Study of Dispersion Rates for Individuals of *Aporrectodea* spp.,

Lumbricus terrestris, and *Lumbricus rubellus*

With and Without Presence of Leaf Litter

Practicum in Field Biology

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Abstract

Attention has been recently cast upon the environmental impact of invasive earthworms into the Northern hardwood forests of the United States. Invading earthworms have been implicated in decreased soil carbon, nitrogen, and phosphorous levels, along with decreased leaf litter, mixing the organic and mineral horizons, and alterations of native flora assemblages. Invasions undergo a succession-like pattern of species abundances, measured with relation with the leading edge of migration. The leading edge of migration is marked by decreased litter mass. Invasion dynamics have been studied for populations, but no research has been done on movement of individuals within earthworm assemblages. Movement of individuals of *Lumbricus terrestris*, *Lumbricus rubellus*, and *Aporrectodea* spp. were studied using visual implant elastomer (VIE). Comparisons were made between plots with and without leaf cover to mimic conditions on either side of the leading edge. *Aporrectodea* spp. moved an average of 16.0 cm, *L. rubellus* moved an average of 44.5 cm, and *L. terrestris* moved an average of 25.8 cm on untreated plots, following relative invasion dynamics for populations. No significant differences in movement between treatments were found for *Aporrectodea* spp. and *L. terrestris*. Non-significant trends were found for *L. rubellus* ($p=0.099$) in which individuals moved further in plots with leaves. Because measured maximum distances for tagged *L. rubellus* and the presence significantly less *L. rubellus* on treated plots, it may be possible that individuals leaf the sampling area, more research needs to be done to determine their movement potentials. *L. rubellus* preferred plots with leaves, and *Aporrectodea* spp. seemed to prefer plots without leaves, mimicking invasion dynamics along the leading edge.

Introduction

Attention has been recently cast upon the environmental impact of invasive earthworms into the Northern hardwood forests of the United States (Bohlen et al. 2004b, Hale et al 2005b, Holdsworth et al. 2007b). It is believed that the most recent glacial period is responsible for the local extinction of native species of earthworms (Bohlen et al. 2004a). Human activity, including fishing, development, and agriculture has contributed to the introduction and spread of earthworm species from Europe and Asia (Keller et al. 2007, Tiunov et al. 2006, Holdsworth et al. 2007a). Earthworm populations have been found to be significantly, negatively correlated with distance from the roads and cabins in the Chippewa and Chequamegon National Forests (Holdsworth et al. 2007a). Given the rate at which earthworm assemblages can migrate, the extent of the effects will only increase with time. Gundale et al. (2005) concluded that 91.7-

98.9% of sugar maple (*Acer saccharum)* dominated regions of the Ottawa National Forest are within the 100 year theoretical invasion distance.

Invasive earthworms affect nutrient cycling and leaf litter mass. Earthworms can decrease the soil C:N ratio and contribute to the loss of phosphorous and nitrogen through burrowed macropores (Bohlen et al. 2004a). The earthworm-induced changes in soil composition have had adverse effects on the plant community, which has affected the distribution of native species. The decreased mass of the forest floor has decreased of the viability of seeds, has led to the mortality of shallow rooted saplings and has increased predation on insect populations (Hale et al. 2005b, Holdsworth 2007b). Earthworm invasion has also facilitated the invasion of exotic plants, further alternating the natural ecosystem (Frelich et al. 2006, Holdsworth 2007b).

The recent understanding of earthworms' ecosystem-wide effects has caused the need to study the invasion dynamics of the earthworm populations in order to learn how they are spreading. This information will provide valuable incite not only about the manner in which they move, but also the rate at which their effects are occurring.

A recent invasion dynamics study was conducted by Hale et al. (2005a) in the woods of Minnesota. Earthworm presence is a recent phenomenon in those woods, and so they were able to study the movement of assemblages through virgin soil. According to the study, the rate of movement and effects of earthworm species appear to be particular to their ecological groups. Three general, descriptive categories are assigned based on relative depth of habitat selection. Epigeic earthworms (e.g., *Dendrobaena* spp.) live in and exclusively feed on the litter layer. Anecic species (e.g., *Lumbricus terrestris*) are deep dwelling earthworms but feed on surface litter (Hendrix et al. 1999). Endogeic earthworms (e.g., *Aporrectodea* spp.) live in the mineral

soil layer and prefer to feed on the organic components of the soil. Because of its unique ability to reduce leaf litter biomass and its habitat selection, *Lumbricus rubellus* is often put in its own ecological group of epi-endogeic (Hale et al. 2005a). *L. rubellus* is found ahead of the leading edge of assemblage migration. *Aporrectodea* spp. and *L. terrestris* are generally found behind the leading edge where leaf litter has already been reduced.

