

Impact of forest type on blacklegged tick (*Ixodes scapularis*) abundance

By

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March, 2025



**Allegheny College
Department of Biology**

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**A Senior Comprehensive Project in Partial Fulfillment of the Requirements
for a Bachelor of Science Degree from Allegheny College**

*I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code
and to maintain the integrity of both myself and the College community as a whole.*

Pledge

Approval by the Senior Thesis Committee:

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Abstract

Forests can vary in composition both within themselves and between other forests. These variations include forest stand and age, tree and understory vegetation species, microclimate, size, and environmental heterogeneity. Differences in these features can lead to variations in animal communities within the forest due to changes in food/resource abundance and shelter availability. Vertebrate populations can vary in abundance due to forest size and food availability/understory growth. Habitat suitability and host availability are two important factors that drive a tick's ability to survive, and both of these factors can change with forest age, vegetation, and density. As such, tick abundance can also vary with shifts in forest features. This project investigated how microclimate variables, and vegetation density and richness impact the abundance of the blacklegged tick (*Ixodes scapularis*). Additionally, it was explored how tick abundance changes over the seasons. To conduct this study, six 75x10 meter plots were set up in forested areas that appear visually unique in their vegetation composition. Each site was inventoried by looking at canopy cover, understory vegetation richness and density, leaf litter depth, ground cover, and abiotic variables. Each plot was also dragged for ticks to see how tick density may relate to variations in the aforementioned variables. After running the biotic variables through a principal component analysis, total and larval tick abundances were found to significantly decrease as tree density and richness increased. Additionally, there were trends in the data suggesting a positive correlation between tick abundance and relative humidity and air temperature, and a negative correlation between tick abundance and sampling date. Microclimate variables, which are largely driven by vegetation composition influence tick abundance suggesting that forest variation is important, but not the strongest predictor of tick abundance.

Introduction

Forests can vary in composition both within themselves and between other forests. Four primary types of trees compose forests, those being angiosperms, gymnosperms, deciduous, and evergreen (Kilgore, 2023). Angiosperms are flowering and fruit bearing, while gymnosperms don't flower, typically growing their seeds in cones or on leaves (Kilgore, 2023). Deciduous trees shed their leaves during colder seasons while evergreen trees retain their leaves year-round (Kilgore, 2023). Coniferous trees are all gymnosperms and can be characterized by their needle-like leaves and production of cones. (Andersson and Lhoir, 2005). These trees compose the four main types of forests, those being temperate, boreal, tropical, and subtropical (Hancock, 2019). Coniferous forests tend to have more leaf litter production and accumulation than deciduous forests (Suh et al., 2005, Min et al., 2006). Deciduous forests tend to have more solar radiation on average, and a larger temperature range in both the soil and atmosphere (Suh et al., 2005). Not only are these tree types used to categorize forests themselves, they scale to some degree with how ecologists categorize biomes. While boreal forests, which experience cold weather year-round, are the most abundant in North America, the northeastern United States is dominated by temperate forests due to the seasonal variation in the area.

In addition to biome and forest type, forests can differ in age and microclimate. They can be evenly or unevenly aged, and there are old-growth and new growth forests (Adalsteinsson et al., 2018). One study found that large trees were absent or uncommon in forest stands approximately 20-40 years old. Alternatively, older forest stands (70-100 years old) had a more even distribution of trees in each class size, including large-class trees (DeWalt et al., 2003). Age has also been shown to impact plant species composition between forest types (Jean et al., 2017). This is likely due to the greater amount of leaf litter accumulation and thicker canopy cover

found in older forests (Jean et al., 2017, Adalsteinsson et al., 2018). Both of these factors impact how well understory vegetation can regrow. A study found that, as forests mature, the density of understory vegetation tends to decrease, and the amount of downed woody debris present increases (DeWalt et al., 2003). Between four stand-age classes ranging from 12 years old to 200+ years, it was found that temperatures in the youngest forests had the hottest median daytime temperature while also having the lowest median, and most variable, nighttime temperatures (Lindenmayer et al., 2022).

Age isn't the only factor impacting climate variables in forests. Oftentimes, the climate within a forest differs from the weather recorded at nearby weather stations (Boehnke et al., 2017). The relative humidity in leaf litter is, on average, greater than the atmosphere outside of the forest (Boehnke et al., 2017). Canopy cover reduces the amount of sunlight and precipitation that reaches the forest floor, and also limits wind intensity (Boehnke et al., 2017). This, in turn, creates what's called a microclimate. Generally, forests have a more narrow temperature range than the outside macroclimate. They tend to have greater minimum temperatures, and lower maximum and mean temperatures (Díaz-Calafat et al., 2023). This leads to forests being cooler in the summer and warmer in the winter compared to temperatures measured outside of the forest (Díaz-Calafat et al., 2023). This effect is dependent on forest density, with offsets between microclimate and macroclimate being larger as tree density and canopy cover increase (Díaz-Calafat et al., 2023). As tree density increases, an increase in the minimum air temperature compared to the surrounding macroclimate has been observed (Díaz-Calafat et al., 2023). One study found that both overstory and midstory canopy cover were inversely related to median daytime temperature within various even-aged forests (Lindenmayer et al., 2022). Seasonal variation and tree composition are also important factors for microclimate variation within

forests. A greater percentage of broadleaf trees has varying effects on minimum air temperature offsets depending on the season. In the summer months, as the percentage of broadleaf trees increased, the difference between the minimum macro and microclimate temperatures also increased (Díaz-Calafat et al., 2023). This suggests that broadleaves retain energy released from the ground better than conifers (Díaz-Calafat et al., 2023). During winter, however, the difference between the minimum temperatures decreases with an increasing percentage of broadleaf trees due to their seasonal loss of leaves (Díaz-Calafat et al., 2023). Within forests, their composition can differ with canopy cover, understory vegetation, and microclimate (Adalsteinsson et al., 2018, Brennan et al., 2023, Talbot et al., 2019). All these factors can be altered in some way with forest age, tree density and diversity, and management techniques (Adalsteinsson et al., 2018, Brennan et al., 2023).

Differences in forest age, tree density, and tree diversity don't only impact forest features, but also population composition and species distribution of organisms that live within forests. Forest populations are largely dependent on environmental factors such as plant diversity and density, and forest age and size (Kent et al., 2014, Sperry et al., 2001, Uusitalo et al., 2022). It's been found that the abundance of certain animal species can change within forests that have differing tree species densities and diversities (Sperry et al., 2001). For example, the abundance of the honeylocust plant bug (*Blepharidopterus chlorionis*) decreased with increasing tree species diversity (Sperry et al., 2001). They also found that the abundance of mimosa webworm (*Homadaula anisocentra*) decreased as honeylocust tree density also decreased (Sperry et al., 2001). As with invertebrates, forest composition influences the vertebrate population. Eastern woodrats (*Neotoma floridana*) and white-footed mice (*Peromyscus leucopus*) were positively associated with the amount of leaf litter and tree density, and negatively associated with low

canopy openness (Hackney and Stancampiano, 2015). Shrews have also been shown to be positively associated with the amount of leaf litter and shrub cover present (Fisher and Wilkinson, 2005). The abundance of white-footed mice is positively associated with vertical vegetation complexity provided by trees and shrubs, canopy cover, and understory vegetation density (Hackney and Stancampiano, 2015). In the spring and summer, white-tailed deer (*Odocoileus virginianus*) primarily forage on green leaves, buds, and twig tips, all of which are more readily available in younger and thinner forests with less canopy cover (Johnson et al., 1995, Bassing et al., 2022). In one study, they found a negative correlation between white-footed mouse density and forest fragment size (LoGiudice et al., 2008). White-footed mice and white-tailed deer are generalist species meaning that they have a broad environmental tolerance and can survive in most habitats (Büchi and Vuilleumier., 2014, Hackney and Stancampiano, 2015). With differing forest size and age, though, the abundance of generalist species will fluctuate. As the abundance of specialist species decline due to habitat loss and fragmentation, the numbers of generalist species tend to increase (Büchi and Vuilleumier, 2014). In the initial stages of regeneration in pine stands razed by forest fires, the small mammal population was predominantly dominated by deer mice before numbers started to decline as the forest aged (Fisher and Wilkinson, 2005).

