The influence of silvicultural manipulations on plethodontid salamanders

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science
In

Fisheries and Wildlife Sciences

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24 April 2024
Blacksburg, VA

Keywords: (*Plethodon cinereus*, terrestrial salamander, forestry, timber harvest, body condition, pit and mound topography, tip-up mound)

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ABSTRACT

Habitat alteration (i.e. degradation, fragmentation, and destruction) is the primary driver of amphibian decline and extinction. Despite their ecological importance and threatened status, very little long-term research has been conducted on how methods of forest management impact salamanders. In this research, I examine how experimental silviculture impacts plethodontid salamander relative abundance and count, and I compare three different body condition indices. Chapter 1 focuses on plethodontid salamander relative abundance 30 years after experimental treatments (including clearcut and shelterwood harvests, understory herbicide, uneven-aged management, and an untreated control) were first applied. I found that plethodontid salamander populations in all silvicultural treatments without stand re-entry have reached pre-harvest relative abundance levels. Chapter 2 describes how artificial tip-up mounds that could be used to mimic old-growth forest characteristics impact plethodontid salamander count. Salamander count significantly declined in treatment units with artificial tip-up mounds but this could be an artifact of the heavy disturbance required for installation. Chapter 3 compares three different body condition indices for plethodontid salamanders. I found that bioelectrical impedance analysis (BIA) is likely not suitable for use with plethodontid salamanders and mass divided by snout-to-vent-length is likely a superior estimate to tail width divided by snout-to-vent-length. These findings further our understanding of how different forest management practices affect salamander populations and provide guidance for evaluating body condition.

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GENERAL AUDIENCE ABSTRACT

Forest understory salamanders play an important role in energy transfer and their position in leaf litter food webs affects multiple ecosystem functions. Despite their ecological importance, very little long-term research has been conducted on how habitat change impacts salamanders. This research investigates how different forest management techniques influence forest-dwelling salamanders and compares three different ways to evaluate salamander health. Chapter 1 focuses on the salamanders 30 years post-harvest. I found that salamander populations in all silvicultural treatments except one had recovered. Chapter 2 described how tipping over trees to mimic old-growth forest characteristics impacts the number of forest-dwelling salamanders. There were significantly fewer salamanders in treatment units after the disturbance created by installing artificial tip-up mounds. Chapter 3 compares three different ways to evaluate forest-dwelling salamander health. I found that bioelectrical impedance analysis (BIA)is likely not suitable for forest-dwelling salamanders, and weight divided by body length is likely a superior estimate to tail width divided by body length. These findings together further our understanding of how different forest management practices affect salamander populations and provide guidance for evaluating body condition.

Chapter 1: The long-term effects of seven different silvicultural treatments on relative plethodontid salamander abundance

The goal of this study is to assess plethodontid salamander populations more than 25 years after the initial implementation of an experiment comparing seven silvicultural treatments. Silviculture is the science of cultivating trees to meet management goals. The original broader goal of the experiment was to evaluate the impacts of silvicultural treatments to facilitate oak regeneration on biodiversity. I compare relative plethodontid salamander abundance to determine if relative abundance returns to levels similar to unharvested mature forest 30 years after the implementation of different silvicultural treatments.

Chapter 2: The impact of artificial tip-up mounds on plethodontid salamander counts in a central Appalachian hardwood forest

My previous research in Michigan suggests that salamanders are more abundant in areas with artificial tip-up mounds. The goal of this study is to determine whether artificial tip-up mounds impact salamander abundance in a central Appalachian hardwood forest and the mechanism behind this impact.

Chapter 3: Evaluating bioelectrical impedance analysis for use on eastern red-backed salamanders and comparing two different body condition indices

The goal of this study is to evaluate the accuracy and precision of a new technique to estimate salamander body composition using bioelectrical impedance analysis (BIA) and to compare a traditional method to estimate body condition, to a less frequently used method.

ACKNOWLEDGEMENTS

Thank you to my advisor, Dr. Carola Haas, for your support, mentorship, advice, feedback, and encouragement throughout these three years. Thank you to my committee members Dr. Nick Caruso, Dr. Holly Kindsvater, and Dr. Mike Aust for giving me sound recommendations and excellent feedback. Thank you to Dr. George Brooks, for all the hours spent guiding and helping me, I couldn't have done this without you. Thank you to my lab mates, graduate student friends, and technicians, for your help in the field, coding fixes, and emotional support. Thank you to my mother for being the one who made this all possible. Your unwavering support and generosity are the sole reason I was able to start graduate school here. Though he cannot read, I must thank my sweet pup, Magne, for providing me with constant companionship, comedic relief, and comfort. Thank you to Zachary Jarvis, for being my safe harbor in the storm that is life and indulging me as I ramble on incessantly about research. Lastly, thank you to the salamanders, you ignite a childlike curiosity and passion inside of me.

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INTRODUCTION

Salamanders are critical members of forest and stream ecosystems and can influence litter decomposition, invertebrate communities, and carbon storage (Laking et al. 2021). They are highly efficient at converting invertebrate prey to biomass (Pough 1980), providing a readily available source of high-quality protein for larger vertebrate predators including mid-sized mammals, birds, reptiles, and fish. Milanovich and Peterman (2016) found that in the Midwest, limiting nutrient estimates were determined by the density of *Plethodon albagula* in the forest, highlighting the importance of their role in nutrient cycling. In the terrestrial ecosystems of the eastern United States, salamanders often comprise the majority of vertebrate biomass (Burton and Likens 1975). In New Hampshire, wet salamander biomass exceeded bird biomass by 2.6 times with higher numbers of salamanders than birds or small mammals (Burton and Likens 1975). Despite their abundance in the eastern U.S., from 1980 to 2009 less than 6% of wildlife research journal space was dedicated to studies of herpetofauna, with reptiles making up the majority of this small percentage (Christoffel and Lepczyk 2012). The underrepresentation of salamanders compared to mammals and birds in modern wildlife research, despite their importance to forest ecosystems, highlights the need to conduct more studies on salamanders.

We are experiencing what many scientists call the sixth global mass extinction (Barnosky et al. 2011), with amphibians being disproportionately affected (Stuart et al. 2004). Amphibians are one of the most threatened taxa in the world with 41% of species considered threatened; the highest among all other vertebrates (IUCN 2021). Furthermore, 15% of all amphibian species are in decline (Ceballos et al. 2017), although it is difficult to determine just how many species are threatened or in decline since 11.4% of amphibian species are data deficient (IUCN 2023). The most common causes of amphibian declines are habitat loss and modification, contamination, introductions of non-native species, infectious disease, and climate change (Houlahan et al. 2000, Blaustein and Kiesecker 2002). Importantly, the decline in amphibians means losing what these species contribute to the ecosystem.

My research focuses on plethodontid salamanders, terrestrial forest-dwelling salamanders. They are the largest and most diverse salamander family in the world (Shen et al. 2016). The family Plethodontidae is a group of lungless salamanders that respire cutaneously, which requires their skin to be moist for gas exchange (Feder 1983). Plethodontid salamanders are sensitive to temperature since this influences their metabolic rate, digestive efficiency, and how quickly they desiccate, so they prefer moderately cool temperatures (Spotila 1972; Bobka et al. 1981; Feder 1983). They can be found in mesic forests or along streams under cover objects such as rocks and logs, which stay cool and wet. Since they are forest-dwelling salamanders, they can be impacted by changes to their habitat such as tree removal. When surface conditions become too hot and dry, they move underground. The main species that occurred at my sites for chapters 1-2 were the eastern red-backed salamander (*Plethodon cinereus*), Alleghany Mountain dusky salamander (*Desmognathus ochrophaeus)* Southern ravine salamander (*Plethodon richmondi*) slimy salamander (*Plethodon cylindraceus/glutinosus*) and Southern two-lined salamander (*Eurycea cirrigera*). I focused on eastern-redbacked salamanders in my third chapter, as they were the most abundant in my study and are one of the most well-studied species of salamander in North America (Liebgold and Cabe 2008; Takahashi and Pauley 2010). However, there are some key differences between these species that could influence my results. Most members of the *Plethodon* genus lay eggs terrestrially and have direct-developing young (Angle 1969; Feder 1983; Kerney 2011). In contrast, most members of the genera *Desmognathus* and

Eurycea lay eggs in streams and have aquatic larvae that metamorphose into terrestrial adults (Bruce 1985). Since some plethodontids rely on streams for reproducing, while others do not, they can vary substantially in the distance from streams they may occur. It is important to note that the taxonomy of plethodontid salamanders is in flux (Wake 2017; Pierson et al. 2023), complicated by many cryptic species (Beamer and Lamb 2020), which is why I focus on the family Plethodontidae as a whole rather than focusing on individual species.

Habitat modification is the primary threat to amphibian populations, including plethodontid salamanders, worldwide (Green et al. 2020). Timber harvest represents one type of habitat modification. It involves the removal of vegetation which can increase surface soil temperature, and air temperature, and reduce soil surface moisture, and leaf litter, which can negatively impact plethodontid salamanders (Childs and Flint 1987; Chen et al. 1993). Previous studies have reported that salamander abundance declines after timber harvest in the short term (Petranka et al. 1993; Ash 1997; Harpole and Haas 1999; Herbeck and Larsen 1999; Cromer et al. 2002; Knapp et al. 2003; Tilghman et al. 2012; O'Donnell 2015). However, timber harvest might not impact salamander abundance in the long term, as many short-term studies claim. Most of these studies have focused on the immediate impacts less than 15 years after harvest and very few studies have directly studied when salamander abundance returns to pre-harvest levels. One of the few other long-term studies of the impacts of silviculture on salamanders, the Missouri Ozark Forest Ecosystem Project (MOFEP) study, found that amphibians took over a decade to return to pre-harvest capture probabilities (Rota et al. 2017). The predicted recovery time for a return to pre-harvest abundance levels for different salamander species ranges from under 11 years to 100 years post-harvest (Morris and Maret 2007; Connette and Semlitsch 2013).

It is important to understand the long-term impacts of harvest on salamander populations in order to accurately evaluate trade-offs associated with different forest management practices.

It is critical for salamander conservation to understand strategies that will help maintain economically viable managed forest lands in ways that can sustain salamander populations over the long term, especially for sensitive or data-deficient species. Currently, several mitigation strategies exist such as retaining fine or coarse woody debris (CWD), especially after biomass harvesting (Fritts et al. 2016), retaining residual trees, and reducing soil compaction, but support for the effectiveness of these strategies is mixed (Otto et al. 2013, Fritts et al. 2016; Romano et al. 2018; Margenau et al. 2023). A review paper emphasized that while some studies have shown that the retention of CWD increased salamander counts only in dry areas, other studies have suggested that only highly decayed CWD increased counts (Otto et al. 2013). However, this could be because salamander populations do not benefit from CWD until after their populations have rebounded after harvest (Otto et al. 2013). Another potential mitigation strategy is the use of alternative silvicultural techniques instead of the more commonly used clearcut. Some examples include partial harvest methods such as group selection where small patches are cut, however, partial harvesting with multiple stand entries can result in higher soil erosion and additional detrimental declines in salamander abundance after the initial stand entry (Hood et al. 2002, Homyack and Haas 2013) and Knapp et al. (2003) found that most silvicultural treatments with canopy removal had similar declines in salamander abundance after the initial harvest.

Timber harvest can impact salamanders in more subtle ways beyond changes in abundance. If harvest impacts body condition, this could lead to a decreased population over time due to less energy being allocated toward reproduction or even decreased survival. Some studies have shown that timber harvest negatively impacts salamander body condition (Veysey

Powell and Babbitt 2015) while other studies have had conflicting results (Homyack et al. 2011). Body condition can be used as a proxy for habitat quality and gives insight into whether or not the habitat is meeting the nutritional needs of the animal (Homyack 2010). These studies utilize a commonly used method to estimate energy stores, the body condition index (BCI), a ratio of mass to length. One of the most commonly used BCI in amphibian studies is the residuals of an ordinary least squares regression of mass on body length (MacCracken and Stebbings 2012). However, there are some problems with using these indices. They are a unitless measure and can only be used to compare individuals within the same study. Also, there have been inconsistencies reported between the BCIs and other measures of habitat quality (MacCracken and Stebbings 2012). Snout-to-vent length, which is typically the body length measurement used to construct the BCI, can be difficult to measure since salamanders often move around in such a way that precise and accurate measurements are challenging to obtain, particularly when utilizing the help of technicians and volunteers with varying levels of experience. Accurate and precise body condition measurements are extremely important because otherwise differences in body condition can be attributed to measurement error and disregarded or false differences could be detected (Luiselli 2005; Bendik and Gluesenkemp 2013). While the current BCI using a ratio of mass to SVL is highly regarded as an accurate measure of fat content (Denoël et al 2002), the scientific community could benefit from exploring new measurement techniques that may be more precise and accurate.

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CHAPTER 1 - THE LONG-TERM EFFECTS OF SEVEN DIFFERENT SILVICULTURAL TREATMENTS ON RELATIVE PLETHODONTID SALAMANDER ABUNDANCE

ABSTRACT

Silvicultural practices can drastically alter the abiotic conditions of forest floor environments. Elevated soil temperatures, reduced humidity, and diminishing leaf litter are all expected following canopy removal. As a result, intensive silvicultural practices are assumed to be detrimental to plethodontid salamanders that rely on cool, moist environments to survive and deep leaf litter to forage. Although many studies have shown declines in salamander abundance immediately following timber harvest, the ability for populations to recover or recolonize harvested areas once they have returned to a forested state is largely unknown. Moreover, recovery may depend on the silvicultural practice employed (e.g., clearcut vs. group selection). Here I analyze 30 years of plethodontid salamander relative abundance data across seven silvicultural treatments on four research sites in southwest Virginia. I measured relative abundance by conducting night-time area-constrained searches only during or after rain events when the ground and leaf litter were saturated, and temperatures were within normal activity levels for plethodontid salamanders. As previously reported, I found that regardless of silvicultural treatment, plethodontid salamander relative abundance decreased in the first decade following any canopy removal, except in the group selection. Populations in the midstory herbicide treatment and the untreated control varied over the years but did not decline in the years post-treatment. Stand re-entry only occurred in the shelterwood treatments at two of the sites. In all cases except the shelterwood, populations began to show signs of recovery 10-20 years post-harvest. After 30 years, relative abundance in all treatments had reached pretreatment

levels. Our findings illustrate the resilience of plethodontid salamander populations; at least to small (2 ha) silvicultural treatments in Appalachian hardwood forests.

INTRODUCTION

The salamander component of the Southern Appalachian Silviculture and Biodiversity (SASAB) project was started by Dr. Carola Haas and Douglas Harpole in 1994 as part of a project developed by David Wm. Smith, Shep Zedaker, and W. Michael Aust, with the Virginia Tech Department of Forest Resources Environmental Conservation in collaboration with David Loftis with the U.S. Department of Agriculture Forest Service. The original goal of the project was to investigate the success of different silvicultural treatments for oak regeneration and the impact on biodiversity. Following the implementation of the project, numerous researchers have investigated how the silvicultural treatments have impacted the vegetative composition, disease vectors, oak regeneration, soil, fuel load, and salamander abundance (Hammond 1997; Harpole and Haas 1999; Wender 2000; Hood 2001; Knapp et al. 2003; Williams 2003; Kelly 2005; Sucre 2008; Atwood et al. 2009; Belote et al. 2009; Homyack 2009; Atwood et al. 2011; Homyack et al. 2011; Harris et al. 2015; Hahn et al. 2021).

Plethodontid salamanders are limited as to where they can survive and when they are able to forage and reproduce, due to their need for moist environments to avoid desiccation (Feder 1983). These salamanders are lungless and respire cutaneously (Feder 1983). Because they breathe through their skin, they need to be wet for gas exchange to occur (Feder 1983). Higher temperatures cause salamanders to dehydrate more quickly (Spotila 1972) and increase the metabolic rate, while decreasing digestive efficiencies, leading to an energy deficit (Bobka et al. 1981, Homyack et al. 2010) so cooler wet environments are more suitable for survival. As a result, they are often restricted to foraging during rainy nights and seek refuge underground or beneath cover when conditions are too hot or dry.

Timber harvesting can alter habitat and make it temporarily unsuitable. The removal of vegetation in clearcutting increases soil temperature and air temperature and reduces soil surface, leaf litter, and air moisture (Childs and Flint 1987; Chen et al. 1993). As a result, salamander abundance usually declines in the short-term following timber harvest (Petranka et al. 1993; Ash 1997; Harpole and Haas 1999; Herbeck and Larsen 1999; Cromer et al. 2002; Knapp et al. 2003; Tilghman et al. 2012; O'Donnell 2015). However, it is possible timber harvest may not have persistent negative effects on salamander abundance since most of our predictions are from short-term studies and chronosequences. It is still unknown when salamander abundance recovers to pre-harvest levels. Homyack and Haas (2009) predicted that it would take 60 years for the salamanders in this study to recover after initial treatments were applied, but many estimates vary because of differences in the harvest type, location, species impacted, methods, time scale, and others (Tilghman et al. 2012). The goal of this study is to evaluate the effects of a range of silvicultural practices for 30 years post-treatment on relative salamander abundance.

OBJECTIVE AND HYPOTHESES

I hypothesized that salamander abundance in each silvicultural treatment will have lower relative abundance 30 years post-harvest compared to pre-harvest abundance levels and compared to the control plots, based on previous predictions from this study estimating at least 60 years for population recovery (Homyack and Haas 2009).

METHODS

STUDY AREA

This study spans a total of six sites in Virginia and West Virginia. Two sites (BB1 and BB2) are located near the town of Blacksburg in Montgomery County, Virginia in the Eastern Divide Ranger District (formerly the Blacksburg Ranger District) of the George Washington and Jefferson National Forest; two sites are located in Wise County, Virginia in the Clinch Ranger District (CL1 and CL2) of the George Washington and Jefferson National Forest; and two sites are located on land that had been part of the Mead-Westvaco Corporation's Wildlife and Ecosystem Research Forest (MWERF) in Randolph County, West Virginia (WV1 and WV2) (Hammond 1997; Wender 2000) but this land has since been sold and harvested. Because no recent data are available from the West Virginia sites, and because the early data had already been published (Homyack and Haas 2009), I did not include data from those two sites in this analysis. The overall climate of this region is temperate and humid with warm summers and cold winters (Table 1.1). The soils at the Eastern Divide Ranger District are mainly Jefferson extremely stony soils and Berks and Weikert soils and the soils at the Clinch Ranger District are mainly Lily gravelly sandy loam and Oriskany very cobbly sandy loam (Soil Survey Staff 2024). Sites had several small intermittent streams running through them. The BB1 and BB2 sites range from 634-725 m in elevation and the CL1 and CL2 sites range from 1,006-1,089 m in elevation. Before silvicultural treatments were implemented, the stands were 76-100 years old (Wender 2000; Homyack 2009) and the overstory consisted mainly of *Quercus prinus*, *Q. rubra,* and *Q. alba* (Hammond 1997). All sites had a southwestern exposure. At each site, seven different silvicultural treatments were randomly assigned and applied from 1994-1998 across adjacent 2-ha experimental units. In order of increasing canopy removal, the treatments were 1) control, in which no treatment was applied, 2) midstory herbicide treatment, in which a methylated seed oil herbicide solution with triclopyr ester or triclopyr and imazapyr was applied to the bottom 15-30 cm of stems to reduce competition to regenerate desirable species, 3) group selection, in which all stems over 2.5 cm diameter at base height (DBH) were removed from 2-3 0.5 ha openings with timber stand improvement used in the remaining stand to remove undesirable trees to promote regeneration of desirable species, 4) shelterwood in which a partial harvest removed 41% of the basal area with the residual shelter trees being dominant and co-dominant stems which were intended to be removed in a subsequent stand re-entry in 2007-2008, however, stand re-entry occurred only at two study sites (BB1 and BB2; Appendix Table 1; Homyack and Haas 2013), 5) leave-tree, in which after harvest, 25-45 high-quality dominant and co-dominant trees over 30 cm DBH were retained per ha, 6) commercial clearcut, in which all merchantable stems were removed but low-quality stems were allowed to remain, and 7) silvicultural clearcut, in which all stems over 5 cm DBH were removed except up to 10 wildlife trees per ha (Wender 2000; Homyack 2009). However, the group selection prescription at all sites and the two shelterwood prescriptions at CL1 and CL2 were unable to be completed.

