson. 2007. Mercury contamination in forest and freshwater ecosystems in the Northeastern United States. *BioScience* 57:17-28.
- Engstrom, D. R. and E. B. Swain. 1997. Recent declines in atmospheric mercury deposition in the upper Midwest. *Environmental Science and Technology* 31:960-967.

- Evans, R. J., J. D. Bails, and F. D'Itri. 1972. Mercury levels in muscle tissues of preserved museum fish. *Environmental Science and Technology* 6:901-905.
- Fisher, S., N. S. Nicholas, and P. R. Scheuerman. 2002. Dendrochemical analysis of lead and calcium in Southern Appalachian American Beech. *Journal of Environmental Quality* 31:1137-45.
- Fitzgerald, W. F., D. R. Mason, and R. P. Nater, and E. A. Nater. 1998. The case for atmospheric mercury contamination in remote areas. *Environmental Science and Technology* 32:1-7.
- Freake, M. J. and E. D. Lindquist. 2008. Geographic pattern analysis of pesticide exposure in salamander populations in the Great Smoky Mountain National Park. *Herpetological Conservation and Biology* 3:231-238.
- Frederick, P. and N. Jayasena. 2011. Altered pairing behavior and reproductive success in white ibises exposed to environmentally relevant concentrations of methylmercury. *Proceedings of Royal Society Biological Sciences* 278:1851-1857.
- Frederick, P. C., B. Hylton, J. A. Heath, and M. G. Spadling. 2004. A historical record of mercury contamination in southern Florida (USA) as inferred from avian feather tissue. *Environmental Toxicology and Chemistry* 23:1474-1478.
- Gibbs, R. H., E. Jarosewich, and H. L. Windom. 1974. Heavy metal concentration in museum fish specimens: effect of preservatives and time. *Science* 184:475-7.
- Grigal, D. F. 2002. Inputs and outputs of mercury from terrestrial watersheds: a review. *Environmental Reviews* 10:1-39.

- Guigueno, M. F., K. H. Elliott, J. Levac, M. Wayland, J. E. Elliott. 2012. Differential exposure of alpine ospreys to mercury: Melting glaciers, hydrology or deposition patterns? *Environment International* 40:24-32.
- Hill, J. J., M. W. Chumchal, R. W. Drenner, J. E. Pinder, S. M. Drenner. 2010. Use of preserved museum fish to evaluate historical and current mercury contamination in fish from two rivers in Oklahoma, USA. *Environmental Monitoring and Assessment* 161:509-16.
- Hopkins, B. C., M. J. Hepner, W. A. Hopkins. 2013. Nondestructive techniques for biomonitoring of spatial, temporal, and demographic patterns of mercury bioaccumulation and maternal transfer in turtles. *Environmental Pollution* 177:164-170.
- Houlahan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian declines. *Nature* 404: 752-5.
- IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.
- Jagoe, C. H., B. Hill, G. M. Yanochko, P. V. Winger, I. L. Brisbin. 1998. Mercury in alligators (*Alligator mississippiensis*) in the southeastern United States. *The Science of the Total Environment* 213:255-262.
- Kelly, T. M., J. D. Jones, and G. R. Smith. 1975. Historical changes in mercury contamination in Michigan walleyes (*Stizostedion vitreum vitreum*). *Journal of Fisheries Research Board of Canada* 32:1745-54.
- Kim, P., Y. Han, T. Holsen, S. Yi. 2012. Atmospheric particulate mercury: concentrations and size distributions. *Atmospheric Environment* 61:94-102.

- Kolker, A., M. L. Olson, D. P. Krabbenhoft, M. T. Tate, and M. A. Engle. 2010. Patterns of mercury dispersion from local and regional emission sources, rural Central Wisconsin, USA. *Atmospheric Chemistry and Physics* 10:4467-4476.
- Krabbenhoft, D. P., B. A. Branfireum, A. Heyes. 2005. Biogeographic cycles affecting the speciation, fate, and transport of mercury in the environment. In M. B. Parsens & J. B. Perival (Eds.), *Mercury: Sources, measurements, cycles, and effects, short course series* (Vol. 34, pp. 139-156). Ottawa: Mineralogical Association of Canada.
- Martins, I., V. Costa, F. M. Porteiro, R. S. Santos. 2006. Temporal and spatial changes in mercury concentrations in the North Atlantic as indicated by museum specimens of glacier lanternfish *Benthoosema glaciale* (Pisces: Myctophidae). *Environmental Toxicology* 21:528-32.
- Miller, G. E., P. M. Grant, R. Kishore, F. J. Steinkruger, F. S. Rowland, V. P. Guinn. 1972. Mercury concentrations in museum specimens of tuna and swordfish. *Science* 175:1121-1122.
- Organ, J. A. 1961. Studies of the local distribution, life history, and population dynamics of the salamander genus *Desmognathus* in Virginia. *Ecological Monographs* 31:189-220.
- Perrault, J., J. Wyneken, L. J. Thompson, C. Johnson, D. L. Miller. 2011. Why are hatching and emergence success low? Mercury and selenium concentrations in nesting leatherback sea turtles (*Dermochelys coriacea*) and their young in Florida. *Marine Pollution Bulletin* 62:1671:1682.
- Peterman, W. E., J. A. Crawford, R. D. Semlitsch. 2008. Productivity and significance of headwater streams: population structure and biomass of black-bellied salamander (*Desmognathus quadramaculatus*). *Freshwater Biology* 53: 347-357.

- Petranka, J. W. 1998. *Salamanders of the United States and Canada*. Washington, DC: Smithsonian Institute Press.
- Pyle, C. and M. P. Schafale. 1988. Land use and history of three spruce-fir forest sites in southern Appalachia. *Journal of Forest History* 32:4-21.
- Rimmer, C. C., E. K. Miller, K. P. McFarland, R. J. Taylor, S. D. Faccio. 2010. Mercury bioaccumulation and tropic transfer in the terrestrial food web of a montane forest. *Ecotoxicology* 19:697-709.
- Ryba, S. A., J. L. Lake, J. R. Serbst, A. D. Libby, and S. Ayvazian. 2008. Assessment of caudal fin clip as a non-lethal technique for predicting muscle tissue mercury concentrations in largemouth bass. *Environmental Chemistry* 5:200-203.
- Sanei, H., F. Goodarzi, P. M. Outridge. 2010. Spatial distribution of mercury and other trace elements in recent lake sediments from central Alberta, Canada: An assessment of the regional impact of coal-fired power plants. *International Journal of Coal Geology* 82:105-115.
- Sorensen, E. M. B. 1991. Metal poisoning in fish: Chapter 8, Mercury. Boca Raton, FL: CRC Press 1991:245-331.
- Sparling, D. W., G. Linder, and C. Bishop eds. 2000. *Ecotoxicology of Amphibians and Reptiles*. Pensacola, FL: SETAC Press.
- Stebbins, R. C. and N. W. Cohen. 1995. *A Natural History of Amphibians*. Princeton, New Jersey: Princeton University Press.
- Stemberger, R. S. and C. Y. Chen. 1998. Fish tissue metals and zooplankton assemblages of north-eastern US lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 55:339-352.

- Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783-6.
- Suarez, A. V. and N. D. Tsutsui. 2004. The value of museum collection for research and society. *BioScience* 54:66-74.
- Thornton, F.C., J. D. Joslin, P.A. Pier, H. Neufeld, J. R. Seiler, and J. D. Hutcherson. 1994. Cloudwater and ozone effects upon high elevation red spruce: A summary of study results from Whitetop Mountain, Virginia. *Journal of Environmental Quality* 23:1158-67.
- Todd, B. D., C. M. Bergeron, W. A. Hopkins. 2012. Use of toe clips as a nonlethal index of mercury accumulation and maternal transfer in amphibians. *Ecotoxicology* 21:882-887.
- Townsend, J. M., C. C. Rimmer, C. T. Driscoll, K. P. McFarland, E. Inigo-Elias. 2013. Mercury concentrations in tropical resident and migrant songbirds on Hispaniola. *Ecotoxicology* 22:86-93.
- Ugarte, C. A., K. G. Rice, and M. A. Donnelly, 2005. Variation of total mercury concentrations in pig frogs (*Rana grylio*) across the Florida Everglades, USA. *Science of the Total Environment* 345:51-59.
- Unrine, J. M., C. H. Jagoe, W. A. Hopkins, and H. A. Brant. 2004. Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenoccephala*) larvae. *Environmental Toxicology and Chemistry*. 23:2964-70.
- USEPA. 1998. *Methods for evaluating solid water, physical/chemical methods, Method 7473*.
- USEPA. 1997. Mercury study report to Congress. Vol. VI: An ecological assessment for anthropogenic mercury emissions in the United States. EPA-452/R-97-008.

- USEPA. 1989. Mountain Cloud Chemistry Project – Wet, Dry, and Cloud Water Deposition. EPA- PB 89-148 597/AS.
- Vong, R. J., J. T. Sigmon, and S. F. Mueller. 1991. Cloud Water Deposition to Appalachian forests. *Environmental Science and Technology* 25:1014-21.
- Wake D.B., and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world amphibians. *Proceedings of the National Academy of Sciences* 105:11466-11473.
- Wiener, J. G. and D. J. Spry. 1996. Toxicological significance of mercury in freshwater fish. In W. N. Beyer, G. H. Heinze, and A. W. Redmon-Norwood eds. *Environmental Contaminants in Wildlife: Interpreting Tissues Concentrations*, pp. 297-339. Boca Raton, FL.
- Woshner, V., K. Knott, R. Wells, C. Willetto, R. Swor, and T. O'Hara. 2008. Mercury and selenium in blood and epidermis of bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, FL; interaction and relevance to life history and hematologic parameters. *EcoHealth* 5:360-70.
- Yu, X., C. T. Driscoll, J. Huang, T. M. Holsen, B. D. Blackwell. 2013. Modeling and mapping of atmospheric mercury deposition in Adirondack Park, New York. *PLoSOne* 8: e59322.

APPENDIX

Table 2.1. Comparison of THg in *D. quadramaculatus* tail tissue from Whitetop Mt., VA (2011-12) and other known amphibian tissue THg from published accounts.

Species	Location	Mean THg Dry wt. (ng/g)	Range THg Dry wt. (ng/g)	Mean THg Wet wt. (ng/g)	Range THg Wet wt. (ng/g)	Advisories	Source
Tail Tissue							
<i>D. quadramaculatus</i>	Whitetop Mt. VA	133.4	8.5 – 1258.3	33.4*	2.1 – 314.6		
(This study)	Whitetop – Big Br.	188.1	54.3 – 1204.0	47.0*	13.3 – 301.0		
<i>P. cinereus</i>	South River VA	~200	63 – 1584	— —	— —	VA Fish	Industrial Point
(Bergeron et al. 2010)							
	Stratton Mt. VT	~110	— —	— —	— —		Atmospheric
(Rimmer et al. 2010)							
Leg Muscle Tissue							
<i>L. catesbeianus</i>	Caddo Lake TX	620	— —	— —	— —	Piscivorous	Atmospheric
(Chumchal et al. 2011)						Predator	
	Cache Creek CA	— —	— —	409	— —	CA Fish	Hg Mining
(Hothem et al. 2010)							
<i>L. grylio</i>	Everglades FL			329	87 - ~2000	Frog	— —
(Ugarte et al. 2005)						Consumption	

* Wet weights (ww) were not calculated but were estimated based on (Burke et al. 2010)

Table 2.2. Comparison of THg in *D. quadramaculatus* liver tissue from Whitetop Mt., VA (2011-12) and other known amphibian liver THg tissue accounts.

Species	Location	Mean THg Dry wt. (ng/g)	Range THg Dry wt. (ng/g)	Mean THg Wet wt. (ng/g)	Range THg Wet wt. (ng/g)	Advisories	Source
<i>D. quadramaculatus</i> (This study)	Whitetop Mt. VA	2783.6	246.6 – 16545.6	695.9*	61.7 – 4136.4		
<i>L. catesbeianus</i> (Chumchal et al. 2011)	Caddo Lake TX	1228	— —	— —	— —	Piscivorous Predator	Atmospheric
(Hothem et al. 2010)	Cache Creek CA	— —	— —	817	— —	CA Fish	Hg Mining
<i>L. grylio</i> (Ugarte et al. 2005)	Everglades FL	— —	— —	— —	253 - 544	Frog Consumption	

* Wet weights (ww) were not calculated but were estimated based on (Burke et al. 2010)

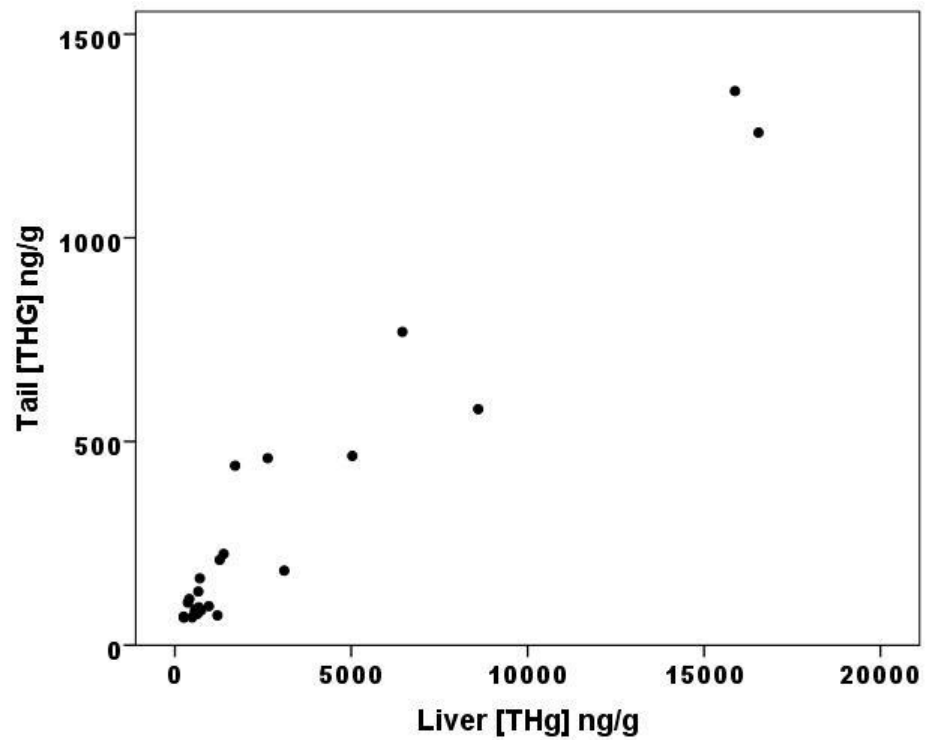


Figure 2.1. Relationship between liver and tail THg concentrations of *Desmognathus quadramaculatus* from Whitetop Mountain, Virginia collected 2011-2012 ($r_s = 0.83$, $P < 0.01$).

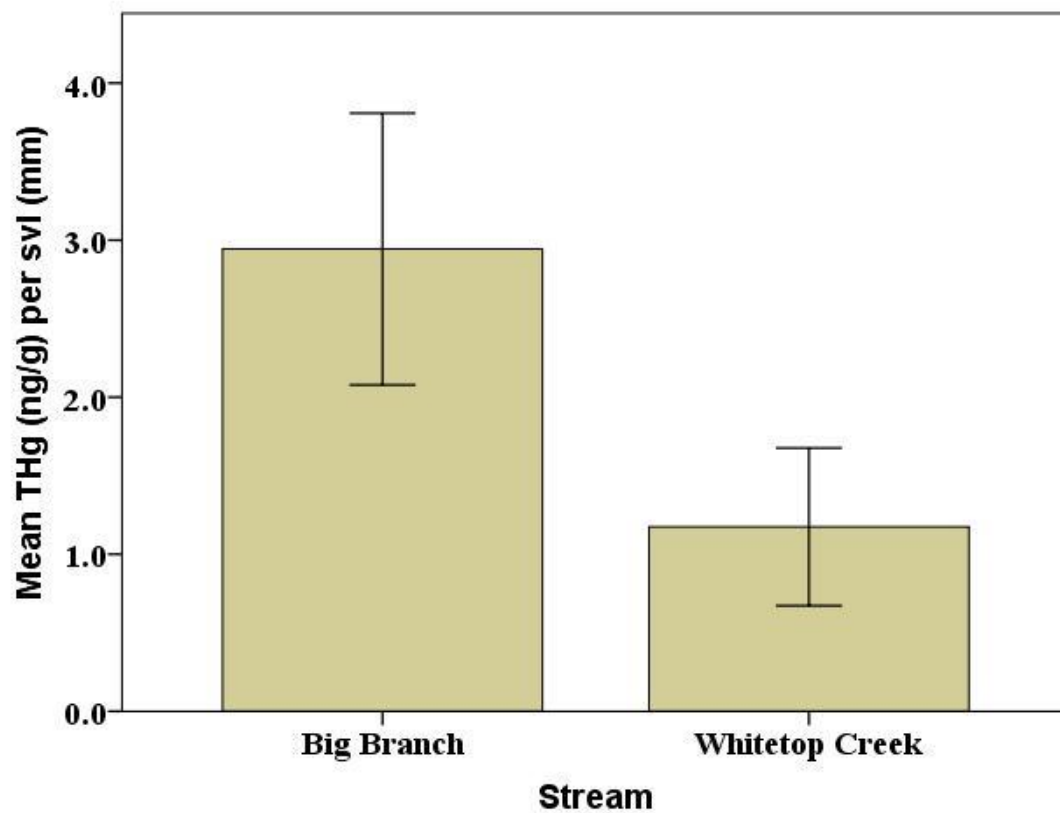


Figure 2.2. Mean total mercury of tail tissue of *Desmognathus quadramaculatus* from Big Branch (northern slope) and Whitetop Creek (southern slope) collected 2011-2. Error bars represent 95% CIs. Raw data are presented in the graph but analysis was conducted on $\log_{10}$ transformed data.

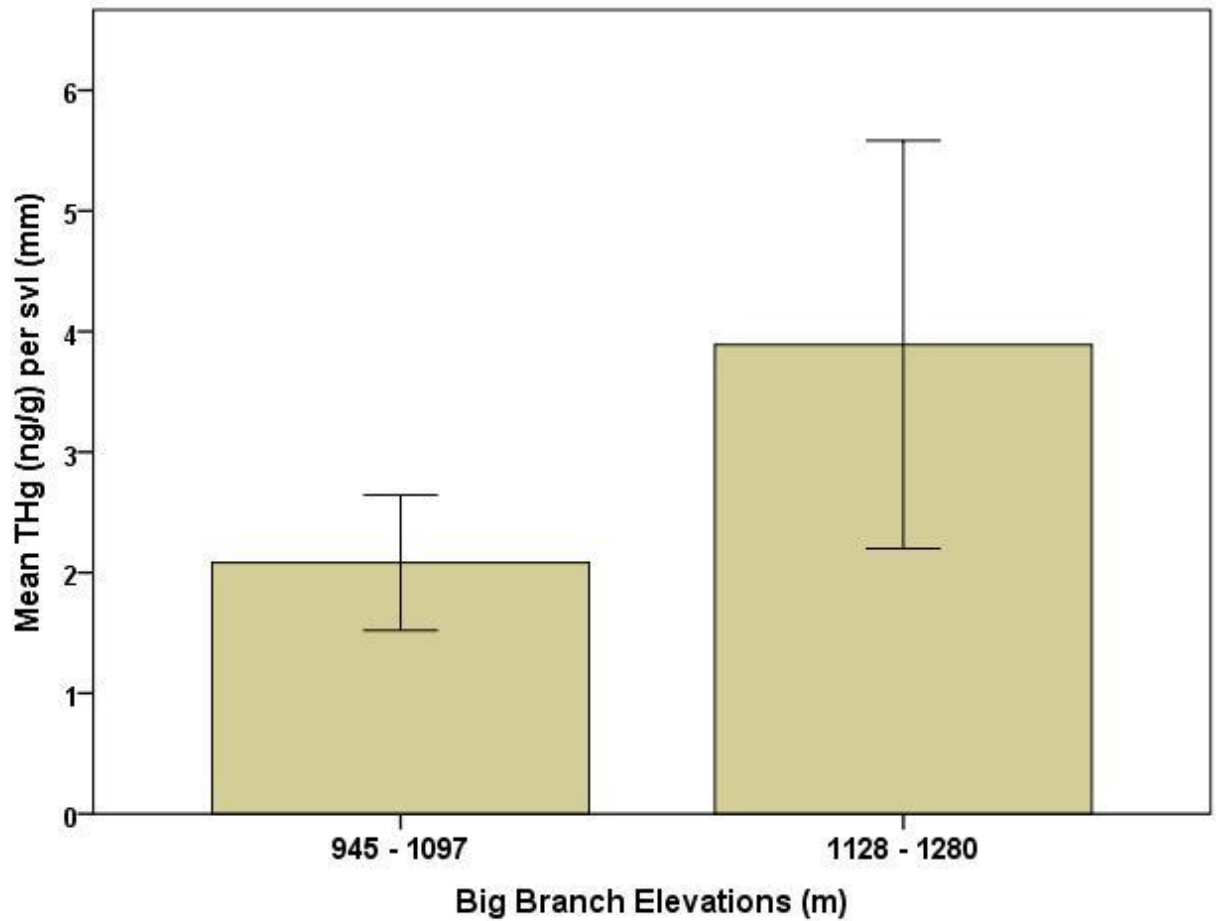


Figure 2.3. Mean total mercury of tail tissue of *Desmognathus quadramaculatus* from low elevations and high elevations on Big Branch, north slope of Whitetop Mountain, Virginia collected 2011-2. Error bars represent 95% CIs. Raw data are presented in the graph but analysis was conducted on $\log_{10}$ transformed data.

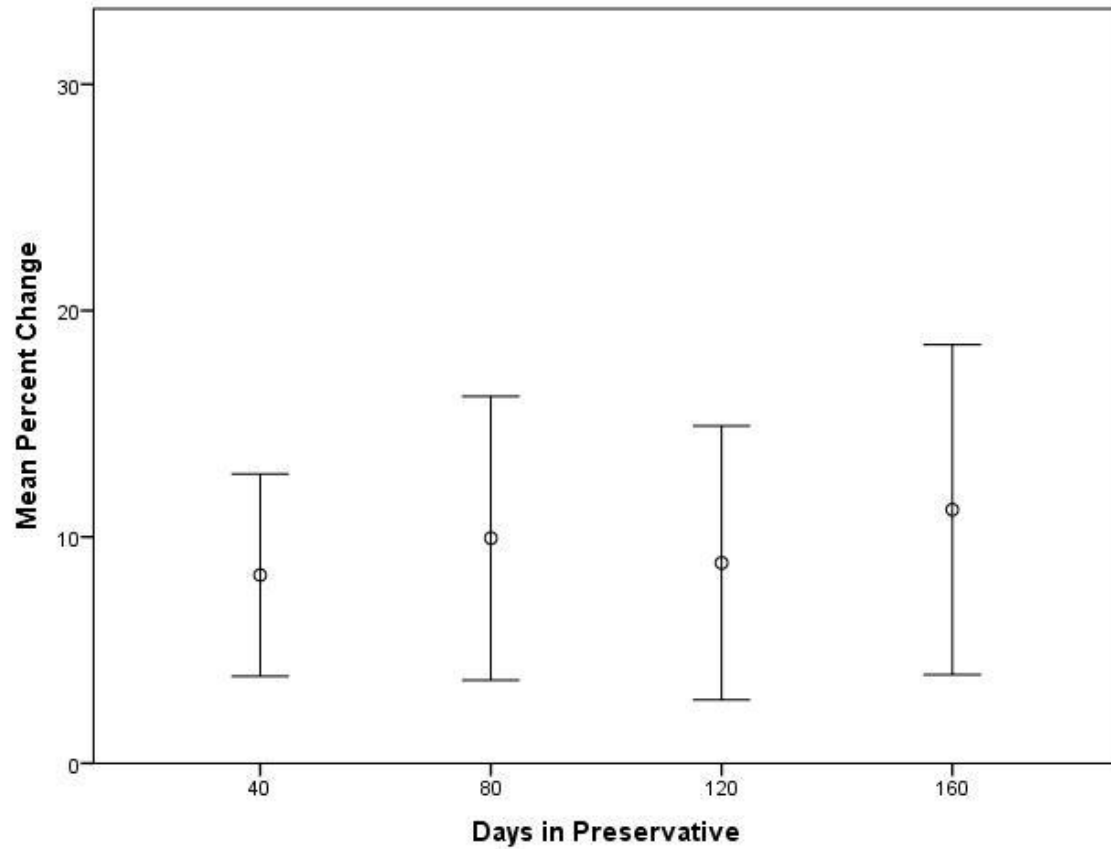


Figure 2.4. Mean percent change of total mercury in preserved tail tissue of *Desmognathus quadramaculatus*, collected from Big Branch north slope of Whitetop Mountain, from initial values stored in 65% ethanol. Error bars represent 95% CIs.

CHAPTER 3: CHANGES IN SALAMANDER DISTRIBUTIONS ALONG AN ELEVATIONAL GRADIENT IN THE MOUNT ROGERS NATIONAL RECREATION AREA

ABSTRACT

Numerous species of plants and animals have shifted their distributions in response to changing climatic conditions with some species experiencing local and even range-wide extirpations. Montane species are especially vulnerable to warming conditions as many are already at the edge of their thermal tolerances. The Southern Appalachian Mountains are home to the greatest diversity of Plethodontid salamanders in the world and many could be vulnerable to climate change as they have limited montane distributions. We investigated potential range changes of Plethodontid salamanders within the Mount Rogers National Recreation Area. In the 1950s and 1990s, Dr. James Organ documented salamander distributions along elevational transects that were sampled every 30.5 m (100'). We surveyed 10 of these original transects from July 2008 – July 2012. Current sampling utilized repeat surveys which allowed us to model detection. Occupancy probabilities for both current and historic sampling were then corrected based on detection probabilities. We also examined regional weather data since 1948 to determine if warming could have influenced observed range changes. Most range limits and midpoint changes were from salamanders in the genus *Plethodon*, where as salamanders in the genus *Desmognathus* experienced few changes. A high elevation endemic, *P. welleri*, shifted lower since 1950 and 1990 surveys. While *P. cylindraceus* experienced range contractions, as lower range limits increased and upper range limits decreased since 1950s surveys. *Plethodon yonahlossee* expanded its range as lower range limits decreased and upper range limits increased. Habitat generalists (e.g. *P. montanus* and *Desmognathus orestes*) had minimal midpoint and no range limit changes since the 1950s and 1990s. Regional temperatures from the Tri-Cities Airport (Kingsport, TN) indicated no significant warming trend for annual, January, and July

mean temperatures since 1948. Our results indicated variability in salamander range changes since the 1950s and 1990s.

INTRODUCTION

Amphibian populations have declined in the past 25 years with more than one-third listed as “globally threatened” and 43% considered to be declining (IUCN 2008). We may be in the midst of the sixth mass extinction with amphibians having the greatest losses (Wake and Vredenburg 2008). Habitat loss, diseases, introduced species, chemical contamination, UV-B radiation, over-exploitation, climate change, and synergistic interactions have been documented to cause declines (Semlitsch 2003, Wells 2007). Climate change has been linked to amphibian declines by altering amphibian habitat due to decreasing leaf litter, intensifying competitive interactions, causing distribution shifts, increasing the effects of diseases and pathogens, and changing breeding phenology (Pounds et al. 2006; Seimon et al. 2007; Kusan and Inoue 2008; Raxworth et al. 2008; Werner et al. 2013).

Effects of climate change have been observed worldwide. In the United States surface temperatures have been rising since the mid-1970s (IPCC 2007) and the past 10 years (2003-2012) have been the warmest on record (NOAA 2012). Global average temperatures have increased by 0.78°C between the periods of 1850-1900 and 2003-12 (IPCC 2013). Temperatures in the Northern Hemisphere for the past 50 years were greater than any 50-year period for the past 500 years ($P > 0.9$) and 1300 years ($P > 0.66$) (IPCC 2007). Western North America has experienced periodic droughts, but precipitation has increased significantly in eastern North America since 1900 (IPCC 2007).

Distribution shifts associated with climate change have the potential to affect species ranges which are often set by tolerances to climatic extremes (Sexton et al. 2009). Ranges limits

for many species have shifted upward in elevation and toward poles following their climatic niche with increasing temperatures (Parmesan 2006; Thuiller et al. 2008; Chen et al. 2011). Plants (Lenoir et al. 2008; Kopp and Cleland 2013), invertebrates (Chen et al. 2011b; Sheldon 2012), fish (Comte and Grenouillet 2013), amphibians (Seimon et al. 2007; Raxworthy et al. 2008; Rovito et al. 2009), reptiles (Raxworthy et al. 2008; Sinervo et al. 2010), birds (Tingley and Beissinger 2013), and mammals (Moritz et al. 2008; Rowe et al. 2010) have shown significant range changes in response to climate change. In the Northern Hemisphere, northern range limits have increased by 6.1 km northward (Parmesan 2006) and upper range limits by 11.0 m upward per decade (Chen et al. 2011). Of species investigated, 41% have experienced range changes due to climate change (Parmesan 2006). However, an increase in precipitation could facilitate downslope movements by montane species even in the presence of warming (Tingley et al. 2012) and increased precipitation might buffer the effects of warming temperatures on soil temperatures (Fridley 2009). Thus, not all range changes in montane habitats have been upward as expected as up to 25% of all species examined have shifted their ranges down slope even in the presence of warming (Parmesan and Yohe 2003; Rowe et al. 2010; Lenoir et al. 2010; Tingley et al. 2012). Lower limits of plants, resurveyed after 100 years, shifted upslope for 66% of species, but shifted downslope for 33% (Lenoir et al. 2008). In a resurvey of butterflies, 17 species raised and 6 lowered their lower range limit (Wilson et al. 2005). Twice the number of moth species shifted upslope as shifted downslope with increasing temperatures (Chen et al. 2009). Members of the *Sorex* genus decreased their lower limit by up to 1000 m in an 80-year resurvey of Yosemite National Park while most other small mammals shifted upward (Moritz et al. 2008).

When examining range changes, determining an organism's niche is critical. Hutchinson (1957) defined a niche as synergistic interactions of abiotic and biotic factors which determine range limits. Range limits of Plethodontid salamanders have been studied extensively to determine whether abiotic or biotic factors are essential for range limits (Hairston 1951; Jaeger 1971; Nishikawa 1985; Highton 1995; Gifford and Kozak 2012). Abiotic factors contribute to range limits as minor differences in temperature and moisture have been linked to range limits in salamanders of the genus *Plethodon* (Kozak and Wiens 2010). Plethodontid salamanders rely entirely on cutaneous respiration and thrive in montane environments with cool and moist conditions (Petranka 1998), where as climate conditions at lower elevations create physiological barriers for respiration and metabolism (Bernardo and Spotila 2006; Gifford and Kozak 2012). Many Plethodontid salamanders may be at their physiological limits at their lowest elevations and any additional warming or drying would create uninhabitable conditions (Bernardo and Spotila 2006). Climatic conditions were also thought to limit upper elevation limits especially for lower elevation species moving higher (Highton 1995). Competition between species has also been shown to influence range limits as competition established lower range limits for many montane Plethodontid salamanders (Hairston 1951; Jaeger 1971; Nishikawa 1985; Highton 1995; Werner et al. 2013). These traditional views of competition and range limits have been reconsidered as lower range limits for *Plethodon jordani*, a salamander inhabiting high elevations, appeared to be influenced by climate, but competitive interactions with *P. jordani* prevented lower elevation species from moving upslope (Gifford and Kozak 2012). Thus lower limits for species inhabiting both high and low elevations are influenced by climate while upper limits for some species may be defined by competition.

Amphibian specialists that inhabit areas with unique montane climates may be particularly vulnerable to climate change and will be most likely to experience range changes (Rowe et al. 2010). The Southern Appalachian Mountains and Highlands of southern Mexico and Guatemala have the greatest diversity of salamanders in the family Plethodontidae (Wells 2007). As global temperatures increase, the distributions of Plethodontid salamanders may become constrained and lead to extirpation of some species (Milanovich et al. 2010). Ecological niche models indicate that many salamander populations at the extreme southern portion of the Appalachian Mountains may be extirpated by 2020 and most by 2080 with projected warming (Milanovich et al. 2010). Movements apparently due to warming have been observed as the range of Southern Appalachian salamander hybrid zones have expanded presumably due to increasing temperatures from 1974 to 1990 (Walls 2009).

In addition to range changes, changing climatic conditions along with other anthropogenic effects could act synergistically to contribute to amphibian declines. Plethodontid salamanders from the Guatemalan Highlands were sampled in the 1930s, 1970s, and 2005-7 (Schmidt 1936; Wake and Lynch 1976; Rovito et al. 2009) and salamanders were found to occupy distinct elevational ranges. No distributional changes were observed between 1930s and 1970s samples. However, in recent (2005-7) surveys, several salamander species from the genus *Bolitoglossa*, have disappeared and other species have experienced 10-fold reductions in abundance (Rovito et al. 2009). Few salamanders tested positive for diseases and pathogens suggesting climate change may have impacted habitat suitability as sites have remained undisturbed by humans (Ravito et al. 2009). Given the similar life history between Plethodontid species in Central America and the Southern Appalachian Mountains, salamanders in Appalachia could experience similar range limit changes and population declines (Milanovich et al. 2010).

Climate change has also been implicated in pathogen emergence which often leads to amphibian declines (Pounds and Puschendorf 2004). The emergence of *Batrachochytrium dendrobatidis* in amphibian populations has been partly attributed to warmer and drier environments (Pounds et al. 2006; Bosch et al. 2007; Seimon et al. 2007; Lips et al. 2008; Rohr et al. 2008). *Bufo boreas* eggs exposed to higher levels of UV-B radiation, due to loss of cloud cover, were more susceptible to the fungus *Saprolegnia ferax* (Kiesecker et al. 2001). Ranavirus infections in Southern Appalachian salamanders were greatest at lower elevations with higher temperatures (Gray et al. 2009). Thus, the susceptibility of amphibians to pathogens appears to be related to ambient temperature and could contribute to declines.

Repeat salamander surveys in the Southern Appalachian Mountains to document salamander declines have been inconclusive as some populations have experienced declines while others have maintained distributions. Additionally, resurveys failed to incorporate methods to account for imperfect detection. A 20-year (1971-1991) resurvey of Plethodontid salamanders in the Great Smoky Mountains National Park and Coweeta Hydrologic Laboratory (North Carolina) found no distributional changes (Hairston and Wiley 1993). However, resurveys from 1960 to 2001 throughout the Southern Appalachian Mountains indicated significant population declines (Highton 2005) and additional resurveys in 2009 within the Great Smoky Mountains National Park also confirmed declining populations (Caruso and Lips 2013). However, repeat surveys to examine changing elevational range limits have not been conducted.

Determining range shifts for a species can be difficult and requires long term data sets (Pechmann et al. 1991). If imperfect detection is not accounted for, apparent range changes could be the result of non-detection instead of actual range shifts (MacKenzie et al. 2002; Moritz et al. 2008; Tingley and Beissinger 2009). Not accounting for imperfect detection has been

shown to overestimate extinction by 59% of actual rates for common species and even greater overestimations for rare species (Kery et al. 2006). Occupancy modeling and detection probability allow for a more accurate comparison of historic and modern samples by correcting for imperfect detection (Bailey et al. 2004; Moritz et al. 2008). Most studies indicating declines in amphibian populations from historical data have not accounted for imperfect detection (Hairston and Wiley 1993; Highton 2005; Rovito et al. 2009; Caruso and Lips 2013). Repeat sampling, which is required for occupancy modeling, has also been shown to be more effective to document amphibian declines (Skelly et al. 2003). Therefore, repeat sampling of historic surveys should include modeling for detection and occupancy to determine if observed changes are products of imperfect detection instead of range changes.

Historic data sets exist for salamander distributions within the Southern Appalachian Mountains (Organ 1961; Hairston et al. 1987; Highton 2005). Dr. James and Della Organ conducted elevational transects to document salamander distributions throughout the Mount Rogers National Recreation Area (MRNA) in southwest Virginia (Organ 1961; 1990; 1991). Initial surveys were made from 1957-59 and repeated from 1990-1 along with additional sites (Organ 1991). Sampling transects were established every 30.5 m (100') of elevation on Mount Rogers and Whitetop, Bluff, and Beech mountains. Both north- and south-facing slopes were sampled with the transect centered on either a creek or ridge top. Dr. Organ and Della each spent one hour collecting salamanders for a total of 2-person hours of sampling effort per elevation. Their work provided baseline data for a 50- and 20-year comparisons. The objective of this project was to determine if salamander distributions have changed since the 1950s and 1990s while examining if regional weather patterns could have influenced changes.

METHODS

We resampled Dr. James Organ's original transects to determine if salamander spatial distribution changes have occurred since the 1950s and 1990s. Organ (1961; 1990; 1991) originally sampled 14 transects throughout the MRNA (Grayson, Smyth, and Washington Counties, VA). However, we only surveyed ten transects located on Whitetop, Beech, and Bluff Mountains from 2008 – 2012. The four transects not resampled were located on Mt. Rogers and within a designated wilderness area with limited access. We identified transects using detailed descriptions in Dr. Organ's field notes, reports, and conversations (Organ 1961; 1990; 1991) and located transects using a Garmin 60CSX GPS unit and personal maps from Dr. Organ. Transect elevations ranged from 914 – 1,646 m (3,000 - 5,400') with 6 transects on northern slopes and 4 on southern slope. Sampling sites were located every 30.5 m (100') in elevation along each transect for a total of 174 sampling sites. We sampled Beech Mt. (July 2009), Bluff Mountain (North slope – July 2008), Bluff Mountain (South slope – August 2010), Big Branch (August 2010), Bills Ridge (July 2011), Byars Creek (May 2009), Dave Ridge (August 2009), Dells Branch (June 2009-11), Pennington Branch (July 2010), and Whitetop Creek (June 2009-11) at the identical time of year as Dr. Organ's sampling which has been shown to minimize potential errors in comparing modern with historic sampling (Tingley and Beissinger 2009).

Transects were positioned perpendicular to the elevational gradient and centered on Dr. Organ's best approximated sampling location. Sampling occurred parallel to the contour thus maintaining the same elevation along transects. We sampled each transect for 2 hours (1 hour on each side of the transect midpoint), which was the identical search effort used by Dr. Organ. We turned all natural cover objects (e.g., rocks, wood, and bark) and searched leaf litter for salamanders. Only a single observer, Kevin Hamed, searched for and captured salamanders.

Once captured, I immediately placed salamanders in individual 1.2-liter plastic bags, and then identified and sexed each salamander if possible. Lastly, we returned each salamander to their approximate capture location.

Inasmuch as 100% detection along transects cannot be assured, we estimated detection probabilities to adjust occupancy estimates (MacKenzie et al. 2002; Bailey et al. 2004).

Estimates of detection require re-sampling (Bailey et al. 2004; MacKenzie et al. 2006) and we sampled each half of the transect (i.e., 1-hr sampling effort) separately. Each half transect was considered a single sampling attempt; thus having a repeat sample at each location during the same site visit (Bailey et al. 2004; Bailey et al. 2007). Habitat on each side of the transect was similar and salamander occupancy should have been the same on each side. Additionally, we sampled 2 transects (Whitetop Creek and Dells Branch, $n = 36$ locations) for 3 consecutive years (2009 – 2011) while continuing to sample one hour on each side of the elevational midpoint. Therefore, we sampled both Whitetop Creek and Dells Branch on 6 occasions during the study. All sites were considered closed as resurveys occurred within one hour of the original survey and since habitat on each side of the transect center was uniform. Whitetop Creek and Dells Branch were also considered closed between years as Plethodontid salamanders have limited home ranges ($< 30\text{m}^2$) and those endemic to the MRNA do not typically migrate, which greatly limits potential immigration or emigration (Petranka 1998). We estimated detection probabilities using AIC model-averaging methods in Program PRESENCE 5.5 (MacKenzie et al. 2006; Hines 2012). Since sampling sites were considered closed, we modeled detection probability using a single-season model (MacKenzie et al. 2002). We constructed competing detection models with the (1) time of year (Julian date), (2) time of year + time of year², and (3) specific transect covariates. Time of year and time of year² were continuous variables and were standardized by

z-transformations. Detection models included covariates that would have been similar for all sampling periods and therefore did not include vegetation data as forest succession and habitat clearing would have changed during the past 50 years. We determined the best model for detection probability, ranked by Akaike's information criterion adjusted for small sample size (AIC_c; Burnham and Anderson 2002), for each species and used the chosen detection probability to model occupancy.

Our sampling effort (2 person-hours) was identical to those of Dr. Organ's, although Dr. Organ's often include two observers (Dr. Jim and Della Organ) who sampled one hour each, for a total of 2 person-hours, which is ideal for modeling detection (MacKenzie et al. 2002), but the data was grouped in both field notes and publications. Therefore, it was not possible to model detection from Dr. Organ's original data. However, Organ (1991) surveyed Whitetop Creek in July 1957 and June 1959 and sites would be considered closed due to lack of potential immigration and emigration, which would allow for modeling detection. Detection during modern surveys was established with 174 locations, but historic detections along Whitetop Creek would have to be established with only 15 locations and several species (e.g. *Plethodon welleri* and *P. richmondi*) are not adequately represented along Whitetop Creek. We used historic detections from Whitetop Creek for historic samples if probabilities of detection were less than modern detections, thus providing a more conservative estimate. For all other species, we applied current levels of detection to Dr. Organ's historic data with the assumption that detection rates would have been equal between time periods. We acknowledge that this assumption leaves room for potential errors, but without historical occupancy applying current detection probabilities is the only option to model occupancy (Tingley and Beissinger 2009). It was also

reasonable to assume a similar detection with the assumption that both the Organs' and Hamed's skill levels were similar.

We once again used the single-season model in Program PRESENCE 5.5 (MacKenzie et al. 2002; Hines 2012) to model for occupancy in both historic and modern sampling periods. We combined Dr. Organ's sampling periods of 1957, 1958, 1959, 1990, and 1991 into a single historic sampling period. This was not the most desirable method of analysis. However, due to incomplete surveys of specific transects, period to period comparisons would not have been possible (Bailey personal conversation). Habitat changes most certainly occurred from 1957 to 2011 and limited covariates were used to model occupancy. Therefore, we constructed (1) elevation, (2) elevation + elevation², (3) aspect (4) elevation + aspect, and (5) elevation + elevation² + aspect models for each species which included the top detection model previously calculated. We standardized covariates by z-transformations. We ranked models by Akaike's information criterion adjusted for small sample size (AIC_c; Burnham and Anderson 2002). We chose models within $\Delta AIC_c \leq 2.0$ for occupancy and if multiple models were selected, we averaged models (Burnham and Anderson 2002). We calculated 85% confidence intervals (CIs) for covariates from each top model and if a CI did not include 0, the covariate was considered to have a strong association with occupancy (Arnold 2010). Instead of 95% confidence intervals, we chose 85% CIs which identify covariates that provide more biologically relevant interpretation and are more appropriate within an AIC framework (Arnold 2010). We reported occupancy probabilities by elevation for each species from historic to modern sampling periods (Figure 1.1 – 1.12).

To compare distributions between time periods we calculated elevation midpoints (centers of distributions) of each species for each transect as the mean elevation between the

lowest and highest location for each time period (e.g. 1950s, 1990s, and modern). A mean and median change in elevation midpoints were calculated for each species between historic and modern sampling periods. A Wilcoxon signed-rank test was used to compare elevation midpoint changes as upward, downward, or unchanged for each species. To determine if life history parameters contributed to midpoint changes between sampling periods, salamanders with terrestrial and aquatic larval periods were also compared with a Wilcoxon signed-rank test. Salamanders from the genus *Plethodon* were combined since they have a terrestrial and direct development life history (Petranka 1998) while salamanders from the genus *Desmognathus* and *Eurycea wilderae* were combined since they have aquatic larval periods or over-winter in aquatic seeps (e.g. *D. organi*; Organ 1961b).

We compared range limits, defined as the lowest and highest elevation in which the species was detected along each transect, among sampling periods. We also calculated and compared median range limit changes for each time period and species. We calculated both mean and median range limits individually for northern and southern facing slopes for each species as well as overall range limit changes that included both northern and southern slopes. We used a chi-square test to determine if changes between terrestrial and aquatic larval periods were associated with range limit changes. We attempted to compare range limits and elevation midpoints with a Wilcoxon signed-rank test to determine if aspect or life history influenced range limit changes. A power analysis (GPower 3.1; Faul et al. 2009) indicated our ability to detect statistically significant change was limited ($\beta = 0.11 - 0.49$). However, we feel many midpoint and range limit changes did have biological significance and are reported. To determine if salamanders had expanded or contracted their distributions, we examined range limits. We compared both range limit and elevation midpoints to occupancy modeling results

and if there was not agreement, we concluded observed changes could have been due to imperfect detection during either sampling period. Life history parameters of terrestrial or aquatic larval periods were once again compared to determine their potential influence on range expansions or contractions as well as the movements of range limits.

We analyzed local weather data to determine if climate change was related to salamander distribution changes. Continuous historic weather data are not available for Whitetop Mountain, but a National Oceanic and Atmospheric Administration (NOAA) weather station at the Tri-cities airport (Kingsport, TN; 36.48°N, -82.41°W; 457 m elevation) has continuous data since 1948. This station is 73 km southwest of Whitetop Mountain. Ideally, a closer weather station and one at a similar elevation to Whitetop Mountain would have been preferred, but the Tri-cities airport station was the closest station with at least 60 years of continuous data. We obtained weather data from the NOAA National Climatic Data Center (2013; www.ncdc.noaa.gov). We obtained mean maximum, mean minimum, highest recorded, and lowest recorded temperatures (°C) as well as total precipitation (cm) for monthly and annual periods from 1948-2011. We also grouped climatic values into 3, 10-year periods with each ending on the last sampling year of each sampling period (e.g. 1950-9; 1982-91; 2002-2011) and calculated that same climatic values as stated above for January, July, and annually in each 10-year period. We compared these values between sampling periods to determine potential differences prior to each sampling period. January and July were chosen as these are traditionally the coldest and warmest months of the year (NOAA 2012). Prior to analysis a Shapiro-Wilk's test indicated data were normally distributed (IBM 2011) and an ANOVA was used to compare mean climate values of each time period with an α -value of 0.05 (IBM 2011). We used linear regression to determine the rate and direction of change for climatic variables over the past 63 years (IBM 2011).

RESULTS

Occupancy modeling was successful for 12 salamander species as *Pseudotriton ruber*, *Gyrinophilus porphyriticus*, *Desmognathus marmoratus*, and *Notophthalmus viridescens* were not encountered frequently enough during historic and/or modern sampling periods to model detection or occupancy. Modern detection probabilities ranged from 0.99 (*Plethodon welleri*, *P. montanus*, and *D. orestes*) to 0.50 (*Desmognathus fuscus*). Only *P. cylindraceus*, *P. cinereus*, *P. yonahlossee*, and *Eurycea wilderae* included the elevation² covariate in their top modern occupancy model(s) (Table 3.1). All other species had elevation and most also included aspect covariates. However, the aspect covariate rarely had 85% CI that did not include zero and parameter estimates (β) were typically < 0.0002 (Table 3.2). Therefore, with the exception of *D. organi*, the aspect covariate was not included in occupancy probabilities as its impact was inconsequential.

Elevation midpoints for each transect and species were compared between time periods (1950s, 1990s, and modern) and no comparison was calculated if a transect was not sampled during the respective historic periods (1950s or 1990s) or if the transect was only partially sampled. In order to standardize descriptions of range changes, a decrease in an elevation was referred to as a downward movement (i.e. going from 1219 m in 1950 to 1067 m in 2010) and an increase in elevation indicated an upward shift. Mean changes in midpoints differed little from median changes in both time periods (Table 3.3). When comparing modern mean elevation midpoints for all 12 species with those from the 1950s, a minimal upward shift trend was suggested (total transects: 15 upslope, 7 downslope, and 4 unchanged). The average change in elevation midpoints from the 1950s samples was 7.6 m (-152.4 – 137.2 m) and the median change was 15.2 m, which equated to a 1.52 m upslope change per decade based on mean

increases. Elevational midpoints between modern and 1990s surveys also suggested a minimal upward shift (total transects: 32 upslope, 23 downslope, and 18 unchanged). The mean midpoint increased 9.7 m (-169.2 – 169.2 m) and the median change was 0. Based on the mean increase, a 4.9 m upward shift per decade was observed.

Plethodon welleri decreased its lower range limit when modern ranges were compared to both the 1950s and 1990s. Median range limits moved 61.0 m and 30.5 m downward when compared to the 1950s and 1990s (Table 3.5). Median elevational midpoints also shifted 30.5 m downward since the 1950s and 25.4 m since the 1990s (Table 3.3). Occupancy probabilities from current surveys were greater at lower elevations than from historic surveys from which we concluded *P. welleri* had shifted downward since historic surveys (Figure 3.1). The greatest downward movement was on Dells Branch and Beech Mountain from the 1990s to modern surveys where the elevation midpoint of *P. welleri* decreased 76.2 m (Table 3.5). Only a single upward movement was detected (Pennington Branch) and the lower range limit shifted upward 30.5 m since 1990s surveys (Table 3.5).

The most consistent range change occurred with *Plethodon cylindraceus* as this salamander constricted its historic range. Comparisons of mean elevation midpoints for *P. cylindraceus* indicated a downward shift since the 1950s, but these average values were skewed as the salamander was only detected at one elevation on 3 transects (Table 3.6). The median lower range limit for *P. cylindraceus* increased 30.5 m with a maximum of 183.0 m. The median upper range limit decreased 121.9 m with a maximum decrease of 304.0 m since the 1950s (Table 3.6). Occupancy probabilities decreased at both upper and lower range limits since historic surveys which indicated a range contraction (Figure 3.2).

Plethodon yonahlossee expanded mean midpoint elevations 68.6 m and 32.7 m upward since 1950s and 1990s surveys (Table 3.7). Upper range limits expanded 15.2 m since the 1950s. Both lower and upper limits expanded since 1990s surveys by 61 m downward and 76.2 m upward respectively (Table 3.7). Occupancy probabilities were greater at both lower and upper range limits than historic surveys, which allowed us to conclude the salamander had expanded its range both up and downslope (Figure 3.3). Dells Branch showed the greatest range limit expansions as the lower limit for *P. yonahlossee* moved 213.4 m downward and the upper limit increased by 304.8 m. All historic comparisons showed an expansion of range limits except Byars Creek where the salamander was not detected at lower elevations.

Mean elevation midpoints for *Plethodon cinereus* increased from the 1950s and 1990s surveys 53.3 and 57.5 m respectively (Table 3.3). Occupancy probabilities were greater at higher elevations and less at lower elevations than historic surveys from which we concluded the salamander had shifted upslope (Figure 3.4). The greatest change was an increase in the upper elevation limit on Dells Branch where *P. cinereus* increased by 365.8 m upward. When we evaluated median range limit changes observed since 1950s and 1990s surveys, the limits did not concur with occupancy (Table 3.8). However, since occupancy modeling incorporated imperfect detection we concluded *P. cinereus* had shifted upslope.

Since the 1990s, *P. montanus* has shifted mean midpoints 13.1 m downward (Table 3.3). Comparisons to 1950s surveys indicated a slight (7.6 m) shift upward but this value was probably due to changes on Byars Creek (Table 3.9). Modern occupancy probabilities were greater than historic probabilities from 914 – 975 m, which indicated *P. montanus* had decreased its lower limit and moved down slope while all elevations above 1097 m had occupancy probabilities > 0.99 in both historic and modern survey periods (Figure 3.5).