Forest composition impacts populations within the forest, which is important when it comes to ticks and their hosts. Tick abundance is linked to host availability and habitat suitability. For example, the abundance of castor bean ticks (*Ixodes ricinus*) and taiga ticks (*Ixodes persulcatus*) have been shown to differ depending on what hosts are present in a population (Uusitalo et al., 2022). One study analyzing the relationship between seven different forest variables against tick density found a significant relationship between dominant tree type

and tick abundance, likely due to tick host preference (Talbot et al., 2019). In one study, they found that the invasion status of forests, specifically regarding the presence of multiflora rose, was positively correlated with infection prevalence in ticks (Adalsteinsson et al., 2018). This study raises the question as to whether ticks, or suitable hosts for ticks, prefer certain species of vegetation. It's been shown that tick abundance can vary between habitats. In one study, they looked at various compositional differences between thinned and unthinned forests to see how they impacted tick abundance. From their research, they found that canopy cover and subsequently microclimate both had significant alterations to tick abundance (Brennan et al., 2023). Thinned plots with less canopy cover, lower relative humidity, and greater average temperatures housed significantly less ticks than the unthinned plots (Brennan et al., 2023). Hard ticks prefer humid habitats with abundant leaf litter. The abundance of questing castor bean ticks, for example, has been shown to increase with average mean temperature (Borşan et al., 2022). Ticks spend a lot of their life either under leaf litter or on understory vegetation since they tend to have a higher relative humidity than the air, creating a favorable environment (Boehnke et al., 2017). If ticks were to prefer certain vegetation, or if certain vegetation creates different habitats, variations in these factors could lead to differences in tick abundance.

Ticks are great reservoirs and vectors for many zoonotic diseases. They aid with the spread of diseases such as Lyme disease (*Borrelia burgdorferi*), human babesiosis (*Babesia microti*), and human granulocytic ehrlichiosis (*Ehrlichia chaffeensis*) (Thevanayagam, 2012). One prime carrier of Lyme disease is the blacklegged tick (*Ixodes scapularis*). Black-legged ticks go through three life stages, those being larval, nymphal, and adult. Lyme disease risk in humans is closely related to nymphal infection prevalence (NIP) and the density of infected nymphs (DIN) since nymphs are the most likely to take blood meals from humans and be

infected with Lyme disease (LoGiudice et al., 2008). They feed on a variety of hosts, and these hosts can both be competent and incompetent. An incompetent reservoir host is unlikely to transmit a pathogen it's infected with to a parasite, while a competent reservoir host will have high success rates in pathogen transmission between them and the parasite. Tick infection prevalence is heavily dependent on host community composition. Larger mammals tend to be incompetent hosts for Lyme disease, but their prevalence and abundance can differ depending on habitat (Răileanu et al., 2021). To attach to a host, these ticks will wait in vegetation or on the ground for a host to brush by them (Thevanayagam, 2012). Typically, ticks will take a blood meal once a year and molt into different life stages following their blood meals. Seasonal activity for when ticks quest for blood meals varies between the life stages. It's been observed that adults have peak questing periods in both April and November, while questing activity for nymphs and larvae generally peaks from June to July (Hamer et al., 2012, Simmons et al., 2015). In their life, a tick will take up to three blood meals. Ticks are parasites and require these blood meals from other organisms, making the presence of available hosts essential to a tick's life cycle. While in the larval and nymphal stages of their life, black-legged ticks will typically take blood meals from small animals such as the white-footed mouse, birds, and other mammals (Thevanayagam, 2012). In their adult stage, these ticks will take their final blood meal primarily from white-tailed deer (Thevanayagam, 2012). Despite being incompetent reservoirs for Lyme disease, deer play a key role in a tick's ability to reproduce (Răileanu et al., 2021, Thevanayagam, 2012).

Objectives

Due to the fact that different forest stand types and vegetation compositions can lead to different microclimates, and since climate is an important driver of a tick's ability to survive, the objective of this study is to see if there is a relationship between forest composition and tick

abundance. Specifically, I tested how the density and diversity of forest vegetation relates to tick density. I predicted that with an increase in vegetation density, temperatures within the forest will generally be cooler and thus be more suitable for ticks. This can also indirectly impact tick numbers by increasing the amount of shelter available to hosts. For vegetation richness, I predict sites that are more heterogeneous will be able to accommodate a greater number of animal species- generalists and specialists alike. In addition to this, I would like to see how ticks differ between seasons, in relation to both abundance and life stage.

From this, I make the following hypotheses:

- Forest patches that differ in their tree density and age will vary in their microclimates.
- Forest patches with differing tree and understory vegetation density will have different densities of ticks.
- Forest patches with differing tree and understory vegetation diversity will have different densities of ticks.

With these hypotheses, I predict that:

- Old growth forests that have a greater tree density will on average be cooler and more humid than forests with less tree density.
- Forest patches that have greater tree and understory vegetation density will have a greater number of ticks than forests with less dense vegetation.
- Forest patches that have a greater diversity of trees and understory vegetation will have a greater number of ticks than forests with a less diverse vegetation composition.

Methods

Experimental Design

This project took place between the end of September and the beginning of November 2024. Data collection was split into two sampling periods. This was to catch the end of the summer season with warmer temperatures, different life stages of ticks questing, and before trees lost their leaves, and to catch the fall season with cooler temperatures and less canopy cover and understory vegetation. The first sampling period of vegetation surveys and tick drags took place at the beginning of September. Approximately two weeks after the first sampling period, the second sampling period of vegetation surveys and tick drags took place. After all the tick drags were completed, ticks were counted at the lab for further analysis.

Study Area

This study took place at two forested sites in northwestern Pennsylvania. These were the Robertson Athletic Complex (*Fig. 1a*) and the Bousson Environmental Research Reserve (*Fig. 1b*), which reside in Meadville and Frenchtown, PA, respectively. Robertson Athletic Complex as a whole is 203 acres, and is a young growth forest that has a high amount of foot traffic. This area is disturbed often due to trail maintenance. Bousson Environmental Research Reserve is 283 acres, and is an unmanaged, old growth forest that is not publicly accessible and is primarily used for research projects. These sites were specifically chosen for their differences in age and management, allowing us to get a range of microclimate variables and differing vegetation types.



Figure 1: Aerial photographs of Robertson Athletic Complex [41°39'36"N 80°08'50"W](a) and Bousson Environmental Research Reserve [41°36'06"N 80°02'10"W](b) where study plots were set up.

