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Investigation of how vegetation, small mammal interactions, and land-use determine blacklegged tick abundance

Claire Hartl
chart8@brockport.edu

Investigation of how vegetation, small mammal interactions, and land-use determine blacklegged tick abundance

By

Claire N. Hartl

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Claire N. Hartl

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_____ Committee Member	_____	_____
_____ Committee Member	_____	_____

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_____ _____
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Abstract

Climate change and shifting land-use patterns have caused the expansion of tick ranges across much of the northeastern United States, which has serious implications for public health. Ticks serve as vectors for numerous tick-borne diseases, which can become more prevalent as tick ranges expand and their populations increase. Western New York is currently experiencing this range expansion as ticks are migrating in from southeastern New York, followed by an increase in incidence of tick-borne disease. The spatial distribution of ticks is dependent on both small-scale and regional-scale ecological interactions. The landscape-level land use mosaic can structure what habitats ticks and their hosts are found in: within those habitats, a moist microclimate provided by ample vegetative cover is necessary to ensure tick survival. My study examined the distribution of ticks in the greater Rochester, NY area at both of these spatial scales.

In the first half of my project, I collected ticks from plots dominated by invasive pale swallowwort and compared their density to tick densities in control sites, where pale swallowwort was absent. In a subset of plots, I used data loggers to study the microclimatic conditions and sampled for white-footed mice, the primary host of juvenile blacklegged ticks. I found that swallowwort was able to alter the microclimate of a site by providing high relative humidities, low vapor pressure deficits, and small ranges for both variables. I found significantly more adult ticks in swallowwort patches as opposed to corresponding bare patches, although I did not

find differences for nymphal ticks. Swallowwort patches seemed to harbor more white-footed mice, and white-footed mice captured in swallowwort plots often had more embedded ticks. Swallowwort seemed to have more of an effect on tick abundance in areas that lacked other vegetative cover in the understory.

In the second part of my study, I collected ticks on public trails in parks across the greater Rochester area to see where ticks were most prevalent and how the landscape was affecting tick density. I found that latitude and longitude were the most important predictor variables for tick density, and that ticks were most likely to be found in the southeastern portion of my study area. I also found that in general, ticks were most likely found in areas with a higher proportion of forests and agricultural areas, and less likely to be found in developed areas.

In summary, my first study provides support for the hypothesis that invasive plants can alter the surrounding microclimate in ways that support tick populations. The second half of my study documents the spatial distribution of ticks in public parks in the Rochester area, supports a northwesterly expansion of ticks across New York State, and suggests that ticks are most likely to occur in areas with high cover of forests and agriculture and less developed areas.

General Introduction

There are four main species of ticks that occur in New York State and are capable of transmitting disease: the lone star tick (*Amblyomma americanum*), the American dog tick (*Dermacentor variabilis*), the woodchuck tick (*Ixodes cookei*) and the blacklegged tick (*Ixodes scapularis*). The blacklegged tick has been the subject of extensive research since it serves as the vector for Lyme disease (*Borrelia burgdorferi*), which is the most common vector-borne disease in the United States (Levy 2013). Lyme disease emerged widely in the 1970s and has been spreading ever since (Tanner *et al.* 2010). This issue is expected to worsen as climate change is expected to expand the range of blacklegged ticks, and by extension, tick-borne diseases (Ostfeld and Brunner 2015). Information on the spatial distribution of blacklegged ticks is important for understanding Lyme disease ecology and can be used to develop risk models for human infection with tick-borne disease (Diuk-Wasser *et al.* 2012).

Infection prevalence in ticks is highly dependent on their lifestage and the hosts that they feed on, and familiarity with tick life cycles may help the public to protect themselves against disease. Host preference and prevalence of *B. burgdorferi* differ depending on the life stage of the individual tick (Levy 2013). Blacklegged ticks go through four instars, or stages of life: egg, larva, nymph, and adult. Adult blacklegged ticks lay their eggs in the early spring, wherever they have detached from their host (Tanner *et al*. 2010). Larval ticks emerge in the summer but are poor dispersers and are usually limited to moving a few meters away from the egg mass as

they quest for a bloodmeal from their first host (Ostfeld *et al.* 1996). White-footed mice (*Peromyscus leucopus*) are the primary hosts for juvenile blacklegged ticks and have a high reservoir potential, meaning that they do a good job of passing the *B. burgdorferi* spirochete to ticks (Ostfeld *et al.* 2006). The following spring, ticks that have successfully fed advance to the nymphal stage, where they quest for their second blood meal. Nymphal ticks are most abundant in the spring and early summer (Bouchard *et al.* 2013), which is when most infection transmission occurs to humans (Wood and Lafferty 2013). Nymphs are very small and humans may be less likely to see them when they become attached, particularly in areas with lower tick densities where people are not as cautious to check themselves or take precautions to avoid contact with ticks.

White-tailed deer (*Odocoileus virginianus*) are principal hosts for adult blacklegged ticks (Bouchard *et al*. 2013). They are a poor reservoir for the Lyme disease spirochete, but indirectly impact Lyme disease prevalence by serving as the primary host for egg-laying ticks (Levy 2013). White-footed mice have an 85% reservoir competence for *Borrelia burgdorferi*, while deer only have a 4.6% competence rate (Brisson *et al*. 2008). In other words, deer do not really make ticks infectious, but they do aid in creating more ticks and transporting ticks to new areas.

