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Effects Of Biomass Harvest On Eastern Red-Backed Salamanders

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EFFECTS OF BIOMASS HARVEST ON EASTERN RED-BACKED SALAMANDERS (PLETHODON CINEREUS)

For the degree of Master of Science

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John B. Dunning Jr.

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Approved by: Robert K. Swihart 11/07/2014

Head of the Department Graduate Program Date
EFFECTS OF BIOMASS HARVEST ON EASTERN RED-BACKED SALAMANDERS

A Thesis
Submitted to the Faculty
of
Purdue University
by
Patrick J. Ruhl

In Partial Fulfillment of the Requirements for the Degree of
Master of Science

December 2014
Purdue University
West Lafayette, Indiana
For my grandfather, Dr. Howard E. Evans, who inspired countless scientists throughout his lifetime. Never stop exploring.
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ABSTRACT


In a typical forest harvest, the volume of coarse woody debris (CWD) increases from nonmerchantable material (i.e., tree-tops, limbs, and small-diameter trees) left on the forest floor. Biomass harvesting removes much of this material for bioenergy production. When removed, ecosystem services associated with CWD, such as seedbed substrate, nutrient cycling, and essential wildlife habitat, is reduced. Woodland salamanders have strict microhabitat and soil moisture requirements that make them especially sensitive to timber harvest practices, particularly those that remove CWD, a primary habitat for the group.

I monitored the abundance of Eastern red-backed salamanders (*Plethodon cinereus*) in response to a gradient of retained CWD following timber and biomass harvesting, a first study assessing the impacts of wood-based biomass harvest on plethodontids. I considered key aspects of salamander physiological health such as standard metabolic rate (SMR) and body condition. Calculating SMR of *P. cinereus* in different harvest regimes has been used as a proxy of forest ecosystem health. In this study I also field-tested the assumptions of current SMR extrapolation techniques.
N-mixture abundance models indicated a positive correlation between the percent of retained CWD and *P. cinereus* abundance. Standard metabolic rate of encountered salamanders was not affected by the CWD retention gradient in any sampling season. However, SMR variance was significantly larger in harvested stands in Fall 2013. Within this sampling season, salamander mass was more variable in harvested stands, and snout vent length was significantly lower in harvested stands. This discrepancy in salamander size between harvested and unharvested stands in Fall 2013 has implications for the current SMR calculation technique, which utilizes the temperature profiles of different harvest treatments and extrapolates SMR for *P. cinereus* based on a standardized one-gram salamander. Although a threshold of resilience for plethodontids based on a gradient of retained CWD was not discernable, this study emphasizes the need for future wildlife research on biomass harvesting in order to ensure appropriate implementation of regulations that provide better protection of the integrity and biodiversity of forest ecosystems.
CHAPTER 1: IMPORTANCE OF COARSE WOODY DEBRIS IN THE CONTEXT OF BIOMASS HARVEST: AN INTRODUCTION

1.1 Timber Harvest

Timber harvests can have both positive and negative effects on forest wildlife. The impact of forest harvest on invertebrate species has ecological relevance to many vertebrate species, especially aerial predatory species. One such invertebrate group, the family Lepidoptera, is especially sensitive to timber harvest incurring drastic decreases in species richness regardless of the specific harvest level (Summerville 2011). Because bat echolocation sequences containing feeding buzzes are highly correlated with counts of Lepidoptera (Morris et al. 2010), decreases in Lepidopteran richness could potentially have negative effects on bats.

In general, bat species are able to forage in stands with varying structural complexity based on their maneuverability. Morris et al. (2010) found that bat species from the genus *Myotis* forage almost exclusively within unmanaged stands or thinned stands whereas larger bats, such as hoary bats (*Lasiurus cinereus*) feed almost exclusively in open areas.

Similarly to the hoary bat dependence on harvested or open stands, some early successional bird species, such as indigo buntings (*Passerina cyanea*), yellow-breasted chats (*Icteria virens*), and prairie warblers (*Setophaga discolor*) have been shown to have increased daily survival rates in recently harvested stands with even-aged management.
schemes (Clawson et al. 2002). This may be due to increased structural diversity of thick understory stems, or higher resource availability in the form of increased arthropod biomass (McDermott and Wood, 2010). But alternatively, some bird species are negatively affected by timber harvest, as some species lose nesting or foraging substrates (e.g. canopy species or cavity nesters).

1.2 CWD Removal

Coarse woody debris (CWD), in the form of logs or standing snags, fulfills several key life-history roles for many different organisms (McKenny et al. 2006, Riffel et al. 2011). Although early successional species often increase in the years following a timber harvest, excess removal of CWD in addition to timber harvest can negatively affect bird communities (Riffel et al. 2011). Invertebrate biomass is linked to the presence of CWD, and research suggests that removal of CWD alters invertebrate density and diversity resulting in less abundant and less diverse bird guilds (Riffel et al. 2011).

Small mammals utilize CWD for cover, nesting, travel corridors, and a source of invertebrate prey, but dependence on CWD varies between species. If CWD is removed in conjunction with a forest disturbance, certain species such as fox squirrels (Sciurus niger) flourish, whereas other species, such as shrews and voles (Sorex and Microtus spp.) decline (Loeb 1999). However, the removal or retention of CWD alone does not affect most small mammal species, and has little effect on overall diversity of small mammal populations (Riffel et al. 2011).

Reptiles and amphibians also utilize CWD throughout many phases of their life history. Intensive harvest techniques involving burning and re-entry have been shown to have a positive impact on lizard abundance and reproduction (Matthews et al. 2010), but
amphibian species tend to experience a negative effect (Semlitsch et al. 2009, Matthews et al. 2010). In a study of 26 species of amphibians by Semlitsch et al. (2009), clearcutting and CWD removal had negative effects on amphibian populations, especially on pond-breeding ambystomatid salamanders. While that study found a few positive effects of timber harvest, these were limited to reproductive behavior and larval characteristics associated with frog species in experimental breeding ponds.

1.3 Biomass Harvest Impacts

In recent years, inflated gasoline prices and fossil fuel depletion have resulted in an increased emphasis on the use of renewable energy sources such as cellulosic ethanol from woody biomass (Janowiak and Webster 2010). Unlike traditional timber harvest techniques, biomass harvest for cellulosic bioenergy involves the removal of otherwise unprofitable wood such as tree-tops, limbs, small-diameter trees, and pre-existing deadwood (Riffel et al. 2011). This material, if removed, can no longer perform ecosystem services such as nutrient cycling, nor can it provide essential habitat for several species of forest wildlife. Because woody biomass harvest is becoming an increasingly popular energy alternative (Janowiak and Webster 2010), ecological impacts of this kind of renewable energy harvest may become more widespread. Guidelines concerning harvest residues in the form of downed CWD and snags must be developed to ensure adequate retention of forest biodiversity (Berger et al. 2013). Most state-specific best management practices (BMPs) include some level of required CWD retention, however these guidelines are often vague, and vary greatly from state to state (Evans et al. 2010).
In the state of Indiana, forests cover about 4.7 million acres (about 20%) of the total land base (Indiana DNR). Of these 4.7 million acres, 85% is privately owned. The remaining 705,000 acres of timberland are owned by federal, state, and local governments. Although forest resources are not as abundant as in other states, such as Minnesota and Maine, timber harvest still provides employment for over 54,000 people annually in forest-based manufacturing jobs throughout the state (Bratkovich et al. 2004). In 2011, annual mortality of growing stock trees accounted for the loss of 80.8 million cubic feet of timber, and annual harvest removals of growing stock trees accounted for another 75.6 million cubic feet of timber. Combined, the removal of growing stock trees accounted for 62 percent of the 259.2 million cubic feet accumulated in annual net growth (Woodall and Gallon 2012).

Because forested land in the state of Indiana is severely fragmented, intensive biomass harvesting involving the additional removal of CWD could potentially have lasting effects on sensitive forest species. Terrestrial salamanders from the family Plethodontidae have been suggested as a bioindicator for mature forest ecosystem health (Welsh and Droege 2001). In addition, Otto et al. (2013) recently highlighted the need for research focused on plethodontid response to woody biomass harvest. In this study I monitored the plethodontid response to a gradient of CWD retention following a biomass harvest in the state of Indiana.

1.4 Research and Chapter Summary

Throughout the course of the study I encountered eight different species of salamanders. However, the eastern red-backed salamander (Plethodon cinereus) represented over 95% of the total captures. Due to its local abundance and proposed
indicator status, I studied the effects of biomass harvest on *P. cinereus*. By manipulating harvest residues in treatment plots to create a gradient of CWD retention, I was able to assess the impacts of CWD retention on both abundance and standard metabolic rate (SMR) of *P. cinereus*.

In chapter two I determine the effects of biomass harvest on *P. cinereus*. I use mark-release-recapture (MRR) data and perform a binomial-poisson (*N*-mixture) model analysis (Royle 2004, Kéry 2010) to explain the effects of site-specific environmental covariates on both abundance and detection. In addition to abundance analyses, I also examined the effects of biomass harvest on *P. cinereus* SMR and body condition. Standard metabolic rate is commonly used as a proxy for the physiological health of plethodontids (Homyack et al. 2011, Careau et al. 2014). Because *P. cinereus* is commonly used in research, methods of species-specific SMR calculation have been developed that enable researchers to better understand the ecological impacts of timber harvest. Homack et al. (2010) developed a predictive multiple regression equation that uses *P. cinereus* mass and temperature to estimate SMR to 95% accuracy. In chapter three I evaluate this lab-based SMR relationship and test its validity in a field setting. Not only does this study contribute to the current body of knowledge concerning the response of *P. cinereus* to timber harvesting, but it also has BMP implications for the state of Indiana. In chapter four, I discuss future directions for *P. cinereus* research, as well as some of the potential limitations of using salamanders as a bioindicator for the ecological study of biomass harvesting.
CHAPTER 2. EFFECT OF BIOMASS HARVESTING ON THE ABUNDANCE AND PHYSIOLOGICAL HEALTH OF EASTERN RED-BACKED SALAMANDERS

Abstract

In a typical forest harvest, the volume of coarse woody debris (CWD) increases from nonmerchantable material (i.e., tree-tops, limbs, and small-diameter trees) left on the forest floor. Biomass harvesting removes much of this material for bioenergy production. When removed, ecosystem services associated with CWD, such as seedbed substrate, nutrient cycling, and essential wildlife habitat is reduced. Woodland salamanders have strict microhabitat and soil moisture requirements that make them especially sensitive to timber harvest practices, particularly those that remove CWD, a primary habitat for the group. I monitored the abundance of Eastern red-backed salamanders (*Plethodon cinereus*) in response to a gradient of 0-100% retained down coarse woody debris (DCWD) following timber and biomass harvesting, a first study assessing the impacts of wood-based biomass harvest on plethodontids. I also considered key aspects of salamander health such as standard metabolic rate and body condition. *N*-mixture models indicated a positive correlation between the percent of retained DCWD and *P. cinereus* abundance, but due to the standard errors associated with the abundance estimates, individual salamander sampling arrays were statistically indistinguishable from one another. Standard metabolic rate of encountered salamanders was not affected by the DCWD retention gradient in any sampling season. However, SMR variance was
significantly larger in harvested stands in Fall 2013. Although a threshold of resilience for plethodontids based on a gradient of retained DCWD was not discernable, this study emphasizes the need for future wildlife research on biomass harvesting to ensure appropriate implementation of regulations that provide better protection of the integrity and biodiversity in forest ecosystems.

