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The Effects of Abiotic Factors Upon the Distribution and Territory Selection of Red-Back Salamanders

Abstract: Abiotic factors soil moisture, soil pH, soil temperature, log decomposition level, log length, and leaf litter height were examined with relation to presence of the Red-back salamander, *Plethodon cinereus* in Northern Michigan forests. Sampling was carried out in four ecologically different sites – the moraine, outwash plain, gorge, and grapevine point – in and around the University of Michigan Biological Station. Two abiotic variables, leaf litter height and log length, were found to be significantly correlated to salamander presence under cover objects. Four abiotic variables –soil pH, soil temperature, soil moisture, and log decomposition – were not significantly correlated with salamander presence. We discuss further possible applications of the research in regard to climate change.

Introduction:

Red-back salamanders (*Plethodon cinereus*)are among the most abundant vertebrates in moist North American temperate forests (Riedel, 2007). Ranging from the Atlantic coast to eastern Minnesota, between the southern United States and southern Canada, Red-backs have a wide North American distribution (Conant and Collins, 1998). Despite their small size, on average 5.7-10 cm (Conant and Collins, 1998) they have been identified as a critical decomposer within their ecosystems (Matthewson, 2009). Their important environmental role led us to inquire what abiotic factors influence territory distribution.

Red-back salamanders are entirely terrestrial (Howard, 2003), as well as lungless, breathing via cutaneous gas exchange across the outer dermal layer. Consequently, all moisture must be absorbed from the immediately surrounding soil and leaf litter (USGS, 1997). This adaptation enables individuals to establish territories farther away from water sources than truly amphibious salamanders. Regardless, moisture requirements limit Red-back salamander distribution to temperate wooded and forested areas, (Conant and Collins, 1998) such as the Maple-Aspen-Oak forests of Northern Michigan (Heatwole, 1962).

Commonly found burrowing under wood, rocks, or leaf litter (Hutchins and Duellman, 2003), the Red-back salamander must remain sheltered during the day to avoid desiccation (Conant and Collins, 1998). Primary food sources in these habitats include earthworms, flies, and small terrestrial arthropods such as mites and beetles (Hutchins and Duellman, 2003). Within a forest ecosystem, Red-backs typically establish small to moderate sized home ranges averaging 0.16-0.33 m^2, each encompassing one or more nesting/feeding sites referred to as activity centers (Zug et al., 2001). In general, a single male will occupy a territory at a time, marking boundaries via pheromones produced by glands near the cloaca (Hutchins and Duellman, 2003).

Many scientists, such as Robert Jaeger, have conducted research on Red-back salamander behaviorisms. As a result, there is an abundance of data on territoriality, mating rituals, and on population density in relation to prey abundance. However, there is a lack of research on the abiotic factors affecting territory choice in the area surrounding UMBS. By examining abiotic factors such as moisture level, pH, and log decomposition level we aimed to determine their relative significance in Red-back salamander territory choice. We predicted that several abiotic factors would show a significant correlation to habitat choice. Soil moisture, soil pH, and soil temperature were expected to affect distribution because of Red-back salamanders' unique circulatory and respiratory mechanisms. The chemical interaction between the soil and logs potentially alter decomposition rates; therefore we expected the extent of log decomposition to influence territory selection. Log size may have affected the rate and extent of decomposition, in turn influencing territory selection. Finally, the accumulation of leaf litter potentially alters decomposition rate, offers protective cover, and provides a source of prey.

Materials and Methods:

To compare the influence of various abiotic factors on salamander distribution, we examined logs at four ecologically different sites to increase the range of the measured abiotic variables. The table below describes the initially observed abiotic differences that led us to choose these research sites.

