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ASSESSING CLIMATE CHANGE EFFECTS ON COMPETITIVE INTERACTIONS
OF THE FEDERALLY ENDANGERED SHENANDOAH SALAMANDER
(Plethodon shenandoah)

By

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A thesis Presented to the faculty of Towson University

in partial fulfillment of the requirements for the degree Master of Science

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August, 2013

TOWSON UNIVERSITY
OFFICE OF GRADUATE STUDIES
THESIS APPROVAL PAGE

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OF THE FEDERALLY ENDANGERED SHENANDOAH SALAMANDER
(PLETHODON SHENANDOAH)**

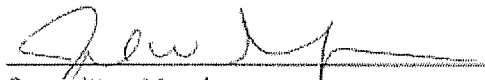
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ACKNOWLEDGEMENTS

My deepest gratitude is extended to Dr. Evan H. Grant for making this thesis research possible, and providing financial support in addition to exceptional guidance. I also extend my sincere gratitude to Dr. Joel Snodgrass for his adaptability and willingness to work around difficult situations, guidance, and inspiration. Additionally, I extend my sincere thanks to Dr. Don Forester for his expertise and counsel.

I thank Jeb Wofford at Shenandoah National Park for providing funding for this project and allowing access to U.S.D.I. National Park lands. I also thank Betty Gatewood and Brett Raeburn at Shenandoah National Park for helping integrate this project into their public outreach efforts. Special thanks to Matt Evans, Brian Gratwicke, James Murphy, Jennifer Sevin, Kimberly Terrell, and Rick Quintero of the Smithsonian Conservation Biology Institute and National Zoological Park for providing experimental resources, guidance, and logistical support whenever needed. Amber Wiewel, David Miller, Anna Deyle, Lacy Rucker, Addison Reid, and Tom Maigret helped collect salamanders in addition to assisting with experimental procedures. Anthony Dallalio, David Nielson, Kayla Nielson, Anatole Suttchenko, Mary Suttchenko, and Toly Suttchenko helped construct the mesocosms. Climatic data used for developing experimental conditions were provided by Steffan De Wekker and Temple Lee at the University of Virginia. Christina Hoh and Garrett Sisson deserve special thanks for their critical roles in assisting with the daily operation of experimental procedures and data collection. Finally, special thanks to Adrienne Brand for guidance and assisting with every aspect of this project.

ABSTRACT

The Shenandoah salamander (*Plethodon shenandoah*) is a federally endangered species with a restricted distribution at high elevation in Shenandoah National Park, presumably limited by competition with the red-backed salamander (*Plethodon cinereus*). Recent research suggests that climate change may exacerbate interspecific competitive interactions at a cost to *P. shenandoah*. An effort to evaluate *P. shenandoah* extinction risk and management options identified uncertainties that must be addressed in order to make a management decision, including the response of *P. shenandoah* to climate change. I investigated the influence of climate on competitive interactions in 3-dimensional mesocosms that mimicked realistic habitats in two phases: (1) a pilot study to ensure the suitability of the experimental venue, and (2) a primary study to investigate the response of *P. shenandoah* to competition with *P. cinereus* under two future climate scenarios. The results suggest that core *P. shenandoah* populations may decline in response to increasing temperatures, particularly if their habitat is getting warmer and drier, though range expansion may occur in areas where it competes with *P. cinereus*.

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CHAPTER 1

ADDRESSING A CRITICAL SCIENCE NEED FOR MANAGEMENT OF A
FEDERALLY ENDANGERED SALAMANDER

The federally endangered Shenandoah salamander (*Plethodon shenandoah*) is found only within the boundaries of Shenandoah National Park, Virginia, and its potential range consists of approximately six square kilometers of northwest facing talus slopes on three of the highest mountains in the park (Highton and Worthington, 1967; Jaeger 1970, 1971a, 1971b; Carpenter et al., 2001). *Plethodon shenandoah* is one of 55 recognized North American species in the Woodland Salamander genus *Plethodon*, a group of salamanders that are among the most abundant vertebrates in forests where they occur (Highton, 2005). *Plethodon* belongs to the lungless salamander family Plethodontidae and many species in the genus, such as *P. shenandoah*, are fully terrestrial with direct development. Plethodontid salamanders depend on cutaneous respiration, relying on moist environmental conditions to permit efficient gas exchange (Feder, 1983; Grover, 1998). A consequence of breathing through the skin is a great potential for water loss, the rate of which depends on environmental conditions and increases with both rising temperatures and decreasing humidity (Feder, 1983). Therefore, the activity patterns of *Plethodon* are limited under dry conditions (Feder, 1983). Accordingly, *P. shenandoah* presence has been found to be strongly influenced by elevation and aspect, presumably in relation to temperature and moisture gradients and associated central and southern Appalachian high elevation forest types (Jaeger, 1971b).

Highton (1995, 2012) hypothesized that most of the species of eastern *Plethodon* arose as a result of isolation on cool, moist mountaintops during the Pliocene Epoch (5.3–

1.8 mya) when warm, dry conditions transformed lowland forests into arid grasslands. These speciation events resulted in four eastern species groups, each with many closely related extant species. *Plethodon shenandoah* is one of 10 known species that form the *Plethodon cinereus* group, which is believed to be the most morphologically primitive group of the eastern *Plethodon*. Species such as the red-backed salamander (*P. cinereus*) appear to have expanded their range dramatically since the Pleistocene, exemplified by its northern spread into territory that was under the last maximum continental ice sheet 21,000 years ago. Other species appear to have persisted only in their restricted mountain habitats. Their ranges currently remain limited, most with distributions that are completely surrounded by *P. cinereus*, as is the case with *P. shenandoah*. Thus, the limited distributions of species, such as *P. Shenandoah*, appear to be a product of changing climate conditions and, more recently, competition with *P. cinereus*.

Evidence suggests that plethodontid salamanders employ an interference competition strategy to defend space associated with conditions that promote optimal foraging (Fraser, 1976; Jaeger, 1981; Wrobel et al., 1980; Maerz and Madison, 2000). Terrestrial salamanders spend a disproportionately greater amount of time in underground retreats than on the surface (Bailey et al., 2004), most likely to avoid desiccation threats at the surface (e.g., wind and solar radiation). When environmental conditions are suitable, plethodontid salamanders move from sub-surface retreats to forage in the forest litter where prey is more abundant than in underground spaces (Fraser, 1976). Thus, *Plethodon* competitive interactions appear to involve territorial behavior that extends from surface to sub-surface habitats. A fair amount of research has investigated the nature of competition between *P. shenandoah* and *P. cinereus* (see summary and citations

in Highton, 1988) and several studies have suggested that the *P. shenandoah* is declining due to competition with *P. cinereus* (Jaeger 1970, 1971b, 1974; Griffiths and Jaeger, 1998). However, few studies have investigated the interaction between climate factors and competition in salamander population dynamics (Cunningham et al., 2009), though it is believed that a coaction between abiotic factors and biotic interactions may alter species densities and distributions (Dunson and Travis, 1991; Bullock et al., 2000; Guo et al., 2005). Even fewer studies have attempted to investigate *Plethodon* behavior in three-dimensional space involving surface and sub-surface habitat.

Climate change is expected to result in temperature and humidity alterations in the Mid-Atlantic in the next few decades and global climate models generally predict warmer and wetter conditions (Hawkins and Smith, 2011), though predictions vary greatly among models (Polsky et al., 2000). Significant changes in climate conditions may have negative consequences for species adapted to high elevation habitats, such as *P. shenandoah* (Highton, 1995). Climate change threats are compounded by the limited range and ability to migrate among mountaintops of many high elevation species. Further, behavioral and physiological functions are influenced by temperature and moisture, and fitness related factors such as foraging activity appear to be limited by environmental conditions, making high-elevation species vulnerable to changes in climate conditions (Deutsch et al., 2008).

The extraordinarily limited distribution of *P. shenandoah* is completely within the boundaries of Shenandoah National Park and National Park Service managers are obligated by federal policy to choose among management actions (including a no action alternative) that might mitigate the effects of potential threats, such as climate change.

The Endangered Species Act (1973) obligates the Park to conserve and restore federally listed species and their ecosystems. Moreover, The National Park Service Organic Act (1916) obligates the preservation of all National Park resources, while providing public access to these resources and keeping them unimpaired for future generations.

Additionally, the National Environmental Policy Act (1969) requires environmental assessments for many federal actions, including impacts of federal management activities. Because meeting the requirements associated with each of these obligations simultaneously can be difficult, resource managers at Shenandoah National Park have initiated a modeling and decision analysis framework that provides a method for developing optimal management strategies for the Shenandoah salamander population.

The framework generally involves identifying management alternatives, investigating the consequences for each alternative using predictive modeling, and analyzing the trade-offs to inform a management decision. Preliminary modeling results indicated that climate change is likely to threaten *P. shenandoah*; populations may decline to low levels, posing an imminent extinction risk. The decision analysis framework is an iterative process, however, and uncertainties remain that must be addressed and incorporated via revisions so that a satisfactory decision can be made. Monitoring and experimental procedures were identified as mechanisms for reducing uncertainty in the relationship between climate variables, competition with *P. cinereus*, changes in distribution of *P. shenandoah*, and the effect of management on *P. shenandoah* persistence.