Previous Experiment

Some of the data utilized for this experiment were collected as part of a prior project. This was a 6-week long project that took place from May to June 2024. Values for tree transect density, tree species present, and subplot tree density were all carried over from data collected over the summer. These data were not expected to change significantly, if at all, between the sample dates, and thus were only collected once. Additionally, plots one, two and three at Bousson, and plots one and five at Robertson were set up prior to this experiment. Data from this experiment is also being used for the seasonal comparison. Due to temporal constraints, data for the summer season was collected as part of the previous experiment.

Plots

From the prior experiment, plots were chosen based on a visual assessment of differing vegetation compositions. This was done in order to get a broad range of vegetation variation and get a better sense of what differences impact tick populations. Three plots were set up at Robertson, and three were set up at Bousson. They are all 75x10 meters, and are set up just off of trail edges. Plots are marked with a flag at each corner. Along the long side of the plot that's

furthest from the trail, flags are placed every 10 meters to assist with consistency in tick dragging. The plots were then subdivided into three 15x10 meter subplots with the center of each subplot marked with a pink flag (*Fig. 2*). This is to help collect a more accurate vegetation survey of the entire plot as a whole.

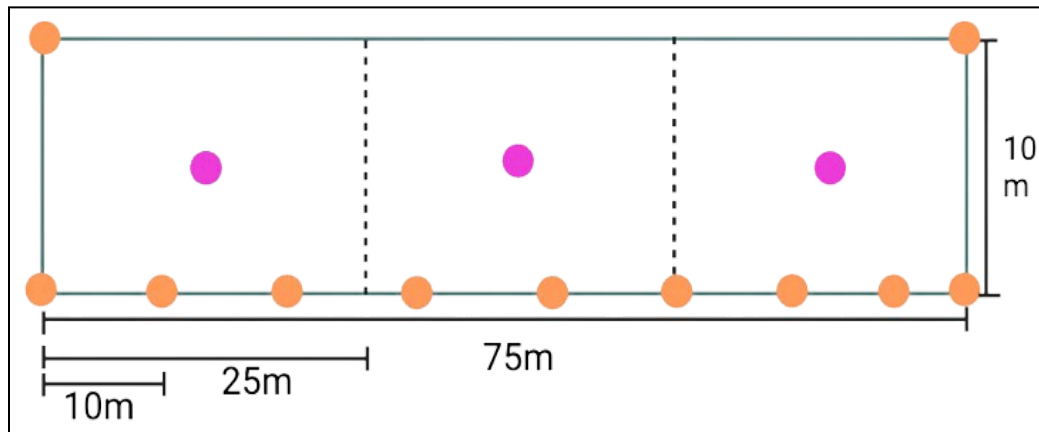


Figure 2: Tick dragging plots were 75x10 meters, and marked every 10 meters. Each plot was divided into three subsections, the center of which was marked with a pink flag. This is where site characterization took place.

Site Characterization

To assess the impact of forest variation on tick abundance, site characterization took place at both the plot and the subplot level. Subplot data was collected twice during the experiment. The first round took place between 9/26/24 and 9/28/24. The second round took place on 11/3/24 and 11/4/24. Data was collected on five main variables: 1. tree and understory vegetation diversity, 2. tree density, 3. canopy cover, 4. understory vegetation density, and 5. abiotic factors.

All of the trees and understory vegetation within the subplot were identified. At the center of the subplot, all the trees within a ~3.6 meter radius from the pink flag were counted, marked, measured for their DBH (diameter at breast height), and identified. In addition, canopy

cover was measured in four different directions from the center of the subplot. Ground coverage was ranked on a scale from 0-5 (*Fig. 3*) for the following categories: total vegetation cover, forbes, grasses, bushes, woody debris, bare ground, and leaf litter. HOBO data loggers were deployed in October to collect temperature, relative humidity, and dew point for the duration of the experiment. Each data logger was installed ~1.5 meters off of the forest floor, and was set to collect data once every hour.

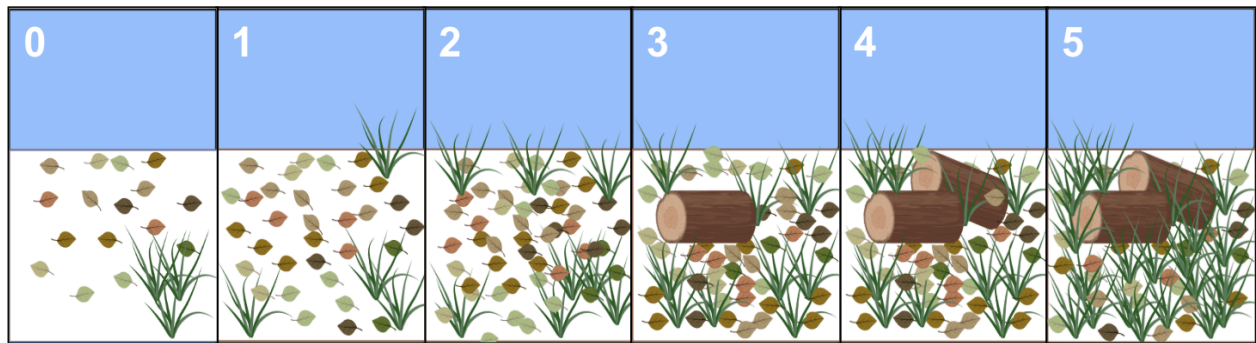


Figure 3: Visual cartoon representation of the 0-5 scale used to determine the various vegetation scores.

At the entire plot level, another tree density survey was conducted. Every tree within a 75x2 meter area was counted, totaled, and identified to get a sense of tree density and richness. Leaf litter depth was measured at each flag along the plot edge. At each flag, a measurement was taken at the flag in addition to four additional measurements one foot away from the flag in four different directions. These measurements were averaged out for the entire plot.

During the experiment, plot 4 was damaged and the vegetation profile was severely altered. To work around this, the plot start was moved 10 meters into the forest to where the plot originally ended. Site characterization was redone for the new location.

Tick Dragging

There were a total of two tick drags. The first round took place between 10/5/24 and 10/12/24. The second round of drags took place between 10/24/24 and 11/04/24. Tick abundance

found in different plots was compared to look at how abundance changes with tree and vegetation density and diversity.

In each plot, dragging proceeded with a 1x1 meter corduroy cloth. Dragging started along the trail edge and moved into the forest. After each 10 meter drag, the cloth was flipped over and checked for ticks. If any ticks were found, they were removed using forceps and placed into a vial of ethanol to be counted, sexed, and sorted later. Ethanol was chosen as it kills the ticks and doesn't denature DNA. The tubes were also labeled to keep track of what site, plot, and date the ticks were collected. Dragging commenced for the entirety of the plot. If there were any obstructions that made dragging the ground or underbrush continuously impossible, then supplemental dragging took place outside of the plot to make up for lost coverage.

Statistical Analysis

Within each site (Bousson, Robertson), I established replicated research plots that were divided into 3 subplots; some data were collected at the level of the plot whereas other data were collected at the level of the subplot. For each replicate plot, I aggregated my data such that I used the mean values for data collected at the subplot level, which allowed me to scale each variable to the level of "plot".

Analysis of Variance

To analyze the climatic differences between sites, average air temperature was log transformed. This was then analyzed against location by using a one-way ANOVA analysis of variance.

Principal Component Analysis

Because my assessment of the biotic and abiotic properties at each plot had more variables than I had replicates (and because many of these variables are correlated with each other; see Methods), I used Principal Components Analysis (PCA) to reduce the dimensionality of the following variables into scores for Principal Component Axis 1 and 2: tree density along the transect, tree density within a subplot, tree species richness within a subplot, plant species richness within a subplot, vegetation cover within a subplot, and tree canopy openness within a subplot (*Fig. 9*).