Suitable habitats for tick hosts, and therefore ticks, are structured by the land-use matrix, and therefore it is important to study ticks at the landscape level. Studies have shown that patterns in the surrounding landscape can be used to predict densities of blacklegged ticks (Khatchikian *et al*. 2012, Ferrell and Brinkerhoff 2018). On the

smaller scale, microclimate is very important for determining tick survival. Ticks' small size make them prone to desiccation, and therefore they need stable areas of high relative humidity to survive (Vail and Smith 1998, Williams and Ward 2010). For this reason, vegetative cover which provides a favorable microclimate is important for determining the distribution of ticks on a small scale. For example, invasive species alter the local microclimate in ways that provide suitable habitat for ticks, and tick abundance is often higher in these areas (Williams *et al*. 2009, Allan *et al*. 2010, Adalsteinsson *et al*. 2016).

My thesis consists of two chapters: the first studies ticks at a local, site level, while the second explores the distribution of ticks at a larger landscape level. In my first chapter, I studied how invasive pale swallowwort (*Vincetoxicum rossicum*) alters the microclimate of a site and affects the abundance of blacklegged ticks and white-footed mice, the primary host for juvenile ticks. In my second chapter, I collected ticks from trails in public parks around Rochester, NY to see where the public was most at risk of getting a tick. I also used land-use data to create predictive models to determine how the landscape was driving density of adult and nymphal ticks.

Literature Cited

Adalsteinsson, S. A., V. D'Amico, W. G. Shriver, D, Brisson, and J. J. Buler. 2016. Scale-dependent effects of nonnative plant invasion on host-seeking tick abundance. Ecosphere. 7: 1-9.

Allan, B. F., H. P. Dutra, L. S. Goessling, K. Barnett, J. M. Chase, R. J. Marquis, G. Pang, G. A. Storch, R. E. Thatch, and J. L. Orrock. 2010. Invasive honeysuckle eradication reduces tick-borne disease risk by altering host dynamics. Proceedings of the National Academy of Sciences. 107: 18523-185-27.

Bouchard, C., G. Beauchamp, P. A. Leighton, R. Lindsay, D. Belanger, and N. H. Ogden. 2013. Does high biodiversity reduce the risk of Lyme disease invasion? Parasites and Vectors. 6: 195.

Brisson, D., D. E. Dykhuizen and R. S. Ostfeld. 2008. Conspicuous impacts of nonconspicuous hosts on the Lyme disease epidemic. Proceedings of the Royal Society. 275: 227-235.

Diuk-Wasser, M. A., A. G. Hoen, P. Cislo, R. Brinkerhoff, S. A. Hamer, M. Rowland, R. Cortinas, G. Vourc'h, F. Melton, G. J. Hickling, J. I. Tsao, J. Bunikis, A. G. Barbour, U. Kitron, J. Piesman and D. Fish. 2010. Human risk of infection with *Borrelia burgdorferi*, the Lyme disease agent in eastern United States. The American Journal of Tropical Medicine and Hygiene. 86: 320-327.

Ferrell, A. M., and R. J Brinkerhoff. 2018. Using landscape analysis to test hypotheses about drivers of tick density and infection prevalence with *Borrelia burgdorferi*. International Journal of Environmental Research and Public Health. 15: 737.

Khatchikian, C. E., M. A. Prusinski, M. Stone, P. B. Backenson, I. Wang, M. Levy and D. Brisson. 2012. Geographical and environmental factors driving the increase in the Lyme disease vector *Ixodes scapularis.* Ecosphere. 3: 85.

Levy, S. 2013. The Lyme disease debate: host biodiversity and human disease risk. Environmental Health Perspectives. 121: 120-125.

Ostfeld, R. S., and J. L. Brunner. 2015. Climate change and Ixodes tick-borne disease of humans. Philosophical Transactions of Royal Society B. 370: 20140051.

Ostfeld, R. S., C. D. Canham, K. Oggenfuss, R. J. Winchcombe and F. Keesing. 2006. Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk. PLOS Biology. 4: 1058-1068.

Ostfeld, R. S., M. C. Miller, and K. R. Hazler. 1996. Causes and consequences of tick (*Ixodes scapularis*) burdens of white-footed mice (*Peromyscus leucopus*). Journal of Mammalogy. 77: 266-273.

Tanner, C. L., F. K. Ammer, R. E. Barry and E. Y. Stromdahl. 2010. Tick burdens on *Peromyscus leucopus* Rafinesque and infection of ticks by *Borrelia* spp. in Virginia. Southeastern Naturalist. 9: 529-546.

Vail, S. G., and G. Smith. 1998. Air temperature and relative humidity effects on behavioral activity of blacklegged tick (Acari:Ixodidae) nymphs in New Jersey. Journal of Medical Entomology. 35: 1025-1028.

Williams, S. C. and J. S. Ward. 2010. Effects of Japanese barberry (Ranculales:Berberidaceae) removal and resulting microclimate changes on *Ixodes scapularis* (Acari:Ixodidae) abundance in Connecticut, USA. Environmental Entomology. 39: 1911-1921.

Williams, S. C., J. S. Ward, T. E. Worthley and K. C. Stafford, III. 2009. Managing Japanese barberry (Ranculales:Berberidaceae) infestations reduces blacklegged tick (Acari:Ixodidae) abundance and infection prevalence with *Borrelia burgdorferi* (Spirochaetales:Spirochaetaceae). Environmental Entomology. 38: 977-984.

Wood, C. L. and K. D. Lafferty. 2013. Biodiversity and disease: a synthesis of ecological perspectives on Lyme disease transmission. Trends in Ecology and Evolution. 28: 239-247.