Research Site	Description			
	Tree Species Present:	Relative Humidity:	Relative Light Availability:	Soil Characteristics:
Moraine	Beech, Maples, Oak, Aspens	High	Low	Relatively moist, small particulate matter
Outwash Plain	Aspens, Pines, Maples, Beech, Oaks	Low	High	Sandy, dry, large particles
Gorge	Birch, Maple, Oak, Beech, Pine	Very high Nearby stream	Intermediate	Extremely moist, small particulate matter
Grapevine Point	Birch, Maple, Oak	Intermediate Nearby lake	Intermediate	Relatively moist, small particulate matter

Logs, abundant at each site, were chosen at random. Salamander presence under a log indicated a preference for that territory. At each log, we measured multiple abiotic factors that might affect salamander territory choice. A total of 60 logs were sampled: 10 from the outwash plain, 10 from the gorge, 10 from grapevine point, and 30 from the moraine. More logs were sampled in the moraine because the other three sites offered fewer logs with salamanders.

The pH of each log was measured using a soil pH testing kit. One to four drops of indicator and a corresponding color chart were used to assess pH. Temperature of the soil directly underneath the log was measured using a soil thermometer. Samples of soil were taken from each cover object, sealed in plastic bags to minimize evaporation and weighed immediately after fieldwork. Samples were then dried in an oven set to 85°C for 72 hours and reweighed. We calculated the percent change in weight to numerically represent soil moisture. Leaf litter height and log length were measured using a small ruler (cm) and tape measure (m), respectively.

Log decomposition rank was decided according to a qualitative scale. Logs were ranked based on appearance (ex: presence of mold) and pliability. The table below details our self-derived scale.

Numerical Decomposition Rank	Description of Log
1 (lowest level)	Hard wood, intact bark, fungus/mold absent
2	Fairly hard wood, most bark intact, little to no fungus/mold
3	Moderately malleable (mild moisture), 50% or less bark intact, can have some fungus/mold
4	Very soft wood (moderate moisture), little to no bark, some disintegration, moderately abundant fungus/mold
5 (highest level)	Extremely soft (high moisture), much of the log has disintegrated, little to no bark, abundant fungus/mold

Soil moisture, which we predicted would have an effect upon salamander distribution, increases with level of log decomposition. We created a manipulative experiment to investigate the hypothesis that log decomposition level determines salamander distribution. For this experiment, we assumed that salamanders chose their territory from a wide array of available cover objects. A high density of logs was placed in a relatively small environment to ensure that salamander habitat choice was based on preference for one log over another, rather than availability. 16 logs of various decomposition levels were evenly spaced 1 meter apart within a grid and marked with colored string. Grid location was selected to provide an accurate representation of the moraine's ecology. After leaving this site undisturbed for seven days, we returned to document the presence or absence of salamanders under each cover object.

Results:

We ran a series of statistical tests on our data using SPSS 15.0. In order to determine whether each variable had an effect on salamander distribution, we compared the distributions or mean values of each variable within our two sample groups: logs with salamanders and logs without salamanders. Of our six variables, five were quantitative: soil temperature, soil pH, leaf litter height, log length, and soil moisture content. The sixth variable, state of decomposition, was a qualitative variable. Decomposition level was not measured numerically; rather, the logs were arranged into qualitative, numbered categories based on their relative state of decomposition.

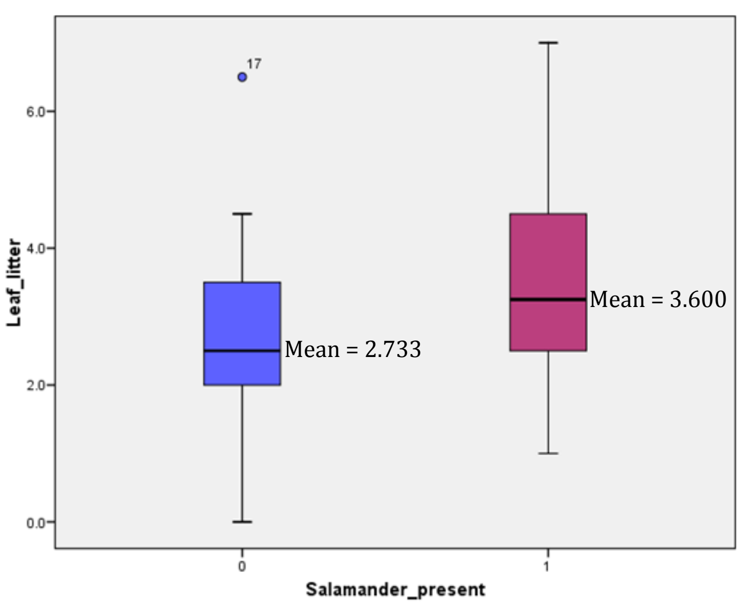
Soil pH, soil moisture, and leaf litter height met the assumptions of independent sampling and normal distribution, so we compared their means between the two sample groups using an independent-samples t-test. Normality was assessed using skewness and kurtosis values. For the data sets that were not normally distributed – soil temperature and log length – we used a less-powerful, non-parametric alternative to compare means: the Mann-Whitney U test. Log decomposition was assessed using a Chi-Square test in order to compare the distribution of a qualitative variable between two sample groups. Our log decomposition data met the two assumptions of a Chi-Square test: independent, random samples and a sufficiently large sample size.