I ran a series of 4 statistical models to evaluate the effects of various biotic and abiotic predictors, as well as site-level differences, and predicted the abundance of larval, nymphal, adult, and total ticks that I collected. For each statistical model, I tested whether the categorical variables of site (Bousson, Robertson) and sample month (September, November), as well as their interaction, significantly predicted the abundance of ticks. I also included the continuous variables of air temperature, average relative humidity, PC1, or PC2 in each statistical analysis.

Results

Abiotic Differences Between Sites

Differences in microclimatic conditions between sites varied in significance. Average temperature was significantly different between sites ($p=0.0005$) with Robertson on average being hotter than Bousson (*Fig. 4*). On average, Bousson was around 10°C, and had a small average temperature range from 9-10°C. Robertson was around 12°C on average and had an average range of temperatures from 10-13°C. Average relative humidity did not vary significantly between sites.

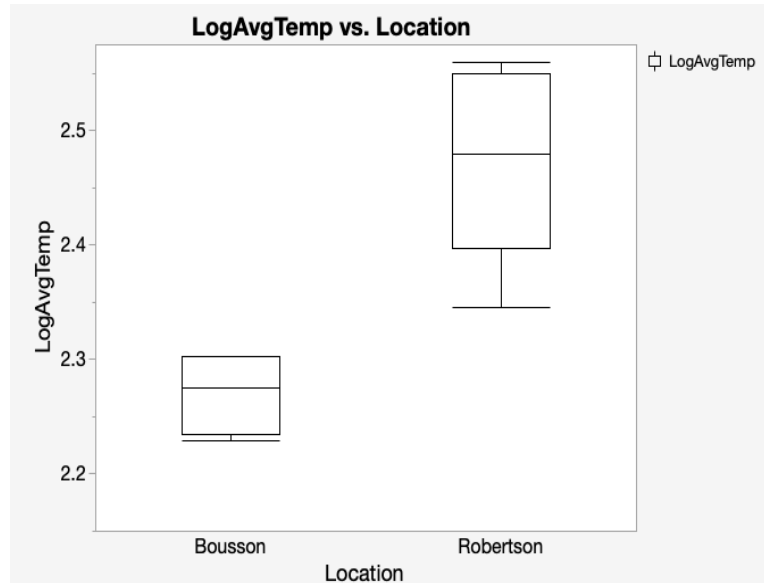


Figure 4: A boxplot showing the relationship between the average air temperature and the two sampling locations used for this experiment. Temperature measurements were collected once every hour from October 5th through November 4th. On average, Robertson was significantly warmer than Bousson ($p=0.0005$).

General Tick Abundance

A total of 318 *Ixodes scapulis* ticks were collected from Bousson Environmental Research Reserve and Roberston Athletic Complex between 10/05/24 and 11/04/24. Of the 318 ticks, 270 were collected during the first set of drags in October and 48 were collected during the second set of drags (Fig. 5). The results of this experiment are driven primarily by the larval tick numbers due to 75.8% of the total ticks collected being larvae. Total and larval abundance trended towards having a significant relationship with categorical sample date. While not statistically significant ($p=0.0840$), total tick abundance generally decreased between September and November tick drags, going from 270 ticks collected in September, to 48 collected in November (Fig. 6a). This trend is also reflected in larval tick abundance ($p=0.0762$) where 231

larval ticks were collected in September, and 10 larval ticks were collected in November (Fig. 6b). Generally, as the seasons progressed, total and larval tick abundance decreased.

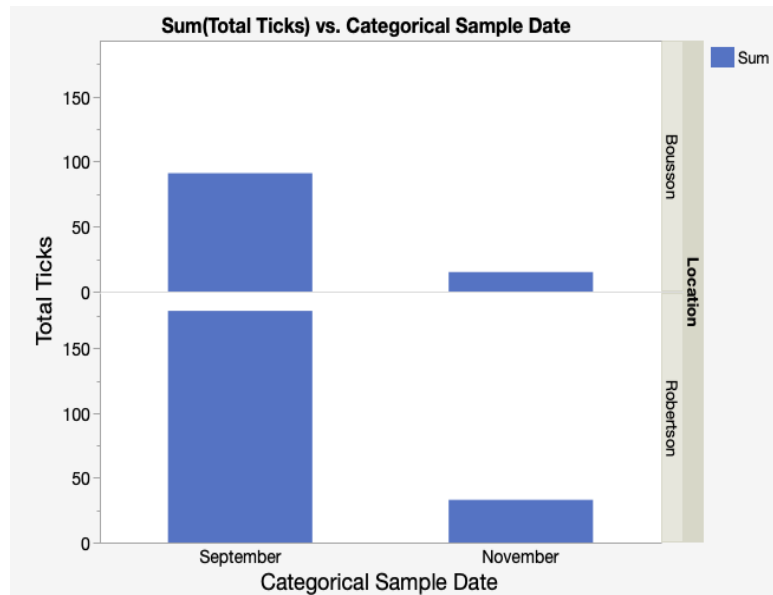


Figure 5: Representation of the total number of ticks collected, broken down by sample date and location. With a total of 318 ticks collected, 106 were from Bousson and 212 came from Robertson. Broken down by sample date, in September 91 and 179 ticks were collected from Bousson and Robertson, respectively, and in November 15 and 33 ticks were collected from Bousson and Robertson, respectively.

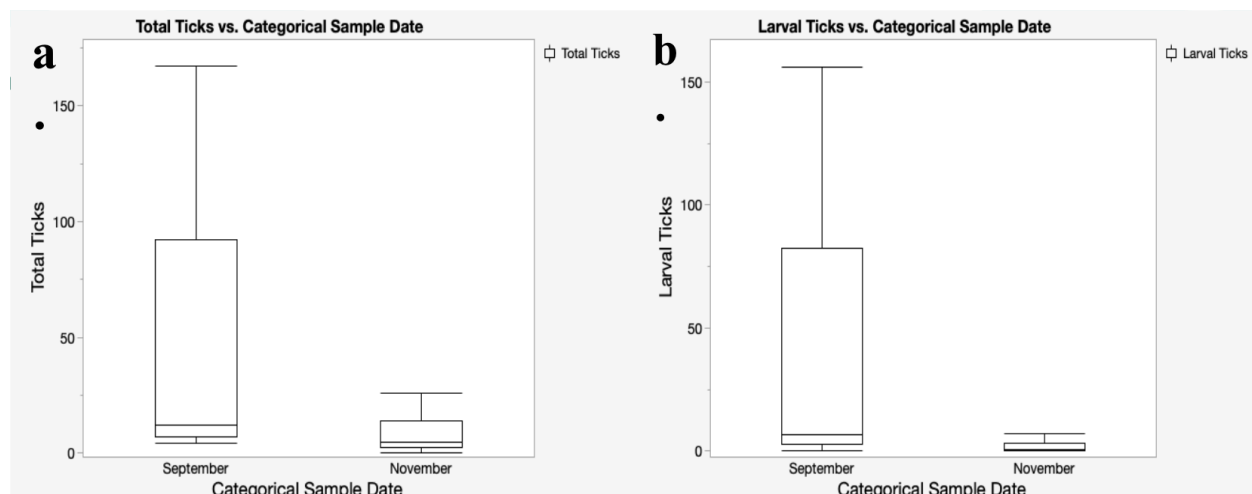


Figure 6: Trend of total ticks (a) and larval ticks (b) between sampling months. Total ticks ($p=0.084$) and larval ticks ($p=0.076$) both tended to decrease between the first and second tick drags.