Part One: Effects of invasive pale swallowwort (*Vincetoxicum rossicum*) on microclimate and the abundance of blacklegged ticks (*Ixodes scapularis*) and their primary host the white-footed mouse (*Peromyscus leucopus*)

Introduction

Blacklegged ticks (*Ixodes scapularis*) are the vector for several diseases including Lyme disease, human granulocytic anaplasmosis, human babesiosis, *Borrelia miyamotoi* disease, and Powassan encephalitis (Nelder *et al*. 2016). Lyme disease is the most common vector-borne disease in North America, with the majority (95%) of Lyme disease cases occurring in the upper Midwest and northeastern United States (Levy 2013). Lyme disease is caused by the spirochete *Borrelia burgdorferi*, which ticks obtain by feeding on infected wildlife hosts, primarily the white-footed mouse (*Peromyscus leucopus*) (Ostfeld *et al*. 1996, Allan *et al*. 2010). Infected ticks pose a public health risk because they can pass these pathogens to humans, livestock, pets, and other animals.

Favorable habitats for blacklegged ticks include deciduous forests with plentiful shrubs and a moist microclimate (Lubelczyk *et al*. 2004). Blacklegged ticks of all life stages have been found to be more common in wooded areas than more open grass-shrub habitats (Ginsberg and Ewing 1989). Cover is crucial for blacklegged tick survival, as ticks have a relatively high surface area to volume ratio, which makes them more prone to desiccation and sensitive to changes in relative humidity (Vail and Smith 1998, Williams and Ward 2010). Blacklegged ticks have low survival rates in areas with high vapor pressure deficit and low relative humidity (Williams and Ward 2010). Leaf litter on the forest floor is another factor that is

crucial to tick habitat. Once a tick has obtained a blood meal, 95% of its life is spent digesting its meal on the forest floor (Ostfeld *et al*. 2006). Leaf litter provides important cover and microclimate, because juvenile ticks are even more subject to desiccation than the adult stage and quest lower to the ground when searching for a host (Stafford 2007).

Abiotic characteristics such as humidity can be altered by exotic plant invasion, which can, in turn, affect vector survival and pathogen transmission rates (Allan *et al*. 2010). Williams *et al*. (2009) found that by altering the microclimate to retain more humidity, the invasive shrub Japanese barberry (*Berberis thunbergii*) supported twice the number of ticks than neighboring forests that lacked Japanese barberry. Adalsteinsson *et al*. (2016) found similar effects of another invasive shrub, multiflora rose (*Rosa multiflora*). That study found that in forests where multiflora rose had invaded, there were twice the number of ticks under rose bushes than in nearby areas. Favorable microclimate and cover provided by another invasive shrub, Amur honeysuckle (*Lonicera maackii*), served as refuges for small mammal hosts and increased lone star tick (*Amblyomma americanum*) abundances, which may increase the risk of human exposure to tick-borne diseases (Allan *et al*. 2010).

The invasive species studied in the context of ticks have all been shrubs; however, Prusinski *et al*. (2006) stated that small mammal-tick interactions may be enhanced by increasing the density of vegetation at the lowest understory strata. Pale swallowwort (*Vincetoxicum rossicum*) is a vine that grows close to the ground, in this stratum. Since its introduction from Ukraine around 120 years ago, pale swallowwort

has been rapidly expanding its range in the northeastern United States and parts of Canada. Swallowwort has achieved its range expansion through polyembrionic seeds, wind-borne dispersal mechanisms, and vegetative and sexual reproductive strategies (Weston *et al*. 2005). Range expansion of swallowwort is an ecological threat since it can be difficult to eradicate and invades a number of different habitats, including pastures, gardens, hedgerows, shrubby thickets, old fields, roadsides, and mixed and deciduous forests. The dense stands formed by swallowwort can suppress native plants, impede forest regeneration, and pose threats to endangered and threatened vegetation (Averill *et al*. 2011). These swallowwort stands may also provide cover for small mammals such as the white-footed mouse. Although the effects of swallowwort on tick abundance have not been studied, dense plant cover at key questing heights for juvenile blacklegged ticks could result in higher Lyme disease infection rates for mammal hosts, thereby posing a greater risk for human health (Prusinski *et al*. 2006).

I hypothesized that swallowwort would provide ideal habitat for ticks by creating areas with high relative humidity. Since the swallowwort grows in dense mats, I hypothesized that the swallowwort plots would have higher relative humidities and lower vapor pressure deficits than control plots. Tick abundance should be greater in swallowwort areas compared to control plots. Additionally, I hypothesized that mouse abundance would also be higher in swallowwort plots, as the growth form would allow protection from predators.

Methods

Site Selection

My project took place at six parks in the greater Rochester area: Oatka Creek Park, Mendon Ponds Park, Horizon Hill Conservation Area, Powder Mills Park, Durand Eastman Park, and Genesee County Park. In each park, I located forested sites that allowed for the paired comparison of 10 x 15 m plots where swallowwort was either present or absent. Some sites allowed for a swallowwort plot and two control plots: one plot that was primarily void of vegetation (bare control) and one plot that was covered in an vegetative layer that did not include any swallowwort (vegetation control). Percent cover of vegetation in the vegetation controls and swallowwort in the swallowwort plots was at least 30%. Vegetation controls could contain any type of other vegetation, including grasses, shrubs, herbs, and exotic or native plants. Some parks allowed for only one control plot. These control plots were later categorized as "bare control" or "vegetation control" based on the vegetation cover within the plot. Within these parks, I studied a total of 27 plots in 11 different sites (Table 1).

Vegetation Data

In each plot, I sampled the tree layer by recording the species and DBH of each tree located within the plot. Later, I converted DBH measurements into total basal area. I also used a densiometer in each plot to estimate canopy cover. I used five 1 x 1 m quadrats in each plot to quantify and characterize the overall herbaceous layer. I placed a quadrat in each corner, 1m in from the edge of the plot, and the last

quadrat was placed in the center of the plot. In each quadrat, I estimated the percent cover of swallowwort, other vegetation and areas void of vegetative cover, adding up to 100%. Beneath the herbaceous layer, I also characterized the litter layer by estimating percent leaf litter, twig, and bare earth, also totaling 100%. Quadrat measurements also included species richness and three measurements of litter depth. Herbaceous layer, litter layer, and tree layer data are shown in Table 2. Forest age was determined using historical photos from Genesee County Web Mapping (Andre 2018) and Mapping Monroe (GIS Services Division 2018).