Even though the dynamics of earthworm population migration are vital to the collective understanding of their environmental effects, the movement of individuals within populations has not been studied. The reason lies in the fact that the population as a whole has a slow annual rate of movement, and more importantly, individuals within a population are difficult to identify (Butt & Lowe 2006). However, with the innovation of a tagging method using visual implant elastomer (VIE), this is now possible. Butt and Lowe (2006) have shown that the intermuscular tag does not interfere with maturation or reproduction, and that the plastic tag can last for at least 12 months.

The objectives of this study are 1) to measure and 2) to compare the dispersion rates of *Lumbricus terrestris* (anecic)*, Lumbricus rubellus* (epi-endogeic) and *Aporrectodea* spp. (endogeic) between area of the forest floor with and without leaf cover. These treatments are designed to estimate rates of movement under specific environmental conditions in front of and behind the leading edge of assemblage migration (Hale et al. 2005a). I hypothesize that both *L. terrestris* and *L. rubellus* move further in areas that do not contain leaves since they primarily feed on decaying leaf matter and would therefore search for recourses. *Aporrectodea* spp. are not expected to travel significantly faster because leaves are not their primary food source. The results of this study could provide a valuable insight into the movement of individual earthworms

within populations, which can reveal a more complete understanding into patterns of migrations as a whole.

Materials and Methods

Earthworms in the study were collected from sites on the UNDERC property using a combination of the electroshocking (Bohlen et al. 1995) and mustard powder technique (Lawrence & Bowers 2002). A portion of *L. rubellus* and *L. terrestris* were also purchased from bait shops. All of the purchased earthworms were sorted and identified for confirmation because of the ambiguity of common earthworm names (Keller et al. 2007). All earthworms were tagged using visual implant elastomer (VIE) (Butt & Lowe 2006). Tags were injected below the clitellum and into muscular tissue to minimize damage. Only viable tagged earthworms were released at the plots.

Ten sites were chosen in a sugar maple (*Acer saccharum*) dominated forest. Sites were selected to have a three meter radius free of large woody stems. All sites were located within a one hectare acre plot and at least thirty meters from the road. The proximity of sites was designed to minimize confounding differences in soil moisture and soil nutrient availability. All leaves and woody debris within a three meter radius were removed from half of the sites (N=5). The remaining sites (N=5) were left undisturbed. At each site 100 tagged *Aporrectodea* spp., fifteen *L. rubellus*, and twenty *L. terrestris* were released simultaneously at the center of the plot.

Eight of the ten locations were chosen haphazardly for sampling after one week. Because of time restrictions not all of the plots could be sampled on the same day. Two sites were sampled sevens days after release, four sites eight days after release, and two sites nine days after release. At each plot, three transects were made using two sets of electroshockers. The first transect started at the release point and extended approximately 1.7 meters in the direction of

magnetic north. The second and third transects also started at the release point but were spaced

120° from north to allow for maximum spacing between the transects.

Each transect was sampled for forty continuous minutes. The orientation of the shockers

was changed halfway through the sampling. In the first orientation, the shockers formed two

parallel lines approximately 1.7 meters in length and 0.5 meters apart. In the second orientation,

the shockers formed four parallel lines 60 cm long (the length of one shocker) and perpendicular

to the first orientation. The same area was sampled in both orientations. During the sampling,

the earthworms were collected by hand and were visually inspected in the field for the presence

of a VIE tag.

To determine whether post-migration earthworm distributions and untagged earthworm

recovery between sites were significantly different, independent-sample t-tests were run.

Results

On the one week plots (Sites 1-8), 44% of tagged *Aporrectodea* spp., 8.1% of *L. terrestris*,

and 12.5% of *L. rubellus* were recovered. The number of untagged *L. terrestris* did not differ

between the treatments; however, the number of untagged *L. rubellus* (p=0.017) and untagged

Aporrectodea spp. (p=0.025) did differ significantly between treatments. Significantly less *L.*

rubellus were sampled at sites without leaf cover (Figure 1a). Inversely, a greater number of

Aporrectodea spp. were sampled at sites without leaves (Figure 1b).

The average movement for *Aporrectodea* spp. on the untreated plots was 16.0cm

(N=148) and 14.4cm on treated plots (N=237). The majority of *Aporrectodea* spp. were

collected five centimeters from the release point (Figure 2). There was no significant difference

in the distance traveled between the two treatments for *Aporrectodea* spp. (p=0.283).

The average distance moved by *L. rubellus* individuals was 44.5cm (N=10) on the untreated plots and 3cm (N=5) at sites without leaves. *L. rubellus* individuals were not recovered past five centimeters from the release point on plots that did not contain leaf cover (Figure 3). For distance traveled by *L. rubellus*, there was a non-significant trend of mean distance moved (p=0.099) and maximum individual distance moved (p=0.073) with *L. rubellus* traveling further on plots with leaf cover (Figure 3).