The purpose of my research was to utilize experimental procedures to address uncertainty in the nature of *P. shenandoah* competitive interactions, particularly with respect to how climate variables (temperature and humidity) may influence competition

with *P. cinereus*. The results of this study will be incorporated into the modeling and decision analysis framework to reduce uncertainty concerning the ecology of *P.*

shenandoah, thereby aiding the process of developing optimal management strategies for the Shenandoah Salamander. Further, this study addressed several recovery tasks described in the *P. shenandoah* Recovery Plan (U.S. Fish and Wildlife Service, 1994) including: task # 3-continue support studies defining relationship/interactions of *P. cinereus* and *P. shenandoah*; task # 17-study abiotic factors affecting distribution; task # 18-study behaviors and activity cycles relevant to management.

In this study, I employed a novel approach to making inferences concerning competition among terrestrial salamander species that incorporates the influence of abiotic factors on competitive interactions among paired individuals within a three-dimensional habitat setting. Because there is a lack of robust research methods in past work on *P. shenandoah*, particularly concerning biotic interactions with *P. cinereus* and the influence that abiotic factors may have on those interactions, I strived for a controlled experimental venue with a primary focus on maintaining interactions in a three-dimensional environment more consistent with conditions in the field. Using a transparent mesocosms coupled with projected climate conditions for high elevation areas in Shenandoah National Park, my objective was to investigate how the ecological relationship between *P. shenandoah* and *P. cinereus* may be altered under future climate change in three-dimensional habitat. To my knowledge, this appears to be the first experiment using vertical habitat mesocosms to investigate competitive interactions among terrestrial salamanders. Because there was a lack of precedent for the suitability of the venue to house salamanders, I tested the response of two *P. cinereus* color

morphotypes to competition under two climate scenarios in the mesocosms before investigating interactions between *P. cinereus* and *P. shenandoah*. The pilot study demonstrated that the experimental venue was suitable for pairs of salamanders over a significant period of time (i.e., 90 days), and it is covered in chapter 2 of this thesis. Chapter 3 covers the primary experiment investigating the response of *P. shenandoah* in terms of growth and behavior to competition with *P. cinereus* under two future climate scenarios.

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CHAPTER 2

RECENT WARMING TRENDS ARE NOT ENOUGH TO AFFECT COLOR MORPH FREQUENCIES IN A HIGH ELEVATION PLETHODON CINEREUS POPULATION

ABSTRACT

Differential adaptive responses to selection pressures may result in fitness differences among color polymorphs in a population. Specifically, polymorphisms may be maintained in a population if each morph has a competitive advantage in a specific part of the species' range. If these competitive advantages are related to differences in climatic conditions, then climate change could lead to changes in morph frequencies or loss of specific morphs and ultimately genetic diversity. I investigated the response of two red-backed salamander (*Plethodon cinereus*) color morphs to competition, in terms of growth and behavior, under two climate scenarios in 3-dimensional mesocosms that mimicked realistic habitats. The results indicated that surface activity and growth were not affected by temperature. Surface activity was mediated by competition however, lending support to behavioral differentiation as a possible mechanism for maintenance of color polymorphism in this species, though the behavioral differences were not strong enough to affect growth.

INTRODUCTION

The rate of evolutionary response to climate variation is sufficient for measuring current and projected responses to climate change for a wide variety of taxa (Skelly et al., 2007). Different morphologies within a single population, such as color polymorphisms,

may experience adaptive trade-offs under climate change. Thus, differential adaptive responses among polymorphs may be evident at the rate that climate change is currently occurring. In addition, adaptive trade-offs may have implications for organisms with color polymorphisms (Gibbs and Karraker, 2006), such as several species of *Plethodon* salamanders. Climate change has received little attention as a factor contributing to the decline of amphibians however, and definitive experiments determining causality of climate change responses are rare (McCarty, 2001; Carey and Alexander, 2003). Further, many strategies that attempt to predict species response to climate change do not consider biotic interactions (Pearson and Dawson, 2003), which are recognized as important to predictive modeling of climate effects on population dynamics (Davis et al., 1998).

The red-backed salamander (*Plethodon cinereus*) is a small, widely distributed terrestrial, lungless salamander inhabiting forest floors from the Great Lakes Region of the United States and Canada, southeast to North Carolina and north to New Brunswick (Petranka, 1998; Lannoo, 2005). There are two color morphotypes found in most *P. cinereus* populations: striped and unstriped. These are interbreeding morphs and the color variations appear to be polygenic and heritable (Highton, 1959; Highton, 1975; Petruzzi, 2006). The color polymorphism appears to be maintained by selection pressures, and several hypotheses have been proposed as the selective mechanism, including climate related factors (Lotter and Scott, 1977; Moreno, 1989; Petruzzi et al., 2006; Anthony et al., 2008; Fisher-Reid et al., 2013), behavioral differences (Venesky and Anthony, 2007), and increased predation pressure (Moreno, 1989). Although widespread population declines have not been reported for *P. cinereus*, the striped and unstriped color morphs exhibit geographic and seasonal variation (Lotter and Scott,

1977). Furthermore, distribution and surface frequency of the unstriped morphs may be correlated with warmer and drier conditions (Burger, 1935; Test, 1952; Lotter and Scott, 1977). Gibbs and Karraker (2006), using a time series of changes in color morph frequencies in free-ranging surface populations, propose that rising temperatures associated with climate change and forest disturbance are positively related to an observed increase in the frequency of unstriped morphs.

Plethodon salamanders may be particularly sensitive to changes in abiotic conditions associated with climate change, as they are typically terrestrial and reliant on humid microhabitats for respiration (Feder, 1983; Bernardo and Spotila, 2006). Further, *Plethodon* salamanders are often found in multispecies communities, where competition is presumed to control distribution patterns (Hairston, 1987). Competitive interactions appear to be governed by abiotic factors (Dunson and Travis, 1991; Bullock et al., 2000) and interspecific differences among closely related species influence how species will respond to climate trends (Aitken et al., 2008). There is a large body of literature that has investigated the nature of intraspecific competitive interactions in *P. cinereus*, including territoriality (Jaeger et al., 1981; Jaeger et al., 1982; Mathis, 1989; Mathis, 1990_{a,b}; Mathis, 1991; Gergits and Jaeger, 1990_a; Jaeger et al., 1995), agonistic behavior (Jaeger, 1981; Jaeger, 1984; Thomas et al., 1989; Gabor and Jaeger, 1995), and mechanisms for mediating territories (Jaeger and Gergits, 1979; Simon and Madison, 1984; Jaeger, 1986; Jaeger et al., 1986; Horne and Jaeger, 1988; Gergits and Jaeger, 1990_b; Jaeger and Forester, 1993). These investigations were largely constrained to sampling the portion of the population that is at the soil surface under constrained laboratory conditions. Terrestrial salamanders spend a larger amount of time in underground retreats than on the

surface; thus, these observations and experimental venues may result in biases associated with neglecting portions of the population distributed vertically within the substrata (Taub, 1961 and Bailey et al., 2004), particularly if the above and belowground populations differ in salient features such as body size (Hyde and Simons, 2001). This has implications for the body of *Plethodon* research that utilizes the observed, soil surface-dwelling portion of the population and behavioral interactions in laboratory arenas for inferring population responses without considering interacting abiotic and biotic factors (Skelly, 2005).

Because *P. cinereus* morphs may be adaptively differentiated, it is possible that between-morph competitive behavior differs from monomorphic competitive behavior. Therefore, the effects of climate change on *P. cinereus* competitive outcomes will depend on how abiotic factors influence intraspecific interactions, including between-morph interactions. Changes to temperature and humidity (e.g., as expected under climate change) have the potential to alter intraspecific interactions (Connell, 1983), thereby influencing expectations of the ratio of *P. cinereus* color morphs within given surface populations throughout their range. Here I test the competitive interactions of *P. cinereus* striped and unstriped morphs using realistic habitat and climate conditions for high elevation areas in Shenandoah National Park. I compared both a proximate response (behavior in terms of surface habitat use) and an ultimate measure (body condition in terms of change in mass) of competition using replicate mesocosms containing realistic fossorial and surface habitats under a climate change scenario. Although I expected to observe a response in the frequency of surface use, I recognize mass change as the ultimate measure of competition because individual mass change will likely affect key

components of population growth rate, fecundity and survival (Heppell et al., 2000; Homyack and Haas, 2009).

MATERIALS AND METHODS

Collection Procedures

On July 21, 2011, I collected 56 striped and 36 unstriped adult male salamanders with intact tails (33 to 44 mm snout-vent-length; 0.48 to 1.59 g mass) from between 950 and 1050 m AMSL on Hawksbill and Stony Man Mountains in Shenandoah National Park, Virginia. Only adult male salamanders were included in the experiment because I expected adult males to show the strongest interactions (Jaeger et al., 1982; Hairston, 1987; Jaeger et al., 1995). Salamanders were immediately transported to the National Zoological Park in Washington D.C. where they were housed in a climate-controlled room. Prior to initiating the 90-day experiment, the salamanders were acclimated for 8 days at 18°C and a 12:12 light:dark photoperiod within individual 23 x 23 cm plastic deli containers with filter paper hydrated with reconstituted reverse-osmosis water. Salamanders were each fed 7 wingless fruit flies (*Drosophila melanogaster*) coated with calcium dust every day, which I calculated was needed to maintain body mass at 18°C (Jaeger, 1980a).