Our independent samples t-tests found that leaf litter height was statistically significant, but soil moisture and soil pH were not significant. We rejected the null hypothesis that leaf litter height does not influence salamander territory selection. However, we could not reject the null hypotheses that soil moisture and soil pH do not affect salamander territory selection. From the Mann-Whitney U test, we rejected the null hypothesis that log length does not affect salamander territory selection, and is a statistically significant influence upon salamander distribution.

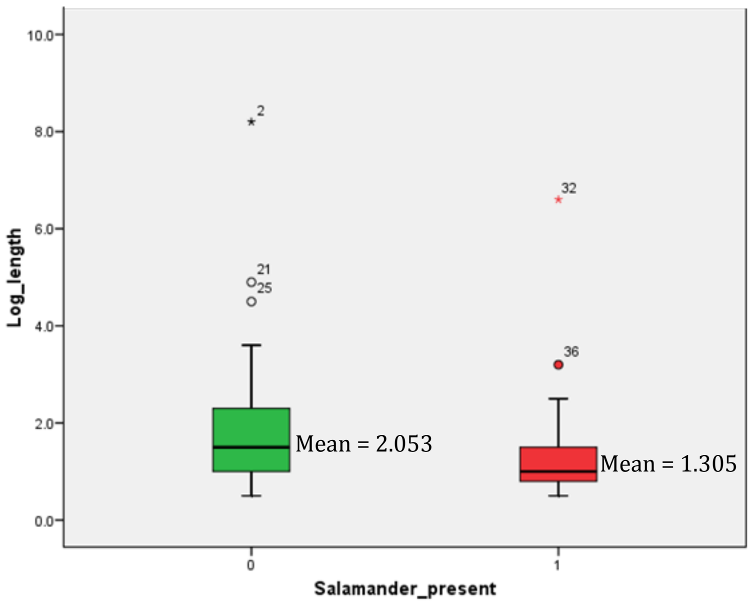
However, we could not reject the hypothesis that soil temperature does not affect salamander territory selection. From the Chi-Square test, we could not reject the null hypothesis that there was no difference in log decomposition distribution between our sample groups. Our manipulative experiment did not yield any significant results.