Tick Collection

I sampled for ticks by standard tick dragging methods to determine abundance in the different plots at the sites. To collect the ticks, I used a 1 m^2 white flannel drag cloth that was weighted down at one end with chain and connected to PVC pipe on the other end. I used a 90º PVC elbow joint to connect the PVC pipe of the drag to a longer piece of PVC, which I used as a handle to pull the tick drag over the vegetation. I used the tick drag to sweep over vegetation in 10 parallel transects throughout each plot to determine tick abundance (Williams and Ward 2010). After each 15m drag, I stopped to inspect both sides of the flannel cloth for the presence of ticks.

I removed any ticks from the flannel using tweezers and placed them in a microcentrifuge tube filled with 100% ethanol. The tubes were transported back to the lab and stored at 4º C until analysis. Tick sampling occurred three times in each plot corresponding with activity peaks for each life stage of the tick: in the fall of

2016 to capture adult ticks, spring of 2017 to capture nymphal ticks, and summer of 2017 to capture larval ticks. This dragging method proved ineffective at collecting larval ticks in swallowwort plots due to swallowwort's tendency to grow in dense patches and the flag's inability to penetrate this thick layer of vegetation and make contact with the ground underneath. For this reason, only the abundances of nymphal and adult ticks were analyzed.

Small Mammal Trapping

To quantify small mammal populations in my plots, I conducted small mammal trapping in a subset of plots in the summer of 2017. This allowed me to determine both the abundance of potential tick hosts and the tick burden on trapped mammals. I trapped at all sites in Oatka Creek Park and Mendon Ponds Park, totaling 18 plots in 7 different sites. Each park was sampled two times throughout the summer of 2017, once in early summer and once in late summer, with three trapping nights per session. Trapping occurred at Mendon Ponds Park from 7 August 2017 to 10 August 2017 and 16 September 2017 to 19 September 2016. At Oatka Creek Park, I trapped from 24 June 2017 to 28 June 2017 and 20 August 2017 to 23 August 2017. The first trapping session at Oatka Creek Park had a one day gap in the schedule due to a tornado warning.

I used five Sherman live traps baited with oats in each plot. Traps were set in the evening and checked the following morning. Any small mammal caught was recorded, but only white-footed mice were analyzed. From captured mice I recorded the sex, age, mass, reproductive condition, and number of ticks embedded on each

individual. Mice were marked using a stainless steel ear tag with an identification number (Style 1005-1P, National Band and Tag Company, Newport, NY) before being released at the point of capture. I recorded the tag number on any mouse captured to avoid double counting individuals. I used the trapping data to estimate white-footed mouse population size in each plot by using the modified Petersen formula

$$Nc = \frac{(S1+1)(S2+1)}{(M+1)} - 1$$

where N_c was the estimated population size, S_1 was the marked sample size from 1st trapping period, S_2 was the marked sample size from the 2nd trapping period, and M was the number of recaptured individuals. Upper and lower confidence intervals were also calculated for each Petersen estimate using the formula CI = N ± 1.96 (S_N) where N was the Petersen estimate and S_N was the standard error. Standard error was calculated using the formula

$$S_N = \sqrt{\frac{N^2(S_2-M)}{(S_2+1)(M+2)}}$$

Microclimate Monitoring

In the same subset of plots used for the small mammal trapping, I set up microclimate stations in the 2017 field season to determine plot suitability for tick habitat. In each plot, I put a Lascar EL-USB-2 data logger (Lascar Electronics, Erie, PA) at ground level in the center of the plot to monitor relative humidity. The loggers were put in the field in mid-May and retrieved in early September, continuously taking readings every 30 min. Several of the data loggers became non-functional at

various points throughout the season, leaving an incomplete data set. To account for this, the data were broken into two different blocks for analysis. Block 1 dates ranged from 18 May 2017 to 28 May 2017 and represented a baseline view of the microclimatic conditions of the area in each plot before seasonal herbaceous growth. Block 2 dates were from 13 July 2017 to 23 July 2017. Since block 2 was later in the summer, it gave swallowwort and other herbaceous forbs time to grow and affect the microclimate of the plots. Analyzing the differences between these two blocks allowed for me to see how vegetation affected my microclimatic variables of interest and analyze the differences between the different plot types.

From the loggers, I was able to calculate daily average relative humidity and daily relative humidity range. Vapor pressure deficit (VPD) was calculated based on relative humidity data obtained from the data loggers. To calculate vapor pressure deficit, I first calculated saturation vapor pressure and actual vapor pressure. Saturation vapor pressure is how much water vapor the air can hold at a given point. Actual vapor pressure is how much water (how humid) is currently in the air at a given site. Vapor pressure deficit is the difference between these two values. A low value for vapor pressure deficit means that the air is near saturation while a high value means that the air can still hold more moisture. Saturation vapor pressure was calculated using the formula

$$e^{\circ}(T) = 0.6108\exp\left[\frac{17.27T}{T + 237.3}\right]$$

where $e^{o}(T)$ is the saturation vapor pressure at a given temperature, T is expressed in C^{o}, and vapor pressure is in kPa (Allen *et al*. 2005). Actual vapor pressure was

calculated using the formula VPactual = (RH/100) * SVP. Once both values were calculated, vapor pressure deficit could be calculated using the formula VPD = SVP-VPactual.