Each salamander was uniquely marked with visual implant elastomer (VIE) so that individuals could be identified without handling or disturbing the salamanders. I established 46 pairs of salamanders (n = 28 same-morph pairs, n = 18 between-morph pairs), with each pair matched to within 1.0 mm snout-vent length to minimize morphological asymmetries. I introduced pairs to each mesocosm simultaneously to

minimize the effect of prior residence (Plenderleith and Forester, 2011; da Silva Nunes and Jaeger, 1989; Figler and Einhorn, 1983; Davies, 1978). Salamanders were allowed to interact undisturbed except when they were removed to obtain mid-point and end-point length and mass measurements.

Experimental Design

I used a two-level factorial design with temperature, competition, and morph as factors: Temperature levels were cool and warm; morph levels were unstriped and striped; and competition levels were two striped or two unstriped (hereafter same-morph), and one striped with one unstriped (hereafter between-morph). Temperature levels were derived from climate projections for high elevation areas of Shenandoah National Park and are described in more detail below. There were unbalanced replicate mesocosms within each treatment, amounting to 42 total mesocosms. The warm temperature treatment had 9 replicates for between-morph pairs of salamanders, 8 replicates for striped same-morph pairs, and 3 replicates for unstriped same-morph pairs. The cool temperature treatment had 9 replicates for between-morph pairs of salamanders, 10 replicates for striped same-morph pairs, and 3 replicates for unstriped same-morph pairs (Table 2.2). Sample sizes were small for unstriped same-morph treatment groups, though an a priori power analysis determined that 4 replicates per group would give us high power (> 90%) to detect a greater than 5% change in mass for 2-way interaction contrasts. Therefore, the power analysis suggested that an ability to detect a difference of 0.09 g was achievable under this design for salamanders weighing approximately 0.90 g.

Mesocosms

My goal was to replicate the natural habitat where *P. cinereus* occurs at high elevation in Shenandoah National Park. Each mesocosm was constructed using a transparent 20.82 l round polycarbonate bucket; I used high-density urethane foam to create underground inter-talus spaces, coating all sides with sand and sphagnum moss using non-toxic silicone (Fig. 2.1). These spaces were connected by two equidistant vertical burrows to the surface where homogenized soil and leaf litter from native sites formed the uppermost 7 cm deep layer, on top of which were two ceramic cover objects (10.2 x 10.2 cm). The lower underground habitat area of each mesocosm was wrapped in black plastic to keep it dark while the surface was exposed to a 12:12 light:dark photoperiod provided by full-spectrum fluorescent lights. An individual misting nozzle was mounted on the lid of each mesocosm that delivered filtered (RO) and reconstituted water from an automated misting system, which drained through a bulkhead in the bottom of the bucket. I designed the mesocosms to have minimal surface area; the territorial home range of adult male *P. cinereus* in Virginia is 0.163 m² (Mathis, 1991b) and the soil surface in each chamber was approximately 0.073m².

The 42 mesocosms were housed in two climate-controlled rooms with identical dimensions and climate control equipment. Temperature and relative humidity levels inside the mesocosms were measured at 30 minute intervals on the surface and in each underground space using U12 Hobo data loggers mounted in 12 of the 42 mesocosms. I also simulated a diurnal thermal cycle within each mesocosm. While the underground temperature of each mesocosm maintained room-level climate conditions, timer-controlled heat cables mounted through each lid increased ambient temperatures in the

atmosphere between the lid and soil during the light cycle. As a result, during the day the belowground habitat retained the cooler room-level temperature, creating a vertical temperature gradient towards the soil surface.

Gibbs and Karracker (2006) suggest that morph frequencies may have changed with warming trends, proposing that a 0.7°C increase in temperature has occurred within the range of *P. cinereus* over the last century, with forest microhabitat disturbances potentially contributing to local increases of 3 to 6°C. I implemented a difference between temperature treatments to simulate a hypothetical warming trend at these high elevation habitats, resulting in a ‘warm’ temperature treatment that corresponded to current conditions. Experimental temperature conditions were determined from climatological measurements at a high elevation location in Shenandoah National Park near the salamander collection sites. Estimates based on climatological measurements from 1970 to 2000 at Big Meadows in Shenandoah National Park suggest the following average July temperature ranges for habitats ranging in elevation from 800 to 1200m for the year 2000: mean maximum = 23.3 to 25.9°C, and mean minimum = 12.9 to 15.4°C (S. De Wekker and T. Lee, *personal comm.*). I implemented the ‘warm’ temperature treatment at $23.92 \pm 0.06^\circ\text{C}$ (n = 11278) to correspond with this range of mean maximum July temperature estimates. The ‘cool’ treatment was set at $20.73 \pm 0.05^\circ\text{C}$ (n = 11598). Underground conditions were maintained by constraining room-level temperatures at 18°C to correspond with daytime summer field measurements of temperatures within the talus habitat matrix near the salamander collection sites.

Humidity in the mesocosms was created via simulated rain events. I controlled humidity with vents that could be opened or closed on the lids, the length of time

between rain events, and the duration of rain events. Relative humidity levels on the surface averaged 96.64 ± 4.05 (± 1 SD, $n = 54$) for all mesocosms throughout the duration of the experiment.

Jaeger (1980a) determined the volumetric amount of food per day needed by *P. cinereus* to maintain a constant body weight at three different temperatures (6.6, 10.0, and 15.5°C). The results give the volume of *D. melanogaster* needed per mm of salamander (snout-vent length) per day that would result in a 0 % weight change at each temperature. Correspondingly, Jaeger (1980a) determined the mean volume of an adult *D. melanogaster* (mean = $1.59 \text{ mm}^3 \pm 0.60$). Using these values with the average snout-vent length of experimental individuals, I calculated the volumetric dietary requirements needed to maintain a constant weight over time. I extrapolated this calculation to match average temperatures in this experiment (i.e., 7 flies per salamander, per day). Salamanders were fed three times per week by introducing prey to the soil surface during each of three separate 12 hr dark cycles. Wingless *D. melanogaster* individuals coated with calcium powder were the prey item for each feeding event.

Response Variables

Mass Change: I measured individual salamanders for snout-vent length (± 0.01 mm), total length (± 0.01 mm), and mass (± 0.01 g) immediately before initiating the experiment, at the 45 d mid-point, and after 90 d at the conclusion of the experiment. The change in mass for the total experimental period was used as the continuous dependent variable for analysis.

Surface Frequency: The location of each salamander was recorded by carefully removing the black plastic covers surrounding the underground habitat and using an ultraviolet light to locate individuals by their VIE marks. Each observation was conducted during a separate 12 hr dark cycle and I used these location data to calculate a nighttime surface frequency history for each salamander ($n = 75$ observations), which was used as the continuous dependent variable for surface activity analysis. A surface observation was recorded when a salamander was located in any part of the habitat matrix above the first underground space, including the upper burrows leading from the first underground space to the soil (Fig. 2.1).

Statistical Analysis

All data were analyzed using the “nlme” (Pinheiro et al., 2012), “car” (Fox and Weisberg, 2011), and “stats” packages of R (R Development Core Team, 2011). I tested whether individuals found more frequently on the surface had higher change in mass using a least squares linear regression. I then used a mixed-effects, three-way analysis of deviance (ANODEV) to test the effects of temperature, competition, and species as independent variables in two analyses; first, with salamander mass change and second with surface frequency as the dependent variable. I estimated fixed effect differences among categories for three groups (temperature; competition; and species). I also included a random effect for mesocosm identity to control for any lack of independence for paired salamanders within each mesocosm and a fixed effect for the initial mass of each salamander. I tested the assumption of homoscedasticity using a Levene’s Test on the distribution of means for each treatment group within each species, for both mass change and surface frequency. I tested the assumption of normality for analysis of

variance using a Shapiro-Wilke's Test on the distribution of means for each treatment group within each species for both mass change and surface frequency as well as visually inspecting model plots of residuals against fitted values. I tested the assumption of normality for the least squares linear regression using a Shapiro-Wilke's Test on the distribution of means for each species for mass change and surface frequency as well as visually inspecting plots of residuals against fitted values. Alpha was set at 0.05 for all analyses.

RESULTS

There was variation in mass between paired salamanders before the start of the experiment, and initial mass significantly influenced mass loss ($\chi^2 = 11.44$, $df = 1$, $P = 0.001$), indicating that larger individuals at the start of the experiment had greater ability to maintain mass than smaller individuals. A least squares linear regression showed that there was a significant positive relationship between surface frequency and mass change ($F = 14.08$, $n = 84$, $P < 0.001$), though it only explained 15% of the variance in mass change. An analysis of deviance showed that temperature ($\chi^2 = 0.02$, $df = 1$, $P = 0.887$), morphotype ($\chi^2 = 0.02$, $df = 1$, $P = 0.893$), and competition ($\chi^2 = 0.32$, $df = 1$, $P = 0.572$) did not have any interactive or independent effects on mass change.

