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Distribution of *Plethodon cinereus* Color Morphs in New England: 40 Years Later

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Bridgewater State University
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1. Abstract

Global climate change is threatening species with habitat loss and extinction, but specific mechanisms driving changes in species abundance are unclear, partly due to a lack of time-series data sets documenting population changes. In order to study these effects on amphibian species, we revisited Lotter and Scott's 1977 study of color morph frequency in relation to climate in *Plethodon cinereus* (eastern red-backed salamander). 25 Massachusetts, New Hampshire, and Vermont localities were resampled during summer 2016, to quantify any changes in morph frequency since 1977. At 19 of the 25 localities, we were able to find ≥ 100 salamanders, and of those 19, five localities had significant changes in morph frequency since 1977. Previous work suggests that the unstriped morph is more heat- and drought-tolerant, and thus should be the morph that increases in relative abundance over time in areas where global climate change results in either higher temperatures, decreased precipitation, more prolonged periods of drought, or some combination of these factors. Collection of fine-scale climate data at each location from 1977 to today was beyond the scope of this study, so I only documented the patterns of morph frequency change. Despite a general warming trend in New England, I found a higher proportion of the unstriped morph at only 1 of the 5 localities with significant change; the others showed an increase in the striped or erythristic morphs. Lotter and Scott also suggested that there was differential mortality between striped and unstriped morphs, with unstriped morphs suffering higher juvenile mortality. We found no evidence of this pattern at 9 MA localities with 2016 age data, suggesting that the current morph frequency distribution is stable. I continue to work with collaborators to resample all 50 Lotter and Scott localities. Once all sites have been resampled, we plan to explore potential climate or land-use changes that may be driving changes in morph frequency.

2. Introduction

2.1 Climate Change and Its Impact on Species

2.1.1 Understanding Climate Change

In 1988, the Intergovernmental Panel on Climate Change (IPCC) was formed as an international attempt to better understand our changing climate and the role of humans (Oreskes 2004). Reports issued by the IPCC and other scientific organizations clearly document the process by which humans are changing Earth's climate (Oreskes 2004). Excess greenhouse gases in the atmosphere are able to absorb heat radiated from the Earth's surface and re-emit it in all directions so that the heat warms the lower atmosphere (Oreskes 2004). These greenhouse gases (e.g., nitrous oxide, carbon dioxide, water vapor, and methane) have increased in atmospheric concentration due to human activity such as industrialization (Oreskes 2004).

In the last 40 years, scientists have seen climate warming affect many aspects of habitat (Niu et al. 2004). For example, in the Tibetan Plateau, the southeast has become warmer and wetter with an increase in rainfall. Temperature and humidity have increased in the center of the plateau, while the north has become warmer and drier (Niu et al. 2004). The climatic changes observed in the Tibetan Plateau have been seen regionally. During a 50 year-long study of the Antarctic Peninsula, the peninsula has experienced major warming, with annual mean surface temperature increasing 1.09°C per decade in the winter (Turner et al. 2005). There have been negative trends in mean pressure recorded, and increased mean wind speeds (Turner et al. 2005). According to Oregon State University's PRISM Group, from 1951-2006 there was a 0.01°C increase in the mean temperature of the continental United States (Figure 1; PRISM 2009). With these shifts in worldwide climate, species everywhere face three possible futures: move to a more suitable climate, adapt rapidly to their new climate, or go extinct. .

2.1.2 Climate Change and Other Factors Impacting Species

Modeling scenarios project that, by 2050, approximately 15-37% of terrestrial species will be “committed to extinction,” meaning entirely or almost extinct (Thomas et al. 2004). The American pika, *Ochotona princeps*, is a small lagomorph species that dwells in the rocky talus at the base of cliffs in the Rocky Mountains. Pikas have a very specific and narrow climatic niche (Beever et al. 2011). In the Great Basin ecoregion of western North America, Beever et al. (2011) compared population records from 1898 to 2008 and found a five-fold increase in the local extinction rate of pikas in the 21st century. Beever et al. (2011) also found an 11-fold increase in the retraction rate of the upslope range between 1998 and 2008, indicating the possibility of a range collapse. In this scenario the lower range limit increases in elevation, as the lower part of the mountain gets warmer, shortening the available niche space for pikas. Two other species from the same ecoregion, the bushy-tailed woodrat (*Neotoma cinerea*) and the shadow chipmunk (*Tamias senex*), are also experiencing range collapse (Moritz et al. 2008). These species are all at a higher risk of extinction than a century ago due to the rapid climate change, likely due to the short period of time over which change has occurred, making adaptation to a new niche difficult, as well as their inability to relocate to a more suitable climate. These climate shifts threaten both plants and animals with significant physiological limitations (e.g., small animals like the pika) and limited dispersal abilities (e.g., plants like trees, which can play a role in forest development and productivity; CCSP 2008; Milanovich et al. 2010).

Additionally, there is increasing evidence that extinctions due to climate change are not just due to unavailability of suitable climate, but may also be caused by changes in species

interactions (Cahill et al. 2013). For example, climate change is predicted to increase the spread of infectious diseases and their vectors, putting species with no previous evolutionary experience with the expanding pathogen at risk (Patz et al. 1996). Mosquito-transmitted diseases that affect people (e.g., dengue, malaria) are particularly likely to experience range shifts or expansion due to climate change, which will directly affect the transmission rates of the diseases being carried (Patz et al. 1996). Chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd* or chytrid fungus), is one of the leading causes of loss of biodiversity in frogs and toads and recent research has shown that its effects are exacerbated with climate warming (Wake & Vredenburg 2008). In 2013, a new chytrid fungus found on salamanders, *Batrachochytrium salamandrivorans* (*Bsal*), was first documented, and now threatens salamander abundance and distribution globally (Grant et al. 2016).