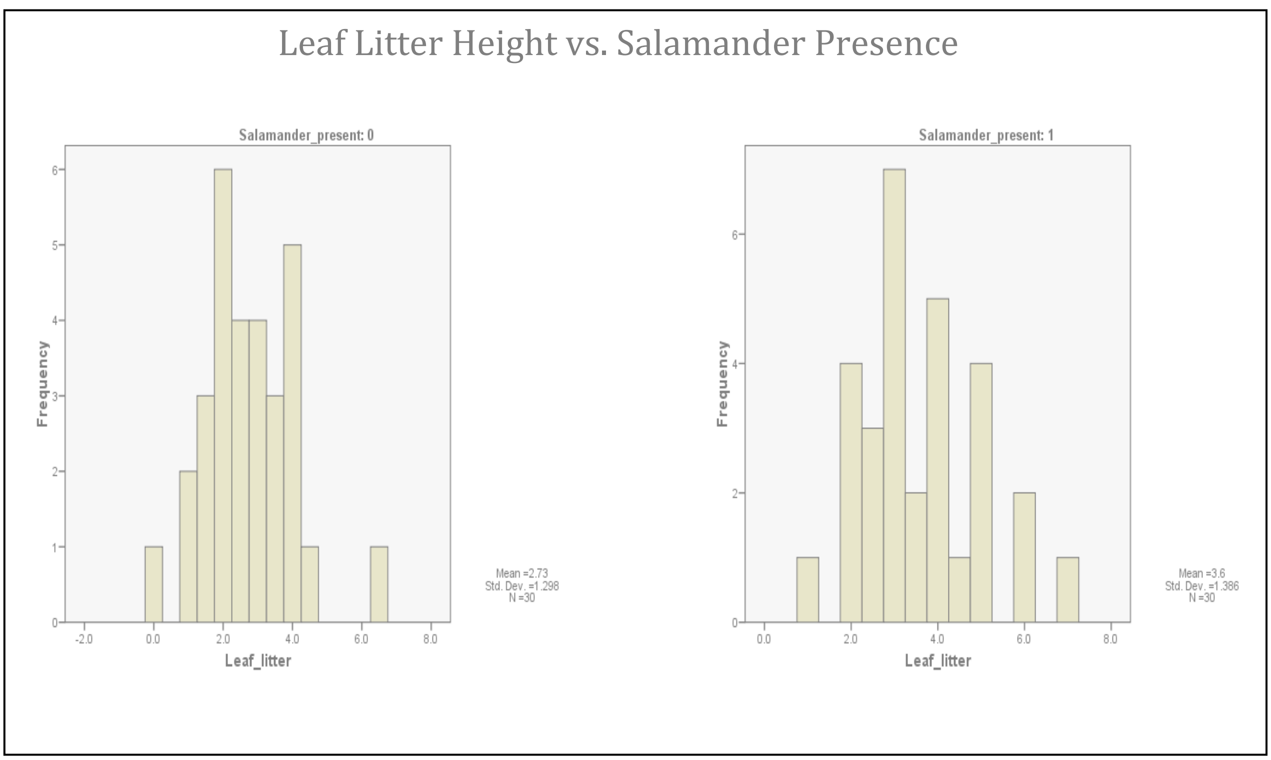
Box plots were generated to visually compare the means and variances of our two statistically significant variables: log length (m) and leaf litter height (cm). Histograms were used to show the distributions of all of our quantitative variables. In addition, pie charts were used to show the distributions of log decomposition, our qualitative variable. In each of the figures below, the x-axis value of 0 indicates salamander absence and 1 indicates salamander presence.