Occupancy probabilities for *Desmognathus organi* slightly decreased from 975 m to 1280 m, which suggested a slight upward shift of lower range limits since historic surveys (Figure 3.6). However, median changes in range limits and elevation midpoints did not indicate similar changes (Table 3.3 & 3.10). Individual range limits changed within 61 m along most transects except Whitetop Creek where the lower range limit decreased by 243 and 213 m since 1950s and 1990s surveys, respectively.

Desmognathus orestes experienced minimal upward shifts for mean midpoints of 7.6 and 6.5 m since 1950s and 1990s surveys (Table 3.3). Median range limit were unchanged from both the 1950s and 1990s (Table 3.11). Occupancy probabilities decreased below 1036 m and remained unchanged at all elevations above 1097 m (Figure 3.7). Occupancy results indicated lower range limits for *D. orestes* have shifted slightly upslope since historic surveys. Lower range limits on 75% of northern facing transects increased 15.2 – 121.9 m, which probably greatly influenced occupancy models.

Desmognathus fuscus displayed a 22.9 m increase in the mean midpoint elevation since 1950s surveys and a 64.8 m decrease since 1990s surveys (Table 3.3). Additionally, median upper range limits increased by 30.5 m since 1990s surveys (Table 3.12). However, occupancy probabilities decreased at the upper range limit which suggested *D. fuscus* has shifted down slope at its highest range limits (Figure 3.8). Salamanders in Pennington Branch experienced the greatest change as the maximum elevation decreased by 167.4 m since 1990.

Occupancy probability trends for *Plethodon richmondi*, *Desmognathus quadramaculatus*, *D. monticola*, and *Eurycea wilderae* were similar at both lower and upper range limits for historic and modern sampling periods (Figures 3.9 – 3.12). Overall occupancy was lower for *D. monticola*, *P. richmondi*, and *E. wilderae*, but occupancy was still the greatest at lower

elevations and decreased with increasing elevations for both historic and modern sampling periods. Based on occupancy modeling we concluded the range limits for these salamanders have not changed since historic sampling. Occupancy probabilities for *D. quadramaculatus* remained unchanged from historic to modern sampling periods, which suggested stable range limits for the past 50 years. Median range limits were more variable (Tables 3.13 – 3.16) as the general trend was a slight (< 45.7 m) upward shift. The lack of agreement with occupancy probabilities was mostly due to correcting historic data for detection.

Only a single regional climate variable, annual lowest temperature, was significantly different between sampling periods, $F(2,27) = 4.03$, $p < 0.03$ (Table 3.17). A Tukey post-hoc analysis indicated a significant increase in mean annual lowest temperatures ($^{\circ}\text{C}$) from 1982-91 (-18.72 ± 5.20) to 2002-11 (-14.41 ± 1.74) by 4.61 $^{\circ}\text{C}$ (95% CI ($0.51 - 8.71$); mean $\pm$ SD; $P < 0.025$). Linear regressions failed to establish a significant relationship between increasing time and climate values. Many parameter (β) estimates were slightly negative for most temperature variables (Table 3.17; Figure 3.13) with the exception of January lowest, July lowest, annual mean maximum, and annual lowest temperatures which were slightly positive. A decreasing trend in annual and July precipitation was observed since 1948, but the trend was not significant ($p > 0.45$; Figure 3.17)

DISCUSSION

Mean elevation midpoints of MRNA salamanders increased by 7.6 m (median = 15.2 m) and 9.7 m (median = 0 m) since 1950s and 1990s surveys respectively. These ranges of change equate to a 1.52 m and 4.9 m increase per decade since 1950s and 1990s surveys. These changes in elevational midpoints seem to be less severe than previously reported for other taxa since

regional weather stations near Whitetop Mountain have shown no warming since 1948. Meta-analysis has shown that terrestrial species are shifting to higher elevations at a median rate of 11.0 meters per decade (Chen et al. 2011). Tropical herptofuana increased 19-51m per decade (Raxworthy et al. 2008). Fish shifted upslope also at a greater rate than MRNA salamanders moving 13.7 m per decade (Comte and Grenouillet 2013). Plants (Lenori et al. 2008) and Southern Appalachian stoneflies (Sheldon 2012) moved upslope at almost 5 times the rate of MRNA salamanders moving 29 and 24 m per decade respectively. Plethodontid salamanders within the MRNA have not experienced as great of elevation midpoint changes as observed in as other taxa.

Plethodon welleri experienced a decrease in occupancy and lower range limits 1950s and 1990s surveys. This salamander prefers cool, high elevation spruce/fir and mesic deciduous forest habitats (Organ 1960; Thurow 1963). On Beech Mountain, *P. welleri* decreased its lower limit 76.2 m since 1990. Beech Mountain lacks spruce trees, and a downward shift in this population could indicate that climate or other factors might be a greater factor than forest type (Organ 1991). Hairston (1951) found that lower limits of montane salamanders were the result of competitive interactions and it has been suggested that down slope movements by taxa are the result of decreased competition pressure or lack realization of their preferred niche (Lenoir et al. 2010). Laboratory trials failed to identify competitive interactions between *P. welleri* and *P. richmondi* (Hamed unpublished data), which would be expected to fill a similar niche as they are approximately the same size and do not co-occur within the MRNA (Organ 1991). Gifford and Kozak (2012) found that climate, not competition, was responsible for the lower limit of *P. jordani*, another high elevation specialist. Regional weather data for the past 60 years suggests that Whitetop Mountain has not been impacted by significant climate change leaving other

factors to explain range changes. A lack of warming could explain the downward movement of *P. welleri*. Lower elevation limits appeared to be conducive to reproduction as *Plethodon welleri* was located nesting 91 m lower than previously documented on Whitetop Mt. and out of the spruce forest (Hamed et al. 2012). Another potential hypothesis for the downward movement of *P. welleri* is an increase in soil acidification due to atmospheric deposition. Both field soil pH values and laboratory trials indicated *P. welleri* preferred substrate with a low pH (Hamed unpublished data). *Plethodon welleri* nests in red spruce logs which are acid and suggests that it might be adapted to these conditions (Organ 1960). As lower elevations become more acidic due to increased deposition (Anderson et al. 1999), Weller's salamanders seemed to move down slope toward its preferred or tolerated soil pH. Previous studies indicated populations of *Plethodon welleri* declined in the Whitetop Mountain area as Highton (2005) noted a decrease in abundance from 10.7 individuals encountered per person during each sampling event from 1957-84 to 1 in 1999. Imperfect detection was not accounted for in those surveys and sampling location could have contributed to the decline. This trend was not observed at any transects where *P. welleri* was detected. *Plethodon welleri* appears to be increasing its distribution in the MRNA by expanding down slope even as other North American montane species are experiencing range contractions.

Plethodon cylindraceus experienced range contractions by decreasing range limits as well as decreased occupancy probabilities at both upper and lower elevations since the 1950s and lower limit since the 1990s. This salamander occurred at the lowest elevations of all historic surveys and would be hypothesized to be impacted by climate change, but regional weather did not indicate warming. Competitive interactions at upper range limits with *P. montanus* could have contributed to upper range limits decreases and prevented its upward expansion (Giffords

and Kozak 2012). Within the Southern Appalachian Mountains members of the *P. jordani* and *P. glutinosus* complexes have displayed varying degrees of competition, but in areas of narrow overlap *P. jordani* limits the occupancy of *P. glutinosus* (Hairston 1951; Hairston et al. 1987). On Whitetop Mt. *P. montanus* often co-occurred with *P. cylindraceus* throughout all sampling periods, but *P. montanus* could have experienced a greater advantage at upper range limits with cooler conditions either due to greater canopy cover from succession. Even though Organ (1961, 1991) did not quantify habitat parameters, many lower elevation transects have experienced habitat loss as forested areas have been converted to Christmas trees farms or residential development and most Plethodontids have experienced declines in these areas. Loss of forest cover has been shown to significantly decrease *Plethodontid* salamander abundance (Petranka et al. 1994). Although range limits were not their focus, Caruso and Lips (2013) documented significant declines in *Plethodon glutinosus* populations within the Great Smoky Mountains National Park from the 1960s to recent sampling but Hairston and Wiley (1993) only documented natural population fluctuations, failing to discover declines from 1971-1991. Highton (2005) reported a similar decrease in abundance of *P. cylindraceus* as the mean number of salamanders encountered, per person during each sampling attempt, dropped from 6.1 to 1.5 from 1960-78 to 1993 samples in the MRNA vicinity. Our data for *P. cylindraceus* supports Highton's declines. Range changes for *P. cylindraceus* could be influenced by local habitat, environmental, and competitive interactions.

Both *Plethodon montanus* and *Desmognathus orestes* were the most abundant and had the widest elevations distribution of any MRNA salamanders during Organ's sampling (1961, 1990, 1991) and our samples. On most transects these salamanders were found at all elevations and their only absences were at extreme lower elevations of a few transects. Occupancy

probabilities suggested a slight shift upslope for *D. orestes* and a slight downward shift for *P. montanus*. Byars Creek and several northern facing transects influenced median elevation midpoints changes, which shifted slightly upward by 7.6 m for both *D. orestes* and *P. montanus* since 1950s surveys, as the lowest 61 m had been cleared for farming or development since 1990 samples. Changes in the distributions of these 2 salamanders could indicate natural population variation or a loss of habitat at the lowest sampling elevations. Highton (2005) noted declines of *P. montanus* from 17.4, per person during each visit, from 1969-74 to 7 in 1999. Based on his description, this sampling site was the head waters to Byars Creek (1,400 m) and no such declines were found in our resurveys. Our results suggests that habitat generalists could be less prone to range changes and populations of *P. montanus* and *D. orestes* appeared stable in the MRNA.

Plethodon yonahlossee expanded its range limits since the 1950s and 1990s as modern occupancy probabilities were greater at both range limits than historic probabilities. However, upward movements should not be interpreted as a species tracking its preferred habitat as *P. yonahlossee* has been found up to 1,737 m within its range, which is 335 m higher than Organ (1961, 1990, 1991) detected (Petranka 1998). On Whitetop Mt., Pope (1950) collected *P. yonahlossee* at 1,668 m, which was 266 m and 114 m higher than Organ (1991) or this survey, respectively. However, Pope (1950) does not provide the exact collection location, which might not have included our historic sites. In comparison to Organ's surveys (1961, 1990, 1991), *P. yonoahlossee* does appear to have increased its range upward. The lowest elevations inhabited by *P. yonahlossee* have shifted down slope as the lowest elevation recorded by Pope (1950) was 1,067 m, which was also Organ's lowest during 1950s surveys (Organ 1991). However, during the 1990s and modern surveys, the lowest recorded elevation was 975 m suggesting a downslope

movement. Even though range limits increased, occupancy models for both historic and modern samples indicated greatest occupancy probabilities at middle elevations (1,036 – 1,402 m). Given the preference for rocky slopes (Pope 1950; Hoffman 1992), habitat characteristics could be a greater determinant for occupancy than current climatic conditions.

Desmognathus organi experienced a decrease in occupancy probabilities since historic surveys at lower range limits (< 1219 m). Median elevational midpoint and range limits indicated a downward movement, but that was due to a single individual along Whitetop Creek. A single *D. organi* was found 244 m and 213 m lower than the lowest record in either the 1950s or 1990s respectively. The lone salamander could have been washed downstream as it was found within 5 m of Whitetop Creek. The upward movement of lower elevations since the 1990s on northern slopes could have been due to habitat clearing for development and farming. Overall the range of *D. organi* appears to be somewhat stable on Whitetop Mountain.

Occupancy probabilities for *Plethodon richmondi* were similar from historic to modern sampling. However, along Byars Creek, which experienced conversion of forested habitat to trees farms since 1950s surveys, *P. richmondi* did increase its lower range limit. Since the 1990s *P. richmondi* decreased its lower range limit 305 m down slope on Beech Mountain, which is most likely due to forest succession of former pasture land and influenced mean decreases in elevation midpoints. Highton (2005) noted declines from 4.7 salamanders for each person collecting per visit from 1969-1974 to 0.5 per visit in 1999 along the headwaters to Byars Creek, but again these declines were not observed in this study.

Occupancy probabilities for *Plethodon cinereus* were the greatest at middle elevations and decreased at both upper and lower elevations. Occupancy probabilities suggest an expansion of upper range limits for transects sampled. However, *P. cinereus* has and currently occupies the

summit of Whitetop Mountain, which was not part of this project. Therefore, *P. cinereus* occupies the lowest and highest elevations on Whitetop Mountain and our observed range expansions could be the result of individual transect conditions and not the entire MRNA.

Most aquatic breeding salamanders followed a similar pattern of distribution changes. *Desmognathus quadramaculatus*, *D. monticola*, *D. fuscus*, and *E. wilderae* had either no median range limit changes or shifted range limits (<45.7 m) higher. Occupancy probabilities were lower for all salamanders compared to historic surveys except for *D. quadramaculatus* which was similar, but no range limit shifts were modeled except for *D. fuscus*. Occupancy modeling indicated these species have not experienced range changes.

Historic occupancy probabilities for *D. fuscus* indicated higher occupancy at lower and higher elevations especially along Whitetop Creek and Pennington Branch, with a slight decrease in middle elevations. However, occupancy at higher elevations was less than historic sampling periods which suggests *D. fuscus* could be declining at upper elevations. The *D. fuscus* complex within the Great Smoky Mountains National Park had a similar distribution, which eventually lead to the discovery of cryptic species, *D. santeetlah* (Tilley 1981). If two separate or subspecies exists within the MRNA range limits might be irrelevant as each population could be changing individually, but as a single species the population has only moved slightly upward. The upper range limit along Daves Ridge moved 274 m higher since 1991. These salamander were discovered where several trees had been uprooted, exposing rocky substrate which in turn held water. *Desmognathus fuscus* was living in and around those small pools of water. Downed trees had experienced minimal decay suggesting the down falls were fairly recent (< 10 years). This suggests that *D. fuscus* had recently colonized the area.

The Appalachian Mountains and especially the Southern Appalachian Mountains have not been thoroughly examined to document species range changes, but the few species examined have shifted their range limits. Lower range limits in Vermont red spruce trees (*Picea rubens*) shifted 67 m upslope from 1962 – 2005 (Beckage et al. 2008). Stoneflies in the Great Smoky Mountains National Park increased their upper range limit 250 m upslope since 1978 in the presence of warming (Sheldon 2012). Hybrid zones of Plethodontid salamander in the Southern Appalachian Mountains have changed as lower elevation species are moving upslope which has increased the elevation of the hybrid zones (Walls 2009). Variations in range changes by different taxa can be observed even within the same physiographic province suggesting regional effects

Regional climatic variables experienced minimal change since 1948 compared to global mean increases of 0.85 °C over the past 120 years (IPCC 2013). Slight increases in minimal temperatures were observed but none of the relationships were significant. A metaanalysis of Southern Appalachian Mountain climate variables indicated no significant changes in temperatures since 1938. An initial cooling was observed followed by a slight warming (Warren and Bradford 2010). However, other areas of the Southern Appalachians have experienced warming. A 0.72 °C warming was documented since 1976 in the Great Smoky Mountains National Park (Sheldon 2012). In the Nantahala Mts. (NC) the mean average temperature from 1974-90 was above the 55 year average suggesting a warming trend (Walls 2009). No significant changes in precipitation were observed over the past 63 years within the Whitetop Mt. region, but we acknowledge that Southern Appalachian mountain peaks receive considerably much more precipitation than lower weather stations and gain even further moisture through cloud water deposition (Vong et al. 1991). We also acknowledge the use of a regional weather

station was only an approximation for what the MRNA would have experienced. Changes experienced on Whitetop Mountain could have been different than the apparent lack of change observed at the Tri-cities Airport weather station given the separation of 73 km between locations. Without continual weather data from Whitetop Mountain, exact climatic changes are unknown. However, weather patterns in the Southern Appalachian Mountains are variable and the impact of climate change might vary across all locations (Gaffin 2012).

Accounting for imperfect detection is necessary for determining range changes from historic samples (MacKenzie et al. 2006; Moritz et al. 2008; Tingley and Beissinger 2009). Our results confirm that not all observed range limit changes were indicated by occupancy modeling and could have been the results of imperfect detection during a sampling period (Tingley and Beissinger 2009). Our occupancy probabilities agreed with 56% of observed changes in ranges limits. Our occupancy confirmations were slightly greater than Moritz et al. (2008) who confirmed 36% of range limits changes with occupancy probabilities of small mammals. If these techniques had not been employed a false pattern of change would have been accepted. Additionally, combining 1950s and 1990s samples into a historic occupancy period appeared to be successful as 10 of 12 species (83%) displayed the same occupancy trend on at least one range limit for both time periods.

CONCLUSIONS

Climatic variables within the MRNA region have experienced minimal changes since 1948 unlike other areas in North America. Salamander range limits underwent both upward and downward range shifts, but no changes were detected for several species. Habitat generalists such as *Plethodon montanus* and *Desmognathus orestes* were still detected at their lowest

elevations and have experienced minimal changes. *Plethodon welleri*, which is thought to prefer cool and moist habitats, moved downslope in the past 50 years. *Plethodon yonahlossee* increased its distribution at both lower and upper range limits, thus expanding its range. However, *P. cylindraceus* experienced a range contraction at both upper and lower limits. *P. cinereus* shifted upslope since historic sampling. No changes in occupancy were detected for *P. richmondi*. Occupancy probabilities changed minimally for salamanders in the genus *Desmognathus* since historic surveys. Our results indicated that some Plethodontid salamanders have experienced ranges changes, but other have not experienced change. Changing climate appears to not be the driving force, but instead habitat modification, competition, acid deposition, or synergistic interactions could be influencing these distributions. Our data now adds to the long term data set originated by Organ in 1957 allowing for continued resurveys and comparisons as climate and habitats change in the future.

LITERATURE CITED

- Anderson, J. B., R. E. Baumgardner, V. A. Mohnen, and J. J. Bowser. 1999. Cloud chemistry in eastern United States, as sampled from three high-elevation sites along the Appalachian Mountains. *Atmospheric Environment* 33:5105-14.
- Arnold, T. W. 2010. Uninformative parameters and model selection using Akaike's Information Criterion. *Journal of Wildlife Management* 74:1175-8.
- Bailey, L. L., T. R. Simons, K. H. Pollock. 2004. Estimating site occupancy and species detection probability parameters for terrestrial salamanders. *Ecological Applications* 14:692-702.
- Bailey, L. L., J. E. Haynes, J. D. Nichols, D. I. MacKenzie. 2007. Sampling design trade-offs in occupancy studies with imperfect detection: examples and software. *Ecological Applications* 17:281-90.
- Beckage, B., B. Osborne, D. G. Gavin, C. Pucko, T. Siccama, and T. Perkins. 2008. A rapid upward shift of a forest ecotone during 40 years of warming in the Green Mountains of Vermont. *Proceedings of National Academy of Science* 105:4197-4202.
- Bernardo, J., J. R. Spotila. 2006. Physiological constraints on organismal response to global warming: mechanistic insights from clinally varying populations and implications for assessing endangerment. *Biology Letters* 2:135-9.
- Bosch, J., L. M. Carrascal, L. Duran, S. Walker, and M. C. Fisher. 2007. Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? *Proceedings of the Royal Society Biology* 274:253-60.
- Burnham, K. P. and D. R. Anderson. 2002. Model selection and multi-model inference. Second edition. Springer-Verlag, New York, NY, USA.

- Caruso, N. M. and K. R. Lips. 2013. Truly enigmatic declines in terrestrial salamander populations in Great Smoky Mountains National Park. *Diversity and Distributions* 19:38-48.
- Chen, I-C, J. K. Hill, R. Ohlemuller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science* 333:1024-6.
- Chen, I-C, J. K. Hill, H-J Shiu, J. D. Holloway, S. Benedick, V K. Chey, H .S Barlow, C. D. Thomas. 2011b. Asymmetric boundary shifts of tropical montane Lepidoptera over four decades of climate warming. *Global Ecology and Biogeography* 20:34-45.
- Chen, I-C, H-J Shiu, S. Benedick, J. D. Holloway, V. K. Chey, H .S Barlow, J. K. Hill, C. D. Thomas. 2009. Elevation increases in moth assemblages over 42 years on a tropical mountain. *Proceedings of the National Academy of Science* 106:1479-83.
- Comte, L. and G. Grenouillet. 2013. Do stream fish track climate change? Assessing distribution shifts in recent decades. *Ecography* 36: 1236-46.
- Faul, F., E. Erdfelder, A. Buchner, and A. G. Lang. 2009. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods* 41: 1149-60.
- Fridley, J. D. 2009. Downscaling climate over complex terrain: high finescale (<1000 m) spatial variation on near-ground temperature in a montain forested landscape (Great Smoky Mountains). *Journal of Applied Meterology and Climatology* 48:1033-49.
- Gaffin, D. M. 2012. *Southern Appalachian Weather*. Vantage Press, New York, NY.
- Gifford, M. E. and K. H. Kozak. 2012. Islands in the sky or squeezed at the top? Ecological causes of elevational range limits in montane salamanders. *Ecography* 35:195-203.
- Gray, M. J., D. L. Miller, J. T. Hoverman. 2009. First report of *Ranavirus* infecting

- lungless salamanders. *Herpetological Review* 40:316-9.
- Hairston, N. G. 1951. Interspecies competition and its probable influence upon the vertical distribution of Appalachian salamanders of the genus *Plethodon*. *Ecology* 32:266-74.
- Hairston, N. G., K. C. Nishikawa, and S. L. Stenhouse. 1987. The evolution of competing species of terrestrial salamanders: niche partitioning or interference? *Evolutionary Ecology* 1:247-62.
- Hairston, H. G. and R. H. Wiley. 1993. No decline in salamander (Amphibia:Caudata) populations: a twenty-year study in the Southern Appalachians. *Brimleyana* 18:59-64.
- Hamed, M. K., M. J. Gray, and B. Parks. 2012. *Plethodon welleri* – nesting location. *Herpetological Review* 43:319.
- Highton, R. 1995. Speciation in eastern North American salamanders of the genus *Plethodon*. *Annual Review of Ecology Systematics* 26:579-600.
- Highton, R. 2005. Declines of Eastern North American Woodland Salamanders (*Plethodon*). Pages 34-46 in M. Lanoo, ed. *Amphibian Declines: Conservation Status of United States Species*. University of California Press, Berkley.
- Hines, J. E. 2012. PRESENCE5.5-Software to estimate patch occupancy and related parameters. USGS-PWRC, Patuxent Wildlife Research Center, Laurel, Maryland, USA.
<http://www.mbr-pwrc.usgs.gov/software/presence/html>
- Hoffman, R. L. 1992. The range of *Plethodon yonahlossee* in Virginia: defined at last? *Catesbeiana* 12:3-8.
- Hutchinson, G. E. 1957. Concluding remarks. Cold Springs Harbor Symposia on Quantitative Biology 22:415-27.

- IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.
- IPCC. 2007. Summary for policymakers. In: Climate Change 2007: Synthesis Report. Contributions of Working Groups I, II, and III to the Fourth Assessment Report of Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R. K. and A. Reisinger (eds.)]. IPCC, Geneva Switzerland.
- IPCC. 2013. Summary for policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of working Group I to the Fifth Assessment Report of Intergovernmental Panel on Climate Change [Stocker, T. F., D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P. M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- IUCN, Conservation International, and NatureServe. 2008. An Analysis of Amphibians on the 2008 IUCN Red List www.iucnredlist.org/amphibians. Downloaded on April 5, 2010.
- Jaeger, R. G. 1971. Competitive exclusion as a factor influencing the distributions of two species of terrestrial salamanders. *Ecology* 52:632-7.
- Kery, M., J. H. Spillman, C. Truong, and R. Holderegger. 2006. How biased are estimates of extinction probability in revisitation studies? *Journal of Ecology* 94:980-6.
- Kiesecker, J., M., A. R. Blaustein, L. K. Belden. 2001. Complex causes of amphibian population declines. *Nature* 410:681-4.
- Kopp, C. W. and E. E. Cleland. 2013. Shifts in plant species elevational range limits and abundances observed over nearly five decades in a western North American mountain range. *Journal of Vegetation Science* 24:1-12.

- Kozak, K. H. and J. J. Wiens. 2010. Niche conservatism drives elevational diversity patterns in Appalachian salamanders. *American Naturalist* 176:40-54.
- Kusano, T. and M. Inoue. 2008. Long-term trend toward earlier breeding of Japanese Amphibians. *Journal of Herpetology* 42:608-14.
- Lenoir, J., J. Gegout, A. Guisan, P. Vittoz, T. Wohlgemuth, N. E. Zimmermann, S. Dullinger, H. Pauli, W. Willner, and J. Svenning. 2010. Going against the flow: potential mechanisms for unexpected downslope range shifts in a warming climate. *Ecography* 33:295-303.
- Lenoir, J., J. C. Gegout, P. A. Marquet, P. de Ruffray, and H. Brisse. 2008. A significant upward shift in plant species optimum elevation during the 20th century. *Science* 320:1768-1771.
- Lips, K. R., J. Diffendorfer, J. R. Mendelson, M. W. Sears. 2008. Riding the wave: reconciling the roles of disease and climate change in amphibian declines. *PLoS Biology* 6:441-54.
- MacKenzie, D. I., J. D. Nichols, J. A. Royle, K. P. Pollock, L. L. Bailey, and J. E. Hines. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press, San Diego, CA, USA.
- MacKenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle, and C. A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83:2248-55.
- Milanovich, J. R., W. E. Peterman, N. P. Nibbelink, J. C. Maerz. 2010. Project loss of a salamander diversity hotspot as a consequence of project global climate change.
- Moritz, C., J. L. Patton, C. J. Conroy, J. L. Parra, G. C. White, S. R. Beissinger. 2008. Impact of a century of climate change on small-mammal communities in Yosemite National Park. *Science* 322: 261-4.

- Nishikawa, K. C. 1985. Competition and the evolution of aggressive behavior in two species of terrestrial salamanders. *Evolution* 39:1282-94.
- National Oceanic and Atmospheric Administration. 2013. National Climatic Data Center. NOAA Asheville, NC. <https://www.ncdc.noaa.gov/crn/> .
- National Oceanic and Atmospheric Administration. 2012. Climate.gov climatic data and services. NOAA. <https://www.climate.gov> .
- Organ, J. A. 1991. Salamander survey of the Mount Rogers Nation Recreation Area section two. Marion, VA: United States Department of Agriculture. 210p. Available from Mount Rogers National Recreation Area, Marion, VA.
- Organ, J. A. 1990. Salamander survey of the Mount Rogers Nation Recreation Area section one. Marion, VA: United States Department of Agriculture. 98p. Available from Mount Rogers National Recreation Area, Marion, VA.
- Organ, J. A. 1961. Studies of the local distribution, life history, and population dynamics of the salamander genus *Desmognathus* in Virginia. *Ecological Monographs* 31:189-220.
- Organ, J. A. 1961b. Life history of the pigmy salamander, *Desmognathus wrighti* in Virginia. *American Midland Naturalist* 66:384-90.
- Organ, J. A. 1960. Studies on the life history of the salamander *Plethodon welleri*. *Copeia* 1960:287-97.
- Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology and Evolution* 37:637-69.
- Parmesan C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421:37-42.

- Pechmann, J. H., D. E. Scott, R. D. Semlitsch, J. P. Cadwell, L. J. Vitt, and J. W. Gibbons. 1991. Declining amphibian populations: The problem of separating human impacts from natural fluctuations. *Science* 253:892-5.
- Petranka, J. W., M. P. Brannon, M. E. Hopey, and C. K. Smith. 1994. Effects of timber harvesting on low elevation populations of southern Appalachian salamanders. *Forest Ecology and Management* 67:135-47.
- Petranka, J. W. 1998. *Salamanders of the United States and Canada*. Washington, DC: Smithsonian Institution Press.
- Pope, C. H. 1950. A statistical and ecological study of the salamander *Plethodon yonahlossee*. *Bulletin of the Chicago Academy of Sciences* 9:79-106.
- Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, B. E. Young. 2006. Widespread amphibian extinctions from epidemic diseases driven by global warming. *Nature* 439:161-7.
- Pounds, J. A. and R. Puschendorf. 2004. Clouded futures. *Nature* 427:107-8.
- Raxworthy, C. J., R. G. Pearson, N. Rabibiso, A. M. Rakotondrazaf, J. Ramanamajato, A. P. Raselimanana, S. Wu, R. A. Nussbaum, and D. A. Stone. 2008. Extinction vulnerability of tropical montane endemism from warming and upslope displacement: a preliminary appraisal for the highest massif in Madagascar. *Global Change Biology* 14:1703-20.
- Rohr, J. R., T. R. Raffel, J. M. Romansic, H. McCallum, P. J. Hudson. 2008. Evaluating the links between climate, disease spread, and amphibian declines. *Proceedings of the National Academy of Science* 105:17436-41.
- Rovito, S. M., G. Parra-Olea, C. R. Vasquez-Almazan, T. J. Papenfuss, D. B. Wake.

2009. Dramatic declines in neotropical salamander populations are an important part of global amphibian crisis. *Proceedings of the National Academy of Science* 106:3231-6.
- Rowe, R. J., J. A. Finarelli, and E. C. Rickart. 2010. Range dynamics of small mammals along an elevational gradient over an 80-year interval. *Global Change Biology* 16:2930-43.
- Schmidt, K. P. 1936. Guatemalan salamanders of the genus *Oedipus*. *Zoological Series of Field Museum of Natural History* 10:135-66.
- Seimon, T. A., A. Seimon, P. Daszak, S. R. P. Halloys, L. M. Schloegel, C. A. Aguilar, P. Sowell, A. D. Hyatt, B. Konecky, J. E. Simmons. 2007. Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. *Global Change Biology* 13:288-99.
- Semlitsch, R. D. 2003. *Amphibian conservation*, ed. Washington, DC: Smithsonian Books.
- Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics* 40:415-36.
- Sheldon, A. L. 2012. Possible climate-induced shift of stoneflies in a southern Appalachian catchment. *Freshwater Science* 31:765-74.
- Sinervo, B., F. Cruz, D. B. Miles, B. Heulin, E. Bastiaans, M. Cruz, R. Lara-Resendiz, N. Martinez-Mendez, M. Calderon-Espinosa, R. Meza-Lazaro, H. Gadsden, L. Avila, M. Lepetz, T. Oksanen, D. Chapple, A. Bauer, W. Branch, J. Clobert, J. Sites. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328:894-99.
- Skelly, D. K., K. L. Yurewicz, E. E. Werner, and R. A. Relyea. 2003. Estimating decline and distributional change in amphibians. *Conservation Biology* 17:744-51.

- Thuiller, W., C. Albert, M. B. Araujo, P. M. Berry, M. Cabeza, A. Guisan, T. Hickler, G. F. Midgley, J. Paterson, and F. M. Schurr. 2008. Predicting global change impacts on plant species' distributions: future challenges. *Perspectives in Plant Ecology, Evolution, and Systematics* 9:137-52.
- Thurrow, G. R. 1963. Taxonomic and ecology notes on the salamander, *Plethodon welleri*. *University of Kansas Science Bulletin* 44:87-108.
- Tilley, S. G. 1981. A new species of *Desmognathus* (Amphibia: Caudata: Plethodontidae) from the southern Appalachian Mountains. *Occasional Papers of the Museum of Zoology, University of Michigan* 695:1-23.
- Tingley, M. W. and S. R. Beissinger. 2013. Cryptic loss of montane avian richness and high community turnover over 100 years. *Ecology* 94:598-609.
- Tingley, M. W. and S. R. Beissinger. 2009. Detecting range shifts from historical species occurrences: new perspectives on old data. *Trends in Ecology and Evolution* 24:625-633.
- Tingley, M. W., M. S. Koo, C. Moritz, A. C. Rush, and S. R. Beissinger. 2012. The push and pull of climate change causes heterogeneous shifts in avian elevational ranges. *Global Change Biology* 18:3279-90.
- Vong, R. J., J. T. Sigmon, and S. F. Mueller. 1991. Cloud water deposition to Appalachian forest. *Environmental Science and Technology* 25:1014-21.
- Wake, D. B. and J. F. Lynch. 1976. The distribution, ecology, and evolutionary history of plethodontid salamanders in tropical America. *Science Bulletin of the Natural History Museum of Los Angeles County* 25:1-65.
- Wake, D. B. and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass

- extinction? A view from the world of amphibians. *Proceedings of the National Academy of Science* 105:11466-73.
- Walls, S. C. 2009. The role of climate in the dynamics of a hybrid zone in Appalachian salamanders. *Global Change Biology* 15:1903-10.
- Warren, R. J. and M. A. Bradford. 2010. Seasonal climate trends, the North Atlantic Oscillation, and salamander abundance in the Southern Appalachian Mountain regions. *Journal of Applied Meteorology and Climatology* 49:1597-1603.
- Wells, K. D. 2007. *The Ecology and Behavior of Amphibians*. Chicago, IL: The University of Chicago Press.
- Werner, P., S. Lotters, B. R. Schmidt, J. O. Engler, and D. Rodder. 2013. The role of climate for the range limits of parapatric European land salamanders. *Ecography* 36:1127-37.
- Wilson, R. J., D. Gutierrez, J. Gutierrez, D. Martinez, R. Agudo, and V. J. Monserrat. 2005. Changes to elevational limits and extent of species ranges associated with climate change. *Ecology Letters* 8:1138-46.

APPENDIX

Table 3.1. Top models explaining occupancy probabilities for historic (1950s & 1990s) and modern (2008-11) sampling of salamanders within the Mount Rogers National Recreation Area, Virginia.

Species and Models	QAIC _c	ΔQAIC _c	w_i
<i>Desmognathus fuscus</i>			
Modern			
Ψ (Elevation)	295.09	0.00	0.4152
Historic			
Ψ (Elevation)	181.31	0.00	0.3386
Ψ (Elevation + Elevation ²)	181.63	0.32	0.2886
Ψ (Elevation + Aspect)	182.90	1.59	0.1529
<i>Desmognathus monticola</i>			
Modern			
Ψ (Elevation)	207.75	0.00	0.7455
Historic			
Ψ (Elevation)	136.30	0.00	0.6362
<i>Desmognathus orestes</i>			
Modern			
Ψ (Elevation + Aspect)	75.16	0.00	0.8844
Historic			
Ψ (Elevation + Aspect)	14.04	0.00	0.5029
<i>Desmognathus organi</i>			
Modern			
Ψ (Elevation)	212.81	0.00	0.7016
Historic			
Ψ (Elevation + Aspect)	150.10	0.00	0.6589

Table 3.1. Continued.

Species and Models	QAIC _c	ΔQAIC _c	w _i
<i>Desmognathus quadramaculatus</i>			
Modern			
Ψ (Elevation + Aspect)	194.86	0.00	0.8428
Historic			
Ψ (Elevation + Aspect)	93.78	0.00	0.7408
<i>Eurycea wilderae</i>			
Modern			
Ψ (Elevation)	397.84	0.00	0.3989
Ψ (Elevation + Aspect)	398.29	0.00	0.3185
Ψ (Elevation + Elevation ²)	399.60	1.76	0.1654
Historic			
Ψ (Elevation + Aspect)	220.71	0.00	0.9999
<i>Plethodon cinereus</i>			
Modern			
Ψ (Elevation + Elevation ²)	406.18	0.00	0.5767
Ψ (Elevation + Elevation ² + Aspect)	406.82	0.64	0.4188
Historic			
Ψ (Elevation + Elevation ²)	229.24	0.00	0.3871
Ψ (Elevation + Elevation ² + Aspect)	229.29	0.05	0.3775
Ψ (Elevation + Aspect)	230.41	0.17	0.2156
<i>Plethodon cylindraceus</i>			
Modern			
Ψ (Elevation + Elevation ²)	206.94	0.00	0.4510
Ψ (Elevation + Elevation ² + Aspect)	207.72	0.78	0.3053
Historic			
Ψ (Elevation + Aspect)	124.44	0.00	1.0000

Table 3.1. Continued.

Species and Models	QAIC _c	ΔQAIC _c	w _i
<i>Plethodon montanus</i>			
Modern Ψ (Elevation)	79.86	0.00	0.5995
Historic Ψ (Elevation)	34.38	0.00	0.6726
Ψ (Elevation + Aspect)	35.82	1.44	0.3274
<i>Plethodon richmondi</i>			
Modern Ψ (Elevation + Aspect)	245.19	0.00	0.9052
Historic Ψ (Elevation + Aspect)	193.50	0.00	0.6192
<i>Plethodon welleri</i>			
Modern Ψ (Elevation)	145.97	0.00	0.6434
Ψ (Elevation + Aspect)	147.15	1.18	0.3566
Historic Ψ (Elevation + Aspect)	94.67	0.00	0.9956
<i>Plethodon yonahlossee</i>			
Modern Ψ (Elevation + Elevation ²)	450.67	0.00	0.7161
Ψ (Elevation + Elevation ² + Aspect)	452.52	1.85	0.2839
Historic Ψ (Elevation + Elevation ²)	132.20	0.00	1.0000

Table 3.2. Model-averaged parameter estimates, SEs, and 85% CIs for variables in AIC candidate models from occupancy probabilities from historic (1950s & 1990s) and modern (2008-11) surveys of salamander from the Mount Rogers National Recreation Area, Virginia.

Species	Variable	$\beta \pm \text{SE}$	85% CI
<i>D. fuscus</i>			
Modern	Elevation	-0.47 ± 0.23	-0.80 to -0.14
Historic	Elevation	-2.25 ± 2.61	-6.01 to 1.51
	Elevation ²	2.01 ± 1.20	0.81 to 3.74
<i>D. monticola</i>			
Modern	Elevation	-2.82 ± 0.82	-4.00 to -1.64
Historic	Elevation	-5.77 ± 2.80	-9.79 to -1.74
<i>D. orestes</i>			
Modern	Elevation	4.64 ± 1.90	1.91 to 7.38
Historic	Elevation	1.80 ± 10.00	-12.6 to 16.2
<i>D. organi</i>			
Modern	Elevation	2.81 ± 0.46	2.14 to 3.47
Historic	Elevation	2.36 ± 0.59	1.51 to 3.21
	Aspect	-1.62 ± 0.50	-2.34 to -0.90
<i>D. quadramaculatus</i>			
Modern	Elevation	-1.92 ± 0.40	-2.49 to -1.34
Historic	Elevation	-2.40 ± 0.47	-3.08 to -1.73
<i>E. wilderae</i>			
Modern	Elevation	-0.63 ± 0.87	-1.88 to 0.62
	Elevation ²	-0.34 ± 0.53	-1.09 to 0.42
Historic	Elevation	-1.11 ± 0.34	-1.60 to -0.63
<i>P. cinereus</i>			
Modern	Elevation	8.67 ± 2.05	5.72 to 11.62
	Elevation ²	-9.30 ± 2.08	-12.29 to -6.31
Historic	Elevation	9.48 ± 3.35	4.66 to 14.30
	Elevation ²	-13.05 ± 2.35	-16.43 to -9.67
<i>P. cylindraceus</i>			
Modern	Elevation	24.35 ± 2.87	20.21 to 28.48
	Elevation ²	-29.79 ± 3.26	-34.47 to -25.10
Historic	Elevation	-4.74 ± 0.61	-5.61 to -3.86

Table 3.2. Continued.

Species	Variable	$\beta \pm \text{SE}$	85% CI
<i>P. montanus</i>			
Modern	Elevation	12.85 ± 3.53	7.76 to 17.93
Historic	Elevation	7.15 ± 0.62	6.25 to 8.04
<i>P. richmondi</i>			
Modern	Elevation	-0.36 ± 0.20	-0.65 to -0.07
Historic	Elevation	-0.24 ± 0.23	-0.57 to 0.10
<i>P. welleri</i>			
Modern	Elevation	3.62 ± 0.61	2.74 to 4.49
Historic	Elevation	4.08 ± 0.62	3.18 to 4.98
<i>P. yonahlossee</i>			
Modern	Elevation	17.17 ± 1.58	14.89 to 19.45
	Elevation ²	-17.97 ± 1.63	-20.32 to -15.62
Historic	Elevation	81.32 ± 2.14	78.24 to 84.39
	Elevation ²	-86.04 ± 2.26	-89.30 to -82.79

Table 3.3. Mean and median changes (meters) in midpoint elevation since historic surveys (1950s & 1990s) for Plethodontid salamanders from Mount Rogers National Recreation Area, Virginia. Values in bold represent changes that were confirmed with occupancy modeling.

Salamander Species	Changes since 1957-9			Changes since 1990-1		
	Mean (m)	Median (m)	SD	Mean (m)	Median (m)	SD
<i>P. welleri</i>	-30.5	-30.5	----	-25.4	-22.9	32.9
<i>P. cinereus</i>	53.4	53.4	118.5	57.5	45.7	62.1
<i>P. cylindraceus</i>	-45.7	-30.5	85.3	54.9	61	55.4
<i>P. yonahlossee</i>	68.6	68.6	53.9	32.7	0	58.0
<i>P. montanus</i>	7.6	7.6	10.8	-13.1	-15.2	16.3
<i>P. richmondi</i>	45.7	61	99.9	-22.6	-15.2	76.9
<i>D. orestes</i>	7.6	7.6	10.8	6.5	0	24.7
<i>D. organi</i>	-106.7	-106.7	---	-8.75	2.7	56.4
<i>D. quadramaculatus</i>	10.2	15.2	8.8	10.2	15.2	15.7
<i>D. monticola</i>	5.1	15.7	17.6	22.9	22.9	44.0
<i>D. fuscus</i>	22.9	22.9	32.3	-64.8	-68.6	103.6
<i>E. wilderae</i>	15.2	15.2	---	31.5	30.5	109.9

Table 3.4. Overall median range limit (meters) changes since historic survey periods (1950s & 1990s) for Plethodontid salamanders from Mount Rogers National Recreation Area, Virginia. Values in bold represent changes that were confirmed with occupancy modeling.

Salamander Species	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
<i>P. welleri</i>	-61	0	-30.5	0
<i>P. cinereus</i>	0	0	0	0
<i>P. cylindraceus</i>	+30.5	-121.9	+30.5	+61
<i>P. yonahlossee</i>	0	+15.2	-61	+76.2
<i>P. montanus</i>	0	0	0	0
<i>P. richmondi</i>	+76.2	+15.2	0	0
<i>D. orestes</i>	0	0	0	0
<i>D. organi</i>	-45.7	+30.5	0	0
<i>D. quadramaculatus</i>	+30.5	0	0	0
<i>D. monticola</i>	0	+30.5	0	+45.7
<i>D. fuscus</i>	0	0	0	+30.5
<i>E. wilderae</i>	0	+30.5	0	+30.5

Table 3.5. Median range limit changes (meters) since each historic time period of *Plethodon welleri* from Mount Rogers National Recreation Area, Virginia (* = transect limit not sampled during that time period; ND=Not detected; ND90= Not detected in 1990s surveys)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	-91.4	0
Bluff Mt.	ND	ND	ND	ND
Byars Creek	ND	ND	*	*
Whitetop Creek	-61	0	ND90	ND90
Median Change	-61	0	-91.4	0
Northern				
Big Branch	-61	*	*	0
Bills Ridge	*	*	0	0
Bluff Mt.	*	*	ND	ND
Daves Ridge	-91.4	*	-30.5	0
Dells Branch	*	*	-152.4	0
Pennington Branch	*	*	+30.5	0
Median Change	-76.2	*	-30.5	0
Median Overall Change	-61	0	-30.5	0

Table 3.6. Median range limit changes (meters) since each historic time period of *Plethodon cylindraceus* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during time period; ND=Not detected; ND2000= Not detected in 2000s surveys)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	+30.5	-61
Bluff Mt.	+61	-15.20	+61	-30.5
Byars Creek	+182.9	-121.9	*	*
Whitetop Creek	0	+30.5	0	+91.4
Median Change	+61	-121.9	+30.5	-30.5
Northern				
Big Branch	0	-304.8	*	*
Bills Ridge	*	*	+61	+91.4
Bluff Mt.	*	*	ND2000	ND2000
Daves Ridge	+30.5	-61	*	+30.5
Dells Branch	*	*	-30.5	+61
Pennington Branch	*	*	+30.5	+243.8
Median Change	+15.2	-182.9	0	+215.2
Median Overall Change	+30.5	-121.9	+30.5	+61

Table 3.7. Median range limit changes (meters) since each historic time period of *Plethodon yonahlossee* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during time period)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	-61.0	+30.5
Bluff Mt.	0	*	0	0
Byars Creek	+213.4	0	*	*
Whitetop Creek	+30.5	+30.5	-91.4	+91.4
Median Change	+30.5	+15.2	-61.0	+30.5
Northern				
Big Branch	0	*	*	0
Bills Ridge	*	*	+91.4	+213.4
Bluff Mt.	*	*	+30.5	+61
Daves Ridge	0	*	*	+30.5
Dells Branch	*	*	-213.4	+304.8
Pennington Branch	*	*	-121.9	+121.9
Median Change	0	*	-45.7	+121.9
Median Overall Change	0	+15.2	-61.0	+76.2

Table 3.8. Median range limit changes (meters) since each historic time period of *Plethodon cinereus* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during time period)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	-30.5	0
Bluff Mt.	+91.4	*	+91.4	0
Byars Creek	+274.3	0	*	*
Whitetop Creek	0	-61	0	+243.8
Median Change	+91.4	-30.5	0	0
Northern				
Big Branch	-121.9	+91.4	*	+30.5
Bills Ridge	*	*	+30.5	-30.5
Bluff Mt.	*	*	+30.5	0
Daves Ridge	-121.9	*	*	-121.9
Dells Branch	*	*	-61	+365.8
Pennington Branch	*	*	-30.5	+182.9
Median Change	-121.9	+91.4	0	+15.2
Median Overall Change	0	0	0	0

Table 3.9. Median range limit changes (meters) since each historic time period of *Plethodon montanus* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	-91.4	0
Bluff Mt.	0	*	0	0
Byars Creek	+30.5	0	*	*
Whitetop Creek	0	0	0	0
Median Change	0	0	0	0
Northern				
Big Branch	0	*	*	0
Bills Ridge	*	*	-30.5	0
Bluff Mt.	*	*	0	0
Daves Ridge	-91.4	*	*	0
Dells Branch	*	*	0	0
Pennington Branch	*	*	-30.5	0
Median Change	-45.7	*	-30.5	0
Median Overall Change	0	0	0	0

Table 3.10. Median range limit changes (meters) since each historic time period of *Desmognathus organi* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period; ND=Not detected; ND50= Not detected in 1950s surveys)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	-30.5	0
Bluff Mt.	0	xx	0	0
Byars Creek	ND50	ND50	*	*
Whitetop Creek	-243.8	+30.5	-213.4	0
Median Change	-121.9	+30.5	-30.5	0
Northern				
Big Branch	0	*	*	0
Bills Ridge	*	*	0	0
Bluff Mt.	*	*	-30.5	-30.5
Daves Ridge	-91.4	*	*	0
Dells Branch	*	*	+61	0
Pennington Branch	*	*	+91.4	0
Median Change	-45.7	*	+30.5	0
Median Overall Change	-45.7	+30.5	0	0

Table 3.11. Median range limit changes (meters) since each historic time period of *Desmognathus orestes* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during time period)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	0	0
Bluff Mt.	0	*	0	0
Byars Creek	+30.5	0	*	*
Whitetop Creek	0	0	0	0
Median Change	0	0	0	0
Northern				
Big Branch	0	*	*	0
Bills Ridge	*	*	+121.9	0
Bluff Mt.	*	*	0	0
Daves Ridge	0	*	*	0
Dells Branch	*	*	+30.5	0
Pennington Branch	*	*	+30.5	0
Median Change	0	*	+30.5	0
Median Overall Change	0	0	0	0

Table 3.12. Median range limit changes (meters) since each historic time period of *Desmognathus fuscus* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period; ND=Not detected; ND2000= Not detected in 2000s surveys)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	ND2000	ND2000
Bluff Mt.	ND	ND	ND	ND
Byars Creek	+91.4	0	*	*
Whitetop Creek	0	0	0	0
Median Change	+45.7	0	0	0
Northern				
Big Branch	0	*	*	+30.5
Bills Ridge	*	*	ND	ND
Bluff Mt.	*	*	+61	+30.5
Daves Ridge	ND	ND	0	+274.3
Dells Branch	*	*	0	-30.5
Pennington Branch	*	*	-30.5	+30.5
Median Change	0	*	0	+30.5
Median Overall Change	0	0	0	+30.5

Table 3.13. Median range limit changes (meters) since each historic time period of *Plethodon richmondi* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period; ND=Not detected; ND90= Not detected in 1990s surveys)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	-304.8	0
Bluff Mt.	+61	*	+61	0
Byars Creek	+274.3	0	*	*
Whitetop Creek	+91.4	+30.5	ND90	ND90
Median Change	+91.4	+15.2	-121.9	0
Northern				
Big Branch	ND50	ND50	ND90	ND90
Bills Ridge	*	*	-30.5	0
Bluff Mt.	*	*	+91.4	-30.5
Daves Ridge	-121.9	*	ND90	ND90
Dells Branch	*	*	0	+30.5
Pennington Branch	*	*	ND	ND
Median Change	-121.9	*	-30.5	-30.5
Median Overall Change	+76.2	+15.2	0	0

Table 3.14. Median range limit changes (meters) since each historic time period of *Desmognathus quadramaculatus* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period; ND=Not detected)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	0	0
Bluff Mt.	ND	ND	ND	ND
Byars Creek	+30.5	0	*	*
Whitetop Creek	0	0	0	0
Median Change	+15.2	0	0	0
Northern				
Big Branch	+30.5	*	+30.5	0
Bills Ridge	*	*	ND	ND
Bluff Mt.	*	*	+30.5	0
Daves Ridge	ND	ND	ND	ND
Dells Branch	*	*	0	+61
Pennington Branch	*	*	0	-30.5
Median Change	+30.5	*	0	0
Median Overall Change	+30.5	0	0	0

Table 3.15. Median range limit changes (meters) since each historic time period of *Desmognathus monticola* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during time period; ND=Not detected)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	0	-61
Bluff Mt.	ND	ND	ND	ND
Byars Creek	+30.5	0	*	*
Whitetop Creek	0	+91.4	0	+91.4
Median Change	+15.2	+45.7	0	+15.2
Northern				
Big Branch	0	+30.5	*	*
Bills Ridge	*	*	ND	ND
Bluff Mt.	*	*	ND	ND
Daves Ridge	ND	ND	ND	ND
Dells Branch	*	*	0	+15.20
Pennington Branch	*	*	+61	0
Median Change	*	*	+30.5	+76.2
Median Overall Change	0	+30.5	0	+45.7

Table 3.16. Median range limit changes (meters) since each historic time period of *Eurycea wilderae* from Mount Rogers National Recreation Area, Virginia. (* = transect limit not sampled during that time period; ND=Not detected; ND90= Not detected in 1990s surveys; ND2000= Not detected in 2000s surveys)

Transect	Change since 1957-9		Change since 1990-1	
	Lower	Upper	Lower	Upper
Southern				
Beech Mt.	*	*	-91.4	+304.8
Bluff Mt.	0	*	0	+30.5
Byars Creek	ND2000	ND2000	*	*
Whitetop Creek	0	+30.5	0	0
Median Change	0	+30.5	0	+30.5
Northern				
Big Branch	0	*	*	-15.20
Bills Ridge	*	*	-61	-243.8
Bluff Mt.	*	*	ND90	ND90
Daves Ridge	0	*	*	-61
Dells Branch	*	*	0	+61
Pennington Branch	*	*	+30.5	+335.3
Median Change	0	*	0	-61
Median Overall Change	0	+30.5	0	+30.5

Table 3.17. Mean Climatic values for each 10-year period prior to last sampling year for historic and modern salamander surveys from the Tri-Cities Regional Airport (Sullivan Co., TN). Values in bold are significant.