2.1 Introduction

In an effort to reduce greenhouse gas emissions, the use of renewable, sustainable energy has become a high priority. When compared to nonrenewable fossil fuels, woody biomass produces less atmospheric CO₂ when burned as an energy source (McKinley et al. 2011). Some studies also suggest that energy from woody biomass harvest could potentially account for up to 10% of the United States’ total energy needs (Perlack et al. 2005, Zerbe 2006). However, the economic benefit of biomass harvest has not been realized to its full potential in the U.S. due to high transportation costs, technological development, and the relatively low prices of fossil fuels (McKinley et al. 2011). These setbacks raise questions as to whether or not biomass harvests on a large-scale would be sustainable and/or greenhouse gas neutral (Schulze et al. 2012). While the overall economic benefits for biomass harvest seem promising, the ecological impacts of biomass harvests have yet to be completely elucidated (Riffel et al. 2011, McKinley et al. 2011). Biomass harvesting removes otherwise nonmerchantable wood such as tree-tops, limbs, small-diameter trees, and pre-existing deadwood (Riffel et al. 2011), thereby reducing harvest-induced contributions to coarse woody debris (CWD). Reduced CWD input may interrupt ecosystem services such as nutrient cycling or providing habitat for forest wildlife.
Biomass harvest best management practices (BMPs) are often vague, and lack science-based guidelines for amphibians (Otto et al. 2013), although research has shown negative effects of CWD removal for pond-breeding ambystomatid salamanders (Semlitsch et al. 2008). The ecological impact of biomass harvest for bioenergy on terrestrial plethodontid salamanders is not fully understood (Otto et al. 2013). Plethodontids are the most abundant forest salamanders, often making up high percentages of the total vertebrate biomass in forest ecosystems of the eastern United States (Burton and Likens 1975b, Jaeger 1979). They play a key role in energy and nutrient cycling, providing an essential trophic link between the numerous, but often energetically inaccessible, invertebrate fauna and their vertebrate predators (Pough 1983). The vulnerability of plethodontid salamanders to management practices (DeMaynadier and Hunter 1998), along with their integral role in the mature forest ecosystem (Burton and Likens 1975a, Pough et al. 1987), makes them an ideal indicator species (Herbeck and Larsen 1999, Welsh and Droege 2001).

A completely terrestrial life-history requires plethodontids to be dependent on moist environments necessary for essential processes such as cutaneous respiration (Feder 1983). Several factors, including leaf litter depth, canopy cover, aspect, slope, and down coarse woody debris ([DCWD]; the most ecologically relevant measure of CWD for plethodontids) play critical roles in providing these specific microhabitats (Spotila 1972, Jaeger 1980b, Pough et al. 1987, Petranka et al. 1994, Grover 1998). Mature forests (>60 years old) allow for the development and stability of these essential microhabitat characteristics, as late seral stage forests have been correlated with the highest diversity and abundance of plethodontids (Pough et al. 1987, Ford et al. 2002).
Because microclimate is so easily disturbed by timber harvest, multiple studies indicate negative effects of forest harvest practices on salamanders (as reviewed in DeMaynadier and Hunter 1995). The presence of dense canopy closure may be the most important habitat characteristics for salamanders (DeMaynadier and Hunter 1998). In fact, Harpole and Haas (1999) and Knapp et al. (2003) could not identify a silvicultural alternative to clearcutting that involved canopy removal but resulted in salamander abundances significantly different from those in clearcuts. Canopy removal increases variability in microclimate (i.e., soil moisture, temperature, leaf litter depth; Zheng et al. 2000), which can impact salamander physiology and body condition (McKenny et al. 2006, Homyack et al. 2011). Along with clearcutting, understory canopy removal (i.e., mechanical and chemical treatments) has also proven to be detrimental to salamander populations (Pough et al. 1987, DeGraaf and Yamasaki 1992, Petranka et al. 1994, Harpole and Haas 1999). Humidity and moisture levels of the forest floor limit plethodontid foraging ability and restrict home range sizes (Jaeger 1978, Kleeberger and Werner 1982, Feder 1983), and the presence of a mature canopy allows for persistent stability of these necessary microhabitat characteristics (Harpole and Haas 1999, Zheng et al. 2000). As environmental moisture levels decrease, plethodontids can no longer remain moist in the forest floor leaf litter, increasing their use of DCWD as a refugium (Jaeger 1980b).

Down coarse woody debris provides several essential components for plethodontid life history, in addition to moisture. It concentrates small invertebrates, which are a major food source of plethodontid salamanders (Jaeger 1980a, Hanula 1995); provides moisture during extended dry periods (Grover 1998, McKenny et al.
and serves as a center for socioecology (Jaeger 1995). In fact, both density and
volume of decaying DCWD are positively correlated with abundance of the eastern red-
backed salamander (*Plethodon cinereus*; Petranka et al. 1994, McKenny et al. 2006).

While abundance of *P. cinereus* has been found to be related to DCWD, it has
been shown that the probability of detecting salamanders under large DCWD (logs >35
cm in diameter) is not significantly different between closed-canopy forests and harvest-
created gaps (Strojny and Hunter 2009). However, most DCWD left after biomass
harvest is smaller than 35 cm in diameter (Herbeck and Larsen 1999). More decayed
DCWD of appropriate size and decay class (3 and 4; Maser et al. 1979) for plethodontid
habitat is commonly crushed and destroyed in the harvesting process (Grialou et al. 2000,
Morneault et al. 2004). Therefore, biomass harvesting, which is more intensive and
leaves only very small DCWD sizes, may have even more pronounced negative impacts
on plethodontids than traditional sawlog or pulpwood harvesting.

Beginning in 2012, Purdue University undertook a multifaceted study examining
the economic, social, and ecological effects of biomass harvest in the Midwestern United
States. The goal of this overall project was to identify the effects and logistics of biomass
harvesting in the state of Indiana, and provide empirical data for the development of
biomass harvesting BMPs. I monitored plethodontid salamanders as a bioindicator
species, as suggested by Otto et al. (2013), to assess ecological impacts of biomass
harvesting for bioenergy. However, because *P. cinereus* made up over 95% of the total
salamander biodiversity at the site, only *P. cinereus* were used in statistical analyses.

Using a combination of mark-release-recapture (MRR) techniques and binomial-
Poisson (*N*-mixture) models, I estimated the abundance of *P. cinereus* in response to a
gradient of DCWD retention (Figure 1) following a biomass harvest in an effort to
delineate a threshold of resilience (as suggested by Semlitsch et al. 2009). I expected a
nonlinear relationship between the DCWD retention gradient and \textit{P. cinereus} abundance
with lower abundance estimates at the low end of the retention gradient, higher
abundance estimates at the higher end of the gradient, and a threshold in the middle. This
result could potentially provide information for BMPs capable of maximizing bioenergy
yields and preserving the ecological diversity of forest systems in the Midwest. In
addition to monitoring the effects of biomass harvest on abundance of \textit{P. cinereus}, I also
measured effects on thermal profile throughout the DCWD retention gradient as well as
on physiological health metrics of \textit{P. cinereus} (i.e., body condition and standard
metabolic rate [SMR]). I predicted that SMR and body condition would worsen by
increasing and decreasing, respectively, towards the low end of the DCWD retention
gradient.

2.2 Methods

2.2.1 Timber Harvest and Creation of DCWD Gradient

The study took place at the Southeast Purdue Agricultural Center (SEPAC),
Jennings County, Indiana, USA. The study site consisted of a 46 ha mature oak-hickory
(\textit{Quercus-Carya}) stand subdivided into 10 treatment plots (mean 3.2 ha, range 2.35-4.61
ha; Figure 2). In October 2012, the timber harvest was conducted such that treatment
plots mimic alternative biomass harvesting approaches. Experimental treatments
included: three treatment plots of biomass harvest without CWD retention (a silvicultural
clearcut with whole-tree removal of all trees greater than 10 cm), three treatment plots of
biomass harvest with CWD retention (a silviculturural clearcut with retention on site of
20-30% of nonmerchantable material [i.e., less than 30 cm diameter] in tree-tops and stems), and three control plots left as unharvested. In addition to these nine focal treatment plots, another treatment plot was harvested with a commercial clearcut, in which only merchantable material greater than 30 cm diameter (i.e., sawlogs) was removed from the site.

Prior to the harvest, I quantified CWD and biomass in all ten treatment plots using a combination of techniques. I used point relascope sampling (PRS; Gove et al. 2001) in a 30×30 m grid of 389 sample points equally distributed throughout the study site to measure volume of CWD with a small-end diameter >10.2 cm. Within artificial cover object arrays (ACO; see below), I quantified CWD more thoroughly by censusing all CWD with a small-end diameter >10.2 cm and length >0.3m within each array and in a 5 m buffer surrounding each array. For all sampled CWD pieces, I recorded species, end diameter, decay class (Maser et al. 1979), length, and percent in contact with the ground.

After harvest, CWD measurements were repeated in the biomass removal areas and across a subset of points in the clearcut. Unharvested controls were not resampled as CWD characteristics under an intact canopy would not change appreciably over the timeframe of this study. These PRS point samples and ACO censuses were then used to determine how the post-harvest residuals should be manipulated in order to create a gradient of CWD percent cover throughout the ACO array within each of the six biomass treatment plots in the study site. I assumed the CWD resembled frustums of cones and used the orthographic projection along the longitudinal axis, in combination with estimates of the proportion in contact with the ground, to estimate the total surface area covered by CWD in each array. Because it is the most ecologically relevant measure of
CWD for plethodontid salamanders, I used percent cover of CWD (hereafter referred to as down coarse woody debris [DCWD]) to create the gradient. Using the traditional clearcut as the 100% retention array, the six remaining arrays consisting of biomass harvest treatments were then manipulated on 28 February 2013 to create a gradient of DCWD (Table 1; Figure 1).

2.2.2 Salamander Sampling

Salamanders were sampled using 25 30×30×5 cm untreated poplar boards as artificial cover objects (ACOs), arranged in 5×5 m arrays. Although salamander abundance and occupancy can be difficult to estimate due to their semi-fossorial life-history characteristics (Bailey et al. 2004b), sampling with ACOs has been shown to provide good estimates of relative abundance and detect changes in population density (Marsh and Goicochea 2003). This technique standardizes the amount of cover between treatments, and reduces habitat disturbance, sampling time, and between-observer variability (Fellers and Drost, 1994). Salamanders sampled with ACOs do not differ in weight, snout vent length (SVL), or sex ratio from those captured under natural cover objects (Monti et al. 2000, Marsh and Goicochea, 2003, Moore 2009). Monti et al. (2000) also showed that *P. cinereus* are not attracted from nearby natural cover objects to ACOs.

