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Colleen Cook
Union College - Schenectady, NY

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The Effects of Soil pH and Texture on the Molting Success and Survival of Blacklegged Ticks (*Ixodes scapularis*): A Field Experiment

By
Colleen Cook

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Submitted in partial fulfillment
of the requirements for
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Abstract

COOK, COLLEEN The Effects of Soil pH and Texture on the Molting Success and Survival of Blacklegged Ticks (*Ixodes scapularis*): A Field Experiment

ADVISOR: Kathleen LoGiudice

The blacklegged tick (*Ixodes scapularis*) is the primary vector of *Borrelia burgdorferi,* the causative agent of Lyme disease. When a tick is not questing or feeding, the majority of its life is spent within the soil. Abiotic factors within soil have been shown to affect tick molting and survival across all life stages. Soil pH, however, has not been heavily investigated. In this field study, I investigated the effects of soil pH and texture on engorged nymphal ticks. Two sites were chosen to encompass the extremes of soil pH in the region; the Albany Pine Bush in Albany, NY has acidic, loam soils and Wolf Hollow in Schenectady, NY has more alkaline, silt-loam soils. In July 2013, four plots, each containing three treatments (undisturbed soil/unaltered pH, disturbed soil/unaltered pH, and disturbed soil/altered pH), were placed at each site. Engorged nymphs were collected from chipmunks and placed within soil cores in each plot. Tick body burden data on the chipmunks was also collected and analyzed, revealing that scrotal males had significantly higher body burdens than non-scrotal males. Tick drags were also conducted at the end of July, confirming that the Albany Pine Bush had higher tick densities. In October 2013, the cores were removed and searched for surviving adult ticks. Although there was significant variation in survival, neither pH nor texture explained the pattern. The disappearances of ticks combined with finding insects within the cores suggest an avenue for further research.

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Introduction

Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is the most common vector-borne infection in the United States (Guerra et al. 2002). The illness is transmitted by a bite from *Ixodes scapularis*, commonly known as the blacklegged tick, and other members of the Ixodidae tick family. The Ixodidae family, or the hard ticks, is the most abundant tick family with over 650 species (Oliver 1989). This family is characterized by the presence of a tough, schlerotized plate on the body surface. Ixodid ticks have three active life stages comprising of a larval, a nymphal instar, and an adult stage. A full life cycle usually takes two years to complete (Sonenshine 1991). The life stages are influenced by the season, with adults becoming active in the fall and depositing eggs in the spring. The larval stage then begins in mid-summer and lasts through the early fall. Fed larvae then overwinter and molt into nymphs the following spring when they feed and molt again in the fall into adults.

Questing, or host-seeking, ixodid ticks are non-nidicolous, or quest for hosts in open and exposed habitats, such as forests and areas of heavy vegetation (Sonenshine 1993). The blacklegged tick is not as common in field habitats due to higher temperatures and lower humidity that could affect water balance in the body (Schulze and Jordan 1996). In comparison to other tick families, ixodids feed relatively slowly, requiring several days to attain a large blood meal from the host. Many *Ixodes* species, including *Ixodes scapularis*, do not exhibit significant host specificity, although immature ticks typically feed on smaller hosts and adults feed on larger hosts (Oliver 1989). *Ixodes scapularis* exhibits a three-host feeding cycle with the larval, nymphal, and adult stages feeding on three separate hosts (Sonenshine 1991), primarily *Odocoileus virginianus*, the white-tailed deer, and *Peromyscus leucopus*, the white-footed mouse (Guerra et al. 2002).

Water balance in non-feeding ticks plays a crucial role in tick survival. Non-feeding ticks must be able to conserve the water in their body, while feeding ticks must be able to eliminate excess water taken in from feeding. Transpiration is the most common way that ticks lose water through the spiracles in their body, and it is usually increased in active, questing ticks. (Sonenshine 1991). The evolution of a cuticle made of a lipid layer has protected the tick against water loss because of the hydrophobic properties of the lipid layer, preventing water from escaping the body. Ticks are also able to reabsorb water from fecal matter stored in the rectal sac and also use guanine for eliminating waste, thus further preventing water loss. Other physiological processes that restore water balance include passive and active water vapor sorption, and metabolic water production. Behavior, such as the opening and closing of spiracles in response to stimuli, movement towards or away from moist locations, and drinking water, can also control the water balance in ticks (Sonenshine 1991). Various factors including temperature and relative humidity are known to affect the water balance in ticks and influence these mechanisms and behaviors.

Ticks spend the majority of their life in soil and leaf litter (Brownstein et al. 2003). Their survival is dependent upon factors such as soil texture, temperature, and humidity, which comprise the microclimate (Sonenshine 1993). Bertrand and Wilson (1996) found that *I. scapularis* longevity was not as robust in field conditions where air and soil temperatures were higher and the humidity was lower. This suggests that tick survival is best in microclimates that have lower air temperatures at about 12°C-24°C, lower soil temperatures at about 12°C-18°C and higher relative humidity. According to Harris (1959), adult blacklegged ticks survive in the lab at an optimum temperature of 21°C and larvae and nymphs fair better at 26°C. It was also suggested that the blacklegged tick does not survive if the relative humidity falls below 80% and survival is optimal when relative humidity is between 90% and 100%. Alekseev and Dubinina (2000) suggested that temperature has an effect on the activation and movement of ticks from the leaf litter in that ticks became more active when surface temperature was higher than the soil temperature. It was also suggested that ticks infected with *Borrelia burgdorferi* behaved differently than uninfected ticks. For example, infected ticks were collected more often from soils at higher temperature gradients than at lower temperature gradients. Guerra et al. (2002) also suggested that tick densities were associated with sand or sandy-loam soils and negatively correlated with clay soils.

While there is substantial research into the environmental factors that affect the distribution and survival of ticks, including soil temperature and humidity, little research has been conducted on how soil pH affects tick survival. pH could affect tick survival by either enhancing or inhibiting the mechanisms used by ticks to regulate water balance. Guerra et al. (2002) and Tack et al. (2012), both express that acidic soils appear to be less favorable for tick survival and densities. In a small pilot study on soil pH and tick molting success conducted by Ahern (2013), however, it was found that acidic soils actually are more favorable for tick survival and development. Kaufman et al. (2010) provides some evidence that the physiological pH of the cuticle plays a significant role during feeding. Low cuticular pH allows for the cuticle to expand more efficiently, accommodating for a large blood meal. There is a slight possibility that environmental pH could influence the maintenance of cuticular pH. With these conflicting reports about the effects of pH and tick survival, there is a need for additional research into the subject to determine whether pH actually has an effect on tick molting and survival as well as what pH is more suitable for the survival of nymphal ticks.