Time Period	Value	1950-9	1982-91	2002-11	F	p
Jan.	Mean Min. (°C)	-1.91	-3.87	-3.5	1.747	0.193
Jan.	Mean Max (°C)	8.87	6.89	7.43	1.056	0.362
Jan.	Mean (°C)	3.46	1.53	1.98	1.404	0.263
Jan.	Maximum (°C)	19.67	17.56	19.83	1.858	0.175
Jan.	Minimum (°C)	-11.72	-15.89	-13.11	1.906	0.168
Jan.	Total Prec. (cm)	11.06	7.13	8.30	1.942	0.163
July	Mean Min. (°C)	18.22	18.12	17.91	0.266	0.768
July	Mean Max. (°C)	30.62	29.79	29.91	0.952	0.399
July	Mean (°C)	24.43	23.98	23.98	0.654	0.528
July	Maximum (°C)	34.67	33.83	33.44	0.972	0.391
July	Minimum (°C)	13.39	13.0	12.72	0.426	0.783
July	Total Prec. (cm)	10.03	11.04	12.16	0.377	0.689
Annual	Mean Min. (°C)	7.40	7.26	7.13	0.598	0.557
Annual	Mean Max. (°C)	19.98	19.53	19.94	1.204	0.316
Annual	Mean (°C)	13.71	13.42	13.56	0.647	0.531
Annual	Maximum (°C)	35.39	34.28	34.56	0.846	0.444
Annual	Minimum (°C)	-15.67	-18.72	-14.11	4.032	0.029
Annual	Total Prec. (cm)	107.58	100.52	107.55	0.339	0.715

Table 3.18. Results from linear regression analysis of climatic variables in response to time from Tri-cities airport (Kingsport, TN) from 1948-2011.

Time	Value	F (1,62)	p	r ²	Equation
Jan.	Mean	0.550	0.461	0.007	$30.622 - 0.014x$
July	Mean	0.110	0.741	0.014	$28.792 - 0.002x$
Jan.	Mean Min.	0.766	0.385	0.004	$29.687 - 0.017x$
July	Mean Min.	0.067	0.797	0.015	$21.305 - 0.002x$
Jan.	Mean Max.	0.365	0.548	0.010	$32.050 - 0.012x$
July	Mean Max.	0.120	0.730	0.014	$36.643 - 0.003x$
Jan.	Highest	0.028	0.868	0.016	$25.789 - 0.003x$
July	Highest	0.549	0.462	0.007	$51.418 - 0.009x$
Jan.	Lowest	0.000	0.987	0.016	$-15.880 + 0.001x$
July	Lowest	0.003	0.957	0.016	$11.424 + 0.001x$
Jan.	Precipitation	0.596	0.443	0.006	$49.230 - 0.020x$
July	Precipitation	0.013	0.908	0.016	$19.793 - 0.004x$
Annual	Mean Min.	1.577	0.214	0.009	$17.724 - 0.005x$
Annual	Mean Max.	0.132	0.718	0.014	$15.978 + 0.002x$
Annual	Mean	0.000	0.983	0.016	$13.516 + 0x$
Annual	Highest	0.613	0.437	0.006	$52.871 - 0.009x$
Annual	Lowest	1.399	0.241	0.006	$-82.980 + 0.033x$
Annual	Precipitation	0.589	0.446	0.007	$284.186 - 0.09x$

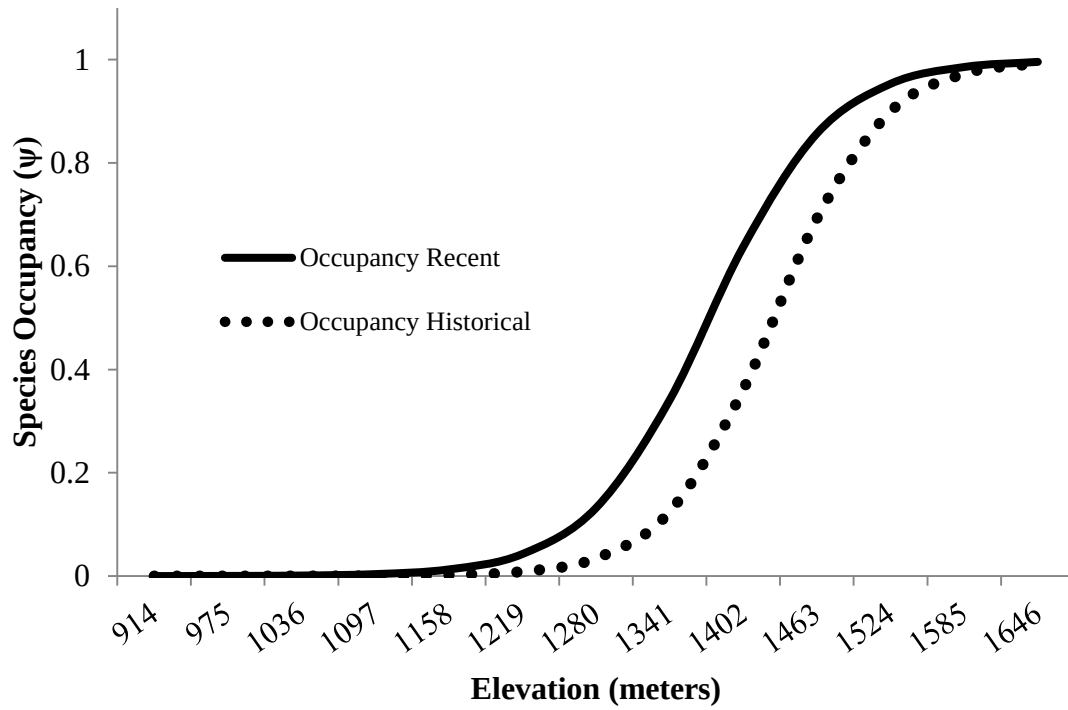


Figure 3.1. Relationship between elevation and occupancy probabilities of *Plethodon welleri* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

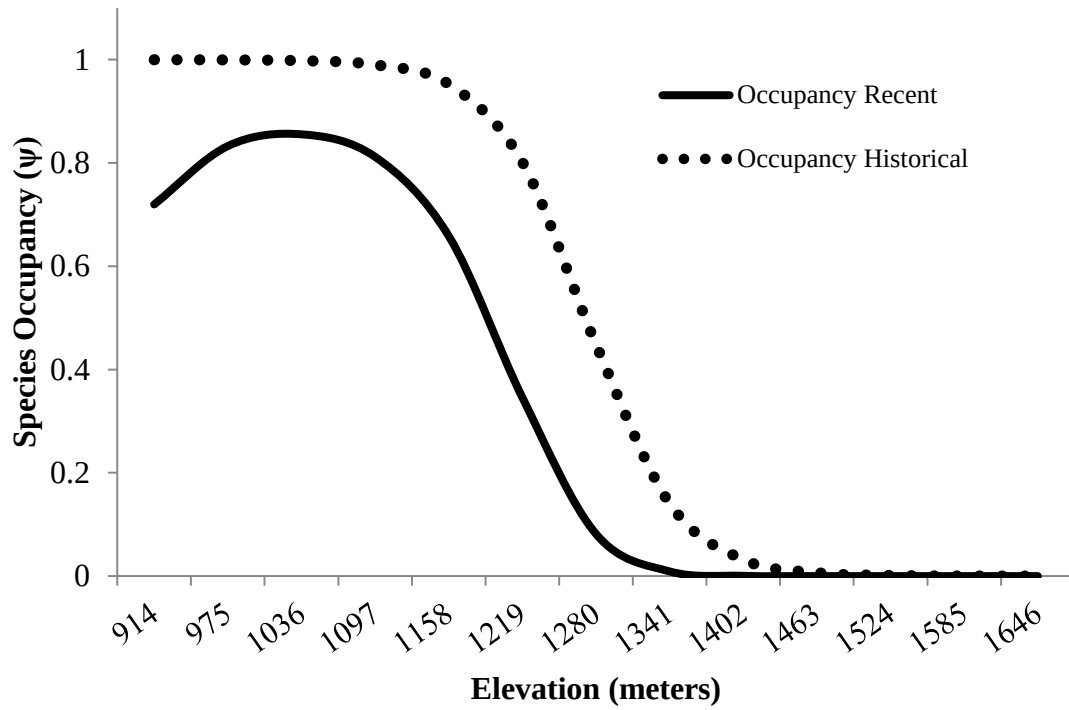


Figure 3.2. Relationship between elevation and occupancy probabilities of *Plethodon cylindraceus* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

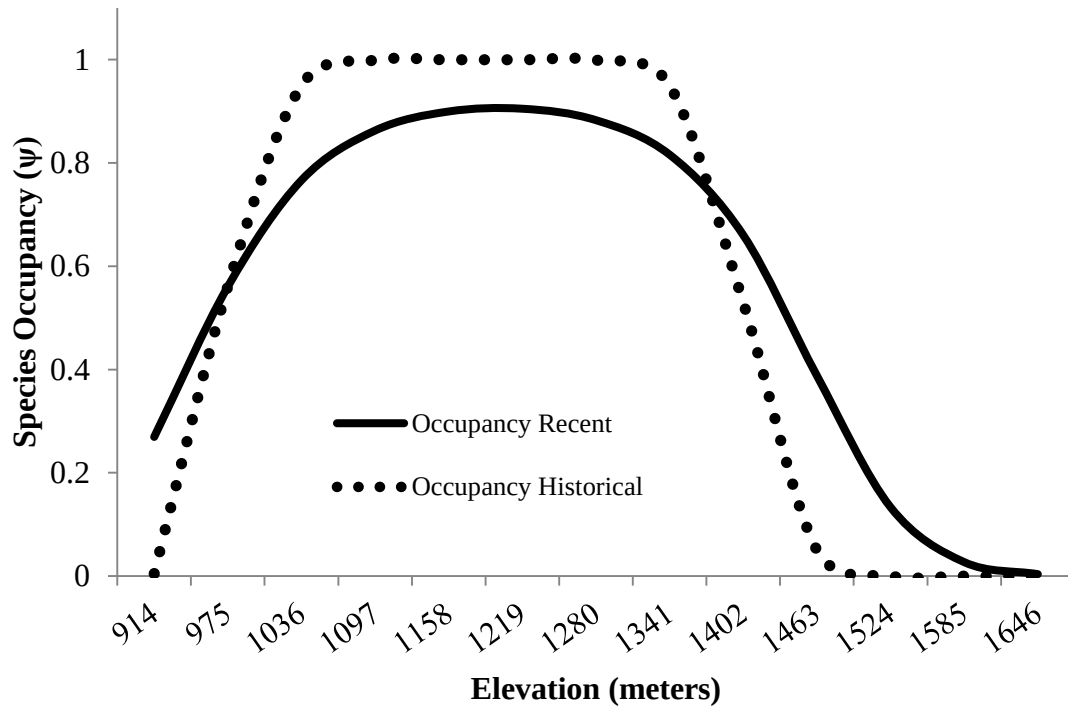


Figure 3.3. Relationship between elevation and occupancy probabilities of *Plethodon yonahlossee* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

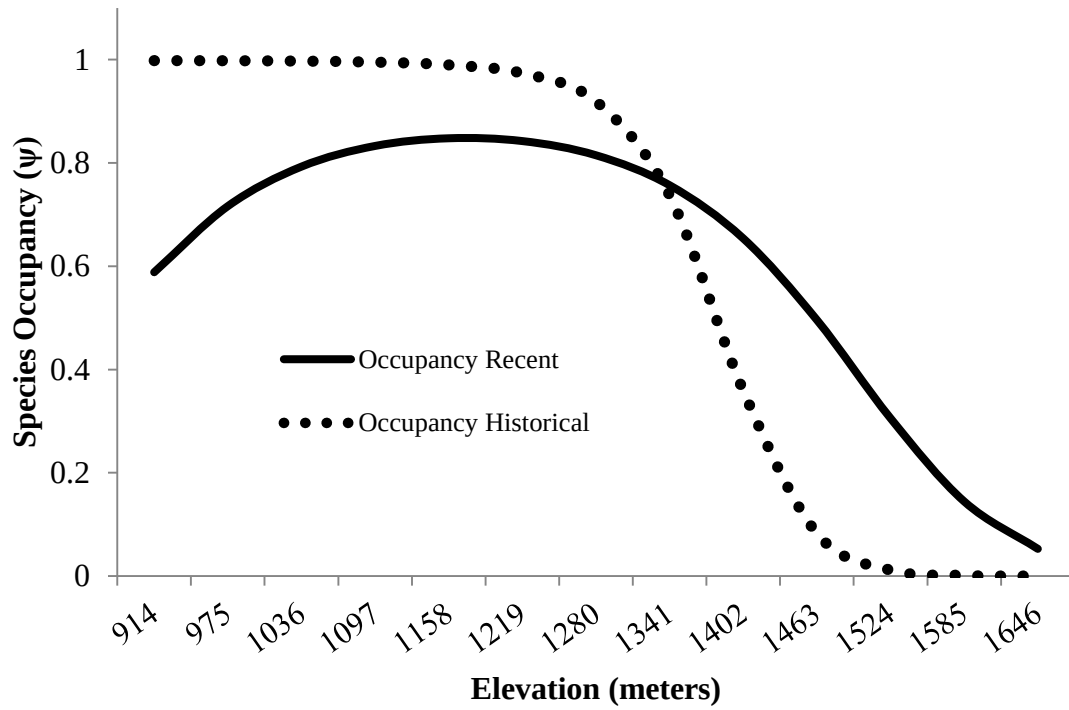


Figure 3.4. Relationship between elevation and occupancy probabilities of *Plethodon cinereus* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

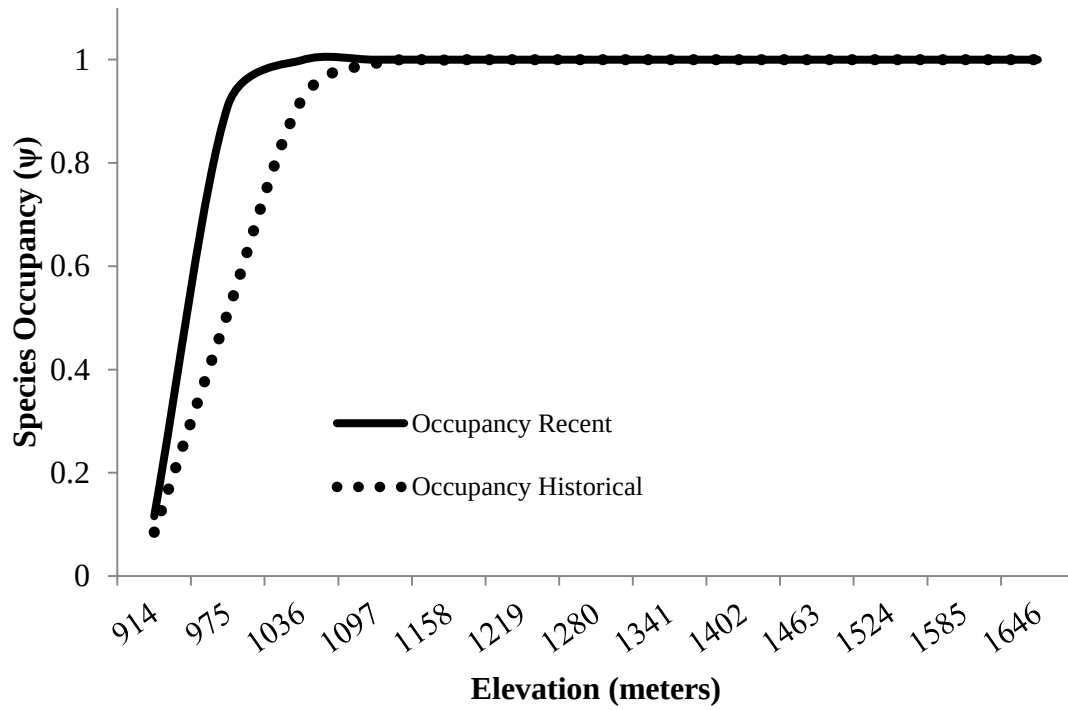


Figure 3.5. Relationship between elevation and occupancy probabilities of *Plethodon montanus* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

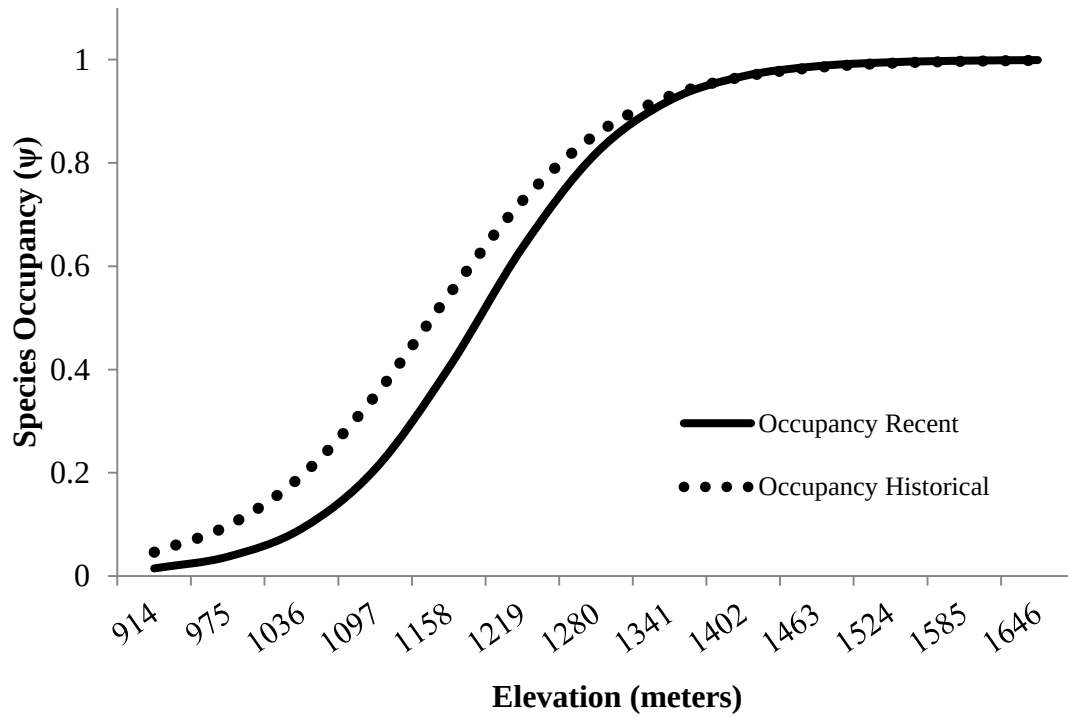


Figure 3.6. Relationship between elevation and occupancy probabilities of *Desmognathus organi* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

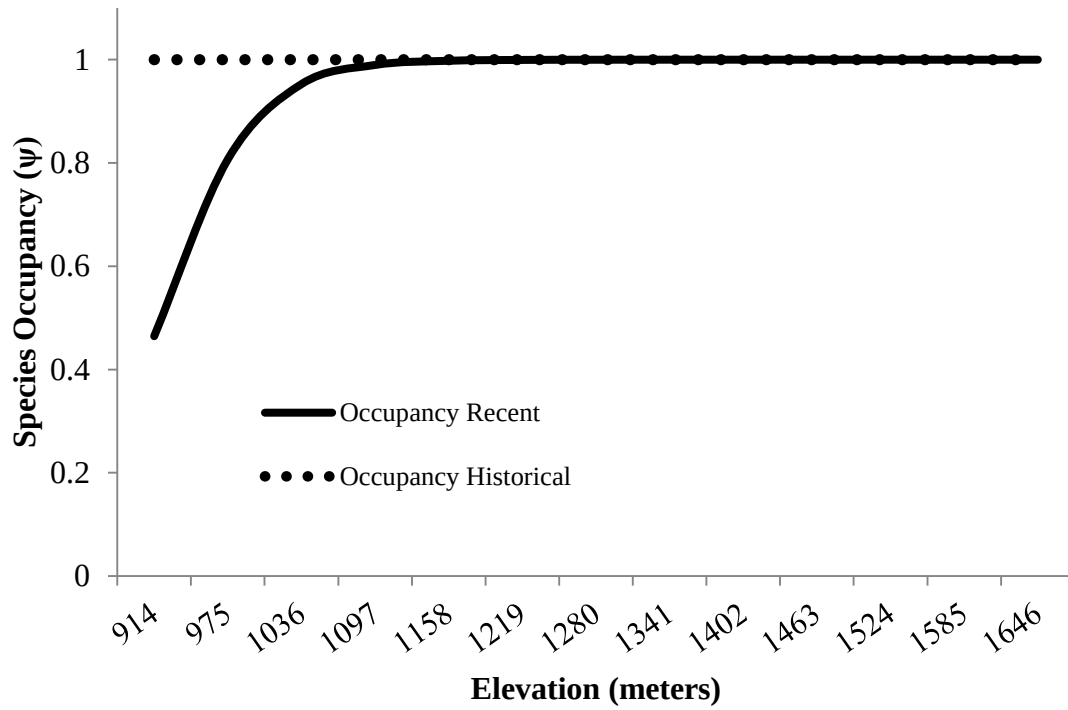


Figure 3.7. Relationship between elevation and occupancy probabilities of *Desmognathus orestes* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

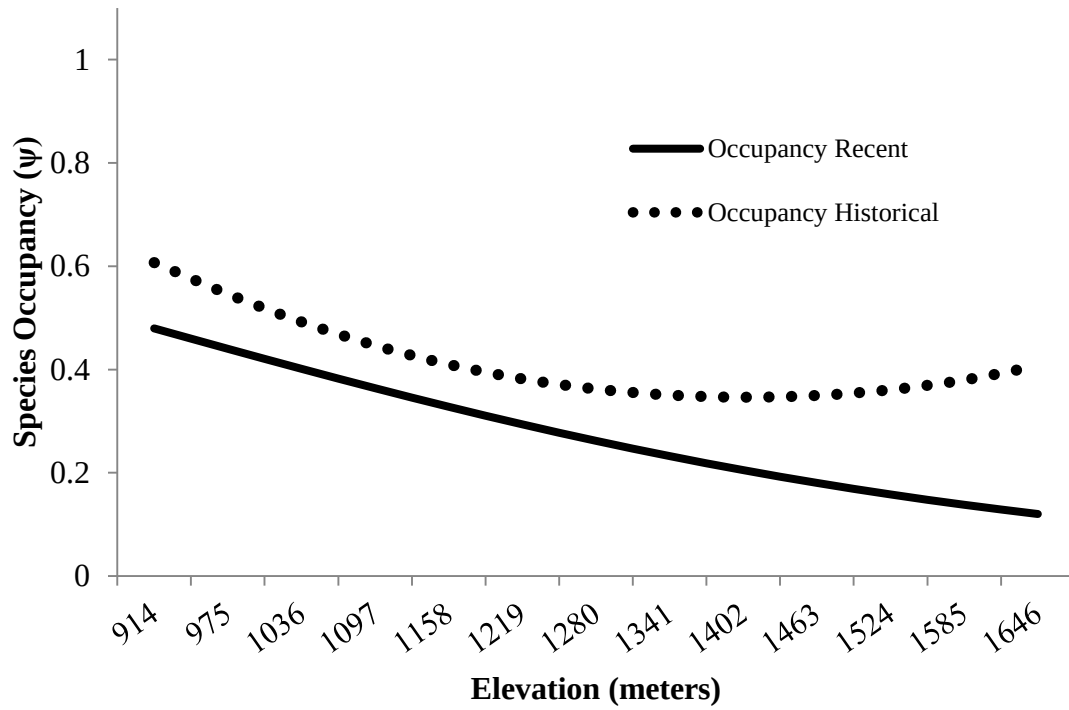


Figure 3.8. Relationship between elevation and occupancy probabilities of *Desmognathus fuscus* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

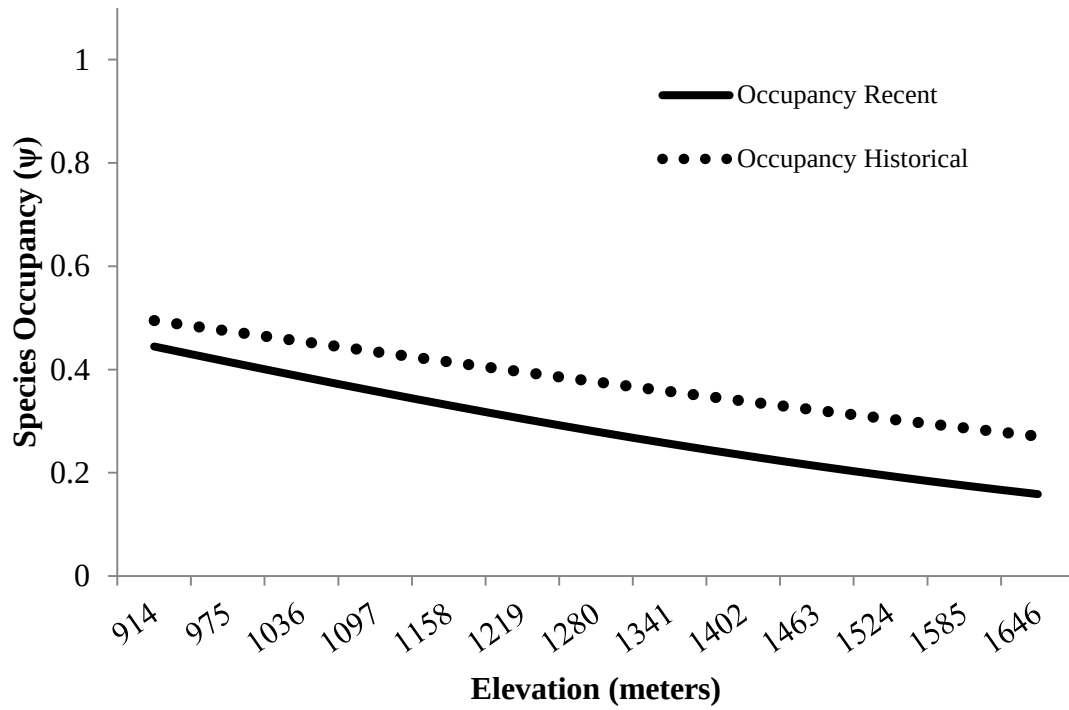


Figure 3.9. Relationship between elevation and occupancy probabilities of *Plethodon richmondi* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

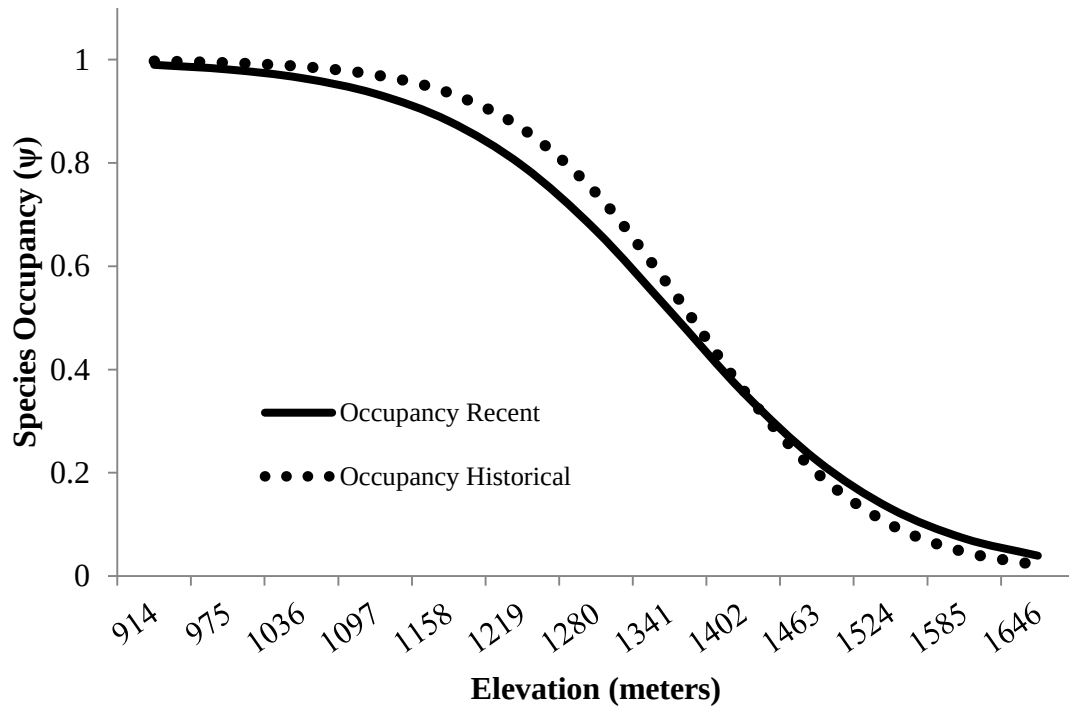


Figure 3.10. Relationship between elevation and occupancy probabilities of *Desmognathus quadramaculatus* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

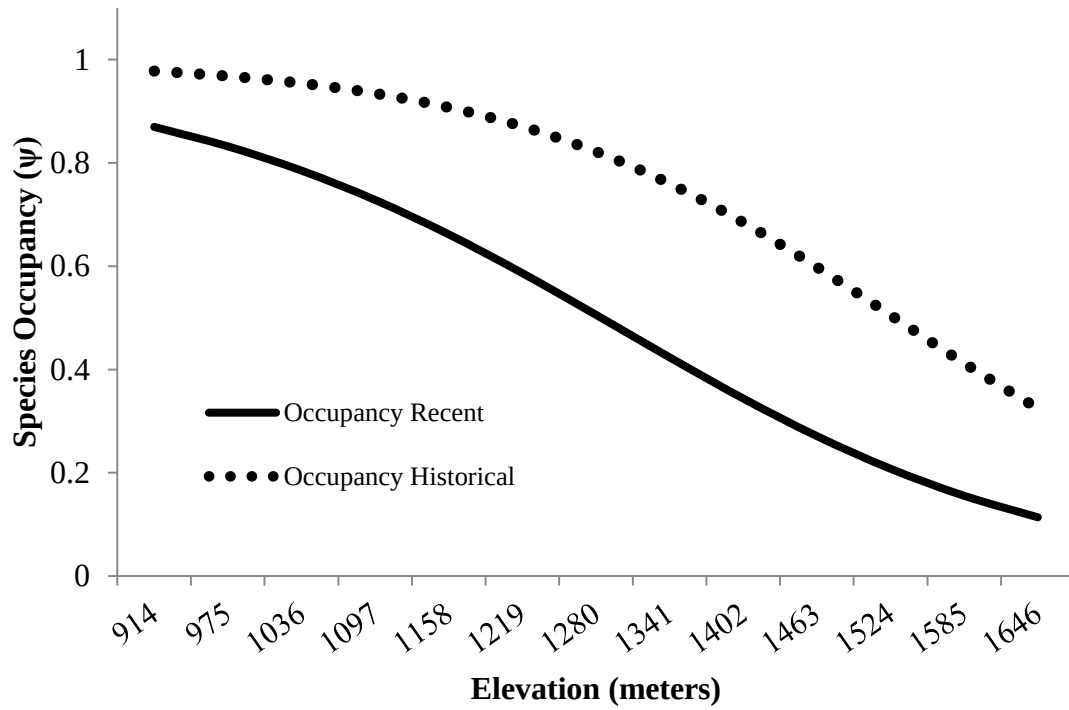


Figure 3.11. Relationship between elevation and occupancy probabilities of *Desmognathus monticola* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

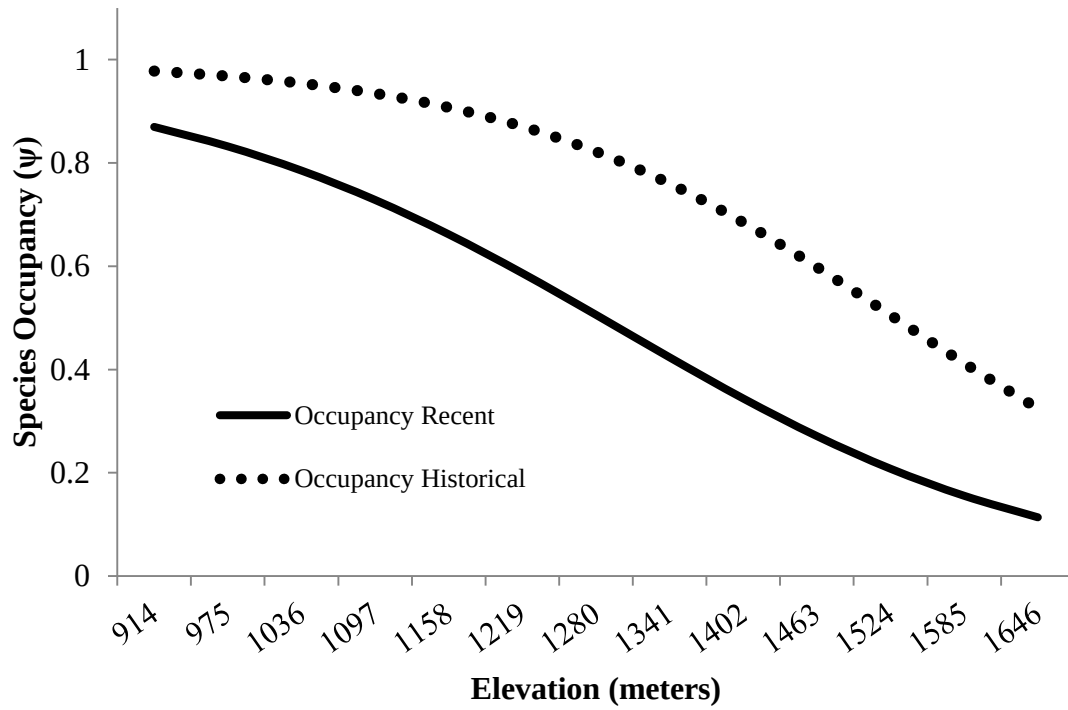


Figure 3.12. Relationship between elevation and occupancy probabilities of *Eurycea wilderae* for historic (1950s & 1990s) and modern surveys (2008-11) from Mount Rogers National Recreation Area, Virginia.

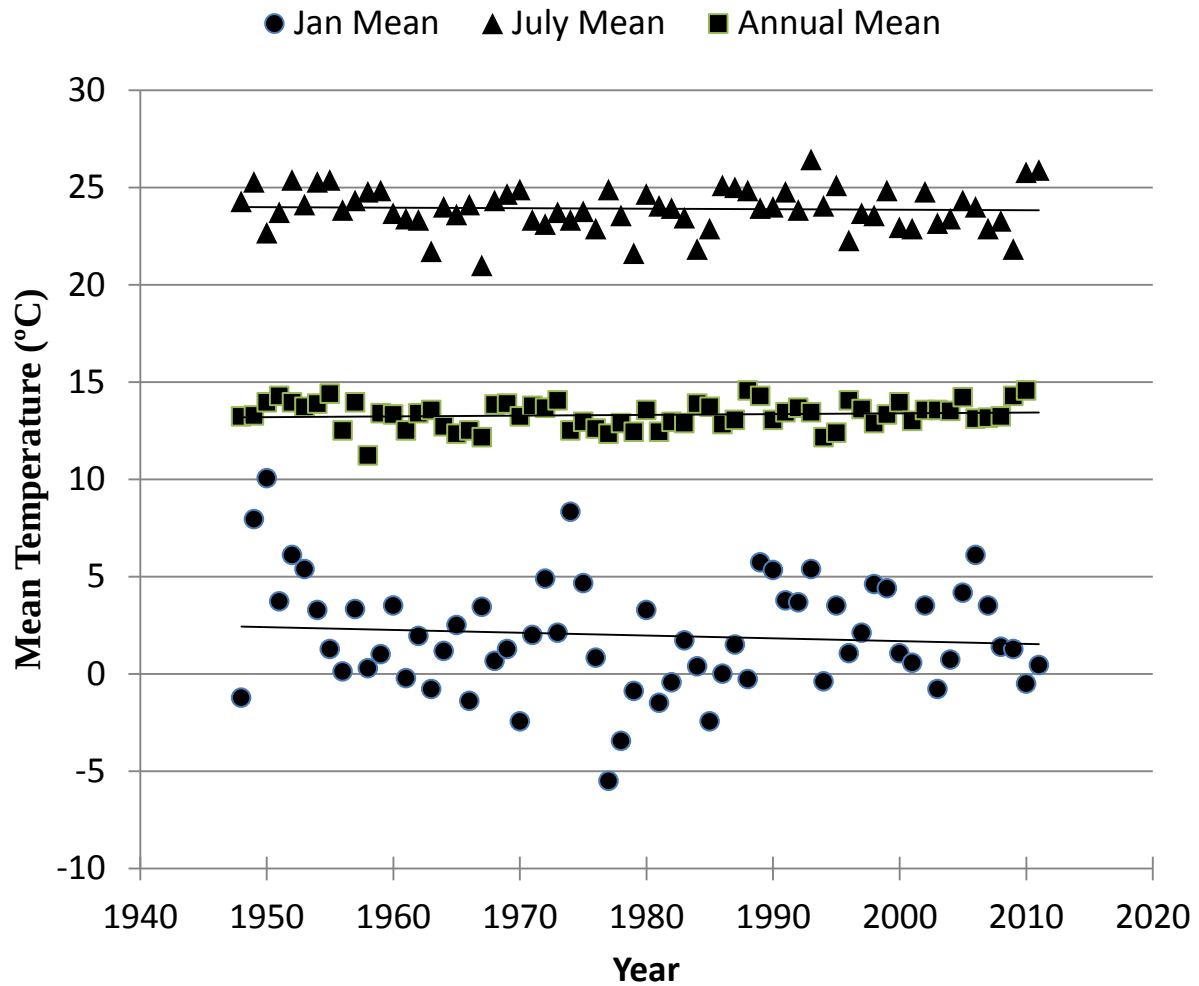


Figure 3.13. Relationship between mean temperature and year of collection (1948-2011) from Tri-cities airport, Kingsport, TN. [(January mean = $30.622 - 0.014x$; $r^2 = 0.007$; $P = 0.461$), (July mean = $28.792 - 0.002x$; $r^2 = 0.014$; $P = 0.741$), and (Annual mean = $13.516 - 0.000x$; $r^2 = 0.016$; $P = 0.983$)]

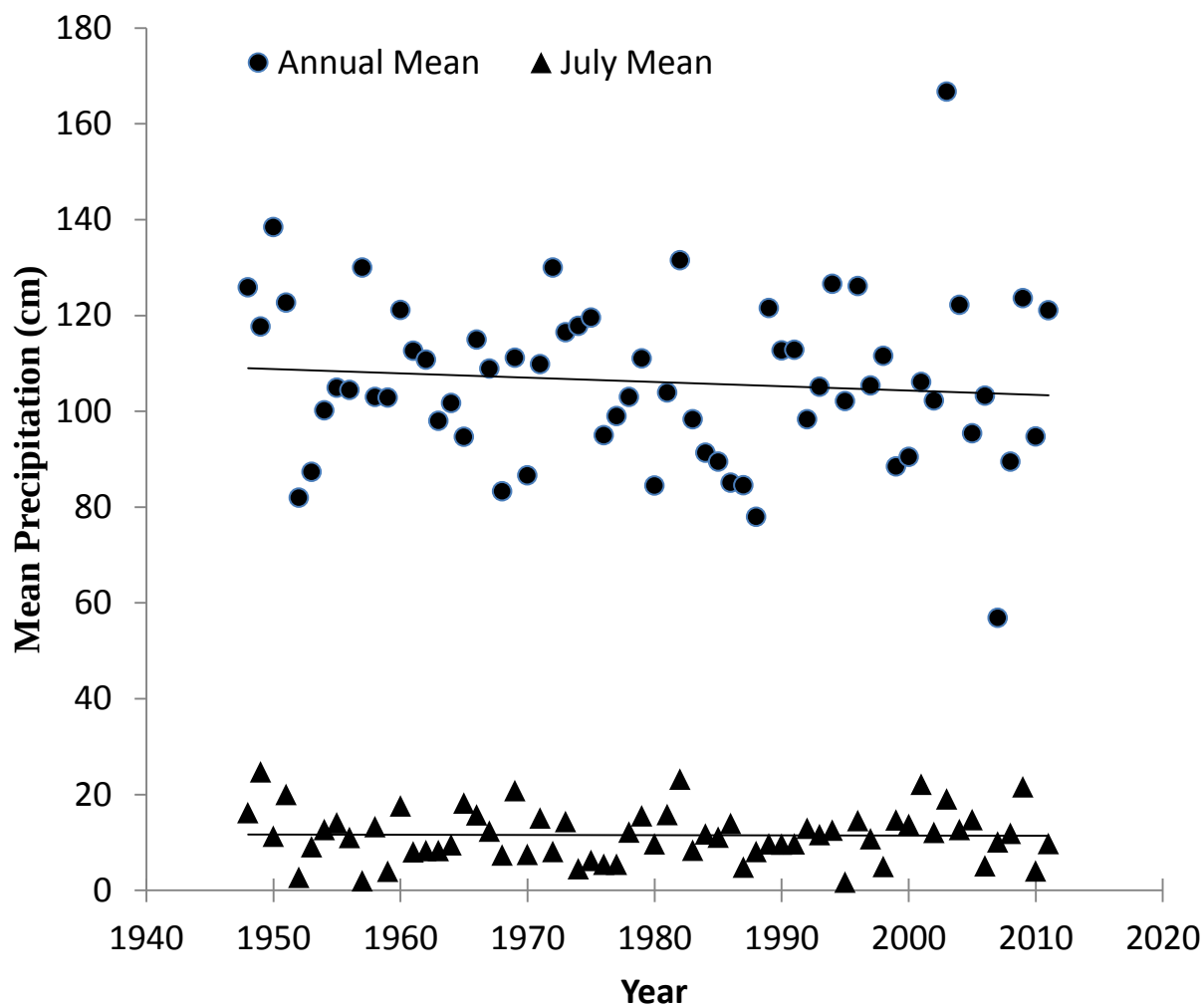


Figure 3.14. Relationship between mean precipitation and sample year (1948-2011) from Tri-cities airport, Kingsport, TN. [(July mean = $19.793 - 0.004x$; $r^2 = 0.016$; $P = 0.908$) (Annual mean = $284.186 - 0.09x$; $r^2 = 0.007$; $P = 0.446$)]

**CHAPTER 4: VEGATATION AND BIOTIC HABITAT
CHARACTERISTICS INFLUENCE PLETHODONTID SALAMANDER
OCCUPANCY WITHIIN THE MOUNT ROGERS NATIONAL
RECREATION AREA**

ABSTRACT

Amphibian populations, especially salamander have declined and almost half of all salamanders are threatened with extinction. Habitat loss is responsible for the decline of 89% of all threatened amphibians. However, there is a lack of knowledge of habitat associations for many amphibians especially caudates. The Southern Appalachian Mountains have one of greatest diversities of Plethodontid salamanders, but relatively little is known of their preferred habitats. We sampled 9 transects at 30.5 m elevational intervals (total samples $n = 149$) from 914 – 1,585 m to determine the presence of 12 Plethodontid salamander species within the Mount Rogers National Recreation Area (MRNA), which has the greatest Plethodontid diversity in Virginia. We used occupancy modeling to determine which vegetation types and abiotic variables were most related to salamander presence while accounting for imperfect detection. We evaluated both abiotic (slope, aspect, elevation, presence of water, and number of rock cover objects) and vegetation (canopy cover, vegetation density, tree basal area, relative frequencies of woody species at all structural levels, and types ground cover) factors. We assessed 116 potential models explaining salamander occupancy. Hypothesized models were constructed based on previous associations with variables in historic salamander descriptions or from closely related taxa. Abiotic variables best explained occupancy for 83% of salamanders whereas vegetation factors best described occupancy for 17%. Top occupancy models for members of the genus *Desmognathus* and *Eurycea wilderae* included the presence of water covariate. Elevation was the leading covariate explaining occupancy for *Plethodon cylindraceus*, *P. montanus*, *P. welleri*, *P. yonahlossee*, *D. organi*, and *D. orestes*. Many vegetation factors such as relative frequency of dominant trees were significant for many species, but were not often the top variable. However, forest types and especially the relative frequency of individual tree

species best explained occupancy for *P. richmondi*, which was associated with decreasing yellow birch relative frequency. Occupancy for *P. cinereus* was strongly related to increased relative frequency of cucumber magnolia trees. Our findings identified vegetation and abiotic factors of important salamander areas within the MRNA. Additionally, managers will have a potential estimation of salamander occupancy for previously unsurveyed areas based on site specific habitat parameters.

INTRODUCTION

Amphibian populations have declined in the past 25 years and salamanders have especially been impacted as over 47% of all salamander species are threatened globally (IUCN 2008; Wake and Vredenburg 2008). Amphibian declines have occurred throughout most habitat types (Adams et al. 2013). Habitat loss, diseases, introduced species, chemical contamination, UV-B radiation, over-exploitation, climate change, and synergistic interactions have been documented to cause declines (Semlitsch 2003, Wells 2007). However, habitat loss and alteration had the greatest impact on amphibian populations (Dodd and Smith 2003) with 89% of all threatened amphibians impacted from habitat loss (Young et al. 2004). In the Southern Appalachian Mountains it is estimated that 14 million Plethodontid salamanders are lost annually to clear cutting in western North Carolina alone (Petranka et al. 1993). Specific habitat changes which contribute to the loss of many amphibians are unknown (Gardner et al. 2007). Conserving habitat will be a key strategy to protecting many amphibians and especially caudates, but for many species there is a lack of knowledge of specific habitat requirements and thus an inability to identify specific areas for conservation and management.

When identifying habitat characteristics, both abiotic and biotic factors should be considered as they both can influence the distribution of species (Brown 1984). Land managers

can actively manage areas to maintain existing amphibian populations and mitigate degraded habitat for population expansion or reestablishment once habitat requirements are identified. Numerous studies have established relationships between habitat variables and a species' niche (Van Horne and Wiens 1991; Larson et al. 2003; Dillard et al. 2008; Martin et al. 2010, and many others). Spatial distributions are often linked to narrow preferences at either the landscape or microhabitat level. Therefore, a fine gradient exists for occupancy (Brown 1984; Dillard et al. 2008 and 2008b).

Plethodontid salamanders typically have small home ranges ($< 30\text{m}^2$) and limited dispersal abilities (Petranka 1998). Therefore, site-specific habitat characteristics greatly influence Plethodontid salamander distributions. Many studies have used GIS to identify and measure spatial variables for salamander habitat preferences (Suzuki et al. 2008; Keyser et al. 2011; Dudaniec and Richardson 2012; Peterman and Semlitsch 2013) while others have measured variables on the ground (Ford et al. 2002; Welsh et al. 2006; Riedel et al. 2008; Dillard et al. 2008 & 2008b). Habitat models that utilize GIS and remote sensing data are often employed for inaccessible areas or large numbers of study sites (Carter et al. 2006). However, these methods might fail to detect slight covariates changes from occupied to unoccupied areas. Within the MRNA, the transition zone from *Plethodon welleri* to *P. richmondi* on Beech Mountain was less than 100 m (Organ 1991). The transition zone was only located through intense survey efforts and slight habitat differences could only be identified on the ground. Therefore, identifying habitat parameters on a small scale may better detect fine-scale environmental gradients for Plethodontid salamanders.

Many forest parameters in relation to timber production and harvesting have been correlated with Plethodontid salamander presence. Forest canopy cover (Herbeck and Larsen

1999; Crawford and Semlitsch 2008), leaf litter depth (Harper and Guynn 1999), and coarse woody debris (Petranka 1998) were associated with salamander presence in and around timber harvest sites. Many early descriptions of habitats where Southern Appalachian salamanders resided were not quantified and served as basic illustrative reports. These early descriptions included dominant tree types (Walker 1934; Pope 1950), woody debris (Dunn 1926), ground vegetation (Netting 1939), and other general descriptors. Many abiotic factors were also identified as contributors to suitable salamander habitat; elevation, aspect, and their associated climatic condition have been shown to greatly influence salamander distributions (Hairston 1949) especially on Whitetop Mountain (Organ 1961, 1990, 1991). Slope (Pope 1950; Wallace 1969) and the presence and distance to water impacted Plethodontid salamander, especially those with aquatic larval periods (Brimley 1944; Hairston 1949; Organ 1961).

Many previous attempts to identify Plethodontid habitat preferences used logistic regression with an information theoretic approach to evaluate hypotheses relating habitat and salamander presence (Suzuki et al. 2008; Dillard et al. 2008, 2008b). Previous attempts only used locations where salamanders were detected, but did not account for imperfect detection (Bailey et al. 2004). Relationships between covariates and salamander presence could have been biased because detection was not considered (Mazerolle et al. 2005). Habitat associations were often skewed as models did not fully incorporate all variables within the salamander's niche due to false absences (Hann et al. 2007). Models better related variables to an organism's true niche when detection probabilities were incorporated into the models (Gu and Swihart 2004; Mazerolle et al. 2005; Bailey et al. 2007). Occupancy modeling accounts for imperfect detection and removes bias associated with covariates (MacKenzie et al. 2002, 2006; Gu and Swihart 2004). Habitat models for reptiles (Luiselli 2006; Steen et al. 2012), mammals (Baldwin and Bender

2008; Martin et al. 2010; Long et al. 2011; Perinchery et al. 2011), and amphibians (Mazerolle et al. 2005; Peterman and Semlitsch 2013) have recently been constructed that incorporate detection and occupancy. Models incorporating occupancy and detection provided different and more accurate conclusions than traditional models (Mazerolle et al. 2005).

The Mount Rogers National Recreation Area (MRNA) has one of the most diverse Plethodontid salamander communities in Virginia (Mitchell and Reay 1999). Whitetop Mountain within the MRNA is inhabited by 15 Plethodontid salamanders (Organ 1961), and 6 species are classified as species of greatest conservation need by the Virginia Department of Game and Inland Fisheries (VDGIF 2005). The U.S. Forest Service established a salamander management zone within the MRNA for all areas above 1,219 m (4,000') on Whitetop and Bluff Mountains and it was recommended to be expanded in 1990 to include Beech Mountain (Organ 1991). However, habitat associations for MRNA salamanders have only been anecdotally described and never quantified (Organ 1960, 1990, 1991). Without the knowledge of known habitat preferences, management mistakes have been made. In the early 1990s the Daves Ridge timber sale allowed timber removal from 1,128 – 1,219 m (3,700 – 4000') in several small plots (<10 acres) adjacent to the MRNA salamander management zone. As of 2011, several species of salamanders have not been detected within timber harvest units since the harvest (Organ unpublished data).

Our goal was to gain an understanding of habitat preferences of Plethodontid salamanders within the MRNA. We modeled occupancy probabilities for 12 species from 9 elevational transects (149 total sampling locations) on 3 different mountains. We constructed competing habitat models based on published and anecdotal habitat associations of MRNA salamander. Occupancy models presented should provide agencies and land managers with a better

understanding of vegetation and abiotic factors associated with salamander occupancy. Additionally, our associations could provide insight on more widely distributed species in the Southern Appalachian Mts. or those with limited distributions (e.g. *Plethodon welleri* and *Desmognathus organi*) residing in habitats ubiquitous to the MRNA.