Surface frequency, however, was affected by competition. An analysis of deviance supported a significant interaction (Table 2.1) of competition by morph ($\chi^2 = 6.24$, $df = 1$, $P = 0.013$), indicating that surface activity was mediated by competitive pairings for each morph (Fig. 2.3). Parsing these results, I found that unstriped individuals spent less time on the surface when paired with the striped morph (mean =

35.17 g; SE = 2.93) than they did when paired with other unstriped morphs (mean = 49.08 g; SE = 5.14). However, striped individuals had similar surface frequencies when paired with the unstriped morph (mean = 43.44 g; SE = 2.73) and other striped morphs (mean = 42.0 g; SE = 2.33).

DISCUSSION

This appears to be the first experiment to utilize 3-dimensional habitat (i.e., surface and underground space) to evaluate adult male *Plethodon* competition in experimental mesocosms. Further, this appears to be the only experiment to investigate the competitive interactions of *P. cinereus* color morphs. Results from amphibian community mesocosm studies have been shown to have high predictability to the field (Dodd, 2010), including competition experiments (e.g., Wilbur, 1972; Parris and Cornelius, 2004). However, there remain doubts whether mesocosm studies are applicable to the real world (Skelly, 2002 and 2005), particularly due to concerns about the potential for variation among replicates. Here, I evaluated the effects of specific temperature differences on intra- and interspecific competition in terms of growth and surface activity within constrained habitat conditions. Each mesocosm was carefully designed to mimic natural talus habitat to reduce variation within treatments and enable the detection of significant differences in response to subtle differences in temperature conditions. Although the use of mesocosms with constrained conditions may undermine the importance of ecological interactions in natural systems (Jaeger and Walls, 1989;

Gascon and Travis, 1992), I was able to directly observe competition in these habitat conditions under specific temperature levels relevant to the natural system.

Because I measured surface frequency as a proximate measure of competitive response that was ultimately associated with salamander mass change, this study was concerned with the relevance of growth results for inferring population responses to competition under temperature conditions in natural communities. Yurewicz and Wilbur (2004) suggest that growth can potentially affect future reproductive success in *Plethodon* because advantages associated with body size and tail condition assist in acquiring and defending high-quality territories (Mathis, 1990; Gabor, 1995; Wise and Jaeger, 1998), thereby improving access to mates (Mathis, 1991) and increasing foraging efficiency (Jaeger et al., 1981). Foraging efficiency may predict body size and this component may be directly related to fitness, where less efficient foragers are thought to experience increased mortality (Gibbons et al., 2005). When potential competitors occur together, one species may be affected more by competition than the other (asymmetric competition), or both species may be equally affected (symmetric competition) (Twomey et al., 2008). In a space limited environment, I assumed competitive interactions between paired individuals would be asymmetrical in the mesocosms, such that one individual would be more efficient than the other at either foraging or allocating energy. Therefore, I used differences in surface activity and body size as measures of foraging success in this experiment, thereby implying a superior competitor in each mesocosm. However, the results of this study should be interpreted cautiously because species do not compete as isolated pairs in natural systems and small sample sizes may have resulted in a reduced

ability to detect growth and surface activity differences among temperature treatments (Table 2.2).

Several lines of evidence have been reported indicating that color polymorphism in *P. cinereus* are maintained by climate related environmental factors: (1) unstriped morphs experience increased mortality in cooler climates (Lotter and Scott, 1977; Moreno, 1989); (2) unstriped morphs retreat from the surface into underground habitat earlier in autumn as temperatures cool (Lotter and Scott, 1977; Moreno, 1989; Anthony et al., 2008); (3) metabolic rates differ, such that unstriped morphs are more active in warmer and drier conditions (Moreno, 1989; Petruzzi et al., 2006); and (4) striped and unstriped morphs are separated geographically on Long Island along environmental gradients, potentially leading to speciation (Fisher-Reid et al., 2013). Other possible mechanisms for the maintenance of color polymorphism in this species backed by evidence are behavioral differences between unstriped and striped morphs (Venesky and Anthony, 2007) and increased predation pressure on unstriped morphs (Moreno, 1989). Venesky and Anthony (2007) reported that unstriped morphs are more mobile than striped morphs in response to a snake predator in laboratory arenas and Moreno (1989) reported that unstriped morphs are more active on the surface than striped morphs due to higher standard metabolic rates, suggesting a greater risk of predation for unstriped morphs. Additionally, higher frequencies of unstriped morphs have been correlated with warmer and drier climate conditions (Burger 1935; Test 1952; Lotter and Scott, 1977), and Gibbs and Karraker (2006) reported increased unstriped morph frequencies associated with rising temperatures and forest disturbance across the species range.

The results of this study do not support a climate related mechanism for polymorphism maintenance in this species. Each salamander pair within the mesocosms showed differential growth over the duration of this experiment. The differences in growth between paired salamanders indicate that the mesocosms provided an environment where paired salamanders interacted in such a way that one was more successful than the other at partitioning space to maximize foraging, suggesting the occurrence of competition. If the unstriped morph is more adapted to warmer conditions than the striped, then the differential growth between paired salamanders demonstrated in this experiment would have shown a pattern biased toward increased mass change for unstriped morphs in the warm temperature treatment. Contrary to these expectations, I failed to find an effect of temperature on surface activity or growth.

However, my results indicated differences in surface activity between the morphs independent of temperature, suggesting that behavioral differences may play a role in maintaining color polymorphism. Surface activity was affected by competition in this experiment, such that competition pairings for striped morphs resulted in nearly equivalent surface frequency between same-morph and between-morph pairings, indicating that striped morph behavior is not affected by unstriped morphs. In contrast, decreased surface frequency when paired with striped morphs suggests that unstriped morphs were greatly affected by the presence of striped morphs (Fig. 2.3). Behavioral differences between morphs in terms of surface activity were not strong enough to affect growth, however, as both morphs showed an equally likely response to the experimental treatments in terms of mass change. My behavioral data are consistent with Venesky and Anthony (2007), such that increased mobility of unstriped morphs may explain how they

spent more time underground than the striped morph in this experiment without growth consequences. The physiological mechanisms underlying their increased mobility may have enabled the unstriped morph to access the surface more frequently from underground spaces at a lower energetic cost than the striped morph, while simultaneously reducing the frequency of costly interactions with the striped morph at the surface. These findings contrast with Moreno's (1989) hypothesis that the unstriped morph is more active on the surface than the striped morph at higher temperatures.

Based on the results of this experiment, I can make inferences for the current high elevation populations of *P. cinereus* in Shenandoah National Park. Although the ratio of morphs at the surface may fluctuate as a consequence of behavioral differentiation, these changes are independent of temperature. Further, any observed decrease in morph surface frequency may not indicate declines, as salamanders may spend more time in subsurface habitat without negative consequences for growth. The results suggest that total morph frequencies may have remained unchanged under recent warming trends in this region (Gibbs and Karraker, 2006), despite behavioral differentiation. These results contrast with previous research suggesting that the proportion of unstriped morphs in a given population may be increasing with warming temperatures (Gibbs and Karraker, 2006).

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Table 2.1 Analysis of deviance for the effect of temperature with competition and morph on surface activity.

Effect	<i>df</i>	χ^2	<i>P</i>
Morphotype	1	0.990	0.320
Competition	1	5.213	0.022
Temperature	1	0.532	0.466
Morphotype x Competition	1	6.238	0.012
Morphotype x Temperature	1	0.460	0.498
Competition x Temperature	1	0.026	0.873

Table 2.2 Number of individuals per treatment group (84 total individuals). Values for column "Between-Morph Pairs" represent the number individuals per species.

Competition				
Striped Same-Morph	Unstriped Same-Morph	Between-Morph Pairs		
20	6	9	Cool	Temperature
16	6	9	Warm	



Figure 2.1 Lateral view of a mesocosm (without lid and heat cables). Sub-surface space and burrows are visible below the soil/litter layer with cover objects.

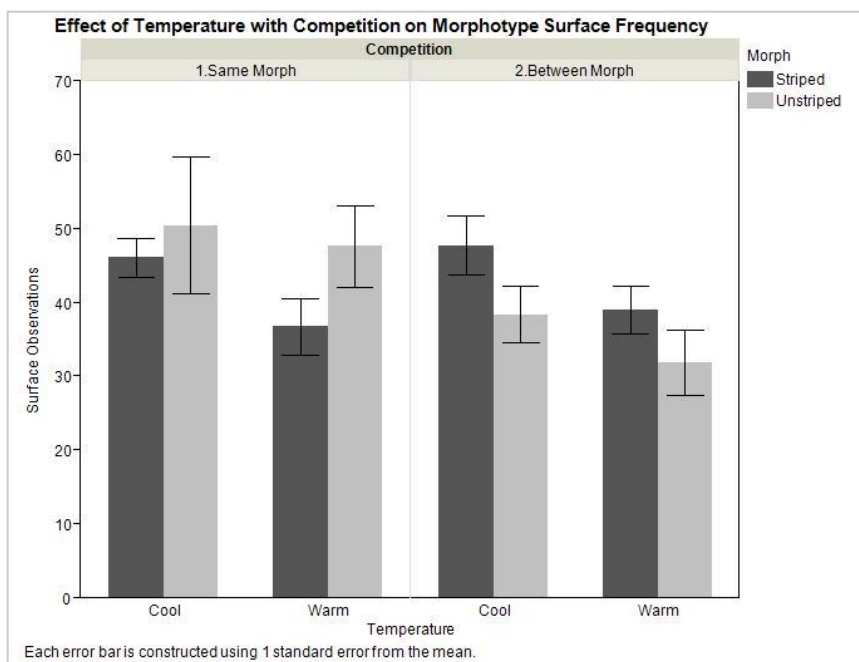


Figure 2.2 Plot showing the mean number of surface observations ($n = 75$) for each morphotype under the competition and temperature treatments.