The research was conducted in the field at two geographically and ecologically different sites; the Albany Pine Bush in Albany, NY and Wolf Hollow in Schenectady, NY. Glaciers once covered the Albany Pine Bush during the Wisconsinan Period of the Pleistocene Age, carrying with them sandy soils that were deposited at the preserve as the glaciers melted and formed Glacial Lake Albany (Albany Pine Bush Preserve 2013). When the lake drained, the soil deposits formed the sand dunes and landscape that is exhibited today (Milne 1985). The Pine Bush is also characterized as having low nutrient, acidic soils with a pH of approximately 4.1 (Seischab and Bernard 1996). Interestingly, although previous research suggests that ticks do not fare as well in acidic soils, the Albany Pine Bush has an extremely high reported tick density. This phenomenon could be due in part to the radical changes the site has experienced due to human activity and the impacts of invasive tree species. With the development of land near the Albany Pine Bush, the disturbances have made it easier for invasive species to become established. Black locust, *Robina pseudoacacia*, became established in the Pine Bush and can devastate much of the native vegetation (Albany Pine Bush Preserve 2013). In a study conducted by Morlando (2008), it was suggested that with the eradication of black locust, tick densities in the restored areas were significantly lower than in areas still containing black locust.

The second site that was investigated was Wolf Hollow in Schenectady, New York. This site is unique in that a fault runs through it and on one side of the fault, the geological make up is predominantly of shale and has a relatively low, acidic pH, while on the other side of the fault, the geological make up is of mainly limestone and is more alkaline (Garver 2013.) The area is heavily forested, a characterization favored by ticks due to the maintenance of low temperatures and high humidity. While very little is known about Wolf Hollow tick densities, there were reports by the private landowners of ticks feeding on household animals. Deer, an important host of *I. scapularis*, were also noted in the area.

The investigation of the effects environmental factors have on ticks could provide insight into the distribution of Lyme disease and help determine what regions would potentially be high-risk areas. This illness is of both medical and veterinary importance. According to the CDC, in the United States, Lyme disease is the most commonly reported vector borne illness and has spread significantly from 2001 to 2011 (CDC 2013). Ticks can contract Lyme disease at any life stage when they feed on an infected host (Ostfeld et al. 1995). The first blood meal is taken during the larval stage and larvae usually feed on smaller mammals, such as the wild rodents, which are considered to be the predominant reservoir of Lyme disease (CDC 2013). The larvae then molt, becoming nymphs, and begin questing for another blood meal. If the larvae took a meal from an infected host, the nymph becomes the vector for Lyme disease. Flat nymphs are very small and go unnoticed by their human hosts until after the blood meal is taken, which aids in the spread of tick-borne diseases (Ostfeld et al. 1995). I hope to understand how pH affects tick development and survival, which could then lead to a better understanding of why certain regions are high-risk areas for Lyme disease. By understanding the environmental factors that favor the survival of ticks, new methods of tick control that target and alter the environment could be developed in order to control the spread of Lyme disease and other tick-borne illnesses.

Methods & Materials

Choosing the Sites

The locations of the experiment, the Albany Pine Bush Preserve in Albany, New York and Wolf Hollow in Schenectady, NY, were chosen due to their differences in soil pH. The Albany Pine Bush has naturally acidic soils, while Wolf Hollow has more alkaline soils. These sites represent the extremes of soil pH in the area. The Albany Pine Bush soil exhibited a pH of about 4.3 and Wolf Hollow soil used for this experiment was about 5.7. To raise the Albany Pine Bush soil pH, 2.0 g/240mL (2.0 g/cup) of calcium carbonate was sufficient to raise and maintain a soil pH that would be similar to the Wolf Hollow soil. Aluminum sulfate did not sufficiently lower the pH of Wolf Hollow soil, thus soil from another site in Wolf Hollow with a low pH was used. The final pHs of the four treatments were about 4.53 and 6.25 for the Albany Pine Bush native and adjusted treatments, respectively, and about 5.66 and 4.65 for the Wolf Hollow native and adjusted treatments, respectively.

Tick Collection and Deployment

In order to collect engorged nymphal ticks, chipmunks were captured at the Kaikout site and Discovery Center at the Albany Pine Bush. Two hours after baiting, the traps were checked for chipmunks. Female chipmunks showing teats, evidence of lactation, or pregnancy were released. The animals were transported to the Union College animal facility and set up in cages that were suspended over moist paper towels to prevent ticks from desiccating. The bins were checked once a day for three days for engorged ticks that had dropped off the chipmunks. Engorged ticks were placed in vials containing moistened plaster of Paris and stored in the refrigerator at 5ºC until deployment. The time ticks resided in the refrigerator ranged from 3 days to 2.5 weeks.

Ticks were deployed in soil cores made of PVC pipe approximately 4 inches in diameter and 2.25 inches deep (Brunner et al. 2012). Each core was placed in a mesh bag and the edges were hot glued to the PVC to prevent ticks from escaping the core and encourage contact with the soil. Cores were set up in plots consisting of 3 cores each, with each plot replicated 4 times, for a total of 12 cores at each site. Treatments in each plot were 1) the native pH with undisturbed soil layers, 2) the native pH but with disturbed soil layers to control for the mixing of the soil required to adjust the pH, and 3) altered pH. Leaf litter from the surrounding area was placed into the cores. The plots were covered with chicken wire cages, to prevent disruption from wild animals, and left to settle into the ground overnight. Ticks from different hosts and of varying refrigerator residence times were randomized and placed in lots of 15 into the cores for a total of 360 ticks for the experiment. The mesh bags containing the soil cores were closed with zip ties to prevent the ticks from escaping the bags. The cores were left at the sites from July to October, when they were dug up and checked for ticks that had either molted or died in the experiment.

Soil Moisture, pH, and Texture

After the soil cores were placed in the field, biweekly checks of soil moisture and pH were conducted. Small soil samples were taken from the native and altered pH areas within the plots and taken back to the lab. Five grams of soil for each treatment was combined with 20 mL of deionized water and vigorously mixed. Organic material and unsuspended soil was filtered from the mixture via an aspirator. The mixture was then placed back into a test tube and the pH measured by a pH meter. From the same sample taken from around the cores, approximately 10 g of soil was measured out using a four-point scale to determine the “wet weight.” The samples were then placed in a drying oven at 60°C for 48 hours to rid the samples of any moisture. The samples were then taken from the oven, placed in a desiccator with desiccant, calcium sulfate, to prevent the samples from taking on moisture and weighed again to determine the “dry weight.” The percent moisture was then calculated by subtracting the dry weight from the wet weight, dividing by the weight wet, and multiplying by 100. Soil samples from both locations were also dried at room temperature and sent to Cornell University for texture analysis.