METHODS

Study Area

The Mount Rogers National Recreation Area is located in southwestern Virginia, lies within Grayson, Smyth, and Washington counties, and is known for its diversity of Plethodontid salamanders (Mitchell and Reay 1999). Over 48,000 ha comprise the MRNA and the two highest peaks in Virginia, Mount Rogers (1,746 m) and Whitetop Mountain (1,684 m), reside in the MRNA. Our study area encompassed Whitetop, Bluff (1,475 m), and Beech Mountains (1,512 m) all located in the western portion of the MRNA. The summit (>1,554 m) of Whitetop Mountain is predominately red spruce (*Picea rubens*) with mountain maple (*Acer spicatum*) and mountain ash (*Sorbus americana*), while the forest below the summit, until approximately 1,158 m, is a northern hardwood forest. Beech Mountain is dominated by American beech (*Fagus grandifolia*) from the summit until 1,463 m, where a northern hardwood forest is established (Organ 1991). Bluff Mountain is covered with trees from both northern hardwood and mixed deciduous forests. The lowest 100 – 250 m of each mountain is located on private properties while the remainder is protected within the U.S. Forest Service MRNA. Northern hardwood forests are dominated by yellow birch (*Betula allegheniensis*), sugar maple (*Acer saccharum*), and American beech (*Fagus grandifolia*). Cove hardwood forests are dominated by American beech, yellow buckeye (*Aesculus flava*), striped maple (*Acer pensylvanicum*), and eastern hemlock

(*Tsuga canadensis*). Mixed deciduous forests are more variable but are typically dominated by red oak (*Quercus rubra*), red hickory (*Carya ovalis*), cucumber magnolia (*Magnolia acuminata*), and chestnut oak (*Quercus prinus*).

Our study transects were established in 1957 and 1990 by Dr. James Organ while documenting elevational distributions for Plethodontid salamanders (Organ 1991). Current transects were resurveyed as part of a larger project to determine historic range changes in salamander distributions. For this project we chose nine transects to relate biotic and abiotic factors to salamander occupancy. Four transects were sampled on the northern slope of Whitetop Mt. [Big Branch (914 – 1,524 m), Bills Ridge (945 – 1,585 m), Daves Ridge (945 – 1,463 m), Dells Branch (945 – 1,585 m)] and another northern facing slope on Bluff Mt. (1,280 – 1,475 m). A total of 4 southern facing slopes were sampled, two on Whitetop Mt. [Byars Creek (1,067 – 1,402 m) and Whitetop Creek (1,158 – 1,585 m)], one on Beech Mt. (957 – 1,512 m), and a single transect on Bluff Mt (1,128 – 1,475 m). Both habitat variables and salamanders were sampled every 30.5 m (100') in elevation along each transect for a total of 149 sampling sites.

Habitat measurements

We sampled habitat parameters from May – September 2011 and sampling time matched the same Julian calendar date of salamander occupancy surveys, which reduced the likelihood of vegetation changes due to seasonal variation (e.g. Beech Mountain was sampled for salamander occupancy from 21 May – 30 May 2009 and vegetation surveys were conducted from 23 May – 26 May 2011). We determined salamander occupancy at each site by sampling on both sides of the transect center for 1-person hour. However, if we had measured habitat variables from the center of each transect and extended in each direction, the impact of factors on the distal ends of transects would be ignored due to the size of the vegetation plot. Therefore, we randomly picked

one side of the transect for sampling by using a table of random numbers. We began habitat sampling at the center of the salamander transect and extended outward for 40 m, which cover the majority of the salamander transect. The shape of sampling plots was modified from traditional square or circular plot shape (Higgins et al. 2005) to a rectangular shape and overall was 10 x 40 m (Figure 4.1). Our plot shape matched our salamander search area. Salamanders detected within the survey plot mostly likely permanently resided in these small areas due to the limited home ranges of most Plethodontid salamander (Petranka 1998). We established plots for ground vegetation and vertical structure 5, 15, 25, and 35 m from the beginning of each transect. Seedling, sapling, and tree sampling occurred at the transect center (e.g. 20 m). The plot for woody seedling vegetation was 40 m², which was rectangular as the longest side (10 m) followed the transect and the width extended 2 m both up and downslope (i.e. total of 4 m) of the transect (Figure 4.1). Sapling and tree plots both extended 5 m up and downslope of the transect which created a 10 m wide path and encompassed all of the salamander search area. Sapling plots were 10 m in length (total = 100 m²) and tree plots were 40 m in length (total = 400 m²) and both were centered at the 20 m mark of the sampling transect thus extending 5 and 20 m on each side of the transect center (Figure 4.1).

Numerous vegetation and abiotic habitat features from other areas in the Appalachians have been associated with Plethodontid salamanders found within the MRNA (Table 4.1). Vegetation and abiotic characteristics at each sampling location were quantified to determine if unique habitat associations existed for each salamander species (Table 4.2). Percent ground cover was determined by implementing a 1-m² Daubenmire frame at four locations along each transect (Higgins et al. 2005; Figure 4.1). We determined visual estimates of percent ground cover for bare earth, dead organic matter, mosses, ferns/allies, rocks, woody vegetation, and

herbaceous vegetation in each frame and calculated a mean value for each transect (Daubenmire 1959). The MRNA herbaceous community in May was considerably different than the community in September. Therefore, we grouped herbaceous plants comprising ground cover into a single category due to the projection sampling period (May 2011 – September 2011). We measured vertical structure of vegetation (visual obscuration) using a Nudds (1977) profile board which was divided into 4 sections (0 = 0 – 0.5 m, 1 = 0.51 – 1 m, 2 = 1.01 – 1.50 m, and 3 = 1.51 – 2.00 m) and each 0.5-m section was divided into 30 - 5x5 cm squares. We made measurements by counting the number of small squares that were at least 50% covered by vegetation by an observer who was 10 m from the board. We estimated vertical structure at the same four locations along each transect used for ground cover, but was 1.5 m upslope, which avoided disruption of ground cover plots (Figure 4.1). We grouped measurements into 2 categories (< 1 m; 1-2 m) and calculated a mean value for the site (Nudds 1977). To estimate overstory canopy cover, we used a spherical densitometer (Forestry Suppliers; Jackson MS) by counting the number of dots in each square (4 dots per square for a total of 96) in which the sky was not visible due to canopy vegetation. We made estimates in the plot center and at each cardinal direction (north, south, east, and west) for a total of five measurements from which a mean value was calculated (Higgins et al. 2005). To determine basal area, we used a 2.5 m²/ha factor prism (Forestry Suppliers; Jackson MS) at the transect center while the observer turned in a 360° circle so that all trees within the plot were visible. We quantified potential cover objects by counting all rocks greater than 161 cm² and all coarse woody debris greater than 11.4 cm² in diameter, along the entire 40 m segment of the transect and within 5 m of each side (400 m² sampling area). We measured transect aspect to the nearest 5° with a compass placed at two locations (5 and 35 m marks) along each transect. We linearized aspect so that cooler and

wetter, northeasterly aspects had lower values and drier southwestern slopes had higher values using the equation: $[1 - \cos(\text{aspect in radians})] + [1 - \sin(\text{aspect in radians})]$ (Dillard et al. 2008). To obtain slope, we placed two observers 10 m apart with one upslope and the other down slope of the transect. We used a clinometer (Suunto; Finland) was used to estimate slope to the nearest 5° by sighting a mark on a Nudds board that was the same height as the observer's eye level. We measured seedling, sapling, and tree density in 3 nested plots (40 m^2 , 100 m^2 , and 400 m^2 respectively) along each transect (Figure 4.1). We identified and quantified all seedlings less than 1.4 m tall in each woody understory plot. We quantified seedlings as those less than 10 cm tall or those 10 cm to 1.4 m tall. A plant was designated as sapling if it was greater than 1.4 m tall but less than 11.43 cm (4.5") diameter at breast height (DBH). We grouped saplings based on DBH and totaled. We measured DBH by using a fabric diameter tape (Forestry Suppliers; Jackson MS) to encircle the tree trunk 1.37 m above the ground. Additionally, we classified saplings as (rhododendron/mountain laurel, red spruce, American beech, deciduous, or mixed) based on the dominant vegetation. These categories were chosen based on discussion with Organ from his 1990-1 surveys. A woody plant was classified as a tree if it was greater than 11.43 cm DBH. We counted trees and measured DBH individually. We established the relative frequency of each tree species in both midstory and overstory plots. We identified plants based on published dichotomous keys and descriptions (Radford et al. 1968; Rheinhardt and Ware 1984; Wofford and Chester 2002; Horn and Cathcart 2005; Evans 2005).

Salamander data

We sampled transects for salamander occupancy from July 2008 – July 2011. Transects sampled were Beech Mt. (July 2009), Bluff Mt. (North slope – July 2008), Bluff Mt (South slope – August 2010), Big Branch (August 2010), Bills Ridge (July 2011), Byars Ck. (May 2009),

Dave Ridge (August 2009), Dells Branch (June 2009-11), Pennington Branch (July 2010), and Whitetop Ck. (June 2009-11). We centered transects on either a ridge or stream and sampled every 30.5 m (100') in elevation. We used a Garmin 60CSX GPS unit was used to establish the correct beginning location. Transects were parallel to the contour which maintained a consistent elevation and we checked the GPS unit every 5 minutes to ensure proper sampling elevation. We conducted sampling within 5 m of either side of the transect which provided for minimal changes in elevation along the entire transect length. Occupancy modeling required repeat sampling (Bailey et al. 2004). We sampled each side of the transect center separately and considered the second side sampled a repeat sample of the first (Bailey personal conversation). Our approach allowed us to model both detection and occupancy as we had a repeat sample. Additionally, we resampled Whitetop Creek and Dells Branch for 3 consecutive years (2009-2011), thus providing a total of 6 surveys for each elevation along those transects. We also considered samples from consecutive years as repeat samples which allowed us to model detect and occupancy. Sampling effort was 2-person hours per transect with one-person hour on each side of the transect center and all salamander sampling was conducted with the same observer (Kevin Hamed). I turned all natural cover objects (e.g., rocks, wood, and bark) and searched leaf litter for salamanders. I captured detected salamanders, placed them in individual 1.2-liter plastic bags, identified the salamander, and returned salamanders to their approximate capture location.

Occupancy modeling

We sampled each elevation along all transects twice except Whitetop Creek and Dells Branch which were sampled for 3 consecutive years for a total of 6 sampling attempts. Sampling sites were considered closed as habitat was essentially uniform on each side of the transect center

and repeat surveys occurred within 1 hour. Consecutive surveys for repetitive years on Whitetop Creek and Dells Branch were also considered closed. Plethodontid salamanders have very limited home ranges ($<30 \text{ m}^2$) and typically have minimal emigration and immigration rates which allowed for a closed assumption from year to year sampling (Petranka 1998). Occupancy modeling required a closed assumption to ensure animals are not immigrating or emigrating between sampling sites (MacKenzie et al. 2006).

Inasmuch as 100% detection along transects cannot be assured, we first estimated detection probabilities to adjust occupancy probabilities (MacKenzie et al. 2002). We used the single-season model (MacKenzie et al. 2002) in Program PRESENCE 5.5 (Hines 2012) to model detection. Since repeat and multi-year surveys were closed due to a lack of migration, single-season models best modeled detection (Steen et al. 2012). Models for detection were (1) null, (2) transect, (3) time of year, (4) time of year + time of year², (5) number of rock cover objects, and (6) number of log cover objects. We standardized covariates by z-transformations prior to analysis. We used the best model of detection as ranked by Akaike's Information Criterion (correct for small sample size; AIC_c) for all further occupancy models (Burnham and Anderson 2002).

We also modeled occupancy in Program PRESENCE 5.5 using single season models (Hines 2012; Steen et al. 2012). Prior to model building we standardized continuous variables by z-transformations. We tested for collinearity by using a Spearman's rank order correlation (SPSS v. 20, IBM 2011) and eliminated variables with strong correlation ($r \geq 0.70$). Understory relative frequency was strongly correlated with overstory relative frequency for $> 85\%$ of all species, therefore only tree relative frequency was used in analysis. Sapling categories also had high levels of correlation with tree relative frequency. Therefore, we chose to use sapling

categories for our analysis. We constructed models based on known preferences for Southern Appalachian Plethodontid salamanders especially those within the MRNA (Table 4.1). A total of 116 competing occupancy models were constructed for each species (Table 4.3). We ranked occupancy models by AIC_c (AIC corrected for small sample size; Burnham and Anderson 2002). We calculated overdispersion parameter ($\hat{c}$) for each species using the global model unless the global model was not valid and then the valid model with the greatest number of parameters was used for calculation (Burnham and Anderson 2002). To correct for overdispersion, we used $\hat{c}$ to correct AIC_c ($QAIC_c$; Burnham and Anderson 2002) and standard errors in occupancy models and reported model weights (w_i). We only used models with $\Delta QAIC_c \leq 2.0$ to identify which covariates were important for habitat models (Burnham and Anderson 2002). If a covariate was present in multiple candidate models, we averaged covariates across models and calculated SEs and 85% confidence intervals (Burnham and Anderson 2002). Biologically significant coefficients are often excluded when 95% confidence intervals are calculated (Arnold 2010). Therefore, we deemed covariates to be informative if 85% confidence intervals did not include zero (Arnold 2010).

RESULTS

Of the 15 Plethodontid salamanders found with the MRNA, three species were not encountered frequently enough to model occupancy. *Desmognathus marmoratus*, *Gyrinophilus porphyriticus*, and *Pseudotriton ruber* were encountered at less than 7 locations and therefore occupancy was not modeled. However, occupancy models related to habitat parameters were constructed for all other Plethodontid salamanders within the MRNA. The landform model received the greatest support for the habitat preference of *Plethodon welleri* ($w_i = 0.40$) and two

additional models (landform + northern hardwood, landform + cove hardwood), each containing landform also received support ($w_i = 0.21$ and 0.19 respectively; Table 4.4). Within the landform models, elevation ($\beta = 7.95 \pm 2.41$; 85% CI = 4.48 to 11.42), slope ($\beta = 3.74 \pm 1.24$; 85% CI = 1.95 to 5.53), and presence of water ($\beta = -3.15 \pm 1.69$; 85% CI = -5.58 to -0.72) all received strong support (Table 4.5). Occupancy probabilities of *P. welleri* increased strongly with elevation (Figure 4.2) and slope (Figure 4.3) while it decreased with the presence of water. Additionally, the relative frequency of American beech and yellow birch (Figure 4.4) were positively associated ($\beta = 1.33 \pm 0.71$; 85% CI = 0.31 to 2.35; $\beta = 1.18 \pm 0.69$; 85% CI = 0.19 to 2.17) and yellow buckeye relative frequency ($\beta = -1.56 \pm 0.99$; 85% CI = -2.99 to -0.13) was negatively associated with the occupancy of *P. welleri* (Table 4.4).

The cove hardwood forest model received the greatest support for occupancy of *Plethodon yonahlossee* ($w_i = 0.58$) while the only other model strongly supported was elevation + elevation² ($w_i = 0.37$; Table 4.4). There was a weak, negative relationship of the four tree species (American beech, yellow buckeye, striped maple, and eastern hemlock; Figures 4.5 – 4.7) we designated as components of a cover hardwood forest and occupancy ($\beta = -0.72$ to -0.51 ; 85% CI = -0.99 to -0.25 ; Table 4.5). Elevation was positively associated ($\beta = 17.19 \pm 1.80$; 85% CI = 14.60 to 19.78) and elevation² ($\beta = -16.97 \pm 1.76$; 85% CI = -18.73 to -15.21 ; Table 4.5 and Figure 4.8) was negatively associated with occupancy which created a quadratic distribution (Moritz et al. 2008) where occupancy increased with elevation to mid-elevations and then decreased with higher elevations.

Occupancy within the MRNA for both *Plethodon montanus* and *P. cylindraceus* appeared to be related to elevation. The elevation model ($w_i = 0.08$) was the only model with support greater than the null model for *P. montanus* (Table 4.4). Slope, percent bare ground, and number

of rock cover objects were models with $\Delta\text{QAIC}_c \leq 2.0$, but since the null model had greater support, their influence was considered irrelevant. Occupancy probabilities increased strongly with elevation for *P. montanus* ($\beta = 12.70 \pm 4.01$; 85% CI = 6.93 to 18.47; Table 4.5 & Figure 4.8). Three top occupancy models for *P. cylindraceus* all contained the elevation covariate. The quadratic distribution model of elevation + elevation², received the greatest support ($w_i = 0.26$) while elevation + aspect and elevation also were supported ($w_i = 0.22$ and $w_i = 0.22$ respectively; Table 4.4). Elevation ($\beta = -6.81 \pm 3.37$; 85% CI = -11.67 to -1.96) and elevation² ($\beta = -25.18 \pm 1.48$; 85% CI = -27.31 to -23.05) were negatively associated with occupancy of *P. cylindraceus* (Table 4.5 & Figure 4.8). Aspects was positively associated with occupancy ($\beta = 1.65 \pm 1.01$; 85% CI = 0.19 to 3.10; Table 4.5 & Figure 4.9) and a positive association indicated a more southwestern facing slope.

Top models explaining the occupancy of *P. cinereus* included several different covariates, but none were strong models with weights < 0.12 , which suggested that occupancy was influenced by multiple characteristics. Striped maple relative frequency ($w_i = 0.12$), cucumber magnolia relative frequency ($w_i = 0.07$), elevation + elevation² ($w_i = 0.06$), vertical strata less than 1 meter ($w_i = 0.06$), percent ground cover of moss ($w_i = 0.05$), and overstory richness ($w_i = 0.05$) all had $\Delta\text{QAIC}_c \leq 2.0$ (Table 4.4). Cucumber magnolia relative frequency ($\beta = 43.30 \pm 7.68$; 85% CI = 32.24 to 54.36; Table 4.5 & Figure 4.10) and overstory species richness ($\beta = 0.63 \pm 0.24$; 85% CI = 0.29 to 0.97; Table 4.5 & Figure 4.11) were positively related to *P. cinereus* occupancy. Striped maple relative frequency ($\beta = -0.68 \pm 0.22$; 85% CI = -1.00 to -0.36; Table 4.5 & Figure 4.6), percent ground cover of moss ($\beta = -0.59 \pm 0.20$; 85% CI = -0.89 to -0.30; Table 4.5 & Figure 4.12), and vertical structure less than 1 meter in height ($\beta = -0.59 \pm 0.22$; 85% CI = -0.91 to -0.27; Table 4.5 & Figure 4.13) were negatively associated with

occupancy. Elevation and elevation² also influenced occupancy as elevation ($\beta = 8.02 \pm 2.29$; 85% CI = 4.72 to 11.32) positively and elevation² ($\beta = -8.13 \pm 2.19$; 85% CI = -11.29 to -5.93) negatively influenced occupancy indicating a quadratic, elevational distribution (Table 4.5 & Figure 4.2)

Only 2 models received strong support for the occupancy of *P. richmondi*. Both the northern hardwood model ($w_i = 0.46$) as well as the combination model of cover, ground vegetation, and northern hardwood ($w_i = 0.28$) had $\Delta\text{QAIC}_c \leq 2.0$ (Table 4.4). The number of rock cover objects ($\beta = 0.86 \pm 0.36$; 85% CI = 0.34 to 1.38; Table 4.5 & Figure 4.14) and the relative frequency of sugar maple trees ($\beta = 0.56 \pm 0.25$; 85% CI = 0.20 to 0.92; Table 4.5 & Figure 4.15) were positively associated with *P. richmondi* occupancy. The relative frequency of yellow birch ($\beta = -1.43 \pm 0.57$; 85% CI = -2.25 to -0.61; Table 4.5 & Figure 4.4) and American beech trees ($\beta = -0.69 \pm 0.32$; 85% CI = -1.15 to -0.23; Table 4.5 & Figure 4.16) in addition to percent ground cover by moss ($\beta = -1.48 \pm 0.60$; 85% CI = -2.34 to -0.62; Table 4.5 & Figure 4.12) were negatively associated with occupancy.

Top models for occupancy were more consistent among members of the genus *Desmognathus*, which rely on water for some aspect of their life history. The presence of permanent water was the top model, or a component of top models for all members of the *Desmognathus* genus except *D. orestes*. Presence of water was the only model with $\Delta\text{QAIC}_c \leq 2.0$ for *D. quadramaculatus* ($w_i = 0.80$; Table 4.4) and occupancy increased strongly with the presence of water ($\beta = 7.53 \pm 1.55$; 85% CI = 5.29 to 9.76; Table 4.5). Landform was the only model explaining the occupancy for both *D. monticola* and *D. organi* ($w_i = 0.62$, $w_i = 0.52$, respectively; Table 4.5). Elevation and the presence of water were the only significant covariates within the landform model for *D. monticola*. The presence of water was positively ($\beta = 4.09 \pm$

0.75; 85% CI = 3.01 to 5.17) and elevation was negatively ($\beta = -1.08 \pm 0.48$; 85% CI = -1.78 to -0.38; Figure 4.17) associated with occupancy (Table 4.4). Presence of water ($\beta = 3.71 \pm 1.26$; 85% CI = 1.89 to 5.52) and elevation ($\beta = 4.59 \pm 1.09$; 85% CI = 3.02 to 6.17; Figure 4.18) were strongly associated with occupancy of *D. organi*, but aspect ($\beta = -1.07 \pm 0.41$; 85% CI = -1.66 to -0.48; Figure 4.9) was negatively associated (Table 4.5). Four models provided support for *D. fuscus* occupancy and 3 contained canopy cover ($w_i = 0.27$, $w_i = 0.12$, $w_i = 0.11$) while the presence of water ($w_i = 0.10$) was the only other model with $\Delta\text{QAIC}_c \leq 2.0$ (Table 4.4). Slope and aspect covariates both had CIs that included 0 and occupancy decreased with canopy cover ($\beta = -2.11 \pm 0.75$; 85% CI = -3.19 to -1.03; Table 4.5 & Figure 4.17). The presence of water was again positively associated with occupancy of *D. fuscus* ($\beta = 1.77 \pm 0.50$; 85% CI = 2.49 to 1.27; Table 4.5). Elevation and elevation + aspect were the only 2 models with $\Delta\text{QAIC}_c \leq 2.0$ to explain the occupancy of *Desmognathus orestes* ($w_i = 0.13$, $w_i = 0.11$; respectively; Table 4.4). Elevation ($\beta = 3.73 \pm 1.72$; 85% CI = 1.25 to 6.21; Figure 4.18) was positively associated and aspect ($\beta = -1.56 \pm 0.82$; 85% CI = -2.74 to -0.38) was negatively associated with occupancy of *D. orestes*, indicating a preference for northeastern facing slope (Table 4.5).

Larvae of *Eurycea wilderae* also require an aquatic environment for larval development. Landform ($w_i = 0.14$), the presence of water ($w_i = 0.12$), landform + northern hardwood forests ($w_i = 0.09$), slope ($w_i = 0.08$), and slope + basal area ($w_i = 0.05$) were the top models explaining occupancy of *E. wilderae* (Table 4.4). Slope ($\beta = -0.95 \pm 0.37$; 85% CI = -1.48 to -0.42; Table 4.5 & Figure 4.3) was negatively associated with occupancy. Water ($\beta = 1.87 \pm 0.67$; 85% CI = 0.91 to 2.83) and the relative frequency of yellow birch ($\beta = 1.15 \pm 0.43$; 85% CI = 0.54 to 1.76; Figure 4.4) were positively associated with occupancy of *E. wilderae* (Table 4.6). Relative

frequency of sugar maples ($\beta = 0.46 \pm 0.32$; 85% CI = 0.01 to 0.92; Table 4.5 & Figure 4.15) was positively associated with occupancy, but it was a weak association.

DISCUSSION

Abiotic factors had the greatest impact on MRNA salamander occupancy as they were the top covariate for 83% (10/12) of salamanders and vegetation factors were the top covariate for 17% (2/12) of salamanders surveyed. Throughout North America a majority of previous habitat studies for Plethodontid salamanders have indicated abiotic factors to be important for occupancy, presence, or abundance. Habitat models for *Plethodon stormi* (Suzuki et al. 2008); *P. albagula* (Peterman and Semlitsch 2013); *P. cinereus*, *Desmognathus ochrophaeus*, *D. monticola*, and *D. fuscus* (Keyser et al. 2011); *D. ocoee* and *P. jordani* (Ford et al. 2002); *P. nettingi* (Dillard et al. 2008) were most impacted by abiotic covariates. Biotic variables alone have not impacted salamander habitat models, but models with a combination of both abiotic and biotic variables were the best models for some species. *Plethodon elongatus* (Welsh et al. 2006), *P. nettingi* (Dillard et al. 2008b), and *P. cinereus* (Riedel et al. 2008) habitat models included both biotic and abiotic covariates. Our results followed previous trends for salamander habitat models with abiotic factors having the greatest impact, but vegetation factors were also influencing several salamander species.

Plethodon welleri was thought to be strictly a resident of high elevation spruce/fir forest (Bishop 1943; Hoffman and Kleinpeter 1948; Organ 1960). However, our models indicated elevation was the best predictor of occupancy, which suggested climatic variables such as temperature or moisture might be a better predictor of occupancy than forest characteristics. The strong association with steep slopes and *P. welleri* occupancy was probably due to the

salamander's preference for talus areas (Petranka 1998). Even though rock cover was not identified as a major contributor to occupancy, most talus areas are in steep terrain within the MRNA. Within the MRNA, Organ (1991) discovered a population of *P. welleri* on Beech Mt., which lacked red spruce and was dominated with American beech. Lower elevation (> 701 m) hardwoods have also been identified as habitat for *P. welleri* (Walker 1934; Organ 1961b; Thurow 1963). Our occupancy models did not indicate a preference for spruce dominated forest, but instead elevation and slope best explained occupancy. The lack of association might have occurred due to our sampling areas. Our sampling was a portion of a larger project to document salamander range changes within the MRNA, and survey sites were replicates of Organ's (1961, 1990, 1991) original study sites, which did not include the highest elevations dominated by spruce (> 1585 m). However, spruce dominated the upper elevations (> 1524 m) of most transects, where *P. welleri* resided and spruce was also found between 1463 – 1524 m, but was not always the dominant tree. Therefore, relative frequencies of other trees better predicted the occupancy of *P. welleri*. A positive relationship between occupancy and both yellow birch and American beech were indicated by models. Anecdotally, *P. welleri* were typically found under yellow birch bark and logs from dead trees if present along transects. Our discovery of a lower elevation nest of *P. welleri* in yellow birch leaf litter and next to a yellow birch log (Hamed et al. 2012) suggested this tree could be important for the natural history and reproduction of *P. welleri*. American beech are the dominate tree on the upper 49 m of Beech Mountain and *P. welleri* was documented there including pairs presumed to be breeding due to the presence of well developed mental glands. Stands dominated by American beech could be an alternative to spruce forests for *P. welleri*. In response to climate change, upper elevation forests are expected to be overtaken by species that typically grow at lower elevations (Beckage et al. 2008). As

American beech trees move higher up Whitetop Mt. due to climate change, the potential impact on *P. welleri* could be minimal since occupancy was high in forest dominated by American beech, as long as thermal tolerances are not exceeded. A strong negative association between occupancy and increased yellow buckeye frequency could have been the result the tree's preference for riparian areas and lower elevations which had lower occupancy for *P. welleri* (Rheinhardt and Ware 1984). The presence of water was also shown to have a negative association with *P. welleri* occupancy. This could be due the lack of water at higher elevation, but Whitetop Creek has flowing water well within the range of *P. welleri*. Organ (1991) conducted linear transects documenting the distances salamanders occurred from streams and found the closest *P. welleri* was to stream margins was 61 m.

Habitat associations of *P. yonahlossee* have included rocky ravines (Bailey 1937; Martof et al. 1980) and talus slopes (Pope 1950). Additionally, *Plethodon yonahlossee* was often found under coarse woody debris (> 25 cm in diameter; Dunn 1926; Pope 1950; Gordon et al. 1962). Hairston (1949) associated *P. yonahlossee* with stream margins as he only located the salamander within 30 m of a stream. However, Petranksa (1998) indicated streams were not a habitat requirement for *P. yonahlossee*. Our top model results did not indicate an association with rocks, coarse woody debris, or the presence of water, but instead elevation had the greatest impact on occupancy. The elevation + elevation² model indicated a quadratic elevational distribution in the MRNA. *Plethodon yonahlossee* has been found at much lower and slightly higher elevations (436 – 1737 m) throughout its range (Pope 1950; Guttman et al. 1978). However, within the MRNA we found this salamander slightly lower (30.5 m) than early surveys (Pope 1950) suggesting that lower elevations had not been suitable habitat for *P. yonahlossee* in the past 60 years and succession or climate changes allowed downward movement. Typically

this salamander was associated with deciduous forest especially oak-hickory (Pope 1950; Guttman et al. 1978; Hoffman 1992). While a positive association with deciduous forest was not indicated with our top models, a slight negative association was indicated with cove hardwood forest and trees that composed this type of forest, such as striped maple which had the greatest negative relationship. Climatic conditions (temperature and moisture) associated with elevation might be a greater contributor for *P. yonahlossee* distributions than habitat variables we measured.

Habitat associations for the slimy salamander complex (*Plethodon glutinosus*) have been examined prior to the separation of individual species. Moist and second growth oak hickory forest have been associated with *P. cylindraceus* (Wells and Wells 1976; Petranka 1998). These salamanders typically inhabited gentle sloping hills with large numbers of downed logs (Wells and Wells 1976). However, within the MRNA, elevation had the greatest influence on occupancy. The top model of elevation + elevation² indicated a peak distribution at mid-elevations with lower occupancy at both the lowest and highest elevations, but when the top models were averaged, occupancy probabilities were high at low elevation and rapid decreased at middle and high elevations. Ford et al. (2002) also found elevation was the greatest covariate explaining the distribution of *P. glutinosus*. This suggested that climate could determine the upper range limit of *P. cylindraceus*. However, recent work with other large bodied Plethodontid salamanders suggested that competition with Plethodontids at higher elevations might have a greater role than climate (Hairston 1980; Gifford and Kozak 2012). Aspect also appeared to influence occupancy of *P. cylindraceus* as occupancy increased as transects faced a more southwestern direction. This also suggested a potential climatic influence as southwestern slopes are typically drier and warmer. Aspect has been shown to also influence other

Plethodontid salamander abundances in edge habitat (Moseley et al. 2009). Since *P. cylindraceus* was a lower elevation species (Petranka 1998) it probably had a greater tolerance for warmer climatic conditions, present on southwestern slopes, than other MRNA salamanders potentially reducing competition and allowing greater occupancy.

Known habitat associations for *P. montanus* were from past studies of *P. jordani* throughout its range and before the species was separated into several geographically distinct species (Highton and Peabody 2000). The species complex preferred cool, mature mesic forests, in addition to second growth forest (Dunn 1917; Grobman 1944; Gordon et al. 1962). The *Plethodon jordani* complex has been shown to be associated with oaks, maples, buckeyes, and spruce dominated forests (Gordon et al. 1962; Walker 1931). However, our models for forest type or tree relative frequencies were not competitive. Elevation ranges for the *P. jordani* complex were from 213 - 1,951 m (Grobman 1944; Hariston 1949), but within the MRNA elevations ranged from 899-1,737 m (Organ 1991), which covered all elevational ranges within the MRNA. Since *P. montanus* was present at almost all transects, individual habitat preferences would have been impossible to distinguish. Therefore, only elevation was positively associated with occupancy, but the model weight was 0.08. Prior models for *P. jordani* also indicated elevation as the main variable for occupancy (Ford et al. 2002).

Plethodon cinereus had been associated with mature forest and closed canopies (Blymer and McGinness 1977; Grover 1998). We found increased occupancy with overstory species richness, but canopy cover was not detected in top models. A negative association with occupancy and increasing percentage of moss ground cover was detected. Moss is typically found growing in moist condition (Crum and Anderson 1981) and *P. cinereus* was absent from wet soils in previous studies (Petranka 1998), which might indicated their avoidance of higher

levels of moss ground cover in our study. We found a strong positive relationship with the relative frequency of cucumber magnolia trees, which was the best predictor of all variables identified, and a negative relationship with the relative frequency of striped maple trees. We classified striped maples as components of cove forest and the avoidance of *P. cinereus* from wet habitat might also explain the negative relationship with striped maples. Cucumber magnolia trees were classified with mixed deciduous forest in our study and mixed deciduous, northern conifer, and mature hardwoods have been shown to be suitable forest types for *P. cinereus* occupancy (Grover 1998; Petranka 1998). *Plethodon cinereus* has also been shown to avoid rocky substrates (Wyman and Hawksley 1987) and presence has been positively associated with herbaceous vegetation (Riedel et al. 2008), but our models within the MRNA did not detect a relationship with either. Other than the relative frequency of cucumber magnolia trees, elevation had the greatest influence on the distribution of *P. cinereus* as it was found to have a quadratic distribution with decreased occupancy at both extreme lower and upper elevations.

Published accounts and anecdotal field observations often described *P. richmondi* from rocky areas (Wallace 1969; Barbour 1971; Green and Pauley 1987; Petranka 1998). Our model indicated a positive association with the number of rock cover objects and occupancy of *P. richmondi*. More than 75% of all *P. richmondi* found within the MRNA were found under rock cover objects. There was a negative association with both yellow birch and American beech trees and occupancy of *P. richmondi*. However, *P. welleri* had a strong positive association with yellow birch and American beech relative frequency. The two species do not co-occur within the MRNA and have only one known co-occurrence across their range overlap (Highton 1971). Either habitat conditions conducive to yellow birch and American beech tree growth or the trees themselves are preferred by these two salamander species. A higher percent ground cover of

moss was negatively associated with occupancy of *P. richmondi*, which could have been due to moister environments required for moss growth and typically *P. richmondi* was found in drier habitats (Netting 1939). Additionally, moss is often found growing on acidic substrates (Crum and Anderson 1981) and laboratory trials suggested that *P. richmondi* preferred less acidic environments (Hamed unpublished data). Thus a negative association with moss could be the result of avoiding acid environments favoring moss growth and not necessary moss itself.

The presence of perennial water was positively associated with *Desmognathus quadramaculatus*, *D. monticola*, *D. organi*, and *D. fuscus* which have all been linked with streams and seeps due to an aquatic portion of their life histories (Brimley 1944; Hairston 1949; Organ 1960 and 1961; Tilley 1980). Therefore, our results were expected as the top model for each species except *D. orestes* contained water. *Desmognathus quadramaculatus*, *D. fuscus*, and *D. monticola* have been observed leaving streams to feed and are thought to need to return to water to prevent desiccation and facilitate gas exchange so they typically must be close to water (Brimley 1944; Camp and Lee 1996). The presence of water was the only variable identified as being associated with *D. quadramaculatus*, which suggested they have a wide range of habitat preferences as long as water was present. In addition to the presence of water, elevation was negatively associated with occupancy for *D. monticola*. Hairston (1949) and Organ (1961) also found *D. monticola* to avoid high elevation streams.

Occupancy for *D. organi* was also influenced by elevation and aspect in addition to the presence of water. *Desmognathus organi* was considered to be a resident of spruce-fir and mesophytic cove forests at high elevations (Weller 1931; Hairston 1949; Organ 1961b, 1990, 1991). Our models confirmed the association with elevation as occupancy increased strongly with elevation, which had the greatest impact on occupancy. Occupancy was also related to

more northeastern aspects. Both elevation and aspect often contributed to cooler and moisture conditions. Aspect has impacted the presence of other small salamanders in the genus *Desmognathus* (Moseley et al. 2009). Additionally, forest parameters were not included in top models, which also suggested that climatic conditions (e.g. temperature and moisture) might have influenced occupancy more than plant communities. Both eggs and adults of *D. organi* have been observed overwintering in seeps (Organ 1961b), which might have accounted for an occupancy increase with water. Just as *P. welleri* was not found to have been reliant on spruce/fir forest, *D. organi* also lacked an association, even though it had previously been associated with spruce/fir.

In addition to the presence of water, a decrease in canopy cover increased occupancy probabilities for *D. fuscus*. Previous habitat associations have indicated a preference for rocky coverage near stream margin (Brimley 1994; Hom 1988), but canopy cover was not associated with *D. fuscus* and our models did not indicate an association with the number of rocks or percent ground cover of rocks. On Whitetop Mt., *D. fuscus* was found at low and high elevations, but was absent at middle elevations (Organ 1991). Lower elevation streams have suffered canopy loss as eastern hemlock trees died due to hemlock adelgid. Higher elevation streams, especially Whitetop Creek, have less canopy cover potentially due to the steep terrain and the loss of trees due to storm damage in the past 15 years. Reduced canopy cover was not indicated for other stream salamanders as they typically occurred on middle elevations where denser canopy cover would have equalized the extreme lower and upper elevations. However, warmer and drier conditions of a more open canopy could have created a competitive advantage for *D. fuscus* or allowed it to choose preferred habitat near streams.

Desmognathus orestes was detected at most elevations along our sampling transects, and was absent from only the lowest elevations. Members of the *D. ochrophaeus* complex, including *D. orestes*, are typically restricted to higher elevations with cooler temperatures and as a member of the complex; *D. orestes* would have been expected in the same conditions (Hairston 1949; Organ 1961; Tilley and Mahoney 1996). Previous habitat surveys for *D. orestes* indicated a close proximity to streams and seeps, except at the highest elevations (Organ 1961; Petranka 1998). However, the presence of water was not the top model, but elevation and aspect best explained occupancy. Both higher elevations and more northern aspects would contribute to cooler temperatures. Presence of *D. ocoee*, another member of the *D. ochrophaeus* complex, also increased with elevation which suggested a preference for cooler temperatures (Tilley and Mahoney 1996; Ford et al. 2002). Since *D. orestes* was found in all forest types within the MRNA an association with particular forest factors would not have been indicated thus leaving elevation as the most descriptive covariate and suggesting a climatic influence.

Eurycea wilderae has been typically found close to water (Petranka 1998) and our results indicated the presence of water was the best explanation of occupancy, which increased strongly with water. Given the aquatic larval period of *E. wilderae*, our findings were expected, but this salamander has also been found greater than 100 m from streams (King 1939; Sever 1999). Occupancy decreased as slope increased, but a correlation between slope and elevation was not indicated, thus the avoidance of steep slopes was not due to increasing elevation. A positive relationship with yellow birch and occupancy of *E. wilderae* was detected. Based on the preference of *P. welleri* for yellow birch habitats, these areas are mostly likely cool and moist. Therefore, *E. wilderae*, a stream salamander, could seek the cooler and moist habitats of yellow birch forest during their terrestrial occupancy.

CONCLUSIONS

Our results identified vegetation and abiotic factors best explaining occupancy probabilities of Plethodontid salamanders within the MRNA. *Plethodon welleri* and *Desmognathus organi* were thought to be strict residents of spruce/fir forests. Our results indicated that occupancy increased with elevation, slope, and relative frequency of yellow birch for *P. welleri*, but red spruce and other high elevation trees species were not selected in top models. Elevation and aspect were strongly related with occupancy for *D. organi* as opposed to vegetation communities. For habitat generalists, *P. montanus* and *D. orestes*, elevation was the main covariate related to occupancy. Occupancy for *Plethodon cinereus*, *P. cylindraceus*, and *P. yonahlossee* was also strongly related to elevation with the greatest occupancy at middle elevations. The presence of water was strongly related to occupancy for most members of the genus *Desmognathus* and *Eurycea wilderae* suggesting that future droughts could greatly impact these salamanders. Our results indicated vegetation factors were related to occupancy for several species. Occupancy for *P. richmondi* decreased and occupancy for *P. welleri* increased with yellow birch relative frequency. Occupancy for *P. cinereus* was strongly related to relative frequency of cucumber magnolia trees. American beech relative frequency was positively related to *P. welleri* occupancy, but was negatively related to occupancy of *P. yonahlossee* and *P. richmondi*. Occupancy for *Eurycea wilderae* and *P. richmondi* was positively associated with sugar maple relative frequency and negatively associated with increasing ground cover of moss, which suggested an avoidance of moisture habitat. Our results also provide insight to salamander community composition in unsurveyed areas of the MRNA. Finally, since abiotic characteristics were the top covariate for 83% of salamander species surveyed, climate might

have a greater influence than forest type as elevation and aspect greatly influence temperature and moisture.

Acknowledgements

This research was completed with funds provided by the Virginia Department of Game and Inland Fisheries through a State Wildlife Grant from the U.S. Fish and Wildlife Service. We thank to J. and D. Organ for providing sampling locations, field notes of 1950s and 1990s sampling, and guidance. A special thanks to B. Parks, G. Poe, and Z. Poulton for field assistance. Additionally, we are grateful to the MRNA staff for project assistance and to many private land owners for access to our study sites. All sampling was approved by the Virginia Department of Game and Inland Fisheries (Scientific Collection Permit #41396) and followed UT Institutional Animal Care and Use Committee protocol #2084-0412.

LITERATURE CITED

- Adams M. J., D. A. W. Miller, E. Muths, P. S. Corn, E. H .C. Grant, L. L. Bailey, G. M . Fellers, R. N. Fisher, W. J. Sadinski, H. Waddle, S. C. Walls. 2013. Trends in Amphibian Occupancy in the United States. PLoS ONE 8(5): e64347.
doi:10.1371/journal.pone.0064347
- Arnold, T. W. 2010. Uninformative parameters and model selection using Akaike's Information Criterion. Journal of Wildlife Management 74:1175-8.
- Bailey, J. R. 1937. Notes on plethodont salamanders of the southeastern United States. Occasional Papers of the Museum of Zoology, Number 364, University of Michigan, Ann Arbor, Michigan.
- Bailey, L. L., J. E. Haynes, J. D. Nichols, D. I. MacKenzie. 2007. Sampling design trade-offs in occupancy studies with imperfect detection: examples and software. Ecological Applications 17:281-90.
- Bailey, L. L., T. R. Simons, K. H. Pollock. 2004. Estimating site occupancy and species detection probability parameters for terrestrial salamanders. Ecological Applications 14:692-702.
- Baldwin, R. A. and L. C. Bender. 2008. Distribution, occupancy, and habitat correlates of American martens (*Martes Americana*) in Rocky Mountain National Park, Colorado. Journal of Mammalogy 89:419-29.
- Barbour, R. W. 1971. *Amphibians and Reptiles of Kentucky*. University of Press of Kentucky, Lexington , Kentucky.

- Beckage, B., B. Osborne, D. G. Gavin, C. Pucko, T. Siccama, and T. Perkins. 2008. A rapid upward shift of a forest ecotone during 40 years of warming in the Green Mountains of Vermont. *Proceedings of National Academy of Science* 105:4197-4202.
- Bishop, S. C. 1943. *A handbook of Salamanders*. Comstock Publishing, Ithac, NewYork.
- Blymyer, M. J. and B. S. McGinnes. 1977. Observations on possible detrimental effects of clearcutting on terrestrial amphibians. *Bulletin of the Maryland Herpetological Society* 13:79-83.
- Brimley, C. S. 1944. *Amphibians and reptiles of North Carolina*. Carolina Tips, Number 7, Elon College, North Carolina.
- Brown J.H. 1984. On the relationship between abundance and distribution of species. *American Naturalist* 124: 255–279.
- Burnham, K. P. and D. R. Anderson. 2002. *Model selection and multi-model inference*. Second edition. Springer-Verlag, New York, NY, USA.
- Camp, C. D. and T. P. Lee. 1996. Intraspecific spacing and interaction within a population of *Desmognathus quadramaculatus*. *Copeia* 1996:78-84.
- Carter, G. M., E. D. Stolen, D. R. Breiningen. 2006. A rapid approach to modeling species-habitat relationships. *Biological Conservation* 127:237-44.
- Crawford, J. A. and R. D. Semlitsch. 2008. Post-disturbance effects of even-aged timber harvest on stream salamanders in southern Appalachian forest. *Animal Conservation* 11:369-76.
- Crum, H.A. and L. E. Anderson. 1981. *Mosses of Eastern North America*. Columbia University Press, West Sussex, England.
- Daubenmire, R. 1959. A canopy-coverage method of vegetation analysis. *NorthWest Science* 33:43-64.

- deMaynadier, P. G. and M. L. Hunter. 1998. Effects of silviculture edges on the distribution and abundance of Amphibians in Maine. *Conservation Biology* 12:340-52.
- Dillard, L. O., K. R. Russell, and W. M. Ford. 2008. Microhabitat models of occurrence for the threatened Cheat Mountain salamander, *Plethodon netting*. *Applied Herpetology* 5:201-24.
- Dillard, L. O., K. R. Russell, and W. M. Ford. 2008b. Site-level habitat models for the endemic, threatened Cheat Mountain salamander (*Plethodon netting*): the importance of geophysical and biotic attributes for predicting occurrence. *Biodiversity and Conservation* 17:1475-92.
- Dodd, C. K., and L. L. Smith. 2003. Habitat destruction and alteration: historical trends and future prospects for amphibians. Pages 94-112 in R. D. Semlitsch, editor. *Amphibian Conservation*. Smithsonian Institution, Washington.
- Dudaniec, R. and J. S. Richardson. 2012. Habitat associations of the coastal giant salamander *Dicamptodon tenebrosus* at its northern range limit. *Herpetological Conservation and Biology* 7:1-15.
- Dunn, E. R. 1926. *Salamanders of the Family Plethodontidae*. Smith College 50th Anniversary Publication. Northhampton, Massachusetts.
- Dunn, E. R. 1917. Reptiles and amphibian collections from the North Carolina mountains, with especial reference to salamanders. *Bulletin of the American Museum of Natural History* 37:593-634.
- Evans, M. 2005. *Ferns of the Smokies*. Great Smoky Mountains Association, Gatlinburg, TN.
- Ford, W. M., M A. Menzel, and R. H. Odom. 2002. Elevation, aspect, and cove size effects on Southern Appalachian Salamanders. *Southeastern Naturalist* 1:315-324.

- Gardner, T. A., J. Barlow, C. A. Peres. 2007. Paradox, presumption and pitfalls in conservation biology: the importance of habitat change for amphibians and reptiles. *Biological Conservation* 138: 166-79.
- Gifford, M. E. and K. H. Kozak. 2012. Islands in the sky or squeezed at the top? Ecological causes of elevational range limits in montane salamanders. *Ecography* 35:195-203.
- Gordon, R. E. and J. A. MacMahon, and D. B. Wake. 1962. Relative abundance, microhabitat and behavior of some Southern Appalachian salamanders. *Zoologica* 47:9-14.
- Green, N. B. and T. K. Pauley. 1987. *Amphibians and Reptiles in West Virginia*. University of Pittsburgh Press, Pittsburgh, Pennsylvania.
- Grobman, A. B. 1944. The distribution of the salamanders of the genus *Plethodon* in eastern United States and Canada. *Annals of the New York Academy of Science* 45:261-316.
- Grover, M. C. 1998. Influence of cover and moisture on abundances of the terrestrial salamanders *Plethodon cinereus* and *Plethodon glutinosus*. *Journal of Herpetology* 32:489-97.
- Gu, W. and R. K. Swihart. 2004. Absent or undetected? Effects of non-detection of species occurrence on wildlife-habitat models. *Biological Conservation* 116:195-203.
- Guttman, S. I., A. A. Karlin, and G. M. Labanick. 1978. A biochemical and morphological analysis of the relationship between *Plethodon longicrus* and *Plethodon yonahlossee* (Amphibia, Urodela, Plethodontidae). *Journal of Herpetology* 12:445-54.
- Hairton, N. G. 1980. The experimental test of analysis of field distributions: competition in terrestrial salamanders. *Ecology* 6:817-26.
- Hariston, N.G. 1949. The local distribution and ecology of the Plethodontid salamanders of the southern Appalachians. *Ecological Monographs* 19:47-73.

- Hamed, M. K., M. J. Gray, and B. Parks. 2012. *Plethodon welleri* – nesting location. Herpetological Review 43:319.
- Haan, S. S., M. J. Desmond, W. R. Gould, and J. P. Ward. 2007. Influence of habitat characteristics on detected site occupancy of the New Mexico endemic Sacramento Mountains salamander, *Aneides hardii*. Journal of Herpetology 41:1-8.
- Harper, C. A. and D. C. Guynn. 1999. Factors affecting salamander density and distribution with four forest types in the Southern Appalachian Mountains. Forest Ecology and Management 14:245-52.
- Harpole, D. N. and C. A. Hass. 1999. Effects of seven silvicultural treatments on terrestrial salamanders. Forest Ecology and Management 114:349-56.
- Herbeck, L. A. and D. Larsen. 1999. Plethodontid salamander response to silviculture practices in Missouri Ozark forest.
- Higgins, K. F., K. J. Jenkins, G. K. Clambey, D. W. Uresk, D. E. Naugle, J. E. Norland, and W. T. Barker. 2005. Vegetation sampling and measurements. Pp. 524-53, In C.E. Braun (Ed.). *Techniques for Wildlife Investigation and Management*. The Wildlife Society, Bethesda, MD. 974 pp.
- Highton, R. 1971. Distributional interactions among eastern North American salamanders of the genus *Plethodon*. In The Distributional History of the Biota of the Southern Appalachians, ed. P. C. Holt, 138-88. Research Division Monographs 4, Virginia polytechnic Institute and State University, Blacksburg, VA.
- Highton, R. and R. B. Peabody. 2000. Geographic protein variation and speciation in salamanders of the *Plethodon jordani* and *Plethodon glutinosus* complexes in the southern Appalachian Mountains with descriptions of four new species. In. Bruce, R. C.,

- R. G. Jaeger and L. Houck (Eds.), *The Biology of Plethodontid Salamanders*. Kluwer Academic/Plenum Publishers, New York.
- Hines, J. E. 2012. PRESENCE5.5-Software to estimate patch occupancy and related parameters. USGS-PWRC, Patuxent Wildlife Research Center, Laurel, Maryland, USA.
<http://www.mbr-pwrc.usgs.gov/software/presence/html>
- Hoffman, R. L. 1992. The range of *Plethodon yonahlossee* in Virginia: defined at last? *Catesbeiana* 12:3-8.
- Hoffman, R. L. and H. I. Kleinpeter. 1948. A collection of salamanders from Mount Rogers, Virginia. *Journal of the Washington Academy of Sciences* 38:106-8.
- Hoffman, R. L. and L. Hulbricht. 1954. Distributional records of two species of *Plethodon* in the southern Appalachians. *Herpetologica* 10:191-3.
- Hom, C. L. 1988. Cover object choice by female dusky salamanders, *Desmognathus fuscus*. *Journal of Herpetology* 22:247-49.
- Homyack, J. A. and C. A. Hass. 2009. Long-term effects of experimental forest harvesting on abundance and reproductive demography of terrestrial salamanders. *Biological Conservation* 142:110-21.
- Horn, D. and T. Cathcart. 2005. *Wildflowers of Tennessee, the Ohio Valley, and the Southern Appalachians*. Lone Pine Publishing, Auburn, WA.
- IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.
- IUCN, Conservation International, and NatureServe. 2008. An Analysis of Amphibians on the 2008 IUCN Red List www.iucnredlist.org/amphibians. Downloaded on April 5, 2010.