Statistical Analyses

Statistical analyses were performed using Minitab (Minitab, Inc., State College, PA) and SPSS (IBM Corporation, Armonk, NY). I used a paired Wilcoxon test to determine any differences between the 2016 and 2017 vegetation data. Since the data were not statistically different, I averaged the 2016 and 2017 data and used that average going forward. Arcsine square-root transformation (ground cover data) and log transformations (adult tick abundance, nymphal tick abundance, total number of ticks on mice) were used to achieve normality. Vegetation data could not be transformed sufficiently to achieve normality.

Vegetation Effects on Microclimate

I ran several paired Wilcoxon tests to determine any differences in microclimatic conditions (relative humidity, relative humidity range, vapor pressure deficit, and vapor pressure deficit range) between the swallowwort plots and bare control plots for both block 1 and block 2. Due to data logger failures, I was only able to explore these analyses at my Oatka 1, Oatka 2, Oatka 3, Mendon 2, and Mendon 3 plots. There were not enough vegetation plots with logger data to include in these analyses.

Vegetation and Ground Cover Effects on Ticks

I also used paired Wilcoxon tests to determine differences in tick abundance between plot types. For this, I ran tests on swallowwort plots vs. bare plots and swallowwort plots vs. vegetation plots for both nymphal and adult ticks. Since not all sites had all three plot types, it was necessary to run paired Wilcoxon tests between swallowwort and each of the control plots separately.

I ran Pearson correlations between the different ground covers (litter, twig, and bare) and different lifestages of ticks (adult and nymph). I also ran a regression analysis on the ability of average litter depth at a site to predict tick abundance, both nymphal and adult, across all plot types and parks. I used a Wilcoxon signed ranks test to determine any differences in average litter depth between the three plot types.

Vegetation Effects on Mice

At each park I trapped (Oatka Creek Park and Mendon Ponds Park), I ran two different Spearman's correlations to determine the effect of swallowwort on mouse/tick interactions: swallowwort abundance vs. the number of mice caught and swallowwort abundance vs. the number of ticks found on captured mice. I ran two paired t-tests to determine any differences in the number of mice caught and the number of embedded ticks on captured mice between swallowwort plots and their paired bare control plots. There were not enough vegetation control plots to include in the analysis.

Results

Vegetation Effects on Microclimate

For block 1 of logger data (18 May 2017 to 28 May 2017), there were not significant differences between swallowwort and control plots for all four microclimatic variables tested (Table 3). In block 2 (13 July 2017 to 23 July 2017), the swallowwort plots were characterized by statistically higher relative humidities and a smaller relative humidity range than their paired control plots (Figure 1). Swallowwort plots also had significantly lower vapor pressure deficits and a smaller vapor pressure deficit range than paired control plots in block 2 (Figure 2). Although the comparison could not be evaluated statistically, vegetation and swallowwort plots were qualitatively similar.

Vegetation and Ground Cover Effects on Ticks

Between the 2016 and 2017 field seasons, I collected 127 nymphs and 203 adults on my plot drags, totaling 330 ticks. There was a significantly greater number of adult ticks found in the swallowwort plots as compared to the bare plots ($p = 0.044$, $W=39.5$, Figure 3). There were no significant differences between the number of adult ticks in vegetation plots versus swallowwort plots ($p=0.528$, $W=13.5$). For nymphal ticks, there was no significant difference between tick abundance in swallowwort and vegetation plots ($p=0.345$, $W=6.0$) or between swallowwort and bare plots ($p=0.905$, $W=21.5$, Figure 4).

There was a significant positive correlation between leaf litter and the total number of nymphs captured at the plots ($p=0.006$, $r=0.513$) and a significant negative

correlation between bare ground and nymph abundance (p=0.006, r=-0.511). For adult ticks, there were similar trends, with a positive correlation with litter, and a negative correlation with bare ground, although they were not significant. Twig cover was not significantly correlated with either adult or nymphal tick abundance. Regression analysis showed that average litter depth at my sites was a significant predictor of total nymph abundance, although it only explained a small percentage of the variance (R^2 = 14.8%, F= 4.33, p = 0.048). There were no significant differences in average leaf litter depth among the three plot types.

Vegetation Effects on Mice

In total, I caught four species of small mammals: 73 white-footed mice (*Peromyscus leucopus*), two eastern chipmunks (*Tamias striata*), one northern short-tailed shrew (*Blarina brevicauda*), and one long-tailed weasel (*Mustela frenata*). At Oatka Creek Park, there were positive trends between swallowwort abundance and both number of individual mice caught (p=0.456, Spearman's ρ=0.238), and the number of ticks on captured mice (p=0.517, Spearman's ρ=0.208) although neither was statistically significant. At Mendon Ponds, there was a significant correlation between swallowwort cover and the number of ticks on captured mice (p=0.050, Spearman's ρ=0.812). The correlation between swallowwort and number of mice caught was also positive and may be biologically significant (p=0.117, Spearman's ρ=0.706).

Across both parks, there were significantly more mice captured in swallowwort plots (mean=5.57, SE=0.922) than in bare control plots (mean=2.43,

SE=0.685) (p=0.010, T=3.67, df=6) (Figure 5). There were more embedded ticks on captured mice from the swallowwort plots (mean=87.0, SE=36.4) than the bare plots (mean = 18.9, SE=8.9), which was significant at a 0.1 threshold (p=0.078, T=2.12, df=6) (Figure 6). I used my trapping data to calculate a modified Petersen's estimate of the population of mice in each plot (Table 4). Some sites showed more ticks in the swallowwort plots versus the control plots, but the results varied.

Discussion

My data suggest that swallowwort is capable of altering the microclimate of areas it invades in ways that have implications for small-scale tick and white-footed mice distributions. This study provided partial support for my hypothesis that there would be more ticks in swallowwort. Adult ticks in this study were more influenced by plot type than were nymphal ticks. There were significantly more adult ticks in swallowwort plots as compared to the bare control plots, which is consistent with studies that have found more adult ticks under invasive vegetation (Williams *et al*. 2009, Allan *et al*. 2010).