Tick Extraction

Beginning in mid-October, replicates 1 and 2 were removed from both the Albany Pine Bush and Wolf Hollow sites and stored for no more than 8 days at 5ºC. Tick extraction occurred in a secured laboratory to ensure ticks would not escape the premises and pose a risk. The cores were placed on white paper that was taped down to a tray and the tape lined with Vaseline to prevent ticks from leaving the tray. The mesh bags of the cores were cut open with scissors and the cores were searched for 45 minutes. The ticks that were found were placed in labeled vials containing moistened plaster of Paris. After the 45-minute search, the soil was then placed into a Burlese funnel apparatus with the funnel placed over a beaker of water. The apparatus was then placed in a tub of water lined with tape and Vaseline with a heat lamp positioned above the funnel. The soil was left under the lamps for 2 hours and the water in the beakers and bins checked for any ticks.

Data Analysis

To determine whether pH and soil disturbance had any effect on tick survival, an ANOVA was conducted that included the survival of ticks in the various treatments (disturbed + native pH, undisturbed + native pH, and disturbed + adjusted pH). Further analysis of the data included t-tests to explore the significance of sex vs. soil disturbance and sex vs. pH. A regression analysis was also conducted to explore a possible relationship between the time the ticks spent in the refrigerator before the cores were searched and survival. Also, a regression analysis was performed to determine whether soil moisture had any significant effect on tick survival. The data were not normally distributed; so all analyses were repeated as rank analyses (i.e., Kruskal-Wallis, etc.).

Results

Upon classification by Cornell Nutrient Analysis Laboratories, the Albany Pine Bush soil was classified as loam and the Wolf Hollow soil was silt-loam. While these classifications are broad, it is evident that these two soils are significantly different as Albany Pine Bush soil is 42% sand and Wolf Hollow soil is only 4% sand. Wolf Hollow also contains more silt than the Albany Pine Bush (Table 1).

Table 1. Calculated percentages of sand, silt, and clay in Albany Pine Bush and Wolf Hollow soil.

Location	% Sand	% Silt	% Clay	Assigned Texture
Albany Pine Bush	41.83	46.35	11.82	Loam
Wolf Hollow	3.88	76.80	19.32	Silt - Loam

A total of 360 engorged nymphal ticks were used in this experiment with 15 ticks placed in each soil core. A total of 230 ticks were recovered, giving a recovery percentage of 63.9%. Of those ticks recovered, there were 227 adult ticks recovered alive, giving the overall molting and survival percentage to be 63.1%. The remaining ticks recovered were dead adults or unmolted, dead nymphs. For the Albany Pine Bush, disturbed soil with low pH, undisturbed soil with low pH, and disturbed soil with high pH had percent survivals of 60%, 58.3%, and 66.7% respectively. For the Wolf Hollow location, disturbed soil with high pH, undisturbed soil with high pH, and disturbed soil with low pH had percent survivals of 70%, 63.3%, and 61.7% respectively. There were no significant effects of pH (p=0.58, ANOVA) or soil disturbance (p=0.74, ANOVA) on tick survival. The ANOVA also determined that soil texture did not produce an effect on the survival of ticks. Overall, soil texture and pH did not have an effect on tick molting or survival (Figure 1).

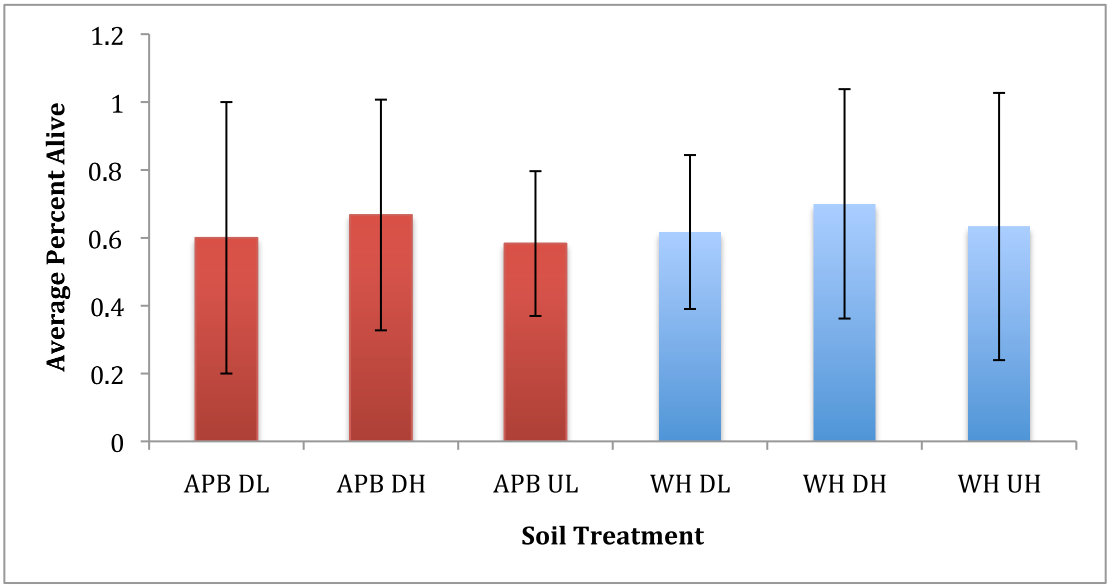

Figure 1. Percent survival of ticks for each soil treatment. Albany Pine Bush (APB),Wolf Hollow (WH), Disturbed-Low (DL), Undisturbed-Low (UL), Disturbed-High (DH), Undisturbed-High (UH).

The relationship between soil moisture and tick survival was also explored, as shown in Figure 2. A regression analysis was conducted to determine if soil moisture played a significant role in tick survival. It was found that minimum soil moisture had a very slight significant and negative effect on tick survival (p=0.049). An ANCOVA was conducted using soil moisture as a covariate and this did not change the results.

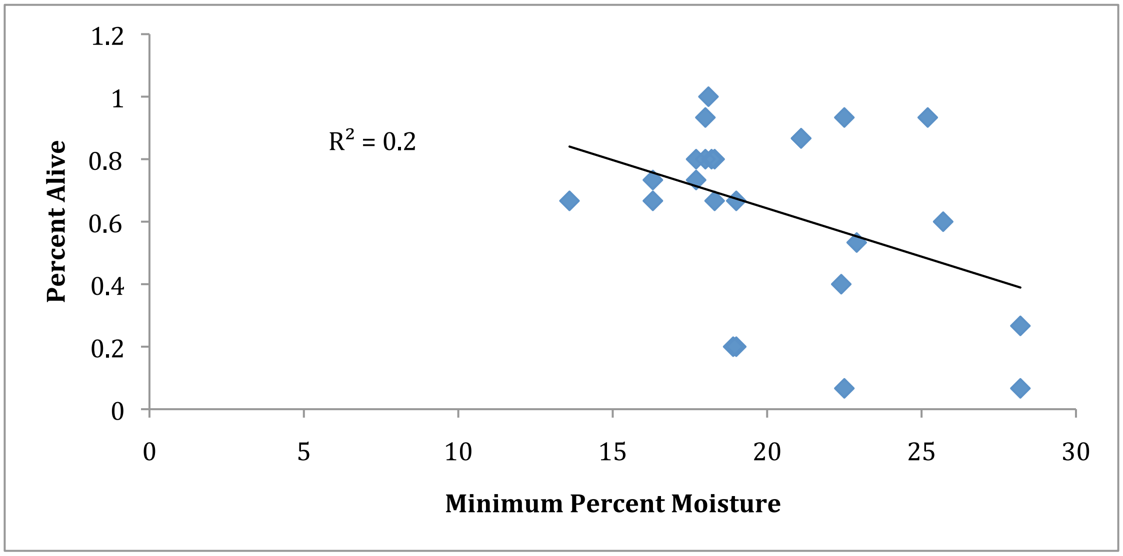

Figure 2. Effect of average percent moisture on percent survival of ticks for each soil core.