FIELD METHODS

I followed the same methods that previous investigators had employed for this study (Harpole and Haas 1999; Knapp et al. 2003; Homyack and Haas 2009). I established nine sampling transects in each experimental unit measuring 2 m by 15 m in a 3 by 3 grid arrangement. While the exact location of many transects has slightly shifted over time due to slash piles, skid trails, fallen trees, or disturbance, all transects were located at least 30 m away from other transects and from the edge of the experimental units to limit edge effects, which could create different microclimate conditions than the interior of the forest (Murcia 1995). I marked transects with a large metal nail in each corner and twine along the edges. I randomly selected one transect per treatment type to be sampled each sampling night and rotated the order in that transects were sampled to reduce bias to ensure the same treatments were not sampled at the same time of night each sampling night to account for potential temporal variation in salamander activity. I sampled transects annually on rainy nights from March-May and September-November at BB1/BB2 and additionally from June-August at CL1/CL2 when temperatures were above 7 degrees C and there was at least 0.25 inches of rain, or the leaf litter and soil were damp prior to sampling. The CL1 and CL2 sites are higher in elevation so I was able to sample salamanders during the summer months. Two observers crawled along the forest floor within each of the selected transects, capturing only surface-active salamanders by hand to avoid disturbing cover objects or leaf litter for repeated long-term sampling. I recognize that I am only sampling a very small subset of the population, but because recapture rates are so low (Smith and Petranka 2000; Bailey et al. 2004a) there is no efficient way to monitor populations across these large, geographically distant sites using mark-recapture. This sampling design helped mitigate some issues associated with variable detection probability by only sampling salamanders under favorable conditions for above-ground activity and sampling all treatments on the same night since weather and sampling effort impact detection probability (Bailey et al. 2004b). After capturing surface active salamanders, I marked each of the salamanders' capture locations with a pin flag corresponding to the bag that the salamander was kept in. After all the selected transects were sampled, I brought the salamanders back to the lab to be measured the following morning. I measured salamander species, the tip of the snout to the posterior edge of the vent length (SVL), tail length (TL), mass, sex, age class (adult or juvenile), if the tail was regenerating, if a female was gravid and if gravid, a count of the number of yolked eggs visible through the ventral surface. After lab measurements, I returned the salamanders to their capture locations.

DATA ANALYSIS

To calculate relative abundance (mean salamanders), I summed the number of salamanders by date, site, plot, and transect (i.e., each unique survey occasion), and then divided by the number of surveys. I created boxplots of relative salamander abundance per survey by decade for each of the four sites to visualize differences in relative salamander abundance between treatments over the three decades of the study. I created a series of seven negative binomial generalized linear models with relative salamander abundance as the response variable and different combinations of the following variables as fixed effects: decade, treatment, site, the interaction of decade and treatment, maximum August temperature, and annual precipitation to investigate the impacts of silvicultural treatment on the relative abundance of salamanders over 30 years. I included maximum August temperature and annual precipitation because the Tilghman et al. (2012) meta-analysis showed that summer maximum temperatures and precipitation influenced salamander abundance declines post-harvest. I used the Akaike Information Criterion corrected for small sample sizes (AICc) for model selection (Hurvich and Tsai 1989). Then, I did two different pairwise post-hoc comparisons to determine statistical differences in relative salamander abundance between decades in each treatment and differences within decades among treatments compared to the control. I used a Tukey adjustment for the pairwise post-hoc comparisons to control for using two post-hoc analyses. I used the top model to generate a predictive plot of relative salamander abundance across decades for all sites combined. I performed all statistical analyses in program R version 4.2.3 (R Core Team 2023) and used the MASS package (Venables and Ripley 2002) for creating negative binomial generalized linear mixed effects models, the AICcmodavg package (Mazerolle 2023) to compute model AICc, the emmeans package (Lenth et al. 2024) for post-hoc pairwise comparisons, and the ggplot2 package (Wickham 2016) and ggpubr (Kassambara 2023) for creating graphics.

RESULTS

From 1994 to 2023, I captured a total of 12,617 plethodontid salamanders across 1,883 sampling nights. Of the species captured, the majority were eastern red-backed salamanders (*Plethodon cinereus;* 60.57%), Alleghany mountain dusky salamander (*Desmognathus ochrophaeus;* 19.84%), Southern ravine salamander (*Plethodon richmondi;* 8.37%), and slimy salamander (*Plethodon cylindraceus/glutinosus;* 4.33%) (Table 1.5). Because not all species in this family occur at all sites (Appendix Table 2), I lumped data for all species in our analyses. Although there is variation in size and proximity to streams across species in this family, all require cool, moist habitats with abundant invertebrates, so the effects of forest management practices that influence solar insolation, wind, leaf litter, and coarse woody debris are likely to have similar effects across species. For all sites, the confidence intervals of the relative abundance of salamanders in each treatment 30 years post-harvest overlaps that of the relative abundance pre-harvest confidence intervals (Figures 1.1, 1.2, 1.3, 1.4). Specifically, at BB1 and BB2, the confidence interval for the relative abundance of salamanders 30 years post-harvest across all treatments overlaps the control confidence interval, except for the shelterwood (Figures 1.1, 1.2). We did not see the same differences in the shelterwood for CL1 and CL2, which did not have a second stand entry in the shelterwood, though it is difficult to determine if relative abundance looks similar or not compared to the control and pre-harvest abundance, due to only one year of data in 2023 (Figure 1.3, 1.4). The best-performing model as evaluated by AICc included decade, treatment, site, the interaction of decade and treatment, and annual precipitation (Table

1.2). The predicted relative abundance of salamanders by survey 30 years post-harvest appears to be equal to the pre-harvest relative abundance (Figure 1.5). All confidence intervals for salamander relative abundance 30 years post-harvest overlapped the control except for the shelterwood treatment (Figure 1.5). Pre-harvest relative salamander abundance was not significantly different from 30 years post-harvest relative salamander abundance ($P > 0.05$; Table 1.3) except in the shelterwood treatment ($P = 0.0322$; Table 1.3). Similarly, 30 years post-harvest none of the relative abundance in each treatment was significantly different from the control ($P > 0.05$; Table 1.4) except in the shelterwood treatment ($P < .0001$; Table 1.4).

DISCUSSION

Silvicultural treatments removing any canopy are thought to have long-lasting negative impacts on plethodontid salamander relative abundance (Homyack and Haas 2009). However, it seems that for nearly all treatments and sites, relative salamander abundance has recovered to the control levels 30 years post-harvest (Figure 1.5). The results refute our hypothesis that salamander abundance would not yet recover 30 years after harvest in all treatments (Table 1.3; Figure 1.5). The only exception was the shelterwood treatment (Table 1.3).

It is likely that salamander abundance in the shelterwood treatments at BB1 and BB2 has not recovered yet because of the second stand entry in 2007-2008. This means that it has only been 15 years since the last canopy removal at those sites. This makes sense because other treatments still had not rebounded after a stand entry 15 years post-harvest (Figure 1.5). Homyack and Haas (2013) found that stand re-entry caused a decline in salamanders captured much like the initial harvest. Shelterwood harvest systems involve a several stand entries typically 3-15 years apart depending on the silvicultural prescription and management goals; group selection typically has a series of stand entries 15-20 years apart. This finding is notable because this means a substantial decline in salamander abundance is likely after each stand re-entry. This suggests it will likely take much longer for salamanders to recover from silvicultural treatments that involve multiple stand entries than a silvicultural treatment such as a silvicultural clearcut, which only involves one stand entry. It seems that numerous partial harvests do not facilitate faster recovery compared to one larger harvest, and these multiple entries depress salamander populations for a longer period of time. Removing less canopy could allow salamanders to recover more quickly due to smaller declines post-harvest (Tilghman et al.2012), but if the prescription calls for multiple stand entries, then the population could decline each time. Other studies have found a range of impacts of shelterwood harvests on plethodontid salamander abundance ranging from severe declines to no impact (Brooks 2001; Duguay and Wood 2002; Mazerolle et al. 2021). These differences are likely due to varying amounts of basal area removed and climate. Tilghman et al. found that warmer summer temperatures and clearcuts led to larger salamander declines post-harvest (2012). Hot, dry areas are more susceptible to larger declines than cool, moist areas. If a site is already hot and dry, small changes could make it completely unsuitable for plethodontid salamanders and could result in mortality whereas if a cool, moist site becomes hotter and dryer, it may still be very suitable for plethodontid salamanders.

Studies have predicted that it would take > 60 years (Homyack and Haas 2013) to 100 years (Connette and Semlitsch 2013) for plethodontid salamanders to recover after harvest. Our study provides the first long-term evidence of a much earlier recovery after silvicultural treatment than previously thought. One of the few other long-term studies of the impacts of silviculture on salamanders is the Missouri Ozark Forest Ecosystem Project (MOFEP) study, which found cave salamander capture probability significantly declined post-treatment in uneven-aged management treatments (single-tree selection and group openings) and did not find an impact for four-toed salamanders (Timm et al. 2020). They also found that amphibians took over a decade to return to pre-harvest capture probabilities (Rota et al. 2017).

However, at a relatively higher elevation (1,380 to 1,500 m), moist (180 cm mean precipitation annually) cool (11.5 °C mean temperature annually) location there was no impact of group selection and shelterwoods on plethodontid salamander abundance two years post-harvest (Ford et al. 2000), which may be due to a lag in salamander decline in response to harvest (Ochs et al. 2022). Mazerolle et al. found there to be no impact of irregular shelterwoods on plethodontid salamander abundance five to six years after harvest (2021).

Some of these differences can be attributed to how much basal area was removed, which can impact how open the canopy gaps from harvest are and can impact soil moisture and litter depth which are important for plethodontid salamanders. Additionally, differences in baseline site conditions like average temperature, precipitation, amount of coarse woody debris, and other refugia known to mitigate some of the negative impacts of harvest could affect whether the silvicultural treatment will result in a decline in salamander abundance.

Otto et al. (2013) found that retaining more coarse woody debris can mitigate some of the negative impacts of harvest on salamanders. At our study sites, Hahn et al. (2021) found a higher load of total woody debris in the shelterwood and control compared to the commercial clearcut and silvicultural clearcut. Of the total woody debris, the control had a higher amount of 1000-hour fuels (woody debris 3-8 inches in diameter) than all other treatments (Hahn et al. 2021). These coarse-woody debris data were collected in 2019. Despite the differences in woody debris between treatments in the 21-30 years post-harvest, salamander relative abundance does not appear to be different (Figures 1, 2). This could suggest that at least several decades after harvest, coarse woody debris is not the primary driver of salamander recovery.

A better understanding of the demographics of plethodontid salamanders could help with models to predict recovery times under different circumstances. Estimates of survival at different life stages, age/size at first reproduction, and clutch size of animals of different sizes are still poorly known for most species in this family (Howard and Maerz 2021). Findings from Ohio that the growth rate of individual eastern red-backed salamanders was higher in an early successional stand compared to in a mature forest stand (possibly because low density reduced competition), can help explain the apparent rapid recovery of populations in this study (Gade et al. 2023).

These findings further our understanding of how different silvicultural techniques impact plethodontid salamander abundance in the long term. The main species captured in this research act as a "case study" to see how other plethodontid salamanders could respond to silvicultural treatments over time. Forest managers should carefully weigh whether or not to implement harvest prescriptions calling for multiple stand entries in areas with sensitive or threatened plethodontid salamander populations due to the potential longer-term negative impacts compared to other silvicultural treatments that only require one stand entry. Additionally, we do not know the mechanism of recovery or decline. Salamanders from the surrounding unharvested forest could have gradually immigrated to the harvested sites, re-emerged from underground to forage more often as the habitat became more suitable, or the remaining salamanders reproduced enough young to eventually replenish the population. So, it is important to note that the recovery rate could potentially be much longer for larger harvested areas (>2-ha) if larger areas take longer for salamanders to recolonize. Future research should aim to investigate the long-term impacts of silviculture in other geographic areas with a variety of site conditions since we know this could impact how salamanders are affected. Overall, this is an encouraging finding and suggests that it is possible to balance some forest management using most silvicultural methods in this study and long-term plethodontid salamander population viability.

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Table 1.1. Mean monthly precipitation (cm) and monthly mean temperature (°C) for the coldest and hottest months for the Southern Appalachian Silviculture and Biodiversity Project study sites by decade (NOAA 2024). The Blacksburg (BB1, BB2) and Clinch (CL1, CL2) sites are located in the George Washington and Jefferson National Forest in southwest VA, USA. BB1, BB2 Pre refers to BB1's pre-harvest year 1994 due to limited available weather data; BB1, BB2 Post 10 refers to years 1995-2004; BB1, BB2 Post 20 refers to years 2005-2014; BB1, BB2 Post 30 refers to years 2015-2023; CL1, CL2 Pre refers to years 1994-1997.

Sites	Mean monthly Precipitation (cm)	Mean Temperature (°C) January	Mean Temperature (°C) August
BB1, BB2 Pre	8.94	-2.11	22.53
BB1, BB2 Post 10	8.51	0.10	21.60
BB1, BB2 Post 20	8.56	0.43	21.79
BB1, BB2 Post 30	9.65	0.58	21.84
CL1, CL2 Pre	10.67	0.44	21.41
CL1, CL2 1998-2008	10.03	1.74	21.79
CL1, CL2 2023	7.70	4.33	21.11

Table 1.2. Parameters (k), AICc, delta AICc, and AICc weight for the negative binomial generalized linear models for relative salamander abundance (mean salamanders) for the Southern Appalachian Silviculture and Biodiversity Project, located in the George Washington and Jefferson National Forest in southwest VA, USA.

Independent Variables	k	AICc	Delta AICc	AICc Weight
Decade + Treatment + Site + Decade*Treatment + Annual Precipitation	33	2226.45	0.00	0.71
Decade + Treatment + Site + Decade*Treatment + Annual Precipitation + Maximum August Temperature	34	2228.29	1.84	0.29
Decade +Treatment + Site + Decade*Treatment + Maximum August Temperature	33	2319.36	92.91	0.00
Decade + Treatment + Site + Decade*Treatment	32	2400.55	174.10	0.00
Decade + Treatment + Site	14	2427.16	200.71	0.00
Decade + Treatment + Decade*Treatment	29	2489.16	262.71	0.00
Decade + Treatment	11	2506.66	280.21	0.00

Table 1.3. Pairwise post-hoc comparison (with Tukey adjustment) of treatment and decade effect on relative salamander abundance estimated marginal mean (estimate) and standard error (SE) for the Southern Appalachian Silviculture and Biodiversity Project, located in the George Washington and Jefferson National Forest in southwest VA, USA.

Treatment and Decade Comparison	Estimate	SE	P-value
Control Pre-harvest - Post10	0.3996	0.226	0.2897
Control Pre-harvest - Post20	0.1855	0.211	0.8165
Control Pre-harvest - Post30	-0.3574	0.216	0.3484
Herbicide Pre-harvest - Post10	0.4610	0.209	0.1204
Herbicide Pre-harvest - Post20	0.7239	0.201	0.0018
Herbicide Pre-harvest - Post30	0.4001	0.210	0.2247
Group Selection Pre-harvest - Post10	0.9236	0.217	0.0001
Group Selection Pre-harvest - Post20	0.8139	0.201	0.0003
Group Selection Pre-harvest - Post30	0.3247	0.208	0.3995
Shelterwood Pre-harvest - Post10	1.0931	0.239	<.0001
Shelterwood Pre-harvest - Post20	1.1004	0.225	<.0001
Shelterwood Pre-harvest - Post30	0.6164	0.226	0.0322
Leave Tree Pre-harvest - Post10	1.5328	0.240	<.0001
Leave Tree Pre-harvest - Post20	1.0285	0.207	<.0001
Leave Tree Pre-harvest - Post30	0.3364	0.210	0.3752
Commercial Clearcut Pre-harvest - Post10	1.1170	0.258	0.0001
Commercial Clearcut Pre-harvest - Post20	0.7235	0.227	0.0078
Commercial Clearcut Pre-harvest - Post30	-0.1355	0.226	0.9323
Silvicultural Clearcut Pre-harvest - Post10	1.4128	0.260	<.0001
Silvicultural Clearcut Pre-harvest - Post20	0.8194	0.221	0.0012
Silvicultural Clearcut Pre-harvest - Post30	-0.0984	0.219	0.9697

Table 1.4. Pairwise post-hoc comparison (with Tukey adjustment) of decade and treatment effect on relative salamander abundance estimated marginal mean (estimate) and standard error (SE) for the Southern Appalachian Silviculture and Biodiversity Project, located in the George Washington and Jefferson National Forest in southwest VA, USA.

Decade and Treatment Comparison	Estimate	SE	P-value
Pre-harvest Control - Herbicide	-0.3805	0.241	0.6951
Pre-harvest Control - Group Selection	-0.3991	0.240	0.6427
Pre-harvest Control - Shelterwood	-0.1110	0.248	0.9994
Pre-harvest Control - Leave Tree	-0.3597	0.241	0.7504
Pre-harvest Control - Commercial Clearcut	0.1319	0.255	0.9986
Pre-harvest Control - Silvicultural Clearcut	-0.0170	0.250	1.0000
Post10 Control - Herbicide	-0.3191	0.160	0.4197
Post10 Control - Group Selection	0.1249	0.172	0.9911
Post10 Control - Shelterwood	0.5825	0.190	0.0349
Post10 Control - Leave Tree	0.7734	0.199	0.0020
Post10 Control - Commercial Clearcut	0.8493	0.203	0.0006
Post10 Control - Silvicultural Clearcut	0.9961	0.212	0.0001
Post20 Control - Herbicide	0.1578	0.135	0.9062
Post20 Control - Group Selection	0.2292	0.136	0.6233
Post20 Control - Shelterwood	0.8039	0.160	<.0001
Post20 Control - Leave Tree	0.4832	0.143	0.0125
Post20 Control - Commercial Clearcut	0.6698	0.149	0.0001
Post20 Control - Silvicultural Clearcut	0.6168	0.148	0.0006
Post30 Control - Herbicide	0.3769	0.150	0.1549
Post30 Control - Group Selection	0.2829	0.148	0.4708
Post30 Control - Shelterwood	0.8628	0.162	<.0001
Post30 Control - Leave Tree	0.3341	0.149	0.2732
Post30 Control - Commercial Clearcut	0.3538	0.150	0.2128
Post30 Control - Silvicultural Clearcut	0.2420	0.147	0.6514

Table 1.5. Plethodontid salamander species captured from 1994-2023 for the Southern Appalachian Silviculture and Biodiversity Project, located in the George Washington and Jefferson National Forest in southwest VA, USA.

