Do Invasive Earthworms Negatively Impact Salamander Abundance along the Credit River Forest?

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Abstract *Lumbricus terrestris* (Common nightcrawler) is an invasive earthworm species that was introduced into North America via European settlers in the early 1800's. As ecosystem engineers, these anecic (deep vertical burrowing) earthworms are able to alter the structure of their habitat by mixing soil layers, altering the conditions of the soil, and consuming leaf litter. In doing so, earthworms impact plant species, invertebrates and vertebrates that also inhabit the same environment. *Plethodon cinereus* (Red-backed salamander), the most common vertebrate in many deciduous forests, also prefers a moist, alkaline soil floor, but unlike earthworms who can burrow into the soil, *P. cinereus* relies on the leaf litter and understory on the forest floor to keep moist and covered. Thus, earthworms have the potential to positively impact salamander habitat by providing burrows, or negatively impact salamander habitat by reducing leaf litter or competing with salamanders for prey items, such as invertebrates. Previous studies have focused on examining this relationship at large (i.e., among-forest) spatial scales. Our pilot study focuses on documenting the abundance and spatial coincidence of earthworms and salamanders within a single deciduous forest fraction (along the Credit River Nature Trail of UTM). A significant positive correlation (R^2=73.1%, P<0.001; Fig. 4) was found between earthworm and salamander abundance, as well as a decrease in both species' population abundance over the summer. The results of our study suggest that *L. terrestris* does impact *P. cinereus*, but further research is needed to determine whether or not this impact is positive or negative. Additional research into this relationship between *L. terrestris* and *P. cinereus* can help with our understanding of ecosystem stability and management at a fine forest scale.

Keywords *Plethodon cinereus*, *Lumbricus terrestris*, Keystone species, Anecic, Ecosystem engineer, Fine-scale forest management

Introduction

We are currently living during the sixth mass extinction event, the Anthropocene, in which human activity is considered one of the main causes of the extinction of species and environmental change. The causes of extinction of species due to human activity can range from habitat destruction to the introduction of invasive species. Globalization of trade and travel has allowed for foreign species to spread to new areas with favorable environmental conditions, where a lack of resource competition allows for the non-native species to thrive (Keller, Cadotte, & Sandiford, 2015). Due to their plasticity, invasive species may also have a more successful evolutionary response to climate change, in which human activity is contributing to an acceleration of global environmental change (Mooney & Hobbs, 2000a). The prevention and management of invasive species has proved difficult, as it requires ecological, ethical, and legal considerations, as well as cost benefit analysis' to consider the positive and/or negative impacts of these invasive species (Mooney & Hobbs, 2000b; Keller, Cadotte, & Sandiford, 2015).

Canada's forests are home to a number of unique species, but due to the globalization of trade aided by shipping vessels, thousands of exotic species have been introduced into our forest ecosystems within the last century, altering many well established ecosystems that have been thriving for thousands of years (Allen & Humble, 2002). These alterations in the ecosystem are further amplified and extended in range by global warming, a topic which Canada has been slow to act on legislatively (Smith et al*.,* 2012). Although this introduction of an invasive species typically results in a negative lasting effect on the ecosystem, such as the Asian Green Horned Beetle in Canada ("Asian long-horned beetle," 2014), there are some invasive species that improve economic relationships and have beneficial impacts within the ecosystem, such as The North American Honey Bee (Butz, 1997). In Canada, one invasive species that is believed to have both positive and negative economic and environmental impacts is the earthworm. One reason why the impact of earthworms is debated is due to its role as an ecosystem engineer. An ecosystem engineer modifies the environmental conditions of its surrounding habitat (Ransom, 2011). The earthworm functions as an ecosystem engineer by modifying soil chemistry, mixing soil layers, and consuming and decomposing leaf litter (Ransom, 2011; Ransom, 2017).