L. terrestris traveled an average of 25.8cm (N=6) on untreated plots and 11.4cm on treated plots (N=7). Even though the difference between the average distance traveled during each treatment was not significant for *L. terrestris* (p=0.313), individuals were captured at a further distance on plots with leaf cover (Figure 4).

Fewer earthworms were recovered at the three week time point. No *Lumbricus* spp. were recovered from either plot. It appears that after three weeks, the *Aporrectodea* spp. on average travel further when no leaves are present (mean=20.4cm), than when leaves are present (mean=15.6cm; Figure 5). Since replicates were not preformed, no statistical inferences could be made. On the treated plot, the three week mode distance (20cm) was further than one week mode distance (5cm; Figure 6). The modes did not differ for the untreated plots between the separate time points (5cm; Figure 7).

Discussion

The hypothesized effects of leaf cover on movement produced mixed results. No difference was expected for *Aporrectodea* spp., and no difference was seen. The movement of *Lumbricus terrestris*, on the other hand, was expected to differ between the plots but did not. *Lumbricus rubellus* was also expected to travel further, but the results suggest that the loss of leaf cover may have hindered its movement. Looking at other data for *L. rubellus* provides an

possible alternative explanation for the observed movement patterns (discussed later). The data

for movement of individuals within a population followed the relative rates of population

movement between the species. The figures, however, were much larger than expected.

Aporrectodea spp. have been shown to have relatively slow population movement rates

1.8-3.3 m/year (Marinissen & van den Bosch 1992). At 'maximum' population expansion rate

of 3.3 m/year earthworms would be expected to move approximately 6.8cm per week assuming

normal conditions. The average movement measured in untreated plots was 16.0cm, which is

approximately 2.5 times greater than Marinissen and van den Bosch's (1992) measurements.

Likewise, the anecic dwelling *Lumbricus terrestris* showed rates almost 3.5 times greater than

the reported colonization rate of about 4 m/year (Marinissen & van den Bosch 1992). *Lumbricus*

rubellus, the earthworm with the quickest colonization rate of 7.5-10 m/year (Hale et al. 2005a

and Marinissen & van den Bosch 1992) showed rates of movement 2.3 times greater than its

upper end colonization rates. These data suggest another factor is driving their extraordinary

displacement. When comparing the one-week and three-week movement figures, a difference

between the total displacements is not observable under either condition. This information

suggests that the earthworms conducted their movement in the first week of study. The

earthworms were placed at a high density of 135 earthworms at the release point. It is likely that

during the first week, they actively spaced themselves to decrease their density.

As predicted, *Aporrectodea* spp. did not show statically different median movement

between the treatments (p=0.283). *Aporrectodea* spp. do not feed on dead leaves, rather

preferring to feed on soil organic matter (Hendrix et al. 1999). Without need to locate resources,

Aporrectodea spp. would not need to increase range of movement. Even though no difference in

movement was measured, significantly more untagged *Aporrectodea* spp. were recovered from

plots that had their leaves removed (Figure 1b). This result was not predicted. Even though

Hale et al. (2005a) showed that *Aporrectodea* spp. biomass was significantly greater behind the

leading edge, these results were based on long-term naturally progressing invasions. This study

was conducted occurred over a one week period. The radius of the transects was approximately

170cm; the radius of the treated area was 300cm. It is therefore unlikely that the *Aporrectodea*

spp. traveled over one meter in a week to the sampled area based on their population movement

rates of 1.8-33 meter/year. In this study the average maximum distance traveled for

Aporrectodea spp. was 68cm, well under the 130cm required for the earthworms to enter the

sampled area. A possible explanation for the difference between the sampling frequency could

be a difference in soil moisture resulting from the exposed plots. Rushton and Luft (1984)

concluded that extract efficiently was most strongly related to soil moisture. Precipitation fell

during the study week, and it may be possible that less water ran off from the exposed soil than

the ground covered by leaves leading to higher soil moisture levels. Without any conclusive

measure, however, it cannot be known whether soil moisture levels were responsible for the

difference.

Opposed to *Aporrectodea* spp., *Lumbricus terrestris* does feed on leaves (Hendrix et al.

1999). Based on the same logic used to discuss *Aporrectodea* spp. movement, *L. terrestris*

would have been expected to travel further on treated plots in search for leafy detritus. The

results of this study do not support this conclusion. *L. terrestris* did not travel further on treated

plots (p=0.313). One possible explanation could be that the time period allowed for movement

was not sufficient to measure a significant result. *L. terrestris* has been shown to move

approximately 4 meter/year (Marinissen & van en Bosch 1992). One week may not be sufficient

for measuring a difference.

