Leaf litter and soil aggregate habitat preferences of *Plethodon cinereus*

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Abstract

During the Pleistocene Ice Age, the Wisconsin glacier covered much of the northwest Great Lakes region. As the glacier moved, it stripped the topsoil from the land and removed earthworms from the area. Recently, earthworms have been introduced to areas of the Northwoods habitat of the northwest Great Lakes region and begun to recolonize it. In the process, they are decreasing the amount of leaf litter and increasing the average size of soil aggregates. *P. cinereus* are living in this region and are sensitive to changes in their habitat that affect moisture, pH, and other environmental factors. This study attempted to determine the preference of *P. cinereus* for soil aggregate size and leaf litter depth as a means to identify possible habitat preferences. It was expected that *P. cinereus* would prefer deep leaf litter and small soil aggregate sizes. Significant differences were found amongst the experimental groups, and it was determined that *P. cinereus* preferred deep over both average and no leaf litter and large over small soil aggregates. *P. cinereus* preferring deep over both average and no leaf litter is likely due to leaf litter trapping moisture and maintaining an ideal microclimate. *P. cinereus* preferring large over small soil aggregates was not expected but could be due to a greater sense of security from the uneven terrain. This knowledge of the preferred habitat of *P. cinereus* is important because it provides the opportunity to examine the effect of earthworms in these habitats and how *P. cinereus* responds to the changes invasive earthworms bring about.

Introduction

Plethodon cinereus, commonly known as the Eastern Red-Back Salamander, is a member of the family Plethodontidae. *P. cinereus* are terrestrial salamanders and can be found throughout the eastern half of the United States and Canada. Plethodontidae are the largest family of salamanders and are lungless. Because they breathe through their skin, *P. cinereus* prefer environments that are moist, shaded, and have a relatively neutral pH

(Sugalski and Claussen 1997). They spend most of their time under cover objects, which trap moisture and offer shade and protection from predators. These cover objects include logs, stones, moss, and other naturally occurring objects (Bishop 1969).

The Wisconsin glacier covered the Upper Great Lakes area approximately 10,000 years ago. As the glacier moved, it stripped the topsoil from the area, which means that today all earthworms found in the area are invasive. At the study site, the University of Notre Dame Environmental Research Center (UNDERC), the most common invasive earthworm species include *Lumbricus terrestris, Dendrobaena octaedra, Lumbricus rubellus,* and *Apporrectodea calignosa* (Costello and Lamberti 2008). These species represent all three functional groups of earthworms: anecic, epigeic, and endogeic. Anecic earthworms dig vertical burrows and return to the surface to feed on the leaf litter layer (Palm *et al*. 2013). Epigeic earthworms dig horizontal burrows but don't have any organized burrow system, and they feed in the topsoil layer (Palm *et al.* 2013). Lastly, endogeic earthworms dig organized horizontal burrows, and they do not return to the surface to feed (Palm *et al.* 2013). All earthworm species have an ecological impact by increasing the rate of leaf litter decomposition, increasing the amount of mineralized nitrogen and phosphorous in the soil, and increasing the average size of soil aggregates (clumps of soil and other organic matter) (Greiner *et al.* 2012).

Numerous experiments have been conducted to examine the relationship between *P. cinereus* and invasive earthworm species. The earthworm populations do not appear to be affected by the presence or absence of *P. cinereus.* On the other hand, *P. cinereus* appears to be affected in different (and sometimes conflicting) ways by the presence of earthworms.

Some studies suggest that *P. cinereus* benefits from the presence of earthworms (Ransom 2012, Ransom 2011). *Plethodon cinereus* has been found to take advantage of earthworm burrows and use them for shelter (Ransom 2012). This has been found to increase the survival rates of *P. cinereus* as it makes them more likely to live through the winter and avoid predation (Ransom 2011). *Plethodon cinereus* has also been found to consume earthworms as prey (Ransom 2012). These two relationships suggest the presence of earthworms does not negatively impact *P. cinereus*.