Though climate change is clearly implicated in modern biodiversity losses, it is not the only factor to blame. Changes in human land use are also a leading cause in the decline in biodiversity. The terms “land cover” and “land use” are often used synonymously though they actually have two completely different meanings (Trisurat et al. 2011). Land cover is the biophysical cover of the Earth's' surface; while land use is the land that is used, maintained, or changed in any way by the human population (Trisurat et al. 2011). When considering extinction, the term that we are most concerned about is land use and how humans may be negatively affecting the land, and indirectly, the biodiversity of organisms, through habitat change. In the last few decades, the need for pastures, plantations, and urban areas has been driven by human population growth and expansion (Foley et al. 2005). With over 7.4 billion people, the needs of humans have multiplied, including increased needs for food, water, clothing, and shelter, driving our use of a large amount of the planet's resources (Foley et al. 2005). Other

negative effects of human land use include: soil erosion, degradation, and salinization (Trisurat et al. 2011). Urbanization, deforestation, and animal husbandry have had additional unforeseen consequences on other ecosystem functions, for example, the availability of fresh water (DeFries et al. 2004).

Between 2000-2010, the United Nations Food and Agricultural Organization found that 13 million hectares of land had been used or destroyed by the human population (Trisurat et al. 2011). Habitat destruction is the leading cause of population decline for wild species (IUCN 2016) and it has been predicted that this decline will continue within the next century (Eglington and Pearce-Higgins 2012). Ectotherms are particularly affected by land use because they typically have narrow and specific ranges of various abiotic factors (e.g., temperature, moisture) in which they are able to successfully survive and reproduce (Corn 2003; Eglington and Pearce-Higgins 2012). Gene flow can also be affected if deforestation leads to habitat fragmentation (Young et al. 1996). Fragmentation limits access to new habitat, and also favors species that are well adapted to the edges of these habitats, as core habitat is reduced but edge habitat increases under fragmentation (Trisurat et al. 2011). Thus, habitat fragments generally experience a decrease in species richness (Trisurat et al. 2011).

2.2 The Need for Long-Term Monitoring and Models

Unfortunately, all species cannot be monitored for ecosystem integrity due to financial and temporal constraints (Welsh & Droege 2001). Much like how we use thermometers while cooking to monitor the progress of the meal, we must use ecological indicators to monitor the integrity of an ecosystem of interest. Using indicator species to deduce the health of a habitat allows ecologists to identify ecosystem stressors that can lead to local extinctions and may

suggest strategies to prevent further loss of species. Any single species, whether it is plant, animal, fungus, or bacterium, is connected both directly and indirectly to several other species due to consumer-resource, mutualistic, or other interactions. As a result, the loss of a single species can have major implications for all other species due to the complexity of the interactions in any given ecosystem (Mills et al. 1993).

The definition of indicator species has been debated. For biological conservation the indicator is often an “umbrella species,” which is a species with a large enough range that when it is protected, a larger group of other species is also covered because of their geographic overlap with the indicator species (Simberloff 1998). Sensitive species with large ranges make good umbrella species on which to focus conservation efforts. There is much debate as to the use of indicator species, but there is agreement that in order to use a species as an indicator, there must be thorough background knowledge on its natural history in order for it to appropriately serve as an indicator (Landres et al. 1988).

2.3 The Eastern Red-backed Salamander

Plethodontidae is the largest salamander family, with approximately 458 species, or roughly 66% of all known salamander species (Amphibiaweb 2017). All plethodontids are lungless, and thus rely solely on cutaneous respiration which requires moist habitats (Petranka 1998). One common plethodontid, the eastern red-backed salamander, *Plethodon cinereus* (Figure 2), is a terrestrial salamander found in northeast North America (Petranka 1998). *Plethodon cinereus* is most commonly found in mature forests with full canopies that keep the forest floor moist and shaded (Petranka 1998). *Plethodon cinereus* has two primary color morphs, striped and unstriped, and a third, rarer, erythristic morph (all red in color; Figure 2).

The striped morph has a red dorsal stripe along its back, while the unstriped does not and is uniformly black in color (Figure 2).

Plethodon cinereus activity has previously been suggested for use as a natural indicator of ecosystem health (Welsh and Droege 2001). Its sensitivity to natural changes of ecosystem processes, like moisture cycling and microclimate, can serve as a signal to ecologists that greater changes are happening in the ecosystem (Welsh and Droege 2001). By studying the responses or behaviors of *P. cinereus*, including changes in the distribution or movement of the salamanders as a result of changes they detect in the environment, ecologists can better understand the health of the broader ecosystem. To efficiently monitor ecosystem variables through salamander responses, it is important to have an ecosystem profile for salamanders, and in particular *P. cinereus*, to most efficiently use them as a model system for other salamanders and amphibians. In previous studies, *P. cinereus* has been found to prefer leaf litter composed of deciduous leaves over coniferous pine needles (Renaldo et al. 2011), but more information is needed as there is range-wide variation in ecological preferences, which results in inconsistencies between studies. For example, Petranka (1998) notes that *P. cinereus* is rare or absent at a $\text{pH} \leq 3.7$, while Moore and Wyman (2010) have found healthy, abundant populations of *P. cinereus* living in soil environments at or just below this apparent limit (mean $\text{pH} = 3.7 \pm 0.4$; Moore and Wyman 2009).

One interesting result to come out of the many habitat studies of *P. cinereus* is that the different color morphs may have different habitat preferences. One hypothesis, addressing why the two main color morphs seem to prefer different climates, suggests that differing climate tolerances of the two morphs allow the species to inhabit more land, or expand its ecological niche (Fisher-Reid & Wiens 2015). Across its range, *P. cinereus* populations are roughly 70%

striped, 30% unstriped; however, there are several places that are different from this general pattern (e.g., Long Island, New York, Fisher-Reid et al. 2013). In previous studies, the striped salamander was more likely to be found in cool and moist climates, while the unstriped salamander was more commonly found in warm and dry climates (e.g., Lotter & Scott 1977; Moreno 1989; summary in Petranka 1998). Other studies have suggested that the unstriped morph also has a higher mortality rate in cooler climates, and retreats back underground earlier than the striped morph when temperatures begin to drop at the end of the fall (Gibbs & Karraker 2006; Anthony et al. 2008). The erythristic morph's climate preferences are very poorly known due to the low sample size across the range of *P. cinereus*. However, these color morph climate preferences are not consistent across the large geographic range of *P. cinereus* (e.g., Petruzzi et al. 2006 contradicts Moreno 1989; and Fisher-Reid et al. 2013 found a higher proportion of unstriped morphs in cooler, drier climates; see also Moore and Ouellet 2014). From 1908-2004, of the species entire range a 0.7 °C warming occurred and the data from 558 sites with over 50,000 salamanders was analyzed for changes in color morphology (Gibbs & Karraker 2006). The probability of an individual being striped was found to increase with latitude, longitude, and elevation, while also decreasing overall by 6% in the years studied (Gibbs & Karraker 2006). Gibbs and Karraker suggest that the results are due to climate warming due to habitat disturbance caused by increased human activity (Gibbs & Karraker 2006; but see Moore and Ouellet 2015).