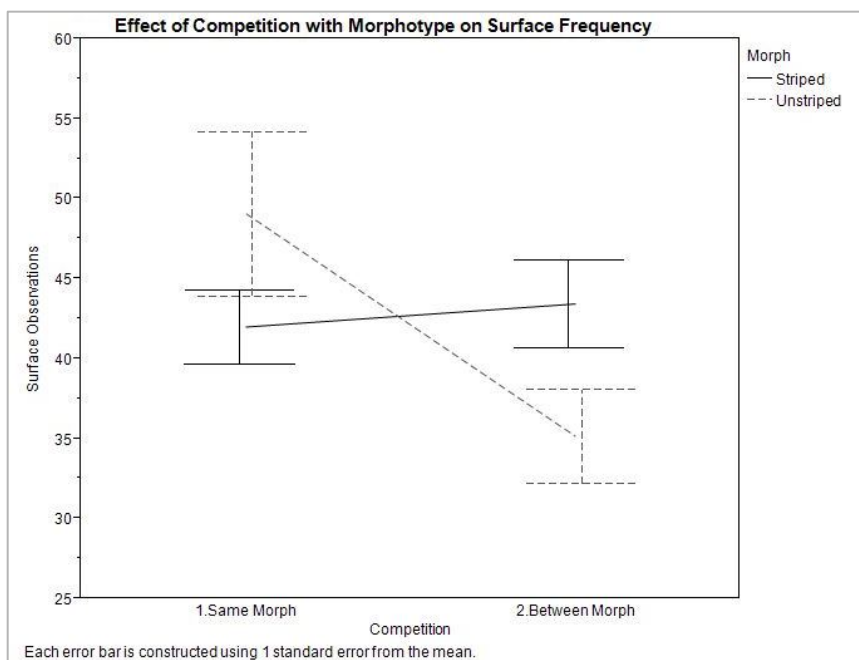


Figure 2.3 Interaction plot showing the mean number of surface observations ($n = 75$) for each morphotype under both competition treatments (conspecific = same-morph pairs; heterospecific = between-morph pairs)

CHAPTER 3

CLIMATE MEDIATES COMPETITION OUTCOMES IN A
HIGH-ELEVATION SALAMANDER COMMUNITY

ABSTRACT

The distribution of the federally endangered Shenandoah salamander (*Plethodon shenandoah*) is presumed to be limited by competition with the red-backed salamander (*Plethodon cinereus*). Species such as *P. shenandoah* with specialized habitat requirements at high elevations have the potential to be severely threatened by climate change. Further, recent research suggests that climate change may exacerbate interspecific competitive interactions at a cost to *P. shenandoah*. I investigated the response of *P. shenandoah*, in terms of growth and behavior, to competition with *P. cinereus* under two future climate scenarios in 3-dimensional mesocosms that mimicked realistic habitats. The results suggest that temperature mediates competitive outcomes between these species in terms of growth, leading to the prediction that core *P. shenandoah* populations may decline in response to increasing temperatures, particularly if their habitat is getting warmer and drier, though *P. shenandoah* may expand its range in areas where it competes with *P. cinereus*.

INTRODUCTION

Plethodon salamanders may be particularly sensitive to changes in abiotic conditions associated with climate change, as they are typically terrestrial and reliant on humid microhabitats for respiration (Feder, 1976; Bernardo and Spotila, 2006). Further,

Plethodon salamanders are often found in multispecies communities, where competition is presumed to control distribution patterns (Hairston, 1987). One such species of particular concern is the Shenandoah salamander (*Plethodon shenandoah*), which is endemic to the highest elevation habitats in Shenandoah National Park, Virginia. The distribution of *P. shenandoah* is strongly influenced by elevation and aspect, presumably in relation to temperature and moisture gradients, and associated central and southern Appalachian high elevation forest types (Jaeger, 1971b). *Plethodon shenandoah*'s environmental tolerances allow for persistence in suboptimal habitat in response to competitive exclusion by *P. cinereus* from surrounding optimal habitats (Jaeger, 1970). This combination of habitat distribution and interspecific competitive behavior appears to have resulted in small, patchily distributed *P. shenandoah* population isolates with margins of co-occurrence with *P. cinereus*.

Between-species differences such as physiological tolerances and dispersal abilities influence how species will respond to climate trends (Aitken et al., 2008). Because range-restricted species such as *P. shenandoah* are often habitat specialists and poor dispersers (Dirnbock et al., 2011) and adaptation is unlikely for species in fragmented ranges with small populations (Aitken et al., 2008), extinction appears to be a realistic outcome for *P. shenandoah* in response to a warming climate. Data from montane studies generally show movement of species upward in elevation (Aitken et al., 2008), while increases in lower elevational limits are correlated with climate warming (Moritz et al., 2008; Chen et al. 2011). The known isolated populations of *P. shenandoah* are generally located above 850-900 m on the summits of the three highest mountains in Shenandoah National Park. Therefore, *P. shenandoah* migration appears to be restricted

in three ways: a lower elevation boundary determined by climate variables, an upper boundary that is fixed by the height of the mountains, and competitive exclusion to sub-optimal habitat patches within these elevation limits. Because options for migration are limited, I focused on the likelihood of extinction via interspecific competition under climate projections in the co-occurrence marginal habitats, where competition between *P. shenandoah* and *P. cinereus* is currently presumed to be strongest and limiting for the distribution of *P. shenandoah* (Jaeger 1980).

Because competitive interactions are frequently governed by abiotic factors (Dunson and Travis, 1991; Bullock et al., 2000), the effects of climate change on *P. shenandoah* and *P. cinereus* competitive outcomes will depend on how these factors influence intra- and interspecific interactions. Although competition between these species has been demonstrated (Jaeger, 1972; Thurow, 1976; Wrobel et al., 1980), changes to temperature and humidity (e.g., as expected under climate change) have the potential to change both interspecific and intraspecific interactions (Connell, 1983), thereby influencing my expectations of the future extinction risk to *P. shenandoah*.

A substantial body of literature has investigated the nature of competitive interactions between *P. shenandoah* and *P. cinereus* (see summary and citations in Highton, 1988). However, most of these studies have been largely constrained to sampling the portion of the population that is at the soil surface, field enclosure experiments that do not account for sex, size, or escapes, and laboratory investigations using unrealistic climate and habitat conditions (e.g., Jaeger, 1971a,b; Jaeger, 1974; Thurow, 1976; Kaplan, 1977; Wrobel et al., 1980). Terrestrial salamanders spend a larger amount of time in underground retreats than on the surface; thus, these

observations and experimental venues may result in biases associated with neglecting portions of the population distributed vertically within the substrate (Taub, 1961; Bailey et al., 2004), particularly if the above and belowground populations differ in salient features such as body size (Hyde and Simons, 2001). This has implications for a body of *P. shenandoah* research in which population and behavioral inferences are conditional on the observed, soil surface-dwelling portion of the population and behavioral interactions in one-dimensional environments without considering interacting abiotic and biotic factors (Skelly, 2005).

Here I tested the competitive interactions of *P. shenandoah* and *P. cinereus* in mesocosms in which we isolated climate and habitat factors of importance for high elevation areas in Shenandoah National Park. Climate factors were derived from downscaled models for the high-elevation habitat, and the design of our mesocosms provided access to belowground habitat similar to structure of habitat in the field. I compared both a proximate response (behavior in terms of surface habitat use) and an ultimate measure (body condition in terms of change in mass) of competition. Although I expected to observe a response in the frequency of surface use, I recognize mass change as the ultimate measure of competition because individual mass change will likely affect key components of population growth rate, fecundity and survival (Heppell et al., 2000; Homyack and Haas, 2009).

MATERIALS AND METHODS

Collection Procedures

I collected 60 adult male salamanders of each species with intact tails (35 to 45 mm snout-vent-length; 0.82 to 1.69 g mass) from between 950-1050 m on Hawksbill (n = 8 transects) and Stony Man (n = 8 transects) mountains in Shenandoah National Park, Virginia on May 10 to 11, 2012. Only adult male salamanders were included in the experiment because I expected adult males to show the strongest interactions (Jaeger et al., 1982; Hairston, 1987; Jaeger et al., 1995). Salamanders were immediately transported to Patuxent Wildlife Research Center in Laurel, Maryland where they were housed in a humidity- and temperature-controlled room. Prior to initiating the 90-day experiment, the salamanders were acclimated for 8 days at 18°C and a 12:12 light:dark photoperiod within individual 23 x 23 cm plastic deli containers with filter paper hydrated with reconstituted reverse-osmosis water. They were each fed 7 wingless fruit flies (*Drosophila melanogaster*) coated with calcium dust every day, which I calculated was needed to maintain body mass at 18°C (Jaeger, 1980).