In Fall 2011, ACO arrays were placed in the anticipated center of each treatment plot to minimize edge effects (DeMaynadier and Hunter 1998) and allow ACOs adequate time for weathering. Artificial cover object placement location was recorded using GPS. All 250 ACOs were removed prior to the start of timber harvest in September 2012, and returned to their original locations after harvest (October 2012). Artificial cover objects
remained in direct contact with the soil and were checked for the presence of salamanders once every two weeks (Marsh and Goicochea 2003) throughout the active season in the spring (March – June) and fall (September – November) during three sampling seasons: Spring 2013, Fall 2013, and Spring 2014. Salamander sampling seasons were truncated to include the maximum number of consecutive productive sampling days in which more than one *P. cinereus* was captured and conditions were conducive for recording environmental covariate data (i.e., the ground was soft enough to allow penetration of the soil moisture probe).

All salamanders were captured and handled in accordance with Indiana DNR Scientific Purposes License # 13-079 and 14-027, and Purdue Animal Care and Use Committee (PACUC) guidelines protocol # 1111000296. I recorded mass (± 0.01 g), snout vent length (SVL), and total length (mm) of each salamander encountered under the ACOs using a digital field scale (American Weigh Scales AT-100) and ruler. Snout vent length was used to classify salamanders into one of three stage classes: young of the year (<25 mm), juvenile (25-32 mm), and adult (>32mm; Moore 2009). Salamanders were uniquely marked using visible implant elastomers (VIEs; Northwest Marine Technology, Inc., Shaw Island, Washington, USA) with body locations and colors determined by a code generator developed by MacNeil et al. (2011). I recorded salamander capture temperature under each ACO with an infrared laser thermometer (± 2 °C; Raytek MT4) immediately following all encounters (Scheffers et al. 2009, Connette and Semlitsch 2013). If the temperature of the salamander could not be recorded (e.g. the salamander tried to escape), I recorded the temperature of the soil where the salamander was sitting prior to capture.
2.2.3 Temperature and Physiological Metrics

In addition to observed measurements of salamander temperature, I also used Thermochron iButton dataloggers (± 0.5°C; model DS1921G-F5, Maxim Integrated Products, Sunnyvale, CA) to record temperature data every three hours (eight measurements per day). I used a total of six dataloggers per ACO array, three were placed under randomly selected ACOs to measure refugium temperature at the interface of the ACO and the soil (Homyack et al. 2011). The remaining three dataloggers were paired with refugium dataloggers, but placed on stakes at approximately board height (5 cm) to measure ambient temperature. I waterproofed each datalogger with red plasti-dip (Plasti-Dip International, Blaine, MN). Datalogger sampling points remained consistent throughout the study to allow for between-season comparisons. I averaged iButton temperature data within each ACO array at every time point for both ambient and refugia datalogger locations. I then calculated daily maximum, minimum, and mean temperatures for each array at both ambient and refugia locations.

I calculated SMR of *P. cinereus* using a predictive multiple regression equation developed by Homyack et al. (2010):

\[ \log_e(\text{SMR}) = 0.102(\text{temperature}) + 0.681 (\log_e(\text{mass})) - 4.849 \].

Salamanders with malformations (e.g. missing limbs, autotomized tails, etc.) were excluded from SMR analyses. As differences in salamander mass (e.g. stage class proportions) between treatments can confound estimates for SMR extrapolation methods based solely on temperature regime (PJR, unpublished data), I only calculated SMR using observed salamander temperature and mass. I also calculated body condition indices by regressing log-transformed mass on log-transformed total length and using the sign of the
residuals (+ or −) to determine good or poor body condition (Homyack et al. 2011).
Gravid females and individuals with malformations (as defined above) were also excluded from these analyses.

2.2.4 Environmental Covariates

I measured leaf litter depth down to the consolidated soil surface (Oe, Oa, and Oi soil horizon layers; U.S. Department of Agriculture 1981), humidity with a Kestrel 3000 Pocket Wind Meter (Nielsen Kellerman, Chester Pennsylvania), and soil moisture with two FieldScout TDR 300 soil moisture meters (Spectrum Technologies, Inc., Aurora, IL) that were calibrated to allow between-season comparison. Environmental covariates were recorded during each day that ACOs were checked, and each covariate was taken at five randomly selected points within each ACO array. These environmental sampling points remained consistent throughout the study to allow for long-term comparisons.

I acquired precipitation data from the Purdue University iClimate weather station located at SEPAC headquarters approximately 4.3 km SW of the study site. I used a proven salamander-relevant precipitation metric (days since last soaking rain; Peterman and Selmlitsch 2013), assuming that any large rain event (≥5 mm) would be relevant for every treatment plot within the relatively small study site. In addition to days since last soaking rain, I also recorded the total precipitation two days prior to sampling (MacNeil and Williams 2013). I measured aspect and slope of each ACO array with a compass and clinometer because north-facing slopes have been found to provide more hospitable microhabitats for terrestrial salamanders (Moseley et al. 2009).
2.2.5 Data Analyses

2.2.5.1 Abundance Estimation

I used a binomial-Poisson $N$-mixture model (Royle 2004, Kéry 2010) to estimate abundance from spatially and temporally replicated count data while accounting for imperfect detection of plethodontid species (Bailey et al. 2004a & b, Connette and Semlitsch 2013, Peterman and Semlitsch 2013). This model enabled me to estimate the true abundance while incorporating specific covariates to see how different array characteristics affected detection on an array by day by season basis. The $N$-mixture model was fit in a Markov chain Monte Carlo (MCMC) approach in a Bayesian framework using program JAGS (Plummer 2003), called from R (R Development Core Team 2013) using the package jags UI (Kellner 2014). I used five MCMC chains run until they reached satisfactory convergence (Rhat values <1.1; Brooks and Gelman 1998). From the resulting posterior distributions for each model parameter, I obtained a 95% credible interval. I considered parameters with 95% credible intervals that did not overlap zero to be statistically significant predictors of abundance and/or detection (Peterman and Semlitsch 2013, Kellner et al. 2013).

Due to the specific microhabitat requirements of $P. \text{cinereus}$ (Spotila 1972, Jaeger 1980b, Grover 1998, Homyack et al. 2010), I included several environmental covariates in the analysis. DCWD, slope, and aspect served as covariates on abundance, whereas leaf litter depth, soil moisture, days since last soaking rain, total precipitation two days prior to sampling, maximum board temperature, humidity, days since first sampling day, and the quadratic form of days since first sampling day served as covariates on detection. The quadratic form of days since first sampling day was added in an attempt to describe
the hyperbolic variability in salamander capture rates throughout each season (high capture rates in the middle and low capture rates at the beginning and end). After each run, the model was modified as needed to remove uninformative parameters (Peterman and Semlitsch 2013).

Using MRR data, individual salamander encounter histories served as a check of the abundance estimates from the N-mixture model. I computed abundance estimates on an array by season basis in R using package RCapture (Baillargeon and Rivest 2007). However, due to low numbers of recaptures in individual array by season tests, I used program MARK (White and Burnham 1999) called from R using package RMark (Laake and Rexstad 2007) to run Pollock’s Robust Design to not only account for the closed nature of salamander populations during the sampling period, but also allow for an open population between sampling periods (Mazerolle et al. 2007, Bailey et al. 2004a). Using data from multiple seasons allowed us to get more accurate estimates of array-specific abundance and detection probabilities. However, due to small sample sizes and low recapture rates in some sampling arrays, I could only use a subsample (four stands from the biomass DCWD retention gradient: 0%, 32%, 52%, 67%, and two control stands) of the array-specific MRR abundance estimates as a check for the N-mixture model, notably those with reasonable standard errors (< ± 2 SE). In order to use all of the arrays in regression comparisons, I used the encounter histories to calculate the minimum number known alive (MNKA) for each array and season (Kellner et al. 2013). I ran regressions of the N-mixture model abundance estimates on both MNKA and Pollock’s robust design estimates as a check of model accuracy.
2.2.5.2 Temperature and Physiological Health

I used R (R Development Core Team 2013) to produce linear models of temperature, SMR, and body condition on DCWD retention. I used normal linear regression with four independent variables to test whether iButton temperature (maximum and range) differed with respect to Julian date, DCWD, aspect, slope and an aspect by slope interaction. This analysis provided a useful perspective of both ambient and refugia temperature regimes. In analyses examining the differences between harvested and control stands, I also included aspect and slope to help account for the microhabitat sensitivity of terrestrial salamanders (Moseley et al. 2009).

We used R (R Development Core Team 2013) and package car (Fox and Weisberg 2011) to perform temperature and SMR analyses. I tested the mean difference of maximum salamander capture temperature between harvested and control stands using Welch’s T-test. Temperature extremes differed among fall and spring sampling periods; therefore, I used the variance of capture temperature and SMR as a more suitable metric for between season comparisons. I also used Levene’s test of homogeneity of variance to test difference in variance of capture temperature, SMR, mass, and SVL between harvested and control stands. Because not all data met assumptions of normality, randomization F-tests and t-tests (based on 10,000 iterations) were used on each specific data set to create null distributions for significance testing.

Because my study design involved resampling ACOs several times over the course of a single season, I encountered some individuals more than once. To avoid violating the assumption of independence, I randomly removed all but one individual recaptures per season and created an adjusted dataset consisting solely of unique
encounters. I performed a side-by-side comparison of analyses using both the complete dataset, and the adjusted dataset. Results from adjusted dataset analyses varied depending on which individual salamanders were randomly retained. However, I performed the analyses several times and with the exception of one F-test, all results and trends remained consistent regardless of whether I used the complete dataset or the adjusted dataset. For the sake of clarity, and consistency, I will report results from the complete dataset, and make explicit the one discrepancy.

2.3 Results

2.3.1 Abundance

During this study I caught a total of 618 *P. cinereus*. After truncation to include the maximum number of consecutive sampling days in each season, the final dataset used for abundance analyses included a total of 456 individual *P. cinereus* encounter histories consisting of 613 captures over three sampling seasons. The final *N*-mixture model included DCWD and aspect as significant covariates on abundance (i.e., 95% credible intervals not containing zero; Table 2). Precipitation, humidity, maximum refugia temperature, days since first sampling day, and days since first sampling day squared were included as covariates on detection; all but days since first sampling day were significant (i.e., Season; Table 2). Regressions using abundance estimates from both MNKA and Pollock’s Robust Design on estimates from the *N*-mixture model served as a check of model accuracy. Due to insufficient recapture rates in some sites, I was unable to get reasonable abundance estimates for every array using Pollock’s Robust Design. However, the six arrays with reliable abundance estimates from Pollock’s Robust Design (< ± 2 SE) were positively correlated with abundance estimates from the *N*-mixture
model (Figure 3). *N*-mixture model abundance estimates were also highly correlated with MNKA, with \( R^2 \) values of 0.65, 0.82, and 0.90 in the Spring 2013, Fall 2013, and Spring 2014 respectively (Figure 4).