Invasive earthworms were introduced into North America via European settlers in the early 1800's. Earthworms can be divided into four groups, known as ecotypes, based on their different ecological services and functions, and how those services and functions affect the overall ecosystem. All four functional variations of earthworms have been found in Canada. There exist compost worms found in rotting vegetation, epigeic worms found on the surface of soil, endogeic worms which make horizontal burrows in the soil, and anecic worms which make vertical burrows in the soil (Earthworm Ecology, n.d.). This study focuses on anecic worms as these earthworms, ranging up to 12.5-20cm long (Great Lakes Worm Watch, n.d.), consume the most amount of leaf litter out of the three types of earthworms, and cause the most amount of soil mixture by dragging the broken-down leaf litter into their burrows. This process drastically changes the leaf litter layer of the forest ecosystem and habitat of organisms that reside within it. One of the most noticeably affected forest floor organisms is the *Plethodon cinereus*, due to the species' prevalent biomass.

Ecosystems are a combination of coexisting and interrelated organisms, of which salamanders have been shown to be an excellent indicator of ecological instability and change. As it impractical to monitor all living organisms that function in a particular environment, salamanders have been used to monitor stress levels of fine scale forest ecosystem processes, due to their function as a predator (of invertebrates) and prey (of various vertebrates, such as snakes and frogs), sensitivity to change in soil and litter conditions, and observable population abundance (Welsh & Droege, 2001). The salamander family Plethontidae have been recorded as the most abundant vertebrate in North American temperate forests (Burton & Likens, 1975; Mitchell et al.,1997; Welsh & Droege, 2001). Within this family, the species *Plethodon cinereus* (Red-backed salamander) has been researched as a strong indicator of forest floor invertebrate populations (Shelford, 1913; Wyman, 1998), and has been used in forest floor management research as a keystone species due to the easily identifiable colour morphs. *P. cinereus* has two different colour morphs. The red-backed colour morphs are grey bodied with a red or orange stripe down the back, while the lead-backs are the same grey colour, but lack the brightly coloured stripe (Cochran, 1911). There is little physical differentiation between age and sex, although juveniles will be smaller during development (Cochran, 1911). In the mixed deciduous forests of Ontario, Canada, which neighbours the United States, the Red-back salamanders have an abundance of shelter provided by leaf litter, tall understory, logs, and other debris. *P. cinereus* require a moist environment and basic soil acidity, where soil levels with a pH lower than 3.7 can be harmful to their sensitive skin, which they use for respiration (Frisbie & Wyman, 1991). For this reason, *P. cinereus* are most active during the night and after rainfall. Where earthworms are ecosystem engineers, salamanders are a keystone species, which makes observing the relationships between these two species a key indicator of forest health and integrity. It has been recommended by Johnson and O'Neil (2001) that monitoring key ecological functions through the observation of a keystone species can be used for biodiversity conservation, something that invasive earthworms threaten to disrupt.

Interactions between *P. cinereus* and *L. terrestris* is less of a predatory relationship, as the small size of *P. cinereus* is an average of 5.7 to 12.7 cm in length (Howard, 2003). This small size means the Red-Backed morphs are unable to eat the larger earthworms, which can typically grow up to 15 cm in length (Great Lakes Worm Watch, n.d.). This large size allows the anecic earthworm to burrow deep below the ground creating large networks of tunnels beneath the leaf litter layer.

Plethodon cinereus (Red-Backed Salamander) is heavily influenced by these ecosystem alterations provided by the invasive earthworm and their burrows. *Plethodon cinereus* is known to utilize the leaf litter layer of a deciduous forest as protection against predation, desiccation, and as a means to hunt small insects (Ransom, 2011). As ecosystem engineers, *L. terrestris* alter the availability of shelter through decreasing the leaf litter mass, but also through decreasing the understory growth by altering the soil structure

(Eisenhauer, Partsch, Parkinson, & Scheu, 2007).

Although the consumption of leaf litter has a negative effect on *P. cinereus*, the argument exists that the burrows provided by *L. terrestris* are large enough for *P. cinereus* to occupy and seek refuge in, reducing the risk of predation and intraspecific competition as well as increasing winter survival rates (Ransom, 2011). It is in this scenario that *L. terrestris* creates a positive effect on *P. cinereus* by simulating the same habitat attributes leaf litter provides but in the form of anecic burrows. This includes protection from predators, protection from desiccation and a space for long term habitation during winter months (Taub, 1961). However, it has also been shown that *P. cinereus* does not distinguish between the burrows of native species (*Eisenoides carolinensis* or *Diplocardia* spp.) and invasive species, such as *L. terrestris* (Ransom, 2012).