- Keyser, P. D., L. A. Williams, K. M. Kelly, C. A. Hass, V. L. Ford, and T. B. Wigley. 2011. An assessment of distribution patterns of terrestrial salamanders in the Central Appalachians using two landscape models. *American Midland Naturalist* 166:194-210.
- King, W. 1939. A survey of the herpetology of the Great Smoky Mountains National Park (Tennessee). *American Midland Naturalist* 21:531-82.
- Larson, M. A., Dijak, W. D., F. R. Thompson, J. J. Millspaugh. 2003. Landscape-level habitat suitability models for twelve wildlife species in Southern Missouri. North Central Research Station, U.S. Forest Service, Department of Agriculture 58p. St. Paul, MN.
- Luiselli, L. 2006. Site occupancy and density of sympatric Gabon viper (*Bitis gabonica*) and nose-horned viper (*Bitis nasicomis*). *Journal of Tropical Ecology* 22:555-64.
- Long, R. A., T. M. Donovan, P. MacKay, W. J. Zielinski, J. S. Buzas. 2011. Predicting carnivore occupancy with noninvasive surveys and occupancy modeling. *Landscape Ecology* 26:327-40.
- MacKenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle, and C. A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83:2248-55.
- MacKenzie, D. I., J. D. Nichols, J. A. Royle, K. P. Pollock, L. L. Bailey, and J. E. Hines. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press, San Diego, CA, USA.
- Martin, J. S. Chamaille-Jammes, J. D. Nichols, H. Fritz, J. E. Hines, C. J. Fonnesebeck, D. I. MacKenzie, and L. Bailey. 2010. Simultaneous modeling of habitat suitability, occupancy, and relative abundance: African elephants in Zimbabwe. *Ecological Applications* 20:1173-82.

- Martof, B. S., W. M. Palmer, J. R. Bailey, J. R. Harrison. 1980. *Amphibians and reptiles of the Carolinas and Virginia*. University of North Carolina Press, Chapel Hill, North Carolina.
- Mazerolle, M. J., A. Desrochers, and L. Rochefort. 2005. Landscape characteristics influence pond occupancy by frogs after accounting for detectability. *Ecological Applications* 15:824-34.
- Mitchell, J. C. and K. K. Reay. 1999. Atlas of amphibians and reptiles in Virginia. Special Publication Number 1, Wildlife Diversity Division, Virginia Department of Game and Inland Fisheries, Richmond, Virginia.
- Moseley, K. R., W. M. Ford, and J. W. Edwards. 2009. Local and landscape factors influencing edge effects on woodland salamanders. *Environmental Monitoring Assessment* 151:425-35.
- Netting, M. G. 1939. The ravine salamander, *Plethodon richmondi* Netting and Mittleman, in Pennsylvania. *Proceedings of the Pennsylvania Academy of Science* 13:50-1.
- Nudds, T. D. 1977. Quantifying the vegetative structure of wildlife cover. *Wildlife Society Bulletin* 5:113-7.
- Organ, J. A. 1991. Salamander survey of the Mount Rogers Nation Recreation Area section two. Marion, VA: United States Department of Agriculture. 210p. Available from Mount Rogers National Recreation Area, Marion, VA.
- Organ, J. A. 1990. Salamander survey of the Mount Rogers Nation Recreation Area section one. Marion, VA: United States Department of Agriculture. 98p. Available from Mount Rogers National Recreation Area, Marion, VA.
- Organ, J. A. 1961. Studies of the local distribution, life history, and population dynamics of the salamander genus *Desmognathus* in Virginia. *Ecological Monographs* 31:189-220.

- Organ, J. A. 1961b. Life history of the pigmy salamander, *Desmognathus wrighti* in Virginia. American Midland Naturalist 66:384-90.
- Organ, J. A. 1960. Studies on the life history of the salamander *Plethodon welleri*. Copeia 1960:287-97.
- Patrick, D. A., M. L. Hunter, and A. J. K. Calhoun. 2006. Effects of experimental forestry treatments on a Maine amphibian community. Forest Ecology and Management 234:323-32.
- Perinchery, A. D. Jathanna, A. Kumar. 2011. Factors determining occupancy and habitat use by Asian small-clawed otters in the Western Ghats, India. Journal of Mammalogy 92:796-802.
- Peterman, W. E. and R. D. Semlitsch. 2013. Fine-scale habitat associations of a terrestrial salamander: the role of environmental gradients and implications for population dynamics. PLoS ONE 8(5): e62184.
- Petranka, J. W. 1998. *Salamanders of the United States and Canada*. Washington, DC: Smithsonian Institution Press.
- Petranka, J. W., M. P. Brannon, M. E. Hopey, and C. K. Smith. 1994. Effects of timber harvesting on low elevation populations of southern Appalachian salamanders. Forest Ecology and Management 67:135-47.
- Petranka, J. W., M. E. Eldridge, and K. E. Haley. 1993. Effects of timber harvesting on Southern Appalachian salamanders. Conservation Biology 7:363-70.
- Pope, C. H. 1950. A statistical and ecological study of the salamander, *Plethodon yonahlossee*. Bulletin of the Chicago Academy of Science 9:79-106.
- Radford, A. E., H. E. Ahles, and C. R. Bell. 1968. Manual of the Vascular Flora of the

- Carolina. University of North Carolina Press, Chapel Hill, NC, USA.
- Rheinhardt, R. D. and S. A. Ware. 1984. The vegetation of the Balsam Mountains of southwest Virginia: a phytosociological study. *Bulletin of the Torrey Botanical Club* 111:287-300.
- Riedel, B. L., K. R. Russell, W. M. Ford, K. P. O'Neill, H. W. Godwin. 2008. Habitat relationship of eastern red-backed salamanders (*Plethodon cinereus*) in Appalachian agroforestry and grazing systems. *Agriculture, Ecosystems and Environment* 12:229-36
- Semlitsch, R. D. 2003. *Amphibian conservation*, ed. Washington, DC: Smithsonian Books.
- Sever, D. M. 1999. *Eurycea wilderae*. Pp. 685.1 – 685.4. *Catalogue of American Amphibians and Reptiles*. Society for the Study of Amphibians and Reptiles, St. Louis, Missouri.
- Steen, D. A., C. J. W. McClue, J. C. Brock, D. C. Rudolph, J. B. Pierce, J. R. Lee, J. Humphries, B. B Gregory, W. B. Sutton, L. L. Smith, D. L. Baxley, D. J. Stevenson, and C. Guyer. 2012. Landscape-level influences of terrestrial snake occupancy within the southeastern United States. *Ecological Applications* 22:1084-97.
- Suzuki, N., D. H. Olson, and E. C. Reilly. 2008. Developing landscape habitat models for rare amphibians with small geographic ranges: a case study of Siskiyou Mountains salamanders in the western USA. *Biodiversity Conservation* 17:2197-2218.
- Thurow, G. R. 1963. Taxonomic and ecology notes on the salamander, *Plethodon welleri*. *University of Kansas Science Bulletin* 44:87-108.
- Tilley, S. G. 1980. Life histories and comparative demography of two salamander populations. *Copeia* 1980:806-21.

- Tilley, S. G. and M. J. Mahoney 1996. Patterns of genetic differentiation in salamanders of the *Desmognathus orchrophaeus* complex (Amphibia: Plethodontidae). *Herpetological Monographs* 10:1-42.
- Van Horne, B. and J. A. Wiens. 1991. Forest Bird habitat suitability models and the development of general habitat models. U. S. Fish and Wildlife Service. *Fish and Wildlife Research* 8.
- Virginia Department of Game and Inland Fisheries. 2005. Virginia's comprehensive wildlife conservation strategy. Virginia Department of Game and Inland Fisheries, Richmond, Virginia.
- Wake D.B., and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world amphibians. *Proceedings of the National Academy of Sciences* 105:11466-11473.
- Walker, C. F. 1934. *Plethodon welleri* at Whitetop Mountain, Virginia. *Copeia* 1934:190.
- Walker, C. F. 1931. Description of a new salamander from North Carolina. *Proceedings of the Junior Society of Natural Sciences* 2:48-51.
- Wallace, J. T. 1969. A study of *Plethodon richmondi* from Mason County, Kentucky, with notes on its distribution within the state. *Transactions of the Kentucky Academy of Science* 30:38-44.
- Weller, W. H. 1931. A preliminary list of the salamanders of the Great Smoky Mts. of North Carolina and Tennessee. *Proceedings of the Junior Society of Natural Sciences of Cincinnati* 2:21-32.
- Wells, K. D. 2007. *The Ecology and Behavior of Amphibians*. Chicago, IL: The University of Chicago Press.

- Wells, P. H. and R. A. Wells. 1976. Patterns and movement in a population of slimy salamander, *Plethodon glutinosus*, with observations on aggressions. *Herpetologica* 32:156-62.
- Welsh, H. H., J. R. Dunk, W. J. Zielinski. 2006. Developing and applying habitat models using forest inventory data: an example using a terrestrial salamander. *The Journal of Wildlife Management* 70:671-81.
- Wofford, E. B. and E. W. Chester. 2002. *Guide to trees, shrubs, and woody vines of Tennessee*. University of Tennessee Press, Knoxville, TN.
- Wyman, R. L. and D. S. Hawksley-Lescault. 1987. Soil acidity affects distribution, behavior, and physiology of the salamander *Plethodon cinereus*. *Ecology* 68:1819-27.
- Young, B. E., S. N. Stuart, J. S. Chanson, N. A. Cox, and T. M. Boucher. 2004. *Disappearing Jewels: The Status of New World Amphibians*. Nature Serve, Arlington, Virginia.

APPENDIX

Table 4.1. Model Terms and published accounts of habitat associations justifying their use in occupancy models for Plethodontid salamanders residing within the Mount Rogers National Recreation Area.

Model Term	Published Accounts
Elevation	Hairston 1949; Organ 1961, 1990, 1991; Ford et al. 2002; Dillard et al. 2008;
Aspect	Hairston 1949; Organ 1961, 1990, 1991; Harper & Guynn 1999; Dillard et al. 2008b; Moseley et al. 2009
Slope	Pope 1950; Wallace 1969; Barbour 1971; Green and Pauley 1987; Petranka 1998; Dillard et al. 2008b
Water	Brimley 1944; Hairston 1949; Organ 1961; Tilley 1980; Petranka 1998; Keyser et al. 2004
Canopy Cover	Hoffman & Hubrich 1954; Blymer & McGinness 1977; Herbeck & Larsen 1999; Crawford & Semlitsch 2008
Basal Area	Harpole & Hass 1999; Homyack & Hass 2009
Vertical Vegetation	Patrick et al. 2006
Ground Cover	
Bare Soil	deMaynedier & Hunter 1998
Leaf Litter	deMaynedier & Hunter 1998; Herbeck & Larsen 1999; Crawford & Semlitsch 2008
Ferns	Martof et al. 1980; Herbeck and Larsen 1999
Rock	Pope 1950; Barbour 1971; Martof et al. 1980; Wyman & Hawksley 1987; Hom 1988;
Moss	Netting 1939; Organ 1960, 1961, 1961b; Martof et al. 1980
Herbaceous	Riedel et al. 2008
Woody Debris	Dunn 1926; Hoffman & Kleinpeter 1948; Pope 1950; Gordon et al. 1962; Petranka et al. 1994; Grover 1998
Red Spruce	Walker 1934; Bishop 1943; Hoffman & Kleinpeter 1948; Hoffman 1953; Organ 1960; Thurow 1963; Dillard et al. 2008
Cove Forest	Hairston 1949; Gordon et al. 1962; Dillard et al. 2008b
Northern Hardwood	Petranka 1998; Harper & Guynn 1999
Mixed deciduous	Pope 1950; Gordon et al. 1962; Wells & Wells 1976; Martof et al. 1980; Petranka 1998

Table 4.2. Covariate descriptions used to model occupancy probabilities of Plethodontid salamanders within the Mount Rogers National Recreation Area (2008-11) and units of measurement.

Variable	Unit of Measurement
Elevation	Elevation in m
Aspect	Mean aspect in °
Slope	Mean slope in °
Basal Area (BA)	Total basal area m ² /400m ²
Canopy Cover (CC)	Mean % canopy cover
Strata 1	Mean % vertical density from 0 to 1 m
Strata 2	Mean % vertical density from 1 to 2 m
% fern ground cover	Mean % ferns in 5 1-m ² plots
% moss ground cover	Mean % mosses in 5 1-m ² plots
% bare ground cover	Mean % bare ground in 5 1-m ² plots
% organic ground cover	Mean % organic cover (leaf litter) in 5 1-m ² plots
% rock ground cover	Mean % rock cover in 5 1-m ² plots
% herbaceous ground cover	Mean % herbaceous vegetation cover in 5 1-m ² plots
# of rock cover objects	Total # of rocks > 161 cm ²
# of log cover objects	Total # of downed logs > 11.4 cm in diameter
Understory species richness	Total # of woody species < 1.4 m tall per 40 m ²
Midstory species richness	Total # of woody sps. > 1.4 m tall & < 11.4 dbh per 100 m ²
Overstory species richness	Total # of woody sps. > 11.4 cm dbh per 400 m ²
Midstory type (categorical)	Majority of midstory veg. within 100 m ² (rhododendron /mountain laurel, red spruce, American beech, deciduous, or mixed)
Presence of water	Presence of perennial water
Red Spruce relative frequency (RF)	Relative frequency of red spruce > 11.4 cm dbh / 400 m ²
Mt. Maple RF	Relative frequency of mt. maple > 11.4 cm dbh / 400 m ²
Mt. Ash RF	Relative frequency of mt. Ash > 11.4 cm dbh / 400 m ²
Yellow Birch RF	Relative frequency of yel. birch > 11.4 cm dbh / 400 m ²
Sugar Maple RF	Relative frequency of sugar map. > 11.4 cm dbh / 400 m ²
American Beech RF	Relative frequency of Am. beech > 11.4 cm dbh / 400 m ²
Yellow Buckeye RF	Relative frequency of yw. buckeye > 11.4 cm dbh / 400 m ²
Striped Maple RF	Relative frequency of striped map. > 11.4 cm dbh / 400 m ²
Eastern Hemlock RF	Relative frequency of hemlock > 11.4 cm dbh / 400 m ²
Red Oak RF	Relative frequency of red oak > 11.4 cm dbh / 400 m ²
Red Hickory RF	Relative frequency of red hickory > 11.4 cm dbh / 400 m ²
Cucumber Magnolia RF	Relative frequency of cucum. mag. > 11.4 cm dbh / 400 m ²
Chestnut Oak RF	Relative frequency of chestnut oak > 11.4 cm dbh / 400 m ²
White Pine RF	Relative frequency of white pine > 11.4 cm dbh / 400 m ²

Table 4.3. Covariates and covariate combinations used to model Plethodontid salamander occupancy probabilities within the Mount Rogers National Recreation Area, Virginia (2008-2011).

Model covariate(s) and combinations

Null
Global
Elevation
Elevation, Elevation ²
Aspect
Slope
Basal Area (BA)
Canopy Cover (CC)
Strata 1 (Vertical Structure 0-1 meters)
Strata 2 (Vertical Structure 1-2 meters)
% fern ground cover
% moss ground cover
% bare ground cover
% organic ground cover
% rock ground cover
% herbaceous ground cover
of rock cover objects
of log cover objects
Understory species richness
Midstory species richness
Overstory species richness
Midstory type (categorical)
Presence of water
Red Spruce relative frequency (RF)
Mt. Maple RF
Mt. Ash RF
Yellow Birch RF
Sugar Maple RF
American Beech RF
Yellow Buckeye RF
Striped Maple RF
Eastern Hemlock RF
Red Oak RF
Red Hickory RF
Cucumber Magnolia RF
Chestnut Oak RF
White Pine RF

Table 4.3. Continued.

Model covariate(s) and combinations

Cover = (Rocks, Logs, % rock ground cover)
Landform = (elevation, aspect, slope, presence water)
Landform2 = (elevation, elevation², aspect, slope, presence water)
Ground vegetation (veg) = (% fern ground cover (GC), % moss GC, % herbs GC, strata 1)
Mistory = (mid story richness, mid story type, strata 2)
Overstory = (CC, BA, Overstory Richness)
Spruce/Fir habitat = (Red Spruce RF, Mt. Maple RF, Mt. Ash RF)
Northern Hardwood = (Yellow Birch RF, Sugar Maple RF, Am. Beech RF)
Cove Forest = (Am. Beech RF, Yellow Buckeye RF, Striped Maple RF, E. Hemlock RF)
Mixed = (Red Oak RF, Red Hickory RF, Cucumber Magnolia RF, Chestnut Oak RF,
White Pine RF)
Elevation, Aspect
Elevation, Elevation², Aspect
Elevation, BA
Elevation, Elevation², BA
Elevation, CC
Elevation, Elevation², CC
Elevation, Slope
Elevation, Elevation², Slope
Aspect, BA
Aspect, CC
Slope, BA
Slope, CC
BA, CC
Elevation, Ground veg.
Elevation, Cover
Elevation, Midstory
Elevation, Overstory
Elevation, Spruce/Fir
Elevation, Northern Hardwood
Elevation, Cove Hardwood
Elevation, Mixed
Elevation, Elevation², Ground veg.
Elevation, Elevation², Cover
Elevation, Elevation², Midstory
Elevation, Elevation², Overstory
Elevation, Elevation², Spruce/Fir
Elevation, Elevation², Northern Hardwood
Elevation, Elevation², Cove Hardwood

Table 4.3. Continued.

Model covariate(s) and combinations

Elevation, Elevation², Mixed
 Landform, Spruce/Fir
 Landform, Northern Hardwood
 Landform, Cove Hardwood
 Landform, Mixed
 Landform2, Spruce/Fir
 Landform2, Northern Hardwood
 Landform2, Cove Hardwood
 Landform2, Mixed
 Landform, Cover
 Landform, Cover
 Landform, Cover
 Landform, Cover
 Landform2, Cover
 Landform2, Cover
 Landform2, Cover
 Landform2, Cover
 Ground veg., Spruce/Fir
 Ground veg., Northern Hardwood
 Ground veg., Cove Hardwood
 Ground veg., Mixed
 Landform, Ground veg., Spruce/Fir
 Landform, Ground veg., Northern Hardwood
 Landform, Ground veg., Cove Hardwood
 Landform, Ground veg., Mixed
 Landform2, Ground veg., Spruce/Fir
 Landform2, Ground veg., Northern Hardwood
 Landform2, Ground veg., Cove Hardwood
 Landform2, Ground veg., Mixed
 Cover, Ground veg., Spruce/Fir
 Cover, Ground veg., Northern Hardwood
 Cover, Ground veg., Cove Hardwood
 Cover, Ground veg., Mixed
 Landform, Cover, Ground veg., Spruce/Fir
 Landform, Cover, Ground veg., Northern Hardwood
 Landform, Cover, Ground veg., Cove Hardwood
 Landform, Cover, Ground veg., Mixed
 Landform2, Cover, Ground veg., Spruce/Fir
 Landform2, Cover, Ground veg., Northern Hardwood
 Landform2, Cover, Ground veg., Cove Hardwood
 Landform2, Cover, Ground veg., Mixed

Table 4.4. Highest supported ($\Delta\text{QAIC}_c < 2$) models explaining Plethodontid salamanders occupancy within the Mount Rogers National Recreation Area, Virginia (2008-11).

Species and Models	QAIC _c	ΔQAIC_c	w_i
<i>Desmognathus fuscus</i>			
Ψ (Canopy cover)	169.87	0.00	0.27
Ψ (Slope + Canopy cover)	171.46	1.59	0.12
Ψ (Aspect + Canopy cover)	171.72	1.85	0.11
Ψ (Water)	171.88	2.00	0.10
<i>Desmognathus monticola</i>			
Ψ (Landform)	173.96	0.00	0.62
<i>Desmognathus orestes</i>			
Ψ (Elevation)	27.08	0.00	0.13
Ψ (Elevation + Aspect)	27.40	0.32	0.11
<i>Desmognathus organi</i>			
Ψ (Landform)	158.39	0.00	0.52
<i>Desmognathus quadramaculatus</i>			
Ψ (Water)	65.98	0.00	0.80
<i>Eurycea wilderae</i>			
Ψ (Landform)	173.43	0.00	0.14
Ψ (Water)	173.74	0.31	0.12
Ψ (Landform + N. Hardwood)	174.31	0.88	0.09
Ψ (Slope)	174.58	1.15	0.08
Ψ (Slope + Basal Area)	175.31	1.88	0.05
<i>Plethodon cinereus</i>			
Ψ (Striped Maple RF)	154.39	0.00	0.11
Ψ (Cucumber Magnolia RF)	155.46	1.07	0.07
Ψ (Elevation + Elevation ²)	155.60	1.21	0.06
Ψ (Strata 0-1)	155.71	1.32	0.06
Ψ (% Moss Ground Cover)	155.88	1.49	0.06
Ψ (Overstory Species Richness)	155.94	1.55	0.05
<i>Plethodon cylindraceus</i>			
Ψ (Elevation + Elevation ²)	96.88	0.00	0.26
Ψ (Elevation + Aspect)	97.20	0.32	0.22
Ψ (Elevation)	97.28	0.40	0.21

Table 4.4. Continued.

Species and Models	QAIC _c	ΔQAIC _c	w _i
<i>Plethodon montanus</i>			
Ψ (Elevation)	15.73	0.00	0.08
Ψ (.)	16.46	0.73	0.06
Ψ (Slope)	17.29	1.56	0.04
Ψ (# of Rock cover objects)	17.34	1.61	0.04
Ψ (% Bare Ground)	17.60	1.87	0.04
<i>Plethodon richmondi</i>			
Ψ (Cover + Ground Vg. + N. hardwood)	203.65	0.00	0.46
Ψ (N. Hardwood)	204.64	0.99	0.28
<i>Plethodon welleri</i>			
Ψ (Landform)	40.42	0.00	0.40
Ψ (Landform + N. Hardwood)	41.70	1.28	0.21
Ψ (Landform + Cove Hardwood)	41.90	1.48	0.19
<i>Plethodon yonahlossee</i>			
Ψ (Cove Hardwood)	410.72	0.00	0.58
Ψ (Elevation + Elevation ²)	411.60	0.88	0.37

Table 4.5. Model-averaged parameter estimates, standard errors, and 85% CIs for vegetation and abiotic variables explaining occupancy of Plethodontid salamanders within the Mount Rogers National Creation Area, VA from top QAIC_c candidate models (2008-11)

Species	Variable	$\beta \pm SE$	85% CI
<i>D. fuscus</i>	Canopy Cover	-2.11 ± 0.75	-3.19 to -1.03
	Slope	-0.25 ± 0.30	- 0.68 to 0.18
	Water	1.77 ± 0.50	1.27 to 2.49
<i>D. monticola</i>	Elevation	-1.08 ± 0.48	-1.78 to -0.38
	Slope	-0.32 ± 0.42	-0.93 to 0.30
	Aspect	0.33 ± 0.34	-0.16 to 0.82
	Water	4.09 ± 0.75	3.01 to 5.17
<i>D. orestes</i>	Elevation	3.73 ± 1.72	1.25 to 6.21
	Aspect	-1.56 ± 0.82	-2.74 to -0.38
<i>D. organi</i>	Elevation	4.59 ± 1.09	3.02 to 6.17
	Aspect	-1.07 ± 0.41	-1.66 to -0.48
	Slope	-0.07 ± 0.53	-1.43 to 0.09
	Water	3.71 ± 1.26	1.89 to 5.52
<i>D. quadramaculatus</i>	Water	7.53 ± 1.55	5.29 to 9.76
<i>E. wilderae</i>	Elevation	-0.20 ± 0.37	-0.73 to 0.33
	Slope	-0.95 ± 0.37	-1.48 to -0.42
	Water	1.87 ± 0.67	0.91 to 2.83
	Sugar Maple RF	0.46 ± 0.32	0.01 to 0.92
	Yellow birch RF	1.15 ± 0.43	0.54 to 1.76
	Am. Beech RF	0.13 ± 0.39	-0.42 to 0.70
<i>P. cinereus</i>	Striped Maple RF	-0.68 ± 0.22	-1.00 to -0.36
	Cucumber Mag. RF	43.30 ± 7.68	32.24 to 54.36
	Elevation	8.02 ± 2.29	4.72 to 11.32
	Elevation ²	-8.13 ± 2.19	-11.29 to -5.93
	Strata 0-1	-0.59 ± 0.20	-0.89 to -0.30
	% Moss Cover	-0.59 ± 0.22	-0.91 to -0.27
	Overstory Richness	0.63 ± 0.24	0.29 to 0.97
<i>P. cylindraceus</i>	Elevation	-6.81 ± 3.37	-11.67 to -1.96
	Elevation ²	-25.18 ± 1.48	-27.31 to -23.05
	Aspect	1.65 ± 1.01	0.19 to 3.10

Table 4.5. Continued.

Species	Variable	$\beta \pm SE$	85% CI
<i>P. montanus</i>	Elevation	12.70 ± 4.01	6.93 to 18.47
	Slope	1.81 ± 0.87	0.56 to 3.06
	# Rock cover objects	8.25 ± 7.23	-2.16 to 18.66
	% Bare Cover	-0.64 ± 0.24	-0.99 to -0.29
<i>P. richmondi</i>	Yellow Birch RF	-1.43 ± 0.57	-2.25 to -0.61
	Sugar Maple RF	0.56 ± 0.25	0.20 to 0.92
	Am. Beech RF	-0.69 ± 0.32	-1.15 to -0.23
	# Rocks	0.86 ± 0.36	0.34 to 1.38
	# Logs	-0.06 ± 0.25	-0.42 to 0.03
	% Moss	-1.48 ± 0.60	-2.34 to -0.62
	% Fern	-0.14 ± 0.28	-0.54 to 0.26
	% Herbaceous	0.08 ± 0.36	-0.44 to 0.60
<i>P. welleri</i>	Strata 0-1	-0.46 ± 0.35	-0.96 to 0.04
	Elevation	7.95 ± 2.41	4.48 to 11.42
	Slope	3.74 ± 1.24	1.95 to 5.53
	Water	-3.15 ± 1.69	-5.58 to -0.72
	Am. Beech RF	1.33 ± 0.71	0.31 to 2.35
	Yellow Birch RF	1.18 ± 0.69	0.19 to 2.17
	Sugar Maple RF	0.30 ± 0.71	-0.72 to 1.32
	Yellow Buckeye RF	-1.56 ± 0.99	-2.99 to -0.13
<i>P. yonahlossee</i>	Striped Maple RF	0.01 ± 0.70	-1.00 to 1.02
	Am. Beech RF	-0.51 ± 0.18	-0.77 to -0.25
	Yellow Buckeye RF	-0.56 ± 0.17	-0.80 to -0.32
	Striped Maple RF	-0.72 ± 0.19	-0.99 to -0.45
	Carolina Hemlock RF	-0.52 ± 0.16	-0.75 to -0.29
	Elevation	17.19 ± 1.80	14.60 to 19.78
	Elevation ²	-16.97 ± 1.76	-18.73 to -15.21

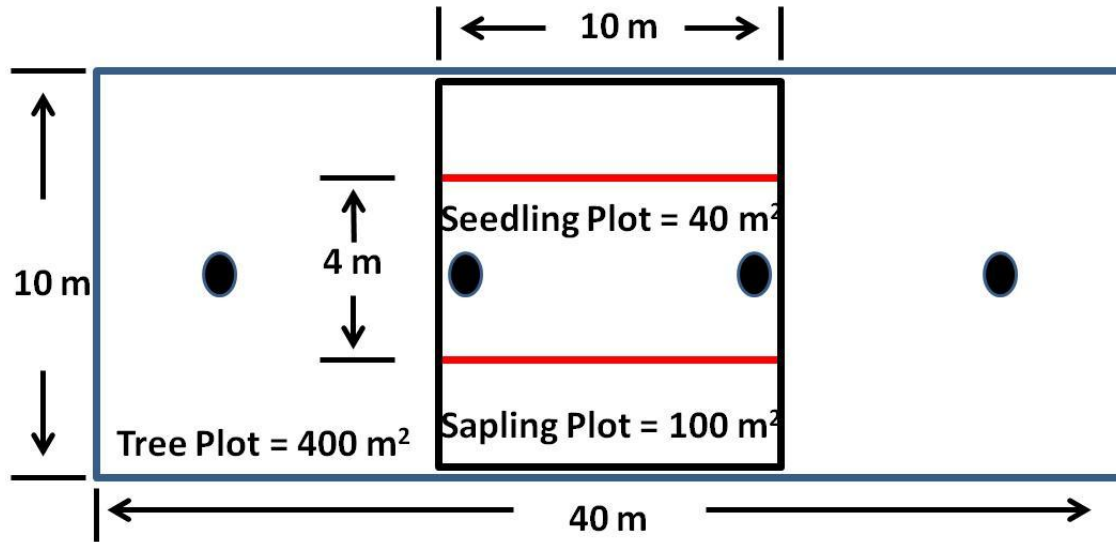


Figure 4.1. Sampling plots for measuring vegetation characteristics for Plethodontid salamanders from the Mount Rogers National Recreation Area, Virginia (2011). Overstory trees were quantified and diameter-breast-height was measured within a 400 m² plot. Saplings were quantified within a 100 m² plot and seedlings were quantified within a 40 m² plot. Circles indicate locations of ground cover and vertical structure measurements. Diagram is not to scale.

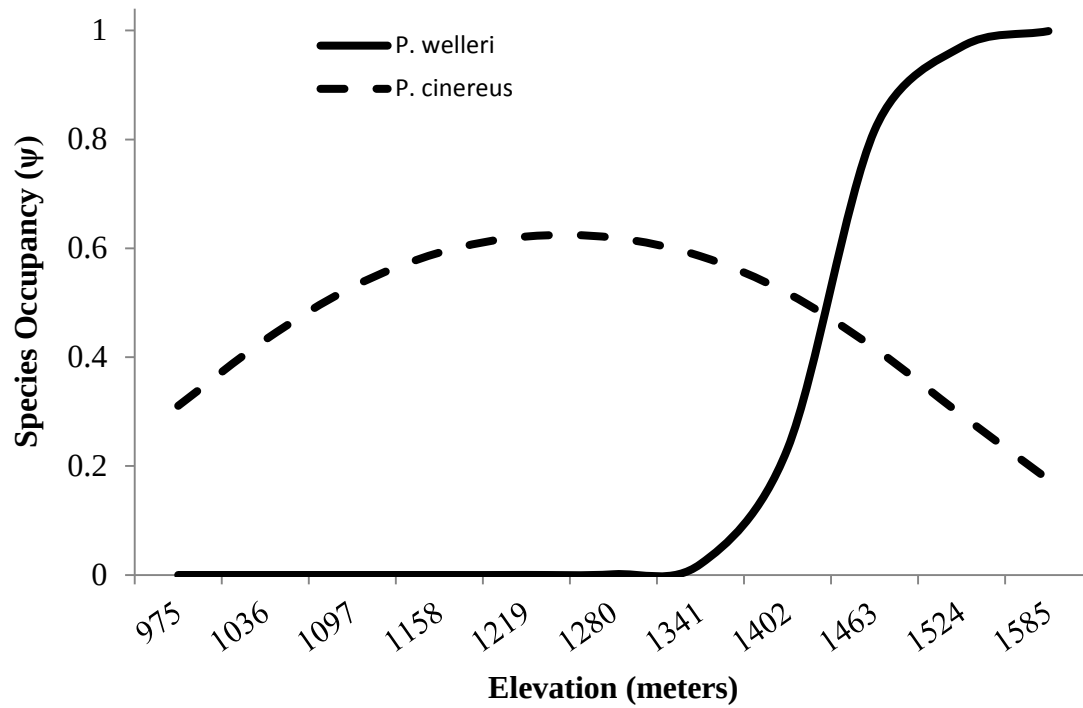


Figure 4.2. Occupancy probabilities of small bodied salamanders of the genus *Plethodon* in relation to elevation from the Mount Rogers National Recreation Area, Virginia (2008-2011).

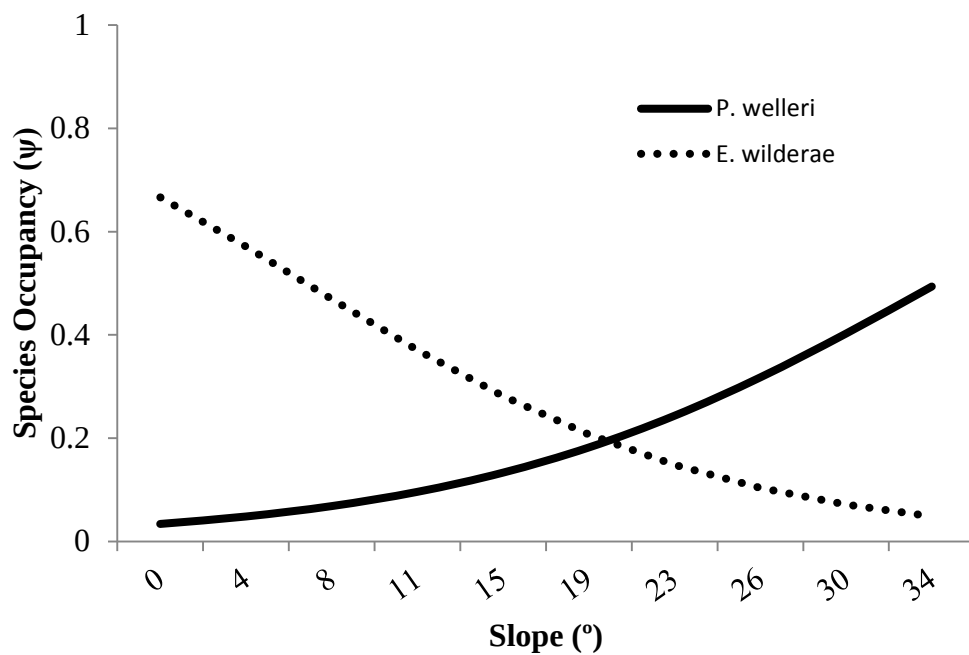


Figure 4.3. Occupancy probabilities of *Plethodon welleri* and *Eurycea wilderae* in relation to slope from the Mount Rogers National Recreation Area, Virginia (2008-2011).

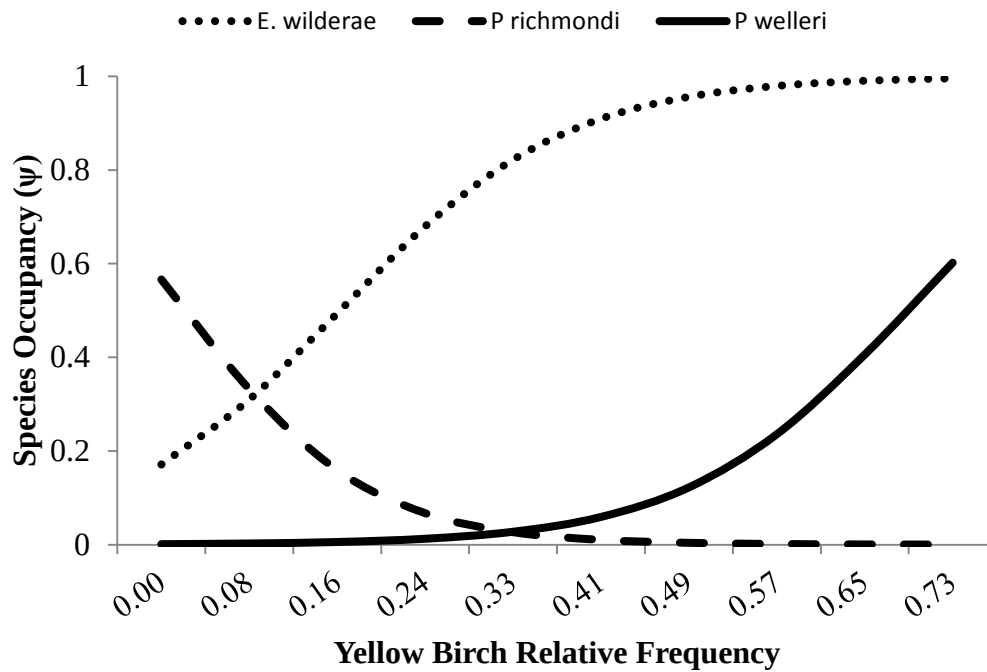


Figure 4.4. Occupancy probabilities of *Eurycea wilderae*, *Plethodon richmondi* and *P. welleri* in relation to yellow birch (> 11.43 cm DBH) relative frequency within 400 m² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).

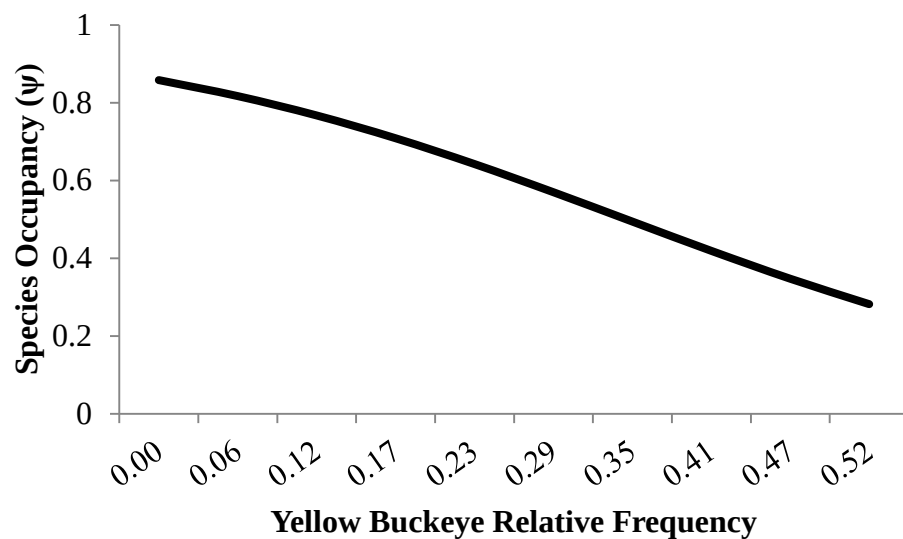


Figure 4.5. Occupancy probabilities of *Plethodon yonahlossee* in relation to yellow buckeye (> 11.43 cm DBH) relative frequency within 400 m² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).

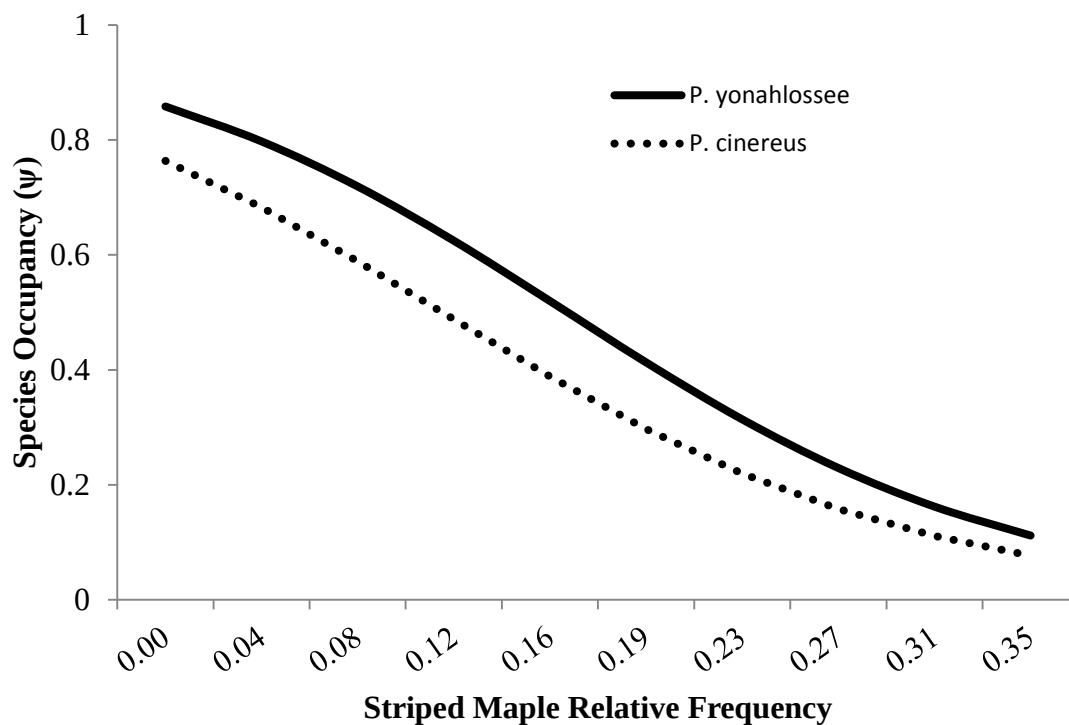


Figure 4.6. Occupancy probabilities of *Plethodon yonahlossee* and *P. cinereus* in relation to striped maple (> 11.43 cm DBH) relative frequency within 400 m² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).

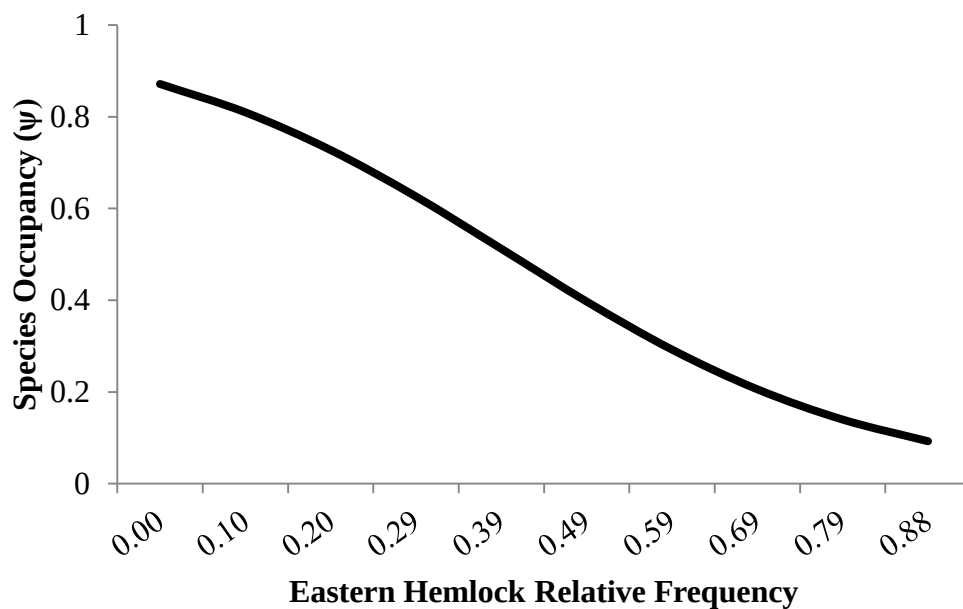


Figure 4.7. Occupancy probabilities of *Plethodon yonahlossee* in relation to eastern hemlock (> 11.43 cm DBH) relative frequency within 400 m² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).

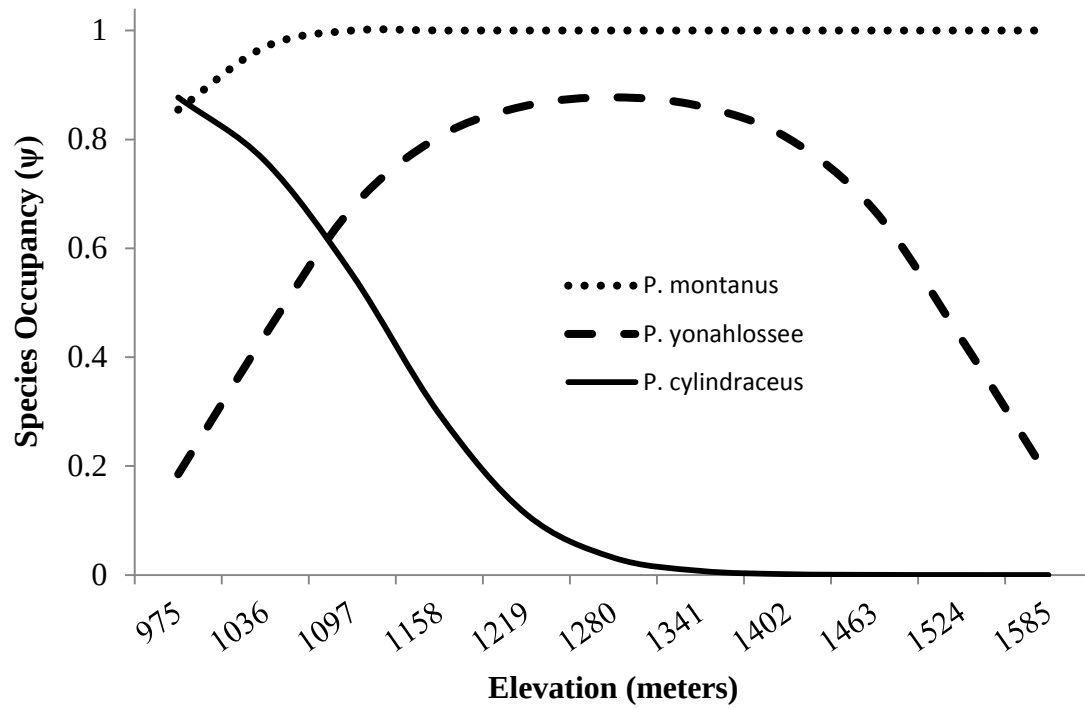


Figure 4.8. Occupancy probabilities of large bodied salamanders of the genus *Plethodon* in relation to elevation from the Mount Rogers National Recreation Area, Virginia (2008-2011).

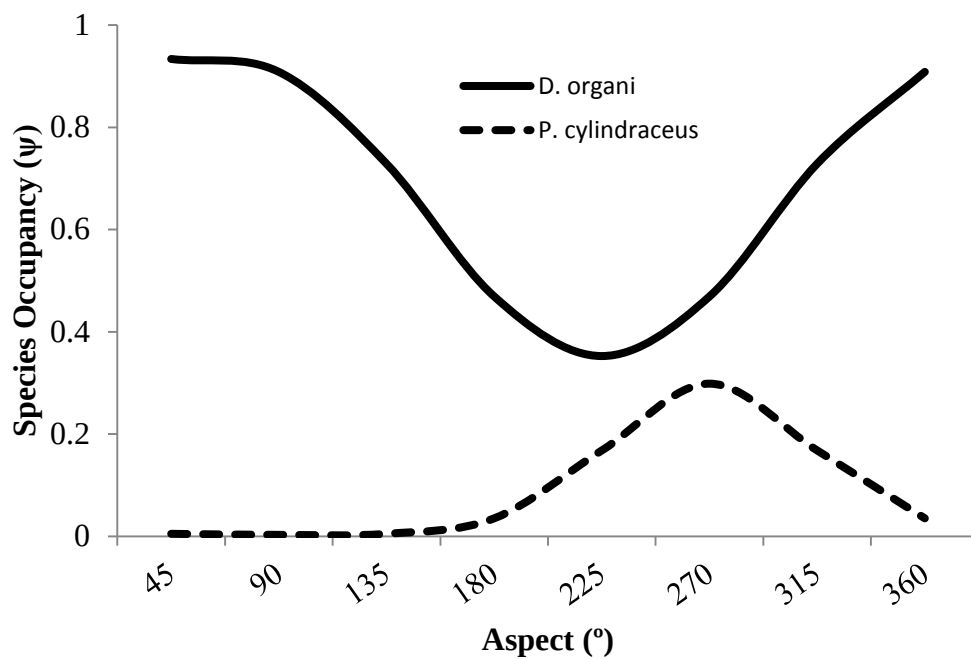


Figure 4.9. Occupancy probabilities of *Desmognathus organi* and *Plethodon cylindraceus* in relation to aspect from the Mount Rogers National Recreation Area, Virginia (2008-2011).

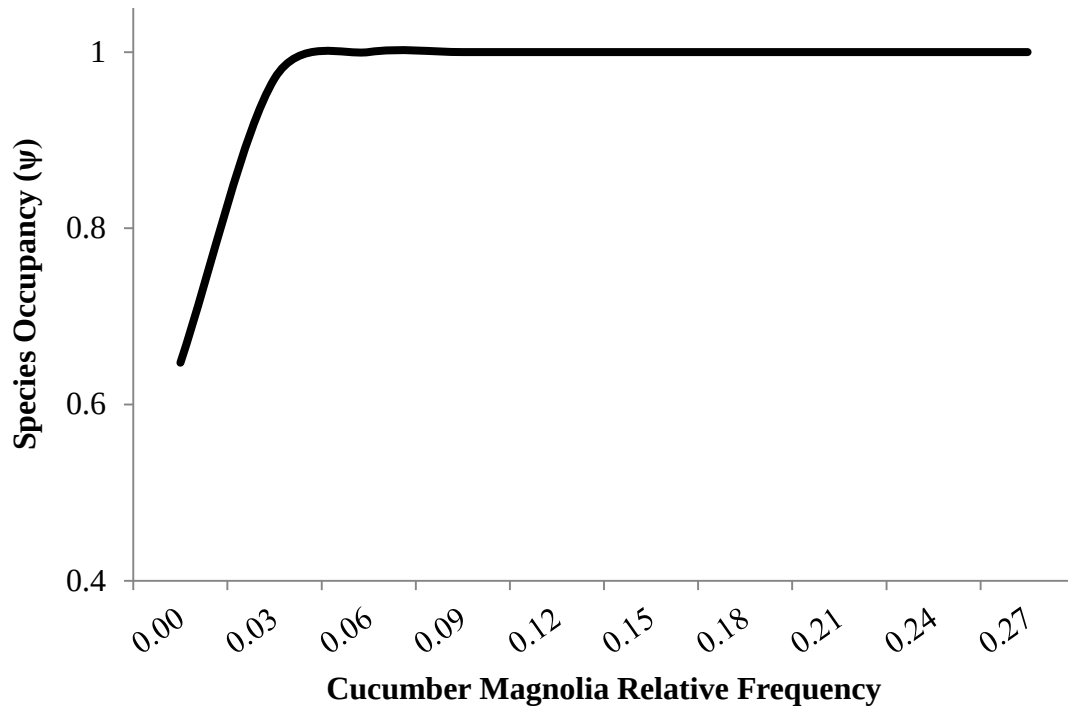


Figure 4.10. Occupancy probabilities of *Plethodon cinereus* in relation to cucumber magnolia (> 11.43 cm DBH) relative frequency within 400 m² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).

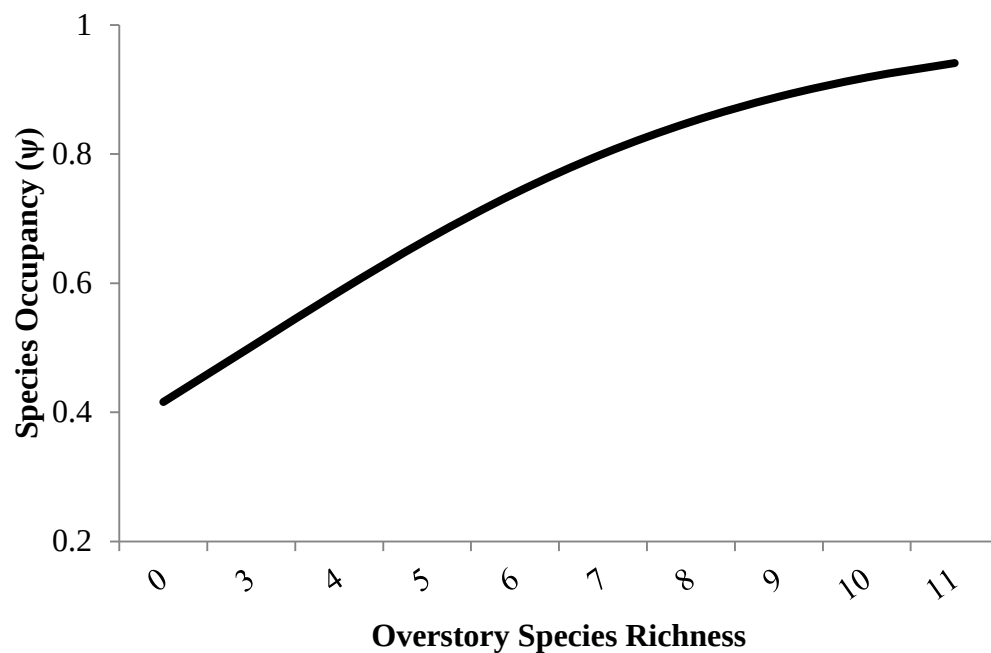


Figure 4.11. Occupancy probabilities of *Plethodon cinereus* in relation to overstory richness from the Mount Rogers National Recreation Area, Virginia (2008-2011).