The percent recovery of tagged individuals was low for the Lumbricus individuals and at the three week time point. Only an average of 1.6 (8.1%) of the tagged *L. terrestris* individuals were recovered. There are three possibilities for the absence of the remaining individuals: 1) they died 2) they were outside of the sampling area 3) they avoided the sampling method. Based on the data that were collected, it is unlikely that *L. terrestris* individuals were outside of the sampling area. The single furthest individual was collected at 55cm from the dispersion point, one third of the sampling distance. It may be possible that some of the anecic earthworms avoided electroshocking, however this is not likely to be the best explanation because of the relative success of the methods for extracting untagged individuals (8.2 *L. terrestris*/plot). It does seems likely that some of the individuals died after being released.

L. rubellus showed a trend between the treatments for average distance traveled (p=0.099) and maximum distance traveled (p=0.073) between the treatments. This tend was in opposition to the hypothesis that *L. rubellus* would travel further on plots that did not have leaves. These data seem to suggest that *L. rubellus* would move further when leaves were present. Another result that must be considered is the significantly fewer untagged *L. rubellus* were captured on sites that did not have leaves (p=0.017). This means that untagged earthworms were removed from the plots. There is a possibility that some *L. rubellus* were removed from the plots during removal of the litter, which could contribute to this result. However, from personal observations when hand-clearing untreated plot to recover earthworms, I believe that it is highly unlikely that an average of thirteen individuals were removed from each the sampling plot. With this information under consideration, it is likely that *L. rubellus* actively removed themselves from the leafless sampling areas.

Since the difference between the treated and untreated distributions probably resulted from movement of *L. rubellus* individuals, conclusions can be made about the distance traveled by the untagged population from the plot area if the distribution is assumed to have been the same as untreated plots. Assuming that the individuals furthest from the release point left the sampling area, than the earthworms would have traveled an average of at least 90.6cm (range=0-120cm) to leave the sampling area. It may be possible that those earthworms left the plot altogether. Knowing that *L. rubellus* individuals can travel 120cm/week (maximum observed distance for tagged *L. rubellus*) and can consistently travel at least 90.6cm/week (range=0-120cm), it may be plausible that the tagged *L. rubellus* could leave the sampling area (radius=170cm) or the entire plot altogether (radius=300cm). The individuals recovered on the treated plot remain around the release point, suggesting that those individuals had not moved during the course of the study. Therefore, a possible explanation for the low recovery rates could have resulted from an extraordinary speed (>170cm/week) in a situation with low resources. *L. rubellus* can crawl over the surface of the soil (Hale 2005a), possibly enabling these large movements. It seems reasonable to speculate that the results could have been biased because sampling was not conducted in area surrounding the plot. Further experimentation is needed to determine if these rates of movement are possible for *L. rubellus*.

Movement dynamics of earthworm individuals is influenced by high density. Even though these densities are unlikely to occur naturally, it is possible that similar densities would be seen when anglers lease unwanted bait. The methods of using a high density release point mimic the release of unwanted bait. Keller et al. (2007) reported the results from a survey showing that 44% of anglers dispose of unused bait on land or in a wastebasket. The initial rate of dispersion of the bait could be higher than average for the first week.

Even though the movement reported in this study resulted from high densities at initial release, the relative movements are still beneficial to individual movement dynamics before and after the leading edge. Even though *Aporrectodea* spp. prefer leafless areas, there movement does not differ significantly before or after the leading edge. *L. terrestris* did not show a difference in movement either, but because recovery rates were low further experimentation should be done for more conclusive data. It may be possible that *L. rubellus* moves at quicker rates behind the leading edge (areas without leaves) to search for leafy matter, but further experimentation would need to be conducted for confirmation. The results did support the conclusions that significantly *L. rubellus* prefer leafy plots, like the areas ahead of the leading edge. This study also supports the claim that more *Aporrectodea* spp. exist behind the leading edge, even though the underlying reason for the difference might not be the same. In order to accurately measure individual dispersion rates with respect to the leading edge of assemblage migration, further experimentation should be done in a way to control for high densities at the release point(s) so that movement is not the result of high population densities.