On the other hand, some populations of *P. cinereus* have been found to decrease in the presence of earthworms. Caceres-Charneco and Ransom (2010) speculate it could be due to the effect earthworms have on the surrounding environment, such as leaf litter decay rates. Populations of *P. cinereus* have been found to decline in the presence of invasive earthworm species as leaf litter decreases (Maerz *et al.* 2009). This could be for several reasons. As organic matter decreases in an area, the density and species richness of arthropods found in the soil and leaf litter decreases as well (Battigelli *et al.* 2004). Decreased leaf litter may cause a smaller moisture and temperature buffer for *P. cinereus* (Maerz *et al.* 2009). As leaf litter is removed from the ground, water evaporates more quickly from the soil causing the environment to be unfavorable for *P. cinereus*. Currently, there have not been studies published examining whether or not the decreased leaf litter has a greater effect on *P. cinereus* through decreased food source or the lack of buffer to the environment.

Identifying the habitat preferred by *P. cinereus* is important for better understanding how landscape modification might affect the habitat selection of *P. cinereus*. The purpose of this experiment is to determine the leaf litter depth and soil aggregate size combinations preferred by *P. cinereus*. The hypothesis is that *P. cinereus* will be found more often in combinations with the greatest leaf litter depth and smallest soil aggregate size over combinations with no leaf litter and large soil aggregates. This hypothesis is expected because the greatest leaf litter depth and smallest soil aggregate size should trap the most moisture, making it the ideal habitat for *P. cinereus*.

Materials and Methods

In this experiment, 20 adult *Plethodon cinereus* collected on the UNDERC property (46' 13' N by 89' 32' W, Gogebic County, MI and Villas County, WI) were tested. Before and after testing, individuals were housed in terrariums with leaf litter and soil from the area where they were collected. The individuals were given food and water *ad libitum*, and were kept on a 12 hour light/dark schedule.

A six-chambered choice chamber was constructed from a steel cattle tank and plexi-glass dividers (see Figure 1). The six sub-chambers consisted of different combinations of leaf litter and soil aggregate size randomly selected. However, it was ensured that no two same leaf litter depths or soil aggregate sizes were side-by-side in the chamber. The large soil aggregate sub-chambers consisted of aggregates of various sizes with the largest ranging from 2 mm to 7 mm. The small soil aggregate sub-chambers consisted of aggregates of various sizes all less than 1 mm. The deep leaf litter sub-chambers contained leaves at a depth of 3.5cm (when the leaves were dry), and the average leaf litter sub-chambers contained leaves at a depth of 1.75cm (when the leaves were dry). All leaves and soil used in the choice chamber were dried in a drying oven for at least six hours, and the soil was then run through a fine sieve to remove large debris, earthworms, seeds, etc. After sifting, the soil in each sub-chamber was mixed with tap water until it measured approximately 50 percent relative saturation (measured using a Kelway soil tester) and a pH of 7.

Prior to each trial, the percent relative saturation was measured in each sub-chamber, and water was added as needed until the reading was approximately 50 with a pH of 7. At 16:30, an individual was weighed, and then held above the center of the chamber. The individual was then allowed to move into a sub-chamber. This initial location was recorded. A nearly transparent screen secured the choice-chamber to prevent any individuals from climbing out of the choice chamber. The individual inside was left to move freely about the choice chamber for 15 hours, and it's location was recorded at 18:30, 20:30, 22:30, 00:30, and 7:30. At 20:30, two dark screens were placed on top of the initial screen to keep the individual on a 12-hour light/dark schedule throughout testing and were removed only to record the individual's location. At 7:30, the following morning, the individual's final location was recorded, and the individual was removed from the choice chamber and weighed. The percent relative saturation and pH were recorded for each sub-chamber. The percent relative saturation and pH values were averaged for the first 18 trials to provide an overall moisture level for the cattle tank, but they were recorded individually for each sub-chamber for the final two trials. After each trial, the chamber was rotated 60^{o} clockwise to control for homing behavior.

Statistical tests were conducted in SYSTAT. The independent variables were leaf litter depth and soil aggregate size, and the dependent variable was the presence or absence of *P. cinereus* in the sub-chamber. Two chi-squared analyses were performed. One chi-squared analysis was performed using the data from the 7:30 observation period only (with the expected value fixed at 20 visits/6 chambers, or in other words, 3.33 visits/chamber). The second chi-squared test incorporated data from all of the observation periods (with the expected value fixed at 120 visits/6 chambers, or in other words, 20 visits/chamber). A two-way ANOVA was also performed on the data from all of the observation periods. A Tukey's post-hoc test was performed to test pairwise comparisons. Finally, a Mann-Whitney U Test was performed on the moisture levels between the large and small soil aggregate sub-chambers for the last two trials. Significant results were identified using an α value of 0.05.

