# The Effects of Soil pH on the Molting Success of Blacklegged Ticks (*Ixodes scapularis*): A Laboratory Experiment

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## Abstract

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*Ixodes scapularis*, or the black-legged tick, is the major vector of Lyme disease in the U.S. *I. scapularis* has expanded its range in recent decades, making the study of factors affecting its distribution a high priority. Studying the effects of various conditions in the soil could help in predicting range expansions, because ticks spend the majority of their lives in contact with the soil. We investigated the effects of soil pH on the molting success of engorged *I. scapularis* nymphs collected from Eastern Chipmunks (*Tamias striatus*). The experiment was conducted in a laboratory to control for covariates such as temperature and moisture. Soil was collected from two sites in upstate NY with different soil textures, the Albany Pine Bush Preserve (loam soil) and Wolf Hollow (silty-loam soil). High (6.0-6.5) and low (4.2-4.5) pH treatments were crossed with the two soil textures in a factorial design. Results suggest that nymphal tick molting success is not affected by soil pH or by soil texture. Additionally the nymphal chipmunk body burdens in this study did not deviate significantly from previous studies. Future experiments could incorporate higher pH values to predict the range expansion of *I. scapularis* to western states which have basic soils.

## Introduction

Ticks are considered second only to mosquitos as the most substantial vector of human and animal diseases, and they transmit a greater variety of pathogens than any other order of arthropods. Many of these pathogens are transmitted by members of the Ixodidae family, or hard ticks, due to their ability to create and feed from an open blood pool for long periods by releasing a plethora of immunosuppressive substances to evade host rejection (Sonenshine, 1991). The hard tick *Ixodes scapularis*, or the black-legged tick, is the major vector of Lyme disease, the most frequently acquired vector-born disease in the United States (Brownstein et al. 2003). Risk of exposure to *B. burgdoreri*, the bacterial spirochete which causes Lyme disease, is dependent in part on tick abundance in areas frequented by humans (Lindsay, 1999). This has made the study of factors affecting the densities and distribution of *I. scapularis* a high priority in recent years.

A map estimating the distribution of *I. scapularis* populations based on climatic conditions, determined by Brownstein et al. in 2003, shows that *I. scapularis* ticks are thoroughly established throughout the northeastern and southeastern states in the United States. The distribution of *I. scapularis* is positively related to deciduous, dry to mesic forests and negatively related to grasslands, conifer forests and wet forests (Guerra et al. 2002). Many effects of wildlife hosts on tick distribution have been observed, including the dependence of ticks on hosts such as white-tailed deer for localized dispersal (Ostfeld et al. 1996). The identity of the host species is also known to affect the abundance of ticks in a habitat, because some species such as opossums and squirrels kill a greater proportion of feeding ticks during self-grooming than other species, such as white-footed mice (Keesing et al. 2009). Additionally, migratory birds have

played a significant role in the range expansion of *I. scapularis* throughout the northeast, to mid-western states and to Canada (Ogden at al. 2008).

Relationships between tick survival and abiotic factors have also proven to be useful tools in mapping future range expansions of this potent disease vector. *I. scapularis* live for longer periods at certain temperatures and relative humidity percentages (Brownstein et al. 2003; Harris, 1959). A model relating the predicted increases in temperature from global climate change to the distributions of *I. scapularis* has shown that a drastic northward expansion is likely to take place in the next two decades, and that the abundance of *I. scapularis* is expected to increase in the northern reaches of its range in Canada (Ogden et al. 2006). The environment of the soil and leaf litter, also strongly impacts tick survival because ticks spend the majority of their lives developing and taking shelter in this microclimate (Sonenshine, 1993).

Abiotic variables in the soil known to affect tick survival include soil texture, soil moisture and soil order. Soil texture is a permanent characteristic that indicates whether the soil particles are primarily large (sand), intermediate in size (silt) or small (clay) (Hillel, 1982). Soil texture impacts soil moisture, with sandy soils draining water more efficiently than clay type soils. *I. scapularis* presence has been found to be favored by sandy soils and disfavored by clay textured soils. Soil order, defined by a combination of factors including organic matter, soil pH and the identity of vegetation in the soil, also influences tick survival although the importance of each individual variable remains unclear (Guerra et al. 2002). The focus of this study was soil pH, a component of soil order classification and a factor of the microclimate which could affect tick mortality rates, but which has only received cursory consideration in previous studies.

Early evidence that soil pH could affect tick survival comes from research on the invasive Japanese stiltgrass, *Microstegium vimineum*, which has been shown to increase both tick mortality (Civitello et al. 2008) and soil pH (Ehrenfeld et al. 2001) as compared to native vegetation. Soil pH is determined by the concentration of $H^+$ ions in the soil solution, which is best known for controlling the availability of micronutrients to plants. High pH can lead to a decrease in the uptake of certain nutrients, while low pH can be toxic to plants by making too many micronutrients available (USDA Web Soil Survey). Similarly, it is possible that ticks may absorb toxic levels of soluble ions at certain pH levels during active sorption of atmospheric moisture. *I. scapularis* have three active life stages, including the larval, nymphal and adult stages. They must ingest a blood meal before molting into the next life stage, and while feeding they have mechanisms by which they excrete excess moisture. However, between blood meals ticks lose water through transpiration, most notably in engorged ticks which cannot close their spiracles to conserve moisture and cannot move to find an ideal microclimate for a period while molting, making water balancing mechanisms necessary. In addition to passive water sorption where water vapor directly enters the cuticle, ticks actively absorb moisture by secreting a salt solution from their salivary glands, which absorbs water and is ingested by the tick (Sonenshine, 2001).

This study focused on soil from two sites in upstate New York with naturally different soil pH values. The Albany Pine Bush Preserve (42.72, -73.88) is characterized by early successional vegetation such as pitch pines, dense shrubs and open grasslands. The Pine Bush has naturally acidic soils (USDA Web Soil Survey), with a sandy texture due to the draining of the large Glacial Lake Albany which had sand deposits on the lake floor (Albany Pine Bush Preserve Commission). Wolf Hollow (42.90, -74.07) developed along the Hoffman's fault due to

erosion and different rock types. The east side of the fault is abundant in shale and sandstone and has acidic clay soil, while the west side of the fault is characterized by limestone and dolostone and has naturally neutral to basic clay soil (Garver).

The question proposed in this study was: does soil pH affect the molting success of engorged *I. scapularis* larvae and nymphs. The pH may affect the amount of soluble ions absorbed by ticks during active water sorption. This could lead to another variable to take into consideration when generating future range expansion predictions, and possible methods for controlling tick densities by changing soil pH in areas where it will not cause detrimental effects on the ecosystem, such as on private residential properties. Engorged ticks were incubated in the field in naturally loam and silty-loam soils to control for the effects of soil texture on tick survival, and the experiment was replicated in the lab to control for the possibility that significant temperature and humidity fluctuations can decrease survival rates (Bertrand et al. 1996). This paper reports the laboratory portion of the experiment.

## Methods

### Experimental Design

This experiment used a 2x2 factorial design with soil texture and soil pH as the independent variables. The treatments included silty-loam textured soil collected from two sites west of the Wolf Hollow fault which have naturally different pH values (high and low), loam textured soil from the Albany Pine Bush (low pH), and pH-altered Pine Bush soil (high pH). High and low pH values were relative for this experiment, with low pH soil defined as very acidic (4.2-4.5 pH) and high pH soil defined as moderately acidic (6.0-6.5 pH). The purpose of

this design was to determine the effects of both soil texture and pH on the molting success of engorged *I. scapularis* nymphs, as well as the influence of one factor on the effects of the other.

**Soil Selection and pH Alterations**

To test the soil pH, approximately 5 g of each soil sample was briefly mixed with 20 ml of deionized (DI) water. Each sample was then filtered through a cheese cloth with an aspirator pump, and tested with a Mettler Toledo pH meter. The pH was recorded when the value on the meter remained constant for at least seven seconds. It was observed that the pH reading did not significantly change if the soil was allowed to settle in the water first.

Pelletized Espoma Organic Garden Lime was used to raise the pH of the Pine Bush soil. On April 19 2013, 2.5 g, 5.0 g, 7.5 g and 10.0 g of lime pellets were each added to 250 ml of Pine Bush soil in a plastic Tupperware container with mesh-covered holes in the lids to allow for air circulation. The samples were incubated until June 7 along with a control (no lime added) as well as 1.5 g and 0.5 g treatments which were added after the first week. Each sample was mixed daily and watered as needed to keep the soil moist. The pH of each treatment was measured and recorded weekly in order to determine that an approximate 1.5-2.0 g. lime pellets: 250 ml soil ratio would achieve the desired high pH range. On June 24, 274 g of lime pellets were added to 32.41 L of Pine Bush soil to be used for this study, and 80 g of lime pellets were added on July 2 to reach the desired pH range.