Abiotic Factors on Tick Abundance

While not statistically significant ($p=0.0998$), as average air temperature increased, larval tick abundance also tended to increase (Fig. 7). Additionally, while again not statistically significant, as average relative humidity increased both total tick abundance ($p=0.0984$) and larval tick abundance ($p=0.0929$) tended to increase (Fig. 8a, 8b).

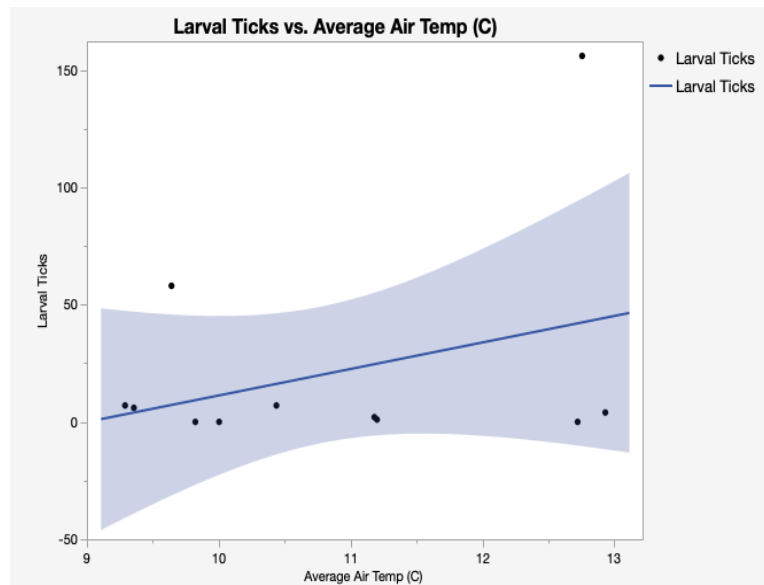


Figure 7: Linear regression plot showing the relationship between larval tick abundance and the average air temperature. Each point on the graph represents one tick drag from one plot. While not significant ($p=0.0998$), as the average air temperature ($^{\circ}\text{C}$) increased, larval tick abundance also increased.

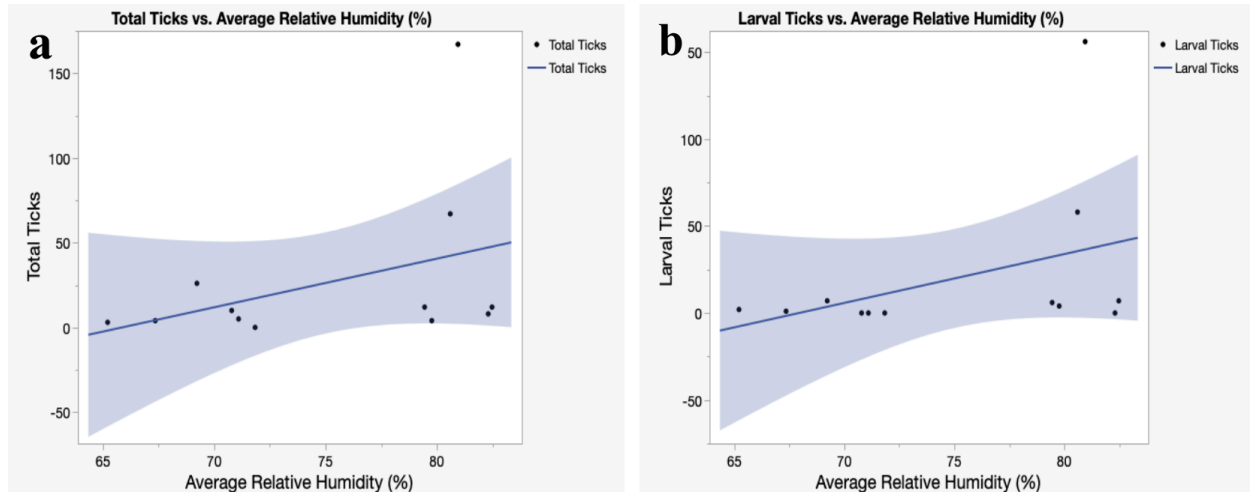


Figure 8: Linear regression showing the relationship between average relative humidity and total tick abundance (a) and larval tick abundance (b). Each point on the graph represents one tick drag from one plot. There is a trend of increasing tick abundance as relative humidity increases. Total ticks ($p=0.0984$) and larval ticks ($p=0.0929$) both tended to increase as the relative humidity increased.

Principal Components

A principal component analysis (PCA) was used to characterize the biotic differences between each site, focusing mainly on vegetation cover and richness (Fig. 9). The first two principal components accounted for 58% of the total variation in the data. PC1, which accounted for 34.8% of the variation between plots, primarily represents the differences in canopy openness, vegetation cover and richness, and tree density. PC1 was not a significant indicator of tick abundance at any life stage. PC2, however, accounted for 23.2% of the variation between plots, and represents tree density and richness. This component was a significant predictor of both total tick abundance ($p=0.0369$) and larval tick abundance ($p=0.0325$). As PC2 increased, the abundance of total ticks and larval ticks significantly decreased (Fig. 10a, 10b).

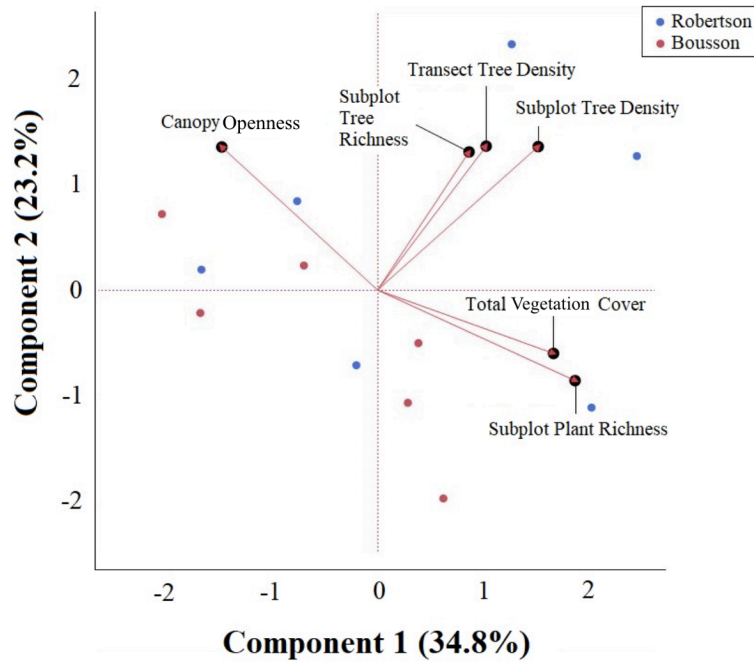


Figure 9: Visual representation of the variables used in the principal component analysis. This shows a projection of the 12 total sampling sessions (two for each of the six plots) against the first two principal components. As plots move horizontally along principal component 1, they go from having a more open canopy to denser and richer canopies. As plots move vertically along principal component 2, they go from greater understory vegetation richness to greater tree density and richness.