Many of my sites had no understory layer, except for the swallowwort that was able to grow there. Swallowwort grows in areas that many native plants cannot, but even in areas where it grows near native vegetation, it presents a threat through allelopathic properties (Douglass *et al*. 2011). Pale swallowwort is able to grow under a wide range of light conditions, including shaded areas, which makes it a problematic invader for forest understories (Smith *et al*. 2006). In these forests, it

seems that swallowwort is providing crucial habitat for ticks, where they otherwise may not have been able to survive.

There were no significant differences between adult ticks in the swallowwort and vegetation controls. In this study, I considered all vegetation control plots as equal, no matter what species were growing in the plots. Some vegetation controls had a diverse mix of native forbs, while other vegetation plots were dominated by invasive species, which may have affected my results. However, swallowwort may be able to alter these habitats further in the future. Smith *et al*. (2006) found that native vegetation is especially at risk in shaded forest understories where pale swallowwort's viney nature allows it to overtop and competitively displace other vegetation.

Abundance of nymphal ticks was more influenced by the ground cover layer than the vegetation layer. Nymphs quest lower to the ground than adult ticks, which may be why the ground cover layer was more important to them in this study (Stafford 2007). Correlation between leaf litter and tick abundance in this study is consistent with studies suggesting that subadult ticks rely heavily on leaf litter for survival (Schulze *et al*. 1995, Bouchard *et al*. 2013, Berger 2014). The litter layer provides a refuge for ticks that buffers against environmental extremes and desiccation (Bouchard *et al*. 2013). I found that litter depth was a significant predictor of nymphal abundance, which is consistent with findings of Adalsteinsson *et al*. (2016) that tick abundance was best predicted by leaf litter volume. It has been shown that when the leaf litter layer is removed, ticks are not able to survive and abundance

of subadult ticks is significantly reduced (Schulze *et al*. 1995). Correlations between leaf litter and ticks were significant for nymphal ticks but not for adult ticks. Adult ticks may not be as limited as juvenile ticks in their microclimatic constraints due to their larger size.

The data supported my hypothesis that swallowwort plots would harbor more white-footed mice than control plots. Swallowwort plots had more mice and more embedded ticks on captured mice than paired control plots. This is consistent with studies on invasive barberry that found understories dominated by barberry were characterized by a greater abundance of subadult ticks embedded on white-footed mice than in neighboring understories that lacked barberry (Williams *et al*. 2009). Swallowwort seemed to have more of an effect on mouse-tick interactions at Mendon Ponds Park than at Oatka Creek Park, likely due to the lack of other vegetative cover at Mendon Ponds Park. At my Mendon Ponds sites, swallowwort was the only available vegetative cover for white-footed mice, so it makes sense that the presence of swallowwort had more effect on mouse/tick interactions there. At Oatka Creek Park, there were abundant patches of other vegetation intermixed with the bare areas and areas covered with swallowwort. Mice would therefore have more options for protective cover and would not need to rely so heavily on the swallowwort patches. In heavily forested areas that may not support much understory vegetation, swallowwort seems to be providing areas of refugia for ticks and their small mammal hosts.

Data from the loggers support my hypothesis that swallowwort should alter microclimate in a manner beneficial for ticks. Swallowwort plots had higher relative

humidities, lower vapor pressure deficits, and smaller ranges in relative humidity and vapor pressure deficit, which are the microclimatic conditions expected to support ticks. This is consistent with Williams and Ward (2010), who found lower VPD levels under invasive barberry. In their study, when barberry was removed, the microclimate shifted and tick abundance was reduced by 60% (Williams and Ward 2010). The small ranges of relative humidity and VPD are important because buffered habitats without large swings in microclimatic variables and preferred for questing blacklegged ticks (Sonenshine 1992, Williams and Ward 2010). In areas with low relative humidities and high VPDs, tick survival is often lower (Bertrand *et al*. 1996).

In addition to its allelopathic properties and ability to grow in many different habitats, swallowwort is additionally able to persist in the environment because there are not any herbivores in North America that use it as a food source. Deer do not eat swallowwort (Ramnujan 2014), but they are still able to use browse-resistant understories for cover (Elias *et al*. 2006). I did not study deer in this project, but I did notice evidence of deer bedding down in my swallowwort plots. Deer, therefore, can contribute to tick disease cycles in two ways: by serving as the primary host for reproductive blacklegged ticks and by altering understory composition through preferential browsing of palatable species, which creates suitable habitat for ticks and their small mammal hosts (Lubelczyk *et al.* 2004).

In this geographic area, high deer populations have led to severe overbrowsing of forest understories. Deer browsing often determines the vegetative species composition of forest understories and can limit native plant regeneration (Averill *et*

al. 2017). Swallowwort is already considered an aggressive invader, and as deer continue to overbrowse forest understories, unpalatable swallowwort may be able to spread into even more forested habitats. Swallowwort's ability to succeed in low light forested areas makes it especially problematic in the context of ticks and their associated diseases.

High abundances of ticks in areas dominated by invasive understory species may pose greater risks for exposure to ticks (Elias *et al*. 2006). Studies on multiflora rose and Japanese barberry have found that tick-borne pathogen prevalence is greater in invaded forest areas than neighboring uninvaded areas (Williams and Ward 2010, Adalsteinsson *et al*. 2018). As swallowwort appears able to influence blacklegged ticks, white-footed mice, and white tailed deer in forested ecosystems, control efforts may be critically important in reducing the impact of tick-borne diseases.