Although the *N*-mixture model indicated that the DCWD retention parameter was positively correlated with abundance of *P. cinereus*, there was not a visible cutoff or threshold value of DCWD retention for optimal *P. cinereus* resilience (Fig 5). Using the 95% credible interval standard errors, abundance estimates were not significantly different in any ACO array in Spring 2014, and the only substantial differences in other seasons were between the 0% and 67% DCWD retention arrays in Spring 2013, the 0% and 67% arrays in Fall 2013, and the 17% and 67% arrays in Fall 2013 (Figure 5). In addition, the abundance estimates were not significantly different between harvested and control stands in any season (Figure 5).

### 2.3.2 Temperature and Physiological Health

The DCWD retention gradient was not a significant predictor of maximum ambient temperature in any season, and it was only significant as a predictor of maximum refugia temperature in Spring 2014 \( (t_1 = -2.36, P = 0.019) \). However, overstory presence (harvested vs. control) did have a significant effect on maximum ambient and refugia temperature (Table 3). There was a significant negative relationship between DCWD retention and temperature range in both ambient and refugia temperature regimes in Fall 2013 and Spring 2014, but not in Spring 2013 (Table 4).

I caught a total of 566 *P. cinereus* that met the requirements for the SMR analysis (318 from harvested stands and 248 from control stands). Using only *P. cinereus* found in the biomass harvest treatments, I ran a linear model with CWD and season as
predictors of SMR. Neither DCWD ($t_1 = -0.169, P = 0.866$) nor season ($t_1 = -0.014, P = 0.989$) contributed significantly to the model. Therefore, SMR did not vary significantly across the biomass harvest gradient. When I included *P. cinereus* from the control stands in an ANOVA with SMR predicted by overstory presence, DCWD, season, and aspect, the effect of season became significant ($F_{5,560} = 12.13, P \leq 0.001$), but overstory presence ($F_{5,560} = 0.99, P = 0.321$) and DCWD ($F_{5,560} = 0.45, P = 0.504$) remained nonsignificant. I then ran a similar ANOVA with overstory presence, DCWD, aspect, and season as predictors of salamander capture temperature; again, season was a significant predictor of capture temperature ($F_{5,560} = 34.25, P \leq 0.001$). In further analyses using Welch’s one-way t-test with randomization as a check, mean *P. cinereus* SMR was not different between harvested and control stands in Spring 2013 ($t_1 = 1.08, P = 0.141$), Fall 2013 ($t_1 = -0.38, P = 0.356$), or Spring 2014 ($t_1 = -0.28, P = 0.390$). Differences in SMR variance, tested using Levene’s test of homogeneity of variance, were also not significant between harvested and control stands in Spring 2013 ($F_{1,156} = 0.30, P = 0.584$) or Spring 2014 ($F_{1,182} = 2.51, P = 0.115$), but I did observe a difference in Fall 2013 ($F_{1,222} = 4.63, P = 0.032$). This difference was significant using the complete dataset, but although this trend was evident using the adjusted dataset, it was not always significant (average $P$-value = 0.069 over 1000 iterations). The variance of salamander capture temperature was significantly different between harvested and control stands in Fall 2013 ($F_{1,222} = 7.50, P = 0.007$), as well as Spring 2013 ($F_{1,156} = 15.56, P \leq 0.001$), but the difference in SMR range in Fall 2013 was most likely caused by a discrepancy in salamander stage class in which harvested stands had a higher proportion of smaller individuals than control stands. Since both temperature and mass are used to calculate SMR in *P. cinereus*, the
differences in range of SMR between harvested and control stands were most likely noticed in the fall, because both the variance of salamander mass ($F_{1,222} = 5.66, P = 0.018$) and SVL ($F_{1,223}= 7.01, P = 0.009$) were significantly larger in harvested vs. control stands in Fall 2013. In fact, mean SVL ($t_1 = -2.21, P =0.014$) was significantly lower in harvested stands in Fall 2013. Body condition of *P. cinereus* was seemingly unaffected by timber harvest. There was no effect of either the DCWD retention gradient ($t_1 = -0.361, P = 0.718$) or overstory presence ($F_{1,496} = 0.117, P = 0.732$) on body condition in any season. The only significant difference was in the variance of body condition between harvested and control stands in the Spring 2013 ($F_{1,130} = 4.31, P = 0.039$).

### 2.4 Discussion

#### 2.4.1 CWD Importance

Studies have demonstrated the importance of DCWD retention for salamander species in mitigating negative effects of timber harvests (Patrick et al. 2006). Because DCWD is an important habitat component of terrestrial plethodontid salamanders (Petranka et al. 1994), and a positive correlation between density and volume of decaying CWD and abundance of *P. cinereus* has been described (Petranka et al. 1994, McKenny et al. 2006), it is essential to identify a threshold of DCWD retention that can support adequate populations of salamanders following a timber harvest (Semlitsch et al. 2009). Plethodontids rely on DCWD as source of forage (Jaeger 1980a, Hanula 1995), moisture (Grover 1998, McKenny et al. 2006), and other essential components of their life history (Jaeger 1995). Although the association between plethodontids and DCWD has been
thoroughly studied, empirical data assessing the effects of DCWD retention within the context of an intensive biomass harvest have been lacking (Otto et al. 2013).

As would be expected, I identified a positive correlation between DCWD retention and *P. cinereus* abundance (Table 2), which supports the previously established relationship (Petranka et al. 1994, McKenny et al. 2006). However, unlike other studies, this correlation was based on a gradient of retained DCWD following an intensive harvest in which large amounts of biomass were removed from the site. Although I was able to demonstrate the importance of DCWD for *P. cinereus*, abundance estimates between individual ACO arrays were statistically indistinguishable from one another (Figure 5). The standard errors associated with the abundance estimates, as well as the lack of a clearly defined pattern or threshold based on the DCWD retention gradient, (Figure 5) prevents me from making any specific recommendations for DCWD retention in BMPs.

### 2.4.2 Difficulties in Abundance Estimation

I used several techniques to estimate the abundance of *P. cinereus* following a biomass harvest. Of these, the *N*-mixture model method was the simplest, and most cost-effective method. Mark-release-recapture methods such as Pollock’s Robust Design are more widely accepted as a reliable technique to estimate population parameters (Bailey et al. 2004a, Mazerolle et al. 2007). However, sampling populations of organisms with low detection probabilities using MRR techniques often proves to be costly, difficult, and time consuming (MacKenzie et al. 2002). Furthermore, these MRR methods do not always give reasonable results. In the present study, some arrays had zero or one recaptured individual over the course of a season. Abundance estimates of these arrays
were therefore unreasonable, and I was unable to use these arrays for robust design estimates. However, because the $N$-mixture model uses count data and array-specific environmental covariates to estimate detection and abundance on a site by season basis, I was able to get meaningful estimates from every array. If possible it would be best to use a combination of these techniques to get a more complete understanding of the system, but since meaningful population estimates could not be gleaned from the entire dataset using a robust design approach, the derived MNKA parameter along with the $N$-mixture model proved to be the best option.

2.4.3 $N$-Mixture Model Covariates

It was not surprising to find a negative correlation between maximum refugia temperature and detection, or a positive correlation between precipitation and detection, reflecting salamander vertical migration lower in the soil column during drier periods and higher following rainfall (Heatwole 1962). It was also not surprising to find a negative correlation between humidity and detection because salamanders should spend more time in the leaf litter if humidity levels are adequately high (Jaeger 1980$^b$) and thus I would not encounter them under ACOs.

The model indicated that a northeast aspect was negatively correlated with abundance. This contradicts the commonly accepted “rule” that salamanders prefer cooler, wetter NE facing slopes (Moseley et al. 2009). This possible contradiction could be due to several factors. First, I incorporated aspect as a binomial variable with a one for NE and zero for SW resulting in six NE and four SW arrays. This can be problematic if arrays have a more SE or NW aspect. Additionally, small sample size ($n = 10$) could potentially allow for bias caused by a random site effect. Terrain is also not solely
accounted for by aspect. Although one of the highest producing salamander arrays occurred on a southwest slope, it was down in a ravine which has been shown to provide a hospitable microclimate for plethodontids (Peterman and Selmlitsch 2013). Finally, the previous studies that have shown the importance of slope and aspect as predictors of salamander abundance have also correlated slope and aspect with differences in temperature and moisture (Moseley et al. 2009, MacNeil and Williams 2013). The lack of a significant difference in maximum temperature or soil moisture between ACO sampling arrays within the DCWD retention gradient suggests that the magnitude of the slope within my study site was not large enough to establish significant differences in salamander habitat on slopes with different aspects. Additionally, slope was not a significant predictor in the model. Therefore, the negative correlation of aspect with abundance in the N-mixture model is most likely a product of small sample size and individual random site effects and is not a reflection of site differences caused by aspect.

Maximum refugia iButton temperature was significantly different between harvested and control stands, and was not related to the amount of DCWD retained on site. However, there was a significant relationship between DCWD and refugia temperature range (maximum - minimum) in both the Fall 2013 and Spring 2014 seasons. A gradient of DCWD on site could result in different levels of albedo (Anderson et al. 2011), but temperature analysis using iButton data is limited to the time interval frequency and ± 0.5°C thermal sensitivity of each datalogger. Thus, iButton data only provides a baseline understanding of temperature regime at the site. Because plethodontid salamanders respond to changes in climate with vertical migrations (Heatwole 1962) and microhabitat selection (Jaeger 1980b), observed temperature
measurements of encountered individuals, rather than average refugium temperature profiles, reflect a more accurate picture of the effect of different harvest treatments on salamander temperature regime.

Because plethodontid abundance is tied to climate variables such as moisture, humidity, and temperature, their distribution often reflects optimal combinations of these characteristics (Peterman and Semlitsch 2013). In a study by Morneault et al. (2004), understory vegetation was not related to salamander abundance prior to harvest. However, after a 50% reduction in overstory canopy cover, a positive correlation between salamander abundance and understory vegetation was observed. In my study, post harvest treatment plots consisted of a patchwork of understory vegetation that was present in different densities. According to Morneault et al.’s (2004) study, this early successional regrowth is able to provide a hospitable microclimate for plethodontids. This further emphasizes the importance of using observed measurements of captured salamanders for temperature and SMR analyses in order to account for potential selection of ACOs with ideal microclimates.