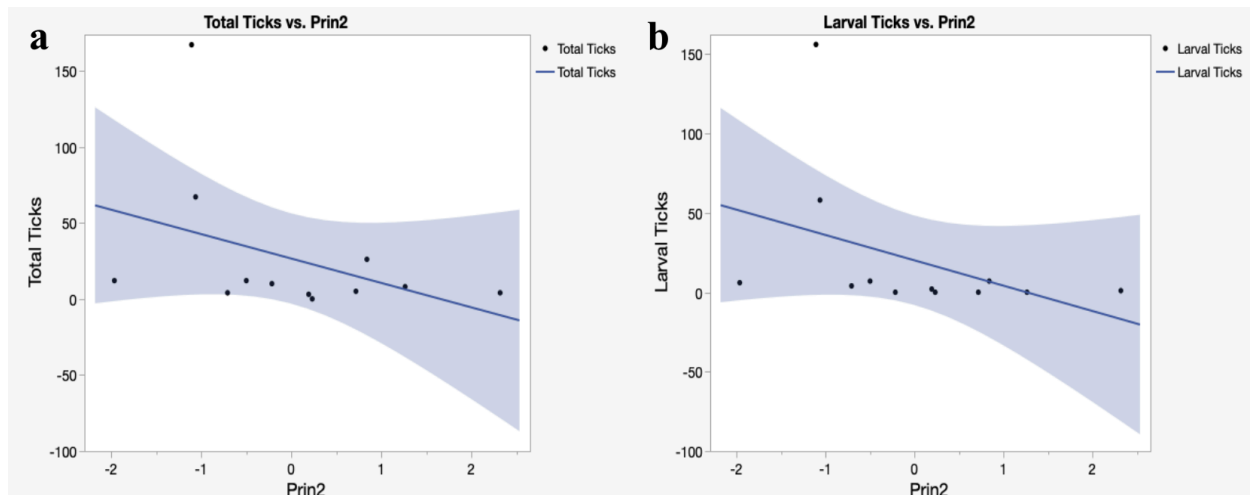


Figure 10: Linear regression representing the relationship between Principal Component 2 (PC2) on total tick abundance (a) and larval tick abundance (b). PC2 represents the tree density and richness at each

plot. Each point on the graph represents one tick drag from one plot. As PC2 increased, the abundance of total ticks ($p=0.0369$) and larval ticks ($p=0.0325$) both significantly decreased.

Discussion

Abiotic Differences Between Sites

In terms of average temperature, Robertson was significantly warmer than Bousson (*Fig. 4*). This suggests that Robertson, the young growth forest with a significantly lower average DBH (*Fig. 11a*), allows more solar radiation into the forest and creates a warmer microclimate as opposed to Bousson. This is consistent with my hypothesis that forests with greater tree densities will be cooler on average. However, I also hypothesized that relative humidity would increase with tree density, and there ended up being no significant difference between the locations in terms of relative humidity and dewpoint (*Fig. 12a, 12b*). The air temperature differences are consistent with previous studies that have found old growth forests to be cooler on average than early succession forests (Lindenmayer et al., 2022). The discrepancy between forest age against average relative humidity could be due to my assumption being based on a study by Brennan et al., (2023) that observed microclimate differences between thinned and unthinned forests. While the trees at Bousson may have had a greater average DBH than Robertson, there was no significant difference between the sites in terms of canopy cover (*Fig. 11a, 11b*). With Brennan et al., (2023), however, they observed significant differences in canopy cover between thinned and unthinned plots, and subsequently found both lower tick abundance and lower relative humidity in thinned plots. This lends itself to how variable forests are from one another, and how management status and practices are important to understand when studying ecological interactions.

General Tick Abundance

Overall, there were 318 ticks collected during this project. The majority (75.8%) were collected from Robertson in September. Additionally, the majority of the ticks found in September were larval (231 larval ticks out of 270 total ticks). In November, however, the majority shifted towards adult ticks (36 adult ticks out of 48 total ticks) as the abundance of larval ticks decreased between September and November (*Fig. 13*). With this information, it could be suggested that around September is either at the end of the peak larval questing period, or that the peak questing season for larval ticks has shifted or been extended. This is inconsistent with previous experiments that show a peak larval questing period between June and July, and a sharp drop off in numbers following August (Gatewood et al., 2009).

Larval Tick Abundance vs Air Temperature

While the results weren't significant, there was a trend of larval tick abundance increasing as average temperature increased. This is inconsistent with my hypothesis that tick abundance would increase as temperature decreased. This could be due to the irregularity of Plot 4 from Robertson. Throughout all of the seasons, plot 4 consistently had the greatest number of ticks per drag despite having relatively consistent biotic and abiotic measurements with other plots from Robertson. One possible explanation for these numbers is the presence of both Japanese barberry and multiflora rose (MFR) in this plot. While all of the plots, except for plots 1 and 2 from Bousson, had Japanese barberry and MFR in some capacity, the plots from Robertson, 1 and 4 especially, had the highest bush cover score (*Fig. 14*). Nothing can be said definitively with this data as we weren't specifically looking at the influence of these two plant species on tick abundance, but field observations suggest that dragging over bushy vegetation

produced a higher number of ticks than when dragging surrounding areas in the same plot. This is supported by Williams et al., (2017), where they found greater tick abundances in study plots with greater Japanese barberry coverage. Not only is this possible correlation important regarding host exposure to ticks, but also regarding exposure to tick-borne diseases such as Lyme disease. Adalsteinsson et al., (2018) found a positive correlation between nymphal infection prevalence (NIP) and the presence of MFR. This could possibly be due to ticks using bushy vegetation as a better vantage point to attach to hosts. Continued research looking at how tick abundance differs with specific plant species should be done in order to better understand how to minimize human exposure to ticks.

Black-legged ticks typically have a two-year life cycle during which they will take three blood meals. In the first year, eggs will hatch larval ticks that will then take their first blood meal, usually during the summer. During the second year, larvae will molt into nymphs, take a blood meal in the spring, molt into an adult, and take a final blood meal in the fall. Finally, these adults will lay more eggs (Thevanayagam, 2012). With global warming, it's possible that this typical life cycle is shifting. In previous studies, larval questing periods typically peaked between June and August. In southwestern Pennsylvania, specifically, one multi-year study found that larval tick activity peaked at the end of July into the beginning of August (Young, 2023). For my project, however, larval tick numbers remained relatively constant between the summer (482 larval ticks collected, average 48.2/plot) and fall (270 larval ticks collected, average 45/plot). The idea of a possible bimodal peak emerging for larval ticks due to climate change is supported by a model created by Wallace et al, (2019). At average historical temperatures in the northeastern US, their model showed larval questing activity to peak around August. At 6°C above historical temperatures, though, a bimodal peak began to form where larval activity

peaked in July before peaking once again at the end of October. With future research, a multi-year study looking at the questing activity of the blacklegged tick could inform if and how tick activity is changing with climate change.

Total/Larval Tick Abundance vs Average Relative Humidity

Total tick and larval tick abundance both tended to increase as relative humidity increased. While this trend wasn't significant, it suggests that ticks prefer/need humid conditions in order to successfully quest (Adalsteinsson et al., 2018). When the humidity is too low, ticks will take shelter in the leaf litter in order to avoid desiccation due to water loss to the air, and/or to avoid starvation due to loss of their lipid stores (Young, 2023). With only the larval and total tick numbers trending towards significance, it is likely that larval ticks are more susceptible to dessication than the more mature tick life stages. This could also be due to the fact that more larval ticks were collected in comparison to adult and nymphal ticks. This is consistent with my prediction that forests that are more humid will have a greater abundance of ticks than those that are drier. This is also consistent with previous research that has found a positive correlation between larvae density and humidity variables (Young, 2023).