2.4 Climate Change and the Eastern Red-Backed Salamander

The International Union for the Conservation of Nature (IUCN) states that global climate change is the cause of elevated extinction risk and threatened status for many species (IUCN 2016). Amphibians in particular are at risk, because they are uniquely sensitive to their climate

(Foden et al. 2013; IUCN 2016). For many species it is difficult to determine how climate change is affecting them, partly because we do not have good time-series data documenting changes in abundance or morphology. Common species are also often ignored in favor of species at more immediate risk (Gaston 2010), who may have idiosyncratic responses that are difficult to generalize.

As discussed above, *Plethodon cinereus* salamanders may be good indicators of climate change (e.g., Gibbs & Karraker 2006) and forest health (e.g., Welsh & Droege 2001; Davic & Welsh 2004). This is in part because *P. cinereus* are easy to find, occur in high densities, eat a large number of forest invertebrates, but, primarily because they are sensitive to changes in temperature and moisture as described above (Petranka 1998). *Plethodon cinereus* also show potential as an indicator species because they are closely related to at-risk amphibian species that cannot be studied as easily (e.g., *P. shenandoah*; Fisher-Reid and Wiens 2011). By studying *P. cinereus*, we can use their responses to understand how global processes, like climate change, may be affecting more at-risk species with similar habitat requirements, as well as the overall health of the forest ecosystem (Welsh & Droege 2001; Davic & Welsh 2004).

2.5 Lotter and Scott 1977 Study

In 1977, Lotter and Scott studied *P. cinereus* color morph frequency across New England. Lotter and Scott (1977) sampled 49 sites uniformly distributed across New England, as well as a 416 specimen collection at the Harvard University Museum of Comparative Zoology which served as the 50th site for color morph analysis. At each site a minimum of 100 individuals were sampled by searching under logs, rocks, and bark fragments in localities of 1-3 km diameter (Lotter and Scott 1977).

Lotter and Scott (1977) also looked at temporal variation in morph frequency by sampling 100 salamanders in Locality 7 six times from March to November in 1973. Finally, they tested for differential mortality between striped and unstriped individuals by comparing the ratios of each color morph among juvenile and adult size classes, using 323 individuals collected from September to October in 1974 at sites adjacent to those used in the temporal variation sites (Lotter and Scott 1977).

Lotter and Scott (1977) found that morph frequency varied according to annual temperature patterns, with Massachusetts containing forests in both the “warm” and “cold” regions (Lotter and Scott 1977; Figure 3). Warm region localities had higher frequencies of unstriped morphs (5-30%), and cold region localities had lower frequencies of unstriped morphs (0-5%; Lotter and Scott 1977). Their temporal variation observations found that the unstriped individuals retreated from the surface earlier in autumn than the striped morph. There was also evidence for greater juvenile mortality in the unstriped morph: the unstriped morph had a higher frequency among juveniles than adults while the striped morph had no significant difference in frequency between age groups (Lotter and Scott 1977).

In this paper, I begin to understand the effects of global temperature change and changes in human land use in the last 40 years on *P. cinereus* populations by revisiting locations sampled by Lotter and Scott in 1977. I compared the abundance of each color morph observed at 25 sites in 2016 to those documented at the same 25 sites by Lotter and Scott (1977). I determined whether there has been a significant change in morph frequency that can be linked to simultaneous changes in environmental factors that might be impacting salamander habitat in Massachusetts, New Hampshire, and Vermont.

3. Methods & Materials

3.1 Site Identification

Prior to sampling, I determined the current status of all Lotter and Scott (1977) localities in Massachusetts. I conducted preliminary site identification using online mapping software (Google Maps and Google Earth) and geographic information systems (GIS) data. For Lotter and Scott (1977) localities that had been disrupted by either urbanization or other forms of habitat destruction, I found the closest public forests within a 5 km radius (Figure 4) of the coordinates in Lotter and Scott (1977, reproduced in Table 1 and Figure 3). I sampled the 15 Massachusetts sites from Lotter and Scott (1977; Table 1). Dr. B. J. Cosentino at Hobart and William Smith Colleges in New York led the resampling effort in the Vermont and New Hampshire localities.

3.2 Data Collection

Each site was visited at least once for data collection between the hours of 0900 and 1700. For each numbered Lotter and Scott (1977) locality, I visited between one and five different forested sites within the 5 km radius described above. More than one site was visited at each locality if a sufficient number of salamanders could not be found at the first site. At each site I searched under logs, bark, rocks, and leaf litter for salamanders. For each salamander found, I recorded color morph (striped, unstriped, or erythristic), the status of the tail (broken or intact), and the size class: juveniles < 35mm and adults > 35mm in snout-to-vent length (SVL; Figure 5). I also recorded the GPS coordinates of the salamander's cover object. I noted the forest type (e.g., deciduous, pine) and the dominant tree species. If few or no salamanders were found at a location, the same site or others within the radius were revisited at a later date until I had sampled a minimum of 100 salamanders for each Lotter and Scott (1977) locality. To avoid

resampling the same individuals on different days, I sampled different, distinct forest patches (e.g., separated by a road) within the radius when returning on a second or third day. At each location, I also recorded the number of juvenile red spotted newts (red efts, *Notophthalmus viridescens*).

3.3 Biological Handling Protocol

Scientific collection permits from the appropriate Divisions of Fisheries and Wildlife were acquired by M. C. Fisher-Reid (with M. Fossella as a subpermittee; permit #024.165SCRA) before data collection began, and research was conducted with approval from Bridgewater State University's Institutional Animal Care and Use Committee (IACUC; Case number 2015-01). Salamanders were captured by hand and if needed contained in open plastic bags for less than 2 minutes to ensure their health was not threatened due to lack of air or moisture. If needed the salamander was gently moved to cause a positional change for measurement. After all data were collected, the salamander was released to the exact spot it was found and covered again by the log, rock, or bark it was found under originally.