Each salamander was uniquely marked with visual implant elastomer (VIE) so that individuals could be identified without handling or disturbing the salamanders. I established 60 pairs of salamanders (n = 24 intraspecific pairs, n = 36 interspecific pairs), with each pair matched to within 0.05 g mass and 2.50 mm snout-vent length to minimize morphological asymmetries. I introduced pairs to each mesocosm simultaneously to minimize the effect of prior residence (Davies, 1978; Figler and Einhorn, 1983; da Silva Nunes and Jaeger, 1989; Plenderleith and Forester, 2011). Salamanders were allowed to

interact undisturbed except for when they were removed to obtain mid-point (45-d) and end-point (90-day) length and mass measurements.

Experimental Design

This was a two-level factorial design with temperature (cool and warm), relative humidity (dry and wet), competition (one of each species, hereafter interspecific; or two of a single species, hereafter intraspecific), and species as factors. Temperature levels were derived from current and future climate projections for high elevation areas of Shenandoah National Park and are described in more detail below. I tested three climate scenarios: current climate (i.e., cool and wet), a warm and wet future climate scenario, and a warm and dry future climate scenario. There were unbalanced replicate mesocosms within each climate scenario: both future climate scenarios had 9 replicates for interspecific pairs of salamanders, 3 replicates for *P. shenandoah* intraspecific pairs, and 3 replicates for *P. cinereus* intraspecific pairs while the current climate scenario had 6 replicates for interspecific pairs, 3 replicates for *P. shenandoah* intraspecific pairs, and 2 replicates for *P. cinereus* intraspecific pairs. Sample sizes were small, particularly for intraspecific treatment groups, and an a priori power analysis determined that 4 replicates per group would give us high power (> 90%) to detect > 7% changes in mass for 3-way interaction contrasts. Therefore, the power analysis suggested that an ability to detect a difference of 0.17 g was achievable under this design for salamanders weighing approximately 1.20 g.

Mesocosms

My goal was to replicate the habitats characterized by sparse ground vegetation cover, dense forest canopy, and moderately shallow soil covering a talus rock matrix

(talus type III in the classification of Jaeger, 1970), where both *P. shenandoah* and *P. cinereus* may co-occur. Each mesocosm was constructed using a transparent 20.82 l round polycarbonate bucket; I used high-density urethane foam to create underground inter-talus spaces, coating all sides with sand and sphagnum moss using non-toxic silicone (Fig. 3.1). These spaces were connected by two equidistant vertical burrows to the surface where homogenized soil and leaf litter from native sites formed the uppermost 7 cm deep layer, on top of which were two ceramic cover objects (10.16 x 10.16 cm). The lower underground habitat area of each mesocosm was wrapped in black plastic to keep it dark while the surface was exposed to a 12:12 light:dark photoperiod provided by full-spectrum fluorescent lights. An individual misting nozzle was mounted on the lid of each mesocosm that delivered reverse-osmosis filtered and reconstituted water from an automated misting system that drained through a bulkhead in the bottom of the bucket. I designed the mesocosms to have minimal surface area; the territorial home range of adult male *P. cinereus* in Virginia is 0.163 m² (Mathis, 1991b) and the soil surface in each chamber was approximately 0.073 m².

The 41 mesocosms were housed in a single climate controlled room. Temperature and relative humidity data were measured at 30 min intervals on the surface and in each underground space using U12 Hobo data loggers mounted in 9 of the 41 mesocosms. Soil moisture was measured every other day between simulated rain events in these same mesocosms using a Model HB-2 Kelway Soil Acidity and Moisture Tester. I also simulated a diurnal thermal cycle within each mesocosm. While the underground temperature of each mesocosm maintained room-level climate conditions, timer-controlled, heat cables mounted through each lid increased ambient temperatures in the

atmosphere between the lid and soil during the light cycle. As a result, during the day the belowground habitat retained the cooler room-level temperature, creating a vertical temperature gradient towards the soil surface (Table 3.3).

Experimental temperatures were estimated from climate models at a high elevation location in Shenandoah National Park near the salamander collection sites. Estimates based on climatological measurements from 1970 to 2000 at Big Meadows in Shenandoah National Park suggest mean maximum July temperature ranges of 23.3 to 25.9°C, and mean minimums of 12.9 to 15.4°C for habitats ranging in elevation from 800 to 1200 m (S. deWekker and T. Lee, *personal comm.*). Projected mean maximum and minimum July temperatures were estimated from analysis of output from the CGCM3 global climate model coupled with the CRCM regional climate model (which assumes SRES A2 emissions scenario) for the year 2041 (Mearns et al., 2009; De Wekker and Lee, *personal comm.*). Accordingly, I estimated an increase of ~3.3°C by 2071 (De Wekker and Lee, *personal comm.*), increasing the average July temperature ranges for my study area for 2041 to a mean maximum of 26.6 to 29.2°C and mean minimum of 16.2 to 18.7°C. During the course of the experiment, I maintained mean daytime warm and cool temperature treatments to correspond with the mean maximum range of projected temperatures for 2041 and mean maximum range of temperatures from 1970-2000, respectively (Table 3.3). Additionally, I maintained nighttime temperatures at 18°C to correspond with daytime summer field measurements of temperatures within the talus habitat matrix near the salamander collection sites. (Table 3.3).

Relative to predictions of temperature increases, there is greater uncertainty associated with predicting future precipitation from the climate models and therefore

greater uncertainty associated with future relative humidity at these locations.

Accordingly, I established two potential future climate scenarios: 'warm wet' or 'warm dry', where the wet treatment had daytime surface relative humidity levels corresponding to current conditions and the dry treatment had daytime humidity levels reduced by approximately 20%. Humidity in the mesocosms was created via simulated rain events. I controlled humidity with vents that could be opened or closed on the lids, the length of time between rain events, and the duration of rain events.

Jaeger (1980a) determined the volumetric amount of food per day needed by *P. cinereus* to maintain a constant body weight at three different temperatures (6.6, 10.0, and 15.5°C). The results give the volume of *D. melanogaster* needed per millimeter of salamander (snout-vent length) per day that would result in a 0% weight change at each temperature. Correspondingly, Jaeger (1980a) determined the mean volume of an adult *D. melanogaster* (mean = $1.59 \text{ mm}^3 \pm 0.60$). Using these values with the average snout-vent length of experimental individuals, I calculated the volumetric dietary requirements needed to maintain a constant weight over time. I extrapolated this calculation to match average temperatures in this experiment and then limited the daily amount by 14% (i.e., 6 flies per salamander, per day), as initial trials suggested that it would be difficult to detect differences in mass change unless food resources were limited (Chapter II). Salamanders were fed three times per week by introducing prey to the soil surface during each of three separate 12 hr dark cycles. Wingless *D. melanogaster* individuals coated with calcium powder were the prey item for each feeding event.

Response Variables

Mass Change: I measured individual salamanders for snout-vent length ($\pm .01\text{mm}$), total length ($\pm .01\text{mm}$), and mass ($\pm 0.01\text{g}$) immediately before initiating the experiment, at the 45 d mid-point, and after 90d at the conclusion of the experiment. The change in mass for the total experimental period was used as the continuous dependent variable for analysis.

Surface Frequency: The location of each salamander was recorded by carefully removing the black plastic covers surrounding the underground habitat and using an ultraviolet light to locate individuals by their VIE marks. Each observation was conducted during a separate 12 hr dark cycle and I used these location data to calculate a nighttime surface frequency history for each salamander ($n = 40$ observations per individual), which were used as the continuous dependent variable for surface activity analysis. A surface observation was recorded when a salamander was located in any part of the habitat matrix above the first underground space, including the upper burrows leading from the first underground space to the soil (Fig. 2.1). On the rare occasion that a salamander was found perched on or climbing the mesocosm wall above the soil, that location was removed from the analysis because behaviorally sub-dominant individuals were periodically observed taking refuge high on the wall to avoid contact with a dominant salamander on or near the surface.

Statistical Analysis

I tested whether individuals found more frequently on the surface had higher change in mass using a least squares linear regression. I then used a mixed-effects, three-way analysis of deviance (ANODEV) to test the effects of temperature, humidity,

competition, and species as independent variables in two analyses; first, with salamander mass change and second with surface frequency as the dependent variable. I estimated fixed effect differences among categories for three groups each in three models: a model with temperature and humidity categorized as climate scenarios (temperature with humidity; competition; and species), a temperature model (temperature; competition; and species), and a humidity model (humidity; competition; and species). I also included a random effect for mesocosm identity to control for any lack of independence for paired salamanders within each mesocosm and a fixed effect for the initial mass of each salamander. I tested the assumption of homoscedasticity using a Levene's Test on the distribution of means for each treatment group within each species, for both mass change and surface frequency. I tested the assumption of normality for analysis of variance using a Shapiro-Wilke's Test on the distribution of means for each treatment group within each species for both mass change and surface frequency as well as visually inspecting model plots of residuals against fitted values. I tested the assumption of normality for the least squares linear regression using a Shapiro-Wilke's Test on the distribution of means for each species for mass change and surface frequency as well as visually inspecting model plots of residuals against fitted values. Alpha was set at 0.05 for all analyses.

All data were analyzed using function "lme" from package "nlme" (Pinheiro et al., 2012), functions "Anova" and "LeveneTest" from package "car" (Fox and Weisberg, 2011), and function "shapiro.test" using program R (R Development Core Team, 2011). Function "Anova" generates restricted Type III sums of squares for unbalanced data sets

and produces p-values that are accompanied by chi-square statistics instead of F-statistics when used with function “lme.”