Results

The chi-squared analysis of the number of visits per chamber at the 7:30 observation period showed a significant difference between the observed and expected values (df=2, p<0.005). The chi-squared analysis of the number of visits per chamber for all observation periods also showed a significant difference between the observed and expected values (df=2, p<0.005). Since there were significant differences, the observed values deviated from the even distribution expected, and a two-way ANOVA was run to determine where the deviations occurred.

In the process of running the two-way ANOVA, a Shapiro-Wilk test was run to determine normality. The data was not normal and was transformed by adding 0.5 and taking the square-root of the sum, and then the ANOVA was run.

Significant differences were observed between leaf litter depth (df=2, F=14.203944, p=0.000003) and soil aggregate size (df=1, F=7.512520, p=0.007) in the two-way ANOVA. No significant interaction was observed between the leaf litter depth and soil aggregate size. Tukey's post-hoc test was run on the leaf litter depth data, and significant differences were found between the different depths. Significant differences were observed between deep and no leaf litter (p=0.000001) and deep and average leaf litter (p=0.0002 Figure 2). No significant differences were observed between average and no leaf litter (p=0.162315). Large soil aggregates had significantly more recordings of *P. cinereus* use per observation period than small soil aggregates (Figure 3).

The Mann-Whitney U Test for the moisture levels between the large and small soil aggregate sub-chambers for the last two trials was not significant (df=1, p=0.342310 Figure 4).

Discussion

Based on the results, the original hypothesis that *P. cinereus* would prefer deep leaf litter and small soil aggregate combinations has been rejected. The current evidence indicates that *P. cinereus* prefers deep leaf litter over both average and no leaf litter, which agrees with the original hypothesis. However, the current evidence also indicates that *P. cinereus* prefers large soil aggregates over small soil aggregates, which disagrees with the hypothesis.

It was expected that *P. cinereus* would prefer deep leaf litter due to the microclimate created by the presence of leaf litter and the protection provided by leaf litter. The leaf litter provides a buffer for *P. cinereus* from extreme temperatures and moisture loss (Maerz *et al.* 2009). *P. cinereus* need to keep their skin moist so they are able to breath. Leaf litter aids them in this because it traps moisture in the soil, which enables the salamanders to stay under the leaf litter and against the moist soil. Additionally, the deep leaf litter provides added shelter for *P. cinereus* and allows them to move around and forage without fear of predation.

The fact that there was not a significant difference between average and no leaf litter could be due to small numbers. If there had been a larger overall sample size, then it is possible that a significant difference would have been found. However, these results could also mean that *P. cinereus* don't have a strong preference between little and no leaf litter, and that they need deep leaf litter in their habitat.

The microclimate created by the trapped moisture has been cited as one reason that *P. cinereus* prefer deep leaf litter, but the importance of the small arthropods found in the leaf litter has also been highlighted in this preference (Maerz e*t al.* 2009). However, in this study, since all leaves and soil used in the choice chambers were dried beforehand, it was possible to exclude food sources as the primary factor in their preference for deep leaf litter.

It was not expected that *P. cinereus* would prefer large soil aggregates because that is not what naturally occurs in their habitat, and the small soil aggregate chambers were expected to retain moisture better. However, the difference in moisture levels between the large and small soil aggregate sub-chambers was not significant, and while this could be due to small sampling size, it could also mean that soil aggregate size does not have a strong affect on moisture retention. The preference for large soil aggregates could be explained predominantly by a perceived sense of security. The sub-chambers that had larger soil aggregates had more small pits and mounds, which might have contributed to a greater sense of security. The individual might have felt better camouflaged when the terrain was uneven rather than when it was flat and even. This agrees with basic observations from the trials. During one particular trial, an individual remained in the no leaf litter chamber for five observation periods sheltered by three large soil aggregates and the barrier to the next chamber.

These preferences for deep leaf litter and large soil aggregates leads to a conflicting view on the effect certain species of earthworms have on *P. cinereus*. *P. cinereus* clearly prefers and needs leaf litter to survive. They are unable to breathe if their skin dries out, so the microclimate leaf litter creates is a necessity. The deeper leaf litter also likely acts as a source of security, which may provide cover from predators while they forage for food. On the other hand, the uneven terrain created by the larger soil aggregates lends itself as a potential source of added security. The pits and mounds created by the larger soil aggregates help to disguise the form of an individual and may eliminate some of the need for leaf litter as protection. These conflicting preferences call for further studies on the matter to continue to shed light on the relationship between *P. cinereus* and the impacts of specific species of earthworms on the forest around them.