Species	Count	Percent of Total Captures
Aneides aeneus	2	0.02
Desmognathus sp	13	0.10
Desmognathus fuscus	177	1.40
Desmognathus monticola	27	0.21
Desmognathus ochrophaeus	2503	19.84
Eurycea cirrigera	67	0.53
Gyrinophilus porphyriticus	12	0.10
Hemidactylium scutatum	1	0.01
Plethodon cinereus	7642	60.57
Plethodon cylindraceus/glutinosus	951	4.33
Plethodon kentucki	75	0.59
Plethodon richmondi	1056	8.37
Pseudotriton ruber	6	0.05
Unknown	80	0.63
Total	12617	

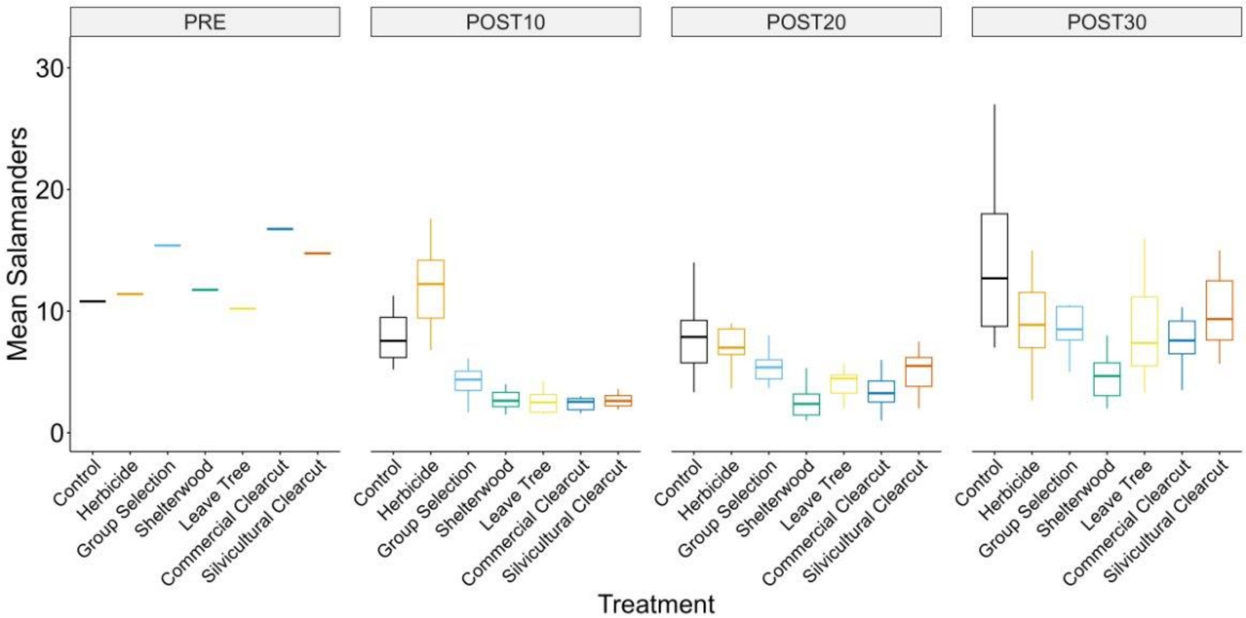

Figure 1.1. Comparison of relative salamander abundance (mean salamanders by survey) for the BB1 site by treatment for pre-harvest (1994), 1-10 years post-harvest (1995-2004), 11-20 years post-harvest (2005-2014), and 21-30 years post-harvest (2015-2023) for the Southern Appalachian Silviculture and Biodiversity Project. The Blacksburg (BB1) site is in Montgomery County in the George Washington and Jefferson National Forest in southwest VA, USA.

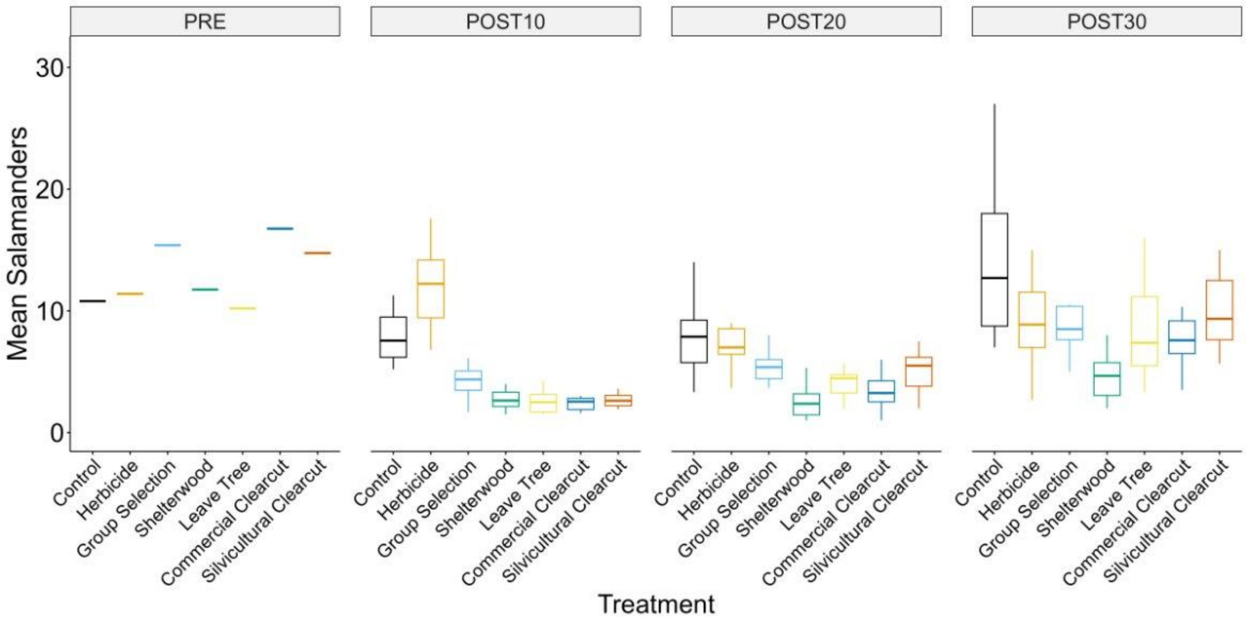

Figure 1.2. Comparison of relative salamander abundance (mean salamanders by survey) for the BB2 site by treatment for pre-harvest (1995), 1-10 years post-harvest (1996-2004), 11-20 years post-harvest (2005-2014), and 21-30 years post-harvest (2015-2023) for the Southern Appalachian Silviculture and Biodiversity Project. The Blacksburg (BB2) site is located in Montgomery County in the George Washington and Jefferson National Forest in southwest VA, USA.

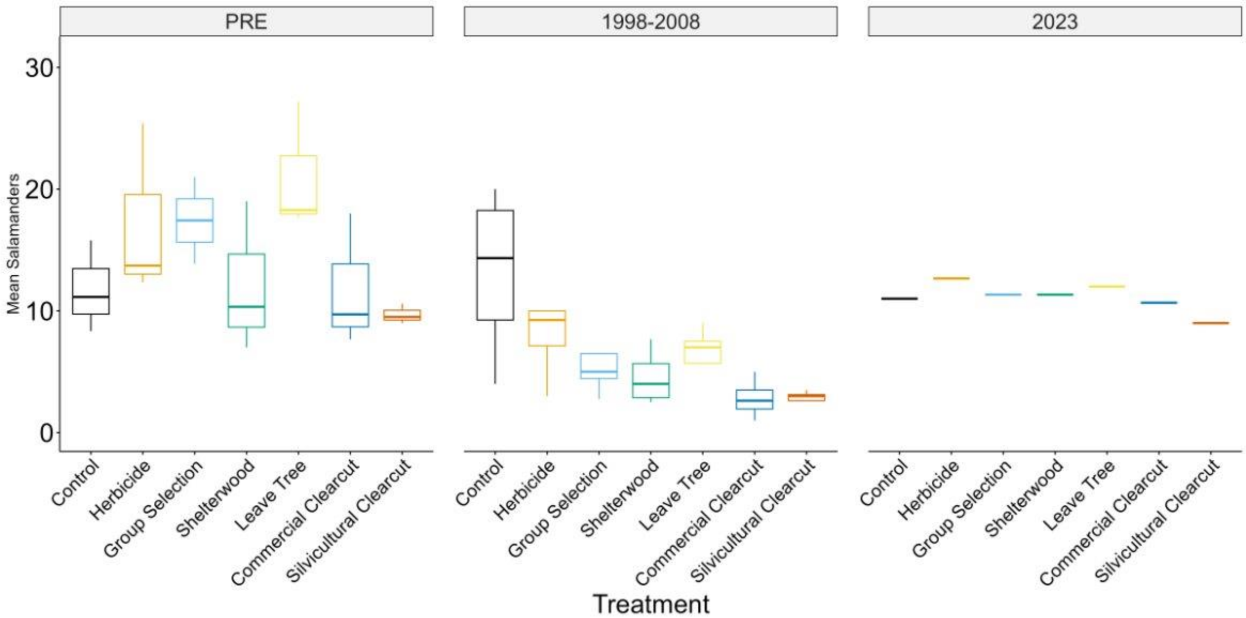

Figure 1.3. Comparison of relative salamander abundance (mean salamanders by survey) for the CL1 site by treatment for pre-harvest (1994-1997), 1-10 years post-harvest (1998-2008), and 30 years post-harvest (2023) for the Southern Appalachian Silviculture and Biodiversity Project. The Clinch (CL1) site is located in Wise County in the George Washington and Jefferson National Forest in southwest VA, USA.

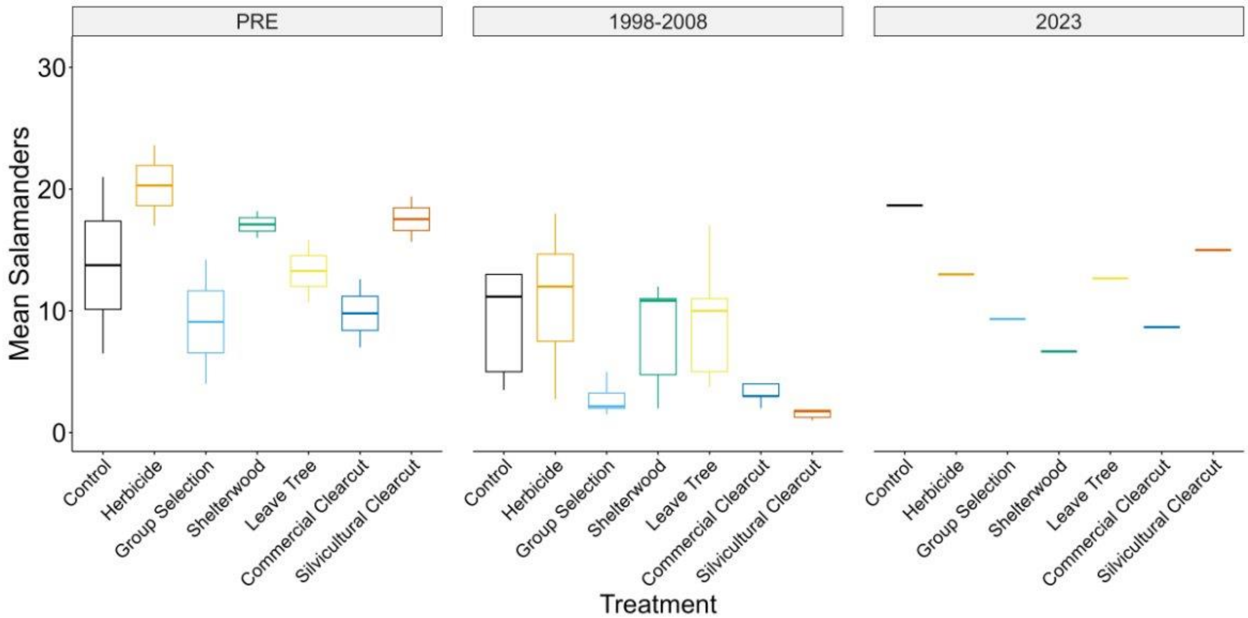

Figure 1.4. Comparison of relative salamander abundance (mean salamanders by survey) for the CL2 site by treatment for pre-harvest (1994-1997), 1-10 years post-harvest (1998-2008), and 30 years post-harvest (2023) for the Southern Appalachian Silviculture and Biodiversity Project. The Clinch (CL2) site is located in Wise County in the George Washington and Jefferson National Forest in southwest VA, USA.

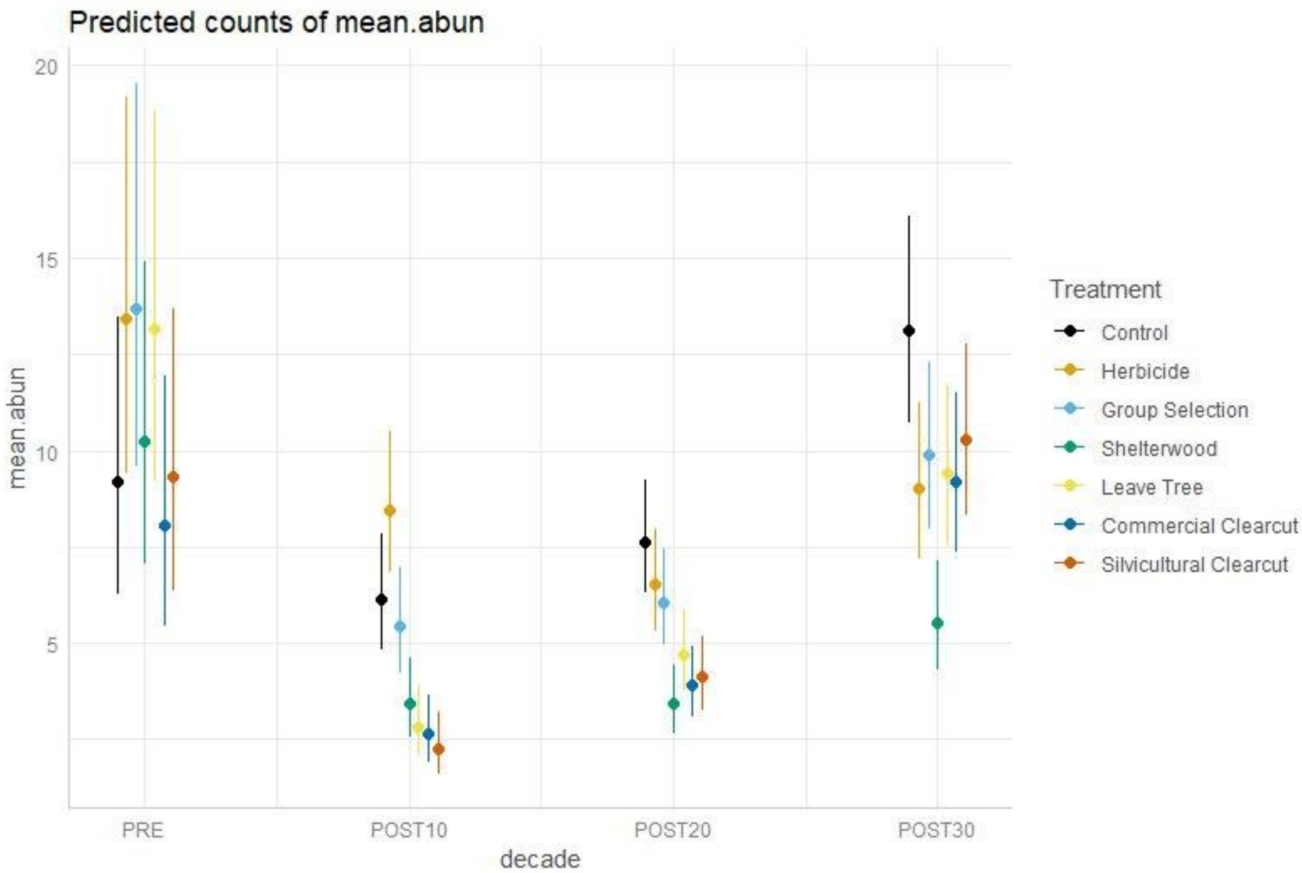

Figure 1.5. Predicted relative salamander abundance (mean number of salamanders) per sampling occasion after harvest by silvicultural treatment and decade for the top model (Decade + Treatment + Site + Decade*Treatment + Annual Precipitation) for the Southern Appalachian Silviculture and Biodiversity Project, located in the Jefferson National Forest in southwest VA, USA. The error bars represent the upper and lower 95% confidence interval of the predicted relative abundance of salamanders.

Ch.2 THE IMPACT OF ARTIFICIAL TIP-UP MOUNDS ON PLETHODONTID SALAMANDER COUNTS IN A CENTRAL APPALACHIAN HARDWOOD FOREST

ABSTRACT

Very little is known about how tip-up mounds, a key feature of old-growth forests, potentially impact plethodontid salamander populations. Prior research suggests that creating tip-up mounds could help increase salamander relative abundance by potentially providing moist refugia. To better understand the impacts of artificial tip-up mounds on plethodontid salamanders in a central Appalachian hardwood forest, I compared plethodontid salamander counts between treatments with tip-up mounds and a control, and compared counts on mounds, pits, tree boles, and flat areas. I hypothesized that salamander counts would be higher in tip-up mound treatments and on mounds and pits compared to flat areas and tree boles. I also hypothesized that soil temperature and soil moisture would be more variable between artificial tip-up mound treatments and other treatments. Results suggest that the creation of artificial tip-up mounds negatively impacts salamander count. However, salamander habitat utilization of mounds, pits, tree boles, and flat areas did not differ. The temperature at the mounds fluctuated much more than the flats and the pits, with the mounds generally hotter than the other locations in the summer and colder in the winter. Further long-term study is needed to discern the impacts of artificial tip-up mounds on these salamanders.

INTRODUCTION

Disturbance is fundamental to forest ecosystems (Attiwill 1994). Disturbances create a mosaic of different successional stages, uneven age classes, and types of habitats within the ecosystem. This structural diversity is essential for promoting a broader diversity of flora and fauna that rely on specific conditions (Viljur et al. 2022). When disturbance opens the canopy and exposes the soil, tree species that require high levels of sunlight and mineral soil can regenerate. Fires, insect pests, invasive plants, and severe weather are just some examples of disturbance (Attiwill 1994).

Tip-up mounds, one of the most important sources of disturbance in eastern oak forests, occur when a tree is pushed over by wind or ice storms, pulling up the root ball and creating long-lasting pit-mound topography (Lorimer and White 2003). When tip-up mounds are formed, the falling tree can damage other surrounding trees, creating more coarse woody debris. Tip-up mounds help form unique pit-mound microtopography with a cool, moist pit (Clinton and Baker 2000) and may provide a habitat for invertebrates (Perry and Herms 2017).

Plethodontid salamanders are an important component of the forest community. They provide crucial ecosystem services such as regulating invertebrate populations and nutrient cycling (Hocking and Babbitt 2014). They act as a key predator of invertebrates, and a food source for mammals, snakes, birds, and other salamanders (Sullivan et al. 2004; Shaw et al. 2023). In some cases, terrestrial salamanders can impact leaf litter decomposition and carbon storage based on the invertebrates they consume (Laking et al. 2021).

Salamanders and other amphibians have experienced serious global population declines over the past several decades (Green et al. 2020). This substantial loss is caused by many interacting factors such as habitat loss and alteration, climate change, disease, pollutants, and introductions of nonnative species, among others (Blaustein and Kiesecker 2002). Notably, these declines could be due to anthropogenic alterations of natural disturbance regimes such as timber harvesting. Harvest removes standing woody vegetation from the harvest area, often leading to a short-term decline in salamander abundance (Harpole and Haas 1999).

My previous findings indicate that in a Michigan northern hardwood forest, artificial tip-up mounds appear to positively impact salamander abundance (Engler et al. In Review). However, there is a significant knowledge gap about why tip-up mounds seem to benefit salamanders and how they affect the broader forest community.

Terrestrial salamanders require moist conditions and invertebrate prey; consequently, they may benefit from the presence of tip-up mounds. They need cool, moist environments and shelter under coarse woody debris or retreat underground during dry conditions to avoid desiccation (Feder 1983). Natural tip-up mounds have a lower soil temperature and higher soil moisture in the pit (Clinton and Baker 2000), may provide habitat for invertebrates (Perry and Herms 2017), can increase plant diversity (Spicer et al. 2018), and may slow leaf litter decomposition when waterlogged (Carlton and Bazzaz 1998). Tip-up mounds could potentially create pockets of suitable habitat for salamanders to take refuge in which could reduce the negative impacts of harvest. The goal of this study was to determine whether artificial tip-up mounds impact salamander abundance in a central Appalachian hardwood forest and the potential mechanism behind any changes.