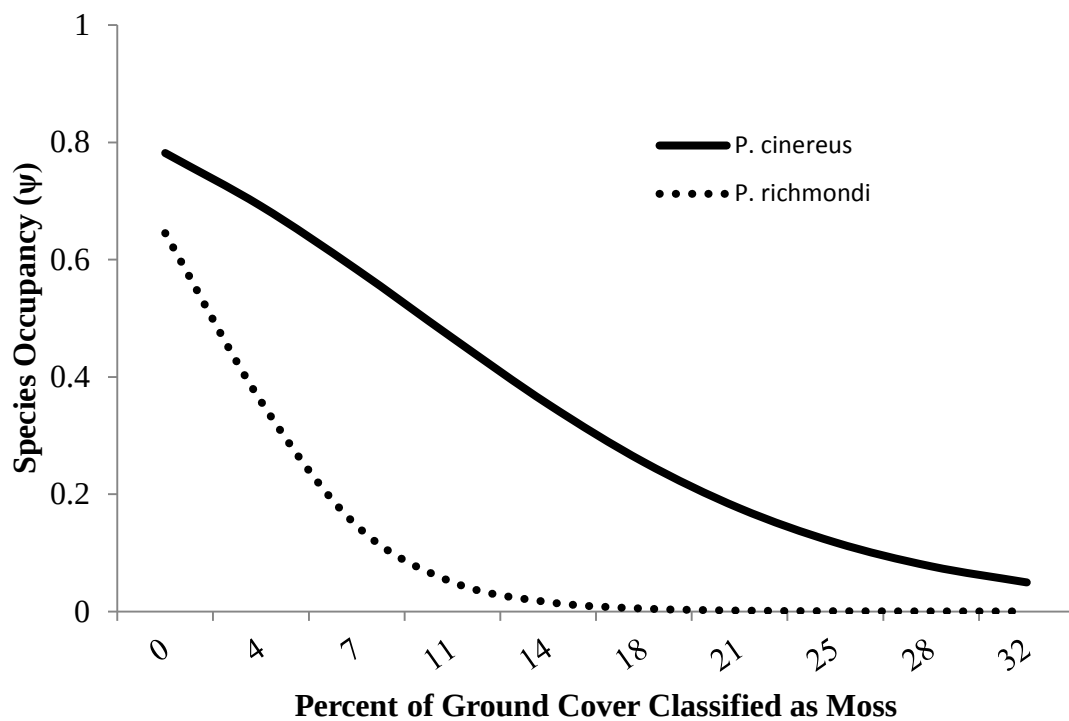


Figure 4.12. Occupancy probabilities of *Plethodon cinereus* and *P. richmondi* in relation to percent ground cover of moss from 4 x 1 m² plots within the Mount Rogers National Recreation Area, Virginia (2008-2011).

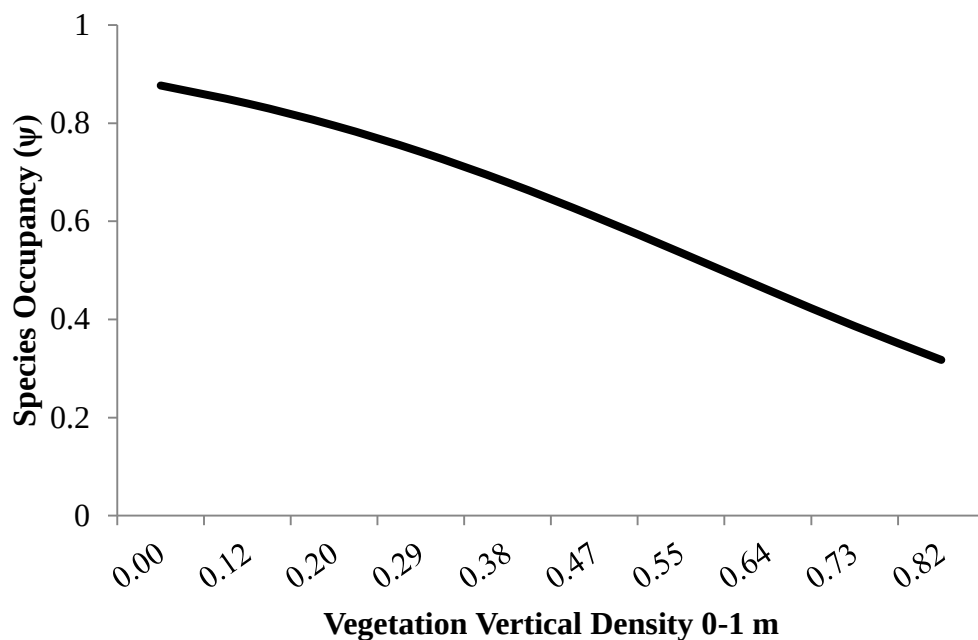


Figure 4.13. Occupancy probabilities of *Plethodon cinereus* in relation to vegetation density < 1 m from the ground surface from the Mount Rogers National Recreation Area, Virginia (2008-2011).

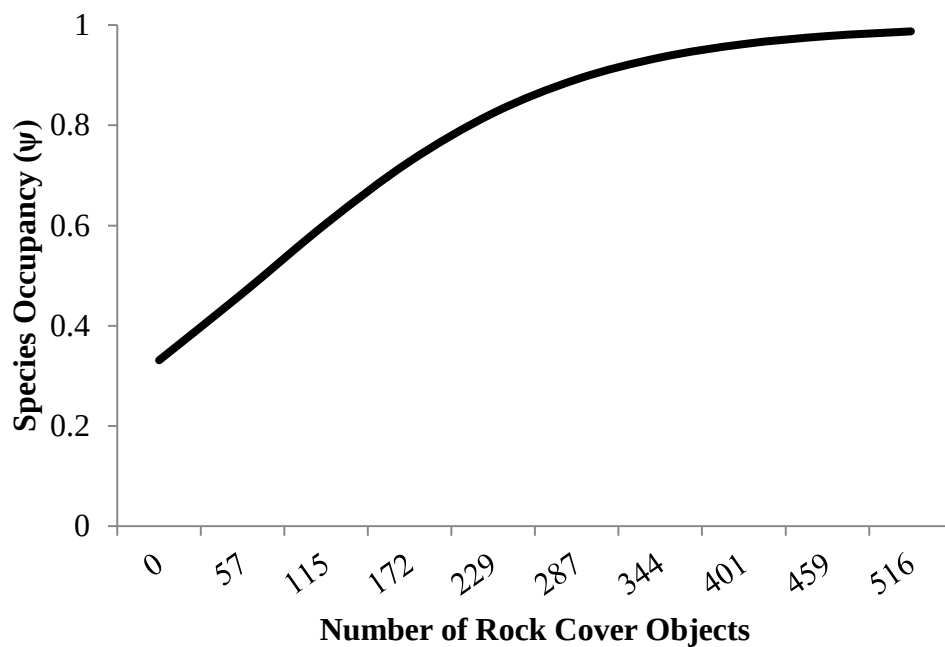


Figure 4.14. Occupancy probabilities of *Plethodon richmondi* in relation to number of rock cover objects within 400 m² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).

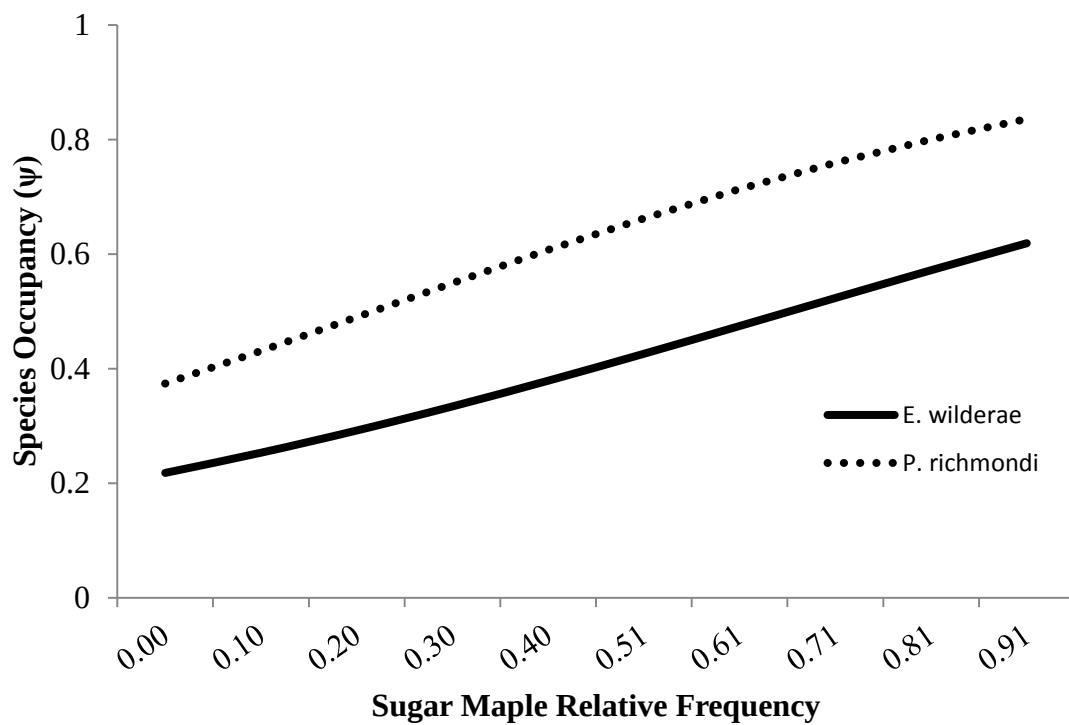


Figure 4.15. Occupancy probabilities of *Eurycea wilderae* and *Plethodon richmondi* in relation to sugar maple (> 11.43 cm DBH) relative frequency within 400 m² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).

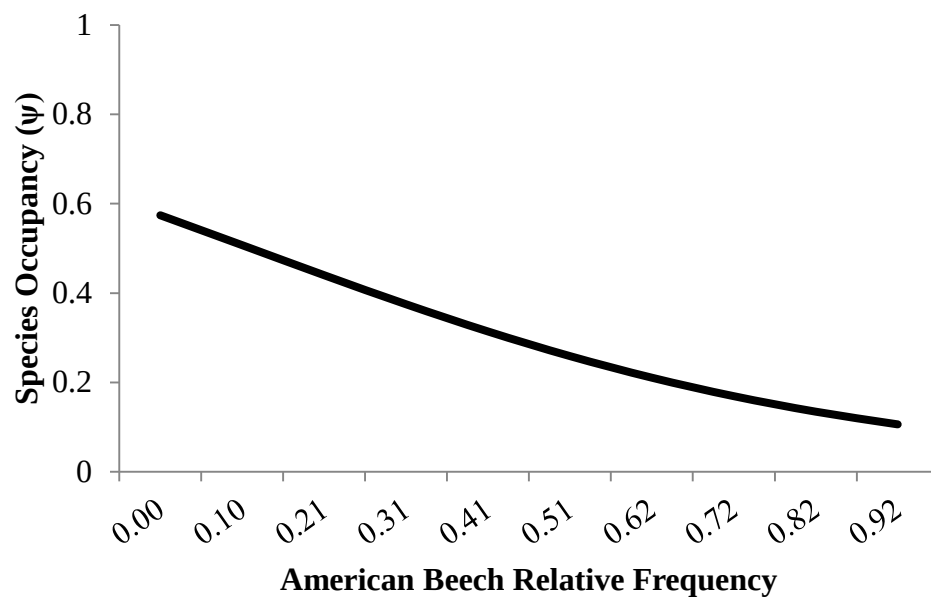


Figure 4.16. Occupancy probabilities of *Plethodon richmondi* in relation to American beech (> 11.43 cm DBH) relative frequency within 400 m² plots from the Mount Rogers National Recreation Area, Virginia (2008-2011).

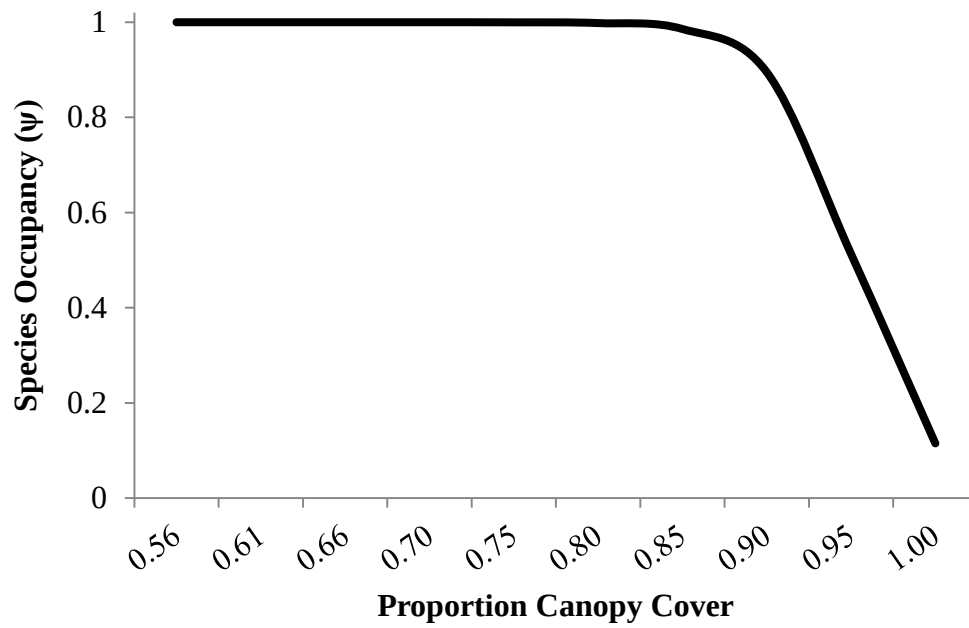


Figure 4.17. Occupancy probabilities of *Desmognathus fuscus* in relation to proportion of canopy cover from the Mount Rogers National Recreation Area, Virginia (2008-2011).

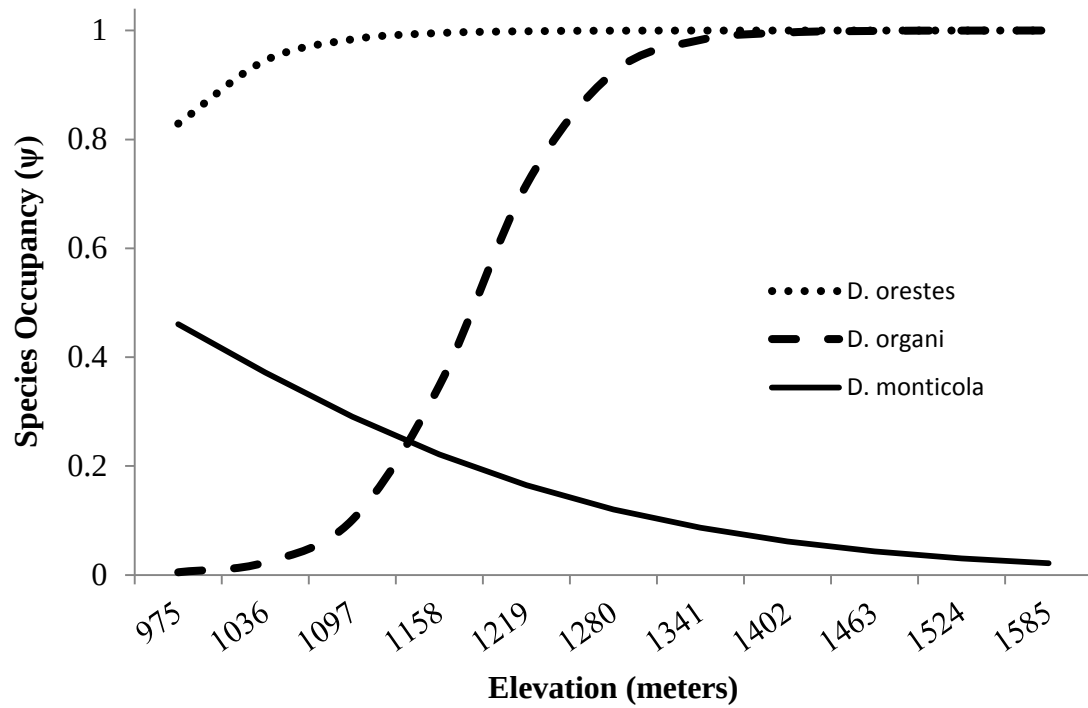


Figure 4.18. Occupancy probabilities of salamanders of the genus *Desmognathus* in relation to elevation from the Mount Rogers National Recreation Area, Virginia (2008-2011).

**CHAPTER 5: FUTURE CHANGES IN SALAMANDER DISTRIBUTIONS
ALONG AN ELEVATION GRADIENT ON WHITETOP MOUNTAIN,
VIRGINIA**

ABSTRACT

Global temperatures are expected to rise by 1.5 to 6 °C by 2100. Numerous species of plants and animals have already shifted their ranges due to recent warming and 28% of amphibians are projected to be at risk of extinction by 2100 due to climate change. The Southern Appalachian Mountains have the greatest diversity and richness of Plethodontid salamanders in the world with many species having limited, montane distributions. Range limits for many Plethodontid salamanders are thought to be determined by climate and competition with other salamanders. The range of Plethodontid salamanders could be altered with changing climate conditions. We determined the current distribution of 12 Plethodontid salamander species on Whitetop Mt., Virginia within the Mount Rogers National Recreation Area. Salamander occupancy was modeled based on mean January and July temperatures and corrected for imperfect detection. We then used the Canadian Centre for Climate Modeling and Analysis Coupled Global Climate Model (CGCM) and the Hadley Centre for Climate Prediction and Research (HAD) global circulation models with 2 different CO₂ emission projections (A1B and B1) to project future temperatures for Whitetop Mountain. Using current temperatures obtained from data loggers at 30.5 m elevational intervals, we downscaled climate predications to each 30.5 m sampling interval from 945 – 1676 m on the north slope and 1158 – 1676 m on the south slope for 2030, 2050, and 2070. All but 3 species of salamanders (*Plethodon welleri*, *Desmognathus organi*, and *Eurycea wilderae*) are projected to occupy Whitetop Mt. by 2070. Both *P. welleri* and *D. organi* are high elevation endemics and would have been hypothesized to be impacted the greatest. Our projections indicated *Plethodon welleri* will be extirpated by 2070 and *Desmognathus organi* would be practically extirpated by 2070. In order to persist the nine

remaining salamander species must be able to move higher in elevation to track their thermal niche.

INTRODUCTION

Amphibian populations have suffered population declines in the past 25 years with more than one-third listed as “globally threatened” and 43% considered to be declining (IUCN 2008). Some have suggested that we are in the midst of the sixth mass extinction with amphibians suffering the greatest losses (Wake and Vredenburg 2008). Habitat loss, diseases, introduced species, chemical contamination, UV-B radiation, over-exploitation, climate change, and synergistic interactions have been documented to cause declines (Wells 2007). Climate change alters amphibian habitat by decreasing leaf litter, intensifying competitive interactions, causing distribution shifts, increasing the effects of diseases and pathogens, and changing breeding phenology (Pounds et al. 2006; Whitfield et al. 2007; Kusan and Inoue 2008; Rovito et al. 2009; Milanovich et al. 2010). Amphibians are projected to be impacted by climate change more than either birds or mammals in North American (Lawler et al. 2009).

Effects of climate change, including drastic variation in temperature and moisture, have been observed worldwide. Surface temperatures have rapidly increased since the mid-1970s (IPCC 2007) and the past 10 years (2003-2012) have been the warmest on record in the United States (NOAA 2013). Global average temperatures have increased by 0.78°C between the periods of 1850-1900 and 2003-12 (IPCC 2013). Temperatures in the Northern Hemisphere for the past 50 years were greater than any 50-year period for the past 1300 years (IPCC 2007). Western North America has experienced periodic droughts, but precipitation has increased significantly in eastern North America since 1900 (IPCC 2007). Current models predict the Southeastern U.S. will become warmer, but many areas will become wetter (Kunkel et al. 2013).

Many species of plants and animals have been documented to shift distributions following their climatic niche in response to changing climatic conditions (Parmesan 2006; Chen et al. 2011). Plants (Kopp and Cleland 2013), invertebrates (Sheldon 2012), fish (Comte and Grenouillet 2013), amphibians (Raxworthy et al. 2009), reptiles (Sinervo et al. 2010), birds (Tingley and Beissinger 2013), and mammals (Moritz et al. 2008) have shown significant range changes in response to climate change. Since predicted climate change will be even greater and at an accelerated rate than past changes (IPCC 2007), species distribution changes are predicted to increase (Thuiller et al. 2005). Many models also predict that the rate of extinction for numerous plant and animal species will rapidly increase during the next century due to climate change (Schneider et al. 2007). In the western U.S. declines have been detected as elevational resurveys of mammal distributions between 60 – 3300 m in Yosemite National Park discovered that many species have shifted their ranges upward, associated with warming temperatures, since 1914-20 (Moritz et al. 2008).

Distribution shifts associated with climate change have the potential to especially affect amphibian communities across large geographic distances (Duellman 1999). Amphibian specialists that inhabit areas with unique climates may be particularly venerable. The Southern Appalachian Mountains and highlands of southern Mexico and Guatemala have the greatest diversity of salamanders in the family *Plethodontidae* (Wells 2007). Plethodontid salamanders are known as lungless salamanders, because they lack lungs and rely entirely on cutaneous respiration. These salamanders thrive in montane environments because cool and moist conditions facilitate cutaneous respiration (Petranka 1998). Climate conditions at lower elevations are physiological barriers for metabolic activities (Bernardo and Spotila 2006) and lower range limits for montane salamander species have been shown to be the results of

physiological stress due to warmer temperatures (Gifford and Kozak 2012). It is hypothesized that as global temperatures increase, distributions of plethodontid salamanders will become constrained and lead to extirpation of some species (Milanovich et al. 2010). Simulations with ecological niche models at broad scales indicate that populations of Southern Appalachian salamanders will be extirpated by 2020 (Milanovich et al. 2010).

Plethodontid salamanders in Central American appear to have already been impacted by changing climate as populations declines have been detected (Rovito et al. 2009). In the 1930s, salamanders were sampled along elevational transects in Guatemala and salamanders were found to occupy distinct elevational ranges (Schmidt 1936). The same transects were resampled in the early 1970s with few documented changes (Wake and Lynch 1976). In recent surveys at the same locations, several salamander species in the genus *Bolitoglossa* have disappeared and other species have experienced 10-fold reductions in abundance (Rovito et al. 2009). Few salamanders tested positive for diseases and pathogens suggesting climate change may have impacted habitat suitability since sites have remained undisturbed by humans (Rovito et al. 2009). Given the similar life history between Plethodontid species in Central America and the Southern Appalachian Mountains, salamanders in Appalachia could also experience similar declines (Milanovich et al. 2010).

Making predictions about how distributions could change in the future is helpful for assessing the impact of climate change and planning conservation programs (Milanovich et al. 2010). Most models make predictions based on an organism's realized ecological niche in relation to climatic conditions (Hutchinson 1957). These bioclimatic models often fail to identify the species' fundamental niche, but can serve as an initial step to begin conservation planning for potential impacts to future range distributions (Lawler et al. 2009; Sinclair et al.

2010). Several modeling approaches have been used to predict future range changes due to climate change. Ecological niche models use habitat, climate, and environment parameters to determine suitable areas for potential occupancy at a large scale, which allows generalizations (Peterson 2001). However, local topography, species interactions, and historical land use can greatly alter and even contradict large scale predictions (Pearson and Dawson 2003; Willis and Bhagwat 2009). Several studies have found when local microclimate is not considered, modeled predictions can be greatly altered (Pearson and Dawson 2003; Botkin et al. 2007; Luoto and Heikkinen 2008; Austin and Van Niel 2011). For European alpine plants, a large scale (16 x 16 km) model predicted the loss of all suitable habitats by the end of the 21st century. However, a smaller scale model (25 m by 25 m) predicted the presence of habitat for all plants in the survey (Randin et al. 2009). Similar results were obtained for California plant communities as smaller scaled distribution models predicted increased future habitat especially in rugged terrain where topography was not accounted for on large scale models (Franklin et al. 2013). Additionally, bioclimatic models often produced flawed future range changes, due to not incorporating detection in current surveys (Sinclair et al. 2010). It would be difficult for any model to predict local genetic variation, but local populations could be adapted to different climatic ranges (Sinclair et al. 2010). Therefore, modeling climatic effects across broad areas could once again misrepresent the realized effects on a local level.

Currently, most species distribution models use bioclimatic variables obtained through GIS layers. Maxent is a commonly used program which utilized maximum entropy to model distributions from presence only data (Phillips et al. 2006). Future range changes for Plethodontid salamanders (Milanovich et al. 2010), plants (Bystriakova et al. 2014), insects (Rochlin et al. 2013), mammals (Johnston et al. 2012), and many other species have been

modeled using Maxent. However, these models predict occurrences on large scales, which have been shown to be problematic. Additionally, false absences are typically not accounted for, which can also skew predications as populations on the range periphery might not be detected, thus failing to indicate all potential future habitat (Sinclair et al. 2010). Climatic data for these models often used WorldClim data, which is downloaded in 1 km grids (Hijmans et al. 2005). This is another disadvantage for local modeling. Many Southern Appalachian mountain peaks could gain more than 500 vertical meters within a 1 km grid, which would create more temperature variation than indicated by the models.

Recently, logistic regression models have been used to model occupancy in relation to climatic variables, on a small scale while accounting for detection, for American pika (*Ochotona princeps*) in 8 U.S. National Parks (Jeffress et al. 2013). This same type of approach will also be used to model site specific, future range changes for pikas and could be used for other montane species such as Plethodontid salamanders (Epps, unpubl. data). Program Presence adjusts site occupancy based on detection and also relates detection to covariates (Hines 2012). This program has been used to relate occupancy to habitat variables and could also be used for climatic variables (Steen et al. 2012). These methods would provide baseline estimates for future species distributions. However, biotic interactions could alter these future predictions as well as those obtained through ecological niche models (Pearson and Dawson 2003).

Our objective was to establish downscaled climate projections at 30.5 m (100') increments for Whitetop Mountain along Whitetop Creek (south slope) and Dells Branch (north slope) for 2030, 2050, and 2070. We then modeled salamander occupancy with mean temperature covariates and corrected with detection probabilities. By applying climate projections we were able to estimate salamander occupancy probabilities at each elevation for

future time periods. Our results will provide land managers with baseline projections of future salamander range changes within the MRNA.

METHODS

Study Site

Our study site was located in the Mount Rogers National Recreation Area in Southwest Virginia (MRNA; Grayson and Smyth Counties). The two highest peaks in Virginia, Mt. Rogers (1,746 m) and Whitetop Mountain (1,684 m), are located within the MRNA. Salamander diversity within the MRNA is the greatest in Virginia and one of the greatest in the Southern Appalachian Mountains (Mitchell and Reay 1999). Sampling occurred along 2 streams on Whitetop Mountain, Dells Branch (north facing stream) and Whitetop Creek (south facing stream). Dells Branch is perennial up to 1250 m while Whitetop Creek is perennial up to 1554 m. The summit (>1,554 m) of Whitetop Mountain is predominately red spruce (*Picea rubens*) with mountain maple (*Acer spicatum*) and mountain ash (*Sorbus americana*), while the forest below the summit, until approximately 1,158 m, is a northern hardwood forest. The forest below 1,158 m is mostly located on private property and predominately mixed deciduous forests consisting of red oak (*Quercus rubra*), red hickory (*Carya ovalis*), cucumber magnolia (*Magnolia acuminata*), and chestnut oak (*Quercus prinus*). We sampled from 1,158 – 1,585 m along Whitetop Creek and 945 – 1,585 m along Dells Branch. Focal salamander species were found along both streams.

Climate Change and Modeling

We estimated existing and modeled future climate conditions using site specific and regional information. We placed Hobo pro v-2 data loggers (Onset Corp, Pocasset, MA) at every 30.5 m of elevation along Whitetop Creek and Dells Branch transects. We attached data loggers to trees with the temperature sensor was < 1 cm above the leaf litter in areas without canopy openings, which measured temperatures salamanders actually experienced on the forest floor as opposed to air temperature. Loggers recorded temperature to the nearest 0.2 C° every 15 minutes. We calculated January and July mean monthly temperatures for each elevation as these months were historically the coldest and warmest for the region (NOAA 2013). We compared mean monthly temperatures from each sampling elevation to recorded temperatures from the closest regional National Oceanic and Atmospheric Administration (NOAA) weather station at the Tri-cities airport (Kingsport, TN; 36.48°N, -82.41°W; 457 m elevation) as weather data was not available for Whitetop Mountain. The Tri-cities airport station is 73 km southwest of Whitetop Mountain. Ideally, a closer weather station and one at a similar elevation to Whitetop Mountain would have been preferred, but no others stations with 40+ years of historical data were available. We obtained current weather data for the Tri-Cities airport from the NOAA National Climatic Data Center (2013; www.ncdc.noaa.gov) and calculated mean differences between each transect elevation and Tri-cities airport, which served as a comparison for MRNA temperatures to the NOAA weather station from July 2009 – January 2012.

We obtained future climate projections through the World Climate Research Programme's (WCRP's) Coupled Model Intercomparison Project phase 3 (CMIP3; Meehel et al. 2007). Data used for projections was compiled for the Intergovernmental Panel on Climate Changes (IPCC; 2007) Fourth Assessment Report (AR4). A single climate projection grid would

have covered a large percentage of the MRNA and elevational ranges. We downscaled projections to represent each elevational transect. We used CMIP3 projections derived from 2 global circulation models (GCM), the Canadian Centre for Climate Modeling and Analysis Coupled Global Climate Model (CGCM3, Flato et al. 2001) and the Hadley Centre for Climate Prediction and Research (HADCM3, Johns et al. 2003). These are two widely used projections and climate analyses using more than a single model are recommended considering each has different spatial resolution and assumptions. For each GCM, we used two CO₂ emission scenarios, A1B and B1 which project moderate and low range emission scenarios (Nakicenovic and Swart 2000). The A1B scenario is the balanced projection, which includes a worldwide balance of energy sources in which one source is not dominant and produces moderate CO₂ emissions (Nakicenovic and Swart 2000). The B1 model is based on a global population peaking in the middle of the century and then declining. This scenario includes global environmental sustainability and the implementation of renewable energy production, which produces the lowest CO₂ emissions of all scenarios (Nakicenovic and Swart 2000). The B1 model was chosen as the best case scenario while the A1B was chosen as a median approach of all potential emission scenarios. We did not choose the A2 or A1FI scenarios as these would have been the worst cases and if drastic changes were detected with the A1B model, the A2 and A1FI would have only produced even worse range changes. For each time period we constructed 4 climate projections (one for each GCM and using both emission scenarios).

We projected mean January and July temperatures for 3 time periods 2030, 2050, and 2070. We used 20 years of temperature projections to calculate the mean temperature for each time period. The mean value established for 2030 comprised temperatures from 2020 – 2039, 2050 was comprised of temperatures from 2040 – 2059, and the mean value for 2070 was

comprised of temperatures from 2060 – 2079. We chose the 20 year range as Plethodontid salamanders are long lived (20+ years) and could potential endure a long time period of the change (Petranka 1998).

To downscale climate projections to each sampling transect on Whitetop Mt., we first determined the accuracy of the CMIP3 model with actual temperatures observed. The CMIP3 projections not only model future temperatures, but also model past temperatures. We modeled temperatures for the Tri-Cities Airport NOAA weather station from 1950 – 1999 and compared those temperatures to actual recorded values (NOAA 2013). Modeled mean temperatures were slightly warmer than observed for both July ($23.95\text{ }^{\circ}\text{C} \pm 1.02\text{ (SD)}$; $23.91\text{ }^{\circ}\text{C} \pm 1.07$; respectively) and January ($2.11\text{ }^{\circ}\text{C} \pm 2.97$; $1.97\text{ }^{\circ}\text{C} \pm 2.95$; respectively) mean temperatures. We assumed that realized future temperatures would also differ slightly than the modeled temperatures and reduced modeled July mean temperatures by $0.04\text{ }^{\circ}\text{C}$ and January mean temperatures by $0.14\text{ }^{\circ}\text{C}$. Next, we modeled past temperatures for Whitetop Mountain and compared those to modeled temperatures for the Tri-cities airport from the same time period to determine the difference between locations. Mean July temperatures from Whitetop Mountain were $5.37\text{ }^{\circ}\text{C} (\pm 0.56)$ cooler than the Tri-Cities Airport and mean January temperatures from Whitetop Mountain were $4.51\text{ }^{\circ}\text{C} (\pm 0.64)$ cooler than the Tri-Cities Airport. These differences allowed us to adjust future modeled temperatures from Whitetop Mt. to those from the Tri-Cities Airport. We then compared observed mean temperatures from January and July at each elevation on Whitetop Creek and Dells Branch to mean temperatures observed from Tri-Cities Airport. Thus, we established how much cooler each elevation was compared to the airport weather station (i.e. Whitetop Creek at 1585 m was 8.49°C cooler than the airport). Given that we had established how much cooler the modeled Whitetop location was from the airport

location, we were now able to calculate the difference between the modeled Whitetop location and each transect elevation. For example, mean July temperatures at the modeled Whitetop location were 5.37 °C cooler than airport and temperatures at 1585 m along Whitetop Creek were 8.49 °C cooler than the airport; therefore, at 1585 m Whitetop Creek would be 3.12 °C cooler than the modeled Whitetop location. From the differences constructed above we determined the difference for each elevation along both Whitetop Creek and Dells Branch. Our sampling transects stopped 1585 m as this was a portion of a larger sampling project, but Whitetop Mt. reaches 1676 m. We established a relationship between elevation and mean temperatures and between 1372 and 1585 m and used the equation generated to project temperatures from 1615 – 1676 m.

Salamander detection and occupancy

We sampled Whitetop Creek and Dells Branch during June from 2009 – 2011 with each transect and elevation sampled at the same time of the month each year. For both transects, the stream was the transect center and the same center location continued upslope even when elevations were above ephemeral streams. Each side of the transect was sampled for one-person hour by the same observer (Kevin Hamed). This allowed each site to be visited twice and sampling was repeated for 3 consecutive years for a total of 6 sampling attempts. We considered sampling sites closed as habitat was essentially uniform on each side of the transect center and repeat surveys occurred within 1 hour of the original survey. Occupancy modeling required repeat sampling (Bailey et al. 2004) and sampling each side of the transect provided a repeat sample. We established starting locations for each transect with a Garmin 60CSX GPS unit (Garmin Int. Olathe, KS) and sampled parallel to and within 5 m of the contour, which maintained a consistent elevation. We checked the GPS unit every 5 minutes to ensure proper

sampling elevation. We turned all natural cover objects (rocks, logs, and leaf litter) along each transect to search for salamanders. Once detected, we placed salamanders in individual 1.2-liter plastic bags to allow for identification and then released salamanders at their point of capture.

We first estimated detection probabilities to adjust occupancy probabilities since 100% detection cannot be assumed (MacKenzie et al. 2002). We used the single-season model (MacKenzie et al. 2002) in Program PRESENCE 5.5 (Hines 2012) to model detection since sampling seasons were closed (Steen et al. 2012). Models for detection were (1) null, (2) transect, and (3) time of year. We standardized covariates by z-transformations prior to analysis and chose the best model of detection as ranked by Akaike's Information Criterion (correct for small sample size; AIC_c) for occupancy models (Burnham and Anderson 2002).

We also modeled occupancy in Program PRESENCE 5.5 using single season models (Hines 2012; Steen et al. 2012). We standardized mean January and July temperatures by z-transformations prior to model building. Models for occupancy were mean July, mean January, mean July + mean July², and mean January + mean January² temperatures. Occupancy models were ranked by AIC_c (AIC corrected for small sample size; Burnham and Anderson 2002). Only models with $\Delta QAIC_c \leq 2.0$ were used to identify which temperature(s) had the greatest impact on salamander occupancy (Burnham and Anderson 2002). We calculated SEs and 85% confidence intervals (CIs), which allows inclusion of variables with greater biological than statistical significance, and temperatures were deemed informative if CIs did not include 0 (Burnham and Anderson 2002; Arnold 2010). We used beta and intercept values from the best model to establish equations to predict future salamander occupancy based on mean temperatures.

RESULTS

Climate Change and Occupancy Modeling

Mean temperatures varied slightly between sampling years as July 2009 and January 2010 were the coolest, while July 2011 and January 2012 were the warmest years. Mean January and July temperatures showed slight variation at the Tri-Cities Airport NOAA station and from data logger on Whitetop Mountain. During the three project years, mean temperatures differences were 3.38 °C (January) and 4.00 °C (July) from the warmest to coolest year at the NOAA station while on Whitetop Mountain mean temperatures differed from 3.25 °C (January) to 3.34 °C (July). The northern slope (Dells Branch) was consistently cooler than the southern slope (Whitetop Creek) at all elevations. Mean July temperatures were 0.62 °C cooler ($p < 0.001$) and mean January temperatures were 3.35 °C cooler ($p < 0.001$) on the northern slope than the southern slope.

The CMIP3 model predictions indicated that mean July temperatures at the reference Whitetop Mt. location would increase to 20.31°C by 2030 and to 20.90 °C by 2070 for the CGCM-B1 model and from 20.86 °C (2030) to 23.44 °C by 2070 for the HAD-A1B model (Table 5.1). Mean January temperatures initially increased by 2030 but then decreased until 2070 under the A1B scenario, but slight increases were predicted through the B1 scenario (Table 5.1). Temperature differences from the CMIP3 reference location to actual elevations ranged from -1.06 to -4.22 °C on Dells Branch and -1.38 to -3.50 °C on Whitetop Creek for July (Table 5.2). Models predicted a 1.1 – 1.8 °C increase on Dells Branch for July mean temperatures (CGCM-B1 and HAD-A1B) by 2030, 1.25 – 3.07 °C by 2050, and 1.69 – 4.30 °C by 2070 from the lowest (945 m) to the highest (1676 m) transects. Mean July temperatures were predicted to

increase 0.8 – 1.71 °C by 2030, 0.96 – 3.03 °C by 2050, and 1.39 – 4.27 °C by 2070 along Whitetop Creek from the lowest (1158 m) to the highest (1676 m) transect.

Occupancy modeling indicated that July mean temperatures were the best predictor of salamander occupancy for 11 of the 12 salamanders investigated (Table 5.3). Models for *Plethodon cylindraceus* were not valid for either mean January or July temperatures; therefore we used the current maximum and minimum temperatures as guides to predict how modeled future temperatures might influence its distribution. *Plethodon richmondi* and *P. yonahlossee* had both mean July and January temperature models with $\Delta AIC_c < 2$, but CIs for coefficients for mean January temperatures included 0 and were deemed not significant, thus only July temperatures were used to model occupancy (Table 5.4). Stream dwelling salamanders, *Desmognathus quadramaculatus* and *D. monticola*, are limited to habitat with somewhat perennial stream conditions (Petranka 1998). Therefore, we used the current maximum elevation of perennial streams as their elevational cutoff even if modeled occupancy was projected to go beyond those limits. Additionally, for all (terrestrial and aquatic) salamanders residing at low elevations, thermal maximums were established as the warmest temperature recorded within their current MRNA distribution. We acknowledge that this is a very conservative estimate and these salamanders might be able to persist at greater temperatures. However, without data specifically related to Whitetop Mountain, our results could not be applied to each elevation, which was the project goal.

Projected Species Range Changes

High elevation endemics *Plethodon welleri* and *Desmognathus organi* are projected to be impacted the greatest from increased warming. By 2030, the lowest elevation for *P. welleri* along Dells Branch would be 1554 m under the CGCM – B1 model, but would be 1767 m under

the HAD – A1B model (Table 5.5; Figure 5.1). By 2050, the HAD model predicts extirpation from Dells Branch, but the CGCM model indicates temperatures would provide a conducive environment down to 1585 m (Table 5.5; Figure 5.2). By 2070, only the CGCM model under the B1 emission scenario has conditions suitable for *P. welleri* and only 1676 m, the summit, has an occupancy probability > 0.50 (Table 5.5; Figure 5.3). The future for Whitetop Creek is much worse as no model provides condition where occupancy was projected to be > 0.50. By 2030, the CGCM model projected temperatures in which occupancy at 1676 m would be 0.34 (Table 5.6; Figure 5.4), but all models had occupancy < 0.19 by 2050 (Table 5.6; Figure 5.5) and < 0.03 by 2070 (Table 5.6; Figure 5.6). Thus, predicting the extirpation of *P. welleri* from Whitetop Creek.

Desmognathus organi will also be negatively impacted by future warming along Dells Branch, but it was not modeled to be extirpated. By 2030, the best climate scenario predicted occupancy probabilities greater than 0.76 from 1402 m upwards and the A1B scenario still provided for occupancy > 0.5 above 1494 m (Table 5.7; Figure 5.7). By 2050, *D. organi* was modeled to have occupancy probabilities > 0.5 above 1402 m under CGCM scenarios, but HAD models decreased occupancy to only 0.12 at 1676 m under the A1B emission scenario (Table 5.7; Figure 5.8). *Desmognathus organi* would be extirpated from Dells Branch under the HAD model by 2070, but the CGCM models still provides occupancy above 1585 m even with A1B emission scenarios (Table 5.7; Figure 5.9). Up to the year 2030 along Whitetop Creek, occupancy remained high (> 0.50) above 1433 m under both CGCM scenarios (Table 5.8; Figure 5.10). By 2050, all models except the HAD model under the A1B emission scenario provided conditions conducive to occupancy from 1615 m upward (Table 5.8; Figure 5.11). However, by

2070 only the CGCM model under the B1 emission scenario indicated occupancy and only above 1646 m (Table 5.8; Figure 5.12).

Eurycea wilderae also appeared to be negatively impacted by future warming. On both transects and throughout the MRNA, *E. wilderae* was typically detected at middle to higher elevations. Thus, occupancy increased with cooler temperatures. Along Whitetop Creek, occupancy was never greater than 0.42 with either climate model or emissions scenario. By 2050, only occupancy models from the CGCM-B1 projection provided a chance of occupancy and it was well below 0.50. *Eurycea wilderae* would be virtually extirpated from Whitetop Ck. by 2070 with all climate projections (Table 5.9). *Eurycea wilderae* along Dells Branch were not as negatively impacted as along Whitetop Ck up to 2050. With the exception of the HAD – A1B projection, occupancy was > 0.50 at 1585 m and above in 2030 (Table 5.10). Occupancy probabilities decreased by 2050, but the salamander was still present with the CGCM – B1 projection. However, by 2070 occupancy probabilities were only > 0.50 at the highest elevation (1676 m) and from the least severe climate projection; thus suggesting extirpation from Whitetop Mt. by 2070 on both northern and southern slopes (Tables 5.9 and 5.10).

For all other salamanders the lower modeled limits were the same as time progressed. We did not establish individual, physiological thermal limits and based their maximum temperature as the highest mean July temperature observed at any elevation and year across the study area, which provided a very conservative estimate. Along Whitetop Creek the minimum elevation with HAD – A1B scenario for 2050 would be 1311 m and by 2070 it would be 1585 m. By 2070 the lowest elevation for occupancy would be 1311 m for HAD – B1. The CGCM projection for both emission scenarios suggests no change in distribution through 2070 along Whitetop Creek. Along Dells Branch, minimum elevations for the HAD – A1B scenario was

1067 m in 2050 and 1463 m by 2070. The HAD – B1 scenario for 2070 indicates the lowest elevation to be 1067 m and the only elevation projected to exceed our established thermal limit for the CGCM model with A1B emission scenario was at 945 m by 2070.

Plethodon montanus and *Desmognathus orestes* were found at all elevations on both transects. Both projections provided for occupancy probabilities of 1.0 except at the lower elevations which exceeded our highest recorded July mean temperatures (see above; Tables 5.11 – 1.14). *Plethodon yonahlossee* and *P. cinereus* were projected to occupy both slopes of Whitetop Mt. through 2070 only shifting upslope. Occupancy for *P. yonahlossee* increased with elevation and time period for each climate projection. By 2070 occupancy probabilities along Whitetop Creek would be >0.90 for all elevations with all climate projections except the CGCM – B1, which has slightly lower (0.89 – 0.83) occupancies at higher elevations due to cooler temperatures (Table 5.15). Occupancy probabilities along Dells Branch would also be > 0.90 except above 1402 m where occupancy probabilities ranged from 0.71 – 0.99, again due to cooler temperatures, across all climate projections and emission scenarios (Table 5.16). *Plethodon cinereus* also increased occupancy with time and elevation. Along Whitetop Creek, occupancy probabilities ranged from 0.96 – 1.00 across all projections and emission scenarios (Table 5.17). Only the previously described uninhabitable lower elevations would not have been occupied. Occupancy at Dells Branch was similar to Whitetop Creek except for a decreased occupancy due to cooler temperatures of the northern slope. Occupancy probabilities were > 0.94 up to 1585 m and decreased to 0.65 by 1676 m for CGCM – B1 projections (Table 5.18).

Aquatic salamanders, *Desmognathus quadramaculatus* and *D. monticola*, were always associated with perennial water within the MRNA. Organ (1991) found both species to inhabit seeps, but those were still close to perennial streams. Therefore, in addition to temperature the

presence of a perennial stream is necessary for occupancy. The current maximum elevation for perennial water along Dells Branch is 1250 m and 1554 m for Whitetop Creek, thus we did not consider any modeled occupancy greater than those elevations to be relevant. Both species have almost identical occupancy projections, which indicated occupancy would persist through 2070 on both slopes except with the HAD – A1B scenario in which temperatures exceeded the maximum July mean we recorded for our study period (Tables 5.19 – 5.22). Occupancy probabilities ranged from 0.95 – 1.00 at all elevations except those previously discussed exceeding our documented maximum temperatures (Tables 5.21 – 5.22). Based on our modeled occupancy probabilities both species will be present on both slopes through 2070.

Plethodon richmondi and *Desmognathus fuscus* are projected to maintain occupancy on both slopes of Whitetop Mt. through 2070. These salamanders were typically found at lower elevations and warming conditions will allow them to move upslope over time. By 2070, *P. richmondi* was modeled to have occupancy probabilities of 0.51 to 0.92 through 1585 m on Whitetop Creek. (Table 5.23) and 0.55 to 0.88 through 1463 m along Dells Branch (Table 5.24) with projections from the CGCM – A1B scenario. *Desmognathus fuscus* had even greater occupancy probabilities ranging from 0.60 to 0.98 on both Whitetop Creek (Table 5.25) and Dells Branch (Table 5.26) with CGCM – A1B projections. However, both species were projected to be absent from lower elevations as demonstrated for other species based on our maximum July temperatures observed by 2070.

Even though we were not able to model occupancy for *Plethodon cylindraceus*, based on the maximum temperature we measured at occupied locations, *P. cylindraceus* will still be present on Whitetop Mt. through 2070. This salamander will have to shift upwards in order to maintain its preferred niche. It would most likely be absent from the same transects that have

been deemed as unsuitable for other lower elevation species due to temperatures exceeding maximum temperatures recorded during our surveys.

DISCUSSION

Plethodontid salamander loss on Whitetop Mt. due to climate change appears to be less severe than predicted by other regional models, but 3 species (*Plethodon welleri*, *Desmognathus organi*, and *Eurycea wilderae*) are predicted to become extirpated by 2070. Salamanders residing in lower latitudes within the Southern Appalachian Mountains were predicted to be extirpated by 2080 by HAD and CGCM models with either A2A or B2A emission scenarios (Milanovich et al. 2010). However, only our HAD model predicted extirpations on Whitetop Mountain, which would occur by 2050 under A1B emission scenarios and by 2070 under A1B and B1 emission scenarios. These extirpations were only for 3 species limited to high elevations. We chose the A1B emission scenario which was similar to the B2A scenario (Nakicenovic and Swart 2000) used by Milanovich et al. (2010). However, our projections only factored mean July temperature changes and did not consider annual or mean temperatures of the wettest quarter as Milanovich et al. (2010). Additionally, we did not consider the impact of precipitation which most likely created more severe impacts (Milanovich et al. 2010), but newest climatic models suggests an increase in precipitation for the MRNA areas (Kunkel et al. 2013) as opposed to models by Milanovich et al. (2010). Of the 12 species models for Whitetop Mt., Milanovich et al. (2010) modeled 11 species including *Desmognathus wrighti* which included *D. organi* found on Whitetop Mt. as the *D. organi* had yet to be described from *D. wrighti* (Crespi et al. 2010). Therefore, we applied their predications for *D. wrighti* to *D. organi*. Projections from the HAD – B2A model predicted > 90% reductions of suitable climatic habitat for 4 salamander

species by 2020 and 6 species by 2080. Suitable climatic habitat losses projected from the CGCM – B2A models were > 90% for 2 salamander species by 2020 and 6 species by 2080 (Milanovich et al. 2010). *Plethodon montanus*, *P. yonahlossee*, *P. welleri* and *Desmognathus quadramaculatus* were projected to experience > 90% loss of suitable climatic habitat with *P. montanus* and *P. welleri* projected to experience 100% loss by 2050 (Milanovich et al. 2010). Our earliest projection of an extirpation was 2050 only for *P. welleri* and by 2070 only 3 species were projected to be extirpated with the HAD model. Differences between our projections and Milanovich et al. (2010) could also be due to our correction for imperfect detection and temperature data recorded at each 30.5 m elevation interval. Milanovich et al. (2010) used climate projections that covered large scales which did not allow for smaller increments of elevation.

Our projections also provide a more realistic representation as Plethodontid salamander on Whitetop Mt. could migrate upward in order to maintain their fundamental climatic niche. Other projections have suggested species would persist due to migrations between mountain peaks, which would most likely not occur given Plethodontid salamanders limited home ranges and dispersal abilities (Petranka 1998). In the Northern Hemisphere upper range limits have increased by 6.1 m upward per decade of all species examined (Parmesan 2006) and Plethodontid salamander would most likely have to move upward at a quicker rate. *Plethodon yonahlossee* was one example of a salamander which would need to move upward on Whitetop Mountain based on our projections. This salamander would be required to move ~152 m upward in the next 40 years to maintain its most desirable thermal niche. We documented *P. welleri* shifting downslope ~130 m in 20 years (1991 – 2011; Hamed, unpubl. data), which suggests Plethodontid salamanders might be able to track their fundamental niche at rates much faster

than observed for other species. However, our models do predict the extirpation of 3 species by 2070 under all but the least intensive warming scenarios and even with the potential of upward migration.