A similar test was performed to attempt to lower the pH of the Wolf Hollow soil with pelletized $AlSO_4$, although the pH could not be significantly altered. Soil samples were collected from different areas west of the Wolf Hollow fault to determine if soil with naturally different

pH values could be used. Two locations had soil with sufficiently high or low pH values for this experiment.

**Tick Enclosures**

The design for the tick enclosures, referred to here as soil cores, was modeled after the enclosures used in Brunner et al 2012. For each soil core, three holes 1.5 cm in diameter were drilled into the side of a cylindrical PVC pipe that was 10.5 cm in diameter and 5-6 cm tall. Hot glue was used to cover the holes with organdy mesh in order to prevent ticks from escaping while still allowing excess moisture to drain out of the soil. The PVC pipe was placed at the bottom of a 35 cm tall organdy mesh bag and kept in place with a ring of hot glue around the top and bottom openings to complete the core.

An 8 cm tall plastic sheet divided each of 8 27 cm wide x 34 cm long x 14 cm tall boxes approximately in half. The sheets were held in place with hot glue, and acrylic latex caulk with silicone was used to create a waterproof seal between each half. Pea gravel was spread 1 layer (1 cm) deep across the bottom of both halves. A straw was glued vertically in each corner of the box with one end in the layer of gravel for adding water to the bottom of the soil. Each box had a plastic lid with an organdy-covered 18 cm x 9 cm hole to allow for air circulation.

After removing as many roots and rocks from the soil as possible, each soil core was filled with soil and leaf litter and placed in one box half, surrounded to the top of the PVC pipe with soil from the same treatment. The top of the soil was kept at least 0.5 cm lower than the 8 cm plastic sheet to prevent soil transfer between the two box halves. The leaf litter in each treatment was representative of the natural leaf litter in that field site, with the Pine Bush soil cores receiving pine needles and the Wolf Hollow soil cores receiving maple leaves. There were

a total of 4 replicates each composed of 2 boxes and 4 soil cores, with the 2 low pH treatments on opposite sides of one box and the 2 high pH treatments in the other box.

**Tick Deployment** – see chapter 2 for tick collection methods.

Ticks from refrigerated vials were divided into the 16 soil cores, with 15 engorged nymphs in each core. For the 12 cores in reps 1, 2 and 3, ticks collected from 17 June to 3 July were randomized by placing an equal number of engorged nymphs collected from each collection date into each core on 3 July. Rep 4 was added on 12 July and consequently had more recently collected engorged nymphs. After leaf litter was placed on top of the ticks in the cores, the mesh bags were sealed with plastic zip ties. DI water was added through the straws into the pea gravel as needed to keep the soil moist by capillary action, which caused a gradient of soil moisture which decreased as the depth of the soil decreased. The boxes with the engorged nymphs were placed in two rows along a counter approximately 1 m from a window, and the order of the replicates moving away from the window was 2, 1, 3 and 4 respectively.

**Soil Data Collection**

The pH of the soil in each replicate was tested on the first day of tick deployment, and once approximately every 2 subsequent weeks until the ticks were removed. The percent moisture in the soil was also calculated every 2 weeks. Approximately 10 g of soil from each replicate was weighed and placed in a separate weigh boat. All 16 soil samples were then dried in an oven at 60 $^{0}$C for two days and placed in an airtight desiccator with desiccant to keep the soil free of moisture until a dry soil weight was obtained. The percent moisture was calculated as the percent difference between the dry soil and the wet soil. The gravimetric water content ($w$) was calculated as the water mass divided by the dry soil mass. For both pH and soil moisture

measurements, soil was taken from no more than 2 cm deep from around each soil core because it was assumed that engorged nymphs were not likely to be found at a greater depth.

Mechanical analysis had to be performed on soil from both sites to measure the soil texture, or percent composition of sand, silt and clay. Mechanical analysis is a delicate procedure involving the separation of soil aggregates and removal of organic matter (Hillel 1982). In order to obtain accurate descriptions of our soil textures, soil samples were sent to Cornell Nutrient Analysis Laboratories for mechanical analysis.

**Removal of Ticks from Cores**

Ticks were collected from soil cores between September 27 and October 11, 2013. Before checking each core, it was examined to ensure that the zip ties were still fastened tightly and that the bags had not been ripped. The core was placed on a white pan lined with Vaseline to prevent ticks from escaping, and the soil and core were searched by hand for 45 minutes. All four treatments from replicate 2 were checked for 80 minutes.

After the 45 minute interval, the soil was placed into a modified Berlese-Tullgren funnel (Barton 1995). Mesh was placed in the bottom of the funnel that had holes small enough to prevent most of the soil from falling through, but large enough to allow ticks to move through it. Water was placed inside the beaker holding the funnel to collect the ticks, and the funnel-beaker apparatus was placed inside a Vaseline-lined plastic bin with water in it. A heat lamp was placed above the funnel to dry the soil and to coerce the ticks into moving down through the soil. The beaker, plastic bin and dry soil were checked for ticks after 2 hours.

## Results

Additions of lime pellets significantly raised soil pH for at least 4 weeks during pH adjustment testing (Figure 1). $AlSO_4$ pellets did not significantly decrease pH for an extended period of time during adjustment testing (Figure 2). There were no notable fluctuations in soil pH in any treatments throughout the molting period (see Appendix).

All ticks that were collected alive from the soil cores had molted. The total molting percentage was 82.9%, with 199 of the original 240 ticks recovered alive. Three ticks were found dead and 38 ticks were not found. The average molting percentages in the Wolf Hollow high and low pH cores were 91.7% and 80% respectively, and the average molting percentages in the Pine Bush high and low pH cores were 78.3% and 81.7% respectively (Figure 3). According to the 2 way factorial ANOVA, the percentage of molted ticks was not significantly different between cores of high and low pH values, or between cores from Wolf Hollow or Pine Bush origin ($p>0.1$). Additionally, there was no significant interaction: the pattern of survival at each pH level was not affected by the soil texture ($p>0.1$). A 2 way factorial ANOVA using the mean rank of the molting percentage in each core was also performed, because the data were not normally distributed. The results of the ranked 2 way ANOVA showed no significant difference in molting percentages between the four treatments ($p>0.1$).

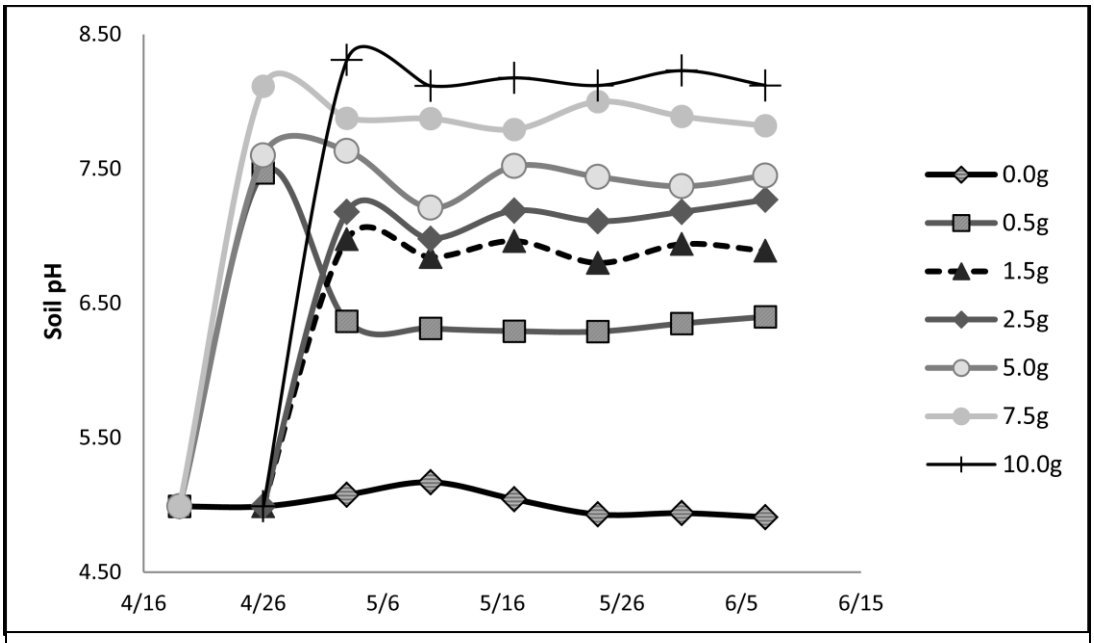


**Figure 1.** Soil pH values during the pH adjustment testing period for raising soil pH. The amount of pelletized lime (g) added to 250 ml of Albany Pine Bush soil for each treatment is shown, along with the pH of each treatment throughout the testing period.