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Figures

A **Untagged Lumbricus spp. Frequency**

B **Untagged Aporrectodea spp. Frequency**
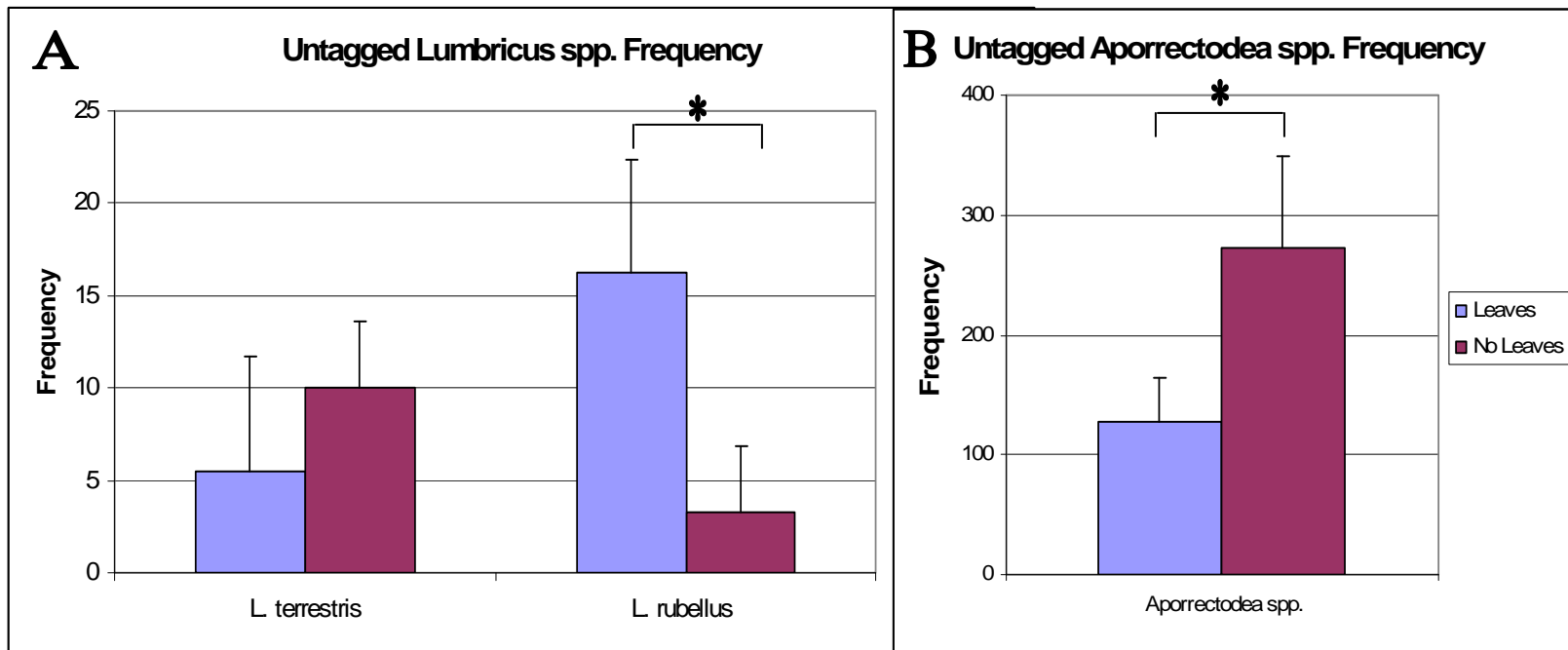
Figure 1. The average frequency of untagged individuals collected per treatment at one week time point. The values are averages of the frequencies recovered per plot, and error bars represent the standard deviation of the average. An asterisk denotes a significant difference (p<0.05). A) Data from the *Lumbricus* spp. B) Data from *Aporrectodea* spp.
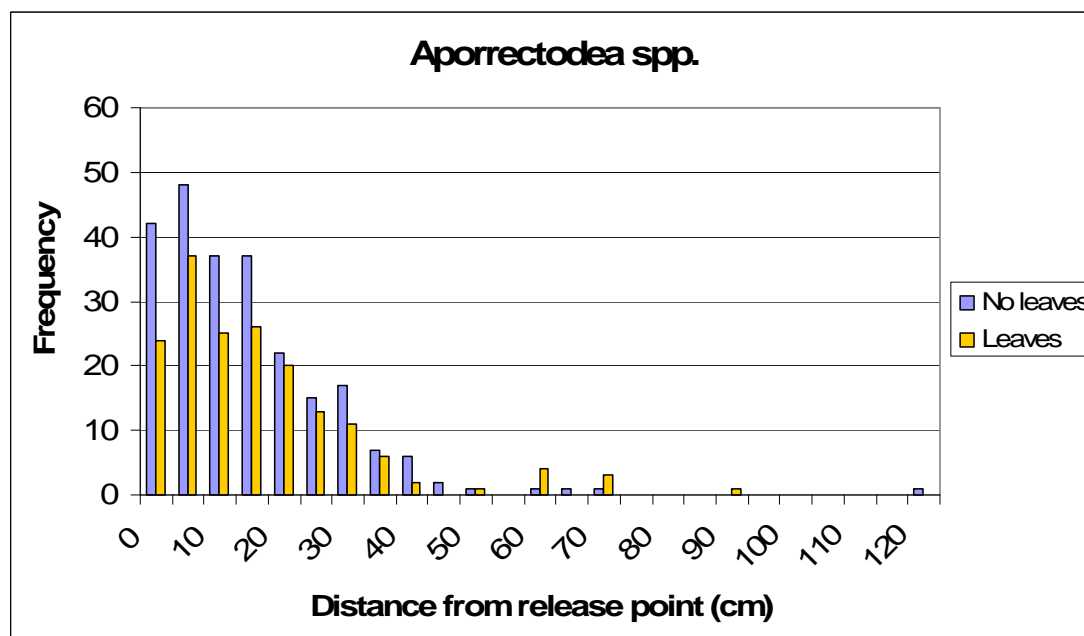