Principal Components

The principal component analysis was used to reduce the dimensionality of all the variables that were collected. This analysis revealed that, as tree density and richness increased, tick abundance decreased. This is not consistent with my hypothesis that forests with greater tree and understory vegetation densities would have a greater abundance of ticks. The decrease in tick abundance with the increase of tree density is also inconsistent with previous literature that found blacklegged tick abundance to have a significant positive relationship with canopy cover

(Talbot, et al., 2019). This could possibly be due to young forests often having greater plant richness due to the availability of sunlight and ease of establishment which could indirectly affect tick numbers due to the increased air temperature (Adalsteinsson et al., 2018). This could also relate to the foraging habits of the typical hosts for blacklegged ticks. White-tailed deer have been found to increase in abundance in thinner habitats with more canopy openness, often due to increased food availability (Johnson et al., 1995, Bassing et al., 2022). While denser areas might have a more favorable microclimate for the blacklegged tick, host availability may be a more powerful predictor of tick abundance than habitat suitability.

Conclusion

In the end, there are many relationships to consider when trying to predict the abundance of the blacklegged tick. My results show a significant negative relationship between tree density and richness, albeit not in line with the direction of my prediction. Microclimate variables, which are heavily influenced by vegetation composition, impact tick abundance, but are not the only important factors to consider.

Future Research

If I were to run this experiment again, I would want to have repeat drags of the same plots. Due to time constraints, only one drag per plot per month was able to be completed. This gives relatively low power to the results found in this study. Additionally, in an ideal scenario, there would be multiple plots that have similar vegetation compositions to allow for duplicates. Another direction I would want to take this experiment in is looking at habitats that aren't restricted to strictly forest settings. Other habitat types such as peri-urban forests, parks, and forest edges also pose a risk of human exposure to ticks, Lyme disease, and other vector-borne

diseases. For example, land in peri-urban areas that is preserved as a way to increase urban living standards can also create an optimal habitat for host and tick populations (Borşan et al., 2022). This also leads into looking at whether forests are managed or not, and the various ways in which they can be managed. Between extensively and intensively managed old growth forests (the former categorized by lots of leaf litter and shrub coverage, and low canopy openness, the latter by greater canopy openness and less leaf litter) one study found differences in the small mammal populations (Suchomel, et al., 2020). Different forest management techniques such as forest thinning, prescribed burning, and clearcutting not only change forest composition, but can lead to shifts in animal communities (Fisher and Wilkinson, 2005). Some of the changes found were the decrease in leaf litter layers, and an increase in grassy understory vegetation as regrowth occurs. These shifts were found to impact mammal populations, being especially favorable to small rodents like the deer mouse and shrews (Fisher and Wilkinson, 2005). Gaining a foundation of knowledge for what forest variables significantly impact tick abundance is essential to further minimize human exposure to Lyme disease.

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Appendix – Supplemental Figures

<u>Total Ticks</u>	F value	p value
Location	2.6216	0.1807
<i>Categorical Sample Date</i>	<i>5.237</i>	<i>0.084</i>
Location*Categorical Sample Date	3.7818	0.1237
Average Air Temp (C)	3.7597	0.1245
<i>Average Relative Humidity (%)</i>	<i>4.6059</i>	<i>0.0984</i>
Prin1	2.4167	0.195
<i>Prin2</i>	<i>9.4856</i>	<i>0.0369</i>

Table 1: Summary statistics for the standard least squares statistical model conducted to look at predictors of total tick abundance. This statistical model included biotic factors accounted for by the principal components (transect tree density, subplot tree density, subplot tree species richness, subplot plant species richness, vegetation covering, and canopy openness), and the abiotic factors (location, categorical sample date, air temperature, and relative humidity.) Variables that are italicized in blue trend towards significance (<0.1), and variables that are bold and italicized in black are significant (<0.05). This table shows that Categorical Sample Date and Average Relative Humidity trend towards significance, and that Prin2 is a significant indicator of total tick abundance.

<u>Larval Ticks</u>	F value	p value
Location	3.4131	0.1384
<i>Categorical Sample Date</i>	<i>5.6547</i>	<i>0.0762</i>
Location*Categorical Sample Date	4.3584	0.1051
<i>Average Air Temp (C)</i>	<i>4.5507</i>	<i>0.0998</i>
<i>Average Relative Humidity (%)</i>	<i>4.8313</i>	<i>0.0929</i>
Prin1	2.7632	0.1718
<i>Prin2</i>	<i>10.3297</i>	<i>0.0325</i>

Table 2: Summary statistics for the standard least squares statistical model conducted to look at predictors of larval tick abundance. This statistical model included biotic factors accounted for by the principal components (transect tree density, subplot tree density, subplot tree species richness, subplot plant species richness, vegetation covering, and canopy openness), and the abiotic factors (location, categorical sample date, air temperature, and relative humidity.) Variables that are italicized in blue trend

towards significance (<0.1), and variables that are bold and italicized in black are significant (<0.05). This table shows that Categorical Sample Date, Average Air Temperature, and Average Relative Humidity trend towards significance, and that Prin2 is a significant indicator of larval tick abundance.

<u>Nymphal Ticks</u>	F value	p value
Location	2.9864	0.159
Categorical Sample Date	3.9646	0.1173
<i>Location*Categorical Sample Date</i>	<i>4.6216</i>	<i>0.098</i>
Average Air Temp (C)	3.257	0.1454
Average Relative Humidity (%)	4.5029	0.1011
Prin1	0.13	0.7367
Prin2	4.3515	0.1053

Table 3: Summary statistics for the standard least squares statistical model conducted to look at predictors of nymphal tick abundance. This statistical model included biotic factors accounted for by the principal components (transect tree density, subplot tree density, subplot tree species richness, subplot plant species richness, vegetation covering, and canopy openness), and the abiotic factors (location, categorical sample date, air temperature, and relative humidity.) Variables that are italicized in blue trend towards significance (<0.1), and variables that are bold and italicized in black are significant (<0.05). This table shows that the Location*Categorical Sample Date interaction trends towards being a significant predictor of nymphal tick abundance.

<u>Adult Ticks</u>	F value	p value
Location	1.0776	0.3579
Categorical Sample Date	0.0198	0.895
Location*Categorical Sample Date	0.1964	0.6805
Average Air Temp (C)	0.4394	0.5437
Average Relative Humidity (%)	0.0651	0.8112
Prin1	0.0112	0.9208
Prin2	0.082	0.7888

Table 4: Summary statistics for the standard least squares statistical model conducted to look at predictors of adult tick abundance. This statistical model included biotic factors accounted for by the principal components (transect tree density, subplot tree density, subplot tree species richness, subplot

plant species richness, vegetation covering, and canopy openness), and the abiotic factors (location, categorical sample date, air temperature, and relative humidity.) Variables that are italicized in blue trend towards significance (<0.1), and variables that are bold and italicized in black are significant (<0.05). This table shows that none of the listed variables trended towards being significant, nor were they significant indicators of adult tick abundance.