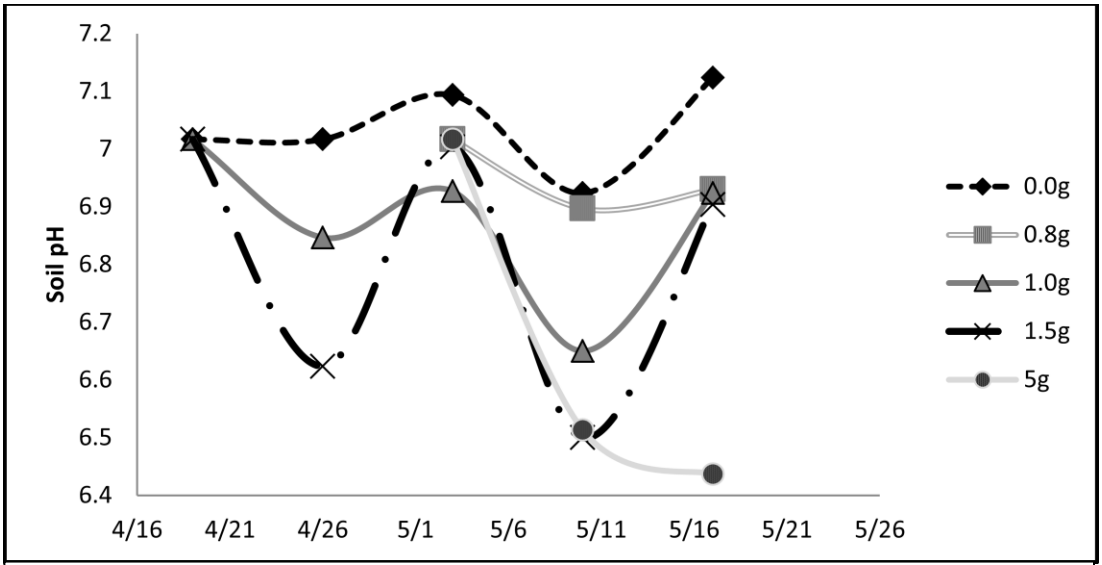


**Figure 2.** Soil pH values during the pH adjustment period for lowering soil pH. The amount of pelletized $AlSO_4$ (g) added to 250 ml of Wolf Hollow soil for each treatment is shown, along with the pH of each treatment throughout the testing period.

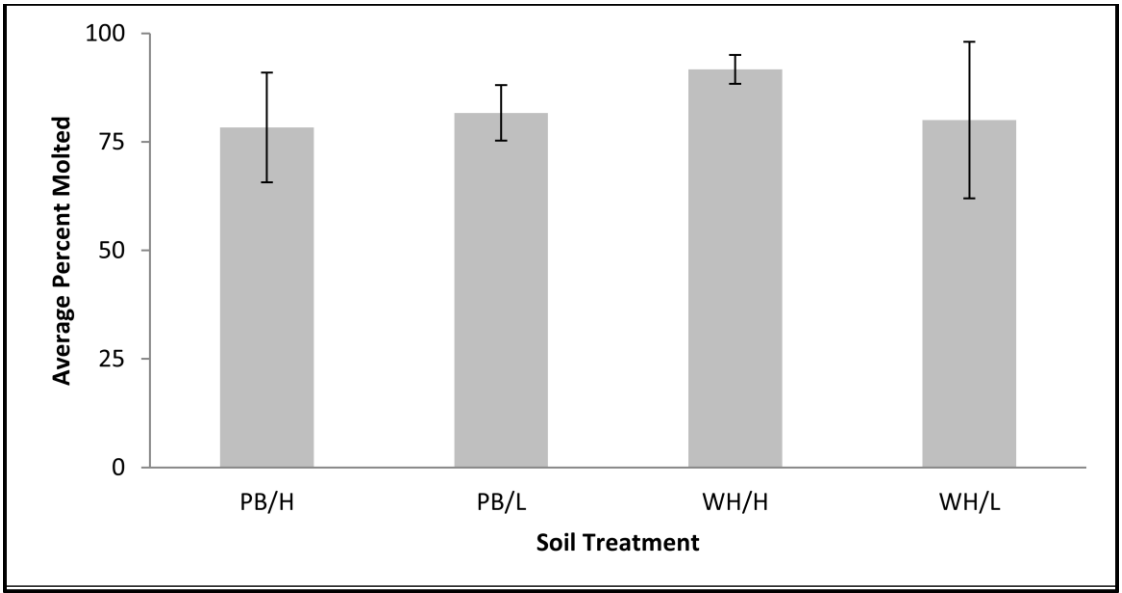


**Figure 3.** The average percent of molted ticks per soil treatment. PB and WH represent Pine Bush and Wolf Hollow. L and H represent low and high pH treatments. Error bars show standard deviations.

The average pH values in the Wolf Hollow cores were 6.48 and 4.36, and the average pH values in the Pine Bush cores were 6.26 and 4.31. Soil moisture in each core did not fluctuate significantly over time, however the average soil moisture in the Pine Bush cores was lower than in the Wolf Hollow cores in both the low pH (t-test; $p<0.001$) and the high pH (t-test; $p<0.001$) treatments. There was no significant correlation between soil pH and molting percent (linear regression; $p>0.1$; Figure 4) or between soil moisture and molting percent (linear regression; $p>0.1$; Figure 6). Similarly, soil moisture and pH were not correlated with the mean ranks of the molting percentages (Figures 5 and 7). When soil moisture was included as a covariate (ANCOVA) these results did not change, indicating that soil moisture did not impact survival ($p>0.1$). In addition, there was no significant difference between the average number of males and females collected in each core (t-test; $p>0.1$), and the date at which the ticks were collected from the cores did not affect molting percent (ANOVA; $p>0.5$) or percent recovery (ANOVA; $p>0.3$).