OBJECTIVE AND HYPOTHESES

If tip-up mounds create beneficial habitats for salamanders, then salamanders will have higher counts in artificial tip-up mound treatment units compared to the control. Based on previous research, I expected to see artificial tip-up mound treatments positively influence salamander counts through potentially increasing moisture in the tip-up mound pits (Engler et al. In Review). I hypothesized that salamanders will more often be found in pits and on mounds than in flat areas. If tip-up mounds create a more varied microclimate, then soil temperature and moisture will have a larger range in tip-up mound treatments compared to controls.

METHODS

STUDY SITE

I worked with the Virginia Tech Department of Forest Resources and Environmental Conservation to establish six replicates each of two different silvicultural treatments in the Fishburn Research Forest, in Montgomery County, Virginia, latitude 37°11’N, longitude 80°29’W with an elevation of 600 to 620 m above sea level (Figure 2.1). Fishburn Research Forest encompasses 526 ha of upland Appalachian oak-hickory forest. The primary tree species consisted of white oak (*Quercus alba*), northern red oak (*Q. rubra*), scarlet oak (*Q. coccinea*), chestnut oak (*Q. prinus*), and white pine (*Pinus strobus)*, and the property was further described by Hook (2010). Virginia Tech acquired the property in the 1960s, and prior to the purchase, the land was selectively logged (Copenheaver et al. 2006). The soils at the sites are mainly Berks-Weikert complex (Soil Survey staff 2024). I expected to primarily find eastern red-backed salamanders (*Plethodon cinereus*) in the Fishburn Research Forest based on pre-study sampling. The treatments were sites where I created artificial tip-up mounds and control sites. Each treatment unit is 0.05 ha in size due to space constraints in the Fishburn Research Forest and the inability of the forestry equipment used for tip-up mound creation to drive on steep terrain. Within each treatment I established a three-by-three cover board array, with each board spaced 1.5 m apart (Figure 2.2). I used 2.5 cm thick white oak boards and cut them to 0.3 m by 0.3 m lengths. I placed the boards at the field site on 25 August 2022 to weather in place for one month before pretreatment sampling. I created artificial tip-ups by using a Takeuchi TB290 compact excavator crane arm to push over eight trees (12.7-30.5 cm DBH) in each tip-up mound treatment unit (a density of 160 trees per ha; Figure 2.3). Some smaller trees were incidentally tipped over. I drove the excavator through some control treatments to access tip-up mound treatment units but tried to minimize disturbance as much as possible. I implemented tip-up mounds during the winter (9-10 January 2023) to minimize disturbance to salamanders active on the surface as well as to reduce soil compaction.

SALAMANDER SAMPLING – TREATMENT EFFECTS

I sampled plethodontid salamander abundance and diversity weekly by checking under the cover boards at each site before tip-up mound treatment implementation for one field season (September-November 2022). During coverboard checks, I collected data on air temperature and humidity at each treatment unit. I also conducted pre-treatment night-time salamander transect surveys after rainfall events by crawling along a 2 m by 15 m transect located 2.5 meters south of each coverboard array from September to November 2022. I marked each individual salamander captured during coverboard and transect surveys with a unique VIE mark with the goal of estimating abundance using mark-recapture. After I implemented the tip-up mounds, I conducted post-treatment salamander sampling using weekly coverboard surveys (March - May 2023, September - November 2023) and night-time transect surveys after rainfall (March-May 2023) and collected air temperature and humidity data during these surveys.

SALAMANDER SAMPLING –HABITAT UTILIZATION

I conducted five night-time area-constrained searches at each pit and mound, and a corresponding flat area and tree bole on rainy nights, defined by any night with precipitation

(September – November 2023), to detect potential differences in microhabitat use. During these events, I had two pairs of observers to observe the four different microhabitats of interest (pit, flat area of forest floor, tip-up mound including exposed tree root ball, and bole of the tree), with one pair observing the pit/and a flat area approximately the size of the corresponding pit, and one pair observing the mound/and a tree bole from ground level to about 2 m up the tree. Each person would record all salamanders observed at the designated location for one minute, and then both pairs of observers would move to the next set of locations, alternating which pair observed the pit/flat area and the mound/tree bole. On each survey night, every set of locations was sampled within each of the six tip-up mound treatment plots. All observations were conducted during or after rain events after sunset.

MICROCLIMATE MEASUREMENTS

During coverboard surveys, I hand-collected air temperature, humidity, and soil temperature data while surveying each treatment unit. I used a Traceable Calibrated Pen-Style Thermohygrometer (±1°C temperature accuracy, ±3.5% relative humidity accuracy) to measure air temperature and humidity at the center of each coverboard array and a Thermco Water Resistant Pocket 4 ½" Stem Digital Thermometer (±1°C accuracy) to measure soil temperature 12 cm below the soil surface at the four corners of the coverboard array. I used an HS2 HydroSense II Handheld Soil Moisture Sensor (±3% water content accuracy) to measure soil moisture 12 cm below the soil surface at the four corners of the coverboard array. During the summer of 2023, I placed temperature loggers in at least one pit, mound, and flat area (2 m to the north of each pit) in each tip-up treatment unit to collect soil temperature data to explain potential differences in salamander count between pits, mounds, and flat areas. I used a marked PVC pipe hammered into the ground to remove soil so that each HOBO logger was placed exactly 10 cm below ground and then replaced the soil on top of the logger. Based on time and financial constraints, I used two different types of temperature loggers; 14 HOBO TidbiT MX Temperature 400' Data Logger MX2203 (±0.2°C accuracy) and 18 HOBO Pendant X Water Temperature Data Logger MX2201 (±0.5°C accuracy). I set each logger to record temperature once an hour starting at 1100 on 10 August 2023 and ending on7 December 2023 at 1400. On 8 September 2023, I rotated each HOBO data logger to a new set of pits/mounds/flat areas within the same treatment unit. I calculated the average temperature by location (pit, mound, flat area) for each hour. I also conducted soil temperature and soil moisture measurements in the pit, mound, and 20-200 cm from the pit in 20 cm increments on 10, 18, and 24 August 2023 to see how far potential temperature and moisture differences extend into the treatment unit using a Thermco Water Resistant Pocket 4 ½" Stem Digital Thermometer (±1°C accuracy) and a HS2 HydroSense II Handheld Soil Moisture Sensor (±3% water content accuracy).

DATA ANALYSIS

Salamander count data were analyzed with a series of five negative binomial generalized linear models with salamander count as the response variable and different combinations of the following variables as fixed effects: treatment, the interaction of treatment and pre or post-treatment, air temperature, and humidity to investigate the impacts of treatment on salamander count. I used the Akaike Information Criterion corrected for small sample sizes (AICc) for model selection (Hurvich and Tsai 1989). For the top models for both coverboard and transect, I conducted a post-hoc pairwise comparison using the package emmeans (Lenth et al. 2024) to determine if there are statistically significant differences between the salamander count in the tip-up mound treatments and control before and after treatment. I used a Tukey adjustment for the pairwise post-hoc comparisons. I also built a negative binomial generalized linear model to investigate if location – pit, mound, flat, or tree (independent variable) impacted salamander count (dependent variable) since these data were collected separately from the treatment effects data and conducted a post-hoc pairwise comparison with a Tukey adjustment using the package emmeans (Lenth et al. 2024) to determine if there are statistically significant differences between the salamander habitat use. I performed all statistical analyses in program R version 4.2.3 (R Core Team 2023) and used the MASS package (Venables and Ripley 2002) for creating negative binomial generalized linear mixed effects models, the AICcmodavg package (Mazerolle 2023) to compute model AICc, and the ggplot2 package (Wickham 2016) and ggpubr (Kassambara 2023) for creating graphics.

RESULTS

I caught a total of 59 plethodontid salamanders over 26 coverboard surveys from 2022 to 2023 and 53 plethodontid salamanders over seven transect surveys within the 12 treatment units (Table 2.1; Table 2.2). Our captures primarily consisted of eastern red-backed salamander (*Plethodon cinereus*; 97% or 57/59), with the remaining captures being Northern slimy salamander (*Plethodon glutinosus*; 2% or 1/59) and Southern two-lined salamander (*Eurycea cirrigera*; 2% or 1/59). Our recapture rate was 4%, so I was unable to estimate abundance using mark-recapture as planned and instead used the total salamander count. Of the salamander recaptures, I had one recapture in the tip-up mound treatment post-treatment (Table 2.3). For night-timed constrained area searches, I captured a total of 52 plethodontid salamanders over five surveys from September to November 2023 (Table 2.4). 88% (46/52) were *Plethodon cinereus*, 4% (2/52) were *Plethodon glutinosus*, and 8% (4/52) were *Eurycea cirrigera*. Salamander count decreased after tip-up mound creation in the tip-up mound treatments for both coverboard and transect surveys (Figure 2.4; Figure 2.6). Our top model included treatment, the interaction between pre/post-treatment and treatment, air temperature, and humidity for coverboard data, and the top model for transect data included only treatment and the interaction between pre/post-treatment and treatment (Table 2.5; Table 2.6). Predicted salamander counts in the artificial tip-up mound treatment significantly differed after treatment implementation for coverboard surveys ($P = 0.0076$; Table 2.7; Figure 2.5), but not for transect surveys ($P = 0.1982$; Table 2.8; Figure 2.7). None of the salamander counts between any location (pits, mounds, flat areas, and tree boles) significantly differed (Table 2.9; Figure 2.8; Figure 2.9). The temperature at the mounds fluctuated much more than the flats and the pits with the mounds generally hotter than the other locations in the summer and colder in the winter (Figure 2.10). From August to mid-September, the pit was consistently cooler than the flat (Figure 2.10). Comparing fine-scale measurements of soil temperature and moisture at pits, mounds, and 20-200 cm away from the pit in 20 cm increments, on all measurement dates, the pit was slightly moister than the mound (ranging from significantly moister to marginally moister) and the pit was always cooler than the mound, however, 20-200 cm from the pit, there were no patterns (Figure 2.11, Appendix Figures 1-3). At both tip-up mound and control treatments, there was no difference in temperature or moisture at coverboard arrays (flat areas) and pits were significantly moister and cooler than mounds (Figure 2.12).

DISCUSSION

Salamander count declined on plots with artificial tip-up mound treatments, but not on control plots, though these differences were only statistically significant for coverboard surveys and not for transect surveys. This refutes the hypothesis that salamanders are more abundant in artificial tip-up mound treatments. The salamander counts in pits vs. mounds vs. flat areas vs. tree boles did not differ. The temperature varied more at mounds seasonally than at flat areas.

This result counters previous findings in Michigan, where eastern red-backed salamanders (the primary species in our study) had a higher relative abundance in artificial tip-up mound treatments (Engler et al. In Review). There were some differences between the two studies that could have contributed to the conflicting results. Firstly, the Michigan study sampled salamanders over a longer period of time (three years post-treatment) compared to this study (one year post-treatment). In the Michigan study, tip-up mound treatments were implemented in the winter of 2017. Salamanders were subsequently sampled post-treatment from 2017 -2019. In this study, I implemented treatments in the winter of 2023 and conducted post-treatment sampling in 2023. It is possible that the initial disturbance from tip-up mound creation results in salamanders moving out of the area and it could take salamanders longer than 1-year post-harvest for tip-up mound treatments to return to being suitable habitat. Alternatively, because plethodontid salamanders have small home ranges, they could have hunkered down and stayed in the habitat despite disturbances from artificial tip-up mound creation but did not return to surface activity until surface conditions improved (Merchant 1972; Kleeberger and Werner 1982). Second, Quinn and Graves found that the natural spatial distribution of Michigan eastern red-backed salamanders across the landscape can differ from those in Virginia (1999). They discovered that Michigan salamanders stayed aggregated together while Virginia salamanders were uniformly distributed (Quinn and Graves 1999). These differences were the same under controlled laboratory conditions (Quinn and Graves 1999). It is possible that in the Michigan study, the eastern red-backed salamanders chose to aggregate underneath coverboards, artificially inflating their counts while in Virginia, the salamanders were more widely dispersed across the study area and did not congregate under coverboards.

Additionally, it is thought that ground compaction during timber harvesting and even silviculture that creates smaller canopy gaps can have a negative impact on plethodontid salamanders (Homyack et al. 2011; Knapp et al. 2003; Margenau et al. 2023). Our study further reinforces that disturbance related to stand entry, either from ground compaction from forestry equipment and/or the creation of small canopy gaps, can have a significant negative impact on salamander counts in the short term. Potential differences in the amount of ground compaction could also have led to differences between our Virginia study and the Michigan study. The climate in Virginia tends to stay warmer and although I created artificial tip-up mounds in the winter to reduce compaction, the temperatures were a low of -5.6 °C and a high of 6.7 °C during the days of tip-up mound creation, so the ground may not have been frozen (NOAA 2024). In some treatments the excavator created deep ruts in the soil, further suggesting the ground was not frozen. Michigan's upper peninsula tends to have a much colder climate during the winter, and it is more likely that the ground was fully frozen with less soil compaction than in the Virginia study. This suggests that ground compaction could be an important factor in how silviculture impacts plethodontid salamanders. Another recent study suggests that by reducing soil compaction, the effects of overstory harvest on eastern red-backed salamanders were lessened, further supporting that soil compaction is influential (Margenau et al. 2023). In addition to winter temperatures, summer temperatures may also have influenced the decline in salamander count in our study. Tilghman et al. (2012) conducted a meta-analysis suggesting that higher summer maximum temperatures led to greater salamander abundance declines post-harvest. At our study site, the maximum temperature of the warmest month was 29.2 °C compared to the Michigan study, which was 26.8 °C (NOAA 2024). This could further explain differences in the two study's findings.

Moreover, there was a large difference in the number of tip-up mounds per hectare between our study and the Michigan study. In our study, I pulled down 160 trees per ha, versus the Michigan study, which only pulled down 14.7 (±2.3) trees per ha (Engler et al. In Review). A meta-analysis found that clearcutting, which removes more trees, resulted in greater salamander abundance declines than partial harvest (Tilghman 2012). I pulled down more trees to create tip-up mounds than in the Michigan study, which increased the number of canopy gaps and amount of disturbance. This could have contributed to differences in how the salamanders in our study responded to the tip-up mound creation. It is possible that there could be a "sweet spot" where under certain conditions (longer time after tip-up mound creation, cooler climate, little to no ground compaction, small number of canopy trees removed per ha) tip-up mounds can benefit salamanders, but immediately after tip-up mound creation under hotter conditions with significant ground compaction and significant canopy opening, tip-up mounds may have a negative effect on salamander abundance.

The number of salamanders found on rainy nights did not differ between pits, mounds, flat areas, and tree boles. However, it is important to note that these measurements all took place in tip-up mound treatment units. Salamanders have very small home ranges (13-24 m^2), so it is entirely possible that they forage very close to their pre-treatment home ranges before the pits and mounds were created (Kleeberger 1982). Of the salamanders left in the tip-up mound treatments, it appears that they utilize each of these locations for foraging evenly.

Compared to flat areas, pits tended to buffer environmental conditions and mounds tended to exaggerate them. Canopy gaps allow more insolation, so that across a plot there was more variation in microclimate within tip-up mound treatments than within control treatments. During the summer, mounds were consistently hotter and drier, and pits were consistently cooler and wetter, than flat areas, similar to published findings (e.g. Clinton and Baker 2000). As the seasons changed from summer to winter, at some treatment units, pits were warmer than flat areas, or than mounds, likely because they retained more moisture and so cooled more slowly, even though they may have been more shaded.

Our results suggest that further study on how tip-up mounds influence salamanders over the long term is needed. The cooler temperatures and higher moisture content in the pits and hotter temperatures and lower moisture content in the mounds provide some insight into how changes in microtopography through the creation of artificial tip-up mounds influence microclimates. As the number of old-growth forests dwindles across our landscape, it could be beneficial to further investigate how some of the key features of mature forests, such as tip-up mounds, could be introduced into young forests to attempt to provide a habitat similar to an older forest. Other researchers should consider the removal of the tree boles after tip-up mound creation so as not to confound the effects of the tip-up mound with the effects of plentiful large course woody debris. Future studies should examine this topic on a much larger scale, across different geographic regions, and for a longer period of time to discern the potential impacts of tip-up mounds on salamanders.

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Table 2.1. Plethodontid salamanders detected under coverboard arrays placed in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA. Coverboards were monitored in six control and six treatment plots before (2022) and after (2023) an experiment to create tip-up mounds by pushing over small trees in January 2023 in the treatment plots.

Treatment	Pre/Post-treatment	Total Salamander Count
Control	Pre	14
Tipup	Pre	16
Control	Post	23
Tipup	Post	6
Total		59

Table 2.2. Plethodontid salamanders detected during night-time transect surveys during or after rain events in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA. Surveys were conducted in six control and six treatment plots before (2022) and after (2023) an experiment to create tip-up mounds by pushing over small trees in January 2023 in the treatment plots.

Treatment	Pre/Post-treatment	Total Salamander Count
Control	Pre	10
Tipup	Pre	18
Control	Post	14
Tipup	Post	11
Total		53

Table 2.3. Plethodontid salamander individual recaptures in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA before (2022) and after (2023) an experiment to create tip-up mounds by pushing over small trees in January 2023 in the treatment plots.

	Pre-treatment	Post-treatment
Control	1	1
Tip-up	1	1

Table 2.4. Plethodontid salamanders detected during night-time constrained-area searches during or after rain events at pits, mounds, flat areas, and tree boles during or after rain events in Virginia Tech Fishburn Forest, Montgomery County, Virginia.