While occupying the same location Earthworms and Salamanders have shown to have their behaviors altered further complicating the relationships between the organisms and their surroundings, specifically territorial patterns and hunting practices changed within mesocosm experiments (Ransom, 2012).

Previous studies (Maerz, Nuzzo & Blossey, 2009; Ransom, 2012; Ziemba, Hickerson, & Anthony, 2016) have focused on examining the relationship between *P. cinereus* and *L. terrestris* at larger spatial scales (ie. in state parks). In contrast, our paper attempts to address a gap in the scientific literature surrounding the small-scale impacts of invasive earthworms on the keystone species *P. cinereus* and its habitat. The results of such research may have far-reaching implications for the sustainable management protocols of fractioned urban forests for municipalities, as well as smaller institutions and conservation organizations. It responsibility of these organizations to consider multiple variables that may affect ecosystems and appropriately prepare for the alterations they may cause. For example, both climatic and non-climatic environmental variables contribute to the survival and abundance of a species. Climate can be manageably observed across broad areas using models such as CLIMEX (Byeon, Jung, & Lee, 2018), but small scale interaction such as resource competition and forest floor composition, have to be observed on smaller scales (Mooney and Hobbs, 2000c). It is with this analysis that the information gathered at a small scale can help management prepare, predict and implement new procedures at a larger operating scale to prevent biodiversity loss in Canadian parks and conservation areas across temperate regions.

This study focuses on the abundance and relationship of *P. cinereus* and *L. terrestris*, the possible forms in which they interact with each other, and how their interactions can be used as a proxy to help determine forest growth and create small scale conservation efforts, that will promote and protect the biodiversity of Canadian municipal parks and forests.

Materials and methods

Site Classification

Research was conducted at the University of Toronto Mississauga campus, ON, Canada. Research was focused along the nature trail located on the campus, which runs along the Credit River, spanning a total of 1.23 Kilometers. The forest around this area is defined principally as a dry-fresh Sugar Maple-Oak deciduous forest type, and part dry-fresh Popular deciduous forest type. The understory vegetation is 0.5-2m in height, and provides a 60% cover (City of Mississauga Natural Areas Survey 2018). The understory vegetation, which along with the leaf litter from the canopy and sub-canopy leaves, provides a habitat for the *P. cinereus*, is comprised of Chokecherry, Witch-hazel, Alternative-leaved Dogwood, Sugar Maple and White Ash saplings (City of Mississauga Natural Areas Survey 2018).

For this series of data collection, we observed the *Plethodon cinereus* and *Lumbricus terrestris*, an anecic (burrowing) worm species, from July to August, 2019.

Establishing Transect Locations Using Google Earth

Transect locations were chosen to extend perpendicular from the Trail into the Forest while being separated at intervals of 30 meters. Using Google Earth Pro (Google, n.d), a path (in red) was drawn over a satellite image of the Nature Trail. This path was then measured using the Google Earth Pro Ruler Tool. Data points representing a potential transect location were placed along the Nature Trail at 30-meter intervals. A grid measuring 1000 meters by 800 meters was then laid over the Nature Trail using Earth Point Tools for Google Earth (Clark, 2019). Each square of the grid was measured out to 200 meters both in length and width. For each field day, transect locations were chosen randomly using a random number generator, to ensure that different sections of the Nature Trail would be surveyed at different times throughout the summer.

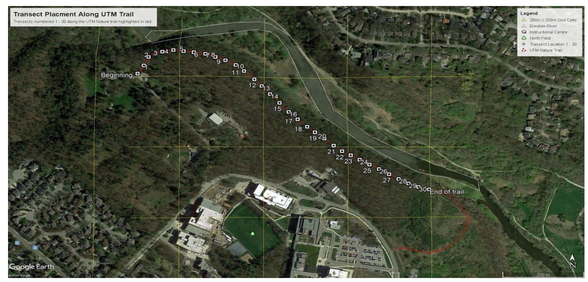

Figure 2: Transect locations along the UTM Nature Trail. The trail is highlighted in red and each white box represents one transect location (30 in total).