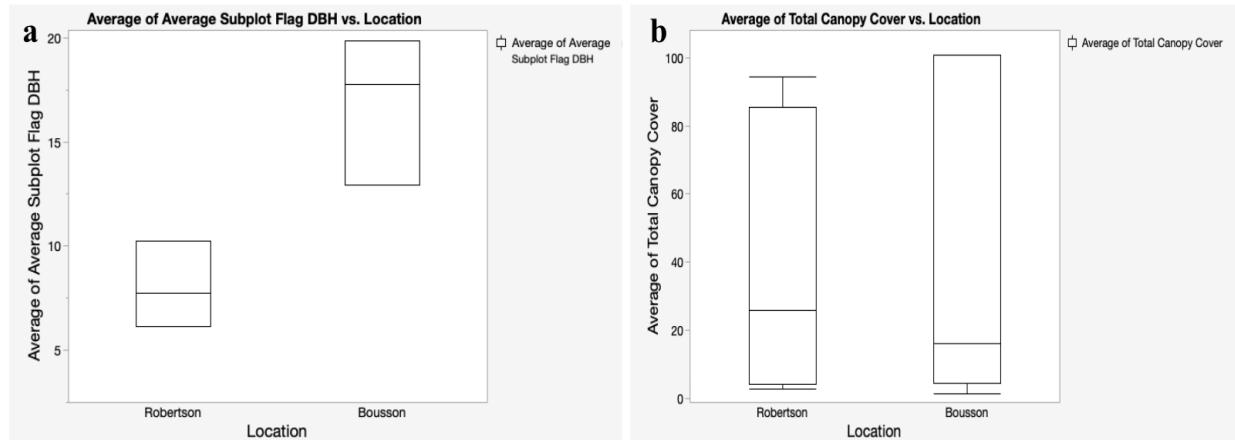


Figure 11: Box and whisker plots showing the average diameter at breast height (DBH) of trees by location (a) and the average canopy openness at each location (b). While Bousson had trees with a greater average DBH than Robertson ($p=0.0002$), the percentage of canopy openness did not significantly differ between locations ($p=0.9716$).

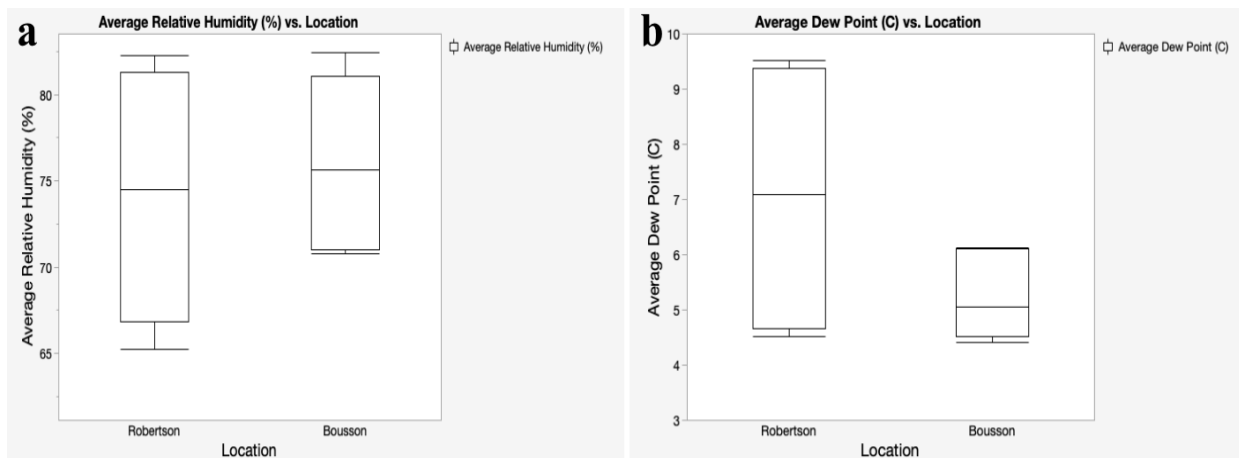


Figure 12: Supplemental box and whiskers plots showing the differences in average relative humidity (a) and average dew point (b) between sampling locations. Neither average relative humidity ($p=0.6290$) nor average dew point ($p=0.1210$) differed significantly between Bousson and Robertson.

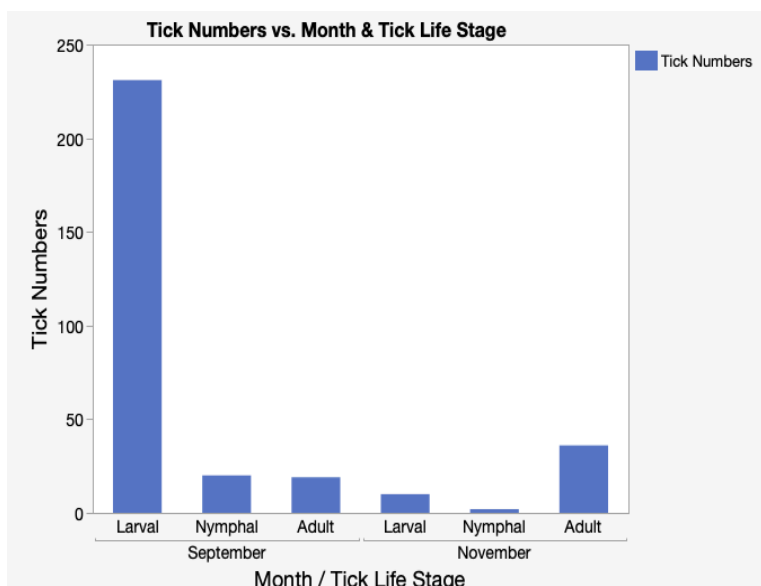


Figure 13: Supplemental graph showing the sum tick numbers found during each sampling session. This is further broken down by tick life stage. In September, there were 231 larval, 20 nymphal, and 19 adult ticks collected. In November, there were 10 larval, 2 nymphal, and 36 adult ticks collected.

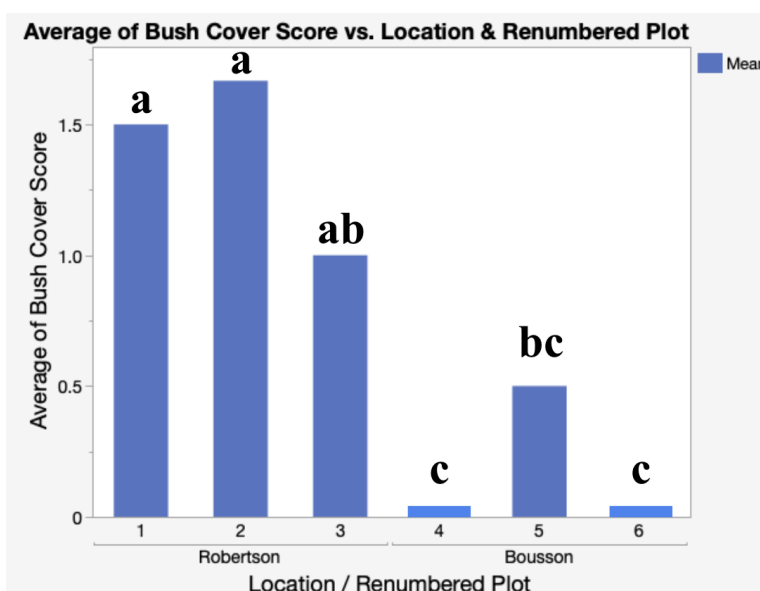


Figure 14: Supplemental graph showing a connected letters report for the bush cover score collected for each plot. Bush score is averaged between the September and November sample dates. Plots with the same letters are not significantly different from each other. Plots 4 and 6 both scored 0 for bush cover.