3.4 Statistical Analyses

I used a χ^2 test of independence to compare morph frequencies over the last 40 years for all sites where the 1977 frequency for each morph was $> 5\%$. For any sites where Lotter and Scott (1977) found less than 5% of a morph, I used Fisher's exact test because it works better for smaller sample sizes and helps reduce type I errors. Any localities which did not have a minimum of 100 salamanders sampled in 2016 were excluded from analyses. In a subset of the data collected (MA localities with 100+ salamanders in 2016 and a minimum of 5 unstriped

salamanders), I compared morph frequencies between the two size classes, adult and juvenile, using a χ^2 test of independence.

I used Spearman's rank correlation to test if there was a relationship between the number of red efts (juvenile newts, *Notophthalmus viridescens*) and the erythristic morph, a purported Batesian mimic of the efts (Petranka 1998). I used Spearman's rank correlation because my data set failed a Shapiro-Wilk test for normality, and Spearman's rank correlation does not assume a normal distribution. All statistical analyses were conducted with R software (version 3.0.3; <https://www.r-project.org/>).

4. Results

In 2016, 25 of the Lotter and Scott (1977) sites were revisited by two teams, one from Bridgewater State University and the other from Hobart and William Smith Colleges. Of these 25, ten Massachusetts, five Vermont, and four New Hampshire localities met the criteria of ≥ 100 salamanders encountered. Of these 19 localities, five had significant differences between morph frequencies in 1977 and 2016 (denoted by bolded P values in Table 1). Two localities in the warm region, Cohasset/Scituate (15) and Ashland (17), had significant changes in frequencies, both with increased striped and decreased unstriped frequencies (Table 1). The cold region's three localities with significant changes were inconsistent in their pattern of change. In New Ashford (42) and Colrain (45), the striped morph became more common, the unstriped remained consistent, and the erythristic decreased in frequency (Table 1). In Westhampton (44) the striped morph decreased, the unstriped increased, and the erythristic remained consistent (Table 1). However, after a sequential Bonferroni test to correct for multiple comparisons, only two localities, 15: Cohasset/Scituate and 44: Westhampton, remained significant (Table 1). One location, 30: Wells, was found to be marginally significant with a $P_{A/B} = 0.0785$ (Table 1). No significant differences were found in morph frequency ratios between the two size classes (all $P > 0.300$). Spearman's rank correlation found a weak, non-significant relationship between number of red efts found and number of erythristic morphs found ($r_s = 0.09$, $P = 0.68$).

5. Discussion

5.1 Morph Frequency

Climate change and land use are increasingly being documented as contributing to morphological change and species extinctions. In this paper, I set out to document the potential effects of climate change and land use on a common amphibian species with known correlations between morphology and climate. By revisiting localities from Lotter and Scott (1977), we were able to document any changes in morphology which might be driven by climate change. Only a few sites tested experienced significant change in morph frequency since 1977 (Table 1), and the direction of change was not only not what we expected, given previously documented climate tolerances of *P. cinereus* color morphs, it was also not consistent across sites.

The warm region localities which experienced significant changes (15 and 17) showed an increase in striped frequencies. Most previous work suggests that striped *P. cinereus* are better adapted to live in wetter and cooler environments (see reviews in Petranka 1998, and the introductions of Anthony et al. 2008; Fisher-Reid et al. 2013). However, some studies point to inconsistencies in this pattern (e.g., Petruzzi et al. 2006; Fisher-Reid et al. 2013; Moore and Ouellet 2015). Our results thus may indicate that the microhabitats of *P. cinereus* in the warm region have become cooler in the last 40 years or that the relationship between color and temperature change due to anthropogenic climate change is not as clear cut as previous studies have assumed. Further examination of local and regional climate change is required to help tease this apart. As described in the Introduction, we expected the cold region, previously defined by Lotter and Scott as the area with ~100% striped, to have more unstriped individuals as the world climate warmed. This occurred at only one locality (#44; Table 1), while the other two cold region localities with significant changes (#42 and #45; Table 1) showed an increase in striped

morphs and decrease in erythristic morphs, with no change in unstriped morphs (persistent absence). These results do not support the hypothesis that with climate warming, the more drought resistant morph (unstriped) would become more common. In sum, our results support no change in the majority of sites, particularly if corrections for multiple comparisons are made. A sequential Bonferroni adjustment only leaves two sites with significant change (#15 Cohasset/Scituate and #44 Westhampton, MA), and a more restrictive alpha value (i.e., of $\alpha = 0.01$ instead of $\alpha = 0.05$), again, only leaves two sites with significant change (#15 Cohasset/Scituate and #44 Westhampton, MA).

According to the PRISM group at Oregon State University, the temperature in New England has varied between (-0.22 and 0.22°C) per year from 1951-2006 (Figure 6). In comparison to Lotter and Scott's determination of the warm and cold regions (Figure 3), the majority of the cold region has experienced at least a -0.03°C temperature change, which is a very small change. Perhaps the climate change in New England has not yet been drastic enough to change the microclimate of *P. cinereus*, or another factor is driving morph frequency changes at these localities, like intermorph competition or intermorph variation in response to changes in land use.

The absence of the unstriped morph in many locations may also be explained by the behavior and physiology of the striped and unstriped morphs. A pair of studies found that the two color morphs had different responses to predation, suggesting that the unstriped morph is more vulnerable to avian predators (Moreno 1989; Venesky & Anthony 2007). A high predatory attack rate on the unstriped morph could explain the lack of increase in the morph that was hypothesized with the increase in temperature, however the present day stability of the unstriped adults and juveniles make this unlikely (see section 5.3).

5.2 Mimicry in the Erythristic Morph

My study also documented the current frequencies of the rare erythristic morph across the 25 sites. In the present study, they are more often found in Western MA, and Southern Vermont and New Hampshire, which are all entirely in the cold region (Table 1). Lotter and Scott (1977) hypothesized that the erythristic morph is a mimic of the terrestrial adult morph of the toxic eastern red-spotted newt, *Notophthalmus viridescens*. If their hypothesis is correct, we expect the erythristic morph would be more common in areas with large red eft populations. However, we found no relationship between number of red efts found and number of erythristic morphs ($r_s = -0.0996$, $P = 0.6849$). Overall, we saw a region-wide decrease and extremely low abundance of erythristic morphs, so further tests of this hypothesis will be difficult.