Location	Total Salamander Count
Flat	16
Mound	17
Pit	8
Tree	11
Total	52

Table 2.5. Parameters (k) and AICc for the negative binomial generalized linear models for salamander count at coverboard arrays in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

Independent Variables	**k**	**AICc**	**Delta AICc**	**AICc Weight**
treatment + pre.post*treatment + air.temp + humidity	7.00	90.06	0.00	0.37
treatment + pre.post*treatment + air.temp	6.00	90.31	0.25	0.33
treatment + pre.post*treatment + humidity	6.00	91.04	0.98	0.23
treatment + pre.post*treatment	5.00	93.38	3.33	0.07
treatment + pre.post*treatment + date	17.00	297.70	207.64	0.00

Table 2.6. Parameters (k) and AICc for the negative binomial generalized linear models for salamander counts at transects in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

Independent Variables	**k**	**AICc**	**Delta AICc**	**AICc Weight**
treatment + pre.post*treatment	5.00	72.60	0.00	0.79
treatment + pre.post*treatment + air.temp	6.00	76.25	3.64	0.13
treatment + pre.post*treatment + humidity	6.00	77.16	4.56	0.08
treatment + pre.post*treatment + air.temp + humidity	7.00	90.06	17.45	0.00
treatment + pre.post*treatment + date	11.00	332.58	259.97	0.00

Table 2.7. Pairwise post-hoc comparison (with Tukey adjustment) of treatment and pre-post effect on salamander count estimated marginal mean (estimate) and standard error (SE) at coverboards in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

Contrast	Estimate	SE	p-value
Control post-pre	-0.289	0.344	0.4004
Tip-up post-pre	-1.305	0.489	0.0076

Table 2.8. Pairwise post-hoc comparison (with Tukey adjustment) of treatment and pre-post effect on salamander count estimated marginal mean (estimate) and standard error (SE) at transects in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

Contrast	Estimate	SE	p-value
Control pre - Control post	-0.069	0.414	0.8677
Tip-up pre - Tip-up post	-0.492	0.383	0.1982

Table 2.9. Pairwise post-hoc comparison (with Tukey adjustment) of location (flat, mound, pit, tree) effect on salamander count estimated marginal mean (estimate) and standard error (SE) in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

Contrast	Estimate	SE	p-value
Flat - Mound	-0.0606	0.477	0.9993
Flat - Pit	0.6931	0.542	0.5764
Flat - Tree	0.3747	0.51	0.8829
Mound - Pit	0.7538	0.539	0.4996
Mound - Tree	0.4353	0.506	0.8252
Pit - Tree	-0.3185	0.568	0.9435

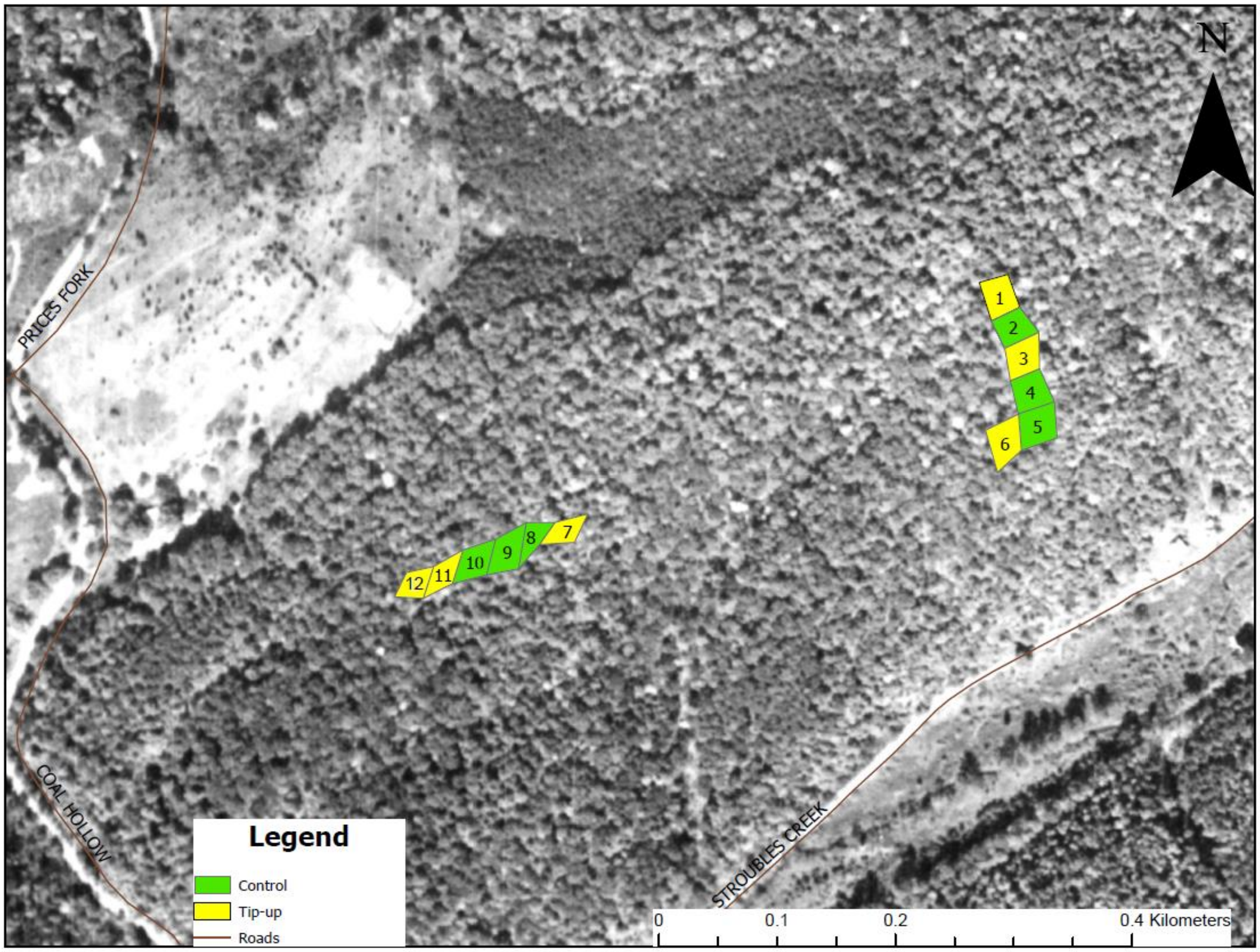

Figure 2.1. Map of Study Site in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA. Each coverboard array is located in the approximate center of each treatment unit (1-12). The green color represents control units, and the yellow color represents tip-up treatment units.

Figure 2.2. Coverboard array layout within each treatment unit in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA. Each cover board array was a three-by-three layout with white oak boards spaced 1.5 m apart.

Figure 2.3. I used a Takeuchi TB290 compact excavator crane arm to push over eight trees (a density of 160 trees per ha) in each tip-up mound treatment unit for artificial tip-up mound creation in Virginia Tech’s Fishburn Forest, Montgomery County, Virginia, USA.

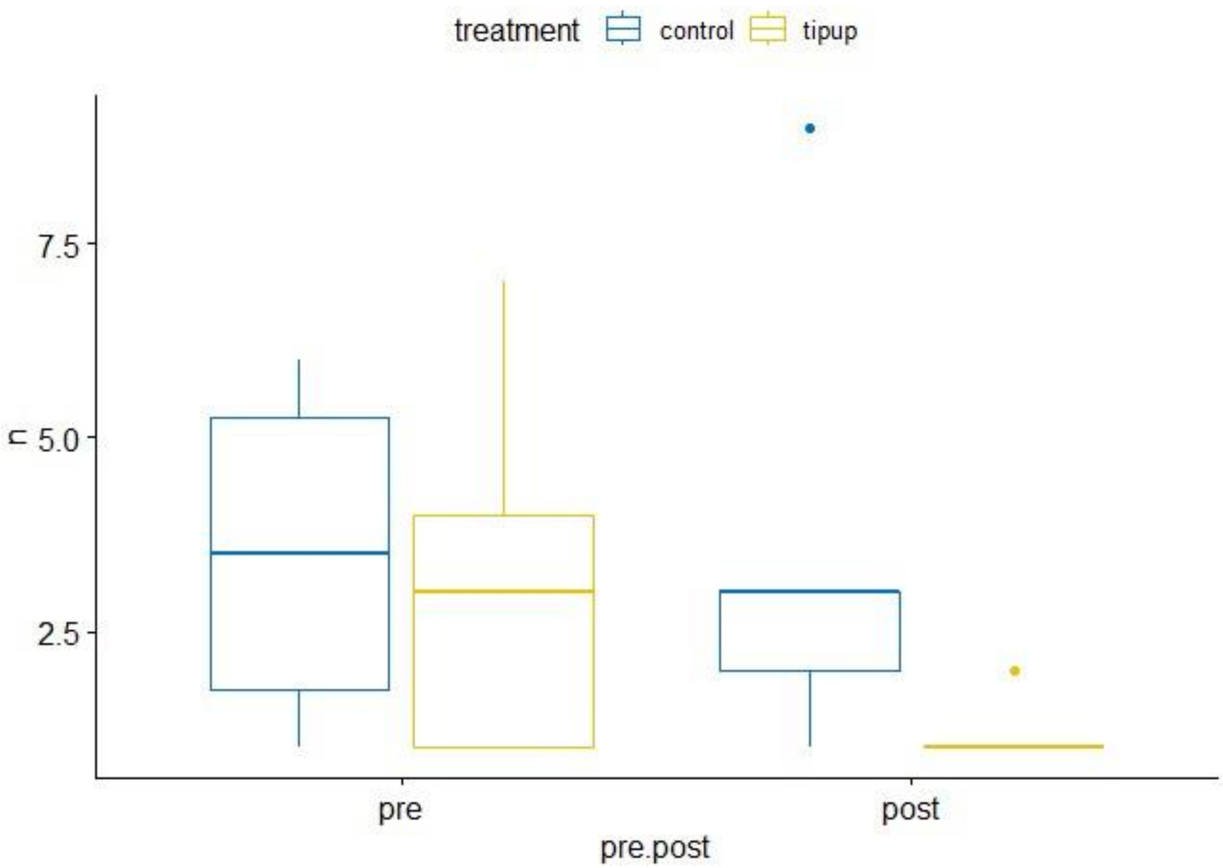

Figure 2.4. Salamander counts at coverboards in control and artificial tip-up mound treatments before and after tip-up mound treatment implementation in Virginia Tech’s Fishburn Forest, Montgomery County, Virginia, USA.

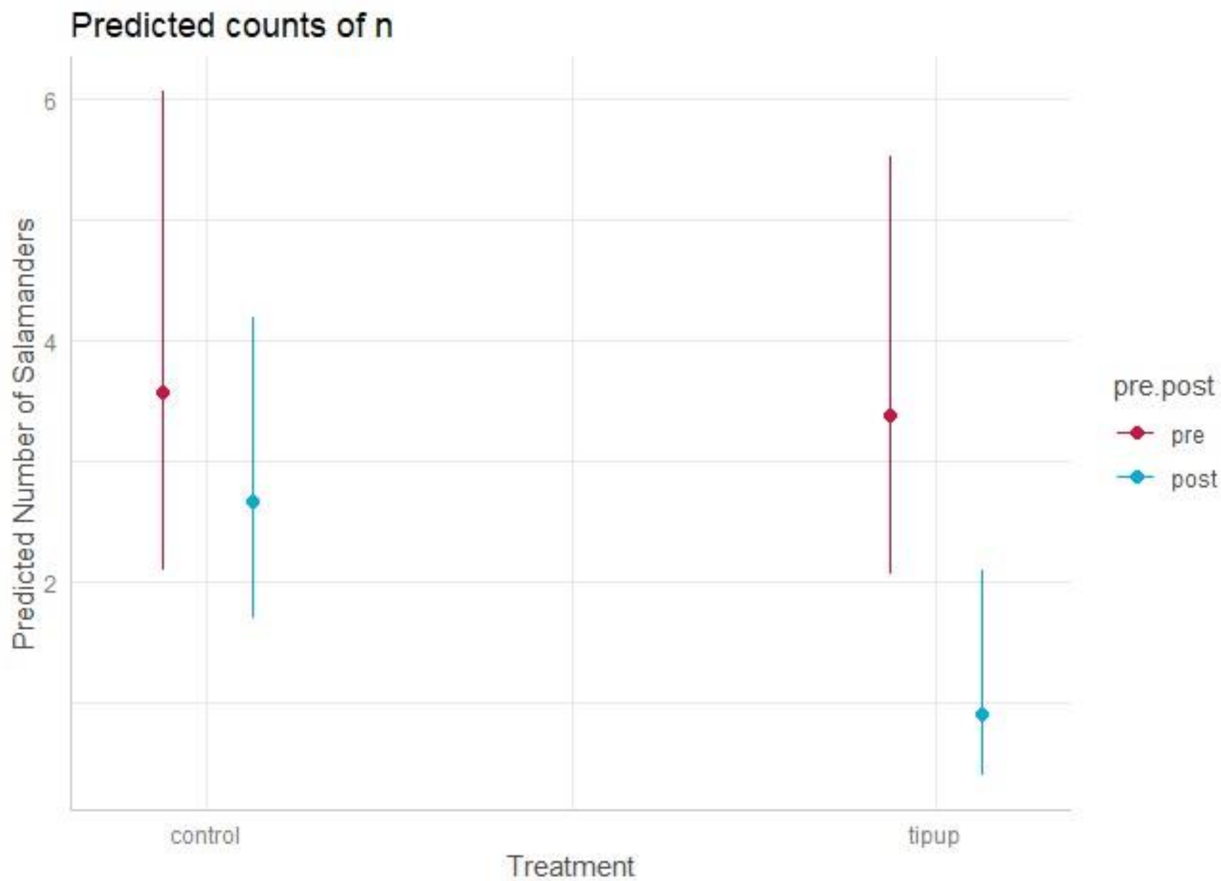

Figure 2.5. Predicted number of salamander counts by treatment and pre/post-treatment at coverboards in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA. The error bars represent the upper and lower 95% confidence interval of the predicted salamander counts.

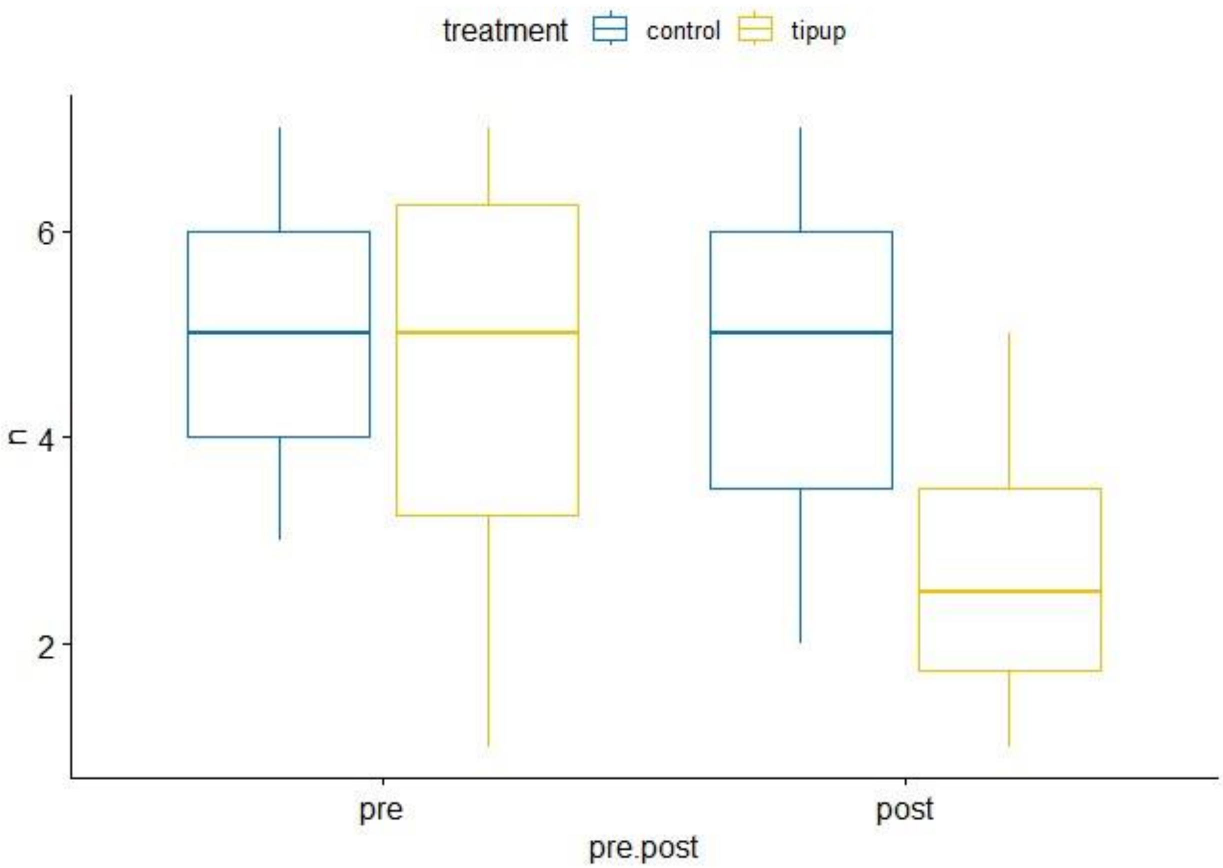

Figure 2.6. Salamander counts at night-time transect surveys in control and artificial tip-up mound treatments before and after tip-up mound treatment implementation in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

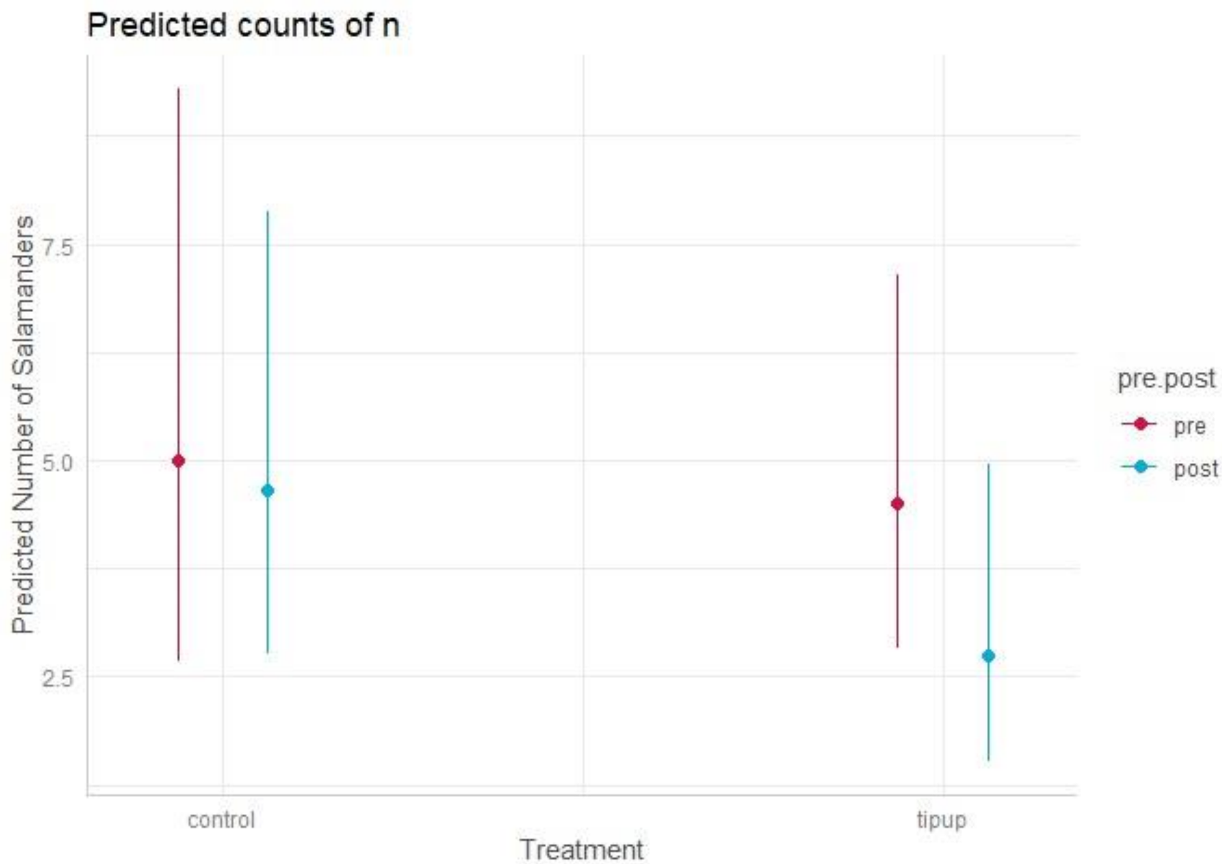

Figure 2.7. Predicted salamander count by treatment and pre/post-treatment at night-time transect surveys in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA. The error bars represent the upper and lower 95% confidence interval of the predicted salamander counts.