Leaf Litter Height (cm) vs. Salamander Presence

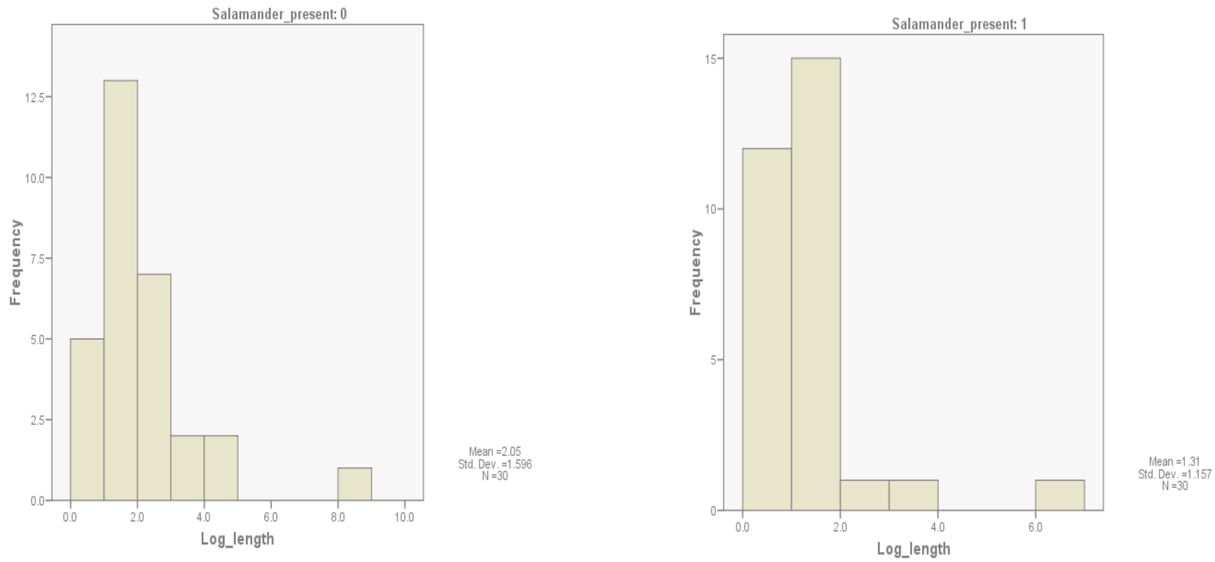


Log Length (m) vs. Salamander Presence

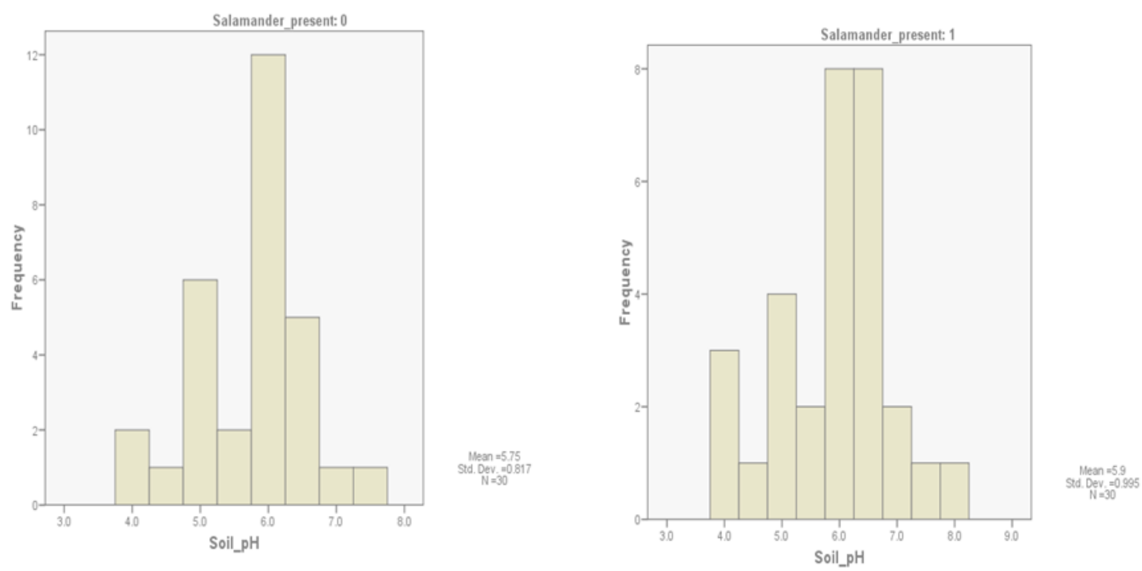




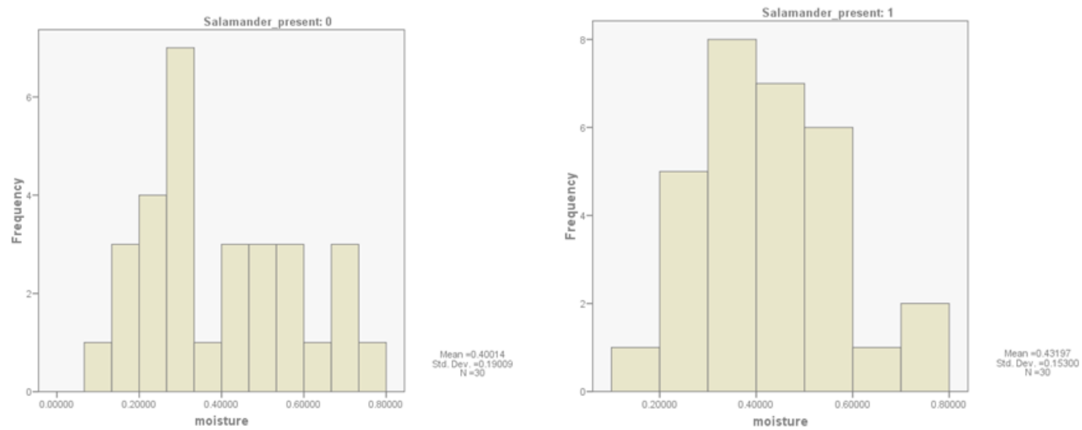
Log Length vs. Salamander Presence



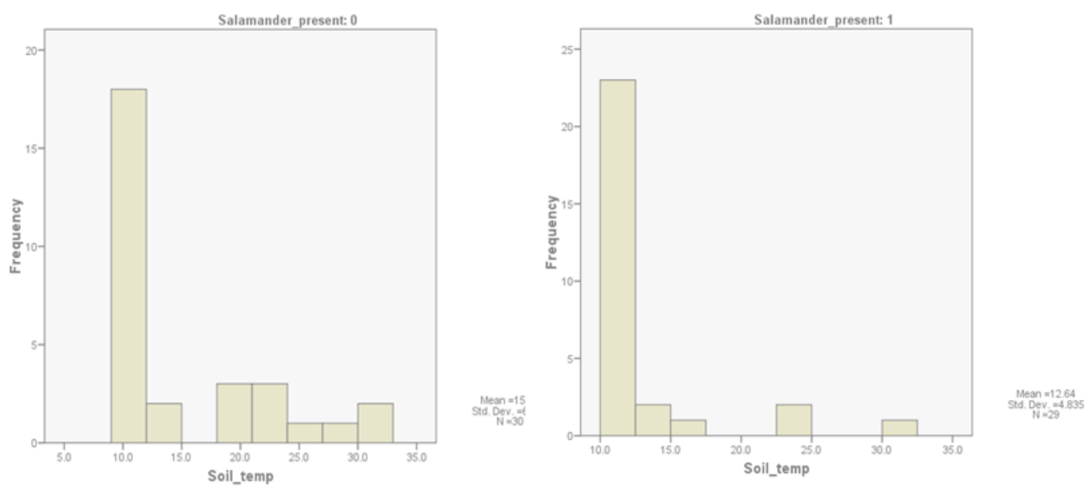
Soil pH vs. Salamander Presence



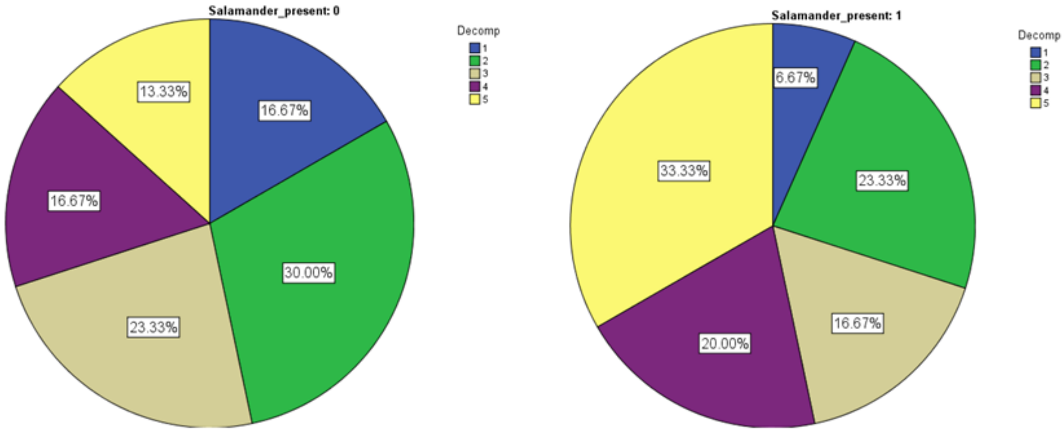
Soil Moisture vs. Salamander Presence



Soil Temperature vs. Salamander Presence



Chi-Square Results Comparing Log Decomposition Distribution



No Salamander Salamander

Statistical Data Analysis

Variable	Salamander	N	Test Statistic	P-value
Soil pH	Y	30	t = -0.638	P = 0.526
	N	30		
Soil moisture	Y	30	t = -0.714	p = 0.478
	N	30		
Soil temperature	Y	29	MWU = 342.000	p = 0.158
	N	30		
Log length	Y	30	MWU = 255.000	p = 0.004
	N	30		
Leaf Litter Height	Y	30	t = -2.500	p = 0.015
	N	30		
Log Decomposition	Y	30	Chi-Square[a] = 2.667	p = 0.615
	N	30	Chi-Square[a] = 5.667	p = 0.225