5.3 Juvenile v. Adult Frequencies

In 1977, Lotter and Scott found that the unstriped morph had a higher juvenile frequency than adult frequency, indicating that there was a higher mortality rate in the unstriped morph compared to the striped morph. They argued that unstriped juveniles had a selective advantage over the unstriped adults, because of their small sizes and ability to blend into dirt with their dark color, they are harder for predators to see (Lotter and Scott 1977). In the present study, there is no significant difference in ratio of the striped and unstriped color morphs between juveniles and adults, meaning we found just as many juveniles as we did adults in both morphs. This suggests that the present day frequencies are stable, and whatever factor was previously leading to juvenile mortality in unstriped morphs is no longer present, or the species has adapted to it. From a biodiversity perspective, the stabilization of the unstriped morph indicates that natural selection is not acting asymmetrically against the unstriped morph, as Lotter and Scott predicted in 1977.

Our results do not support Lotter and Scott's (1977) hypothesis that there is variation in juvenile mortality between the morphs.

5.4 Conclusions

Further studying of particularly temperature-sensitive species, such as *P. cinereus*, is necessary to assess the loss of biodiversity that is occurring due to the changes in climate over the last 40 years (Figure 1). The decrease of the unstriped morph in all locations is interesting and indicates that the morph may be lost in some populations. Current conservation efforts must be strengthened to protect the current biodiversity of the planet. Efforts can be strengthened by continuing to map species occurrences, assessing population size, and species viability (Schmitz et al. 2015). Forecasting efforts must be made to analyze the potential effects of climate change on landscapes, pests, and invasive species (Schmitz et al. 2015). Ecological connectivity must be restored in areas of human fragmentation (Schmitz et al. 2015). Areas of refugia for species at risk of displacement due to climate change must be identified and protected (Schmitz et al. 2015). As explained in the framework by Schmitz et al. (2015), three main steps need to be taken to combat the loss of climate driven biodiversity: (1) establish a baseline of current environmental conditions, (2) identify ecological features vulnerable to climate change, and (3) deduce the future needs of the most vulnerable and at risk species, ecosystems, and landscapes.

6. Future Work

As of right now, we have only a few pieces of this puzzle. My work is part of a larger collaborative effort lead by Dr. B. J. Cosentino at Hobart and William Smith Colleges in New York to resample all 50 Lotter and Scott sites. Resampling will be completed in the next year or two. Once all of the sites have been resampled, we will have complete data on overall regional change, and be able to test the impact of climate and land use as potential drivers of the observed change.

7. Acknowledgements

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9. Figures and Tables

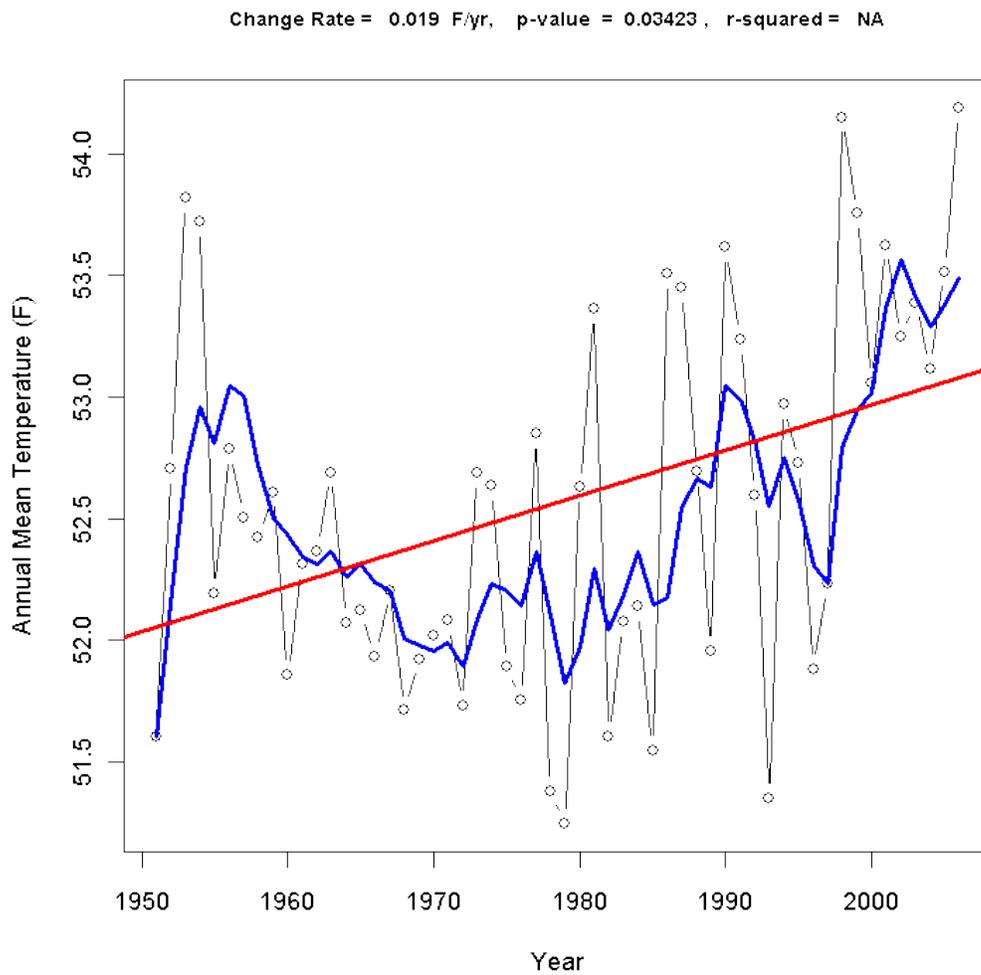


Figure 1. The annual mean temperature of the United States of America from 1950-2000. Map produced by Climate Wizard, University of Washington and the Nature Conservancy (2009).



Figure 2. The three most common color morphs of *P. cinereus*. From left to right: unstriped, striped, and the all red erythristic morph. Photos used with permission from M.C. Fisher-Reid.