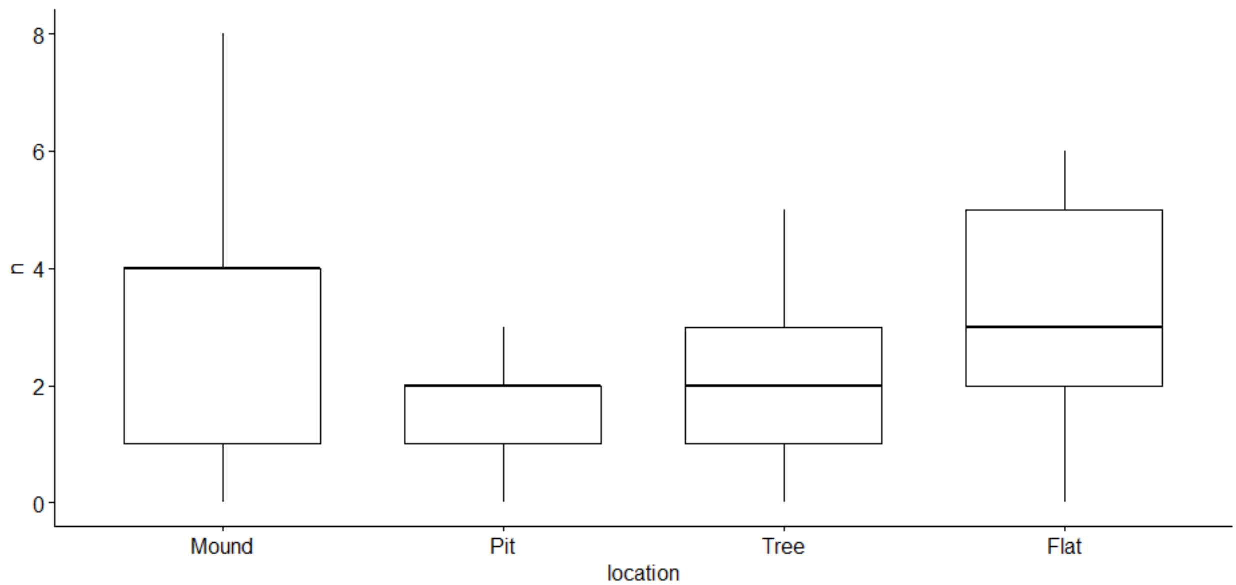

Figure 2.8. Salamander counts at mounds, pits, tree boles, and flat areas from night-time constrained area searches in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

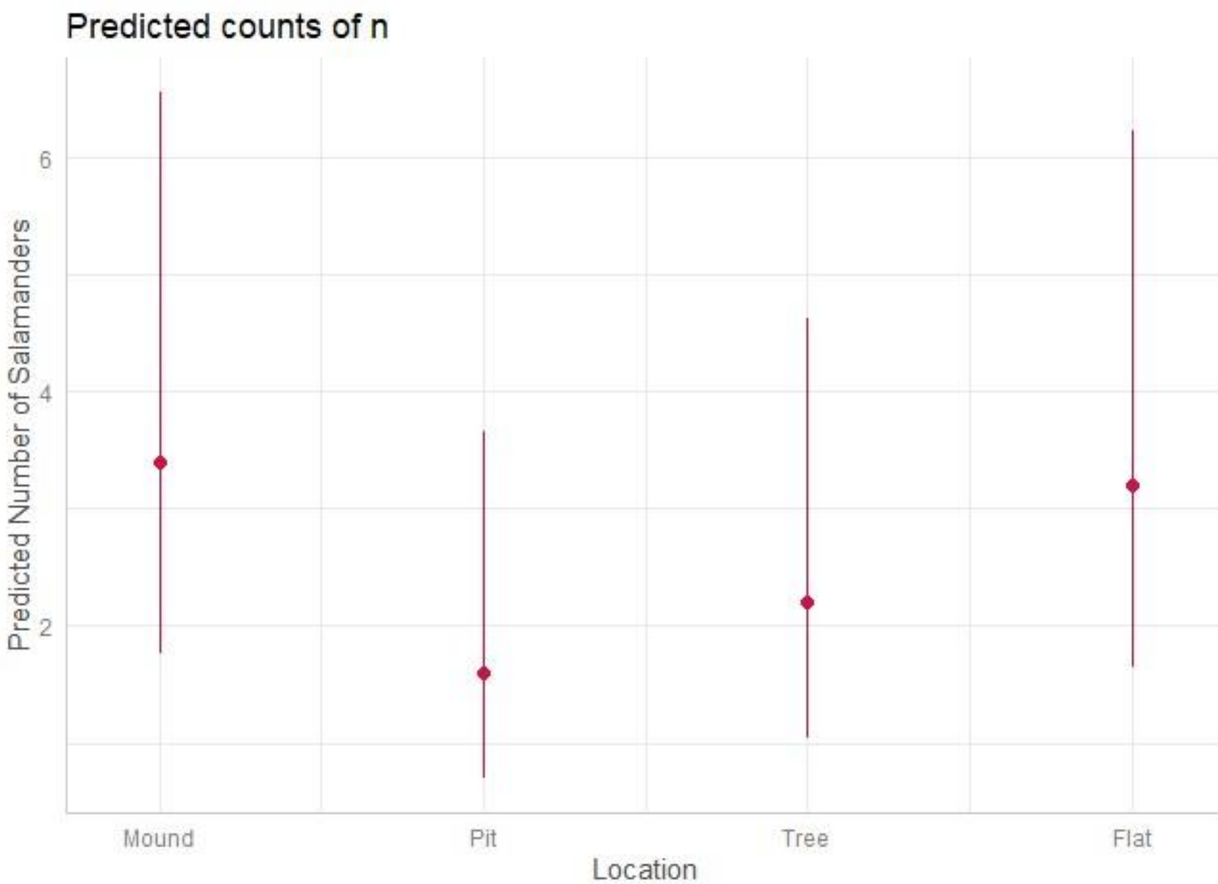

Figure 2.9. Predicted salamander count by location (mound, pit, tree, flat) from night-time constrained area searches in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA. The error bars represent the upper and lower 95% confidence interval of the predicted salamander counts.

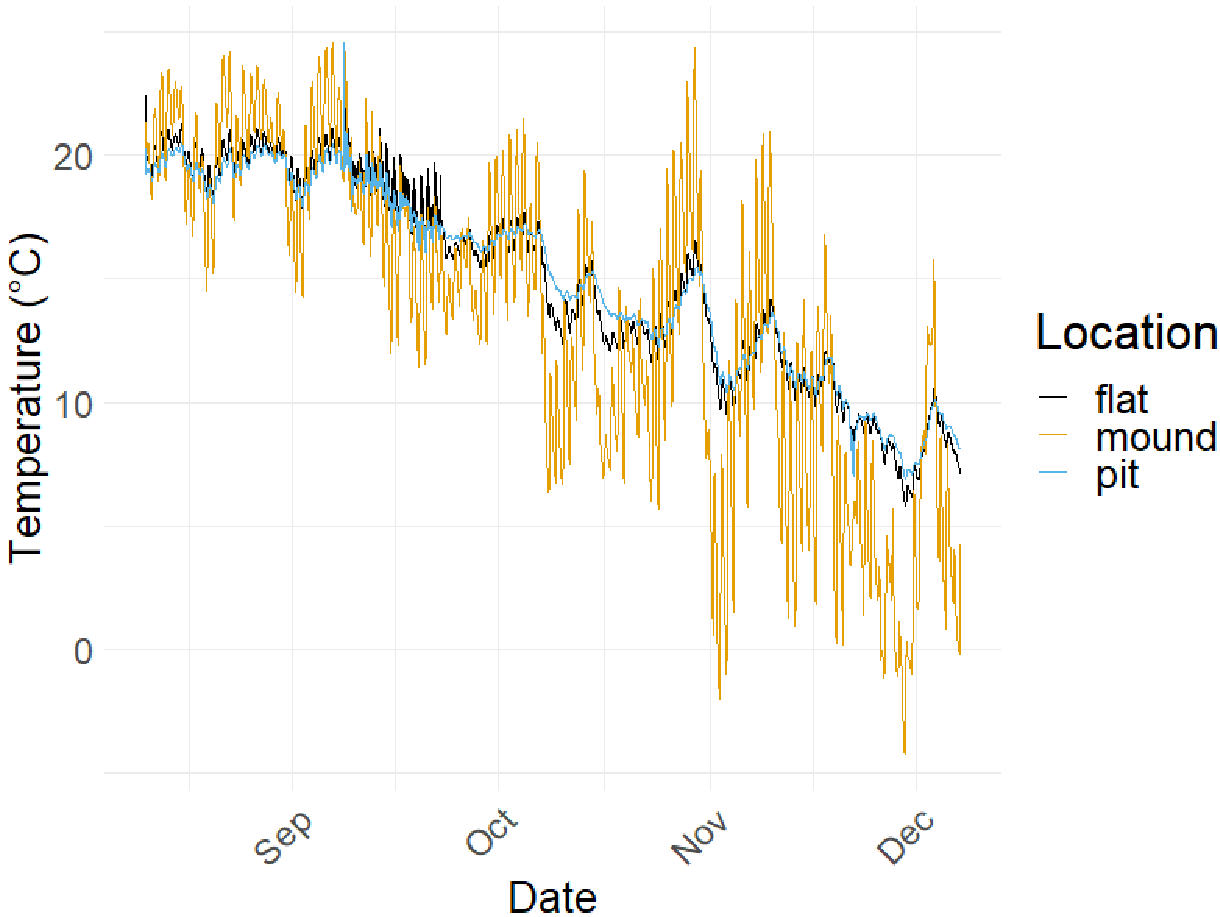

Figure 2.10. Temperature (°C) from HOBO data loggers buried 10 cm in the ground averaged over all treatment units at flat areas, mounds, and pits for each hour in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

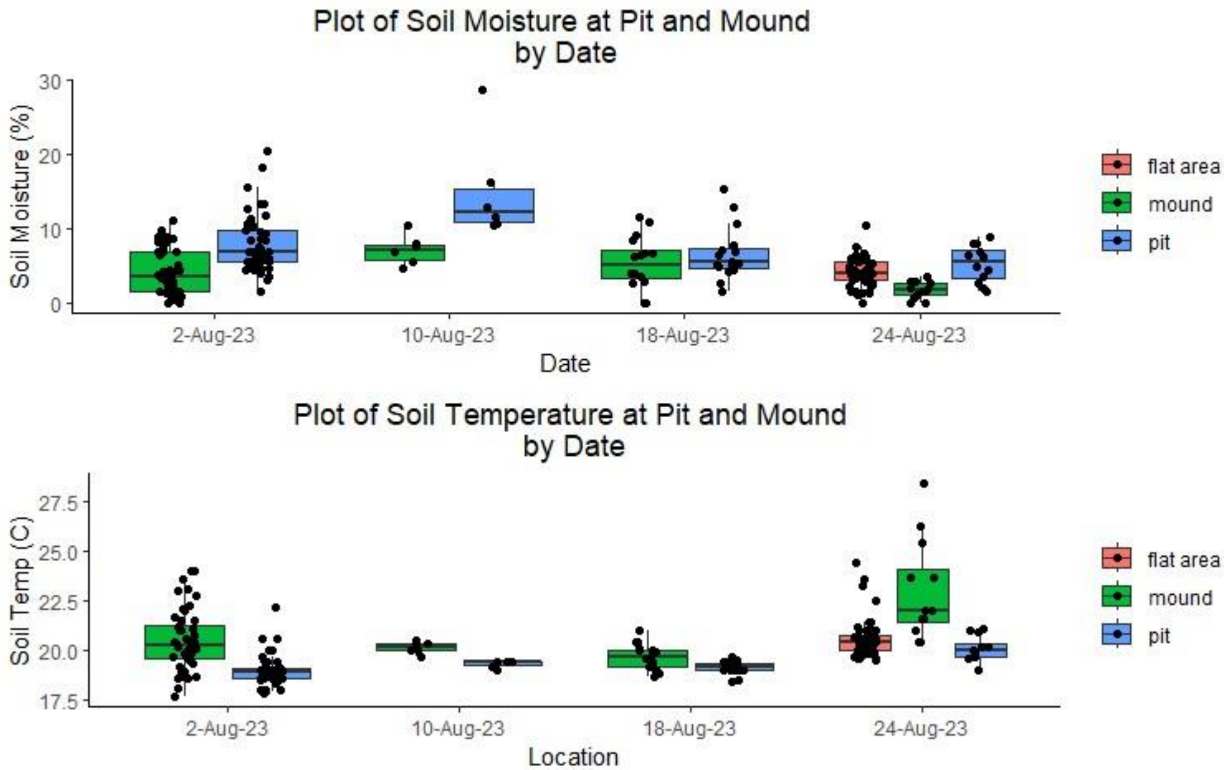

Figure 2.11. Hand-collected soil moisture and temperatures at pits, mounds, and adjacent to coverboard arrays (flat area) in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

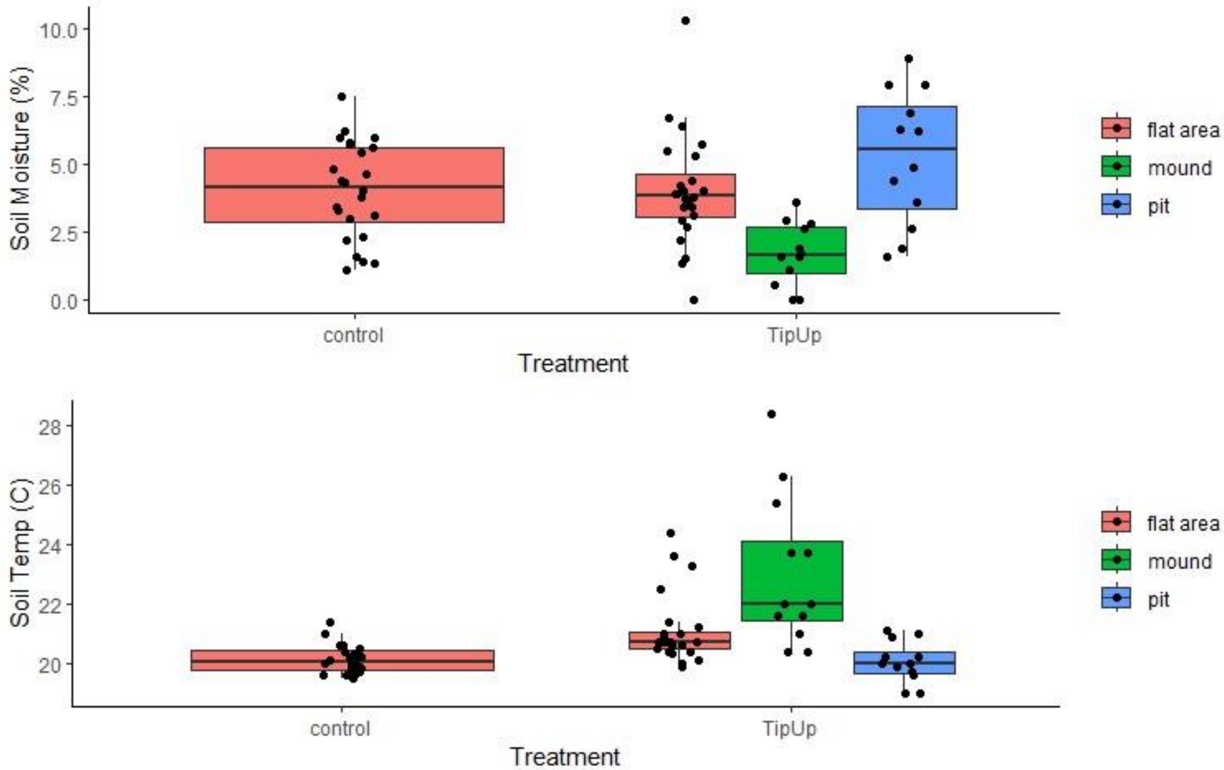

Figure 2.12. Hand-collected soil moisture and temperature adjacent to coverboard arrays (flat area) compared to pits and mounds in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

CH.3 EVALUATING BIOELECTRICAL IMPEDANCE ANALYSIS FOR USE ON EASTERN RED-BACKED SALAMANDERS AND COMPARING TWO DIFFERENT BODY CONDITION INDICES

ABSTRACT

Body condition estimates can reveal whether the environment is able to meet an organism's nutritional needs. A higher body condition suggests high quality habitat while a lower body condition suggests poorer quality habitat. Testing the reliability and comparing different plethodontid body condition estimates to determine the one with the least variability is useful to the herpetological scientific community. I tested three different body condition indices: bioelectrical impedance analysis (BIA), mass divided by snout-to-vent-length (SVL), and tail width (TW) divided by SVL. I found that SVL is likely the more repeatable measurement than TW and BIA estimates are very unreliable, so I recommend that researchers use mass divided by (SVL) as the primary body condition estimate for eastern red-backed salamanders. Future research could test BIA for use in humidity-controlled lab conditions.

INTRODUCTION

Amphibians use lipids as the primary reserves of energy, which are stored in the abdomen, between carcass muscles, and in the tail of salamanders (Fitzpatrick 1976). One study found that newts that were unfed were not able to regenerate or maintain fat reserves (Adams and Rae 1929). Another study found that salamanders fed less food regenerated tails with lower mass than those fed more food (Jamison and Harris 1992). Consumption of more food generally leads to larger fat reserves. These lipid stores can be used to assess the body condition of salamanders. One study found that female salamanders in a high-food treatment with higher lipid stores had larger clutch sizes than females in a low-food treatment with lower lipid stores (Scott and Fore 1995). Body fat is particularly important for salamanders because females that are in poorer condition may delay reproduction (Bernardo 1994).

Body condition is a common metric in wildlife studies for assessing lipid storage. It can be used as a proxy for habitat quality and gives insight into whether the habitat is meeting the nutritional needs of the animal (Homyack 2010). Researchers often use some measure of length and mass to calculate a body condition estimate. For these indices, positive values indicate animals that have a greater mass for their body size compared to the average for a given population whereas negative values indicate animals with less mass for a given body size.

Currently, most scientists use the residuals of an ordinary least squares regression of mass on body length to estimate salamander body condition (MacCracken and Stebbings 2012) or the scaled mass index (Pieg and Green 2009) but some have also used the residuals of an ordinary least squares regression of tail width (TW) on body length or have only used TW (Bendik and Gluesenkamp 2012; Gutierrez et al. 2018; Nissen and Bendik 2020; Pierce 2022; Pierce and Gonzalez 2019). Some researchers use TW because salamanders use their tails to store fat (Fraser 1980). Notably, only one prior study (Rosa et al. 2021) has compared the residuals of an ordinary least squares regression of mass on snout-vent length (SVL) to the residuals of an ordinary least squares regression of TW on SVL of salamanders. They found that the index using TW was not reliable (Rosa et al. 2021). However, several other studies have already used TW as a method to estimate body condition (Table 3.2), so the reliability of these two body condition indices warrants more comparison.

Regardless of how body condition is calculated, it requires accurate and repeatable measurements. Obtaining accurate measurements from salamanders can be challenging because their bodies curve and contort, and their soft tissue changes shape when handled (Margenau et al. 2018; Walston and Mullin 2005; Wise and Buchanan 1992). Many research projects utilize help from volunteers and technicians with varying levels of experience and skill so there could be large discrepancies between the same observers and different observers in their measurements. Accurate and precise body condition measurements are extremely important because otherwise differences in body condition can be attributed to measurement error and disregarded or false differences could be reported (Bendik and Gluesenkemp 2013; Luiselli 2005). Lastly, these body condition indices have several strong assumptions, 1) mass or tail width increases in a linear relationship with body length, 2) actual body condition is not related to body length, and 3) body length is an accurate measure of actual size, which may be violated (Green 2001). Aside from these assumptions, this index is a unitless measure, which prevents comparisons of animal body condition between studies unless all of the data from both studies are combined and a new body condition index is recalculated.

Bioelectrical impedance analysis (BIA) is one potential alternative to this traditional method of estimating body condition. BIA utilizes four electrodes to send an electrical current through an organism to measure resistance, with higher lipid content leading to more resistance (Hartman et al. 2015). Historically, BIA has been used to assess hydration, fat-free mass, and body-water mass in humans (Hartman et al. 2015). In the early 2000s, BIA began to be used in fisheries science to estimate the body condition of fish (Hartman et al. 2015). BIA is considered to be a new and developing method to accurately estimate fish body condition (Hafs and Hartman 2011; Champion et al. 2020). Cox and Hartman (2005) found that BIA measurements were strongly correlated with total body fat ($r = 0.9223$). However, few studies have examined the influence of BIA on mortality. One study found that out of the three fish species in their study, Bonytail (*Gila elegans*) exhibited 14% mortality following needle BIA measurements, in contrast to Humpback Chub (*G. cypha*) and Roundtail Chub (*G. robusta*) which exhibited 0% and 1% mortality respectively, suggesting that mortality is species-specific (Dibble et al. 2017). To my knowledge, BIA has never been used to estimate amphibian body condition. It is unknown whether amphibian BIA measurements would be correlated with actual body fat and whether there would be any mortality. BIA has the potential to be a more accurate and precise method to estimate body condition in salamanders, highlighting the importance of investigating its viability. Additionally, only one other study has compared the residuals of an ordinary least squares regression ratio of mass to SVL to the residuals of an ordinary least squares regression ratio of TW to SVL, even though several other studies have already used TW as a method to estimate body condition, so this warrants more comparison.