Figure 2. Histogram of total frequency over all of the one week time point plots of tagged *Aporrectodea* spp.
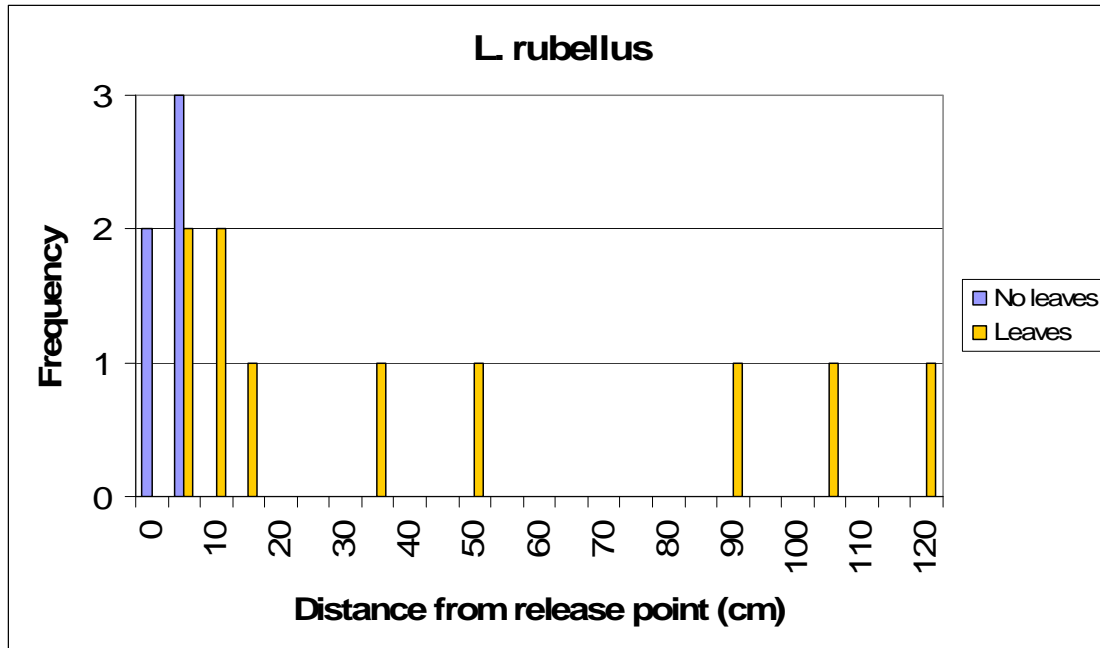
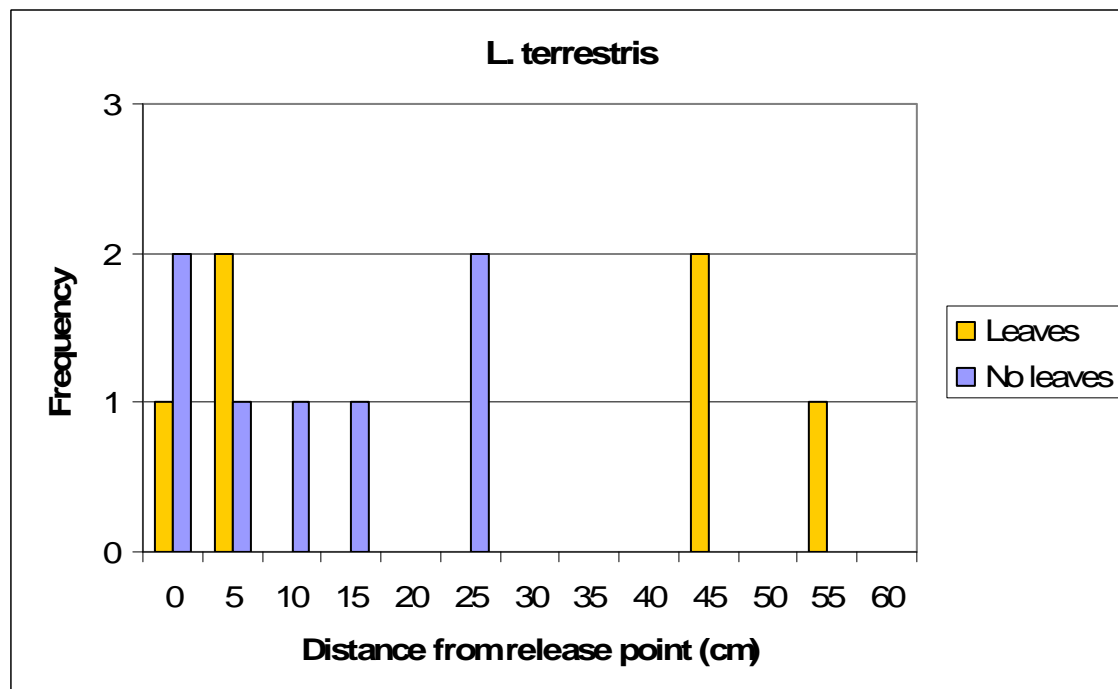Figure 3. Histogram of total frequency over all of the one week time point plots of tagged *L. rubellus*.