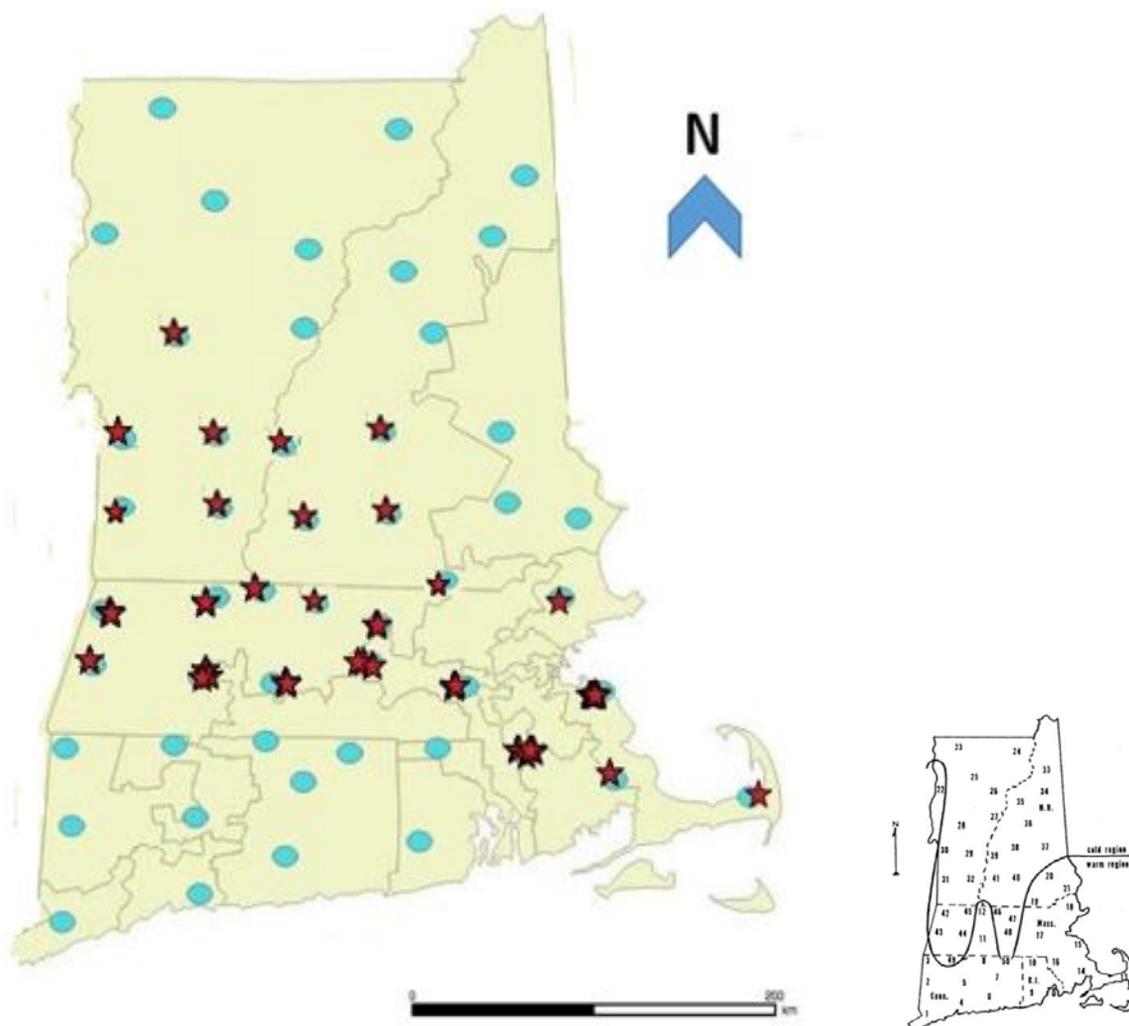


Figure 3. Map of New England. The light blue dots represent all 50 localities used in the Lotter and Scott (1977) study (coordinates from Lotter & Scott Table 1). The 25 red stars represent the locations sampled in the present study. Map made with QGIS software. The small subset map shows the warm and cold regions as defined by Lotter and Scott, regions were based on the morph frequency composition, with the upper “cold” region being ~100% striped and the lower “warm” region being ~5-30% unstriped (Lotter & Scott, Figure 1).