My goal was to evaluate several methods of estimating salamander body condition (BIA, the residuals of an ordinary least squares regression of mass on SVL, and the residuals of an ordinary least squares regression of TW on SVL) to determine which methods have the least amount of variability and if the results from different indices agree with each other. I also evaluated variability among observers, to assess whether comparisons should be made only between animals measured by the same observer.

OBJECTIVES AND HYPOTHESES

I hypothesized that BIA would provide a less variable body condition estimate than the residuals of an ordinary least squares regression ratio of mass to SVL. Because SVL measurements can be highly variable due to varying levels of technician or volunteer experience and the movements of the salamanders during the measurement, I would expect BIA to be more accurate and precise. In human children, Talma et al. (2013) found that BIA test-retest mean differences range from 7.5-13.4% of the total percent body fat, but this has not been calculated for salamanders and no known comparisons have been made between BIA and mass/SVL in the literature. It is thought that BIA does not require skilled technicians and only minor errors can be attributed to the observer (González-Correa and Caicedo-Eraso 2012). I hypothesize that the residuals of an ordinary least squares regression ratio of mass to SVL will provide a less variable body condition estimate than the residuals of an ordinary least squares regression ratio of TW to SVL. Salamanders tend to weigh the same when placing them on the scale multiple times while measuring TW involves more measurement error. Lastly, I hypothesized that variation (measurement error) within observers would be less than that between observers.

METHODS

LAB METHODS

I measured SVL, TW (Figure 3.1), and mass of 49 eastern red-backed salamanders (Plethodon cinereus) that were scheduled for euthanasia at the completion of another study that involved feeding trials (Holguin et al. In Review). Because salamanders had been maintained in similar laboratory conditions for the previous 12 months, I expected that differences due to microclimate or recent feeding activity would be minimized relative to variation arising in salamanders captured in the wild. However, the salamanders were fed at two different food levels during their experimental treatments, so differences in body condition among salamanders were expected. The salamanders were housed individually using plastic containers (Dart CH48DEF 47 oz Tamper-Resistant Clear Hinged Containers). To ensure that a moist environment was maintained, plastic containers were filled with moist paper towels and placed in one of two growth chambers. The chambers were also equipped with built-in thermostats and two backup thermometers (Temp Stick Model TEMP-STICK-TH-W-FBA, and ThermoPro TP50).

I measured all salamanders on the same day with the help of eight total observers with varying levels of experience. For each animal, four observers were randomly assigned. Two observers were randomly assigned to measure SVL and two observers were randomly assigned to measure TW. Each observer measured SVL/TW twice but without knowing whether it was the same animal. I measured mass once. For SVL and TW measurements, I placed each salamander in a plastic zip-closure bag and gently pushed the animal to the edge of the bag to flatten and straighten it and then used calipers. For mass, I placed each salamander in a weight boat and used an analytical balance (U.S. Solid Model: USS-DBS15-1: Accuracy 0.001 g/1 mg). Between measurements, I placed salamanders in an individual plastic container on wet paper towels to rehydrate.

For BIA measurements, I used an RJL Systems hand-held compression BIA electrode probe with the RJL Systems Quantum X Body Composition Analyzer. I secured the BIA electrode probe to a ring stand and used a dry sponge with a slit cut into it to restrain the salamander. I attempted to measure the salamanders by laying them supine in the sponge slit and sliding them underneath the BIA electrode probe with the electrodes positioned in the center of the abdomen. Shortly after beginning measurements, two salamanders perished so I immediately halted BIA measurements.

DATA ANALYSIS

I constructed a series of scatter plots of measurements by a single observer and measurements by two different observers on the same salamander and looked at the correlation. I also compared the standard deviation of centered and scaled SVL measurements to centered and scaled TW measurements to determine which measurement had more variation. I centered and scaled these measurements because SVL is always larger than TW and this ensured each variable contributed equally to the analysis. I plotted the residuals of a linear model for log(mass) and log(average SVL) to the residuals of a linear model for log(average TW) and log(average SVL) to compare both body condition estimates. I took the log of mass, average SVL, and average TW because they are not linear, but needed to be in a linear model for the body condition indices. I calculated the residuals by subtracting the predicted value from the linear model from the actual value. I performed all statistical analyses in program R version 4.2.3 (R Core Team 2023).

RESULTS

The two SVL measurements by a single observer were highly correlated ($R = 0.90$), indicating repeatability between measurements (Figure 3.2A). SVL measurements of the same salamander by two different observers were slightly less correlated ($R = 0.82$), but still very consistent (Figure 3.2B). TW measurements by a single observer are also correlated ($R = 0.66$) but less so than SVL measurements by a single and two different observers (Figure 3.3A). TW measurements of the same salamander by two different observers were not very correlated ($R = 0.45$; Figure 3.3B). Overall centered and scaled TW measurements were more variable than centered and scaled SVL measurements since TW standard deviation (mean = 0.56; [0.48, 0.63]) was significantly different from SVL standard deviation (mean = 0.29; [0.23, 0.35]; $P < 0.05$) and more TW standard deviations were larger than SVL standard deviations, when comparing standard deviation of SVL to standard deviation of TW with a line with a slope of one (Figure 3.4). Lastly, when comparing the residuals of a linear model for log(mass) and log(average SVL) to the residuals of a linear model for log(average TW) and log(average SVL), these two indices only agreed 70.83% of the time, meaning that 29.17% of the time sometimes a salamander with "good" body condition under the log(mass) and log(average SVL) sometimes had a "bad" body condition under the log(average TW) and log(average SVL) method or vice versa (Figure 3.5).

BIA measurements took much longer than expected and did not seem reliable due to drastic differences between BIA phase angles (Table 3.1). The meter took a very long time to settle on one resistance value. Furthermore, this process resulted in the mortality of two animals out of the seven animals measured using this methodology. Of the two mortalities, one salamander had undergone three BIA measurements and the other salamander had undergone one BIA measurement.

DISCUSSION

Measurements by a single observer are more repeatable (Figure 3.2; Figure 3.3) so if researchers are worried about small differences between measurements, it could be helpful to use only a single observer. SVL is much more precise and repeatable than TW so it would be best to avoid using TW measurements when possible.

Some salamanders deplete their carcass (body) lipid stores during times of stress before their caudal (tail) lipid stores and appear emaciated (Fitzpatrick 1976). This means a salamander that had just undergone overwintering, brooding, or another stressor, and had depleted its carcass fat reserves, but not its caudal fat reserves, would be classified as having a very high body condition under the TW and SVL estimation. Yet the mass and SVL estimation would take into consideration both the tail weight and the body weight and would likely generate an estimate classifying this as a salamander with moderate body condition. In other words, the mass and SVL estimation are more likely to reflect changes in total body lipid stores.

Far fewer studies have used TW as an estimate of body condition rather than mass (Table 3.2) and only one other study compared the two (Rosa et al 2021). Measurements using a scale tend to be far more consistent (e.g., our scale had a repeatability error of ± 0.002 g) than length measurements, where sometimes the measurement error can be even larger than changes from growth (Holguin et al. In Review). Tail width and mass represent two different condition indices, and apart from their measurability, one may be more indicative of body condition than the other. More studies have used mass and SVL, so it is preferable to utilize an index that is better known and understood. Therefore, I recommend that researchers use mass rather than TW when calculating plethodontid salamander body condition.

It is possible that the BIA electrical current from this methodology did not cause the mortality, but rather the dry sponges that the animals were restrained with for measurements. The animals were juveniles with a mean SVL of 26.49 mm. Due to their small body size and propensity to lose water quickly, as well as being squeezed by a dry sponge that soaked up moisture, it is possible that these animals died from dehydration. Unfortunately, when choosing a flexible but firm structure to keep the salamander in place during measurements, I failed to consider how absorbent a sponge would be. In retrospect, I should have used something such as a pool noodle to contain the animals during processing. Attending the hydration state of salamanders is always an important consideration, and especially for a measurement such as BIA in which water to lipid ratio in the body determines the electrical impedance. I regret my mistakes in this process and describe them here in hopes that others can avoid them. Despite my doubts that the actual electrical current from BIA harmed the salamanders in any way, I cannot recommend this methodology for use on plethodontid salamanders. Because of cutaneous respiration in plethodontid salamanders, they can quickly gain and lose moisture through their skin. Plethodontid salamanders held in a lab for 12-24 hours without a damp substrate can lose 8-33% of their body mass. BIA measures the speed at which a mild electrical current travels through the body, and with water having much greater conductivity than fat, this is more a measure of how much water is in the salamanders rather than estimating their body condition. Therefore, I suggest that the traditional measures of SVL and mass to calculate a body condition index are likely more appropriate for plethodontid salamanders. However, the bioelectrical impedance analysis methodology could still be investigated for use on other amphibians, or on plethodontid salamanders in a humidity-controlled lab setting with a better device for containing animals during the process.

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Table 3.1. BIA electrical phase angle (phase angle (°) = (arctan($\frac{X_c}{R}$))x$\frac{180°}{\pi}$) as a body condition index where X_c is reactance and R is resistance. Possible values range from 0°-90° where higher values suggest good body condition.

Salamander ID	**BIA phase angle 1 Observer A**	**BIA phase angle 1 Observer B**	**BIA phase angle 2 Observer B**
307	82.71193952	NA	NA
390	89.99136528	NA	NA
332	NA	2.507952177	NA
397	89.96941535	NA	NA
313	81.17962045	7.136684758	NA
314	82.36666941	5.030801926	NA
416	78.93495355	5.271208013	81.36735302

Table 3.2. Scientific literature that uses the residuals of a linear model for log(mass) and log(SVL), the residuals of a linear model for log(TW) and log(SVL), and compares both methods as an estimate of salamander body condition.

Mass and measure of body length	TW and measure of body length	Compares Both
Davenport and Lowe 2016	Gutierrez et al. 2018	Rosa et al. 2021
Flynn et al. 2021	Nissen and Bendik 2020	
Gabor 1995	Pierce and Gonzalez 2019	
Iglesias-Carrasco et al. 2017	Pierce 2022	
Liles et al. 2017		
Lowe et al. 2006		
Maldowan et al. 2022		
Peig and Green 2009		
Riedel et al. 2012		
Unger et al. 2021		
Whiteman et al. 2012		
Zhang et al. 2014		

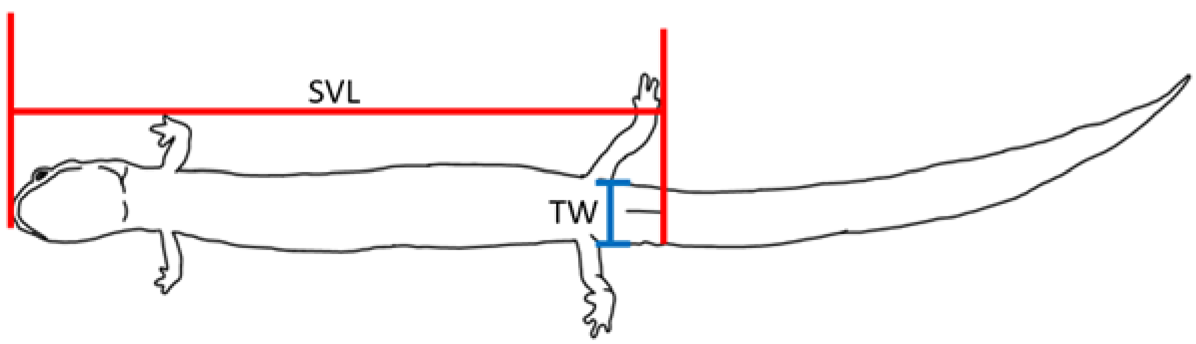

Figure 3.1. Diagram showing where I measured the snout-vent length (SVL) and tail width (TW) of each salamander.

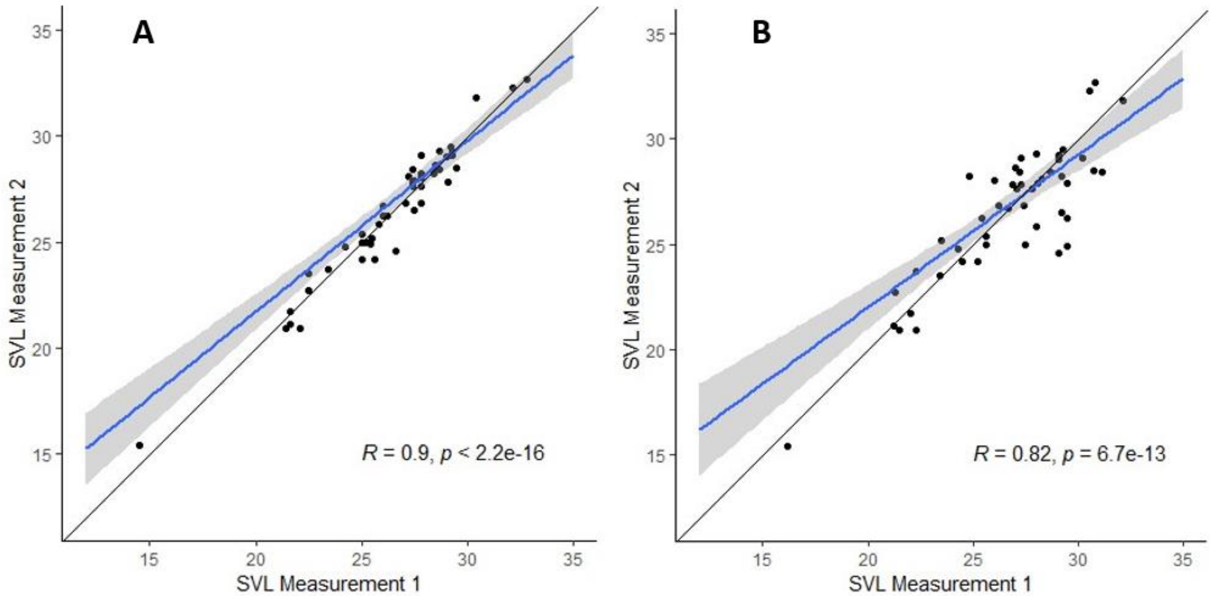

Figure 3.2. Comparing two snout-vent length (SVL) measurements of *Plethodon cinereus* by a single observer (A) with two measurements by two different observers (B) on the same salamander with a gray 95% confidence band around a line with a slope of 1. Each dot indicates measurements for a single salamander.

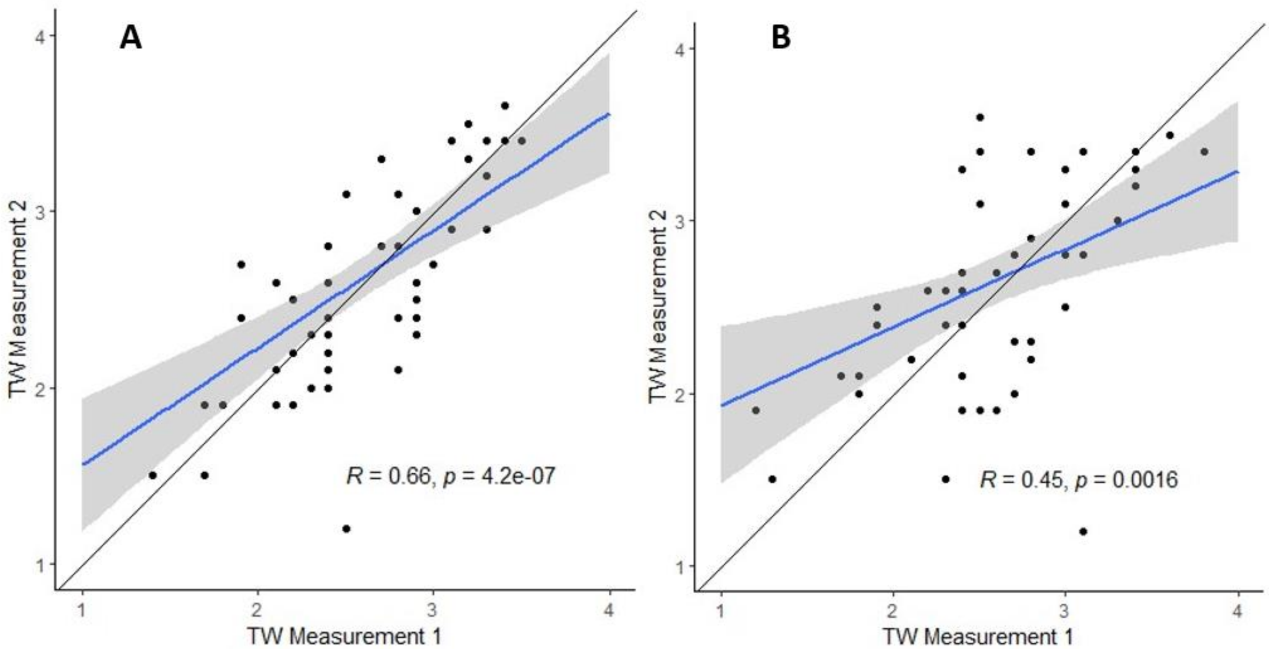

Figure 3.3. Comparing tail width (TW) measurements of *Plethodon cinereus* by a single observer (A) with measurements by two different observers (B) on the same salamanders with a gray 95% confidence band around a line with a slope of 1. Each dot indicates measurements for a single salamander.

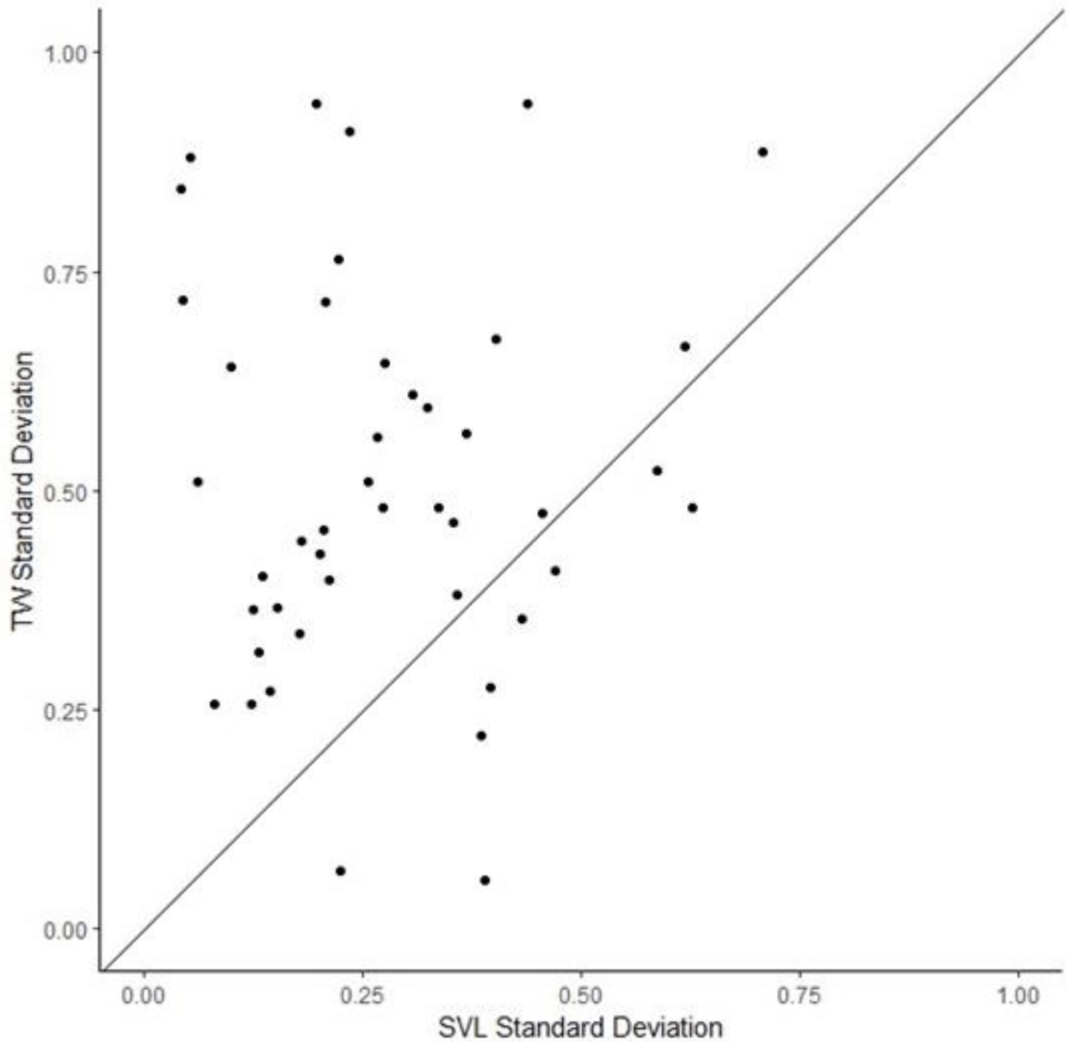

Figure 3.4. Comparison of variation in centered and scaled snout-vent length (SVL) measurements to variation in centered and scaled tail width (TW) measurements with a line with a slope of 1.