RESULTS

There was little variation in mass between paired salamanders before the start of the experiment, and initial mass did not influence mass loss in the model with only the effect of temperature, species, and competition ($\chi^2 = 1.06$; $df = 1$; $P = 0.304$) or the model with only the effect of humidity, species, and competition ($\chi^2 = 0.76$; $df = 1$; $P = 0.383$). I was able to closely match paired *P. shenandoah* individuals (mean = 1.17 g; SD = 0.15) and *P. cinereus* individuals (mean = 1.16 g; SD = 0.15). Although same-species pairs were matched with equal precision, intraspecific *P. shenandoah* individuals (mean = 1.51 g; SD = 0.08) were larger than intraspecific *P. cinereus* individuals (mean = 0.94 g; SD = 0.09).

A least squares linear regression showed that there was a significant positive relationship between surface frequency and mass change ($F = 16.95$; $n = 82$; $P < 0.001$), though it only explained 18% of the variance in mass change. The model with temperature and humidity categorized as a climate factor did not fit the surface frequency data well, and the interaction of climate with competition was not significant ($\chi^2 = 0.025$; $df = 1$; $P = 0.873$). However, the model with only the effect of humidity, species, and competition indicated that individuals were more likely to be observed on the surface under the high humidity treatment ($\chi^2 = 4.201$; $df = 1$; $P = 0.040$; Table 3.2). Temperature did not have any effect on surface activity, and there were no differences

among competition treatments, species, or the interactions among the climate variables, species, and competition.

Combined climate factors similarly had no effect on mass change; the model with temperature and humidity combined as a climate factor did not fit the mass change data well, and the interaction of climate scenario with competition was not significant ($\chi^2 = 0.621$; $df = 1$; $P = 0.431$). Correspondingly, the model with only humidity, species, and competition as factors did not result in any significant effects on mass change, indicating that competitive behavior was not mediated by humidity in terms of growth rates. On the other hand, temperature affected mass change profoundly. An analysis of deviance supported a significant 3-way interaction of competition with temperature by species ($\chi^2 = 5.737$; $df = 1$; $P = 0.017$), indicating that each species responded differently to temperature, and the response was mediated by competitive pairings (Table 3.1). Parsing these model results (Fig. 3.2), I found that *P. shenandoah* individuals had more negative mass change (mean = -0.37 g; SE = 0.1) than *P. cinereus* (mean = -0.06 g; SE = 0.04) when paired with a heterospecific under current temperatures. Under future temperatures, however, mass change was similar for *P. shenandoah* (mean = -0.24 g; SE = 0.04) and *P. cinereus* (mean = -0.22 g; SE = 0.05) when paired with heterospecific individuals. When individuals were paired with individuals of the same species, temperature affected *P. shenandoah* and not *P. cinereus*: same-species *P. shenandoah* pairs had greater mass loss under future temperatures (mean = -0.45 g; SE = 0.07) than under current temperatures (mean = -0.31 g; SE = 0.05), while same-species *P. cinereus* pairs had similar mass change under future (mean = -0.1 g; SE = 0.04) and current temperatures (mean = -0.13 g; SE = 0.08).

DISCUSSION

This appears to be the second experiment to utilize three-dimensional habitat (i.e., surface and underground space) to investigate adult male *Plethodon* competition in experimental mesocosms, preceded only by a pilot for this study (Chapter II). Although results from amphibian community mesocosm studies have been shown to have high predictability to the field (Dodd, 2010), including competition experiments (e.g., Wilbur 1972; Parris and Cornelius, 2004), the potential for variation among replicates casts doubt on whether mesocosm studies are applicable to the real world (Skelly, 2002; 2005). Here, I investigated the potential for climate change to induce competitive asymmetries, which would affect the persistence of *P. shenandoah* populations. Under a changing climate, species' interactions may change expectations of future extinction risk, especially in high-elevation habitats where the potential for migration to track suitable conditions is limited (Walther, et al. 2002; Stuart et al., 2004; Pounds et. al., 2006). Therefore, I investigated how future climate scenarios for high-elevation habitats in the Blue Ridge Mountains of Shenandoah National Park affect competitive interactions of *P. shenandoah* and *P. cinereus* in experimental mesocosms that mimic natural talus habitat. Each mesocosm was carefully designed to reduce variation within treatments and enable the detection of significant differences in response to subtle differences in temperature and relative humidity conditions. Although the importance of ecological interactions in natural systems may be undermined by the use of mesocosms with constrained conditions (Jaeger and Walls, 1989; Gascon and Travis, 1992; Wilhelm et al., 2000), I was able to directly observe competition in these habitat conditions under specific climate conditions relevant to the natural system. The results demonstrate the importance of considering the

impact of future climate conditions for real landscapes on competitive interactions, and find that temperature mediates interspecific competitive outcomes. Although current climate conditions favored *P. cinereus* when paired with *P. shenandoah*, increased temperatures associated with climate change may reduce the competitive dominance of *P. cinereus* on *P. shenandoah*. However, the results of this study should be interpreted cautiously because species do not compete as isolated pairs in natural systems and small sample sizes may have resulted in a reduced ability to detect growth and surface activity differences among temperature treatments (Table 3.4).

In contrast to Jaeger (1971), who found that both species responded strongly to soil moisture, I found that temperature was a more important determinant of competitive interactions, though the response depended on species' pairings. Unlike previous experiments, the subsurface habitat in the mesocosms provided a cool, humid refuge from the dry soil surface between simulated rain events, which possibly explains our lack of effect of surface relative humidity or soil moisture on mass change or surface activity. Accordingly, the results suggest that the salamanders behaviorally avoid harsh moisture conditions by moving underground where relative humidity levels are more stable than at the surface; this observation is confirmed in the field, and in surveys of other high-elevation *Plethodon* (Spotila, 1972). In my experiment, however, utilization of the subsurface habitat did not mitigate the effects of varying surface temperatures on competitive interactions. Overall, I find that environmental temperature has a greater influence on competition outcomes. This is useful for understanding future extinction risk to *P. shenandoah*, as predictions of changes in precipitation (and therefore humidity) have greater uncertainty (Christensen et al., 2007). While relative humidity does not

appear to affect the outcome of competition, it does appear to affect the elevation limits of *P. shenandoah*, though not *P. cinereus*.

Intraspecific pairings of *P. cinereus* resulted in nearly equivalent mass loss between current and future conditions, indicating that *P. cinereus* may have a greater physiologic breadth in its tolerance of temperature. In contrast, the larger body size of *P. shenandoah* is a liability under future increased temperatures. This leads to the prediction that the smaller *P. cinereus* may be more tolerant of thermal changes, while *P. shenandoah* may be more sensitive to changes. This result is not unexpected, considering the wide range of habitats where *P. cinereus* occurs in the eastern U.S. relative to the small fragmented range where *P. shenandoah* occurs (Adams et al., 2007). Highton (1995; 2012) hypothesized that *P. shenandoah* is a product of allopatric speciation in high elevation forest refugia due to lineage isolation during dry periods of the Pliocene, expanding its distribution more widely during cooler periods of the Pleistocene before being restricted by competition with *P. cinereus* (Highton and Worthington, 1967; Highton and Larson, 1979; Highton, 1995). *Plethodon shenandoah* appears to be a relict form adapted to cooler climate conditions and the current fragmented range is probably a product of both competition with *P. cinereus* and changing climate conditions (Highton and Worthington, 1967), which is confirmed in the present experiment. Additionally, although *P. shenandoah* has experimentally been shown to tolerate drier habitats (Jaeger, 1971), the higher temperatures under future climate scenarios may exceed the physiological limits of *P. shenandoah* where they currently occur. Thus, intraspecific populations of *P. shenandoah* in these habitats may experience future declines under warmer temperatures.

Under current climate conditions, interspecific competition in terms of mass change was asymmetrical in favor of *P. cinereus* (Fig. 3.2). *Plethodon shenandoah* had greater mass loss when it was paired with *P. cinereus*, corroborating the findings of previous studies suggesting that *P. cinereus* is competitively superior to *P. shenandoah* in the habitats where both species occur (Jaeger 1971a; 1971b). However, under future climate warming interspecific competition was symmetrical (Fig. 3.2). Relative to the intraspecific pairing, interspecific mass change was more negative for *P. cinereus*, and less negative for *P. shenandoah*, indicating that resource utilization changed for both species under interspecific pairings in future conditions and that the change was costly for *P. cinereus*. These results suggest that interspecific competition may not increase the extinction risk for *P. shenandoah* under future climate conditions. One caveat is that climate change may precipitate changes in habitat suitability for these salamanders, which were not considered in my experimental design, such as changes to the composition or productivity of the vegetation community, or the probability and types of disturbance such as fire. Further, uncertainty among global climate models is large (Polsky et al., 2000), particularly for precipitation, and potential changes in moisture conditions, though they are not expected to influence the competitive outcomes directly, may have an impact on the ecosystem and affect resource availability (McCain and Colwell, 2011).

Based on the results of this experiment, I can make limited predictions for changes in distribution of *P. shenandoah* in a warmer world. Under projected future temperatures in the next 60 years, *P. shenandoah* may colonize areas where *P. cinereus* occurs; if food resources remain constant or decrease in response to high-elevation

warming, intraspecific *P. shenandoah* population densities may decrease from current levels. Thus, accurately forecasting the future distribution of *P. shenandoah* is not straightforward, and must consider multiple biotic and abiotic constraints on the range, and future extinction risk, of *P. shenandoah*.