To determine whether or not the number of days the cores were in the refrigerator after removal from the field had any significant effect on tick survival, a regression analysis was performed and it was found that the number of days spend in the refrigerator had no significance on tick survival (p=0.17; Figure 3).

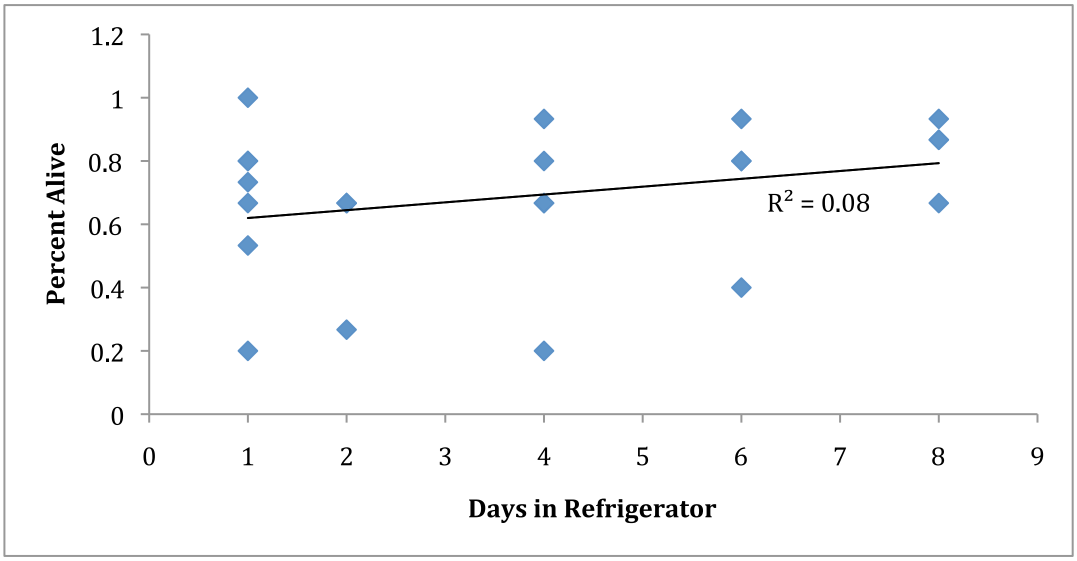

Figure 3. Effect of number of days the soil cores spent in the refrigerator on percent survival.

Neither soil disturbance nor soil pH had a significant impact on the sex ratio of the adult ticks (p=0.46 and p=0.44, respectively).

Discussion

Based on this experiment, it was found that soil pH does not have an effect on tick molting and survival. It was hypothesized that soil pH could potentially affect the cuticles of engorged ticks, subsequently affecting water balance and survival of ticks. It appears that this was not the case for the pH ranges tested in this experiment. Perhaps the hypothesis could be upheld for soils with different pH ranges, such as in western United States where soils are more alkaline and *I. scapularis* densities are low. There could also be a threshold effect where pH does not affect ticks until pH is either too high or too low. The pilot project Ahern (2013) found that ticks had significantly higher survival rates in acidic soil. Those results are most likely products of flaws in the design, small sample sizes, and limited number of replicates in the Ahern (2013) study. There is evidence that endogenous, cuticular pH has an effect on engorged nymphs. Kaufman et al. (2010) found that cuticular flexibility increased with declining pH. This suggests that an acidic, physiological pH would aid in the expansion of the body during a large blood meal. However, if the cutciular pH were more alkaline, the tick possibly wouldn't be able to expand with a large blood meal. There could be a slight possibility that environmental pH, particularly extreme pH, could affect the maintenance of physiological pH in ticks.

Our study found that the soil textures used from the two sites did not have a significant impact on tick molting and survival. Wolf Hollow soil was considerably more packed when wet than the Albany Pine Bush, leading to the hypothesis that perhaps it was more difficult for ticks to navigate through the Wolf Hollow soils. Since the Albany Pine Bush soil was mainly sandy loam and the Wolf Hollow soil was silt loam, the drainage of the soils is most likely different. Water would drain more easily in sandy soils due to the large particle size when compared to silt soils, potentially making it easier for ticks to move within the soil. However, molted ticks were found distributed in cores from both sites. These results are generally consistent with Guerra, et al. (2002) because they found that tick densities were positively correlated with areas associated with sand/loam and sandy soils, similarly to how this study found that ticks faired well in that sandy-loam soils of the Albany Pine Bush. Tick drags of the Albany Pine Bush also found high tick densities, further supporting the findings of Guerra et al. (2002). While the soil textures used in this experiment did not produce significant impacts on tick molting and survival it is possible that other, more extreme, soil types may affect tick development.

Soil disturbance also did not have an effect on tick molting and survival success. It was hypothesized that the soil structure in an undisturbed core might allow ticks more or less access to the lower soil horizons, allowing them to find favorable microclimates in the heat of the summer. However, ticks did not survive significantly differently in the intact-soil cores vs. the disturbed-soil cores. The ticks were placed on top of leaf litter and on the O soil horizon of the intact soil cores. Most of the ticks were recovered questing on leaf litter while some were found as deep as 5 cm. It cannot be assumed that the ticks remained on the leaf litter for the full three months, as they most likely would have died from desiccation and exposure. It is remotely possible that some ticks, which were kept in the refrigerator for up to 2.5 weeks until deployed in the cores, may have reached the point of immobility when they were used. This would have prevented them from finding a favorable microclimate, leading to their deaths from factors unrelated to soil pH or texture. This seems unlikely, since ticks in the cores were stratified by collection date, such that each core had ticks with approximately the same age profiles. Some ticks were also collected from lower soil horizons, suggesting that the ticks were mobile and were able to adjust their positions within the soil accordingly.