Discussion:

After using SPSS to analyze the data collected in the field, we found that only two of the abiotic environmental factors examined showed a significant correlation to salamander presence: leaf litter height (cm) and log length (m). These correlations implicate both leaf litter height and log length as abiotic factors capable of influencing *P. cinereus* abundance. From this, we made two extrapolations regarding salamander habitat preference. First, Red-back salamanders prefer to inhabit the areas surrounding UMBS with relatively high leaf litter. This finding is congruent to a similar experiment conducted at UMBS in 1994, which also found this to be a significant factor, as well as a preference for higher leaf litter (Jarvis et al., 1994). Another study by Riedel states that the abundance of salamanders increases as herbaceous vegetation and leaf litter increase (Riedel, 2007). These findings led us to conclude there may be a minimum leaf litter height required for salamanders to inhabit an area. Distribution may therefore be restricted to ecosystems where leaf litter can accumulate. Second, Red-back salamanders territory choice may be limited to ecosystems in which relatively small logs are available as cover objects.

A preference was demonstrated for high leaf litter and relatively small logs. To explain the existence of these preferences, we returned to the natural history of Red-back Salamanders. Recalling that Red-backs are entirely terrestrial and breathe via cutaneous gas exchange, we hypothesized that leaf litter height plays a role in the salamander's ability to regulate both body temperature and moisture levels. Red-back salamanders are burrowing amphibians and seek relief from atmospheric temperature stress on the surface by retreating deeper into the soil (Hutchins et al., 2003). Leaf litter, as opposed to sand or rocks, is a substrate through which *P. cinereus* can easily burrow in order to avoid desiccation. Leaf litter accumulation enhances their predatory success. Red-back salamanders, primarily nocturnal hunters, are commonly found foraging on the surface of leaf litter (Heatwole, 1962).

The abundance of prey species on this substrate creates an ideal foraging environment (Riedel, 2007). These behavioral adaptations led us to conclude that Red-back salamanders prefer higher leaf litter. There is no research to explain why we found a correlation between log length and salamander presence. The Red-back's preference for log length may have been the result of a preference for tree species with rates of decomposition resulting in logs of a smaller size. It is also important to note that our physical limitations only allowed us to sample smaller logs.

The other abiotic variables we measured – soil pH, soil moisture, soil temperature, and decomposition level – all failed to display significant correlations to Red-back salamander presence under logs. From this data, we demonstrated that none of these variables influence the salamanders' distribution, nor do they play a role in an individual salamander's territory choice. These results were contrary to our predictions given the Red-back salamander is lungless and dependent upon its surrounding environment for moisture. In addition, high rates of decomposition are indicative of moisture-rich environments. For these reasons, we originally predicted that moisture and decomposition levels would show a strong correlation to habitat preference.

Although our SPSS analysis of the data did not display a significant correlation between these abiotic factors and Red-back salamander habitat preference, we did observe a trend in the log decomposition level data. Given that we sampled equally among all levels of decomposition, and assuming that decomposition level was irrelevant to territory preference, the number of individuals we expected to find under each log (6 individuals) should match the observed values. Instead, we observed a large disparity between the expected and observed values. Of the 30 salamanders found in the field, 10 were found beneath highly decomposed level 5 logs, while only 2 were found inhabiting slightly decomposed level 1 logs. Based on these results, salamanders appeared to prefer logs of a higher decomposition level. However, the number of logs sampled was not large enough to show a statistically significant correlation.