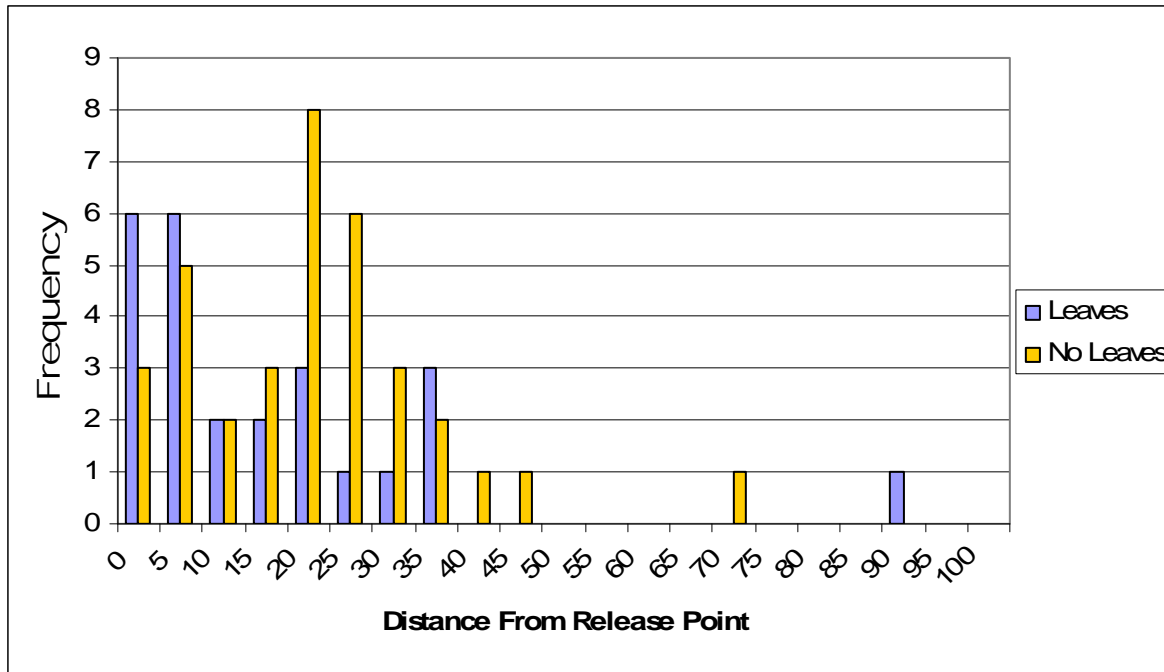Figure 4. Histogram of total frequency over all of the one week time point plots of tagged *L.terrestris*.