An interesting area for further study would be to look at how the presence and absence of cover objects influences *P. cinereus*' preference for leaf litter depth. If a cover object is present in a chamber with little leaf litter and no cover object is present in a chamber with deep leaf litter, would the cover object be preferred by the individual over leaf litter? This would provide a better idea of *P. cinereus*' tolerance for disruption to their habitat. Another interesting area of study would be to do population estimates of *P. cinereus* on UNDERC property in areas of high earthworm density and low earthworm density, as well as, to take measurements of soil aggregate size and leaf litter depth in those areas. This would give a better idea of how the earthworms might already be affecting populations of *P. cinereus* at UNDERC and elsewhere in the Great Lakes area.

As determined by this study, *P. cinereus* prefers deep leaf litter and large soil aggregates. This knowledge is important because it will allow future studies that focus on these ideal habitats and how *P. cinereus* responds when earthworms bring about changes that deviate from their preferred leaf litter depth and soil aggregate size. Earthworms are continually invading the Great Lakes area and will continue to increase in density and affect the forest floor ecosystem. Further studies in this area will continue to advance our knowledge of what the future of these two organisms are and whether they will be able to coexist or whether one will have to migrate elsewhere.

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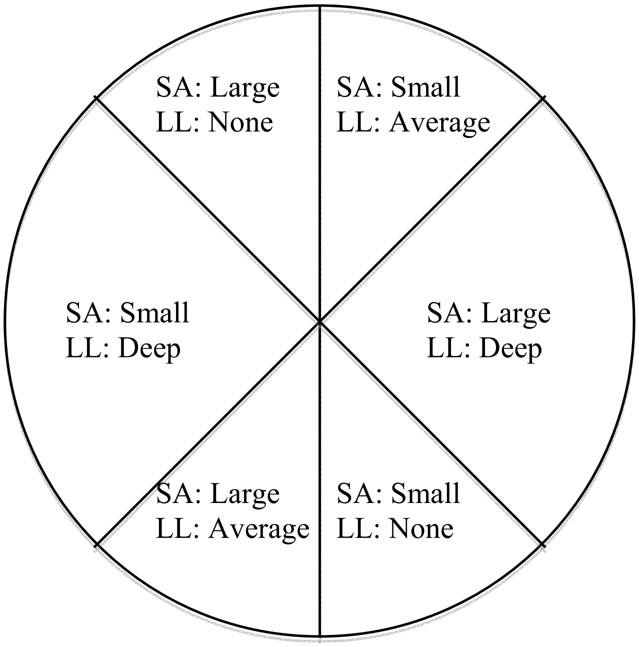

Figure 1. Diagram of choice chamber for *P. cinereus*. SA represents soil aggregate size. LL represents leaf litter depth.

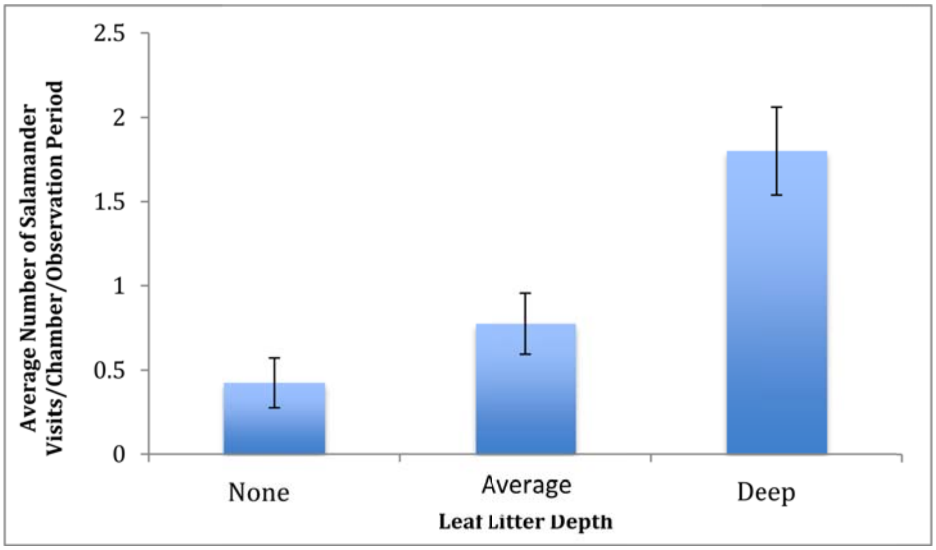

Figure 2. The average number of *P. cinereus* visits per chamber in an observation period based on leaf litter depth. Significant differences between deep and no leaf litter (p=0.000001) and deep and average leaf litter (p=0.000280) were observed. No significant differences were observed between average and no leaf litter.