Swallowwort can alter forest understories in ways that impact ticks and their hosts by providing cover and a favorable microclimate. Future studies should consider further exploring the relationship between swallowwort and tick/host interactions to determine tick-borne disease implications. Carbon dioxide traps have been used to collect ticks in vegetation where tick drags are not practical, such as multiflora rose, and should be considered when sampling swallowwort for ticks in the future (Adalsteinsson *et al.* 2016, 2018). Control for swallowwort is often labor-intensive, difficult and time consuming. Land managers should take aggressive action when swallowwort is first located to prevent large-scale infestations from forming. In places where swallowwort is already established, efforts should first be focused on

areas where swallowwort is some of only understory cover, to eliminate cover for ticks and their hosts.

Literature Cited

Adalsteinsson, S. A., V. D'Amico, W. G. Shriver, D, Brisson, and J. J. Buler. 2016. Scale-dependent effects of nonnative plant invasion on host-seeking tick abundance. Ecosphere. 7: 1-9.

Adalsteinnson, S. A., W. G. Shriver, A. Hojgaard, J. L. Bowman, D. Brisson, V. D'Amico, and J. J. Buler. 2018. Multiflora rose invasion amplifies prevalence of Lyme disease pathogen, but not necessarily Lyme disease risk. Parasites and Vectors. 11: 1- 10.

Allan, B. F., H. P. Dutra, L. S. Goessling, K. Barnett, J. M. Chase, R. J. Marquis, G. Pang, G. A. Storch, R. E. Thatch, and J. L. Orrock. 2010. Invasive honeysuckle eradication reduces tick-borne disease risk by altering host dynamics. Proceedings of the National Academy of Sciences. 107: 18523-185-27.

Allen, R. G., I. A. Walter, R. Elliott, T. Howell, D. Itenfisu, and M. Jensen, editors. 2005. The ASCE standardized reference evapotranspiration equation. Environmental and Water Resources Institute of the American Society of Civil Engineers. Rep.

Andre, C. 2018. Genesee County Web Mapping. [Online]. Genesee County Department of Planning, Batavia, NY. Available at: http://cogeneseeny.maps.arcgis.com/apps/webappviewer/index.html?id=0600d51c145b44c1b13dfb773063af03

Averill, K. M., A. DiTommaso, C. L. Mohler and L. R. Milbrath. 2011. Survival, growth, and fecundity of the invasive swallowworts (*Vincetoxicum rossicum* and *V. nigrum*) in New York State. Invasive Plant Science and Management. 4: 198-206.

Averill, K. M., D. A. Mortensen, E. A. H. Smithwick, S. Kalisz, W. J. McShea, N. A. Bourg, J. D. Parker, A. A. Royo, M. D. Abrams, D. K. Apsley, B. Blossey, D. H. Boucher, K. L. Caraher, A. DiTommaso, S. E. Johnson, R. Masson, and V. A. Nuzzo. 2017. Annals of Botany Plants. Special issue: Interactions between white-tailed deer and invasive plants in North American forests.

Berger, K. A., H. S. Ginsberg, L. Gonzalez, and T. N. Mather. 2014. Relative humidity and activity patterns of *Ixodes scapularis* (Acari: Ixodidae). Journal of Medical Entomology. 51: 769-776.

Bertrand, M. R., and M. L. Wilson. 1996. Microclimate-dependent survival of unfed adult *Ixodes scapularis* (Acari:Ixodidae) in nature: life cycle and study design implications. Journal of Medical Entomology. 33: 619-627.

Bouchard, C., G. Beauchamp, P. A. Leighton, R. Lindsay, D. Belanger, and N. H. Ogden. 2013. Does high biodiversity reduce the risk of Lyme disease invasion? Parasites and Vectors. 6: 195.

Douglass, C. H., L. A. Weston, and D. Wolfe. 2011. Phytotoxicity and potential allelopathy in pale (*Cynanchum rossicum*) and black swallowwort (*C. nigrum*). Invasive Plant Science and Management. 4: 133-141.

Elias, S. P., C. B. Lubelczyk, P. W. Rand, E. H. Lacombe, M. S. Holman and R. P. Smith. 2006. Deer browse resistant exotic-invasive understory: an indication of elevated human risk of exposure to *Ixodes scapularis* (Acari: Ixodidae) in southern coastal Maine woodlands. Journal of Medical Entomology. 43: 1142-1152.

Ginsberg, H. S. and C. P. Ewing. 1989. Habitat distribution of *Ixodes dammini* (Acari: Ixodidae) and Lyme disease spirochetes on Fire Island, New York. Journal of Medical Entomology. 26: 183-189.

GIS Services Division. 2018. Interactive historic photo map. [Online]. Monroe County Department of Environmental Services, Rochester, NY. Available at: https://mappingmonroe.monroecounty.gov/Html5Viewer2/index.html?viewer=Historic

Levy, S. 2013. The Lyme disease debate: host biodiversity and human disease risk. Environmental Health Perspectives. 121: 120-125.

Lubelczyk, C. B., S. P. Elias, P. W. Rand, M. S. Holman, E. H. Lacombe, and R. P. Smith, Jr. 2004. Habitat associations of *Ixodes scapularis* (Acari:Ixodidae) in Maine. Environmental Entomology. 33: 900-906.

Nelder, M.P., C. B. Russell, N. J. Sheehan, B. Sander, S. Moore, Y. Li, S. Johnson, Samir N. Patel, and D. Sider. 2016. Human pathogens associated with the blacklegged tick *Ixodes scapularis*: a systematic review. Parasites and Vectors. 9: 1-14.

Ostfeld, R. S., C. D. Canham, K. Oggenfuss, R. J. Winchcombe and F. Keesing. 2006. Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk. PLOS Biology. 4: 1058-1068.