Salamander Abundance Survey Using Transects

Each transect was measured out to a total of 30 metres. Because the *Plethodon cinereus* require moisture and therefore live primarily under leaf litter, logs, and in burrows (Howard, 2003), only areas with logs or leaf litter that completely covered the ground were surveyed. In these areas, surveying was done within a metre of the transect line, on either side of the transect. Color morph (red-backed or lead-backed) and life stage (juvenile or adult) were recorded, along with the number on the transect line where the salamander had been found. Because both sides of the transect line were used, the number of the transect line would be recorded as positive if the salamander was observed on the right hand side, or negative if the salamander was observed on the left hand side of the transect.

Worm Abundance Survey Using Quadrats and The Mustard Protocol

Anecic worms were sampled (counted) along the same transect at the same time as the salamanders. Only two points along the transect were used to survey worms. A random number generator was used to arbitrarily choose those two points. Since both sides of the transect were used, the random number generator contained numbers from 0-61, where 0-29 corresponded to numbers on the right hand side, and numbers 30-61 corresponded to numbers on the left hand side of the transect. Once a number was selected, a quadrat was placed within 2 metres from the transect line. We used a 1 square foot circular quadrat as our sample plot. To count the population of *L. terrestris* within the sample plot, we used a standard liquid extraction, known as the Mustard Protocol ("Research Methods," n.d.). The mustard solution percolates into the soil and irritates the skin of the earthworms forcing them to the surface. The procedure involves preparing the mustard solution which requires 40 grams of ground yellow mustard seed (powder) dissolved into 4 litres of water contained in a gallon jug ("Research Methods," n.d.). The mixture was shaken well to homogenize the solution.

Within the quadrat, we cleared away any surface litter such as leaves, wood, etc and slowly poured half of the solution over the area. We waited two minutes, timed on our phones, to allow as many worms to emerge. This method allows for worms to be observed immediately ("Research Methods," n.d.). The worms that emerged to the surface were removed from the quadrat and placed on a plastic sheet to be counted and identified. The number of anecic worms and life stage (adult or juvenile) were recorded.

We identified the worm as anecic from their color, length, and presence or absence of a clitellum, which would indicate if the worm was an adult or juvenile. The adult worms have a greyish-white clitellum, located close to the head, whereas the juveniles do not. Anecic worms appear darker at the head and lighter at the tail (Earthworm Ecology, n.d.).

The Protocol is most effective during moderate temperatures and after recent rainfall, when the worms are most active ("Research Methods," n.d.). Otherwise, the worms are in a state of aevestation (hibernation). Additionally, poor soil structure and compaction will cause a poor response from the worms because the solution will not seep into the soil to reach the worms ("Research Methods," n.d.).

Figure 2: *Plethodon cinereus* (Red-backed salamander) found under a log during field surveying.

Figure 3: Anecic earthworm in 12in diameter quadrat used for worm sampling. Picture taken after leaf litter had been cleared and the mustard solution had been poured.

Results

We found a significant positive correlation between Earthworm and Salamander abundance, indicating an effect of earthworm presence on salamander abundance ($P<0.001$, $R^2=73.1\%$; Fig. 4). Of the thirteen transects observed, six showed no sign of anecic worms. Of those six, two also showed no signs of salamanders.

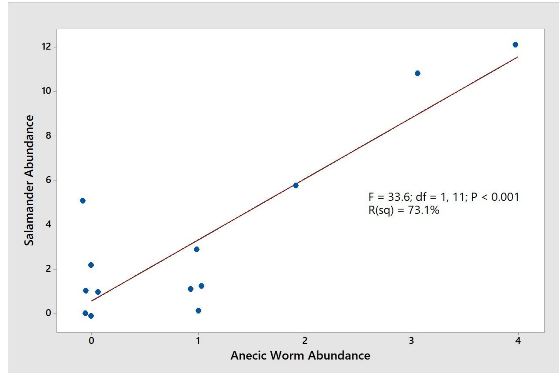

Figure 4: Regression analysis of Salamander abundance to Anecic worm abundance. Number of salamanders per transect are positively correlated with number of Anecic worms per transect. Jitter was used to reduce overlap of points.

We found that both earthworm and salamander abundance decreased over the summer observational period (July 12-August 1, Fig. 5).