Similar to the findings of Homyack et al. (2011), SMR in this study was affected by canopy removal. The data indicated larger variances in SMR in harvested stands than control stands in the Fall 2013 field season. In my study, body condition did not differ between salamanders captured in harvested vs. control stands. In contrast, Homyack et al. (2011) documented a difference in *P. cinereus* body condition between harvest treatments. This discrepancy could be due to a number of differences including, but not limited to: smaller scale, smaller sample size, or different geographic location of my study. More research is needed to further elucidate the effects of timber harvest on *P.*
*cinereus* body condition. In my study, body condition and SMR were unaffected by the DCWD retention gradient within the biomass harvest treatment plots. Although DCWD is vital for many plethodontid life-history requirements (Jaeger 1980*bc*, Hanula 1995, Grover 1998, McKenny et al. 2006), my data suggest that DCWD retention is not necessary for *P. cinereus* to maintain adequate body condition and SMR. However, the DCWD gradient in this study was composed entirely of harvest residues, (decay class 1 material) which are not as ecologically relevant to salamanders (Petranka et al. 1994). Therefore, perhaps differences in body condition and SMR may become more pronounced several years following the harvest, when the gradient of retained DCWD becomes more ecologically important.

In this study, down coarse woody debris retention was an important predictor of *P. cinereus* abundance, but due to the standard errors associated with the abundance estimates, individual ACO arrays were statistically indistinguishable from one another. Because of the lack of ecological relevance for salamanders associated with DCWD of decay class 1, trends and differences may become more pronounced several years following the harvest. The DCWD retention gradient did not have an overwhelming effect on temperature and associated physiological health of salamanders. However, I did find overstory presence (i.e. harvested vs. control stands) to be a critical factor in determining differences in temperature, SMR, and salamander stage class. This study emphasizes the importance of DCWD for *P. cinereus* abundance, but additional research is needed to identify a specific threshold level of plethodontid abundance in response to a gradient of retained DCWD.
Figure 1. Down Coarse woody debris (DCWD) retention gradient created in artificial cover object (ACO) arrays. Zero percent DCWD retention represents complete DCWD removal as done in biomass harvest; 100% retention contains same amount of DCWD as commercial clearcut with no biomass removal. Intermediate levels were created by removing DCWD from ACO arrays to reach desired % retention levels.
Figure 2. Southeast Purdue Agricultural Center (SEPAC; Jennings County, IN) Biomass Harvest Down Coarse Woody Debris (DCWD) Retention Gradient:

DCWD retention plots (N = 7): black (0%), white (100%), gray (intermediate %)

Unharvested (control) plots (N = 3): crosshatch pattern
Table 1. Characteristics of coarse woody debris (CWD) and down coarse woody debris (DCWD) within each artificial cover object salamander sampling array (+ 5m buffer) before and after experimental manipulations.

<table>
<thead>
<tr>
<th>Plot Number</th>
<th>Harvest Treatment Plots</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total surface area of CWD within array + buffer (m²)</td>
<td>13.4, 26.6, 42.8, 16.5, 48.4, 23.9, 74.7</td>
</tr>
<tr>
<td>2. Total surface area of DCWD within array + buffer (m²)</td>
<td>8.6, 9.5, 9.6, 10.1, 13.1, 16.0, 19.4</td>
</tr>
<tr>
<td>3. Average large end diameter of DCWD (cm)</td>
<td>20.0, 22.1, 17.7, 23.4, 18.3, 26.0, 17.7</td>
</tr>
<tr>
<td>4. Average % DCWD of total CWD</td>
<td>14%, 24%, 27%, 8%, 24%, 16%, 21%</td>
</tr>
<tr>
<td>5. Total surface area of DCWD relative to clearcut array (plot 7; % of 19.4 m²)</td>
<td>44%, 49%, 49%, 52%, 67%, 82%, 100%</td>
</tr>
<tr>
<td>6. Final gradient of DCWD</td>
<td>0%, 17%, 32%, 52%, 67%, 82%, 100%</td>
</tr>
</tbody>
</table>
Table 2. N-mixture model parameter estimates: Credible Intervals that do not overlap zero are considered to be statistically important predictors of abundance or detection. Negative values indicate negative correlation of parameter with either detection or abundance.

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>Mean</th>
<th>2.5 %</th>
<th>97.5 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Season a</td>
<td>0.032</td>
<td>-0.088</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>Season²</td>
<td>-0.770</td>
<td>-0.965</td>
<td>0.589</td>
</tr>
<tr>
<td></td>
<td>Max Refugia Temp</td>
<td>-1.067</td>
<td>-1.295</td>
<td>0.858</td>
</tr>
<tr>
<td></td>
<td>Humidity</td>
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<td>-0.418</td>
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</tr>
<tr>
<td></td>
<td>Precipitation</td>
<td>0.200</td>
<td>0.062</td>
<td>0.342</td>
</tr>
<tr>
<td>Abundance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DCWD</td>
<td>0.344</td>
<td>0.203</td>
<td>0.485</td>
</tr>
<tr>
<td></td>
<td>Aspect</td>
<td>-0.297</td>
<td>-0.518</td>
<td>-0.076</td>
</tr>
</tbody>
</table>

a The number of days since the first sampling day of each season
Figure 3. Linear regression of seasonal salamander abundance estimates from \(N\)-mixture model and reasonable estimates (\(< \pm 2 \text{ SE}\)) from Pollock’s Robust Design. A – Spring 2013; B – Fall 2013; C – Spring 2014.
Figure 4. Linear regression of seasonal abundance estimates from $N$-mixture model and minimum number known alive (MNKA). A – Spring 2013; B – Fall 2013; C – Spring 2014.
Figure 5. \( N \)-mixture model salamander abundance estimates (± SE) for each artificial cover object array (DCWD retention gradient and unharvested stands) in Spring 2013 (white), Fall 2013 (black), and Spring 2014 (hatched).
Table 3. Results of ANOVAs accounting for the effect of overstory presence (OP; harvested vs. unharvested) on maximum ambient and refugia temperature by season:

(Temperature Range = OP + Julian Date + Aspect + Slope + Aspect:Slope)

*P < 0.05, ** P < 0.01, *** P < 0.001

<table>
<thead>
<tr>
<th>Location/Season</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient Spring 2013</td>
<td>39.85</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>Refugia Spring 2013</td>
<td>74.91</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>Ambient Fall 2013</td>
<td>7.43</td>
<td>0.007**</td>
</tr>
<tr>
<td>Refugia Fall 2013</td>
<td>0.49</td>
<td>0.482</td>
</tr>
<tr>
<td>Ambient Spring 2014</td>
<td>20.49</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>Refugia Spring 2014</td>
<td>31.04</td>
<td>&lt; 0.001***</td>
</tr>
</tbody>
</table>
Table 4. Results of individual linear models accounting for the effect of the down coarse woody debris (DCWD) retention gradient on ambient and refugia temperature range by season:

\[(\text{Temperature Range} = \text{DCWD} + \text{Julian Date} + \text{Aspect} + \text{Slope} + \text{Aspect:Slope})\]

*P < 0.05, **P < 0.01

<table>
<thead>
<tr>
<th>Location/Season</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>P-value</th>
</tr>
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<td>Ambient Spring 2013</td>
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<td>0.006</td>
<td>-0.505</td>
<td>0.614</td>
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<td>0.011</td>
<td>-3.022</td>
<td>0.003 **</td>
</tr>
<tr>
<td>Refugia Fall 2013</td>
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<td>0.004</td>
<td>-2.815</td>
<td>0.005 **</td>
</tr>
<tr>
<td>Ambient Spring 2014</td>
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<td>0.016</td>
<td>-2.071</td>
<td>0.039 *</td>
</tr>
<tr>
<td>Refugia Spring 2014</td>
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<td>0.007</td>
<td>-2.046</td>
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</tbody>
</table>
Plethodontid salamanders often make up high percentages of vertebrate biomass in forest ecosystems. Due to their abundance, diversity, and sensitivity to disturbance, they are often monitored as indicator species for mature forest ecosystem health. In addition to relative abundance, researchers often monitor physiological changes in salamander body condition, such as standard metabolic rate (SMR), in response to timber harvest. Nearly ubiquitous in forested stands throughout the northeastern United States, the eastern red-backed salamander (*Plethodon cinereus*) is often the focal species of such studies. In 2010, a predictive multiple regression equation was developed that calculates SMR of *P. cinereus* to 95% accuracy using salamander mass and temperature. This method of SMR estimation has been implemented in field studies as a measure of salamander health. In these studies, temperature regime is often the only variable measured, and SMR is calculated by extrapolation based on a one-gram salamander. Because this equation does not factor in soil moisture or other microhabitat characteristics that are present within a field setting, extrapolations on the effects of silvicultural practices on plethodontid populations may not be justified. In this study, I measured the mass and temperature of every salamander encountered in harvested and
unharvested stands, and compared the current SMR extrapolation techniques with SMR calculated using salamander capture temperature and mass data. I found larger temperature variability in harvested stands using both methods, but did not identify a similar trend in seasonal SMR analyses. The data indicated that mass may be the driving factor in SMR calculation. Noticeable differences in mass and SVL between harvested and unharvested stands in Fall 2013 suggest that researchers should use caution when making claims based on differences in calculated mean SMR when data on salamander mass has not been taken.

3.1 Introduction

Researchers often monitor the abundance and diversity of salamanders as a surrogate for monitoring overall habitat quality within a complex forest ecosystem (Welsh and Droege 2001). Lungless salamanders, from the family Plethodontidae (hereafter referred to as “plethodontids”), are the most abundant forest salamanders, often making up high percentages of the total vertebrate biomass in forest ecosystems (Burton and Likens 1975b, Jaeger 1979). Plethodontids play a key role in energy and nutrient cycling by providing an essential trophic link between the numerous, but often energetically inaccessible, invertebrate fauna and the plethodontids’ vertebrate predators (Pough 1983). Along with their integral role in forest ecosystems, the sensitivity of plethodontid salamanders to management practices has led some researchers to nominate them as ideal indicator species for mature forest ecosystem health (Herbeck and Larsen 1999, Welsh and Droege 2001).

Dependence on temperature and microclimate stability can make plethodontids vulnerable to timber harvest. In fact, multiple studies have shown negative effects of
forest harvest practices on salamanders (as reviewed in DeMaynadier and Hunter 1995). Mature forests (>60 years) allow for the development and stability of essential microhabitat characteristics and have been shown to support the highest diversity and abundance of terrestrial salamanders (Pough et al. 1987, Ford et al. 2002). Although forest harvesting impacts several components of salamander habitat (Grialou et al. 2000, Morneault et al. 2004), the presence, or absence, of dense canopy closure seems to be one of the most important habitat characteristics for salamanders. Indeed, Harpole and Haas (1999) and Knapp et al. (2003), while investigating alternatives to clearcutting, were not able to identify significant differences in salamander abundances among any of the management practices that involved canopy removal, including clearcutting itself. This is most likely due to the excess variability in microclimate caused by canopy removal (Zheng et al. 2000), which then has an impact on physiology and body condition of plethodontids (McKenny et al. 2006, Homyack et al. 2011). Some studies suggest that thinning, selection systems, and other silvicultural practices that maintain high residual canopy cover have limited or no negative effects on salamanders (Pough et al. 1987, Messere and Ducey 1998, Brooks 1999, Grialou et al. 2000, McKenny et al. 2006).