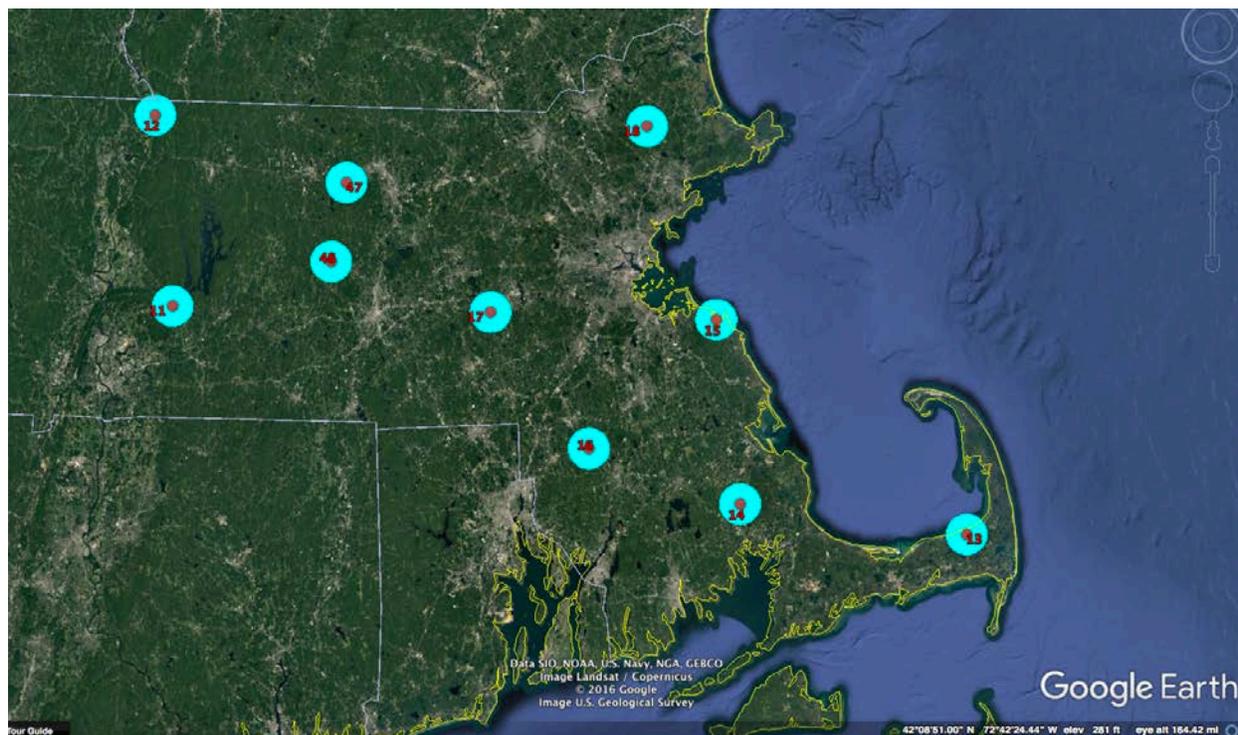


Figure 4. A Google Earth image of 10 localities sampled in Massachusetts. The red dots represent the original coordinates Lotter and Scott samples. The 5 km sampling radius that each locality had is represented by the light blue filled circles.

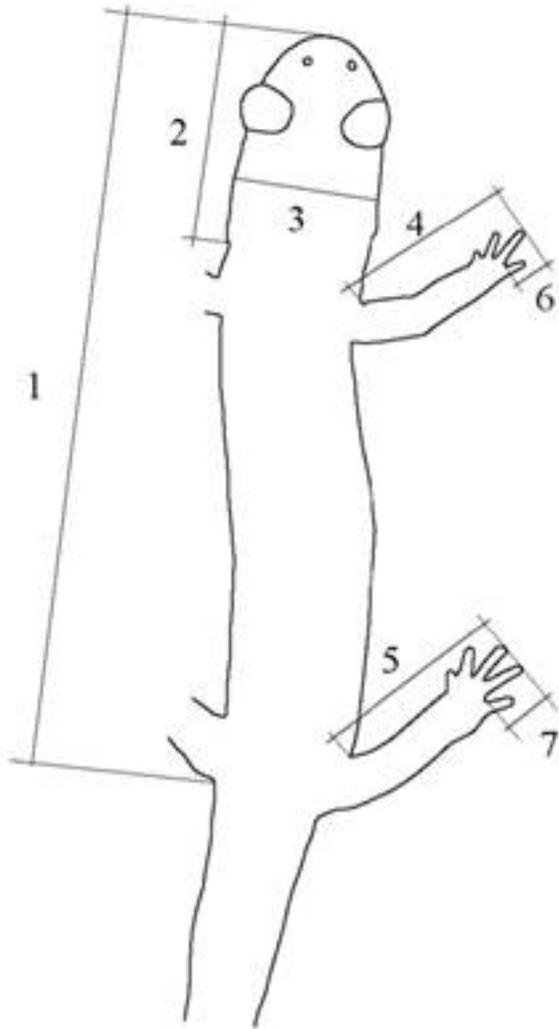


Figure 5. Common length measurement on salamanders. SVL is the distance from the tip of the rostrum (snout) to the anterior edge of the cloacal vent (bottom of the core); (1) SVL (2) Head or snout-gular length (3) Head width (4) Forelimb length (5) Hindlimb length (6) Third finger length (7) Fourth toe length (figure adapted from Alexandrino et al. 2005, Figure 2).