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Table 3.1 Analysis of deviance for the effect of temperature with competition and species on mass change.

Effect	<i>df</i>	χ^2	<i>P</i>
Species	1	0.2132	0.6442
Competition	1	1.0517	0.3051
Temperature	1	2.1824	0.1396
Species x Competition	1	1.427	0.2323
Species x Temperature	1	1.4216	0.2331
Competition x Temperature	1	4.7199	0.0298*
Species x Competition x Temperature	1	5.7365	0.0166*

Table 3.2 Analysis of deviance for the effect of humidity with competition and species on surface frequency.

Effect	<i>df</i>	χ^2	<i>P</i>
Species	1	0.044	0.8338
Competition	1	0.3466	0.5561
Humidity	1	4.2012	0.0404*
Species x Competition	1	0.0182	0.8926
Species x Humidity	1	0.0267	0.8702
Competition x Humidity	1	0.7281	0.3935
Species x Competition x Humidity	1	0.1022	0.7492

Table 3.3 Mean night and day temperature (°C) and relative humidity (RH) measures at the surface, first underground space, and second underground space (± 1 standard deviation), and mean relative saturation (RS) of the soil (± 1 standard deviation) for each of the three climate treatments.

	Daytime Climate Conditions					
	Cool Wet		Warm Wet		Warm Dry	
	Temperature	Humidity	Temperature	Humidity	Temperature	Humidity
Surface	25.64 \pm 2.18°C	84.58 \pm 5.59%RH	27.31 \pm 2.47°C	81.99 \pm 7.66%RH	28.47 \pm 2.47°C	62.73 \pm 7.16%RH
1st Underground Space	21.3 \pm 1.41°C	94.54 \pm 3.6%RH	21.75 \pm 1.22°C	93.5 \pm 4.0%RH	21.81 \pm 1.5°C	86.1 \pm 14.61%RH
2nd Underground Space	19.68 \pm 1.2°C	91.62 \pm 7.97%RH	20.21 \pm 0.95°C	91.06 \pm 10.72%RH	19.81 \pm 1.29°C	94.82 \pm 6.81%RH
	Nighttime Climate Conditions					
	Cool Wet		Warm Wet		Warm Dry	
	Temperature	Humidity	Temperature	Humidity	Temperature	Humidity
Surface	17.61 \pm 1.19°C	96.73 \pm 5.3%RH	17.67 \pm 1.14°C	95.35 \pm 5.69%RH	17.55 \pm 1.22°C	81.65 \pm 8.52%RH
1st Underground Space	17.74 \pm 1.11°C	89.86 \pm 12.18%RH	17.89 \pm 1.12°C	89.07 \pm 11.71%RH	17.5 \pm 1.1°C	79.87 \pm 19.05%RH
2nd Underground Space	17.48 \pm 1.14°C	85.69 \pm 15.5%RH	17.58 \pm 1.15°C	84.26 \pm 16.65%RH	17.04 \pm 1.27°C	83.27 \pm 19.21%RH
	Soil Moisture					
	Cool Wet		Warm Wet		Warm Dry	
	72.04 \pm 31.98%RS		66.84 \pm 18.37%RS		26.12 \pm 24.2%RS	
Surface						

Table 3.4 Number of individuals per treatment group (84 total individuals). Values for column "Interspecific" represent the number individuals per species.

Competition				
Pshen Intraspecific	Pcin Intraspecific	Interspecific		
6	4	6	Cool Wet	Climate
6	6	9	Warm Wet	
6	6	9	Warm Dry	



Figure 3.1 Lateral view of a mesocosm (without lid and heat cables). Sub-surface space and burrows are visible below the soil/litter layer with cover objects.

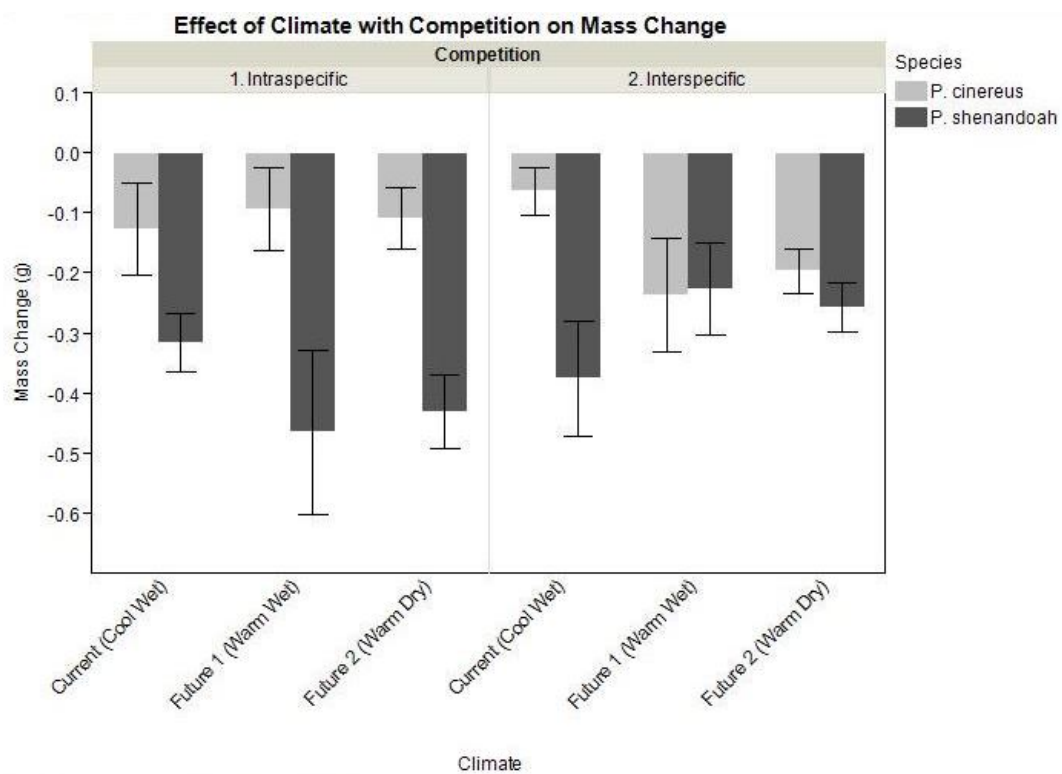


Figure 3.2 Mean change in mass (± 1 standard error) for each species under competition in current and future climate conditions.

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Works in Progress:

Dallalio, E.A., J.W. Snodgrass, and E.H.C. Grant. (In Prep). Recent warming trends are not enough to affect color morph frequencies in a high-elevation *Plethodon cinereus* system. (to be submitted to Journal of Herpetology in Fall, 2013).

- Dallalio, E.A., A.B. Brand, and E.H.C. Grant. (In Prep). Climate mediates competition outcomes in a high-elevation salamander community. (to be submitted to Ecology in Fall, 2013).
- Dallalio, E.A. and B.A. Maxell. (In Prep). Local and landscape habitat characteristics of the Idaho giant salamander (*Dicamptodon aterrimus*) in Western Montana. (to be submitted to Northwestern Naturalist in Fall, 2013).
- Dallalio, E.A. and E.H.C. Grant. (In Prep). Differential physiological characters play a role in mediating competition outcomes between the red back salamander (*Plethodon cinereus*) and Shenandoah salamander (*Plethodon shenandoah*) under climate change. (to be submitted for publication in Winter, 2013).

Professional Reports:

- Dallalio, E.A. and J. Horn. 2003. Wilderness Campsite Inventory for Admiralty National Monument. Report to the Juneau Ranger District-Admiralty National Monument of Tongass National Forest, Region 10 Office of the U.S. Forest Service. 30pp.

Presentations:

- Climate and competition variety shape expectations of extinction risk for a federally endangered species (*Plethodon shenandoah*) in a high-elevation salamander system. Paper presentation at The Virginia Herpetological Society Annual Meeting, Smithsonian National Zoo, Washington D.C. October 20, 2012. Co-authored by E.A. Dallalio, J.W. Snodgrass, and E.H.C. Grant. Presented by Eric A. Dallalio.
- Climate change and competition: implications for a federally endangered salamander. Poster presentation at The Wildlife Society Annual Meeting, Portland, Oregon. October 13-18, 2012. Authored by E.A. Dallalio. Presented by Eric A. Dallalio.
- Interspecific competition under climate change: addressing a critical science need for management of the federally endangered Shenandoah Salamander (*Plethodon shenandoah*). Paper presentation at The Northeast Partners in Amphibian and Reptile Conservation Annual Meeting, Bretton Woods, New Hampshire. July 24-26, 2012. Co-authored by E.A. Dallalio, J.W. Snodgrass, and E.H.C. Grant. Presented by Eric A. Dallalio.
- Climate change and amphibians: Northeast ARMI's approach. Poster presentation at The Northeast Partners in Amphibian and Reptile Conservation Annual Meeting, Millersville, Maryland. August 17-18, 2011. Co-authored by E.H.C. Grant E.A., A.B. Brand, and E.A. Dallalio. Presented by E.H.C. Grant, A.B. Brand, and E.A. Dallalio.