Many plethodontids have a completely terrestrial life history. They are dependent on specific microhabitat characteristics (i.e. leaf litter depth, canopy cover, aspect, slope, and coarse woody debris [CWD]) for temperature control, osmo-regulation, and cutaneous respiration (Spotila 1972, Jaeger 1980a, Pough et al. 1987, Petranka et al. 1994, Grover 1998). Plethodontid foraging is limited to periods of higher humidity and higher forest floor moisture levels (Jaeger 1978, Feder 1983). Leaf litter depth, as one characteristic associated with forest floor microclimate, has been considered to be the
best indicator of aboveground salamander activity (Pough et al. 1987, DeGraff and Yamasaki 1992). Additionally, if leaf litter remains moist, salamanders (mostly nocturnal feeders) can continue to forage into the day (Jaeger 1980b).

Because they are ectothermic poikilotherms, plethodontid body temperature varies with the temperature of their surrounding environment (Feder 1983), resulting in different physiological consequences associated with different temperature regimes (Careau et al. 2014). Lunglessness also adds to standard metabolic rate (SMR) sensitivity with respect to changes in temperature and moisture (Feder 1983). Because plethodontids rely on cutaneous respiration for volumetric oxygen consumption, moisture and temperature greatly affect the efficiency of this process, thus influencing SMR (Careau et al. 2014). Spotila (1972) found that eastern red-backed salamanders (Plethodon cinereus) preferentially select habitat at a specific temperature (~16.2 °C) as opposed to simply avoiding temperature extremes. Homyack et al. (2010) developed a predictive multiple regression equation estimating SMR of the eastern red-backed salamander (Plethodon cinereus) based on salamander temperature and body mass:

\[
\text{Log}_e(SMR) = 0.102(\text{temperature}) + 0.681(\text{Log}_e(\text{mass})) - 4.849.
\]

This equation is useful for quantifying potential ecological consequences of surface-active salamanders at different temperature regimes (Homyack et al. 2011). Since P. cinereus is sedentary for a large majority of the time, SMR makes up a large portion of its daily energy budget (Spotila 1972). As a result, the ability to calculate SMR in a field study could provide a useful gauge of physiological health. Although Homyack et al. (2011) used this equation to estimate the potential impacts of varying surface temperatures caused by different timber harvest regimes on SMR, they developed
and verified the SMR method in a lab setting, without field validation. No one has yet verified the method in situ where soil moisture or other microhabitat characteristics could effect SMR.

I compared the Homyack et al. (2010) method, which uses remotely sampled temperature regimes and extrapolates SMR based on a one-gram salamander, with an alternate method that uses observed capture temperature and mass measurements from salamanders encountered in a field setting. Confirmation of the current technique would allow for researchers to continue to use thermal profile data of different harvest regimes to extrapolate SMR of *P. cinereus* as a proxy of forest health.

3.2 Methods

This study took place at the Southeast Purdue Agricultural Center (SEPAC), Jennings County, Indiana, USA. The study site consisted of a 46 ha mature oak-hickory (*Quercus-Carya*) stand subdivided into 10 separate treatment plots (mean 3.2 ha, range 2.35-4.61 ha). Timber on seven of the treatment plots was harvested in Fall 2012 as part of a multidisciplinary project through Purdue University in which harvest residues were removed to mimic a biomass harvest for cellulosic bioenergy. The three remaining treatment plots were left as unharvested stands.

Salamander Sampling and Data Collection

Salamanders were sampled using 25 30×30×5 cm untreated poplar boards as artificial cover objects (ACOs), arranged in 5×5 m arrays. In Fall 2011, ACO arrays were placed in the field to provide sufficient time for weathering. Artificial cover object arrays were placed in center of each treatment plot in order to minimize future edge effects after the harvest (DeMaynadier and Hunter 1998). Location of ACO arrays was
recorded using GPS. All 250 ACOs were removed prior to the timber harvest in September 2012, and replaced in their original locations following the harvest. Artificial cover objects remained in direct contact with the soil and were checked once every two weeks (Marsh and Goicochea 2003) throughout the active season in the spring (March - June) and fall (September - November) during three sampling seasons: Spring 2013, Fall 2013, and Spring 2014. Because the purpose of this study was to compare SMR extrapolation methods based on temperature regime with SMR calculations from surface active salamanders in a field setting, I truncated each collection season to include only sampling days with 10+ salamander captures. This ensured that I only made comparisons during a period when I could assume that salamanders were surface active.

All salamanders were captured and handled in accordance with Purdue Animal Care and Use Committee (PACUC) guidelines protocol # 1111000296. I recorded mass (± 0.01 g), snout vent length (SVL), and total length (mm) of each salamander encountered under the ACOs using a digital field scale (American Weigh Scales AT-100) and ruler. Snout vent length was used to classify salamanders into one of three stage classes: young of the year (<25 mm), juvenile (25-32 mm), and adult (>32 mm) (Moore 2009). Temperature of the salamander/soil was also recorded manually under each ACO with an infrared laser thermometer (± 2 °C; Raytek MT4) immediately following all captures (Scheffers et al. 2009, Connette and Semlitch 2013).

I used three randomly placed Thermochron iButton dataloggers (± 0.5 °C; model DS1921G-F5, Maxim Integrated Products, Sunnyvale, CA) per ACO array to record temperature data every three hours (eight measurements per day). These dataloggers were placed centrally underneath ACOs to measure refugia temperature at the interface of
ACO and the soil (Homyack et al. 2011). I waterproofed dataloggers with red plasti-dip (Plasti-Dip International, Blaine, MN) prior to use in the field. Datalogger sampling points remained consistent throughout the study to allow between-season comparisons.

I averaged iButton refugia temperature data within each ACO array at every time point, and calculated daily maximum and minimum temperatures for each array. I used these refugia temperature dataloggers at the interface of the soil and ACOs to extrapolate seasonal SMR trends based on a one-gram surface-active salamander using the predictive multiple regression equation developed by Homyack et al. (2010). In addition to using iButton refugia thermal profile, I also calculated SMR using observed salamander temperature and mass for comparison. Salamanders with malformations (e.g. missing limbs, autotomized tails, etc.) were excluded from these analyses.

3.2.1 Data Analysis

I used R (R Development Core Team 2013) and package car (Fox and Weisberg 2011) to perform temperature and SMR analyses. I compared (using Welch’s T-test) daily maximum refugia temperature between harvested and unharvested stands as an indicator of the most extreme temperature that could be encountered by a surface-active salamander throughout each season. Since temperature extremes are different between the fall and spring field seasons, I also tested differences in the mean and variance of daily temperature range (maximum − minimum) of refugia temperature as a metric for between-season comparisons. I used Levene’s test of homogeneity of variance to test for differences in the variance of refugia temperature. I also used Welch’s T-test and Levene’s test of homogeneity of variance to test for differences in the means and variance of capture temperature, SMR, salamander mass, and SVL between harvested and
unharvested stands. Because not all data met assumptions of normality, results were confirmed using a randomization F-test and t-test, which used each specific data set to create a new null distribution based on 10,000 iterations to determine whether or not the test statistic was significantly different from the randomized null distribution. I ran an Analysis of Variance (ANOVA) with harvest type (harvested or unharvested), season, and aspect (NE vs. SW) as predictors of observed SMR and capture temperature. I included aspect in these analyses because north-facing slopes have been found to provide more hospitable microhabitats for terrestrial salamanders (Moseley et al. 2009, MacNeil and Williams 2013).

Because my study design involved resampling ACOs several times over the course of a single season, I encountered some individuals more than once. Although this paper does not present results on abundance analyses, all encountered salamanders were marked with visible implant elastomers (Northwest Marine Technology, Inc., Shaw Island, Washington, USA) as part of another mark-release-recapture study. To avoid violating the assumption of independence, I randomly removed all but one encounter of each individual per season and created an adjusted dataset consisting solely of unique encounters. I performed a side-by-side comparison of analyses using both the complete dataset, and the adjusted dataset. Results from adjusted dataset analyses varied depending on which encounters of individual salamanders were randomly retained. However, I performed the analyses several times and with the exception of one F-test, all results and trends remained consistent regardless of whether I used the complete dataset or the adjusted dataset. For the sake of clarity and consistency I report results from the complete dataset, and make explicit the one discrepancy.
3.3 Results

I encountered a total of 670 *Plethodon cinereus* over three sampling seasons (Spring 2013, Fall 2013, Spring 2014). After removing malformed and injured individuals, the sample size consisted of 566 salamander encounters made up of 443 unique individuals.

3.3.1 Refugia Temperature

Mean maximum refugia temperature was higher in harvested stands than unharvested stands in Spring 2013 ($t_1 = 6.30, P \leq 0.001$), and Spring 2014 ($t_1 = 3.65, P \leq 0.001$), but it was not different in Fall 2013 ($t_1 = -0.43, P = 0.332$; Table 5). The variance of maximum refugia temperature was larger in harvested stands than unharvested stands in all three seasons: Spring 2013 ($F_{1,588} = 15.15, P \leq 0.001$), Fall 2013 ($F_{1,448} = 6.35, P = 0.01$) and Spring 2014 ($F_{1,448} = 4.35, P = 0.038$). I used the refugia temperature thermal profiles for each season, and the method described by Homyack et al. (2010) to calculate SMR by extrapolation with a one-gram salamander. The mean maximum SMR test statistics and $P$-values were identical to those of the refugia temperature analysis between harvested and unharvested stands due to the use of a standardized mass conversion factor.

Mean daily refugia temperature range was significantly higher in harvested stands in all three seasons: Spring 2013 ($t_1 = 11.15, P \leq 0.001$), Fall 2013 ($t_1 = 10.72, P \leq 0.001$), Spring 2014 ($t_1 = 9.04, P \leq 0.001$; Figure 6). Levene’s test for homogeneity of variance also confirmed that the variation of daily temperature range in harvested stands was significantly larger in all three seasons: Spring 2013 ($F_{1,588} = 20.86, P \leq 0.001$), Fall 2013 ($F_{1,448} = 35.64, P \leq 0.001$), Spring 2014 ($F_{1,448} = 29.20, P \leq 0.001$; Figure 6).
3.3.2 Salamander Capture Temperature

Salamander capture temperature was not different between harvested and unharvested stands in Spring 2013 ($t_1 = 1.61, P = 0.056$), Fall 2013 ($t_1 = 0.56, P = 0.288$), or Spring 2014 ($t_1 = 0.04, P = 0.516$). However, the variance of capture temperatures did vary between harvested and unharvested stands in Spring 2013 ($F_{1,156} = 15.56, P \leq 0.001$), and Fall 2013 ($F_{1,222} = 7.50, P = 0.007$) sampling seasons (Figure 7).