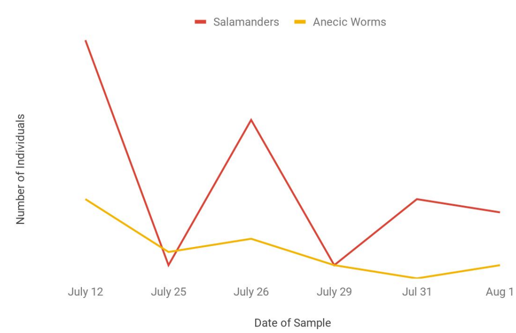

Figure 5: Temporal Line graph of amount of worm and salamander individuals found between July 12th and August 1st over 6 field surveying days.

Discussion

Our primary objective of this research study was to observe the coincidence of earthworms and salamanders through a survey of their abundance and study the possible influences of *L. terrestris* on *P.*

cinereus. We sampled (counted) for earthworms and salamanders along 13 randomly selected transects stretching across the UTM nature trail. We found a positive correlation between earthworm and salamander abundance, meaning where earthworms were observed, salamanders too were also observed (Fig.3).

This trend suggests large abundances of cohabitation throughout the late summer specifically in August. The dates transect data was recorded was used to create figure 5, in which we see a declining trend in both Salamander and Worm individuals as the late summer began. This overall declining temporal trend is consistent with other literature (Ziemba *et al.,* 2015) as Salamanders and Earthworms both aestivate during the warm summer days. As both species aestivate at the same time, earthworms can effectively hide through a burrow, whereas the salamanders depend on the leaf litter layer, which has been progressively destroyed by the earthworms during their active season (temperate and wet).

Since Salamanders and Earthworms are coincident with each other, both species are bound to interact (Ransom, 2012), but more research is needed to determine if this burrowing process is positive or negative. If the presence of *L. terrestris* is negative for *P. cinereus* then the consumption of leaf litter will leave *P. cinereus* more vulnerable to predators and desiccation (Ransom, 2012). As for competition, it is known that *L. terrestres* and *P. cinereus* do not compete for food (Ransom, 2011), thus the main disruption *L. terrestris* can cause is the consumption of leaf litter. If the relationship is positive however, *P. cinereus* will be able to use the earthworm burrows as a suitable location to hide from predators and prevent desiccation. To determine whether this relationship is positive or negative is crucial to understanding how Canadian forests operate on an ecological level.

Addressing the research gap between large scale and small scale earthworm and salamander interactions is important because small scale research can lead to small scale conservation efforts, which have been increasingly recognized by conservation organizations as being vital to the long-term sustainability and protection of ecosystems, as many of them have adopted a multi-scale management approach to the maintenance of ecosystem functions (Poiani, Richter B., Anderson, & Richter H., 2000). To effectively enact conservation efforts within an ecosystem, it is crucial to understand the connections within that system. Without an understanding of multi-scale ecological dynamics (ie., only observing large or fine scale) it may not be possible to have a holistic understanding of ecological changes, thus affecting the ability to manage conservation efforts effectively on a regional scale (Turner, Gardner, & O'Neill, 1995; Smith & Wishnie, 2000; Fischer & Lindenmayer, 2002).

Although *P. cinereus* and *L. terrestris* were coincident at 6 transects, 4 transects yielded salamanders but no anecic worms (Fig. 4). This could be due to the ineffectiveness of the mustard protocol which works best at moderate temperatures or after a recent rainfall which is when the earthworms are most active. Otherwise, they are in a state of aevestation (hibernation) during which they can penetrate to a depth of 2 meters ("Great Lakes Worm Watch," n.d) into the soil where the mustard solution does not seep deep enough to irritate the worms (Edwards & Bohlen, 1996). Additionally, there were 2 transects where we did not find any earthworms or salamanders which could have been caused by dry surface conditions that cause the salamanders to aestivate. The temporal graph (Fig. 5) which shows the decline in both earthworm and salamander abundance over the duration of the summer months further supports the effect of late summer conditions on these organisms.

Future research and data collection should include measurements of temperature and soil moisture to explore what effects these variables have on the organisms as it has been found (Bailey et al., 2004) that the probabilities of salamanders near the surface vary due to changing environmental conditions as salamanders tend to be found less frequently during hot and dry conditions.