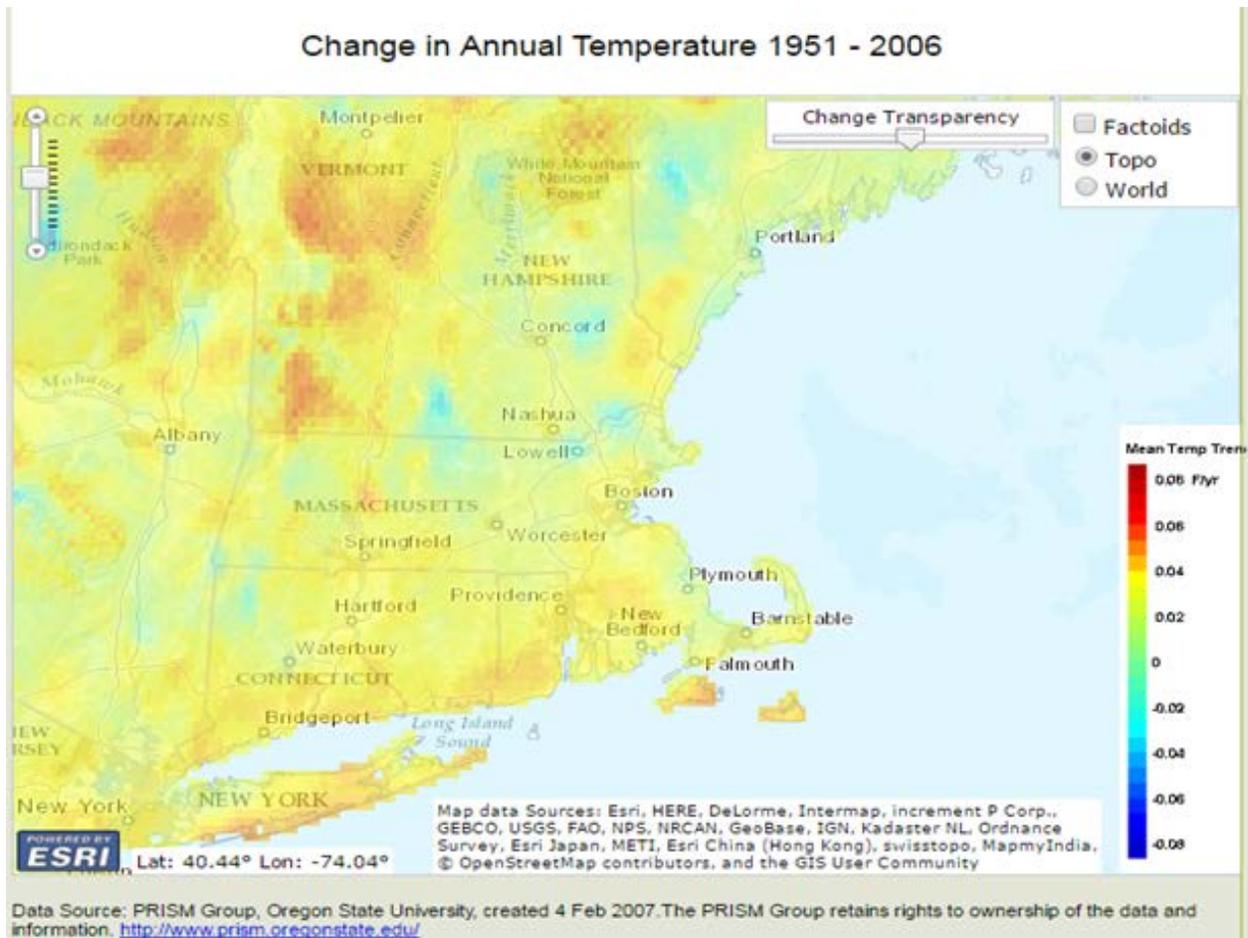


Figure 6. Change in Annual Temperature from 1951-2006 in New England. The orange and yellow colors represent an annual increase in temperature and the blue colors represent a decrease in temperature. Across New England there are increases and decreases, but overall there is a slight increase. Map was made by Prism Group at Oregon State University and has incorporated statistical confidence. Gray areas are areas of low confidence (imagery from PRISM Climate Group, Oregon State University 2007).

Table 1: χ^2 contingency tables showing changes in color morph frequencies from 1977 and 2016 sampling. Following Lotter and Scott (1977), all data are reported to the nearest 1% for all localities with sample sizes ≥ 100 . *P*-values in bold represent significant differences between the 1977 and 2016 frequencies at the $\alpha = 0.05$ level. After a sequential Bonferroni correction, only Locality 15: Cohasset/Scituate remains significant.

Locality 11: Belchertown, MA	1977	2016
Unstriped	24	29
Striped	76	71
Erythristic (all red)	0	0
$\chi^2 = 0.64$, $df = 2$, $P = 0.726$		
Locality 12: Northfield, MA	1977	2016
Unstriped	12	22
Striped	88	78
Erythristic (all red)	0	0
$\chi^2 = 8.87$, $df = 2$, $P = 0.119$		
Locality 15: Cohasset/Scituate, MA	1977	2016
Unstriped	18	1
Striped	80	98
Erythristic (all red)	2	1
Fisher's Exact Test: $P_A = 0.000022$; $P_B = 0.000016$ Significant after Bonferroni Correction		
Locality 16: Norton, MA	1977	2016
Unstriped	9	17

Table 1 (Continued)		
Striped	88	82
Erythristic (all red)	2	1
Fisher's Exact Test: $P_A = 0.2357$; $P_B = 0.2357$		
Locality 17: Ashland, MA	1977	2016
Unstriped	18	5
Striped	82	95
Erythristic (all red)	0	0
$\chi^2 = 8.3$, $df = 2$, $P = 0.016$		
Locality 28: Robinson, VT	1977	2016
Unstriped	0	0
Striped	100	100
Erythristic (all red)	0	0
$\chi^2 = 0.00$, $df = 2$, $P = 1.00$		
Locality 29: Ludlow, VT	1977	2016
Unstriped	0	0
Striped	100	100
Erythristic (all red)	0	0
$\chi^2 = 0.00$, $df = 2$, $P = 1.00$		

Table 1 (Continued)		
Locality 30: Wells, VT	1977	2016
Unstriped	2	4
Striped	98	92
Erythristic (all red)	0	4
Fisher's Exact Test: $P_A = 0.0785$; $P_B = 0.0785$		
Locality 31: Arlington, VT	1977	2016
Unstriped	0	0
Striped	99	100
Erythristic (all red)	1	0
Fisher's Exact Test: $P_A = 1.0$; $P_B = 1.0$		
Locality 32: Townshend, VT	1977	2016
Unstriped	0	0
Striped	100	97
Erythristic (all red)	0	3
$\chi^2 = 3.05$, $df = 2$, $P = 0.2176$		
Locality 38: Wilmot Flat, NH	1977	2016
Unstriped	1	0
Striped	99	100

Table 1 (Continued)		
Erythristic (all red)	0	0
Fisher's Exact Test: $P_A = 1.0$; $P_B = 1.0$		
Locality 39: Claremont, NH	1977	2016
Unstriped	0	1
Striped	100	99
Erythristic (all red)	0	0
$\chi^2 = 1.01$, $df = 2$, $P = 0.6035$		
Locality 40: Deering, NH	1977	2016
Unstriped	0	0
Striped	100	100
Erythristic (all red)	0	0
$\chi^2 = 0.00$, $df = 2$, $P = 1.00$		
Locality 41: Gilsum, NH	1977	2016
Unstriped	0	0
Striped	87	85
Erythristic (all red)	13	15
$\chi^2 = 0.17$, $df = 2$, $P = 0.9185$		
Locality 42: New Ashford, MA	1977	2016

Table 1 (Continued)		
Unstriped	0	0
Striped	92	100
Erythristic (all red)	8	0
$\chi^2 = 8.33, df = 2, P = 0.0155$		
Locality 43: Lenox, MA	1977	2016
Unstriped	0	1
Striped	100	98
Erythristic (all red)	0	1
$\chi^2 = 2.02, df = 2, P = 0.364$		
Locality 44: Westhampton, MA	1977	2016
Unstriped	1	12
Striped	99	88
Erythristic (all red)	0	0
Fisher's Exact Test: $P_A = 0.0025; P_B = 0.0014$ Significant after Bonferroni Correction		
Locality 45: Colrain, MA	1977	2016
Unstriped	0	0
Striped	82	95
Erythristic (all red)	18	5

Table 1 (Continued)

$\chi^2 = 8.3, df = 2, P = 0.0158$		
Locality 48: Rutland, MA	1977	2016
Unstriped	0	0
Striped	100	100
Erythristic (all red)	0	0
$\chi^2 = 0.00, df = 2, P = 1.00$		