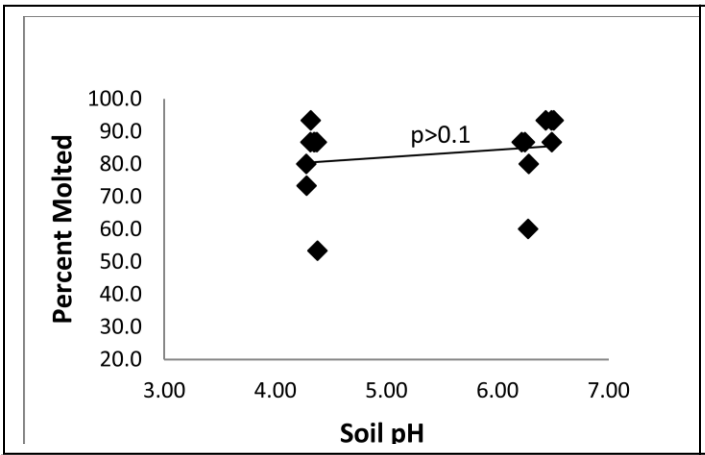


**Figure 4.** Percent of molted ticks at each pH level. Each diamond represents one replicate.

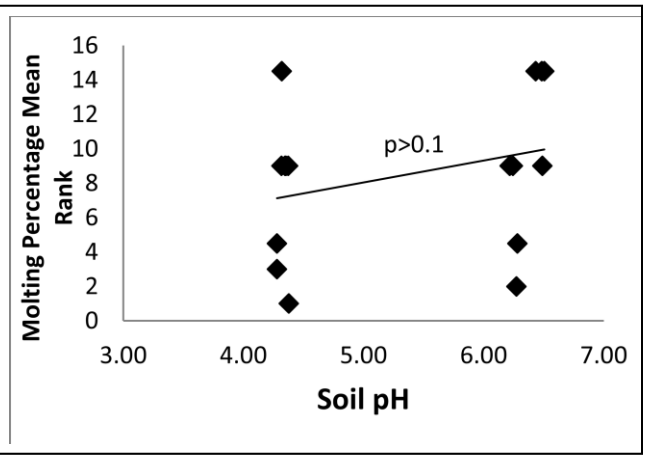


**Figure 5.** Molting percentage mean ranks at each soil pH level. Each diamond represents one replicate.

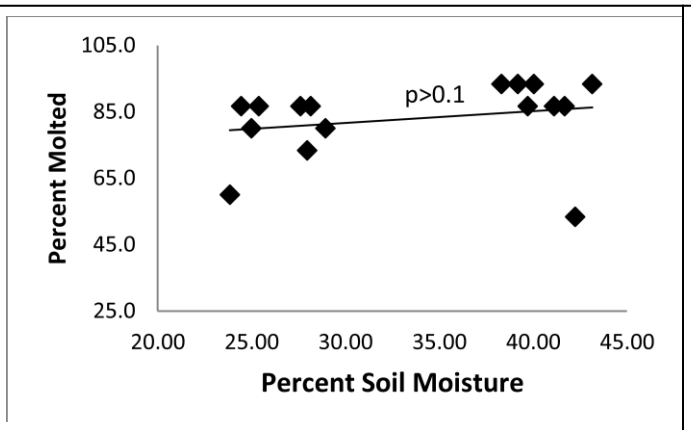


**Figure 6.** Percent of molted ticks vs. soil moisture. Each diamond represents one replicate.

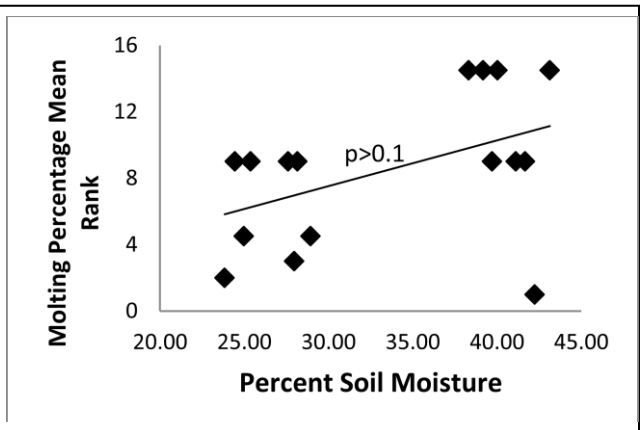


**Figure 7.** Molting percentage mean ranks vs. soil moisture. Each diamond represents one replicate.

## Discussion

The data suggest that soil pH at the levels tested does not affect the molting success of engorged *I. scapularis* nymphs. The pH range in this experiment reflects the high and low soil pH values which could be identified in upstate NY. There could be soil pH values that impede tick development but which were not identified in this study. It is possible that pH may affect tick fitness in ways that were not detected here such as the time it takes to molt, the size of the molted adult or its questing behavior. Other factors which were kept relatively constant such as providing adequate moisture, and providing leaf litter which helps ticks find favorable microclimates within the soil profile, could affect survival more than pH (Brunner et al. 2012). This could consequently have made any effects of pH on molting undetectable with this experimental approach and sample size. In order to plan future experiments and understand these results, it is necessary to review the relationships between the physical characteristics of the soil pH and soluble ion concentrations.

Clay particles and organic humus influence most of the physical behavior and control most of the chemical reactions and nutrient exchanges in soil. Clay particles have high surface area to mass ratio, with about 10,000 times the surface area of an equal weight of sand (Brady, 1974). Water adsorbs, or adheres, to the surface of clay and humus. The negative charge of clay particles attracts a layer of cations into the adsorbed water, which coupled with the negatively charged surface forms an electrostatic double layer. These cations are exchangeable with cations in the water in the surrounding pores, or soil solution (Hillel, 1982; Brady, 1974). The water content and nutrient concentration in the soil solution is freely available to organisms in the soil. The total quantity of exchangeable cations that can be adsorbed for a given mass of soil is the soil's cation exchange capacity. By adsorbing cation-rich water, clay and humus prevent cations

from leaching so that they can be steadily exchanged into the soil solution (Hillel, 1982; Brady, 1974).

Soil pH influences the concentrations of specific ions in adsorbed water, and consequently the availability of ions in the soil solution. The $H^+$ concentration in solution represents the acidity which the ticks are directly exposed to. The $H^+$ concentration adsorbed to clay particles and organic matter is commonly referred to as reserve acidity. If the $H^+$ ions are leached from solution, then they will be replaced by reserve $H^+$ ions until the reserve and active concentrations equalize, allowing the solution to change in pH. This inherent resistance to sudden and drastic changes in pH is known as buffering (Brady, 1974; Gerrard, 2000). At low pH values, $H^+$ ions adsorb tightly to soil particles and are not easily replaced. As a result, the cation exchange capacity increases as the soil pH increases and $H^+$ ions are more easily replaced by basic cations, such as $Ca^{+2}$, $Mg^{+2}$, $K^+$ and $Na^+$. These cations, along with other ions that also vary in concentration at exchange sites at different pH values, represent the soluble ions available to ticks during active and passive sorption of moisture (Brady, 1974).

The disparity between the high and low soil pH ranges in this study may not have been great enough. Many ions such as iron, manganese and zinc are less available at pH values above 7.5, whereas we did not obtain any basic pH values. Future studies could have different results by finding soil with naturally basic pH, or by adding more lime to increase the pH if possible. We were hesitant to add additional lime because we believed the lime itself could harm the ticks, which may not be true. As $Ca^{+2}$ and $Mg^{+2}$ ions from the lime replace $H^+$ ions at the exchange sites, the soil solution correspondingly adjusts to the new concentration of ions and the pH is raised. The exchangeable $Ca^{+2}$ and $Mg^{+2}$ ions will replace those in solution as they leach from the soil over time (Brady, 1974). Since the lime functions as the supply of $Ca^{+2}$ and $Mg^{+2}$, the pH

is an indirect measure of how much lime is in solution. If more lime is added and the pH is not raised any higher, then the additional lime must have leached from solution. High pH alone indicates the presence of the same or similar basic cations in solution. Any effects of liming the soil on tick molting success could be from the pH change caused by the lime rather than the lime itself.

Similarly, we did not attempt to lower the WH soil with aluminum sulfide after 7g $AlSO_4$/250ml soil did not lower the pH significantly. We wanted to avoid aluminum toxicity harming the ticks and affecting the results. As the pH gets lower than about 5.0, hydrogen ions dominate exchange sites. As a result the previously tightly adsorbed $Al^{+3}$ ions, as well as iron and manganese, are soluble in concentrations high enough to become toxic to plants. Also, low pH causes the normally insoluble aluminum hydroxide in soil solution to become soluble $Al^{+3}$. However this is recognized as a problem in all highly acidic soils regardless of whether aluminum pellets were added to the soil. Heavy rainfall can even increase the amount of soluble $Al^{+3}$ enough to cause aluminum toxicity (Brady, 1974; Gerrard, 2000). It was likely that the pH of the Wolf Hollow soil did not remain low because it was strongly buffered. In the future, a higher concentration of aluminum sulfide pellets could be used to deplete the reserve acidity and lower the pH. This would allow for the use of high and low pH soil treatments that originate from the same location. Using soil from two locations in Wolf Hollow that had naturally different pH values could have resulted in textural, water retention and microbial community differences.

In future studies, measurements of wet and dry soil volumes can be used to calculate the degree of saturation and the air-filled porosity of the soil at different levels of water content. Water content can then be controlled to provide moist conditions for ticks while not significantly

reducing soil aeration. Anaerobic soil environments often induce metabolic processes in microorganisms that include denitrification, manganese reduction, iron reduction and sulfate reduction. Many products of these processes are toxic to plant roots, and may also be toxic to engorged ticks with perpetually open spiracles (Hillel, 1982). Soil respiration is also influenced by temperature, so future experiments should include soil temperature logs (Lloyd and Taylor 1994).

The methods of measuring soil moisture and gravimetric water content used in this experiment are prone to error. Water can still be strongly adsorbed to the soil after oven drying, and conversely organic matter can decompose at high temperatures. Methods including measuring soil volume as opposed to mass, electrical resistance or neuron scattering would have provided more accurate data. Also, soil is usually dried at 105$^{o}$C for this calculation, so our soil (dried at 60$^{o}$C) probably retained adsorbed water (Hillel, 1982). In order to avoid decomposition of organic matter, soil should be dried at 86$^{o}$C, which a recent study has shown to be the optimum temperature for determining water content (O'Kelly 2005).