Similarly, the weight of leaf litter should be measured to determine whether consumption of leaf litter has significant negative effects on the salamanders' habitat (See Supplement Research). If it does, then the different earthworm species' abundance should be measured as well since anecic worms along with the epigeic and endogeic worms all actively feed on leaf litter and compete with salamanders for invertebrates. If this were to be determined to be true, then the negative effect on salamanders could be a cumulative effect of the three earthworm species and not anecic worms alone.

There is also the possibility that salamanders are using burrows created by other species, this could be why salamanders were found at transects where no earthworms were found. Other species such as *Eisenoides carolinensis* or *Diplocardia* spp. create burrows like *L. terrestris* in such a way that *P. cinereus* could use as habitat (Ransom, 2012). It has been found that *P. cinereus* does not differentiate between native and invasive species or their burrows despite differences in burrow shape and size. Further research is needed to examine the net effects of both native and non-native earthworms on salamanders.

We sampled (counted) for salamanders along the entire 30 meter transect but sampled for worms within a quadrat at two random points along the transect. With this method, we are bound to find more salamanders compared to earthworms because the two quadrats are not representative of how many anecic earthworm species are actually present along the transect. Variability changes drastically along a 30 meter transect as some areas are much wetter or drier than others. This could explain why no earthworms were observed per transect but within the same transect many more salamanders than worms were found as the salamanders were counted along the entire 30 meters. The methods can be revised to produce a more accurate representation of both the species' abundance by taking a more concentrated sampling method in a smaller location.

Conclusion

L. terrestris and *P. cinerus* share a common habitat and temporal behaviors. As an ecosystem engineer, the modifications made by *L. terrestris* to the forest ecosystem will impact *P. cinereus*. The positive and negative effects that *L. terrestris* can have on *P. cinereus* is of interest because salamanders are a keystone species within forest ecosystems (Ransom, 2012). The effects of earthworms on salamanders help link above and below-ground ecosystems, especially because of the increasing introduction of non-native earthworms across North America. Previous studies (Maerz, Nuzzo & Blossey, 2009; Ransom, 2012; Ziemba, Hickerson, & Anthony, 2016) have focused on the relationship between *P. cinereus* and *L. terrestris* at large (i.e., among-forest) spatial scales (Hale *et al.*, 2005; Ransom, 2011), but more research into the relationship of *L. terrestris* and the keystone species *P. cinereus* will need to be conducted on smaller spatial scales (i.e., among fractionated forest). Other effects of earthworms, such as leaf litter consumption, and burrow use by salamanders, should be considered in order to conclude whether or not the net effect of the *L. terrestris* is positive or negative, which in turn, will provide insight into the overall health of temporal forest ecosystems. This study, and more studies conducted on a fractioned scale, can help define the relationship between earthworms and salamanders, and can be used as a catalyst for all levels of conservation management to help preserve and protect many of Canada's parks and forests.

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Supplement Research

Establishing Transect Locations Using Google Earth

Several iterations of the map were produced using various GPS coordinate sampling methods. The first method was used via a GPS tracker which used cell phone location (GPS Coordinates, n.d). Although this program served to be inaccurate at best and the data provided proved difficult to use for an accurate map. The phone app version Google Earth Pro was used to create data points for the current map as the program provided satellite imagery of GPS locations in real time, along with GPS coordinates.

Worm Abundance Survey Using Quadrats and The Mustard protocol

Initially, for the Mustard Protocol, we mixed 50 grams of 'hot' mustard powder with 100mL of water to create a paste and allowed it to sit overnight. On the day we wanted to use the Protocol, we diluted the paste with 7L of water. At the site, we simply sprinkled some of the solution over the sample plot enough to wet the surface of the soil, however, we did not find the earthworms to emerge at all. We modified this Protocol to what is found in the methods section.