Soil moisture was found to have a slight negative impact on tick molting and survival ($p=0.05$; $R^2=0.17$). It was found that the higher the moisture level within the cores, the lower the survival rate. This suggests that there could be a point where too much moisture prevents the ticks from properly maintaining water balance, that they were too saturated with water. Subak (2003), however, found that tick survival is negatively impacted by drought conditions, that there was a decrease in nymphal tick densities when conditions are dry. Subak (2003) also suggested that excessive moisture, from large rainfall events for example, could have detrimental effects on tick survival, agreeing with the results of our study. These studies suggest that ticks are sensitive to moisture while they are in the engorged state. It also suggests possible thresholds for soil moisture, where too much or too little moisture negatively impacts tick survival. Soil moisture could have been high due to the presence of leaf litter within the confined space. Leaf litter may aid in retaining moisture in the soil of the cores, thus preventing desiccation but also possibly holding in too much moisture (Schulze et al. 1995).

The mean recovery rate (live molted adults, dead molted adults, and unmolted nymphs) for the 40-minute searches was approximately 64%. There is a slim possibility that the ticks were able to escape the cores through small tears in the mesh that could have developed in the field (although few to none were seen) or were able to slip through an opening where the zip tie was tightened. This seems unlikely, as ticks are not as active in the engorged state and in the heat of the summer. In addition, the approach used in our study closely follows the protocol of Brunner et al. (2012), which had a recovery rate of 90-95% of ticks deployed. That study used flat nymphs, which are smaller and more active when compared to the less active engorged nymphs and larger adults used in our study.

There were also various insects and earthworms contained within the cores, most likely trapped within the soil at the beginning of the study. This raised the question of whether or not some of these invertebrates in the cores were potential predators of the ticks since they went undocumented and unclassified. Some birds and reptiles are known to be predators of ticks, but it is not well known whether there are arthropods or annelids that act as predators to ticks. Samish and Alekseev (2001) reviewed data on the subject, finding that ants, beetles, and spiders were the most common predators of *Ixodes* ticks. It was also suggested that engorged ticks were most likely to become prey than unfed, flat ticks, possibly due to lack of mobility. They also suggested the possibility of cannibalism among ticks.

It is also possible that entemopathogenic fungi (such as *Beauveria bassiana*), nematodes, or bacteria could have led to the death and subsequent decomposition of unrecovered ticks. Low concentrations of *B. bassiana* spores have been shown to induce 80% mortality in engorged ticks. This high mortality and vulnerability is most likely due to the distension of the cuticle, suggesting that the fungi may inhibit cuticle function (Kirkland et al. 2004). Ostfeld et al. (2006) reviewed data on the effects of entomopathogenic fungi and reported that infection appears to reduce tick body mass and egg mass size, reducing tick fitness. Keesing et al. (2011) explored the effects of the invasive herb garlic mustard (*Alliaria petiolata*) on entomopathogenic fungi and found that garlic mustard inhibits the growth of *B. bassiana*, which could then lead to the success of the blacklegged tick within garlic mustard infested locations. The Albany Pine Bush and Wolf Hollow sites had garlic mustard present, but the plot sites that were chosen were devoid of surrounding garlic mustard. Thus, there is the possibility that entomopathogenic fungi were able to proliferate in the cores and kill some ticks.

Future studies are necessary to fully understand the forces behind tick molting and survival. Research regarding potential predators of the blacklegged tick is very minimal and could be a strong candidate for future research. In our study, there were various soil cores from which we were unable to recover all of the ticks, both alive and dead. However, we found various other insects within the cores that survived the three months, suggesting the possibility that the engorged ticks used in the study could have fallen prey to the other insects. Upon identification of the insects, engorged larval and nymphal ticks could be collected and placed within vials containing the insects. The replicates would include a control without any other insects within the vial and other vials containing engorged ticks with one type of insect for each replicate. This procedure could then be moved to the field to address the more complicated relationships the insects and ticks may have in a more natural environment.

This research suggests that soil pH, texture, and disturbance in the Albany-Schenectady region do not have an impact on the molting and survival of engorged nymphal ticks. While these factors do not play a significant role in this region, it cannot be assumed that they play no role in the distribution of ticks in other regions with different pH ranges and soil textures. Understanding these factors could improve predictions of regions associated with increased risks of tick-borne illnesses. Such knowledge could eventually lead to control methods to prevent the expansion of tick populations and reduce tick densities, thus decreasing the risk of exposure to the various tick-borne diseases such as Lyme disease.

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Tick Densities and Host Body Burdens

Introduction

While conducting the study on the effects of pH on tick molting and survival in Cook (2014), body burden data from the chipmunks was also collected during the trapping and 72-hour holding period. Tick density data was also collected at the Albany Pine Bush and Wolf Hollow.

The eastern chipmunk, *Tamias striatus*, is a small, brown, omnivorous rodent with dark and buff stripes down the back and sides of its body and face. Adult chipmunks typically weigh between 70 and 110 g (Cornell Cooperative Extension 1983). Their short tail is covered in fur but is not as bushy as their squirrel relatives. Eastern chipmunks are found in eastern United States and Canada, as far west as Manitoba and extending south to Louisiana Their habitat is variable, usually consisting of deciduous and mixed forests, particularly those containing hardwood trees with an open area beneath (Adirondack Ecological Center 2014). Chipmunks are relatively solitary mammals with a single chipmunk occupying an underground burrow and territory, with the exception of a mother chipmunk and her young. The entrance to the burrow is usually covered by brush to provide camouflage from predators (Cornell Cooperative Extension 1983).

Chipmunks usually do not exhibit large movements out of their territories, but immature and male chipmunks are capable of travelling longer distances (as far as 857 m Snyder 1982). These mass movements are likely due to foraging for food sources or seeking out female chipmunks in estrus during the breeding seasons (Cornell Cooperative Extension 1983). Eastern chipmunks are diurnal, exhibiting peak behaviors during the mid-morning and mid-afternoon hours with most activity on warm, bright spring and summer days (Snyder 1982). During winter, chipmunks enter torpor, where their metabolism is reduced about 85% and they rely heavily on their food stores within the burrow (Snyder 1982). The Eastern chipmunk typically reemerges in mid-March for the first period of breeding in the season (Cornell Cooperative Extension 1983).

Ixodes scapularis, or the blacklegged tick, is the vector responsible for the transmission of Lyme disease, caused by the bacterium *Borrelia burgdorferi* (CDC 2013). These ticks are part of the Ixodidae, or hard tick family (Oliver 1989). The blacklegged tick has a two-year life cycle beginning with eggs hatching into larvae. Larval ticks are the smallest life stage and are often found closest to the ground questing for hosts. Preferred hosts of larval ticks include the white-footed mouse (*Peromyscus leucopus*) and other small rodents. After feeding on a host, the engorged larval ticks drop off and proceed to molt in the soil and leaf litter. The newly molted nymphs then begin questing for a new host on which to feed. Upon completion of a second blood meal, nymphs drop off their host and undergo the molting process again within the soil and leaf litter. The newly molted adult ticks once again begin questing for yet another host, commonly deer, to take a meal from before mating (Sonenshine 1991). Mannelli et al. (1993) found that larval and nymphal ticks have different molting success rates when allowed to feed upon the same species, such as on raccoons (*Procyon lotor*). Female adult ticks take a meal, lay an egg mass, and die (Oliver 1989). Larvae exhibit peak activity between July and October and nymphs have peak activity from late spring to early summer. Adults are usually most active in the month of November as well as April through June (Sonenshine 1993).