Standard metabolic rate calculated from observed salamander mass and capture temperatures did not differ between harvested and unharvested treatments in any season. Using the complete dataset, the variance of SMR was larger in harvested stands in Fall 2013 ($F_{1,222} = 4.63, P = 0.032$; Figure 8). However, after removing individuals randomly to avoid violating the assumption of independence, this trend, although still evident in the adjusted dataset (Figure 9), was no longer significant (mean $P$-value of 0.069, only significant 490 of 1000 randomized iterations).

The variance of salamander capture temperature was significantly different in Spring 2013 and Fall 2013, but the variance of SMR only showed this trend in Fall 2013 (although not in the adjusted dataset). Therefore, I examined salamander mass and SVL to see if changes in seasonal salamander size assemblages could potentially influence SMR calculations. Mass was not significantly different between harvested and unharvested stands in Spring 2013 ($t_1 = -0.15, P = 0.441$) or Fall 2013 ($t_1 = -1.23, P = 0.109$). The variance of mass was also not significantly different between harvested and unharvested stands in Spring 2013 ($F_{1,156} = 0.10, P = 0.752$; Figure 10). However, there was a difference in the variance of salamander mass between harvested and unharvested stands in Fall 2013 ($F_{1,222} = 5.66, P = 0.018$; Figure 10). Snout vent length was also
significantly lower ($t_1 = -2.21, P = 0.014$) with a significantly larger variance in harvested stands ($F_{1,222} = 7.01, P = 0.009$), in Fall 2013, whereas mean and variance of SVL were not different in harvested and unharvested stands in Spring 2013 ($t_1 = 1.13, P = 0.130; F_{1,156} = 1.56, P = 0.214$).

Season was a significant predictor of salamander SMR ($F_{4,561} = 12.10, P \leq 0.001$), but harvest type and aspect had no effect on SMR ($F_{4,561} = 0.04, P = 0.839; F_{4,561} = 2.70, P = 0.101$ respectively). Similarly, season was a significant predictor of salamander capture temperature ($F_{4,561} = 34.29, P \leq 0.001$), but harvest type and aspect had no effect ($F_{4,561} = 1.52, P = 0.219; F_{4,561} = 0.58, P = 0.446$ respectively).

3.4 Discussion

Researchers have suggested that plethodontids are good candidates for use as bioindicator species for mature forest ecosystems (Welsh and Droege 2001); therefore, methods that assess their health are beneficial for conservation science. Upon investigation of the field implementation of SMR calculation using an equation developed by Homyack et al (2010), this method has the potential to serve as a non-invasive monitoring technique, but not as currently applied. In previous studies, this equation has been used to extrapolate SMR of surface-active salamanders based on different temperature regimes resulting from different timber harvest treatments (Homyack et al. 2011). However, these temperature regimes were collected during the summer months, representing the most severe temperatures encountered by a surface-active P. cinereus. In this study, I truncated seasons to include only sampling days when at least 10 P. cinereus were captured. Although SMR estimates from iButton temperature dataloggers and estimates from salamander capture temperature and mass
measurements were different, similar trends in variability were noticeable using both methods. However, my results suggest that mass may be the driving factor in SMR calculation, and researchers should use caution when making claims based on differences in calculated mean SMR when data on salamander mass has not been taken.

Temperature dataloggers exhibited a noticeable difference in temperature regime between harvested and unharvested stands in all three seasons. Subsequently, when I extrapolated SMR based on the predictive regression equation, the results showed identical differences in SMR for harvested and unharvested stands in all three seasons. However, I did not see this same trend in observed SMR based on salamander capture temperature and mass measurements. Observed SMR was not significantly different between harvested and unharvested stands in any season.

The iButton dataloggers indicated consistently larger variance of maximum refugia temperature in harvested stands than in unharvested stands in all three seasons. This was expected, because harvested forest stands are known to have a larger variability in climate (Zheng et al. 2000). Variance of salamander capture temperature was also larger in harvested stands in both Spring 2013 and Fall 2013. However, this difference was not as extreme as suggested by the iButton data, and it was also not seen in Spring 2014. Temperature dataloggers were placed underneath three randomly selected ACOs per array in order to record the thermal profile of each array. Perhaps this small subsample of the available ACOs did not provide an adequate thermal profile of the array, and thus the discrepancy between refugia and capture temperature data is due to salamander selection of more hospitable microclimate under cooler ACOs. Also, by Spring 2014 the study site had more herbaceous and woody ground cover, which has
been shown to provide moisture and shade in recently harvested stands (< 5 years) (Morneault et al. 2004) potentially mitigating effects of harvest on temperature regime.

Although the variance of salamander capture temperature was larger in harvested stands in both Spring 2013 and Fall 2013, observed SMR only seemed to follow this trend in Fall 2013 (in adjusted dataset $P = 0.069$). Upon further analysis, both mass and SVL also had significantly larger variance in harvested stands in Fall 2013, and mean SVL was significantly lower in harvested stands during that year. Capture temperature also differed between harvested and unharvested stands in Spring 2013, however, mass and SVL did not. Mass, therefore, may be the driving factor in SMR calculation, and if salamander mass has the potential to differ between harvest treatments, extrapolation based on a one-gram salamander might not be a valid method of SMR estimation.

Because *Plethodon cinereus* mass is a transient measurement, the average difference in mass of recaptured salamanders fluctuated by 0.086 g within a season. Plethodontid mass is subject to soil moisture levels (Heatwole 1960), and can also change by as much as 0.1 g in one meal (P. J. Ruhl, Purdue University, personal observation). Because of the intrinsic problems with mass measurements of an organism that varies so much with one meal, it is impossible to ensure that all salamanders measured in a field environment are in a standardized post-absorptive or hydrated state. However, SVL is a reliable measurement of *P. cinereus* stage class (Moore 2009). In addition to the differences in the variance of *P. cinereus* mass, differences in mean SVL (and thus stage class; Figure 11) between harvested and unharvested stands call into question the validity of SMR extrapolation using a one-gram salamander as an accurate proxy of ecosystem health.
There are several potential explanations for the discrepancy in salamander mass and stage class (SVL) observed in Fall 2013, but perhaps territoriality may be the most parsimonious. *Plethodon cinereus* is known to have a complex socioecology in which mature adults defend their territory and exclude juveniles from high quality cover objects (Jaeger 1979, Jaeger 1995). Artificial cover object searches have been shown to perform comparably to (if not better than) transect, quadrat, and natural cover object searches (Monti et al. 2000, Moore 2009, Hesed 2012). Salamanders sampled with ACOs do not differ in weight, SVL, or sex ratio from those captured under natural cover objects (Monti et al. 2000, Marsh and Goicochea 2003, Moore 2009). In addition, *Plethodon cinereus* are not drawn from nearby natural cover objects to ACOs (Monti et al. 2000).

While some studies do not identify a territoriality bias between ACO sampling and other salamander sampling techniques (Monti et al. 2000), Hesed (2012) suggests that ACOs may effect a size bias toward larger individuals. Perhaps in the unharvested stands, smaller individuals were pushed into the surrounding leaf litter, but in the harvested stands ACOs were not considered to be high quality cover objects, and were not defended as such. Due to the nature of this study, there was a higher proportion of coarse woody debris (CWD) in the harvested stands than the unharvested stands since the harvested stands were subject to a replicated biomass harvest resulting in the creation of a CWD retention gradient. Although previous research supports the use of ACOs as a reliable sampling method (Monti et al. 2000, Moore 2009, Hesed 2012), perhaps a combination of the sheer volume of excess CWD created by timber harvesting in some harvested stands, along with the harsh environment created by the biomass harvest,
effectively lowered the value of ACOs as high quality territory, thus allowing higher proportions of smaller individuals to occupy them.

Jaeger (1979) also noted that social boundaries and territoriality are more extreme in harsher climates and conditions that are often associated with the drier summer months. It is plausible that territoriality was not consistent between spring and fall field seasons, and could have accounted for some of the discrepancy in stage class. Another possibility may be that adverse conditions in harvested stands may simply cause the territoriality framework to break down (A. Mathis, Missouri State University, personal communication). I often found two salamanders together under the same ACO in the unharvested stands (typical of a breeding pair), but as many as four salamanders were found under one ACO in the harvested stands. Regardless of the cause of the discrepancy in stage class between harvested and unharvested stands, our data suggest that researchers should take caution when using Homyack et al.’s (2010) method to extrapolate SMR of *P. cinereus* in timber stands that have been subject to different harvest treatments.
Table 5. Mean (± SD) and associated \( P \)-values of \( t \) and \( F \) tests for differences in mean and variance of iButton maximum refugia temperature (°C), salamander capture temperature (°C), natural log of standard metabolic rate (lnSMR), natural log of salamander weight (lnMass) and snout vent length (SVL) (mm) between harvested and unharvested stands. Statistical analyses were performed on datasets including all salamanders encountered.

<table>
<thead>
<tr>
<th>Season</th>
<th>Parameter</th>
<th>Harvested Mean ± SD</th>
<th>Unharvested Mean ± SD</th>
<th>Difference in Means ((P\text{-value}))</th>
<th>Difference in Variance ((P\text{-value}))</th>
</tr>
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<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>iB Max Temp</td>
<td>15.37 ± 5.72</td>
<td>12.64 ± 4.39</td>
<td>( \leq 0.001 ) ***</td>
<td>( \leq 0.001 ) ***</td>
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<td>0.001 ***</td>
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<tr>
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<td>lnSMR</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>iB Max Temp</td>
<td>12.94 ± 4.50</td>
<td>13.12 ± 3.68</td>
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<td>0.012 *</td>
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<td>10.69 ± 3.54</td>
<td>0.288</td>
<td>0.007 **</td>
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<td>lnSMR</td>
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<td>-4.32 ± 0.53</td>
<td>0.355</td>
<td>0.032 * ( a )</td>
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<td>-0.38 ± 0.48</td>
<td>0.109</td>
<td>0.018 *</td>
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<tr>
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<td>SVL</td>
<td>35.08 ± 7.15</td>
<td>37.07 ± 6.18</td>
<td>0.014 *</td>
<td>0.009 **</td>
</tr>
<tr>
<td>Spring 2014</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>iB Max Temp</td>
<td>14.18 ± 5.31</td>
<td>12.36 ± 4.62</td>
<td>( \leq 0.001 ) ***</td>
<td>0.038 *</td>
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<tr>
<td></td>
<td>Cap Temp</td>
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</tbody>
</table>