Measuring Leaf Litter Decomposition

Before a quadrat was cleared for worm sampling, four piles of leaf litter were taken at four opposing points along the quadrat using a 2 cm diameter soil core. The leaf litter was removed from the soil core and placed in a paper bag. The four sample piles were placed in the same bag to ensure a random but homogenized sample of the leaf litter within each quadrat. Each bag was labelled with the coordinates of the transect, the number along the transect line where the pile had been taken from the quadrat, and the date. The leaf litter samples were taken back to the lab, weighed, and left to air dry (see Table 1). For future leaf litter collection, a method should be established to ensure all of the piles are left to dry for the same amount of time before processing it into a solution, to ensure consistency within the measurements. Each bag containing the collected leaf litter were transferred into a plastic dish and weighed, respectively.

Because the study focuses on anecic worms, we believe that future methods used to analyze leaf litter decomposition should use calcium as the natural element of leaf litter (instead of, or as well as Carbon:Nitrogen ratios) because according to Holdsworth, Frelich, and Reich (2008), *Lumbricus* prefer leaf litter with high amounts of calcium, and will pull these leaves down into their burrows: "litter calcium is the best predictor of mass loss when litter is accessible to *Lumbricus*". The other possible method to observe leaf litter decomposition via nutrients concentration would be to prepare the leaf litter using a sodium hydroxide solution, and determine the Carbon to Nitrogen ratios using UV-Visible recording spectrometer (Hasanuzzaman & Hossain, 2014).

Originally we planned on measuring both leaf litter mass and quality, but since the purpose was to measure leaf litter that was already present on the surface floor, we were unable to design a method that could control for the amount of leaf litter. We couldn't use mesh or nylon bags, because the purpose was not to measure the mass of leaf litter collected over a period of time, but the leaf litter already present on the surface floor. We tried to measure the same leaf litter we collected from the soil core, but each core yielded very little leaf litter, and it would be mixed in with the soil, therefore it was not possible to accurately measure the leaf litter itself using the soil core.

Table 1: Leaf Litter Collected and Weighed per Quadrat

Date	Weight (g)	Transect Data Point (m)	Quadrat Point Along Transect (m)
Aug. 13	0.017	17	11L
Aug. 13	0.032	17	26R
Aug. 13	0.134	15	5R
Aug. 13	0.043	15	25L
Aug. 1	0.214	5	22L
Aug. 1	0.538	5	29R
Aug. 01	1.104	8	16L
Aug. 01	0.672	8	6R
Jul. 31	0.16	13	11L
Jul. 31	0.128	13	6L
Jul. 31	0.324	10	9L
Jul. 31	0.315	10	8L
Jul. 31	0.514	6	7R

Jul. 31	0.293	6	19L
Jul. 29	0.127	23	2R
Jul. 29	0.204	23	11L
Jul. 29	0.130	24	23R
Jul. 29	0.214	24	23L
Jul. 26	0.215	11	5L
Jul. 26	0.426	11	24L
Jul. 26	0.074	16	5R
Jul. 26	0.049	16	5L
Jul. 25	0.625	3	28L
Jul. 25	0.752	3	11R
Jul. 25	0.559	14	29L
Jul. 25	0.278	14	13R

*L=left, R=right

Sampling the Effects of Leaf Litter and Understory Density as a Factor in Salamander Habitat Selection Using Cover boards

Cover boards were placed in contrasting population densities in areas of thick and thin understory. A stratified sampling method was used to assess percent plant coverage with a focus on tree seedlings and shoots. Naturally occurring woody debris was avoided as it is known to be the primary habitat of *P. cinereus.* We used 32 1ft² engineered untreated oak panels, 16 for denser and 16 for sparser areas, and ranked seedling percent coverage in a 5ft² area surrounding the cover-board locations as follows: <25, 25-50, 50-75, 75-100. Each cover-board location was marked with an orange flag and its coordinates were recorded.

iButtons were ordered online, but did not arrive in time to be used during our research. In the continuation of this research, iButtons should be installed to the bottom of each cover board to record the temperature and humidity levels under the coverboards, as the Red-backed salamanders are sensitive to these environmental variables for habitat preference. Cover boards and iButton information should be checked bi-weekly, as checking cover boards more than once a week may be harmful to the salamanders and may dry out the soil underneath the coverboards (Marsh & Goicochea, 2003).

Cover-boards in general, and oak in particular are known to retain less moisture than natural cover and results in approximately 30% fewer salamanders (Houze & Chandler, 2002), which should be factored into any future analysis.