The blacklegged tick can be found throughout northeastern North America, extending down the east coast of the United States, even reaching as far west as parts of Texas. This species is also found in the states of Michigan, Wisconsin, and parts of Minnesota (CDC 2013). *I. scapularis* typically inhabits deciduous forests that are brushy and have many shrubs. Schulze and Jordan (1996) found that adult ticks reached higher densities in forests containing a mix of oak and pine trees when compared to pine-dominated forests. Questing ticks can be found at the lower canopy levels of forests, often questing in the leaf litter for smaller hosts, or on the leaves or branches of shrubs.

The ranges and distributions of chipmunks and the blacklegged tick often overlap, with both organisms being present in the eastern part of North America. Ostfeld et al. (1996) found that ticks had a higher probability of interacting with mouse hosts when mice densities were high. The same could be assumed for chipmunks, as the more available hosts there are, the more likely the ticks would be able to feed and survive. Keesing et al. (2009) suggested that the chipmunk was an intermediate quality of a host for larval ticks with a mean body burden of about 22 ticks per chipmunk with a feeding success of 24.3%. LoGiudice et al. (2003) found Eastern chipmunks with mean body burdens of 36 larval ticks per chipmunk and those ticks experienced a 41.2% molting success.

Multiple studies have been conducted to determine the forces influencing tick body burdens on various hosts. Vor et al. (2010) investigated the possible factors driving tick burden on European roe deer and found that adult tick burden was positively correlated with body mass, age, and hind foot length. Seasonality was also found to have an effect, with more ticks found on deer in the spring than in the fall. Through the use of predictive modeling, it was indicated that larval ticks may have a preference for younger, smaller deer, and higher deer body mass was positively correlated with adult tick burdens (Vor et al. 2010). Shaw et al. (2003) explored factors that possibly influence body burdens among rodents, finding that larval ticks per host were higher in mice than in chipmunks and that 62% of larval ticks exhibited a preference towards mice for a host. It was also found that larval ticks had more success feeding on chipmunks than mice, possibly a consequence of grooming behaviors or anti-tick host immunity.

Overall, Shaw et al. (2003) suggest that mice have higher body burdens due to utilizing habitats infested with ticks, thus attracting ticks more frequently.

Brunner and Ostfeld (2008) also continued the investigation of the possible causes of tick burdens on hosts, suggesting that densities of ticks and host densities had an effect on tick body burdens. Specifically, larval and nymphal body burdens on chipmunks increased with an increase in chipmunk density. Individual host characteristics were not effective at predicting nymphal body burdens, but were sufficient for predicting larval body burdens. Chipmunk body mass had an effect on larval body burden, with the smaller chipmunks having more larval ticks than nymphal ticks. Brunner and Ostfeld (2008) also indicated that males had higher larval body burden than females and that subadults had larger burdens overall than adults and juveniles.

There is a lack of literature and data on nymphal body burdens on host species. Brunner and Ostfeld (2008) found that nymphal burdens were positively correlated with chipmunk population density. Vol et al. (2010) found a similar pattern with roe deer. There may be more focus on larval body burdens because Lyme disease and other tick-borne illnesses are transmitted to the larvae, which then molt into infected nymphs that proceed to successfully feed on an infect humans. Nymphs are also able to become infected and molt into infected adults. However, adults transmit Lyme disease to humans less frequently than nymphs, which could possibly be due to the size of the nymphs and how difficult it is to notice them. When hosts that are competent disease reservoirs have high larval burdens, the infection prevalence in nymphs is likely to be higher.

The data collected and analyzed during this phase of the study could potentially lead to a better understanding of areas with high Lyme disease risks. Lyme disease, caused by the bacteria *Borrelia burgdorferi*, has become one of the most prominent vector-borne diseases in the United

States (CDC 2013). It is transmitted through the bite of a tick that had become infected after feeding on an infected host. The most common reservoir for *B. burgdorferi* is the white-footed mouse (*Peromyscus leucopus*), although the Eastern chipmunk has proven to be a reservoir as well. The white-footed mouse has a reservoir competency of about 92% compared to the Eastern chipmunk competency of 55% (LoGiudice et al. 2003). According to Mannelli et al. (1993), reservoir competence may be dependent on host habitat and geographic locations. Regions with higher chipmunk populations may see chipmunks acting as more competent reservoirs while areas with higher white-footed mice populations may see the mice as more competent reservoirs.

The LoGiudice et al. (2003) study on the importance of host biodiversity revealed that nymphal infection prevalence increased when host biodiversity decreased. When alternative hosts are not available, the potential for ticks to feed off the white-footed mouse and contract Lyme disease, and thus transmit Lyme disease to humans, is higher than if there is more than one possible host to feed from. Considering the white-footed mouse is a very good reservoir and the Eastern chipmunk is an intermediate reservoir for Lyme disease, areas with these two rodents possibly pose a higher to Lyme disease risk to humans. Diluting these populations could lead to lower prevalence of Lyme disease and lead to lower health risks. Regions with high Lyme disease risk could be predicted by understanding the possible effects that hosts have on tick body burdens and subsequent survival success. Such knowledge could also aid in formulating control methods that could reduce tick densities and tick-borne diseases in high-risk regions.

Methods and Materials

The experimental portion exploring the effects of pH on tick survival and development began on June 17, 2013 and yielded supplemental data such as host body burden data and tick density data. Chipmunks were trapped at two sites in the Albany Pine Bush, the Kaikout barrens and the Discovery Center. Sherman traps were set in the morning and were subsequently checked every two hours. Each captured chipmunk was weighed, sexed, and marked with Monel type, numbered ear tags. Tagged chipmunks were brought back to Union College and held for 72 hours in cages suspended over bins with moistened paper towels to collect engorged ticks. The number of engorged larval and nymphal ticks for each chipmunk was recorded. Thus, a true body burden is not determined, rather a 72-hour collection. Body burden data were not normally distributed therefore parametric and nonparametric statistics were used. Statistical analysis of the data included t-tests, Spearman rank correlation, and ANOVA.

Tick drags to determine larval tick density were also conducted in early August at each study site, the Albany Pine Bush and Wolf Hollow. Cloths were dragged for approximately 60 m before being checked for ticks. At Wolf Hollow, a total of 9 drags were performed, while at Albany Pine Bush, only 4 drags were conducted due to the higher tick densities encountered at this site.