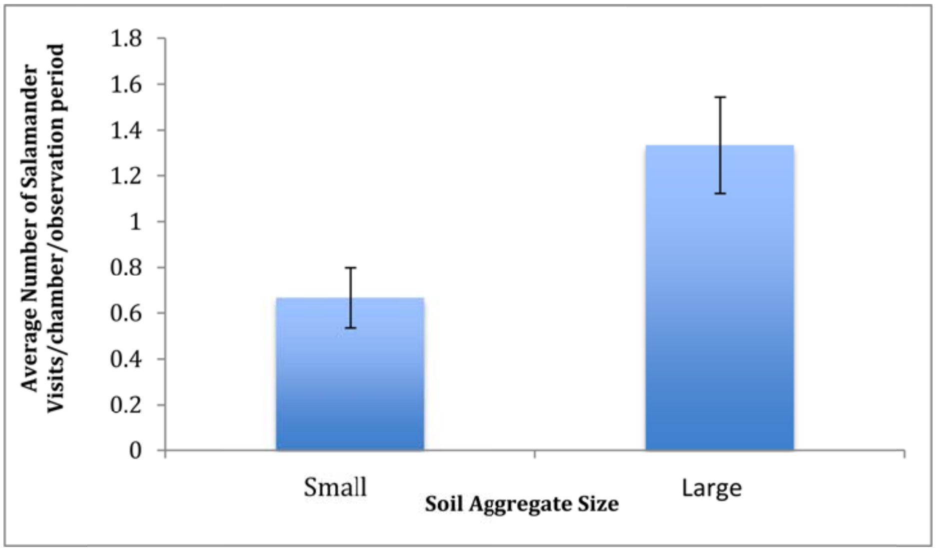

Figure 3. The average number of *P. cinereus* visits per chamber in an observation period based on soil aggregate size. Significant differences between large and small soil aggregates were observed (p=0.007125).

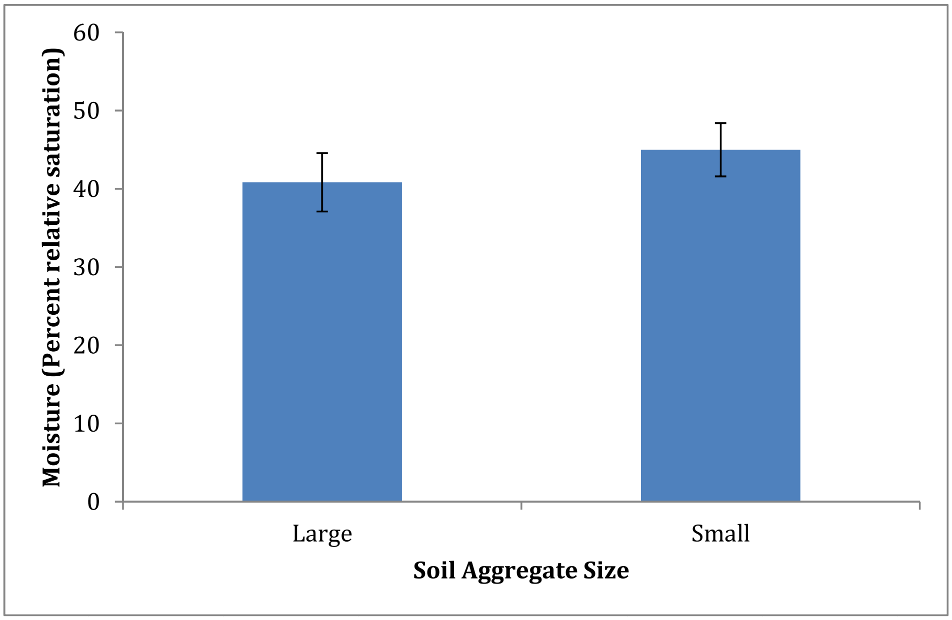

Figure 4. The average moisture levels of large and small aggregate sub-chambers for the last two trials. Differences between large and small soil aggregates was not significant (p=0.342310).