Plethodontid salamanders have shifted upward in the past to maintain their fundamental temperature niche. Many high elevation Plethodontid salamanders are derived from salamanders that were originally inhabiting cooler and lower elevations (Kozak and Wiens 2010). Due to warming these original species shifted upslope and speciation ensued as they became isolated on mountain peaks, but the upward migration occurred over millions of years (Kozak and Wiens 2010). Recently, Southern Appalachian salamanders have been exposed to additional warming not only caused by climate change, but due to habitat loss. Canopy removal during timber harvesting increases forest floor soil temperatures (Homyack et al. 2011). In the late 1800s and early 1900s a large percentage of the Great Smoky Mountains National Park was clear cut. This area currently has one of the higher diversities of Plethodontid salamanders in the world, which persisted through the inevitable warming due to loss of canopy. In many historic clear cut areas the abundance and richness is less than pristine areas, but salamanders still exist (Dodd 2004). If those salamanders could persist through such a dramatic warming and habitat loss, they might be able to persist even longer than projected by our models.

Plethodon welleri, *Desmognathus organi*, and *Eurycea wilderae* are listed as “species of greatest conservation need” by the Virginia Department of Game and Inland Fisheries and all were projected to become extirpated due to the loss of their fundamental climate niche by 2070 on Whitetop Mt. (VADGIF 2005). The MRNA is recognized as having some of the richest populations of both *P. welleri* and *D. organi* in the Southern Appalachian Mountains. Both species are high elevation endemics, reaching their northern most limits within the MRNA, and

have a very limited distribution. If Whitetop Mt. populations experience extirpations, more southern populations could also perish even before Whitetop Mt. populations. *Plethodon welleri* was thought to be limited to spruce/fir forest, but we found occupancy to be related to elevation and not habitat, which suggested climatic conditions, had a greater influence on occupancy than habitat (Hamed, unpubl. data). The northern slope of Whitetop Mt. will be a refuge for *P. welleri* until 2070 as the CGCM with the lowest CO₂ emission scenario (B1) indicated occupancy probabilities > 0.50 at the highest elevation. Our projections for *P. welleri* were less severe than Milanovich et al. (2010) which predicted 100% loss of suitable climatic habitat by 2020 under the CGCM-B2A scenario and by 2050 under the HAD-B2A predictions. Discrepancies between the two predications were previously discussed. Even though we did not model other areas, if more southern areas also experience similar warming, this northern population could be one of the last remaining populations in the Southern Appalachian Mountains. Although we did not factor diseases and pathogens into our model, *P. welleri* has been reported to be infected with ranavirus (Hamed et al. 2013) and ranavirus prevalence has been suggested to increase with warmer temperatures (Gray et al. 2009). Therefore, other factors could work synergistically to negatively impact *P. welleri*.

Our models suggested *Desmognathus organi* would persist in warmer temperatures slightly better than *P. welleri*. Along Dells Branch, climatic conditions within the fundamental niche of *D. organi* would be present through 2070 above 1554 m with the CGCM and lower B1 emission scenarios. The salamander would also persist with even greater CO₂ emission scenarios as occupancy probabilities would be ≥ 0.66 above 1585 m. Only the HAD projection with A1B emissions predicted the extirpation of *D. organi*. Whitetop Mountain could potentially have the richest population of *D. organi* within its current range (Organ, unpubl. data). Given

our projections, it appears that this salamander might be able to persist with projected warming until 2070. Even though moisture was not built into our projections, current models predict a precipitation increase in the Southern Appalachian Mountains (Kunkel et al. 2013). Given the reliance of *D. organi* on seeps and other areas of moisture, which have been shown to have less temperatures increases than drier slopes (Fridely 2009), *D. organi* might be able to persist even longer than we projected. Again our projections for *D. organi* were more optimistic than Milanovich et al. (2010) which predicted a 92% and 86% loss of suitable climatic habitat by 2020 with HAD and CGCM – B2A models.

Eurycea wilderae was the final Plethodontid salamander projected to become extirpated by 2070. The impact on the entire species might not be as severe as for other high elevation species on Whitetop Mountain as *E. wilderae* has a much large distribution throughout the Southern Appalachian Mountains. Our projections indicated extirpation from Whitetop Creek. by 2050 and from Dells Branch by 2070. *Eurycea wilderae* relies on streams to complete its reproductive cycle and any changes in precipitation could impact the population. However, current models are suggesting an increase in moisture for the Southeastern U.S. (Kunkel et al. 2013). Additionally, *E. wilderae* migrate from streams after nesting and remain in more terrestrial habitat. Any changes in forest floor moisture due to loss of canopy could also provide additional negative impacts (Petranka 1998). Our projections for *E. wilderae* were similar to those from Milanovich et al. (2010) which predicted an 85-89% loss by 2020 under CGCM – B2A and HAD – B2A respectively, but our models needed an additional 30 years before extirpations would occur.

Our models only indicated species occupancy probability based on fundamental temperature niche. However, Plethodontid salamanders have life history traits that might allow

them to persist even longer than projected with warming temperatures. Estimates indicated that only 20% of salamanders present in a given area will be above the surface at a given time (Petranka 1998). Organ (1991) found different species of Plethodontid salamanders do not emerge to the surface at the same time at night, but instead partition time above the surface, presumable to avoid resource competition. Given the extensive use of sub-terrain space and somewhat flexible time of emergence, Plethodontid salamander might be able to alter their use of above ground habitat to avoid warm temperatures. It has been suggested that species might alter the timing of activities to avoid increasing temperatures and to be active within their fundamental temperature niche with warming temperatures (Sinclair et al. 2010). *Plethodon richmondi* currently uses a similar strategy in which it engages in courtship and nesting in early March/April or late October each season, virtually remaining below the surface during warmer summer months (Duellman 1954). If *P. welleri*, *D. organi*, or *E. wilderae* were able to adapt to a similar strategy these species might be able to persist even longer in the presence of warming temperatures and once again our models presented would be a worst case scenario.

Competitive interactions between Plethodontid salamanders have also been shown to intensify with climatic extremes and even lead to local extirpations. *Plethodon cinereus* and *P. shenandoah* occur parapatrically in Shenandoah National Park, Virginia. *Plethodon shenandoah* is restricted to dry isolated talus slopes while *P. cinereus* is found throughout the forest surrounding the rock talus areas (Jaeger 1971). *Plethodon cinereus* appears to be a better competitor for food and space in the leaf litter leaving *P. shenandoah* to live and forage in the talus areas where *P. cinereus* experienced reduced survival (Jaeger 1971b). Interspecific competition has been shown to cause local extirpation of a subpopulation of *P. shenandoah* as extreme drought conditions created inhospitable conditions in talus slopes, but due to

competition, *P. shenandoah* was not able to move into surround leaf litter and experienced mortality (Griffis and Jaeger 1998). Other larger Plethodontid salamanders have also been documented to experience competition within distributions. *Plethodon glutinosus* extends its distribution to much higher elevations when *P. jordani* is absent, even on cooler, north facing slopes (Hairston 1951). In experimental plots in areas of overlap, removing *P. jordani* created significant increases in the number *P. glutinosus* (Hairston 1980). Gifford and Kozak (2012) documented the potential movement upslope by *P. teyahlae* was prohibited by *P. jordani*, a high elevation endemic. Thus, species interactions obviously affect Plethodontid salamander distributions. The presence of salamanders on Whitetop Mt. with increasing temperatures depends on the salamander's ability to move upslope based on our models. With the exception of 3 high elevation species all other Plethodontids must move upslope to survive. If competition with a higher elevation species prevents or even slows the upward movement, the species could become extirpated, which was not predicted by the model. *Plethodon richmondi* would need to move upslope by 122 m within 40 years to maintain occupancy probabilities > 0.50 based on our models. This rate should be obtainable based on movement rates of *P. welleri* previously discussed. However, if competition with another Plethodontid delays this movement, optimal conditions could exceed (move higher) the salamander's current occupancy and lead to extirpation. Again our models should be considered a baseline for managers as competition was not included.

Our models did not account for habitat changes that could potentially limit upslope movement or species interactions that could affect range changes. However, habitat models for MRNA salamanders did not indicate a preference for a specific habitat type for most species (Hamed, unpub. data), but general loss of canopy due to vegetation changes could have a great

impact. On both Whitetop Creek and Dells Branch temperatures were projected to be greater at 1615 m than either 1646 m or 1676 m. We feel this was probably due to the angle/degree of slope. Both areas have a change in slope at this elevation as 30 m below is much steeper. The change to a less steep slope creates a flatter angle in relation to the sun and increases the amount of thermal energy striking the surface. Transects above 1615 m are slightly steeper, receive less direct sunlight, and thus are cooler. Even though the areas above 1615 m might have higher occupancy probabilities, the warm conditions at 1615 m could be a barrier to upslope migration. Again, our models did not account for how species will handle these changes or what natural conditions might mitigate the additional warmth such as increased woody debris from the loss of vegetation due to warming. In forest clear cuts, salamanders have been able to persist longer if additional woody debris is left after cutting (Semlitsch et al. 2009). Additionally, our models did not account for potential changes in precipitation. Plethodontid salamanders would be just as sensitive to moisture as temperature due to their semi-permeable skin that lacks protective covering and the necessity of moist skin to facilitate gas exchange (Spight 1968). Within the Great Smoky Mountains National Park, increased moisture has been shown to decrease the effects of warming air temperatures on soil temperatures (Fridley 2009). Current precipitation models do indicated an increase in moisture for most of the Southern Appalachian Mountains including the MRNA (Kunkel et al. 2013). If precipitation does increase, our observed impacts might be less than predicted by models.

Our models projected a conservative forecast for lower limits which were established as the warmest mean temperature we detected salamanders to inhabit over the 3 years of the project. With the exception of the 3 species in which lower limits were established based on current distributions (*P. welleri*, *D. organi*, *E. wilderae*), it is very likely that most salamanders on

Whitetop Mountain are not at their thermal limits and could persist at lower elevations than we indicated. Organ (1990; unpubl. data) conducted critical thermal maximum laboratory trials for MRNA salamanders from the genus *Plethodon*. He found *P. welleri* was the most sensitive with critical thermal maximum temperatures of 32.91 °C (± 1.05) and *P. cylindraceus* was the least sensitive with maximum thermal limits of 35.18 °C (± 0.49). We are not suggesting these salamanders could tolerate mean or even maximum monthly temperatures at their critical thermal maximums, but it is likely the lower limits of our sampling were not their maximum temperatures. However, our focus was to present land managers and agencies with data for baseline planning. Members of the *Plethodon glutinosus* complex range into Northern Florida and obviously are able to survive greater temperatures than within the MRNA (Petranka 1998). Individuals have been selected for higher thermal tolerances in warmer climates, but *P. cylindraceus* could also be selected for slightly warmer conditions than currently exist at the lowest elevation we sampled on Whitetop Mountain, allowing it to persist even longer. Other MRNA salamanders also have subspecies or complex members that occur at lower latitudes and these species could also have greater genetic variability.

It has been suggested that the coldest temperatures could be the factor that best relates to a species ability to move upslope in the presence of warming conditions (Nakawatase and Peterson 2006). However, models with mean July temperatures had the lowest ΔAIC_c values and models with January mean temperatures often had parameter estimates with CIs that included 0. Our modeled January temperatures, even at the lowest elevations, were warmer than temperatures predicted by the CMIP3 model for our reference location, which was not the pattern suggested by mean July temperatures. Warmer January temperatures were most likely due to snow cover as we measured temperature on the leaf litter surface and a snow pack of only

4 cm would have insulated the data logger from cooler air temperatures. However, the insulating effect would have also been experienced by salamanders below ground. This insulating effect has been shown to keep soil temperatures much warmer than air temperatures immediately above the snow (Decker et al. 2003). January temperatures could change greatly if warming alters the timing, duration, or amount of snow cover on Whitetop Mt. and have a much greater impact on MRNA salamanders in the future.

CONCLUSIONS

Our models used moderate emission scenarios for Whitetop Mountain salamanders and by 2070 all but 3 species of salamander will still remain on Whitetop Mountain. However, if future emissions are greater and follow a pattern predicted by more severe scenarios (e.g. A1FI or A2) effects could be much worse than predicted. Our modeled predications were not as severe as others which have projected a major decline in Southern Appalachian salamanders by 2080 (Milanovich et al. 2010). Results from our study indicated the greatest losses will be with *Plethodon welleri* and *Desmognathus organi* which are both at the northern most limits of their distributions. *Plethodon welleri* will be extirpated along Whitetop Creek by 2050 and Dells Branch by 2070 for all but the least severe warming projection (CGCM – B1). The impact on *D. organi* is projected less severe as the species will persist until 2070 along Dells Br. under all but the most extreme warming scenarios (HAD – A1B). The potential loss of *Eurycea wilderae* on Whitetop Mountain will not have as great of an impact on the species since it has a much greater distribution. Suitable temperatures for all other Plethodontid salamanders on Whitetop Mountain are projected to persist through 2070. However, many salamander species will be required to shift their distributions upslope. Our models could not identify potential barriers to dispersal, but

as long as they are not preventative and species movements shifting upward on the mountain can keep pace with changing temperatures, these species should still occupy Whitetop Mountain.

Acknowledgments

This research was completed with funds provided by the Virginia Department of Game and Inland Fisheries through a State Wildlife Grant from the U.S. Fish and Wildlife Service. We acknowledge the modeling groups, the Program for Climate Model Diagnosis and Intercomparison (PCMDI) and the WCRP's Working Group on Coupled Modelling (WGCM) for their roles in making available the WCRP CMIP3 multi-model dataset. Support of this dataset is provided by the Office of Science, U.S. Department of Energy. We thank J. and D. Organ for providing sampling locations and guidance. A special thanks to B. Parks and G. Poe for field assistance. Additionally, we are grateful to the MRNA staff for project assistance and to many private land owners for access to our study sites. All sampling was approved by the Virginia Department of Game and Inland Fisheries (Scientific Collection Permit #41396) and followed UT Institutional Animal Care and Use Committee protocol #2084-0412.

LITERATURE CITED

- Arnold, T. W. 2010. Uninformative parameters and model selection using Akaike's Information Criterion. *Journal of Wildlife Management* 74:1175-8.
- Austin, M. P. and K. P. Van Niel. 2011. Improving species distribution models for climate change studies: variable selection and scale. *Journal of Biogeography* 38:1-8.
- Bailey, L. L., T. R. Simons, K. H. Pollock. 2004. Estimating site occupancy and species detection probability parameters for terrestrial salamanders. *Ecological Applications* 14:692-702.
- Bernardo, J. J. R. Spotila. 2006. Physiological constraints on organismal response to global warming: mechanistic insights from clinally varying populations and implications for assessing endangerment. *Biology Letters* 2:135-9.
- Botkin, D. B., H. Saxe, M. B. Araujo, R. Betts, R. H. W. Bradshaw, T. Cedhagen, P. Chesson, T. P. Dawson, J. R. Etterson, D. P. Faith, S. Ferrier, A. Guisan, A. S. Hansen, D. W. Hilbert, C. Loehle, C. Margules, M. New, M. J. Sobel, and D. R. B. Stockwell. 2007. Forecasting the effects of global warming on biodiversity. *BioScience* 57:227-36.
- Burnham, K. P. and D. R. Anderson. 2002. Model selection and multi-model inference. Second edition. Springer-Verlag, New York, NY, USA.
- Bystriakova, N., S. W. Ansell, S. J. Russell, M. Grundmann, J. C. Vogel, and H. Schneider. 2014. Present, past, and future of the European rock fern *Asplenium fontanum*: combining distribution modeling and population genetics to study the effects of climate change on geographic range and genetic diversity. *Annals of Botany* 113:1-8.
- Chen, I-C, J. K. Hill, R. Ohlemuller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science* 333:1024-6.

- Comte, L. and G. Grenouillet. 2013. Do stream fish track climate change? Assessing distribution shifts in recent decades. *Ecography* 36: 1236-46.
- Crespi, E. J., R. A. Browne, L. J. Rissler. 2010. Taxonomic revisions of *Desmognathus wrighti* (Caudata: Plethodontidae). *Herpetologica* 66:283-95.
- Decker, K. L. M., D. Wang, C. Waite, and T. Scherbatskoy. 2003. Snow Removal and Ambient Air Temperature Effects on Forest Soil Temperatures in Northern Vermont. *Soil Science Journal of America* 67:1234-43.
- Dodd, C. K. (2004). *The amphibians of Great Smoky Mountains National Park*. Knoxville, TN: Univ. of Tennessee Press.
- Duellman, W. E. 1954. The salamander *Plethodon richmondi* in southwestern Ohio. *Copeia* 1954:40-5.
- Duellman, W. E. 1999. *Patterns of Distribution of Amphibians*. McGraw-Hill, New York, NY.
- Flato, G.M. and G. J. Boer. 2001. Warming asymmetry in climate change simulations. *Geophysical Research Letters* 28:195-8.
- Franklin, J., F. W. Davis, M. Ikegami, A. D. Syphard, L. E. Flint, A. L. Flint, and L. Hannah. 2013. Modeling plant species distributions under future climates: how fine scale do climate projections need to be? *Global Change Biology* 19:473-83.
- Fridley, J. D. 2009. Downscaling climate over complex terrain: high finescale (<1000 m) spatial variation on near-ground temperature in a mountain forested landscape (Great Smoky Mountains). *Journal of Applied Meteorology and Climatology* 48:1033-49.
- Gifford, M. E. and K. H. Kozak. 2012. Islands in the sky or squeezed at the top? Ecological causes of elevational range limits in montane salamanders. *Ecography* 35:195-203.

- Gray, M. J., D. L. Miller, J. T. Hoverman. 2009. First report of *Ranavirus* infecting lungless salamanders. *Herpetological Review* 40:316-9.
- Griffis, M. R. and R. G. Jaeger. 1998. Competition leads to an extinction-prone species of salamander: interspecific territoriality in a metapopulation. *Ecology* 79:2494-502.
- Gifford, M. E. and K. H. Kozak. 2012. Islands in the sky or squeezed at the top? Ecological causes of elevational range limits in montane salamanders. *Ecography* 35:195-203.
- Hairston, N. G. 1951. Interspecies competition and its probable influence upon the vertical distribution of Appalachian salamanders of the genus *Plethodon*. *Ecology* 32:266-74.
- Hairston, N. G. 1980. The experimental test of analysis of field distributions: competition in terrestrial salamanders. *Ecology* 6:817-26.
- Hamed, M. K., M. J. Gray, and D. L. Miller 2013. First report of ranavirus in Plethodontid salamanders from the Mount Rogers National Recreation Area, Virginia, USA. *Herpetological Review* 44:455-7.
- Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25:1965-78.
- Hines, J. E. 2012. PRESENCE5.5-Software to estimate patch occupancy and related parameters. USGS-PWRC, Patuxent Wildlife Research Center, Laurel, Maryland, USA. <http://www.mbr-pwrc.usgs.gov/software/presence/html>.
- Homyack, J. A., C. A. Hass, and W. A. Hopkins. 2011. Energetics of surface-active terrestrial salamanders in experimentally harvested forest. *Journal of Wildlife Management* 75:1267-78.

- Hutchinson, G. E. 1957. Concluding remarks. Cold Springs Harbor Symposia on Quantitative Biology 22:415-27.
- IPCC. 2007. Summary for policymakers. In: Climate Change 2007: Synthesis Report. Contributions of Working Groups I, II, and III to the Fourth Assessment Report of Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R. K. and A. Reisinger (eds.)]. IPCC, Geneva Switzerland.
- IPCC. 2013. Summary for Policymakers. In: *Climate Change 2013: The Physical Science Basis. Contributions of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T. F., D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley (eds.)]. Cambridge Univ. Press, Cambridge, United Kingdom and New York, NY, USA.
- IUCN, Conservation International, and NatureServe. 2008. An Analysis of Amphibians on the 2008 IUCN Red List www.iucnredlist.org/amphibians. Downloaded on April 5, 2010.
- Jaeger, R. G. 1971. Competitive exclusion as a factor influencing the distributions of two species of terrestrial salamanders.
- Jaeger, R. G. 1971b. Moisture as a factor influencing the distributions of two species of terrestrial salamanders. *Oecologia* 6:191-207.
- Jeffress, M. R., T. J. Rodhouse, C. Ray, S. Wolff, and C. W. Epps. 2013. The idiosyncrasies of place: geographic variation in the climate-distribution relationship of the American pika. *Ecological Applications* 23:864-78.
- Johns, T.C., J. M. Gregory, W. J. Ingram, C. E. Johnson, and A. Jones. 2003.

- Anthropogenic climate change for 1860 to 2100 simulated with the HadCM3 model under updated emissions scenarios. *Climate Dynamics* 20:583:612.
- Johnston, K. M., K. A. Freund, and O. J. Schmitz. 2012. Projected range shifting by montane mammals under climate change: implications for Cascadia's National Parks. *Ecosphere* 3:1-58.
- Kopp, C. W. and E. E. Cleland. 2013. Shifts in plant species elevational range limits and abundances observed over nearly five decades in a western North American mountain range. *Journal of Vegetation Science* 24:1-12.
- Kozak, K. H. and J. J. Wiens. 2010. Niche conservatism drives elevational diversity patterns in Appalachian salamanders. *American Naturalist* 176:40-54.
- Kunkel, K.E, L.E. Stevens, S.E. Stevens, L. Sun, E. Janssen, D. Wuebbles, C.E. Konrad II, C.M. Fuhrman, B.D. Keim, M.C. Kruk, A. Billet, H. Needham, M. Schafer, and J.G. Dobson. 2013. Regional Climate Trends and Scenarios for the U.S. National Climate Assessment. Part 2. Climate of the Southeast U.S., NOAA Technical Report NESDIS 142-2, 94 pp.
- Kusano, T. and M. Inoue. 2008. Long-term trend toward earlier breeding of Japanese Amphibians. *Journal of Herpetology* 42:608-14.
- Lawler, J. J, S. L. Shafer, D. White, P. Kareiva, E. P. Maurer, A. R. Blaustein, and P. J. Barlein. 2009. Projected climate-induced faunal change in the Western Hemisphere. *Ecology* 90: 588-97.
- Luoto, M., R. K. Heikkinen. 2008. Disregarding topographical heterogeneity biases species turnover assessment based on bioclimatic models. *Global Change Biology* 14:483-94.
- MacKenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle, and C. A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are

- less than one. *Ecology* 83:2248-55.
- Meehl, G. A., C. Covey, T. Delworth, M. Latif, B. McAvaney, J. F. B. Mitchell, R. J. Stouffer, and K. E. Taylor, 2007: The WCRP CMIP3 multi-model dataset: A new era in climate change research. *Bulletin of the American Meteorological Society* 88:1383-94.
- Milanovich, J. R., W. E. Peterman, N. P. Nibbelink, J. C. Maerz. 2010. Project loss of a salamander diversity hotspot as a consequence of project global climate change.
- Mitchell, J. C. and K. K. Reay. 1999. Atlas of amphibians and reptiles in Virginia. Special Publication Number 1, Wildlife Diversity Division, Virginia Department of Game and Inland Fisheries, Richmond, Virginia.
- Moritz, C., J. L. Patton, C. J. Conroy, J. L. Parra, G. C. White, S. R. Beissinger. 2008. Impact of a century of climate change on small-mammal communities in Yosemite National Park. *Science* 322: 261-4.
- Nakawatase, J. M., and D. L. Peterson. 2006. Spatial variability in forest growth – climate relationships in the Olympic Mountains, Washington. *Canadian Journal of Forest Research* 36:77-91.
- Nakicenovic, N. and R. Swart. 2000. IPCC Special report on emissions scenarios. Cambridge: Cambridge, Univ. Press 599 p.
- National Oceanic and Atmospheric Administration. 2013. National Climatic Data Center. NOAA Asheville, NC. <https://www.ncdc.noaa.gov/crn/> .
- Organ, J. A. 1991. Salamander survey of the Mount Rogers Nation Recreation Area section two. Marion, VA: United States Department of Agriculture. 210p. Available from Mount Rogers National Recreation Area, Marion, VA.
- Organ, J. A. 1990. Salamander survey of the Mount Rogers Nation Recreation Area

- section one. Marion, VA: United States Department of Agriculture. 98p. Available from Mount Rogers National Recreation Area, Marion, VA.
- Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology and Evolution* 37:637-69.
- Pearson, R. G. and T. P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography* 12:361-371.
- Peterson, A. T. 2001. Predicting species' geographic distributions based on ecological niche modeling. *The Condor* 103:599-605.
- Petranka, J. W. 1998. *Salamanders of the United States and Canada*. Washington, DC: Smithsonian Institution Press.
- Phillips, S. J., R. P. Anderson, R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modeling* 190:231-59.
- Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, B. E. Young. 2006. Widespread amphibian extinctions from epidemic diseases driven by global warming. *Nature* 439:161-7.
- Randin, C.F., R. Engler, S. Normand, M. Zappa, N. E. Zimmermann, P. B. Pearman, P. Vittoz, W. Thuiller, and A. Guisan. 2009. Climate change and plant distribution: local models predict high-elevation persistence. *Global Change Biology* 15: 1557-69.
- Raxworthy, C. J., R. G. Pearson, N. Rabibiso, A. M. Rakotondrazaf, J. Ramanamajato, A. P. Raselimanana, S. Wu, R. A. Nussbaum, and D. A. Stone. 2008. Extinction vulnerability

- of tropical montane endemism from warming and upslope displacement: a preliminary appraisal for the highest massif in Madagascar. *Global Change Biology* 14:1703-20.
- Rochlin I., D. V. Ninivaggi, M. L. Hutchinson, A. Farajollahi. 2013. Climate Change and Range Expansion of the Asian Tiger Mosquito (*Aedes albopictus*) in Northeastern USA: Implications for Public Health Practitioners. *PLoS ONE* 8(4): e60874.
doi:10.1371/journal.pone.0060874
- Rovito, S. M., G. Parra-Olea, C. R. Vasquez-Almazan, T. J. Papenfuss, D. B. Wake. 2009. Dramatic declines in neotropical salamander populations are an important part of global amphibian crisis. *Proceedings of the National Academy of Science* 106:3231-6.
- Schmidt, K. P. 1936. Guatemalan salamanders of the genus *Oedipus*. *Zoological Series of Field Museum of Natural History* 10:135-66.
- Schneider, S.H., S. Semenov, A. Patwardhan, I. Burton, C.H.D. Magadza, M. Oppenheimer, A.B. Pittock, A. Rahman, J.B. Smith, A. Suarez and F. Yamin, 2007: Assessing key vulnerabilities and the risk from climate change. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden and C.E. Hanson, Eds., Cambridge University Press, Cambridge, UK, 779-810.
- Semlitsch, R. D., B. D. Todd, S. M. Blomquist, A. J. K. Calhoun, J. W. Gibbons, J. P. Gibbs, G. J. Grater, E. B. Harper, D. J. Hocking, M. L. Hunter Jr., D. A. Patrick, T. A. G. Rittenhouse, B. B. Rothermel. 2009. Effects of timber harvest on amphibian populations: understanding mechanisms from forest experiments. *BioScience* 59:853-62.

- Sheldon, A. L. 2012. Possible climate-induced shift of stoneflies in a southern Appalachian catchment. *Freshwater Science* 31:765-74.
- Sinclair, S. J., M. D. White, and G. R. Newell. 2010. How useful are species distribution models for managing biodiversity under future climates? *Ecology and Society* 15:1-8.
- Sinervo, B., F. Cruz, D. B. Miles, B. Heulin, E. Bastiaans, M. Cruz, R. Lara-Resendiz, N. Martinez-Mendez, M. Calderon-Espinosa, R. Meza-Lazaro, H. Gadsden, L. Avila, M. Lepetz, T. Oksanen, D. Chapple, A. Bauer, W. Branch, J. Clobert, J. Sites. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328:894-99.
- Spight, T. M. 1968. The water economy of salamanders: Evaporative water loss. *Physiological Zoology* 41:195-203.
- Steen, D. A., C. J. W. McClue, J. C. Brock, D. C. Rudolph, J. B. Pierce, J. R. Lee, J. Humphries, B. B Gregory, W. B. Sutton, L. L. Smith, D. L. Baxley, D. J. Stevenson, and C. Guyer. 2012. Landscape-level influences of terrestrial snake occupancy within the southeastern United States. *Ecological Applications* 22:1084-97.
- Thuiller, W., S. Lavorel, M. B. Araujo, M. T. Sykes, I. C. Prentice. 2005. Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Science* 102: 8245-50.
- Tingley, M. W. and S. R. Beissinger. 2013. Cryptic loss of montane avian richness and high community turnover over 100 years. *Ecology* 94:598-609.
- Virginia Department of Game and Inland Fisheries. 2005. Virginia's comprehensive wildlife conservation strategy. Virginia Department of Game and Inland Fisheries, Richmond, Virginia.
- Wake, D. B. and J. F. Lynch. 1976. The distribution, ecology, and evolutionary history of

- plethodontid salamanders in tropical America. *Science Bulletin of the Natural History Museum of Los Angeles County* 25:1-65.
- Wake, D. B. and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Science* 105:11466-73.
- Wells, K. D. 2007. *The Ecology and Behavior of Amphibians*. Chicago, IL: The University of Chicago Press.
- Whitfield, S. M., K. E. Bell, T. Philippi, M. Sasa, F. Bolanos, G. Chaves, J. M. Savage, and M. A. Donnelly. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of National Academy of Science* 104:8352-6.
- Willis, K. J. and S. A. Bhagwat. 2009. Biodiversity and climate change. *Science* 326:806-7.

APPENDIX

Table 5.1. Coupled Modeled Intercomparison Project phase 3 (CMIP3) model January and July mean monthly temperature predictions for 2030, 2050, and 2070 from the Whitetop Mountain, VA reference location.

<u>Year/Month</u>	<u>CGCM (°C)</u>		<u>HAD (°C)</u>	
	<u>A1B</u>	<u>B1</u>	<u>A1B</u>	<u>B1</u>
<u>January</u>				
2030	-1.17	-1.68	0.76	-1.93
2050	-0.41	-1.08	-0.17	-0.74
2070	-0.64	-1.14	-0.32	0.07
<u>July</u>				
2030	20.31	20.31	20.86	20.43
2050	20.86	20.46	22.21	21.01
2070	21.65	20.90	23.44	22.13

Table 5.2. Mean temperature differences from the Coupled Modeled Intercomparison Project phase 3 (CMIP3) model reference location to each Whitetop Mountain, VA transect for January (2010-12) and July (2009-11) along Dells Branch (northern slope) and Whitetop Creek (southern slope).

<u>Elevation(m)</u>	<u>Dells Branch</u>		<u>Whitetop Creek(°C)</u>	
	<u>January</u>	<u>July</u>	<u>January</u>	<u>July</u>
945	0.62	-1.06	---	---
975	0.69	-1.67	---	---
1006	1.23	-1.56	---	---
1036	1.33	-1.64	---	---
1067	0.87	-1.86	---	---
1097	0.30	-1.96	---	---
1128	0.73	-1.95	---	---
1158	0.98	-2.00	1.06	-1.38
1189	-0.01	-2.35	1.15	-1.34
1219	0.01	-2.14	1.66	-1.21
1250	0.53	-2.26	1.98	-1.42
1280	-0.14	-2.33	1.19	-1.55
1311	0.60	-2.08	1.09	-2.01
1341	0.85	-2.38	0.93	-2.04
1372	0.17	-2.49	0.64	-2.30
1402	1.01	-2.88	0.57	-2.38
1433	0.26	-2.95	0.50	-2.68
1463	1.25	-3.04	0.01	-2.59
1494	0.35	-3.24	0.01	-2.76
1524	0.01	-3.28	0.10	-2.73
1554	-1.77	-3.61	-0.57	-2.91
1585	0.43	-4.14	0.32	-3.17
1615	0.21	-3.84	-0.34	-3.22
1646	0.17	-4.03	-0.47	-3.36
1676	0.13	-4.22	-0.61	-3.50

Table 5.3. Highest supported models describing salamander occupancy with mean January and July temperatures from Whitetop Mountain, VA from July 2009 – January 2012. Only models with $\Delta AIC_c < 2.0$ are listed.

Species and Models	AIC _c	ΔAIC_c	w_i
<i>Desmognathus fuscus</i> Ψ (Mean July)	161.64	0.00	0.70
<i>Desmognathus monticola</i> Ψ (Mean July)	122.41	0.00	1.00
<i>Desmognathus organi</i> Ψ (Mean July)	107.79	0.00	0.99
<i>Desmognathus quadramaculatus</i> Ψ (Mean July)	116.82	0.00	0.97
<i>Eurycea wilderae</i> Ψ (Mean July)	68.25	0.00	1.00
<i>Plethodon cinereus</i> Ψ (Mean July)	195.83	0.00	1.00
<i>Plethodon richmondi</i> Ψ (Mean July)	66.53	0.00	0.38
Ψ (.)	66.95	0.42	0.31
Ψ (Mean Jan)	67.58	1.05	0.23
<i>Plethodon welleri</i> Ψ (Mean July)	54.39	0.00	1.00
<i>Plethodon yonahlossee</i> Ψ (Mean July)	226.63	0.00	0.41
Ψ (Mean Jan)	227.23	0.60	0.30
Ψ (.)	227.30	0.67	0.29

Table 5.4. Model-averaged temperature, parameter estimates, standard errors, and 85% confidence intervals for variables explaining occupancy for Whitetop Mountain, VA salamanders from July 2009 – January 2012 in top QAIC_c candidate model.

Species	Variable	$\beta \pm \text{SE}$	85% CI
<i>D. fuscus</i>	July mean temperature	0.82 ± 0.42	0.22 to 1.42
<i>D. monticola</i>	July mean temperature	7.57 ± 3.26	2.88 to 12.26
<i>D. organi</i>	July mean temperature	-3.97 ± 1.59	-6.26 to -1.68
<i>D. quadramaculatus</i>	July mean temperature	2.37 ± 0.79	1.23 to 3.51
<i>E. wilderae</i>	July mean temperature	-2.21 ± 0.74	-3.28 to -1.14
<i>P. cinereus</i>	July mean temperature	2.47 ± 1.03	0.99 to 3.95
<i>P. richmondi</i>	July mean temperature	0.88 ± 0.52	0.13 to 1.63
<i>P. welleri</i>	July mean temperature	-4.92 ± 1.81	-7.52 to -2.31
<i>P. yonahlossee</i>	July mean temperature	0.68 ± 0.45	0.03 to 1.33

Table 5.5. Future occupancy probabilities for *Plethodon welleri* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
945	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
975	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1036	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1067	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1097	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1128	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1158	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1189	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1219	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1280	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1311	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1341	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1372	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1402	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1433	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1463	0.02	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1494	0.07	0.00	0.07	0.03	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
1524	0.09	0.00	0.09	0.04	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
1554	0.50	0.02	0.51	0.31	0.02	0.00	0.26	0.00	0.00	0.00	0.02	0.00
1585	0.98	0.42	0.98	0.95	0.47	0.00	0.93	0.15	0.00	0.00	0.39	0.00
1615	0.83	0.08	0.83	0.68	0.10	0.00	0.62	0.02	0.00	0.00	0.07	0.00
1646	0.95	0.25	0.95	0.89	0.29	0.00	0.86	0.07	0.00	0.00	0.23	0.00
1676	0.99	0.55	0.99	0.97	0.60	0.00	0.96	0.23	0.01	0.00	0.52	0.00

Table 5.6. Future occupancy probabilities for *Plethodon welleri* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1189	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1219	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1280	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1311	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1341	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1372	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1402	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1433	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1463	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1494	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1524	0.01	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1554	0.03	0.00	0.03	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1585	0.09	0.01	0.09	0.05	0.01	0.00	0.05	0.00	0.00	0.00	0.01	0.00
1615	0.11	0.01	0.11	0.07	0.01	0.00	0.06	0.00	0.00	0.00	0.01	0.00
1646	0.20	0.02	0.20	0.13	0.02	0.00	0.11	0.01	0.00	0.00	0.01	0.00
1676	0.34	0.03	0.34	0.22	0.03	0.00	0.19	0.01	0.00	0.00	0.03	0.00

Table 5.7. Future occupancy probabilities for *Desmognathus organi* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
945	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
975	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1036	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1067	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1097	0.02	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1128	0.02	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1158	0.02	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1189	0.14	0.01	0.14	0.08	0.01	0.00	0.06	0.00	0.00	0.00	0.00	0.00
1219	0.05	0.00	0.05	0.03	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
1250	0.09	0.00	0.09	0.05	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00
1280	0.13	0.01	0.13	0.07	0.01	0.00	0.06	0.00	0.00	0.00	0.00	0.00
1311	0.04	0.00	0.04	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
1341	0.16	0.00	0.16	0.09	0.01	0.00	0.07	0.00	0.00	0.00	0.01	0.00
1372	0.26	0.01	0.26	0.16	0.02	0.00	0.13	0.00	0.00	0.00	0.01	0.00
1402	0.76	0.11	0.76	0.62	0.13	0.00	0.58	0.04	0.00	0.00	0.10	0.00
1433	0.82	0.16	0.82	0.71	0.18	0.00	0.67	0.06	0.00	0.00	0.15	0.00
1463	0.88	0.24	0.88	0.80	0.26	0.00	0.76	0.09	0.00	0.00	0.22	0.00
1494	0.96	0.49	0.96	0.92	0.53	0.00	0.91	0.24	0.01	0.00	0.46	0.00
1524	0.97	0.54	0.97	0.94	0.57	0.00	0.92	0.27	0.02	0.00	0.51	0.00
1554	0.99	0.88	0.99	0.99	0.90	0.00	0.99	0.71	0.09	0.00	0.87	0.01
1585	1.00	0.99	1.00	1.00	0.99	0.09	1.00	0.98	0.66	0.00	0.99	0.12
1615	1.00	0.96	1.00	1.00	0.97	0.02	1.00	0.89	0.26	0.00	0.96	0.02
1646	1.00	0.99	1.00	1.00	0.99	0.05	1.00	0.96	0.51	0.00	0.99	0.06
1676	1.00	1.00	1.00	1.00	1.00	0.12	1.00	0.99	0.75	0.00	0.99	0.17

Table 5.8. Future occupancy probabilities for *Desmognathus organi* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1189	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1219	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1280	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1311	0.02	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1341	0.03	0.00	0.03	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1372	0.11	0.01	0.11	0.06	0.01	0.00	0.05	0.00	0.00	0.00	0.00	0.00
1402	0.16	0.01	0.16	0.09	0.01	0.00	0.08	0.00	0.00	0.00	0.01	0.00
1433	0.51	0.04	0.51	0.35	0.05	0.00	0.31	0.01	0.00	0.00	0.04	0.00
1463	0.39	0.02	0.39	0.25	0.03	0.00	0.21	0.01	0.00	0.00	0.02	0.00
1494	0.61	0.06	0.61	0.45	0.07	0.00	0.40	0.02	0.00	0.00	0.05	0.00
1524	0.57	0.05	0.57	0.41	0.06	0.00	0.36	0.02	0.00	0.00	0.05	0.00
1554	0.79	0.13	0.79	0.66	0.15	0.00	0.62	0.05	0.00	0.00	0.12	0.00
1585	0.94	0.38	0.94	0.89	0.42	0.01	0.87	0.17	0.01	0.00	0.36	0.00
1615	0.95	0.45	0.95	0.91	0.49	0.01	0.90	0.21	0.01	0.00	0.43	0.00
1646	0.98	0.64	0.98	0.96	0.68	0.02	0.95	0.37	0.02	0.00	0.62	0.00
1676	0.99	0.80	0.99	0.98	0.82	0.05	0.98	0.56	0.05	0.00	0.78	0.00

Table 5.9. Future occupancy probabilities for *Eurycea wilderae* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1189	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1219	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1280	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1311	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1341	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1372	0.02	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1402	0.02	0.00	0.02	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1433	0.05	0.01	0.05	0.04	0.01	0.00	0.03	0.00	0.00	0.00	0.01	0.00
1463	0.04	0.01	0.04	0.03	0.01	0.00	0.03	0.00	0.00	0.00	0.01	0.00
1494	0.07	0.01	0.07	0.05	0.01	0.00	0.04	0.01	0.00	0.00	0.01	0.00
1524	0.06	0.01	0.06	0.04	0.01	0.00	0.04	0.01	0.00	0.00	0.01	0.00
1554	0.11	0.02	0.11	0.08	0.02	0.00	0.07	0.01	0.00	0.00	0.02	0.00
1585	0.21	0.04	0.21	0.15	0.05	0.00	0.14	0.02	0.00	0.00	0.04	0.00
1615	0.23	0.05	0.23	0.17	0.05	0.00	0.16	0.03	0.00	0.00	0.05	0.00
1646	0.32	0.07	0.32	0.25	0.08	0.00	0.23	0.04	0.01	0.00	0.07	0.00
1676	0.42	0.11	0.42	0.33	0.12	0.00	0.31	0.06	0.01	0.00	0.10	0.00

Table 5.10. Future occupancy probabilities for *Eurycea wilderae* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
945	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
975	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1036	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1067	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1097	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1128	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1158	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1189	0.02	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1219	0.01	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1250	0.02	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1280	0.02	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1311	0.01	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1341	0.02	0.00	0.02	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
1372	0.03	0.01	0.03	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00
1402	0.10	0.02	0.10	0.07	0.02	0.00	0.06	0.01	0.00	0.00	0.02	0.00
1433	0.12	0.02	0.12	0.08	0.02	0.00	0.08	0.01	0.00	0.00	0.02	0.00
1463	0.15	0.03	0.15	0.11	0.03	0.00	0.10	0.02	0.00	0.00	0.03	0.00
1494	0.25	0.05	0.25	0.19	0.06	0.00	0.17	0.03	0.01	0.00	0.05	0.00
1524	0.27	0.06	0.27	0.21	0.06	0.00	0.19	0.03	0.01	0.00	0.05	0.00
1554	0.51	0.15	0.51	0.42	0.16	0.00	0.39	0.08	0.02	0.00	0.14	0.00
1585	0.84	0.47	0.84	0.79	0.49	0.01	0.77	0.32	0.08	0.00	0.46	0.02
1615	0.67	0.26	0.67	0.59	0.27	0.01	0.56	0.16	0.03	0.00	0.25	0.02
1646	0.79	0.39	0.79	0.72	0.41	0.01	0.70	0.25	0.05	0.00	0.37	0.02
1676	0.87	0.53	0.87	0.82	0.55	0.02	0.81	0.38	0.09	0.00	0.52	0.02

Table 5.11. Future occupancy probabilities for *Plethodon montanus* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
945	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00
975	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1006	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1036	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1067	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1097	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1128	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1158	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1189	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1219	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1250	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1280	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1311	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1341	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1372	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1402	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1433	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1463	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1494	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1524	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1554	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1585	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1615	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1646	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1676	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 5.12. Future occupancy probabilities for *Plethodon montanus* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1189	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1219	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1250	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1280	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1311	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1341	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1372	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1402	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1433	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1463	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1494	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1524	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1554	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1585	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1615	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1646	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1676	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 5.13. Future occupancy probabilities for *Desmognathus orestes* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012

Elevation (m)	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
945	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00
975	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1006	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1036	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1067	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1097	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1128	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1158	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1189	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1219	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1250	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1280	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1311	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1341	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1372	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1402	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1433	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1463	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1494	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1524	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1554	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1585	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1615	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1646	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1676	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 5.14. Future occupancy probabilities for *Desmognathus orestes* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1189	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1219	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1250	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1280	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1311	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1341	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1372	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1402	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1433	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1463	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1494	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1524	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1554	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1585	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1615	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1646	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1676	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 5.15. Future occupancy probabilities for *Plethodon yonahlossee* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	0.95	0.97	0.95	0.96	0.97	0.00	0.96	0.98	0.99	0.00	0.97	0.00
1189	0.96	0.97	0.96	0.96	0.97	0.00	0.96	0.98	0.99	0.00	0.97	0.00
1219	0.96	0.98	0.96	0.96	0.98	0.00	0.97	0.98	0.99	0.00	0.98	0.00
1250	0.95	0.97	0.95	0.96	0.97	0.00	0.96	0.98	0.99	0.00	0.97	0.00
1280	0.95	0.97	0.95	0.95	0.97	0.00	0.95	0.97	0.98	0.00	0.97	0.00
1311	0.92	0.95	0.92	0.93	0.95	0.99	0.93	0.96	0.98	0.00	0.95	0.98
1341	0.92	0.95	0.92	0.93	0.95	0.99	0.93	0.96	0.98	0.00	0.95	0.98
1372	0.90	0.94	0.90	0.91	0.94	0.98	0.91	0.95	0.97	0.00	0.94	0.98
1402	0.89	0.93	0.89	0.90	0.93	0.98	0.90	0.94	0.97	0.00	0.93	0.98
1433	0.86	0.91	0.86	0.87	0.91	0.97	0.87	0.93	0.96	0.00	0.91	0.97
1463	0.87	0.92	0.87	0.88	0.92	0.98	0.88	0.93	0.96	0.00	0.92	0.97
1494	0.85	0.91	0.85	0.86	0.90	0.97	0.87	0.92	0.95	0.00	0.91	0.97
1524	0.85	0.91	0.85	0.87	0.91	0.97	0.87	0.92	0.95	0.00	0.91	0.97
1554	0.83	0.89	0.83	0.84	0.89	0.97	0.85	0.91	0.95	0.00	0.89	0.96
1585	0.79	0.87	0.79	0.81	0.86	0.96	0.81	0.89	0.93	0.99	0.87	0.96
1615	0.78	0.86	0.78	0.80	0.86	0.96	0.81	0.88	0.93	0.99	0.86	0.95
1646	0.76	0.84	0.76	0.78	0.84	0.95	0.78	0.87	0.92	0.98	0.85	0.95
1676	0.73	0.83	0.73	0.76	0.82	0.94	0.76	0.85	0.91	0.98	0.83	0.94

Table 5.16. Future occupancy probabilities for *Plethodon yonahlossee* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

Elevation (m)	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
945	0.97	0.98	0.97	0.97	0.98	0.00	0.97	0.98	0.00	0.00	0.98	0.00
975	0.94	0.96	0.94	0.95	0.96	0.00	0.95	0.97	0.99	0.00	0.97	0.00
1006	0.95	0.97	0.95	0.95	0.97	0.00	0.95	0.97	0.98	0.00	0.97	0.00
1036	0.94	0.97	0.94	0.95	0.96	0.00	0.95	0.97	0.98	0.00	0.97	0.00
1067	0.93	0.96	0.93	0.94	0.96	0.99	0.94	0.97	0.98	0.00	0.96	0.99
1097	0.92	0.95	0.92	0.93	0.95	0.99	0.93	0.96	0.98	0.00	0.95	0.99
1128	0.92	0.95	0.92	0.93	0.95	0.99	0.93	0.96	0.98	0.00	0.95	0.99
1158	0.92	0.95	0.92	0.93	0.95	0.99	0.93	0.96	0.98	0.00	0.95	0.98
1189	0.89	0.93	0.89	0.90	0.93	0.98	0.91	0.95	0.97	0.00	0.94	0.98
1219	0.91	0.95	0.91	0.92	0.94	0.98	0.92	0.95	0.97	0.00	0.95	0.98
1250	0.90	0.94	0.90	0.91	0.94	0.98	0.92	0.95	0.97	0.00	0.94	0.98
1280	0.89	0.94	0.89	0.90	0.93	0.98	0.91	0.95	0.97	0.00	0.94	0.98
1311	0.91	0.95	0.91	0.92	0.95	0.98	0.92	0.95	0.97	0.00	0.95	0.98
1341	0.89	0.93	0.89	0.90	0.93	0.98	0.90	0.94	0.97	0.00	0.93	0.98
1372	0.88	0.93	0.88	0.89	0.92	0.98	0.89	0.94	0.96	0.00	0.93	0.98
1402	0.83	0.90	0.83	0.85	0.89	0.97	0.85	0.91	0.95	0.00	0.90	0.97
1433	0.82	0.89	0.82	0.84	0.89	0.97	0.84	0.91	0.94	0.00	0.89	0.96
1463	0.81	0.88	0.81	0.83	0.88	0.96	0.83	0.90	0.94	0.99	0.88	0.96
1494	0.78	0.86	0.78	0.80	0.86	0.96	0.80	0.88	0.93	0.99	0.86	0.95
1524	0.77	0.85	0.77	0.79	0.85	0.95	0.80	0.88	0.92	0.99	0.86	0.95
1554	0.71	0.81	0.72	0.73	0.81	0.94	0.74	0.84	0.90	0.98	0.81	0.93
1585	0.60	0.72	0.60	0.63	0.72	0.90	0.63	0.76	0.84	0.97	0.72	0.89
1615	0.67	0.78	0.67	0.69	0.77	0.92	0.70	0.81	0.88	0.98	0.78	0.92
1646	0.63	0.74	0.63	0.65	0.74	0.91	0.66	0.78	0.86	0.97	0.75	0.90
1676	0.58	0.71	0.58	0.61	0.70	0.89	0.62	0.74	0.83	0.96	0.71	0.89

Table 5.17. Future occupancy probabilities for *Plethodon cinereus* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012

<u>Elevation</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1189	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1219	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1250	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1280	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1311	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1341	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1372	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1402	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1433	0.98	1.00	0.98	0.99	1.00	1.00	0.99	1.00	1.00	0.00	1.00	1.00
1463	0.99	1.00	0.99	0.99	1.00	1.00	0.99	1.00	1.00	0.00	1.00	1.00
1494	0.97	1.00	0.97	0.98	1.00	1.00	0.98	1.00	1.00	0.00	1.00	1.00
1524	0.98	1.00	0.98	0.98	1.00	1.00	0.99	1.00	1.00	0.00	1.00	1.00
1554	0.96	0.99	0.96	0.97	0.99	1.00	0.97	1.00	1.00	0.00	1.00	1.00
1585	0.90	0.99	0.91	0.93	0.98	1.00	0.94	0.99	1.00	1.00	1.00	1.00
1615	0.89	0.98	0.89	0.92	0.98	1.00	0.93	0.99	1.00	1.00	0.98	1.00
1646	0.83	0.97	0.83	0.88	0.97	1.00	0.89	0.99	1.00	1.00	0.97	1.00
1676	0.74	0.96	0.74	0.81	0.95	1.00	0.83	0.98	1.00	1.00	0.96	1.00

Table 5.18. Future occupancy probabilities for *Plethodon cinereus* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
945	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00
975	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1006	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1036	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1067	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1097	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1128	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1158	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1189	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1219	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1250	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1280	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1311	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1341	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1372	0.99	1.00	0.99	0.99	1.00	1.00	0.99	1.00	1.00	0.00	1.00	1.00
1402	0.96	0.99	0.96	0.97	0.99	1.00	0.98	1.00	1.00	0.00	0.99	1.00
1433	0.95	0.99	0.95	0.97	0.99	1.00	0.97	1.00	1.00	0.00	0.99	1.00
1463	0.93	0.99	0.93	0.96	0.99	1.00	0.96	1.00	1.00	1.00	0.99	1.00
1494	0.88	0.98	0.88	0.91	0.98	1.00	0.93	1.00	1.00	1.00	0.98	1.00
1524	0.86	0.96	0.86	0.90	0.98	1.00	0.91	1.00	1.00	1.00	0.98	1.00
1554	0.66	0.93	0.66	0.75	0.93	1.00	0.77	1.00	1.00	1.00	0.94	1.00
1585	0.24	0.70	0.24	0.32	0.68	1.00	0.35	0.97	1.00	1.00	0.71	0.99
1615	0.47	0.87	0.47	0.58	0.86	1.00	0.61	0.99	1.00	1.00	0.88	1.00
1646	0.32	0.77	0.32	0.41	0.76	1.00	0.44	0.98	1.00	1.00	0.78	1.00
1676	0.19	0.64	0.19	0.27	0.62	0.99	0.29	0.96	1.00	1.00	0.65	0.99