Figure 5. Histogram of the frequency of distance moved by tagged *Aporrectodea* spp. at three week time point.
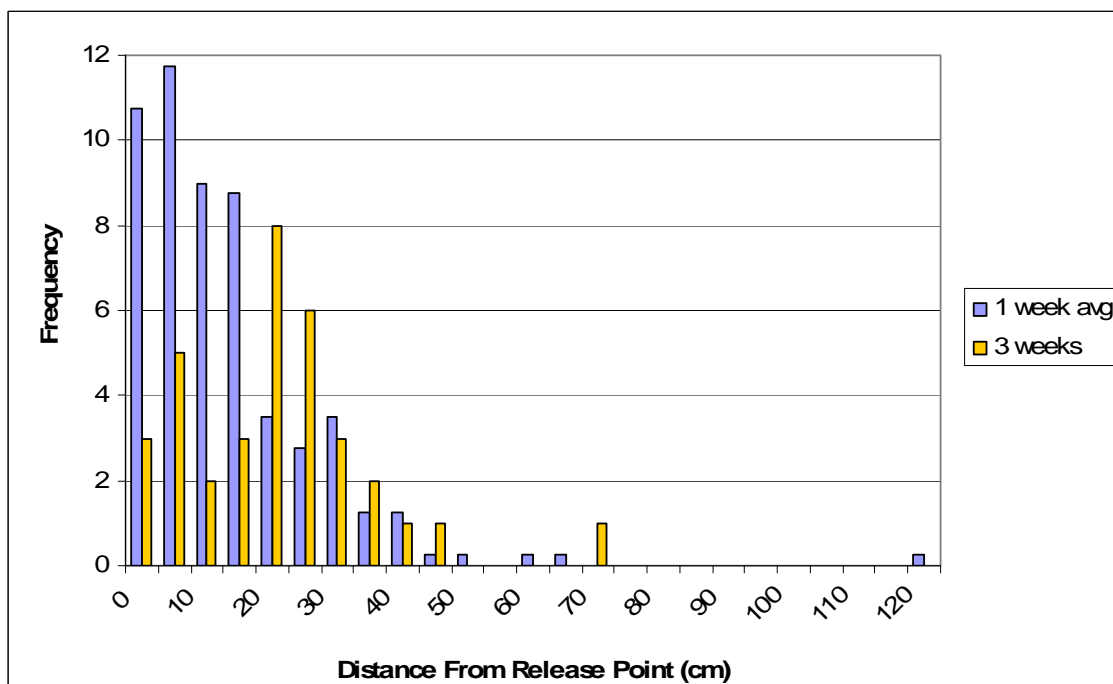

Figure 6. Histogram for plots without leaves at one week and three week time points for tagged *Aporrectodea* spp.. The one week values are an average of the frequencies (N=4) of treated plots at each distance. The three week time point is the total reported treated distribution (N=1).
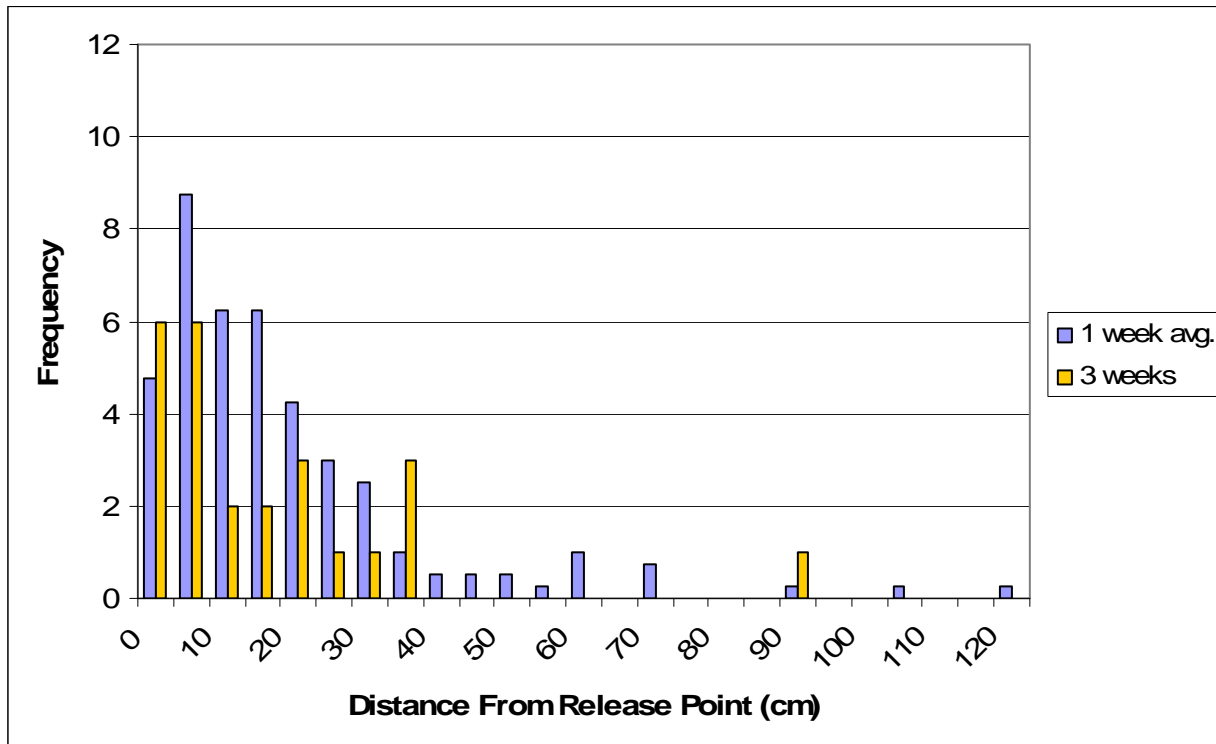
Figure 7. Histogram for plots with leaves at one week and three week time points for tagged *Aporrectodea* spp.. The one week values are an average of the frequencies (N=4) of treated plots at each distance. The three week time point is the total reported treated distribution (N=1).