Our finding that pH did not significantly influence Red-back salamander presence is contradictory to the results of several other studies: a four-year New York salamander census study by Wyman indicated that Red-back salamander distribution and density is affected by soil pH (Wyman and Hawksley-Lescault, 1987). A laboratory experiment on the salamanders found that growth and respiration were greatly reduced in soils below a pH of 4 (Wyman 1988). None of the territories we sampled had soil with a pH below 4, so it was never a limiting factor for the Red-back salamanders we found. This provided a possible explanation for why we failed to observe a correlation between pH and salamander presence.

Similar correlative studies conducted on Red-back salamanders have found that although these amphibians demonstrate a preference for moist soil (Jarvis et al., 1994), they are also able to tolerate a wide range of moisture levels (Messere and Ducey, 1997) (Hawksley-Lescault and Wyman, 1987). The ability of Red-back salamanders to survive in environments that vary in moisture content may explain why we did not find a significant correlation between percent soil moisture and salamander presence. The same two studies also found that distribution was not affected by temperature (Messere and Ducey, 1997) (Hawksley-Lescault and Wyman, 1987). These patterns appeared to indicate that this salamander is a generalist whose physiological tolerances enable it to survive in many different environments.

In addition to our correlative studies, we also conducted a manipulative experiment. Unfortunately, we did not obtain significant results. The most significant limitation was our week-long time constraint. After a single week, the soil remained unaffected by the log decomposition level. The lack of results may have also been due to our high level of impact on the area. For example, salamanders may have sensed disturbances as we arranged the grid. An alternate explanation is that salamanders were present in our plot, but had burrowed beneath the soil. We did not acquire a license to handle vertebrates and were therefore unable to search beneath the top soil layer. A final possible explanation for our lack of results is that we chose an atypical area for the experiment.

Research Limitations/Future Research:

In the course of our research, we encountered several possible confounding variables. One possible confounding variable is weather. According to a study by Williams and Berkson, higher temperatures led to a lower likelihood of finding salamanders in the daytime (Williams and Berkson, 2004). Thus, on those days when the temperature was higher, salamanders may have been less abundant in our samples. Williams and Berkson's study states that salamanders are more active during rainfall (Williams and Berkson, 2004). Consequently, on rainy days we may have encountered more salamanders solely because of the high top soil moisture content. Our perceptual limitations may have created other confounding variables. For example, olfactory cues, imperceptible to humans, may have influenced territory choice. Females use olfactory cues from male pheromones during the mating season to seek mates and choose a territory (Zug et al. 2001). Our study was conducted during the typical *P. cinereus* mating season (Zug et al. 2001) which may have affected our sample size.

As with any experiment, human limitations and error can skew results. There are two types of limitations: perceptual and logistic. Perceptual limitations included the fact that we could only sample the surface as opposed to beneath the soil or inside the logs. Another limitation is the fact that humans cannot perceive the chemical cues of a Red-back salamander. Our first logistical limitation was a lack of time for gathering samples. A second logistical problem was our inability to search under larger, heavier logs. Possible sources of error were length and weight measurement errors, contamination of the pH kit, and technology malfunctions (ex: scales).

There are many possible avenues of continued research based on what we learned in this three-week study. For example, researchers could examine preference for a particular type of wood and how canopy cover affects log decomposition rates. This would enable scientists to determine how changes in forest biodiversity can affect salamander distribution. In the face of rising global temperatures, it may be important to further examine the ratio of moisture content within the different soil layers as it relates to salamander burrowing behavior. As temperature increases, the stratification of moisture throughout soil levels may be disproportionately altered. As of now, the IUCN lists Red-back salamanders as "Least Concern" (IUCN 2004). However, if the predictions of the IPCC report are accurate, Red-back salamanders could be negatively impacted by climate change. Increased temperatures may lead to a loss of habitat, and possibly even a biome shift in the Northern Hemisphere. These changes could substantially alter their range of inhabitable territory. As more research is conducted on the ecological relationship between abiotic habitat characteristics and Red-Back salamanders, we continue to learn how to both preserve the *P. cinereus* species and its ecosystem in the face of global environmental changes. 32% of amphibian species are currently threatened; further research into Red-back salamander natural history can prevent them from ever being added to this list.

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