The higher gravimetric water content ($w$) in the Wolf Hollow cores as compared to Pine Bush cores could be due to differences in texture and organic material. Fine textured soils generally have more pore space than coarse textured soils (Brady, 1974). Water also adsorbs to clay particles with much more tension than to silt and sand particles (Hillel, 1982; Brady, 1974). As a result the saturation water content, or the amount of water needed to occupy all of the pore space for a given volume of soil, is generally higher in more clay soil (Hillel, 1982). Additionally, soil with a high percentage of organic matter has much higher saturation water content than soil that is more dominated by minerals, allowing for a high $w$ in heavily organic soils (Hillel, 1982; Brady, 1974). The higher clay content of the Wolf Hollow soil explains at least part of the reason

for its high *w* as compared to Pine Bush soil. However the Wolf Hollow soil may also have more organic matter than Pine Bush soil. Organic content increases exchange capacity. High exchange capacity causes high buffering capacity, which provides further evidence that the attempt to change the pH of the Wolf Hollow soil was hindered by buffering (Brady, 1974).

Soil can contain thousands of species of bacteria and fungi, which can alter characteristics of the soil such as its ability to form aggregates of particles. The microbial composition of the soil is in part dependent on the soil pH, the soil moisture and the amount of organic material present in the soil (Hillel, 1982). Since the Wolf Hollow and Pine Bush soils differed in organic material and soil moisture, and because they came from different habitats, the microbial floras were presumably dissimilar between soils from each site. The pH may also have affected microbial communities between treatments. Also magnesium is usually severely lacking in soils in the eastern United States (Brady, 1974). The lime used to raise the pH in this study contains a substantial proportion of magnesium, and as a result we may have added a previously absent nutrient which could have an impact on the microbial community. However, the non-significant differences in the data suggest that variations in soil microbial communities between treatments did not affect molting success.

A source of error in the data that needs to be addressed is the 38 unrecovered ticks. No openings were found in the organdy mesh enclosures and the zip ties were securely fastened, so escape from the cores is a highly unlikely explanation. Hornbostel et al. (2004) observed 87% molting success in nymphs placed in humid and temperature controlled vials with mesh tops. Our molting percentages were very similar to this, despite the numerous additional potential factors that are introduced with the soil and leaf litter. Assuming that these are normal nymphal molting percentages when most external factors are held constant, it is possible that none of these

factors including soil microbial community, moisture, texture and pH affected molting success in any significant way. If so, then possibly many of the missing ticks failed to molt and were too difficult to find in the soil.

The area with the immobile tick could have dried out, and then the tick desiccated as a result. Ticks could have become too difficult to find, or become brittle and fallen apart after desiccation. The soil atmosphere generally has higher relative humidity than the air above it, reaching as high as 100 percent (Brady, 1974). The regular watering of the soil in this experiment should have been favorable for tick survival, because they thrive best in high relative humidity. Rodgers et al. (2007) suggests that flat nymphs can survive a dry atmosphere for long periods of time, although this is probably not the case with engorged ticks which cannot close their spiracles in order to conserve water (Sonenshine, 1991).

Predation of ticks could also have accounted for this error. A worm was observed in each of two different cores; however the presence of organisms in the soil other than ticks was not strictly observed or recorded. Other species of arthropods and insects such as ants, beetles, mites and spiders have been known to prey on ticks (Samish and Alekseev 2001; Samish et al. 2004; Samish and Rehacek 1999). On rare occasions adult ticks have shown cannibalistic behavior with males feeding on females; however this was improbable in our study because cannibalism is uncommon in Ixodid ticks as compared to Argasid ticks, and we did not observe a difference in the number of males and females (Samish and Alekseev 2001, Samish and Rehacek 1999).

There could also have been unidentified tick pathogens present in the soil. Viruses as well as several species of bacteria, helminthes and fungi are suspected or documented pathogens of ticks. Most of these pathogens are only effective at high humidity, which the ticks were

provided with for this experiment (Samish and Rehacek 1999). Two notable entomopathogenic fungi include *Metarhizium anisopliae* and *Beauveria bassiana*, which have large geographic ranges and can cause high tick mortality rates (Samish et al. 2004, Kirkland et al. 2004). Fungal spores are commonly found in forest soils and leaf litter. If spores come into contact with a tick, they release enzymes including lipases, proteases and chitinases which allow the spore to release active cells through the cuticle and inside the tick, where the fungus grows and ultimately kills the tick. Occasionally fungi are easily noticed after killing a tick, however often electron microscopy or molecular techniques are required for identification (Tuininga et al. 2009, Kirkland et al. 2004).

Further studies are needed to determine if soil pH has any effect on *I. scapularis* ticks. One treatment should have truly basic pH (>7.0) because availability of certain nutrients changes drastically at high pH values. The soil textures were also very similar in this experiment. In the future, one of the textures should have higher clay content because clay influences the physical and chemical behavior of the soil more than the other particle classes. Clay also helps to form more stable aggregates of particles, which affect porosity and aeration (Hillel, 1982). Organic material should also be factored into the statistical analysis because it accounts for a significant portion of ion adsorption and buffering capacity. If soil pH does not affect engorged *I. scapularis* nymphs enough to prevent molting, then studies could test whether pH affects the molting incubation period or adult body mass.

# Chapter 2: Chipmunk Body Burden Analysis

## Introduction

Eastern chipmunks (*Tamias striatus*) are one of the most important hosts for *I. scapularis* ticks, along with white-footed mice (*Peromyscus leucopus*) and deer (Ostfeld et al. 1996). In New York State, *P. leucopus* are the major host for larvae whereas *T. striatus* are the major host for nymphs (Schmidt et al. 1999). *P. leucopus* are generally considered to have the highest reservoir competence, or the greatest likelihood of transmitting *Borrelia burgdorferi* spirochetes to black-legged ticks, with chipmunks having the second highest reservoir competence (LoGiudice et al. 2003; Giardina et al. 2000; Mather et al. 1989). While studies have found that chipmunks have the highest reservoir competence in natural habitats in Illinois and Minnesota, no such findings have been observed in the northeast (Mannelli et al. 1993; Johnson et al. 2011). As a result, factors affecting mouse body burdens could indirectly affect Lyme disease risk.

The prevalence of *B. burgdorferi* in a given habitat should decrease as species diversity increases. According to the dilution effect hypothesis, the decrease in tick burdens among the most competent disease reservoir should decrease the prevalence of infected ticks, and consequently decrease the Lyme disease risk in that area. While the tick burdens on mice have been shown in some cases not to be affected by increased diversity in residential habitats, the dilution effect hypothesis is strongly supported by previous research in natural wooded habitats (Schulze et al. 2005; LoGiudice et al. 2003; Ostfeld and Keesing 2000; Bouchard et al. 2011).

It is feasible that the tick body burdens for a chipmunk population are correlated with the body burden of the mouse population in the same habitat, given that chipmunk and mouse densities are strongly correlated (LoGiudice et al. 2003). Previous findings suggest that in Castle Rock State Park, Illinois, there was a significant correlation between larval body burdens on

mice and on chipmunks (Slajchert et al. 1997). If high nymphal burdens on chipmunks decreased the burdens on mice, then the infection prevalence of ticks could decline with increasing species diversity. Observing chipmunk body burdens could also help in determining the overall health of the chipmunk population, because tick burden may be indicative of overall parasite burden (O'Connor 2010).

Mean nymphal and larval body burdens observed on chipmunks vary considerably according to the season in which trapping was performed. The peak of questing larval activity is during the summer, and the peak of the nymphal season is late spring to early summer (Ostfeld et al. 1996). This seasonal activity is consistent in most studies including those performed in New York, Illinois, Wisconsin and Quebec, Canada, all of which found that nymphal abundance peaks in June and larval densities peak in mid to late August (LoGiudice et al. 2003; Slajchert et al. 1997; Mannelli et al. 1993; Bouchard et al. 2011). However, some habitats exhibit different seasonal patterns. Some studies have observed larval densities that peak in spring, corresponding with the nymphal peak, in addition to the larval peak in summer (Bouchard et al. 2011; Lindsay et al. 1993). In Minnesota, Johnson et al. (2011) observed peak abundances of both life stages occurring 1-2 months earlier than in all the above papers.

Individual differences between chipmunks could also explain some variation in body burdens. Boyer et al. (2010) found that body burden is related to a chipmunk's personality. They suggest that *Ixodes ricinus*, the primary vector of *B. burgdorferi* in Europe, preferentially infested Siberian chipmunks (*Tamias sibiricus*) in France that displayed higher levels of activity and exploration and consequently had a wider home range. Boyer et al. (2010) also found that male chipmunks had significantly higher body burdens than female chipmunks, and that body burden was positively related to a chipmunk's body mass. In contrast, studies from New York

indicate that the *I. scapularis* burden on an eastern chipmunk is not related to sex or body mass (Schmidt et al. 1999; Brunner and Ostfeld 2008). Brunner and Ostfeld (2008) also observed that chipmunks who fed more nymphs also fed more larvae, suggesting that there is an unidentified factor that causes an individual chipmunk to have a greater body burden.