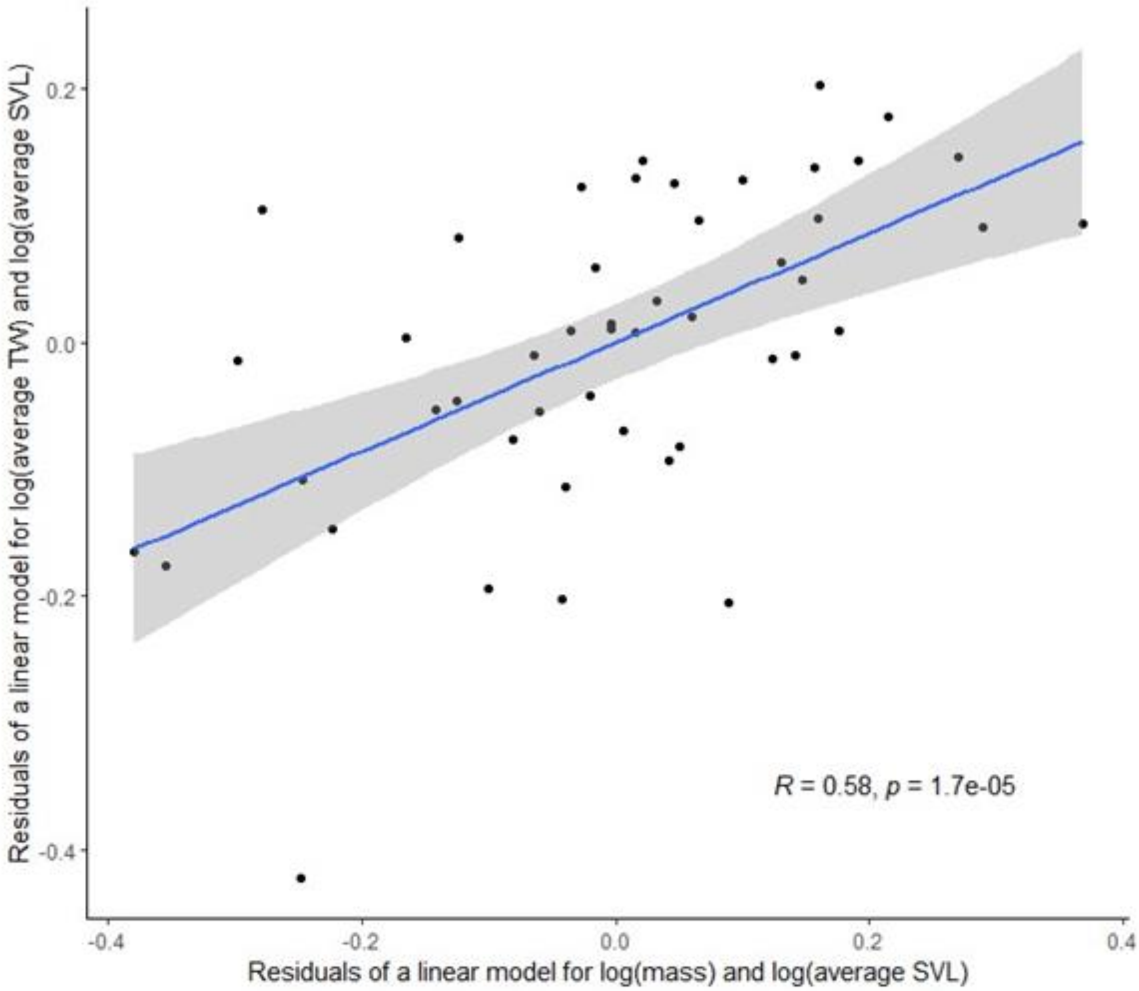

Figure 3.5. Comparison of the residuals of a linear model for log(mass) and log(average SVL) to the residuals of a linear model for log(average TW) and log(average SVL) with a gray 95% confidence band. Acronyms: Snout-vent length (SVL), tail width (TW)

CONCLUSIONS

Plethodontid salamanders are ecologically significant members of the forest ecosystem. They are apex predators of invertebrates but are important food sources for other animals including other salamanders, snakes, birds, and mammals (Sullivan et al. 2004; Shaw et al. 2023). Importantly, these forest-dwelling salamanders influence the distribution of limiting nutrients for plant growth (Milanovich and Peterman 2016). Although some plethodontid salamander species are plentiful in central Appalachian forests, species like the *Plethodon Shenandoah* (Shenandoah salamander), *Plethodon amplus* (Blue Ridge gray-cheeked salamander), and *Plethodon hubrichti* (Peaks of Otter salamander), are endangered or vulnerable (IUCN 2023). Broadly, amphibians are one of the most threatened and endangered groups of animals due to human activities causing habitat alteration, fragmentation, and destruction, climate change, disease, pollution, non-native species, and more (Blaustein and Kiesecker 2002; Houlahan et al. 2000). It is essential that we determine how different human activities such as forest management techniques impact plethodontid salamanders both in the short-term and long-term in order to make more informed management decisions. Few studies have examined the impacts of silviculture over a longer time scale and until now it was unknown when populations would fully recover.

In this work, I evaluated plethodontid salamander relative abundance 30 years after experimental silvicultural treatments (Chapter 1), described the short-term impacts of artificial tip-up mounds on plethodontid salamander counts (Chapter 2), and tested three different body condition indices for use on plethodontid salamanders (Chapter 3).

My results suggest that silvicultural treatments may not be as detrimental to long-term plethodontid salamander populations as originally thought. After 30 years, salamander relative abundances had returned to pre-harvest levels except in the shelterwood treatment which had an overwood removal 16-17 years ago. This suggests that shelterwood harvests with multiple stand entries may have a longer-lasting negative impact on salamander populations than other silvicultural treatments, even those that remove more canopy than the shelterwood, but during only one stand entry. Earlier results from this study found that over a regional scale, salamander declines would be lower using clearcuts than the other techniques simply because a smaller acreage would be disturbed to harvest the same amount of wood (Knapp et al. 2003). The results from this study show that the population declines that result from repeated stand entries (documented by Homyack and Haas, 2013) persist for at least 17 years. Although there may be benefits to other taxa from some techniques that require more frequent stand entries, plethodontid salamanders do not. Salamander count decreases in the short-term after artificial tip-up mound implementation. The warm climate of Appalachian central hardwood forests and ground compaction could have contributed to this result. Lastly, I found that SVL measurements are less variable both between the same and different observers than TW measurements, so I suggest that scientists use mass divided by SVL as a body condition index for plethodontid salamanders. BIA was not a reliable estimate for use with plethodontid salamanders.

Future studies should examine the long-term impacts of experimental silviculture including artificial tip-up mounds on plethodontid salamanders in different geographic locations, site conditions, harvest sizes, and with different species since these could influence the results and how long recovery takes. This research adds especially long-term results to the existing body of literature on the effects of silviculture on salamanders and shows that full recovery in 30 years is possible.

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APPENDIX

Table 1. Stand age at treatment, date of harvest completion and date of shelterwood overstory removal for Blacksburg 1 (BB1), Blacksburg 2 (BB2), Clinch 1 (CL1), and Clinch 2 (CL2) sites for the Southern Appalachian Silviculture and Biodiversity Project, located in the George Washington and Jefferson National Forest in southwest VA, USA. Table adapted from Jessica A. Homyack's Dissertation.

	Study Site			
	Blacksburg 1	Blacksburg 2	Clinch 1	Clinch 2
Age at Treatment	100	99	111	76
Date of Harvest Completion	Mar 1995	Jun 1996	Oct 1998	Mar 1998
Date of Shelterwood Overstory Removal	Winter 2007-2008	Winter 2007-2008	NA	NA

Table 2. Plethodontid salamander species occurrence at sites from 1994-2023 for the Southern Appalachian Silviculture and Biodiversity Project, located in the George Washington and Jefferson National Forest in southwest VA, USA.

	BB1	**BB2**	**CL1**	**CL2**
Aneides aeneus				X
Desmognathus sp	X		X	X
Desmognathus fuscus	X	X	X	X
Desmognathus monticola	X	X		
Desmognathus ochrophaeus	X	X	X	X
Eurycea cirrigera	X	X	X	
Gyrinophilus porphyriticus	X		X	X
Hemidactylium scutatum				X
Plethodon cinereus	X	X		
Plethodon cylindraceus/glutinosus	X	X	X	X
Plethodon kentucki			X	X
Plethodon richmondi			X	X
Pseudotriton ruber	X	X		

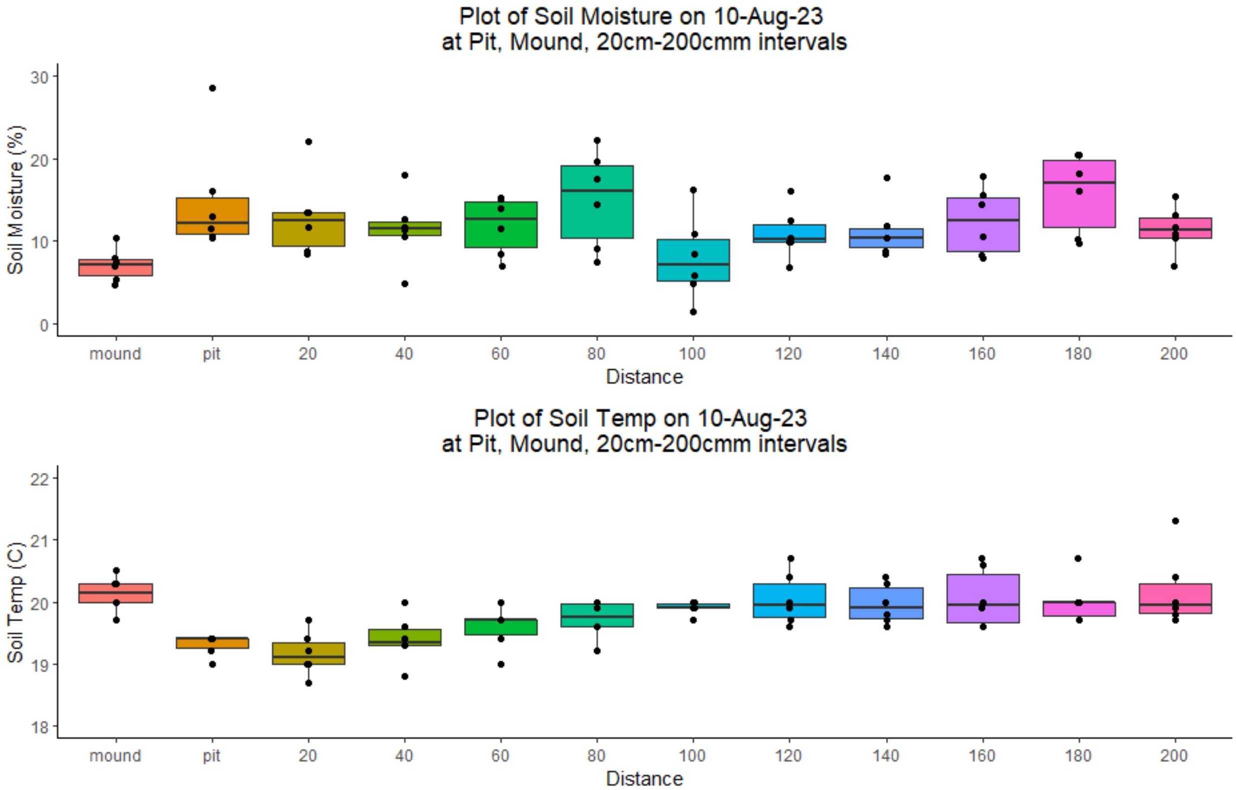

Figure 1. Hand-collected soil moisture and temperature at mounds, pits, and 20-200 cm from the center of the pit on 10 August 2023 in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

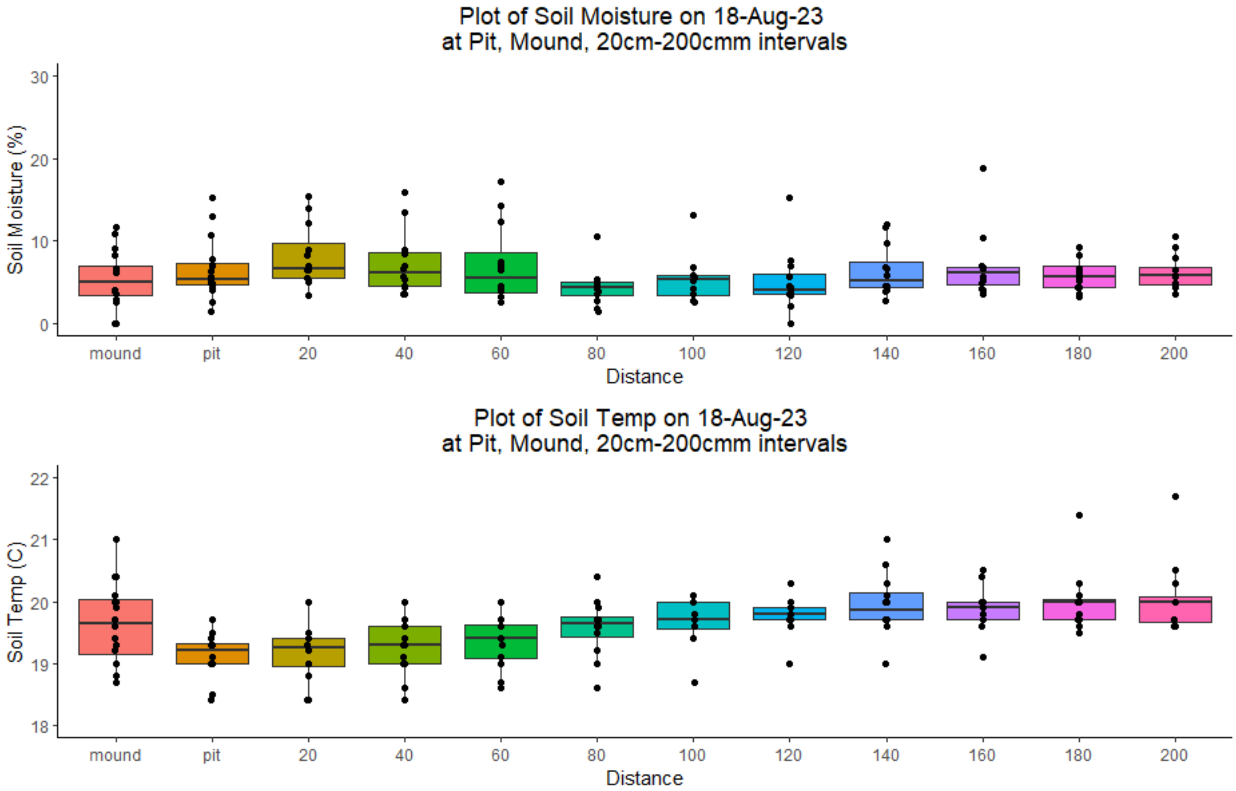

Figure 2. Hand-collected soil moisture and temperature at mounds, pits, and 20-200 cm from the center of the pit on 18 August 2023 in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.

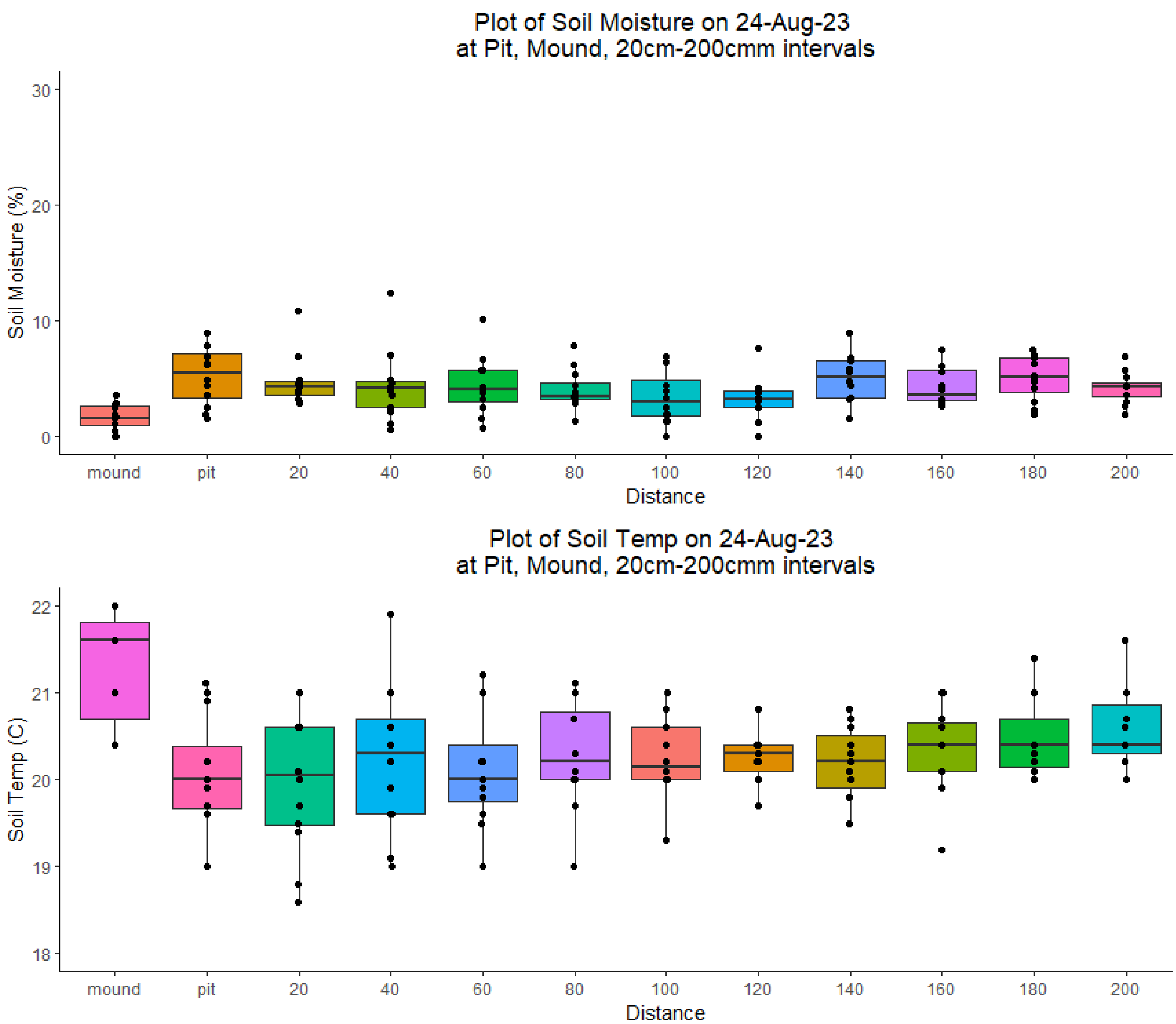

Figure 3. Hand-collected soil moisture and temperature at mounds, pits, and 20-200 cm from the center of the pit on 24 August 2023 in Virginia Tech's Fishburn Forest, Montgomery County, Virginia, USA.