Results

The body burdens of the chipmunks were determined from the collection of engorged ticks that had dropped off from the chipmunks while they were held for 72 hours. The mean nymphal body burden was 21.5 (±22.7) per chipmunk and the mean larval body burden was 1.1 (±2.0) per chipmunk. There was not a significant difference in total body burdens between male and female chipmunks (t-test, p=>0.05), nor were there differences between the sexes in larval or nymphal burdens independently (t-test, p=>0.05) with female mean burdens of 0.76 (± 1.7) and 14 (± 15), for larvae and nymphs, respectively and male mean burdens of 1.4 (± 2.1) and 30 (± 27). There was a positive, non-significant relationship between chipmunk body weights and nymphal body burdens.

There were significantly more nymphal ticks on scrotal males, 35 (± 28) ticks per chipmunk, than non-scrotal males, 10 (± 10) ticks per chipmunk, (t-test, p= 0.03; Figure 1).

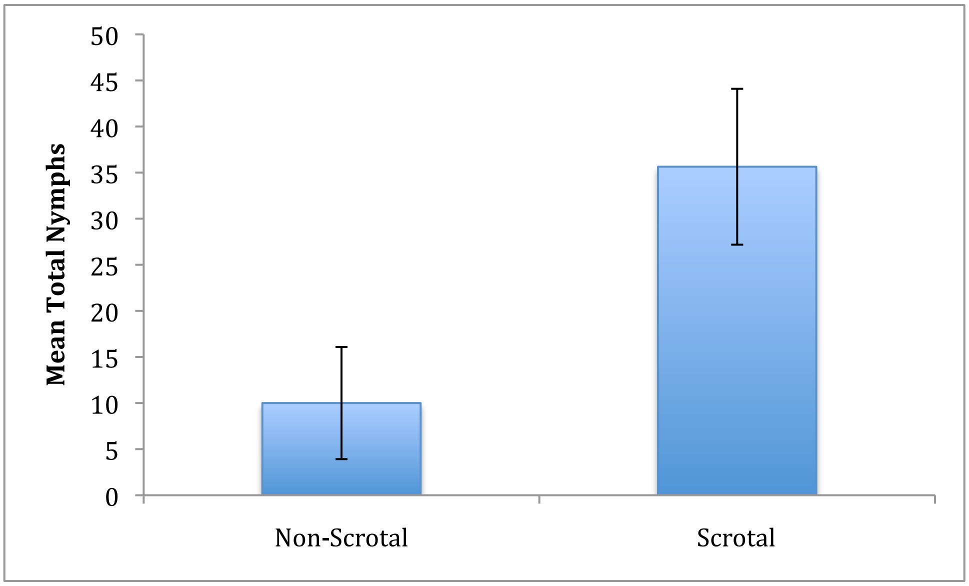

Figure 1. Mean total nymphs on scrotal and non-scrotal males. Error bars represent standard deviation from the mean.

A positive, yet non-significant Spearman rank correlation was found between scrotal male weights and body burden as shown in Figure 2.

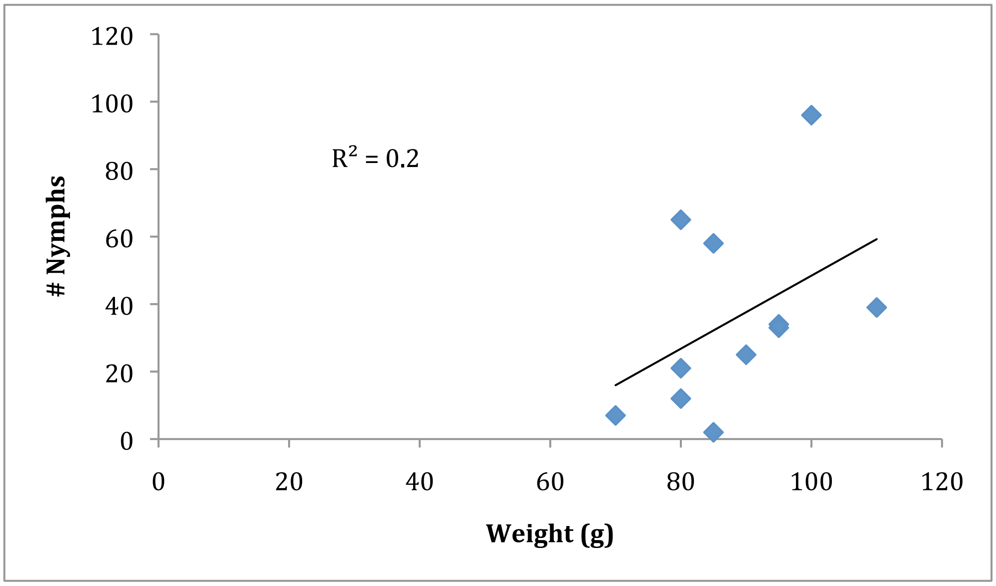

Figure 2. Number of nymphs found on scrotal males vs. weight of scrotal males. p= 0.11.

There were no differences between 2013 body burdens and chipmunk weights and 2012 body burdens and chipmunk weights. Combining the data from the two years also failed to produce any additional patterns in the data.

There were significantly more nymphal ticks than there were larval ticks on chipmunks during the months of June and July. Towards the end of July, however, there was a sudden drop off in the number of nymphal ticks and a slight increase in the number of larval ticks infesting the chipmunks, as shown in Figure 3.

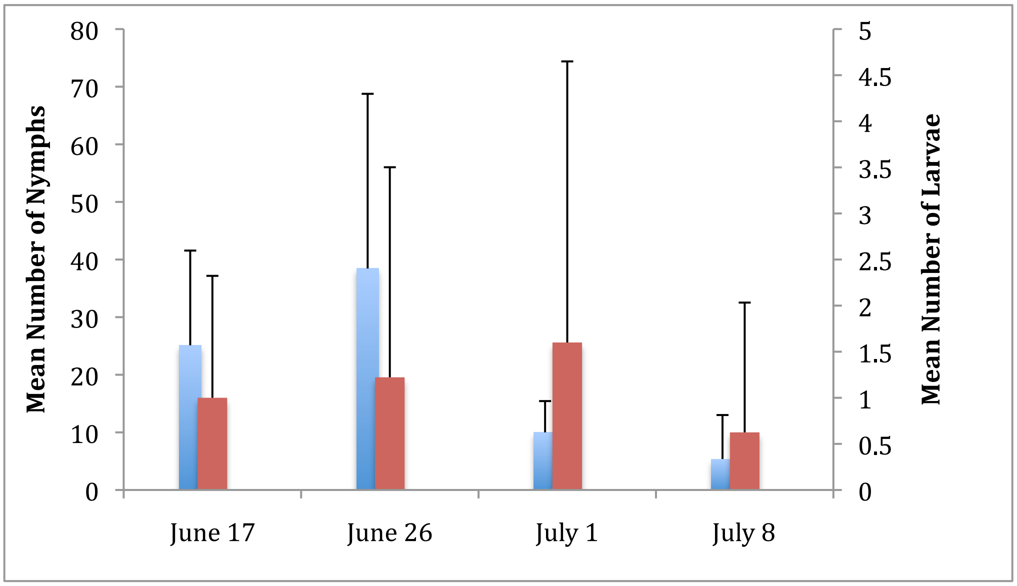

Figure 3. Mean number of nymphs and larvae per chipmunk in each of 4 weeks from 6/17/13- 7/8/13. Blue represents nymphs; red represents larvae.