I present the relevant results from trapping eastern chipmunks for *I. scapularis* collection from the Albany Pine Bush, NY in the summer of 2013 during the nymphal season. The nymphal body burdens of chipmunks are compared both qualitatively and quantitatively with those found in the literature from trapping sessions that occurred during the same season, in order to determine whether these results were consistent with past findings. I also determine if the date of the nymphal peak in our data matches that of other studies, and if my data show any significant relationships between nymphal body burden and sex or body mass.

## Methods

Chipmunks were captured from the Albany Pine Bush Kaikout site (42.71103, -73.8713) and from near the Pine Bush Discovery Center (42.71837, -73.8603) using Sherman traps. From 17 June to 9 July, chipmunks were captured, sexed, weighed and tagged. Pregnant and lactating females were immediately released. Each chipmunk was held for 3 days in a wire cage suspended inside a box over wet paper toweling to provide a moist environment for engorged ticks that fell off the chipmunk. Three days of engorged tick collection data represents the chipmunk's body burden (LoGiudice et al. 2003). Each box was checked once per day for *I. scapularis* ticks.

## Results

A total of 26 chipmunks were held, with 5 animals held on two occasions separated by at least 2 weeks. Recapture of a chipmunk did not affect its body burden (t test; p=0.133). Seventeen female and 14 male chipmunks were captured. The difference in nymphal body burdens between male (mean=30.1; SD=5.49) and female (mean=14.3; SD=15.7) chipmunks was just near significant (t test; p=0.052). There was no significant difference between male and female body mass (t-test; p=0.094). There was also no significant difference between the body mass of chipmunks captured in this study and those captured in the pilot project from the previous summer (Ahern 2012; t-test; p>0.1).

A linear regression suggested no significant relationship between chipmunk body mass and body burden (p=0.11). However, according to a Spearman rank correlation, there was a significant positive correlation between chipmunk body mass and body burden after ranking the data to make it normally distributed (p=0.02; Figure 1). The number of nymphs per chipmunk declined during the course of the capture period from 17 June to 9 July, with the peak mean nymphal burden of 55.2 nymphs per chipmunk on the 26 June capture date (Figure 2). The overall mean nymphal body burden was 21.5 (SD=22.7), and the mean larval body burden was 1.1 (SD=1.9). The mean nymphal burden was compared with those from published studies which were also performed during the nymphal season (Figure 3). Larval burdens were not compared with published studies because our capture period did not extend into the larval season, resulting in significantly lower larval burdens than those in published papers.

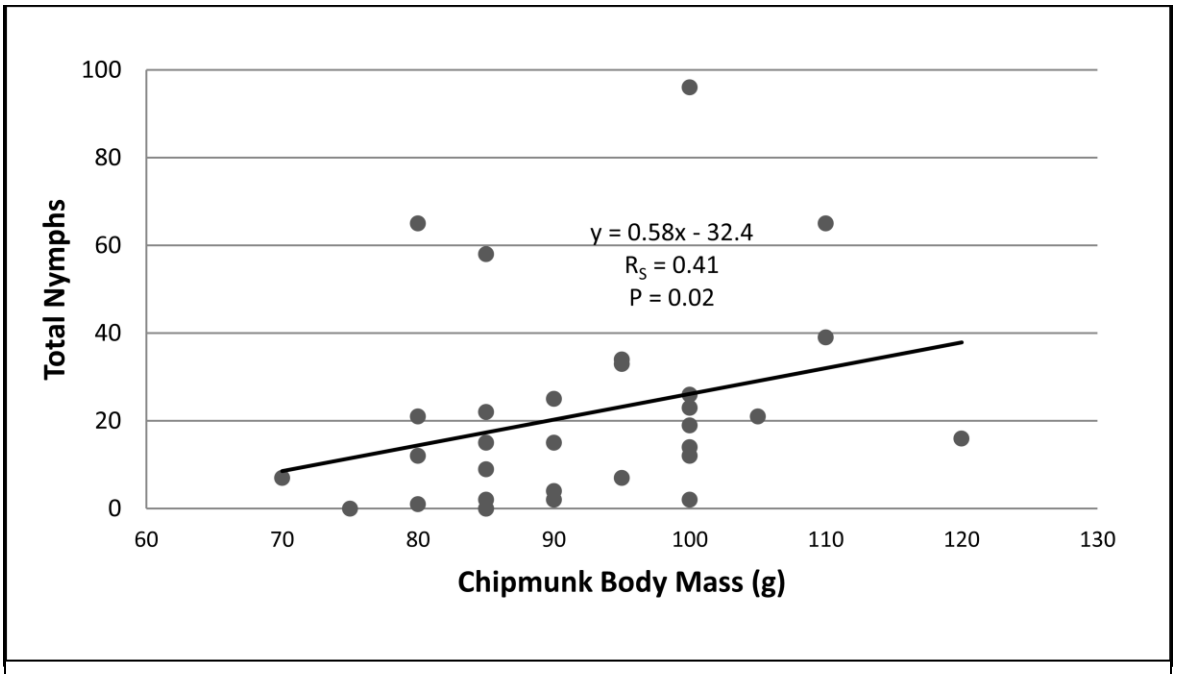


**Figure 1**. Total nymphs per chipmunk vs. chipmunk body mass (g). Each data point shows the mass and nymphal burden of one chipmunk. P and $R_s$ values were calculated using the Spearman rank correlation.

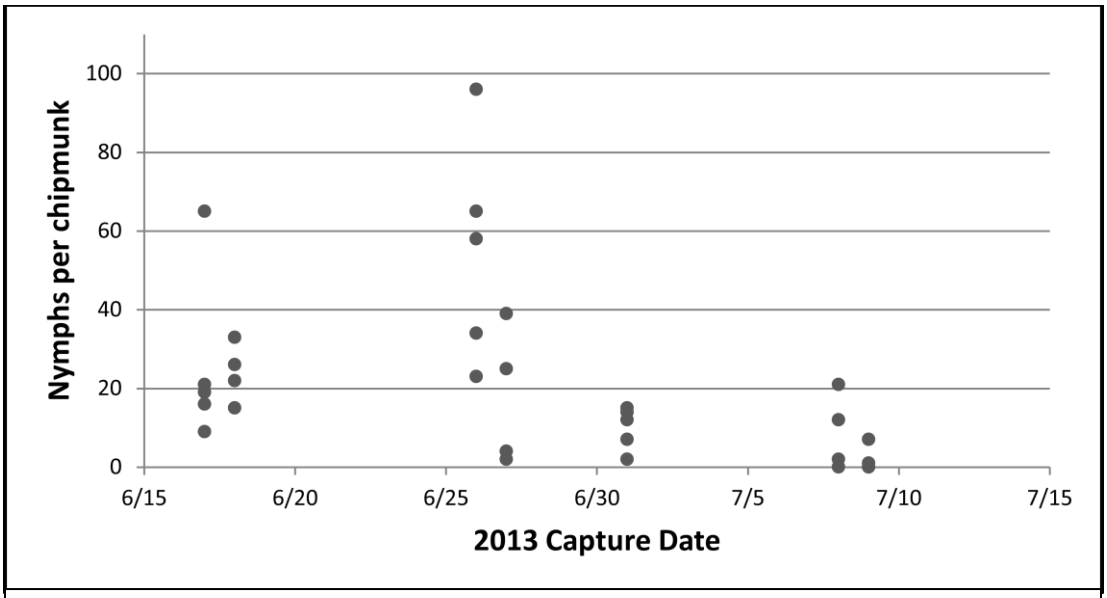


**Figure 3.** Average nymphs per chipmunk vs. capture date. Each data point represents the nymphal burden and capture date of one chipmunk.