\( a \) If adjusted dataset consisting of only unique individuals in each season was used, variance of SMR in Fall 2013 was no longer significant, but it showed a similar trend (Figure 9)
Figure 6. Refugia temperature range (maximum – minimum; °C) between harvested and unharvested stands in Spring 2013 (A.), Fall 2013 (B.), and Spring 2014 (C.).
Figure 7. Salamander capture temperature (Capture temp) in harvested and unharvested stands in Spring 2013 (A.; n = 158), Fall 2013 (B.; n = 225) and Spring 2014 (C.; n = 184).
Figure 8. Observed lnSMR of all captured individuals in harvested and unharvested stands Spring 2013 (A.; n = 158), Fall 2013 (B.; n = 225), and Spring 2014 (C.; n = 184).
Figure 9. Observed lnSMR of randomly selected unique individuals in harvested and unharvested stands in Spring 2013 (A.; n = 136), Fall 2013 (B.; n = 199), and Spring 2014 (C.; n = 167). Although variance of SMR in Fall 2013 is no longer significant ($P = 0.069$), trend of non-homogeneity of variance is still evident.
Figure 10. Natural log of salamander weight (lnMass) of captured salamanders in harvested (left) and unharvested (right) stands in Spring 2013 (n = 158), Fall 2013 (n = 225) and Spring 2014 (n = 184).
Figure 11. Proportion of total salamanders encountered in both harvested (black) and unharvested (white) treatments based on stage class (juvenile [≤ 32mm], adult [>32mm]) in Spring 2013 (n = 158), Fall 2013 (n = 225), and Spring 2014 (n = 184).
CHAPTER 4: CONCLUSIONS

4.1 Summary

In this study, I assessed the effects of woody biomass harvest on *Plethodon cinereus*. Although a threshold of abundance based on a gradient of retained CWD was not discernable, N-mixture models identified a positive correlation between CWD retention and *P. cinereus* abundance. I monitored the effects of woody biomass harvest (as well as the difference between harvested and unharvested stands) on standard metabolic rate SMR of *P. cinereus* as a proxy of forest ecosystem health. *Plethodon cinereus* SMR exhibited no discernable trend with respect to the biomass harvest CWD retention gradient, but differences were noticeable between harvested and unharvested stands. In addition, I also tested the validity of a lab-based *P. cinereus* SMR estimation technique in a field setting. Based on my results I suggest that researchers use caution when extrapolating SMR of *P. cinereus* using Homyack et al.’s (2010) equation without accounting for differences in mass between harvest treatments. In this summary, I suggest areas of future research as well as discuss some of the intrinsic problems with designation of indicator (or keystone) species.
4.2 Future Research Directions

4.2.1 Mark-Release-Recapture Studies of Plethodontids

Mark-release-recapture (MRR) studies using plethodontids have been attempted, but due to the difficulty and time associated with marking small salamanders, researchers often resort to using counts of surface-active salamanders as a gauge of population size (Smith and Petranka 2000). However, detection probabilities have been shown to vary between sites and seasons, and raw count data are no longer an acceptable substitute for abundance (Dodd and Dorazio 2004). N-mixture models that account for site-specific differences in detection probability are becoming increasingly popular in salamander studies (Connette and Semlitsch 2013), but if researchers want to get a more complete idea of population dynamics such as survival and recruitment, MRR studies may still be best.

Before visible implant elastomers (VIEs) became the most common marking method for plethodontid research (Bailey 2004, MacNeil et al. 2011), toe clipping and photographs were the two main methods used to identify recaptured individuals (Burton and Likens 1975b, Gill 1978; Tilley 1980). Until recently, VIEs were thought to be a less invasive and more efficient way of compiling encounter histories for individual salamanders (Davis and Ovaska 2001). However, with recent developments in computer aided photo ID software, researchers no longer need to go over hundreds of photographs by hand in order to compile encounter histories.

Bendik et al. (2013) found that a computer assisted photo identification software: Wild-ID (ver. 1.0.1 [13]; http://www.dartmouth.edu/~envs/faculty/bolger.html), outperformed VIEs for recognition of recaptured salamanders. This software has been
proven effective for identification of *Eurycea* spp (Bendik et al. 2013), but no research has been done on the effectiveness of this software on terrestrial plethodontids. *Plethodon cinereus* does not have the unique head patterning common to many species of *Eurycea*, but perhaps the variation of stomach pigmentation could serve as a potential ID focus for plethodontid species (C. Anthony and N. Bendik, personal communication). More research should be done to see if implementation of this software could potentially replace VIEs as a less-invasive MRR method for terrestrial plethodontid salamanders.

### 4.2.2 Standard Metabolic Rate

The predictive regression equation developed by Homyack et al. (2010) enables researchers to use temperature and mass to calculate SMR of *Plethodon cinereus* to 95% accuracy. Currently this equation is used in field studies by acquiring thermal profiles of study sites and calculating site differences of SMR by extrapolation, assuming a one-gram salamander as a size standard (Homyack et al. 2011). Through my research I have demonstrated potential problems associated with assuming a one-gram salamander in SMR calculations, but there may be other problems with this method.

In my study, the variance of *P. cinereus* body mass was larger in harvested stands vs. unharvested stands in Fall 2013. Mean snout vent length (SVL) was also significantly smaller in harvested stands vs. unharvested stands in Fall 2013 indicating a discrepancy in stage class proportion between harvested and unharvested treatments in that season. These differences in size suggest that salamanders were using harvest treatments differently, in that more small individuals, presumably of young age classes were found in harvested stands, and the implementation of the currently accepted method of SMR calculation would be inaccurate. Although the current method of SMR calculation in
field studies is not meant to accurately predict SMR of every individual in the population, it is designed to serve as a surrogate for salamander health in field studies. Therefore, ignoring a key coefficient, by assuming standard mass, opens the door for inaccuracies and invalid conclusions.

In addition to potential differences in *P. cinereus* mass between harvest types, study site location may pose another potential problem for use of this specific regression equation to calculate SMR in field studies. *Plethodon cinereus* has a relatively large geographic range spanning most of the northeastern United States (Gibbs 1998). However, due to their relatively small home ranges (Kleeberger and Werner 1982) and limited dispersal ability (Marsh et al. 2004), genetic studies have shown that populations of *P. cinereus* become increasingly different with increasing distance (Cabe et al. 2007). In a species distribution as large as that of *P. cinereus*, there may be the potential for different levels of volumetric oxygen consumption in separate populations throughout their range, and thus different relationships between mass and temperature, based on location and/or elevation.

All of the salamanders used to develop the predictive regression equation were caught in the Jefferson National Forest, Montgomery County, VA (Homyack et al. 2010). This study site is approximately 730 km away from my study site. Because of this geographic separation, microhabitat composition and forest structure were undoubtedly different at these two locations. The consistency of the relationship between mass and temperature to predict SMR across such a vast geographic range may not be a valid assumption. In the future, Homyack’s experiment should be replicated with several
populations of *P. cinereus* sampled throughout its range in order to validate the use of this equation in other parts of the country.

In addition to the potential problems on a large scale, these problems may even persist with this assumption at the individual study-site level. Researchers have detected population differentiation of *P. cinereus* at scales as small as 200m (Cabe et al. 2007). Recent research has also demonstrated a significant amount of individual variation in thermal sensitivity for SMR in another plethodontid species, *Plethodon albagula* (Careau et al. 2014). If SMR calculation can vary between different treatments within a single study site, then using SMR as a metric of salamander health will need to involve specific lab calculations for every treatment. More research is needed to fully elucidate the relationships between mass and temperature to predict SMR at both large and small scales throughout the geographic range of *P. cinereus*.

4.3 Using Plethodontids as a Bioindicator Species

Although plethodontids have long been suggested as bioindicator species for forest ecosystem health (Wyman 1998; Welsh and Droega 2001) there has been some disagreement on the effectiveness of using solely *Plethodon cinereus* as a valid bioindicator. It is widely accepted that plethodontid abundance and diversity are directly associated with microhabitat characteristics associated with late seral stage forests (DeMaynadier and Hunter 1995). However, many studies are unable to examine the repercussions of forest harvests on multiple plethodontid species due to lack of adequate sample sizes. Because *P. cinereus* is nearly ubiquitous and highly resistant to forest fragmentation, it often makes up more than 95% of the plethodontid diversity in many eastern forests (Gibbs 1998, Harpole and Haas 1999, Homyack and Haas 2013), as was
the case in my study. In these locations, where the majority of plethodontid diversity is composed of one or two species, plethodontid abundance may or may not be indicative of forest health.

It has been suggested that *P. cinereus* plays an integral role in forest ecosystem services such as nutrient and energy cycling, and soil decomposition rates (Burton and Likens 1975a, Wyman 1998). However, at this time the research is still largely inconclusive. In 1998, Wyman demonstrated the top-down effects of salamanders as a predator in detrital food webs. His research showed that the presence of *P. cinereus* in the environment resulted in the reduction of leaf fragmenters, resulting in a reduced rate of leaf decomposition by between 11 and 17% in salamander-occupied treatments.

However, using the same litter type, Hocking and Babbitt (2014) did not find any effect of the removal of *P. cinereus* on various ecosystem functions. Other studies have also looked at the effects of *P. cinereus* as a forest-floor regulator species, but results were inconclusive (Walton et al, 2006). *Plethodon cinereus* has also been described as a keystone species (Wyman 1998), but conflicting and inconclusive evidence (Walton et al. 2006; Hocking and Babbitt 2014) suggests that making strong conclusions about forest ecosystem health solely based on the abundance of *P. cinereus* may be questionable.

The idea of a keystone species has traditionally been in conflict with food-web theory, which suggests the relative importance of conservation of all species within an ecological community (Mills et al. 1993). Additionally, keystone species is a term that is often loosely attributed and poorly defined (Mills et al. 1993, Bengtsson 1997). In fact, deer have been described as keystone species because of their vast impact as herbaceous grazers (Waller and Alverson 1997), but this classification seems to contradict the
traditional definition of keystone species. Generally, the best understood definition of a keystone species is one whose preservation should be crucial for maintaining ecological organization and diversity within a specific community (Mills et al. 1993). Although species are often labeled keystone species to illustrate their importance within the ecosystem, the lack of empirical data on species interactions as well as the broad use of the term can make it difficult to determine whether or not this label is justifiable.

Many species have been labeled as keystone species, but empirical data do not always support these claims. Prairie dogs (*Cynomys* spp.) and beavers (*Castor canadensis*) are widely accepted as keystone species, because the physical disturbance caused by their respective burrowing and damming behaviors creates habitat that supports higher biodiversity (Naiman et al. 1986, Augustine and Baker 2013). However, while species diversity is generally increased in the presence of prairie dogs and beavers, species distributions of associated species do not strictly follow those of the keystone species. Thus, even in two of the most accepted examples of keystone species, the continued existence of associated species may not be completely dependent on keystone survival or persistence.

There is disagreement within the scientific literature as to whether or not *Plethodon cinereus* acts as a bioindicator for ecosystem health and should be considered a keystone species (Wyman 1998, Welsh and Droge 2001, Walton et al. 2006, Hocking and Babbitt 2014). My research shows that *P. cinereus* can persist on recently harvested stands with 0% CWD remaining. Although my data do show that abundance of *P. cinereus* decreases with decreased CWD retention after the harvest, I cannot make any claims about the bioindicator status of this species. Full acceptance of *P. cinereus* as a
bioindicator (or keystone) species will require implementation of surrogate validation procedures as described by Murphy et al. (2011). Future research should focus on empirical confirmation of the plethodontid salamander bioindicator status in order to validate its classification as a keystone species.
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