A tick drag conducted at the end of July produced vast numbers of larval ticks and few nymphal ticks. The tick drags were conducted at the two study sites and the results of the tick drags indicated higher tick densities at the Albany Pine Bush than at Wolf Hollow (Appendix).

Discussion

This study did not find a significant effect of sex on chipmunk body burden. This could indicate that ticks do not have a preference for the sex of a host or that male and female chipmunks show equal opportunities to acquire ticks in their behaviors. Vor et al. (2010) suggested host sex did not play a significant role in tick body burdens, while Brunner and Ostfeld (2008) and Mannelli et al. (1993) found that males had higher tick burdens than females. Chipmunks, male and female, establish territories and defend them when an invading chipmunk enters, resulting in chasing behaviors that may drive the chipmunk to travel to the edge of their territory or further, potentially running through leaf litter and brush that may contain questing ticks (Snyder 1982). Ostfeld et al. (1995) suggested that potential hosts played a role in tick densities through their movement patterns in their habitats. Areas that are high in host populations often have high tick populations as well, most likely due to ticks having blood meal sources available to them (Ostfeld et al. 1996). Such movements in both sexes could provide an explanation for why sex of the host does not affect tick body burden.

Scrotal males had higher tick burdens than non-scrotal males. This could be due to the fact that these chipmunks were captured during a breeding season and with breeding season come certain behaviors not seen during non-breeding seasons. Scrotal males are potentially more likely to travel larger distances looking for not only food to sustain themselves, but also females to mate with, thus having an increased chance of entering tick infested spaces and acquiring more ticks (Snyder 1982). The breeding time of chipmunks and peak activity of nymphal ticks overlap, supporting the possibility that sexually active males may be more prone to acquiring ticks (Mannelli et al. 1993).

Observations during this study noted that the majority of ticks collected from chipmunks were in the nymphal stage, while only a few were in the larval stage. There were not any adult ticks found on the chipmunks during the trapping period. As the summer progressed into July, there was a steady decline in the number of nymphs found on the chipmunks. This reinforces findings in other studies and literature that nymphal ticks peak in the late spring and early summer before declining. Vor et al. (2010), for example, found that life stages comprising body burdens on roe deer changed with the season. Observations made during the tick drag at the end of July showed that nymphal tick populations had declined significantly while the larval tick population had increased dramatically. Mannelli et al. (1994) conducted tick drags to estimate tick densities and found that nymph densities were highest in mid-June before declining into July and that larval densities increased from the end of June through July. Overall, our findings support the data of Vor et al. (2010) and Mannelli et al. (1994).

The tick drags at the Albany Pine Bush and Wolf Hollow showed significant differences in tick densities at the two sites. This large contrast could be due to either environmental and habitat factors or possibly host densities. Schulze and Jordan (1996) suggests that habitat compositions likely have effects on tick density and distribution, for it was found that questing adult ticks had high densities in pine/oak forests than just pine stands. Tick body burdens on white-footed mice were found to be highest in habitats associated with wooded sites while mice in grassy sites had lower burdens (Adler et al. 1992). Guerra et al. (2002) suggested that soil pH and texture has an effect on tick density as well with ticks existing at high densities in areas with sand and loamy/sand textures and existing at low densities in areas with acidic soils. Areas of high host populations serve as optimal locations due to having adequate access to nutritional sources. These data will provide a useful basis for comparison between years and sites in the future.

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Appendix

Tick Densities

A t-test was performed on the data from the tick drags from the Albany Pine Bush and Wolf Hollow. While the statistical analysis indicated a non-significant difference between the tick densities of the two sites, observational data reveals considerable differences in tick densities. Table 1 displays the raw data for each tick drag and Figure A1 depicts the mean ticks per 60 m drag, standard deviation from the means, and a non-significant p value of 0.06.

Table A1. Ticks Collected per 60 m drag at the Albany Pine Bush and Wolf Hollow.

Replicate	Albany Pine Bush	Wolf Hollow
1	**100**	**0**
2	**355**	**0**
3	**67**	**0**
4	**275**	**0**
5	**----------**	**2**
6	**----------**	**2**
Mean	**199.3**	**0.7**

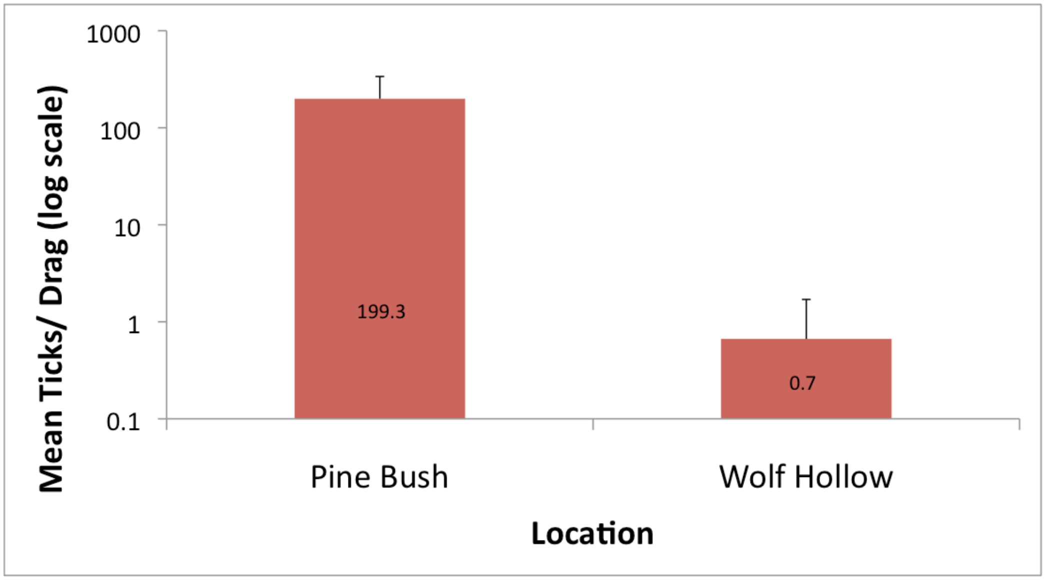

Figure A1. Mean larval, nymphal, and adult ticks combined per 60 m at Albany Pine Bush and Wolf Hollow. Error bars represent standard deviation from the mean. p= 0.06.