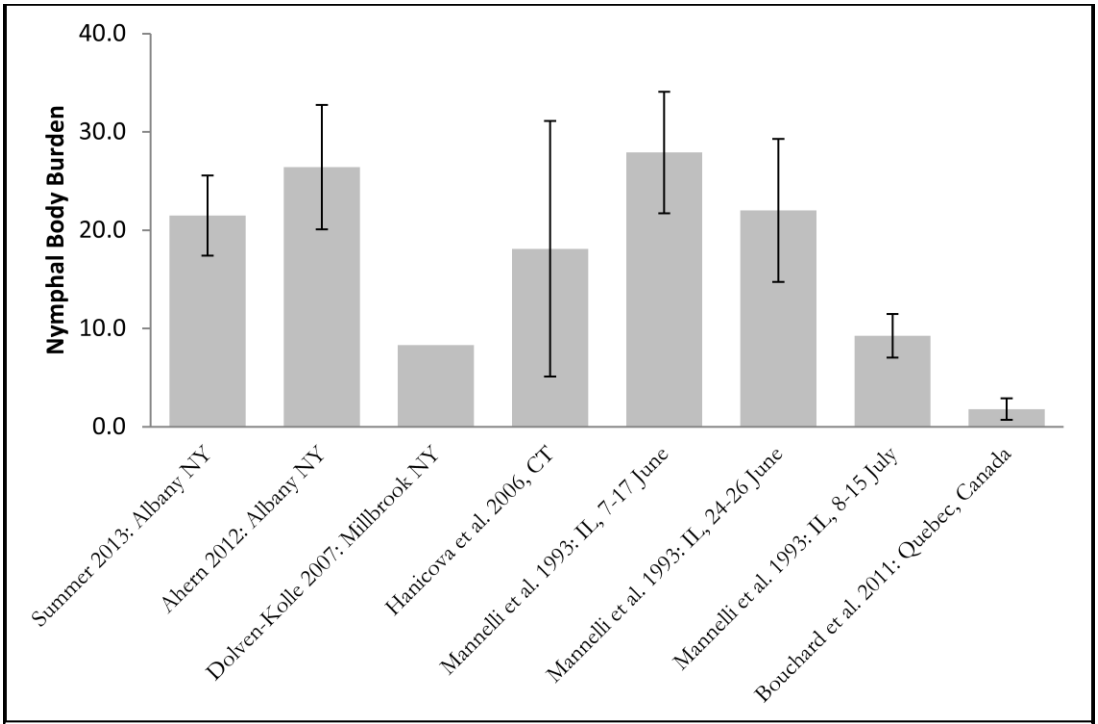


**Figure 4.** Nymphal body burdens from the summers of 2012 and 2013, as well as from published work. Each bar represents the mean nymphal burden on chipmunks in the given paper, with the location and capture period shown where applicable. Error bars represent standard error where it was available.

## Discussion

The non-significant difference between male and female body burdens is consistent with other research performed in the northeast. However, these data conflict with Boyer et al. (2010), which could be due primarily to species-specific differences in behavior between *T. striatus* and *T. sibiricus*. Boyer et al. (2010) found that males had both significantly higher activity levels and larger home ranges than females, and they suggest that the difference in home range size was a major factor in males having greater body burdens. Our data suggest that either home range is not as strong of a factor in determining body burden in the Albany Pine Bush, or the difference in personalities between *T. striatus* males and females is less pronounced than in *T. sibiricus*.

The positive relationship between chipmunk body mass and nymphal body burden conflicts with Brunner and Ostfeld (2008). Using a much larger dataset, they found no relationship between body mass and body burden in New York. However these data do agree with Boyer et al. (2010), who found a positive relationship between body mass and body burden in France. They also suggested that this was due to the increased surface area on the heavier chipmunks, which potentially agrees with the finding of Brunner and Ostfeld (2008) that a host has a limit to the number of ticks that can infest them at any time. Since both papers have different results regarding mass and body burden, and because these data agree with those from a different geographical region and focus on different host and vector species, it is more likely that the unidentified factor mentioned in Brunner and Ostfeld (2008) is causing these significant results as opposed to weight.

The nymphal peak in late June and subsequent decrease in nymphal body burdens in early July are consistent with published research (Slajchert et al. 1997; Mannelli et al. 1993; Bouchard et al. 2011; Ostfeld et al. 2006). This suggests that there were no severe unidentified circumstances affecting the normal seasonal activity of the nymphs, which may otherwise have skewed our data. Johnson et al. (2011) suggests that variations in seasonal activity reported in Minnesota are due to the lower minimum temperatures as compared to the northeast, which could also explain the slight variation in the seasonal activity in Quebec (Bouchard et al. 2011). The trapping period in our study did not extend far enough into the larval season to compare to published larval burden data.

Our chipmunk nymphal body burden data reasonably corresponds with most published data in New York, Connecticut and Illinois (Figure 3). The body burdens in Quebec are probably low because that region is just beginning to establish tick populations (Bouchard et al. 2011).

The only unexpected results were the low nymphal burdens reported in Dolven-Kolle (2007), which were observed in New York. This inconsistency is probably not due to limited sample size, because Dolven-Kolle (2007) captured 55 chipmunks. Additionally they used the same tick collection methods during the same season as previous experiments. There was likely another factor, such as generally low nymphal density at the Institute of Ecosystem Studies in the spring of 2007, which caused those results.

It should also be noted that body burden has previously been correlated with chipmunk personality. Chipmunks which were determined to be more active were also both more prone to being captured and more heavily infested with ticks (Boyer et al. 2010). If this holds true in the Albany Pine Bush, then observed body burdens may not be representative of the entire chipmunk population.

Based on a review of and comparison with previous studies, no serious deviations in body burdens were discovered. Nymphal body burdens and seasonal fluctuations in abundance were similar to what would be predicted based on the literature, and should not strongly affect the major findings of this study regarding the effects of soil pH and texture on molting success. Future studies can add to this compilation of data to help establish what is considered a "normal" nymphal body burden for an eastern chipmunk in a given habitat and during a given season.

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## APPENDIX

**Table 1. Soil pH and percent moisture data during the nymphal molting period.**

| Soil Origin | Replicate | Treatment | Date (2013) | pH | % Moisture | Soil Origin | Replicate | Treatment | Datev (2013) | pH | % Moisture |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PB | 1 | High | 10-Jul | 6.29 | 25.06 | WH | 1 | High | 10-Jul | 6.5 | 40.44 |
| PB | 1 | High | 23-Jul | 6.24 | 26.53 | WH | 1 | High | 23-Jul | 6.51 | 41.20 |
| PB | 1 | High | 23-Aug | 6.17 | 23.22 | WH | 1 | High | 23-Aug | 6.5 | 38.65 |
| PB | 1 | High | 13-Oct | 6.19 | 22.96 | WH | 1 | High | 13-Oct | 6.44 | 39.83 |
| PB | 1 | Low | 10-Jul | 4.62 | 28.27 | WH | 1 | Low | 10-Jul | 4.79 | 43.34 |
| PB | 1 | Low | 23-Jul | 4.38 | 29.43 | WH | 1 | Low | 23-Jul | 4.46 | 42.36 |
| PB | 1 | Low | 23-Aug | 4.14 | 27.78 | WH | 1 | Low | 23-Aug | 4.24 | 42.20 |
| PB | 1 | Low | 13-Oct | 4.15 | 26.34 | WH | 1 | Low | 13-Oct | 4.22 | 38.79 |
| PB | 2 | High | 10-Jul | 6.21 | 24.67 | WH | 2 | High | 10-Jul | 6.47 | 40.43 |
| PB | 2 | High | 23-Jul | 6.18 | 24.73 | WH | 2 | High | 23-Jul | 6.5 | 39.77 |
| PB | 2 | High | 23-Aug | 6.4 | 22.66 | WH | 2 | High | 23-Aug | 6.48 | 40.14 |
| PB | 2 | High | 13-Oct | 6.35 | 23.30 | WH | 2 | High | 13-Oct | 6.51 | 38.47 |
| PB | 2 | Low | 10-Jul | 4.53 | 29.02 | WH | 2 | Low | 10-Jul | 4.68 | 42.76 |
| PB | 2 | Low | 23-Jul | 4.5 | 29.76 | WH | 2 | Low | 23-Jul | 4.52 | 44.08 |
| PB | 2 | Low | 23-Aug | 4.23 | 25.86 | WH | 2 | Low | 23-Aug | 4.28 | 41.30 |
| PB | 2 | Low | 13-Oct | 4.25 | 25.78 | WH | 2 | Low | 13-Oct | 4.2 | 40.77 |
| PB | 3 | High | 10-Jul | 6.22 | 25.19 | WH | 3 | High | 10-Jul | 6.43 | 37.05 |
| PB | 3 | High | 23-Jul | 6.28 | 27.06 | WH | 3 | High | 23-Jul | 6.43 | 41.67 |
| PB | 3 | High | 23-Aug | 6.33 | 23.49 | WH | 3 | High | 23-Aug | 6.41 | 39.82 |
| PB | 3 | High | 13-Oct | 6.31 | 24.19 | WH | 3 | High | 13-Oct | 6.47 | 34.69 |
| PB | 3 | Low | 10-Jul | 4.48 | 28.44 | WH | 3 | Low | 10-Jul | 4.61 | 40.29 |
| PB | 3 | Low | 23-Jul | 4.45 | 30.06 | WH | 3 | Low | 23-Jul | 4.52 | 41.60 |
| PB | 3 | Low | 23-Aug | 4.12 | 27.56 | WH | 3 | Low | 23-Aug | 4.22 | 42.63 |
| PB | 3 | Low | 13-Oct | 4.32 | 26.52 | WH | 3 | Low | 13-Oct | 4.19 | 39.91 |
| PB | 4 | High | 10-Jul |  | 26.30 | WH | 4 | High | 10-Jul |  | 39.77 |
| PB | 4 | High | 23-Jul | 6.26 | 28.30 | WH | 4 | High | 23-Jul | 6.53 | 41.31 |
| PB | 4 | High | 23-Aug | 6.24 | 22.86 | WH | 4 | High | 23-Aug | 6.47 | 37.63 |
| PB | 4 | High | 13-Oct | 6.24 | 24.04 | WH | 4 | High | 13-Oct | 6.52 | 38.01 |
| PB | 4 | Low | 10-Jul |  | 25.68 | WH | 4 | Low | 10-Jul |  | 41.73 |
| PB | 4 | Low | 23-Jul | 4.47 | 30.98 | WH | 4 | Low | 23-Jul | 4.55 | 43.97 |
| PB | 4 | Low | 23-Aug | 4.15 | 30.05 | WH | 4 | Low | 23-Aug | 4.24 | 47.01 |
| PB | 4 | Low | 13-Oct | 4.28 | 29.01 | WH | 4 | Low | 13-Oct | 4.24 | 39.85 |