Table 5.19. Future occupancy probabilities for *Desmognathus quadramaculatus* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
945	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00
975	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1006	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1036	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1067	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1097	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1128	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1158	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1189	0.99	1.00	0.99	0.99	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1219	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1250	0.99	1.00	0.99	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1280	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1311	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1341	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1372	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1402	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1433	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1463	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1494	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1524	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1554	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1585	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1615	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1646	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1676	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5.20. Future occupancy probabilities for *Desmognathus monticola* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
945	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00
975	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1006	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1036	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1067	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1097	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1128	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1158	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1189	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1219	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1250	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00
1280	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1311	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1341	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1372	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1402	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1433	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1463	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1494	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1524	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1554	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1585	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1615	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1646	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1676	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5.21. Future occupancy probabilities for *Desmognathus quadramaculatus* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1189	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1219	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1250	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1280	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1311	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1341	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1372	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1402	0.99	1.00	0.99	0.99	1.00	1.00	0.99	1.00	1.00	0.00	1.00	1.00
1433	0.97	1.00	0.97	0.98	1.00	1.00	0.98	1.00	1.00	0.00	1.00	1.00
1463	0.98	1.00	0.98	0.99	1.00	1.00	0.99	1.00	1.00	0.00	1.00	1.00
1494	0.97	0.99	0.97	0.98	0.99	1.00	0.98	1.00	1.00	0.00	1.00	1.00
1524	0.97	1.00	0.97	0.98	1.00	1.00	0.98	1.00	1.00	0.00	1.00	1.00
1554	0.95	0.99	0.95	0.96	0.99	1.00	0.97	1.00	1.00	0.00	0.99	1.00
1585	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1615	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1646	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1676	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5.22. Future occupancy probabilities for *Desmognathus monticola* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1189	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1219	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1250	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1280	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
1311	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1341	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1372	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1402	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1433	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1463	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1494	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1524	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1554	0.98	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
1585	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1615	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1646	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1676	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5.23. Future occupancy probabilities for *Plethodon richmondi* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>		<u>2050</u>		<u>2070</u>							
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	0.64	0.78	0.64	0.67	0.78	0.00	0.68	0.82	0.90	0.00	0.79	0.00
1189	0.65	0.79	0.65	0.68	0.78	0.00	0.69	0.83	0.91	0.00	0.79	0.00
1219	0.69	0.82	0.69	0.72	0.81	0.00	0.72	0.85	0.92	0.00	0.82	0.00
1250	0.63	0.77	0.63	0.66	0.77	0.00	0.67	0.82	0.90	0.00	0.78	0.00
1280	0.59	0.75	0.59	0.63	0.74	0.00	0.64	0.79	0.88	0.00	0.75	0.00
1311	0.45	0.62	0.45	0.49	0.62	0.89	0.50	0.68	0.81	0.00	0.63	0.89
1341	0.44	0.61	0.44	0.48	0.61	0.89	0.49	0.67	0.81	0.00	0.62	0.88
1372	0.36	0.54	0.36	0.40	0.53	0.86	0.41	0.60	0.75	0.00	0.54	0.84
1402	0.34	0.51	0.34	0.37	0.50	0.84	0.38	0.57	0.73	0.00	0.52	0.83
1433	0.26	0.42	0.26	0.29	0.41	0.79	0.30	0.48	0.65	0.00	0.42	0.77
1463	0.28	0.45	0.28	0.31	0.44	0.80	0.32	0.51	0.67	0.00	0.45	0.79
1494	0.24	0.40	0.24	0.27	0.39	0.77	0.28	0.46	0.63	0.00	0.42	0.75
1524	0.25	0.41	0.25	0.28	0.40	0.78	0.29	0.47	0.64	0.00	0.41	0.76
1554	0.21	0.35	0.21	0.24	0.34	0.74	0.24	0.41	0.58	0.00	0.36	0.72
1585	0.16	0.28	0.16	0.19	0.28	0.67	0.19	0.34	0.51	0.90	0.29	0.65
1615	0.15	0.27	0.15	0.17	0.26	0.66	0.18	0.32	0.49	0.90	0.28	0.63
1646	0.13	0.24	0.13	0.15	0.23	0.62	0.16	0.29	0.45	0.88	0.24	0.59
1676	0.11	0.21	0.11	0.13	0.20	0.57	0.13	0.25	0.41	0.86	0.21	0.55

Table 5.24. Future occupancy probabilities for *Plethodon richmondi* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

Elevation (m)	2030				2050				2070			
	A1B		B1		A1B		B1		A1B		B1	
	CGCM	HAD	CGCM	HAD	CGCM	HAD	CGCM	HAD	CGCM	HAD	CGCM	HAD
945	0.72	0.84	0.72	0.75	0.83	0.00	0.76	0.87	0.00	0.00	0.85	0.00
975	0.55	0.72	0.55	0.59	0.71	0.00	0.60	0.76	0.87	0.00	0.72	0.00
1006	0.59	0.74	0.59	0.62	0.74	0.00	0.63	0.79	0.88	0.00	0.75	0.00
1036	0.56	0.72	0.56	0.59	0.72	0.00	0.61	0.77	0.87	0.00	0.73	0.00
1067	0.50	0.67	0.50	0.53	0.66	0.91	0.54	0.72	0.83	0.00	0.67	0.90
1097	0.46	0.64	0.46	0.50	0.63	0.90	0.51	0.69	0.82	0.00	0.64	0.89
1128	0.47	0.64	0.47	0.50	0.63	0.90	0.51	0.70	0.82	0.00	0.64	0.89
1158	0.45	0.63	0.45	0.49	0.62	0.90	0.50	0.68	0.81	0.00	0.63	0.89
1189	0.35	0.52	0.35	0.38	0.51	0.85	0.39	0.58	0.74	0.00	0.53	0.84
1219	0.41	0.58	0.41	0.44	0.58	0.88	0.45	0.64	0.78	0.00	0.59	0.87
1250	0.37	0.55	0.37	0.41	0.54	0.86	0.42	0.61	0.76	0.00	0.55	0.85
1280	0.35	0.53	0.35	0.39	0.52	0.85	0.40	0.59	0.74	0.00	0.53	0.84
1311	0.43	0.60	0.42	0.46	0.59	0.89	0.47	0.66	0.80	0.00	0.61	0.88
1341	0.34	0.51	0.34	0.37	0.50	0.84	0.38	0.58	0.73	0.00	0.52	0.83
1372	0.31	0.48	0.31	0.34	0.47	0.82	0.35	0.54	0.70	0.00	0.48	0.81
1402	0.22	0.36	0.22	0.24	0.35	0.74	0.25	0.42	0.59	0.00	0.36	0.72
1433	0.20	0.34	0.20	0.23	0.33	0.73	0.23	0.40	0.57	0.00	0.35	0.71
1463	0.19	0.32	0.19	0.21	0.31	0.70	0.22	0.37	0.55	0.66	0.32	0.68
1494	0.15	0.26	0.15	0.17	0.26	0.65	0.18	0.32	0.48	0.61	0.27	0.63
1524	0.15	0.19	0.15	0.16	0.25	0.64	0.17	0.31	0.47	0.60	0.26	0.62
1554	0.10	0.11	0.10	0.11	0.18	0.54	0.12	0.23	0.37	0.49	0.19	0.52
1585	0.06	0.15	0.06	0.06	0.10	0.38	0.07	0.13	0.24	0.34	0.11	0.36
1615	0.08	0.12	0.08	0.09	0.14	0.47	0.09	0.18	0.31	0.42	0.15	0.45
1646	0.06	0.09	0.06	0.07	0.12	0.41	0.08	0.15	0.26	0.37	0.12	0.39
1676	0.05	1.00	0.05	0.06	0.09	0.36	0.06	0.12	0.22	0.32	0.10	0.34

Table 5.25. Future occupancy probabilities for *Desmognathus fuscus* from Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
1158	0.89	0.94	0.89	0.91	0.94	0.00	0.91	0.95	0.98	0.00	0.94	0.00
1189	0.90	0.95	0.90	0.91	0.94	0.00	0.91	0.96	0.98	0.00	0.95	0.00
1219	0.91	0.95	0.92	0.92	0.95	0.00	0.93	0.96	0.98	0.00	0.95	0.00
1250	0.89	0.94	0.89	0.90	0.94	0.00	0.91	0.95	0.97	0.00	0.94	0.00
1280	0.88	0.93	0.88	0.89	0.93	0.00	0.89	0.95	0.97	0.00	0.93	0.00
1311	0.80	0.89	0.80	0.83	0.89	0.97	0.83	0.91	0.95	0.00	0.89	0.97
1341	0.80	0.89	0.80	0.82	0.88	0.97	0.83	0.91	0.95	0.00	0.89	0.97
1372	0.75	0.85	0.75	0.77	0.85	0.96	0.78	0.88	0.93	0.00	0.85	0.96
1402	0.73	0.84	0.73	0.75	0.83	0.96	0.76	0.87	0.93	0.00	0.84	0.96
1433	0.65	0.79	0.65	0.68	0.78	0.94	0.69	0.82	0.90	0.00	0.79	0.94
1463	0.68	0.80	0.68	0.71	0.80	0.95	0.71	0.84	0.91	0.00	0.81	0.95
1494	0.63	0.77	0.63	0.66	0.77	0.94	0.67	0.81	0.89	0.00	0.77	0.93
1524	0.64	0.78	0.64	0.67	0.77	0.94	0.68	0.82	0.89	0.00	0.78	0.94
1554	0.59	0.74	0.59	0.62	0.73	0.93	0.63	0.78	0.87	0.00	0.74	0.92
1585	0.52	0.68	0.52	0.55	0.67	0.91	0.56	0.73	0.84	0.98	0.68	0.90
1615	0.50	0.66	0.50	0.54	0.66	0.90	0.55	0.71	0.83	0.97	0.67	0.89
1646	0.46	0.63	0.46	0.50	0.62	0.89	0.51	0.68	0.80	0.97	0.63	0.88
1676	0.42	0.59	0.42	0.46	0.58	0.87	0.47	0.64	0.78	0.97	0.59	0.86

Table 5.26. Future occupancy probabilities for *Desmognathus fuscus* from Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012.

<u>Elevation (m)</u>	<u>2030</u>				<u>2050</u>				<u>2070</u>			
	<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>		<u>A1B</u>		<u>B1</u>	
	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>	<u>CGCM</u>	<u>HAD</u>
945	0.93	0.96	0.93	0.93	0.96	0.00	0.94	0.97	0.00	0.00	0.96	0.00
975	0.86	0.92	0.86	0.87	0.92	0.00	0.88	0.94	0.98	0.00	0.92	0.00
1006	0.87	0.93	0.87	0.89	0.93	0.00	0.89	0.94	0.97	0.00	0.93	0.00
1036	0.86	0.92	0.86	0.88	0.92	0.00	0.88	0.94	0.97	0.00	0.93	0.00
1067	0.83	0.91	0.83	0.85	0.90	0.98	0.85	0.92	0.97	0.00	0.91	0.98
1097	0.81	0.89	0.81	0.83	0.89	0.98	0.84	0.91	0.96	0.00	0.90	0.97
1128	0.81	0.90	0.81	0.83	0.89	0.98	0.84	0.92	0.95	0.00	0.90	0.97
1158	0.81	0.89	0.81	0.83	0.89	0.97	0.83	0.91	0.95	0.00	0.89	0.97
1189	0.74	0.84	0.74	0.76	0.84	0.96	0.77	0.87	0.93	0.00	0.85	0.96
1219	0.78	0.87	0.78	0.80	0.87	0.97	0.81	0.90	0.94	0.00	0.87	0.97
1250	0.75	0.86	0.75	0.78	0.85	0.97	0.79	0.88	0.94	0.00	0.86	0.96
1280	0.74	0.85	0.74	0.76	0.84	0.96	0.77	0.87	0.93	0.00	0.85	0.96
1311	0.79	0.88	0.79	0.81	0.88	0.97	0.82	0.90	0.95	0.00	0.88	0.97
1341	0.73	0.84	0.73	0.76	0.84	0.96	0.76	0.87	0.93	0.00	0.84	0.96
1372	0.70	0.82	0.70	0.73	0.82	0.95	0.74	0.85	0.92	0.00	0.82	0.95
1402	0.60	0.74	0.60	0.63	0.74	0.93	0.64	0.79	0.88	0.00	0.75	0.93
1433	0.58	0.73	0.58	0.61	0.72	0.93	0.62	0.77	0.87	0.00	0.73	0.92
1463	0.55	0.71	0.55	0.59	0.70	0.92	0.60	0.75	0.86	0.98	0.71	0.91
1494	0.50	0.66	0.50	0.53	0.65	0.90	0.54	0.71	0.82	0.97	0.66	0.89
1524	0.49	0.65	0.49	0.52	0.64	0.89	0.53	0.70	0.82	0.97	0.65	0.89
1554	0.39	0.56	0.39	0.42	0.54	0.85	0.43	0.61	0.75	0.96	0.56	0.84
1585	0.26	0.40	0.26	0.29	0.40	0.76	0.29	0.46	0.62	0.93	0.41	0.74
1615	0.33	0.49	0.33	0.36	0.48	0.82	0.37	0.55	0.70	0.95	0.50	0.80
1646	0.28	0.44	0.28	0.31	0.43	0.78	0.32	0.49	0.65	0.94	0.44	0.77
1676	0.24	0.38	0.24	0.27	0.38	0.74	0.28	0.44	0.60	0.92	0.39	0.73

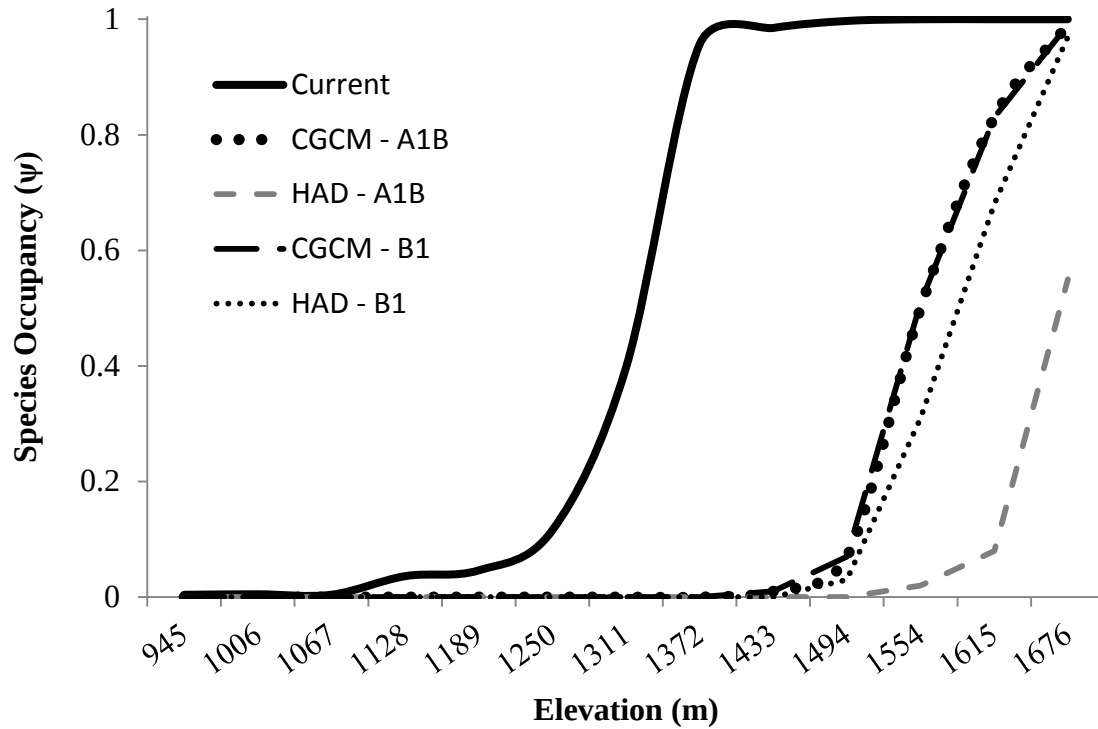


Figure 5.1. Future occupancy probabilities for *Plethodon welleri* by 2030 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

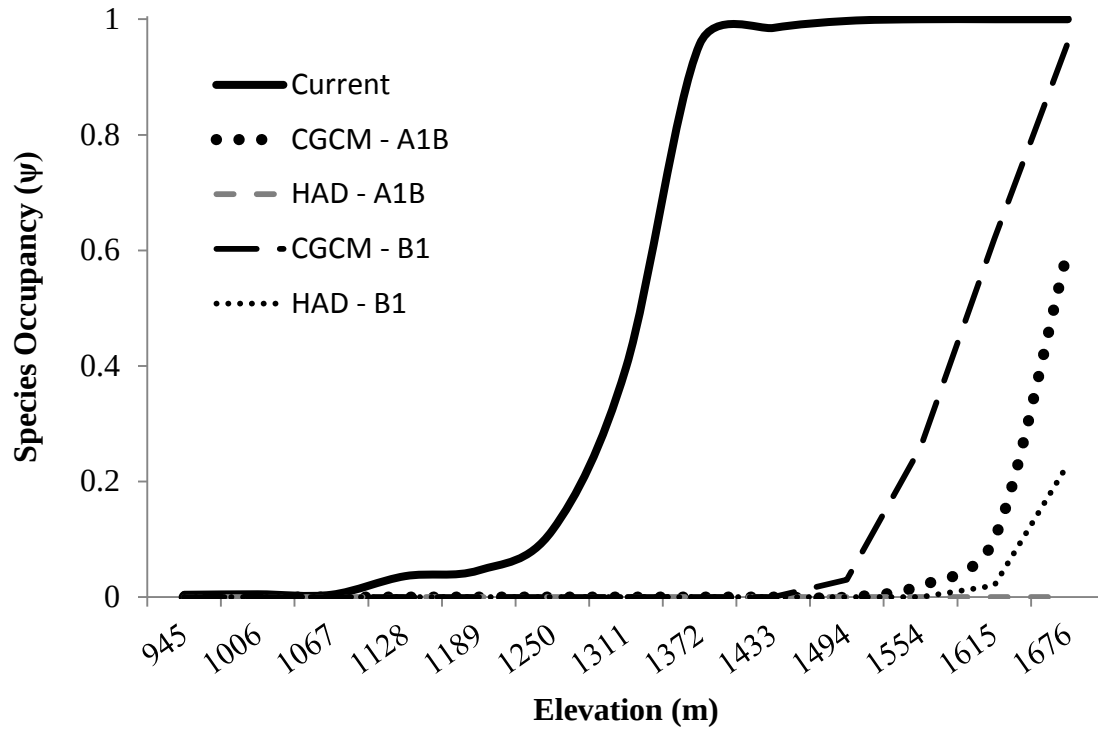


Figure 5.2. Future occupancy probabilities for *Plethodon welleri* by 2050 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

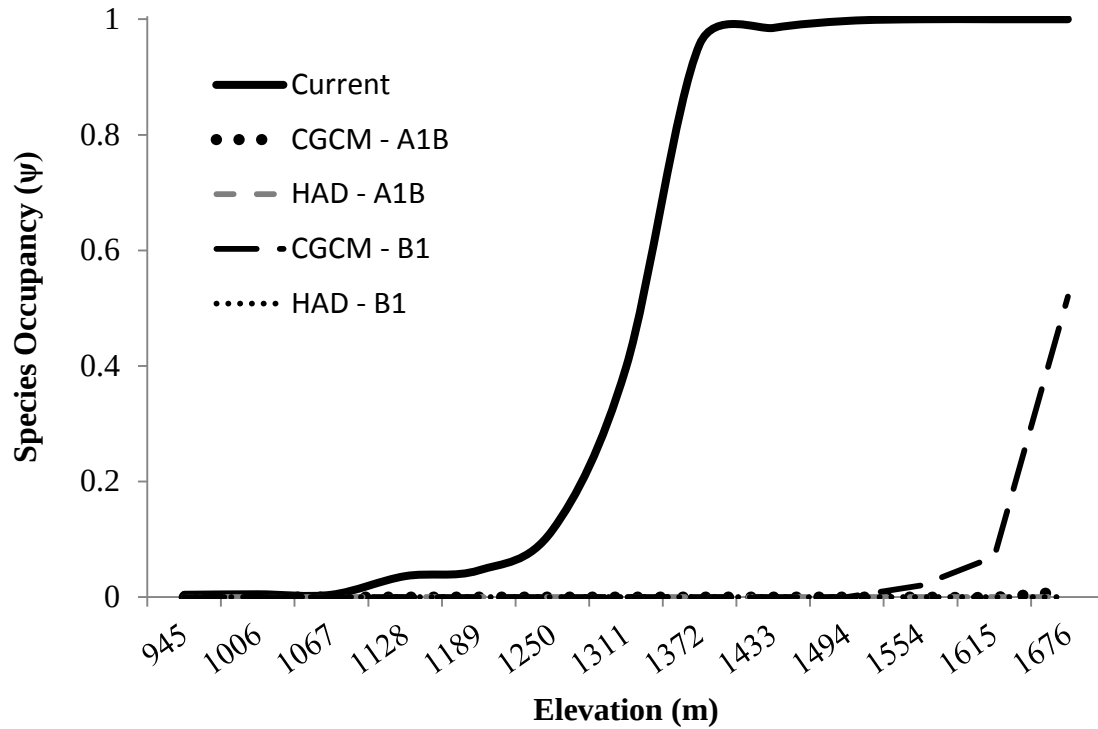


Figure 5.3. Future occupancy probabilities for *Plethodon welleri* by 2070 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

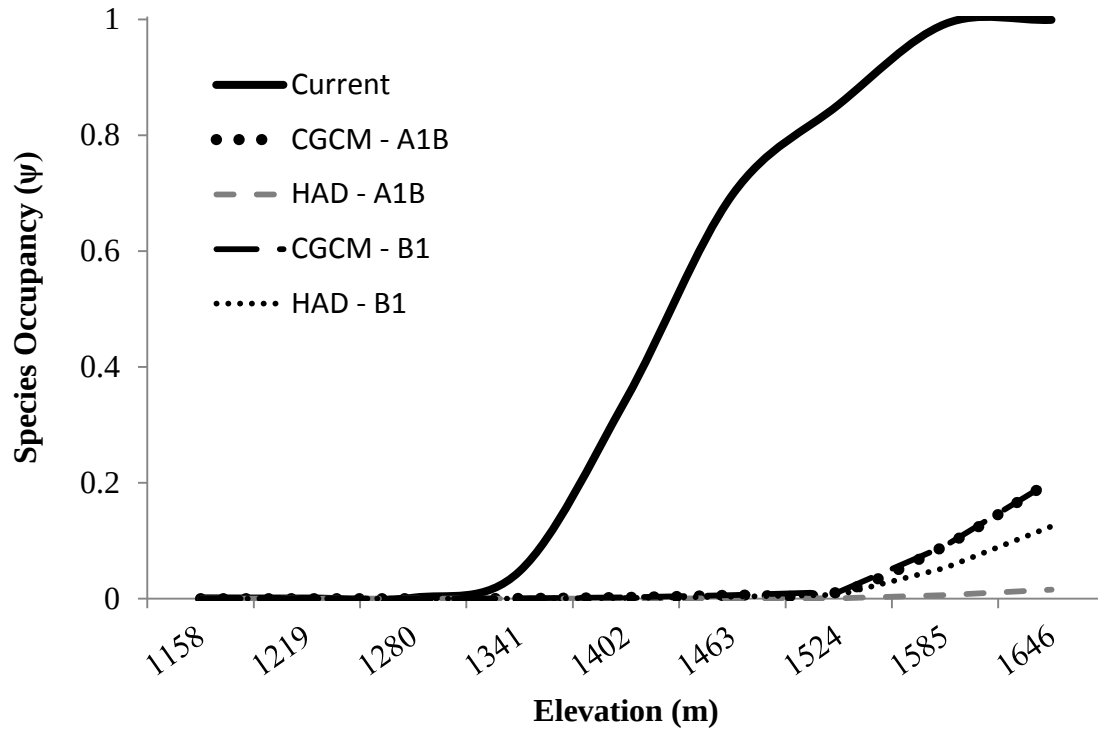


Figure 5.4. Future occupancy probabilities for *Plethodon welleri* by 2030 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

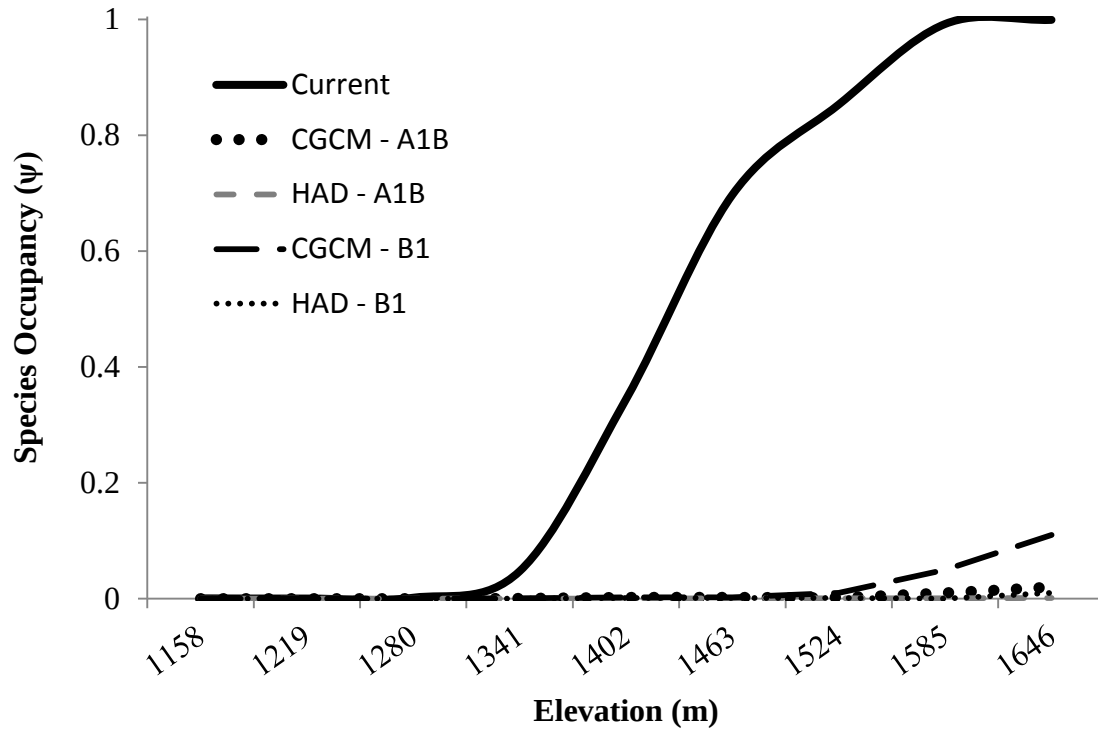


Figure 5.5. Future occupancy probabilities for *Plethodon welleri* by 2050 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

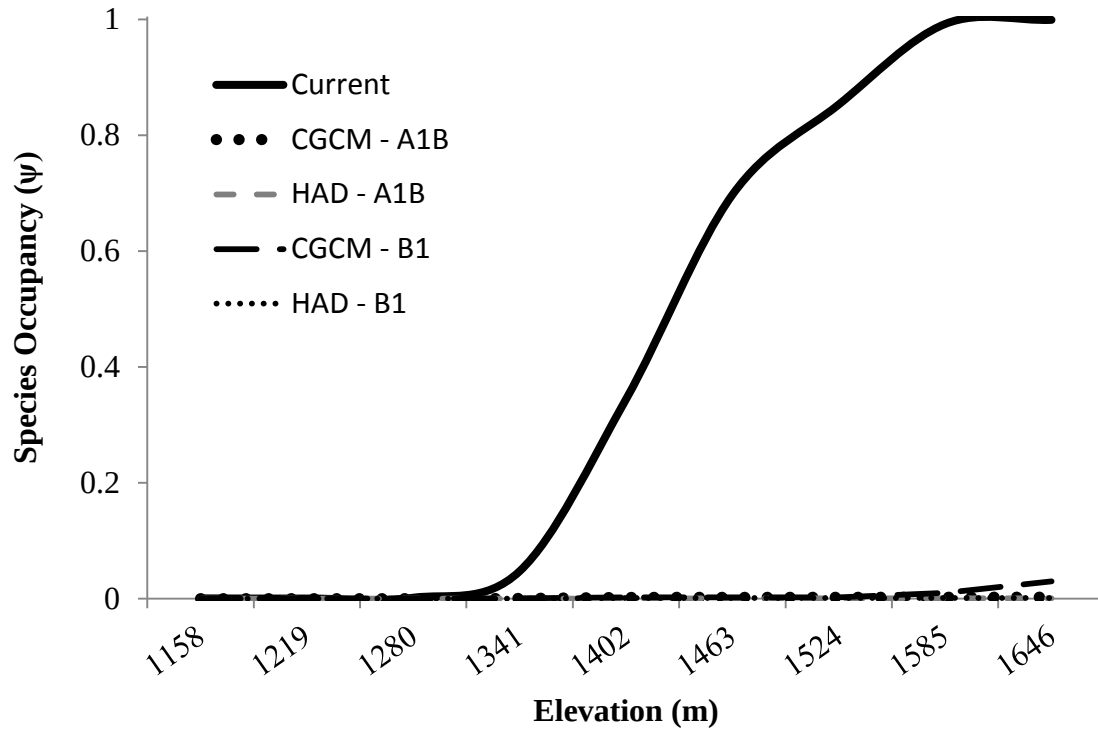


Figure 5.6. Future occupancy probabilities for *Plethodon welleri* by 2070 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

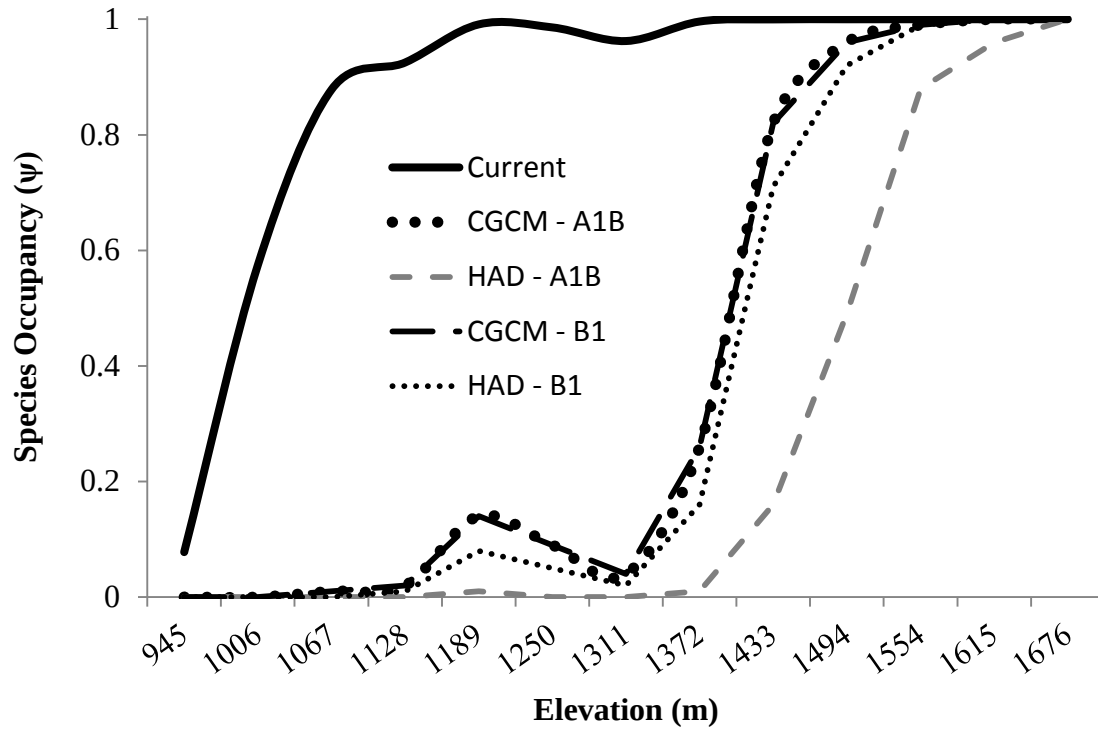


Figure 5.7. Future occupancy probabilities for *Desmognathus organi* by 2030 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

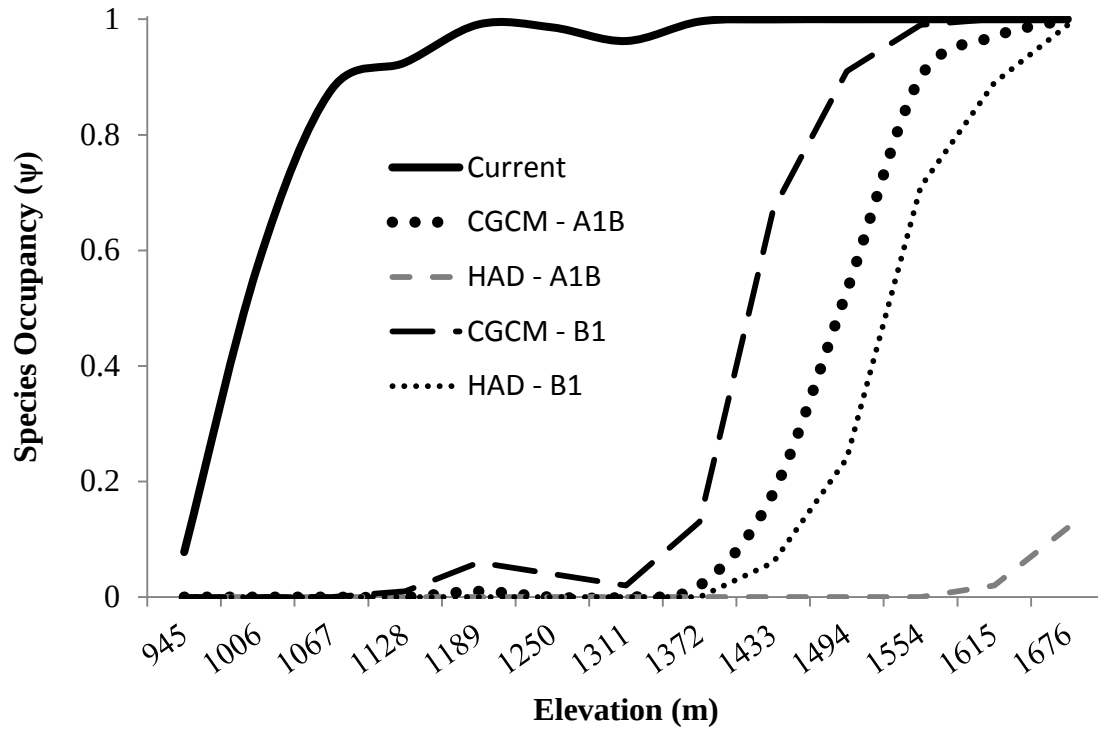


Figure 5.8. Future occupancy probabilities for *Desmognathus organi* by 2050 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

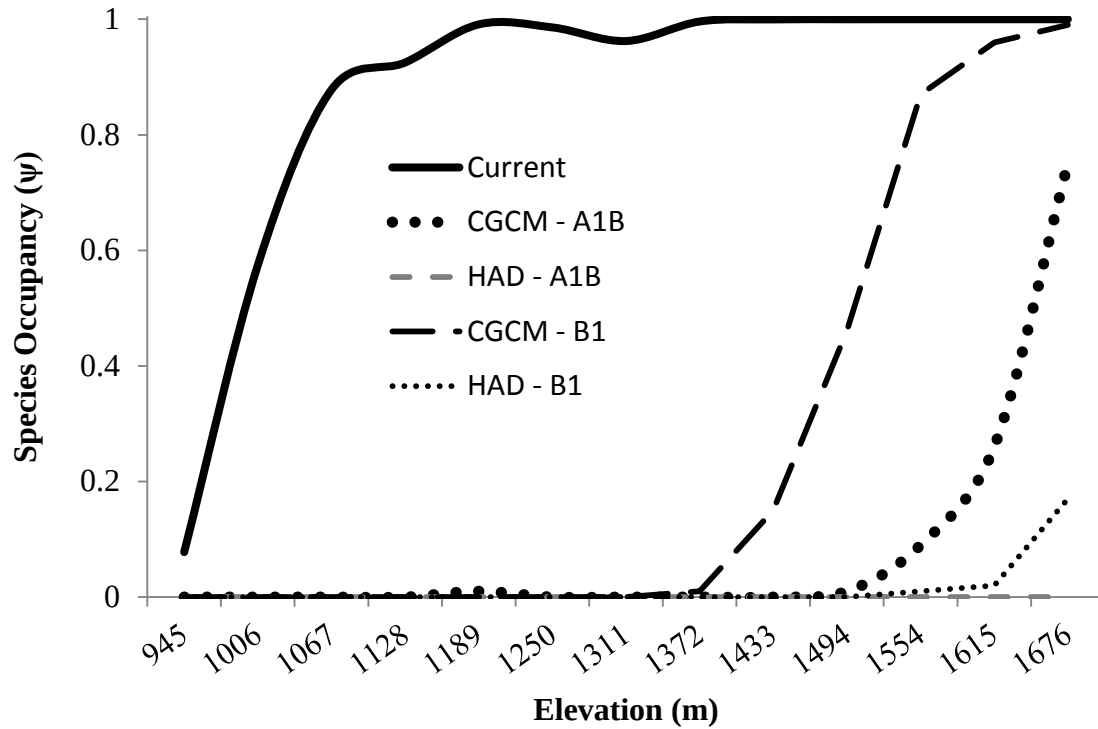


Figure 5.9. Future occupancy probabilities for *Desmognathus organi* by 2070 on Dells Branch (north slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

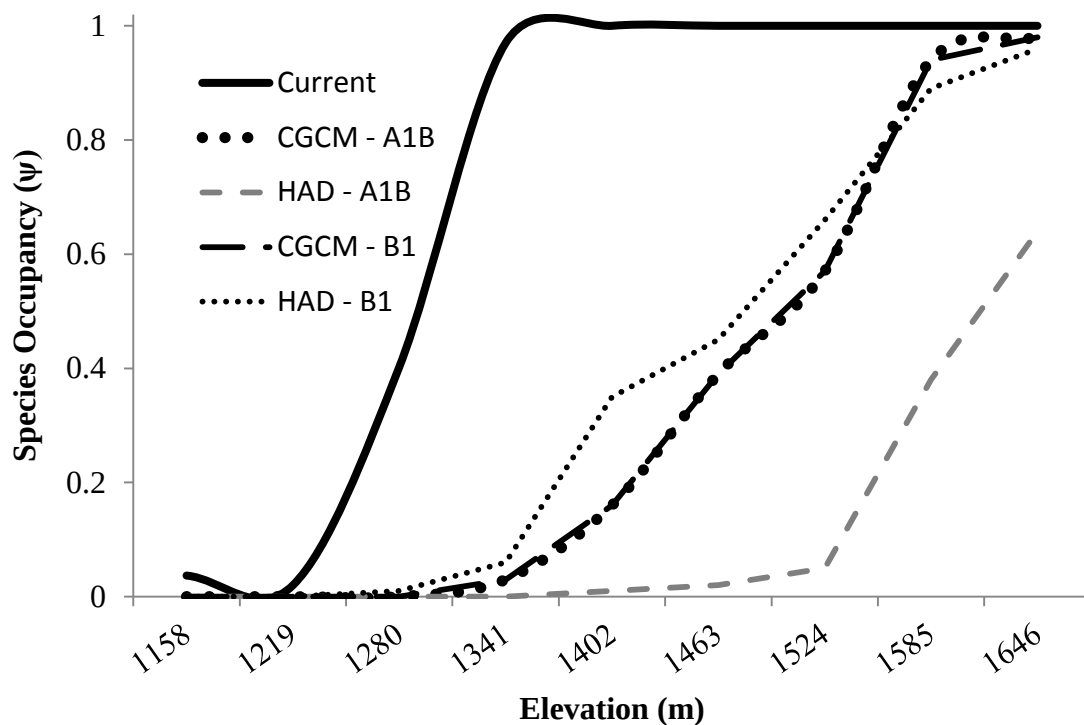


Figure 5.10. Future occupancy probabilities for *Desmognathus organi* by 2030 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

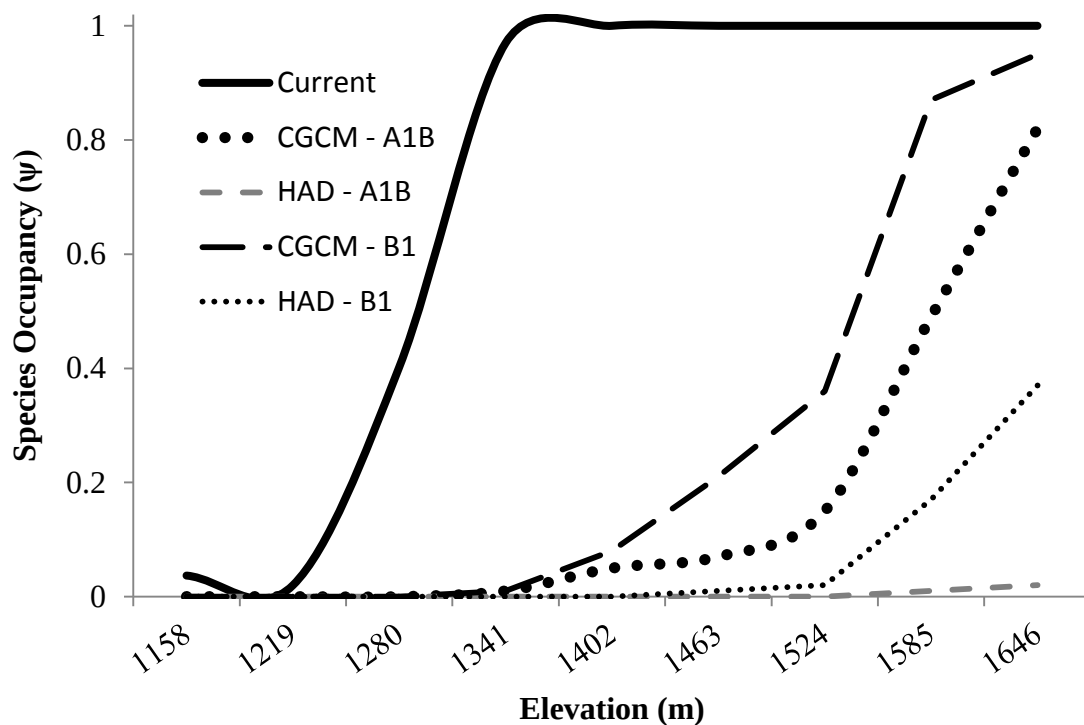


Figure 5.11. Future occupancy probabilities for *Desmognathus organi* by 2050 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

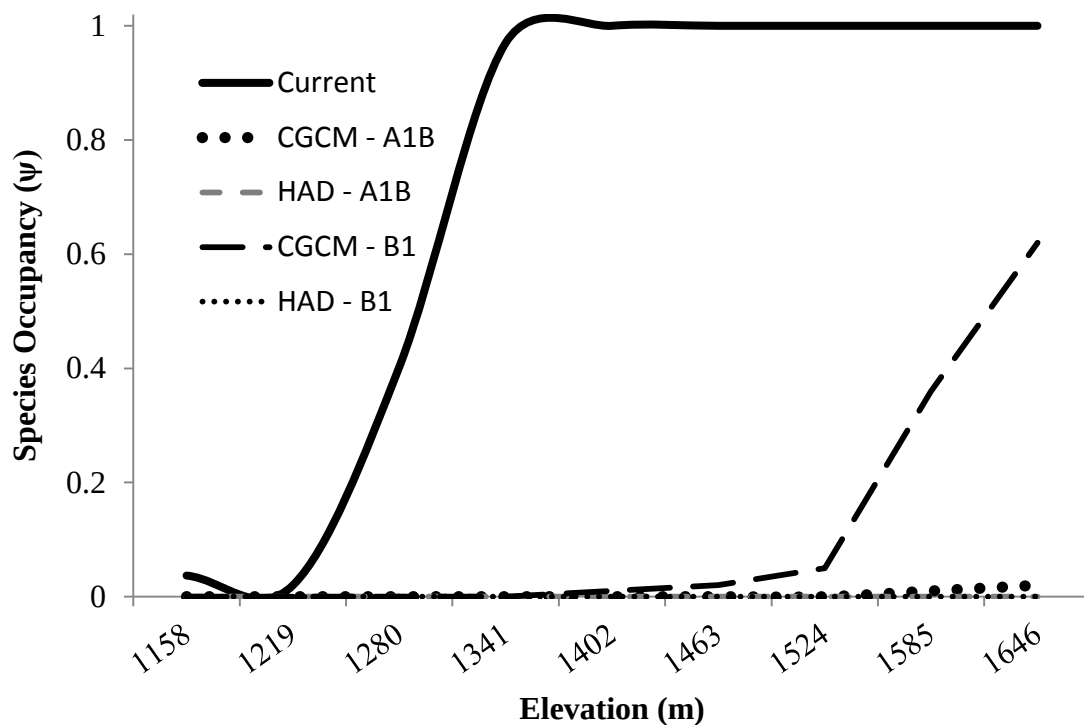


Figure 5.12. Future occupancy probabilities for *Desmognathus organi* by 2070 on Whitetop Creek (south slope of Whitetop Mountain, Virginia) as calculated from current occupancy probabilities established from July 2009- January 2012. Probabilities established from climate predictions utilizing the Canadian Center for Climate Modeling and Analysis Coupled Global Climate Model and the Hadley Centre for Climate Prediction and Research Model using A1B and B1 emission scenarios.

CONCLUSIONS

The goal of this dissertation was to examine the impacts of human land use, mercury contamination, and climate change on Southern Appalachian Plethodontid salamanders. We first investigated the impact of power line right-of-way mowing on four-toed salamanders. We found mowing of ROWs negatively impacted both four-toed salamander embryonic and larval survival. Embryonic survival significantly decreased in the ROW for 3 years after mowing compared to adjacent nests within the forest. Larval survival in ROW mesocosm pools was significantly less than pools at the edge or within the forest for 2 years after mowing. The greatest impact was found in pools 9 m into the ROW. After 4 years of regrowth, pools 9 m into the ROW had greater survival than pools in the annual mowing treatment. Four-toed salamander nesting success was reduced for up to 3 years during the current 5 year mowing cycle.

We then investigated the potential of mercury contamination in black-bellied salamanders residing at high elevations on Whitetop Mountain. Black-bellied salamanders, serving as apex predators, had elevated Hg tissue levels. We found black-bellied salamander liver and tail muscle tissue to be equal and even greater than levels in other herpetofauna from known point sources and areas with atmospheric deposition. If black-bellied salamanders on Whitetop Mountain could be consumed by humans, they would fall within Environment Protection Agency (USEPA) mercury human consumption guidelines. Additionally, levels also fell within the mammalian piscivorous predator warnings. Black-bellied salamanders from the northern slope, which receives the predominate wind direction, had significantly greater Hg levels. To establish a non-lethal sampling method for black-bellied salamanders, we established an association between liver and tail total Hg levels. Based on the rate of change we observed in preserved salamander tissue, we feel preserved museum specimens can provide an accurate

approximation of past mercury levels. When current black-bellied salamanders were compared to museum specimens collected over 50 years ago, mercury levels appear to have significantly declined.

To determine if Plethodontid salamanders have experienced range changes in the past 50 years, we compared current salamander distribution with those from 50 and 20 years ago within the Mount Rogers National Recreation Area (MRNA). We investigated regional climatic data to determine if climate change could be influencing range changes. We were not able to detect any statistical differences in regional mean and maximum temperatures in addition to mean precipitation since 1948. *Plethodon welleri* expanded its lower range limit since 1950s and 1990s surveys, but *P. cylindraceus* contract both its upper and lower range limits. *Plethodon yonahlossee* expanded both lower and upper range limits from historic surveys. Occupancy modeling indicated that members of the genus *Desmognathus* experienced no range changes in either historical sampling period. Our results suggest minimal range changes for Plethodontid salamander in the past 50 years.

Limited data exists for the association of vegetation characteristics and abiotic factors and Plethodontid salamander occupancy. Therefore, we investigated which factors best explained occupancy for Plethodontid salamanders within the Mount Rogers National Recreation Area. *Plethodon welleri* and *Desmognathus organi*, both species associated with spruce forest, were found to have the greatest association with elevation and not vegetation type. This suggests that climatic conditions might be a better predictor than vegetation type. Occupancy probabilities for habitat generalists, *Plethodon montanus* and *Desmognathus orestes* were also associated with elevation. Members of the *Desmognathus* genus had the greatest association with the presence

of water. Our results indicated that for most Plethodontid salamander on Whitetop Mountain, abiotic factors were most associated with occupancy.

Lastly, to determine the potential of impact of climate change on Plethodontid salamanders within the MRNA, we modeled current occupancy with mean January and July temperatures. Based on climate projections from the Canadian Centre for Climate Modeling and Analysis Coupled Global Climate Model (CGCM) and the Hadley Centre for Climate Prediction and Research (HAD) global circulation models with A1B and B1 CO₂ emission projections, we modeled future ranges. By 2070, *Plethodon welleri*, *Desmognathus organi*, and *Eurycea wilderae* would be extirpated within the Mount Rogers National Recreation Area. All other Plethodontid salamanders currently residing within the MRNA would still persist, but many would be required to shift upslope to maintain their desired thermal niche.

The results from this dissertation indicated Plethodontid salamanders are being impacted from anthropogenic factors in the Southern Appalachian Mountains. Habitat loss and change due to right-of-way mowing, mercury contamination, and future climate change will impact these salamanders in the future. Additionally, we identified many abiotic and some vegetation factors that influence Plethodontid salamander occupancy, which will provide land managers with a tool to potentially identify salamander communities in areas that had not been surveyed. We hope future work will expand on these projects to better understand the impact on populations and provide potential mitigation efforts.

VITA

Michael Kevin Hamed was born and raised in Bristol, TN. From an early age, Kevin was always interested in nature, especially herpetofauna. He earned his bachelor's of science degree in biology from Tennessee Technological University in 1995. After graduating, Kevin served as the Steele Creek Park Nature Center Manager for the City of Bristol, TN. Shortly after accepting his position, Kevin began a research program to study Southern Appalachian amphibians. While working full-time, Kevin earned his master's of science degree in biology from East Tennessee State University. His thesis focused on the life history of the Tennessee dace. In 2003, Kevin accepted a position as a biology instructor at Virginia Highlands Community College in Abingdon, VA. Kevin incorporated his research with amphibians, fish, and small mammals into his undergraduate teaching allowing him to expose students to natural history. At the 2008 Southeastern Partners in Amphibian and Reptile Conservation Conference, Kevin and Dr. Matt Gray discussed Ph.D. opportunities at the University of Tennessee. Under Dr. Gray's guidance, Kevin began his course work for his Ph.D. in August 2008, but his dissertation research began in the spring of 2008. During the 2009-10 academic year, Kevin was in residence at the University of Tennessee after being awarded the Virginia Community College Systems Chancellor's Faculty Fellowship. Kevin was promoted to Associate Professor of Biology in 2010 and remains on the faculty at Virginia Highlands Community College.