**Table 2.** **Tick collection and mean soil pH and moisture data.**

| Site | pH Hi/Low | Rep | Average pH | Date collected (2013) | Live Female | Live Male | Dead Unmolted | Percent Alive | Avg. Soil Moisture (%) | Gravimetric Water Content (*w*) |
|---|---|---|---|---|---|---|---|---|---|---|
| PB | High | 1 | 6.22 | 8-Oct | 7 | 6 | 0 | 86.7 | 24.44 | 32.40 |
| PB | High | 2 | 6.28 | 27-Sep | 6 | 3 | 0 | 60.0 | 23.84 | 31.32 |
| PB | High | 3 | 6.28 | 10-Oct | 4 | 8 | 2 | 80.0 | 24.98 | 33.34 |
| PB | High | 4 | 6.25 | 11-Oct | 9 | 4 | 0 | 86.7 | 25.38 | 34.11 |
| PB | Low | 1 | 4.28 | 9-Oct | 4 | 7 | 0 | 73.3 | 27.96 | 38.84 |
| PB | Low | 2 | 4.36 | 27-Sep | 6 | 7 | 0 | 86.7 | 27.61 | 38.22 |
| PB | Low | 3 | 4.32 | 10-Oct | 7 | 6 | 0 | 86.7 | 28.15 | 39.22 |
| PB | Low | 4 | 4.28 | 11-Oct | 6 | 6 | 0 | 80.0 | 28.93 | 40.82 |
| WH | High | 1 | 6.49 | 8-Oct | 7 | 7 | 0 | 93.3 | 40.03 | 66.79 |
| WH | High | 2 | 6.49 | 8-Oct | 7 | 6 | 0 | 86.7 | 39.70 | 65.87 |
| WH | High | 3 | 6.43 | 10-Oct | 8 | 6 | 0 | 93.3 | 38.31 | 62.40 |
| WH | High | 4 | 6.51 | 11-Oct | 9 | 5 | 0 | 93.3 | 39.18 | 64.52 |
| WH | Low | 1 | 4.37 | 8-Oct | 6 | 7 | 0 | 86.7 | 41.67 | 71.59 |
| WH | Low | 2 | 4.38 | 11-Oct | 4 | 4 | 0 | 53.3 | 42.23 | 73.18 |
| WH | Low | 3 | 4.35 | 10-Oct | 10 | 3 | 1 | 86.7 | 41.11 | 69.86 |
| WH | Low | 4 | 4.32 | 11-Oct | 6 | 8 | 0 | 93.3 | 43.14 | 76.27 |

**Table 3.** **Soil mechanical analysis from Cornell Nutrient Analysis Laboratories.**

| Soil Origin | Sand (%) | Silt (%) | Clay (%) | Texture |
|---|---|---|---|---|
| Albany Pine Bush | 41.83 | 46.35 | 11.82 | Loam |
| Wolf Hollow | 3.88 | 76.8 | 19.32 | Silty-Loam |

**Table 4.** **Larval (L) and Nymphal (N) *I. scapularis* collection and *T. striatus* body burden data.**

| Capture Date | Sex | Weight (g) | Day 1 L | Day 1 N | Day 2 L | Day 2 N | Day 3 L | Day 3 N | Total L | Total N | Recapture Y/N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6/17/2013 | F | 120 | 0 | 1 | 0 | 0 | 0 | 15 | 0 | 16 | N |
| 6/17/2013 | F | 100 | 1 | 1 | 0 | 7 | 0 | 11 | 1 | 19 | N |
| 6/17/2013 | F | 110 | 0 | 7 | 1 | 38 | 0 | 20 | 1 | 65 | N |
| 6/17/2013 | M | 105 | 1 | 3 | 0 | 7 | 3 | 11 | 4 | 21 | N |
| 6/17/2013 | M | 85 | 0 | 2 | 0 | 3 | 0 | 4 | 0 | 9 | N |
| 6/18/2013 | F | 85 | 0 | 1 | 0 | 8 | 1 | 13 | 1 | 22 | N |
| 6/18/2013 | F | 100 | 0 | 7 | 0 | 11 | 0 | 8 | 0 | 26 | N |
| 6/18/2013 | F | 85 | 0 | 3 | 2 | 5 | 0 | 7 | 2 | 15 | N |
| 6/18/2013 | M | 95 | 0 | 3 | 0 | 15 | 0 | 15 | 0 | 33 | N |
| 6/26/2013 | F | 100 | 0 | 1 | 0 | 14 | 0 | 8 | 0 | 23 | N |
| 6/26/2013 | M | 95 | 0 | 7 | 0 | 11 | 0 | 16 | 0 | 34 | N |
| 6/26/2013 | M | 100 | 0 | 13 | 0 | 38 | 0 | 45 | 0 | 96 | N |
| 6/26/2013 | M | 80 | 5 | 20 | 2 | 28 | 0 | 17 | 7 | 65 | N |
| 6/26/2013 | M | 85 | 1 | 9 | 1 | 32 | 0 | 17 | 2 | 58 | N |
| 6/27/2013 | F | 100 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 2 | N |
| 6/27/2013 | F | 90 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 4 | N |
| 6/27/2013 | M | 90 | 0 | 2 | 1 | 13 | 0 | 10 | 1 | 25 | N |
| 6/27/2013 | M | 110 | 0 | 3 | 1 | 17 | 0 | 19 | 1 | 39 | N |
| 7/1/2013 | F | 100 | 0 | 3 | 0 | 9 | 0 | 0 | 0 | 12 | Yes |
| 7/1/2013 | F | 100 | 0 | 1 | 0 | 8 | 0 | 5 | 0 | 14 | N |
| 7/1/2013 | F | 90 | 2 | 0 | 5 | 4 | 0 | 11 | 7 | 15 | N |
| 7/1/2013 | M | 85 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 2 | Yes |
| 7/1/2013 | M | 70 | 0 | 1 | 0 | 4 | 0 | 2 | 0 | 7 | Yes |
| 7/8/2013 | F | 90 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 2 | Yes |
| 7/8/2013 | F | 85 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N |
| 7/8/2013 | M | 80 | 1 | 4 | 0 | 11 | 0 | 6 | 0 | 21 | Yes |
| 7/8/2013 | M | 80 | 4 | 3 | 0 | 6 | 0 | 3 | 4 | 12 | N |
| 7/9/2013 | F | 85 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N |
| 7/9/2013 | F | 80 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | N |
| 7/9/2013 | F | 95 | 0 | 2 | 0 | 1 | 0 | 4 | 0 | 7 | N |
| 7/9/2013 | M | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N |