Ostfeld, R. S., M. C. Miller, and K. R. Hazler. 1996. Causes and consequences of tick (*Ixodes scapularis*) burdens of white-footed mice (*Peromyscus leucopus*). Journal of Mammalogy. 77: 266-273.

Prusinski, M. P., H. Chen, J. M. Drobnack, S. J. Kogut, R. G. Means, J. J. Howard, J. Oliver, G. Lukacik, P. B. Backenson and D. J. White. 2006. Habitat structure associated with *Borrelia burgdorferi* prevalence in small mammals in New York State. Environmental Entomology. 35: 308-319.

Ramanujan, K. 2014. Invasive vines swallow up New York's natural areas. [Online.] Cornell Chronicle, Ithaca, New York. Available at: http://news.cornell.edu/stories/2014/04/invasive-vines-swallow-new-yorks-natural-areas

Schulze, T. L, R. A. Jordan and R. W. Hung. 1995. Suppression of subadult *Ixodes scapularis* (Acari: Ixodidae) following removal of leaf litter. Journal of Medical Entomology. 32: 720-733.

Smith, L. L., A. DiTommaso, J. Lehmann and S. Greipsson. 2006. Growth and reproductive potential of the invasive exotic vine *Vincetoxicum rossicum* in northern New York State. Canadian Journal of Botany. 84:1771-1780.

Sonenshine, D. E. 1992. Biology of ticks, volume 1. Second edition. Oxford University Press. New York, New York.

Stafford III, K. C. 2007. Ticks of the Northeastern United States [Online.] Connecticut Agricultural Experiment Station, New Haven, Connecticut. Available at http://www.ct.gov/caes/lib/caes/documents/publications/bulletins/b1010.pdf.

Vail, S. G., and G. Smith. 1998. Air temperature and relative humidity effects on behavioral activity of blacklegged tick (Acari:Ixodidae) nymphs in New Jersey. Journal of Medical Entomology. 35: 1025-1028.

Weston, L. A., J. N. Barney, and A. DiTommaso. 2005. A review of the biology and ecology of three invasive perennials in New York State: Japanese knotweed (*Polygonum cuspidatum*), mugwort (*Artemisia vulgaris*) and pale swallow-wort (*Vincetoxicum rossicum*). Plant and Soil. 277: 53-69.

Williams, S. C. and J. S. Ward. 2010. Effects of Japanese barberry (Ranculales:Berberidaceae) removal and resulting microclimate changes on *Ixodes scapularis* (Acari:Ixodidae) abundance in Connecticut, USA. Environmental Entomology. 39: 1911-1921.

Williams, S. C., J. S. Ward, T. E. Worthley and K. C. Stafford, III. 2009. Managing Japanese barberry (Ranculales:Berberidaceae) infestations reduces blacklegged tick (Acari:Ixodidae) abundance and infection prevalence with

Borrelia burgdorferi (Spirochaetales:Spirochaetaceae). Environmental Entomology. 38: 977-984.

Tables and Figures

Table 1. Breakdown of the 11 sites and the corresponding control plots location that each site offers, as compared to swallowwort plots.

Park	Site	Plot Comparison		
Oatka Creek Park	1	Swallowwort	Veg Control	Bare Control
	2	Swallowwort	Veg Control	Bare Control
	3	Swallowwort	Veg Control	Bare Control
	4	Swallowwort	Veg Control	Bare Control
Mendon Ponds Park	1	Swallowwort		Bare Control
	2	Swallowwort		Bare Control
	3	Swallowwort		Bare Control
Horizon Hill	1	Swallowwort		Bare Control
Powder Mills Park	1	Swallowwort	Veg Control	Bare Control
Durand Eastman Park	1	Swallowwort		Bare Control
Genesee County Park	1	Swallowwort	Veg Control	

Table 2. Average species richness and percent cover of the herbaceous layer, percent cover of the ground cover layer, litter depth and tree cover data for each plot.

PARK	SITE	PLOT	AVG SR OF FORBS	% SW	% OTHER VEG	% NO VEG	% LITTER	% TWIG	% BARE	AVG LITTER DEPTH	AVG CANOPY COVER	BASAL AREA	SR OF TREE LAYER
Oatka	1	SW	3.9	73.9	5.45	22.7	82.0	3.70	15.8	2.62	73.5	40.0	2
Oatka	1	VEG	6.2	0.00	35.8	64.3	65.1	5.30	29.7	2.09	81.0	14.0	3
Oatka	1	BARE	3.1	0.00	1.90	98.1	80.9	11.2	7.85	2.31	75.0	70.4	4
Oatka	2	SW	5.5	72.0	4.23	23.8	93.3	4.10	2.60	1.27	64.1	16.1	2
Oatka	2	VEG	7.3	0.10	48.0	52.0	93.2	5.80	1.00	2.03	71.9	0.00	0
Oatka	2	BARE	5.2	0.00	2.35	97.7	79.4	13.8	4.80	1.50	71.4	41.0	4
Oatka	3	SW	4.5	66.8	1.96	30.2	55.2	12.6	32.7	1.25	76.6	0.00	0
Oatka	3	VEG	5.5	0.00	79.0	21.0	79.5	6.10	14.4	2.15	74.0	0.00	0
Oatka	3	BARE	2.5	0.00	1.31	98.7	72.0	20.7	7.30	3.45	80.2	0.00	0
Oatka	4	SW	2.8	50.0	1.43	38.5	43.8	10.3	45.9	1.13	75.0	0.00	0
Oatka	4	VEG	7.5	0.00	28.4	71.6	66.7	6.80	22.5	2.08	68.3	57.0	7
Oatka	4	BARE	6.4	0.58	4.30	95.1	38.5	10.5	50.9	0.80	73.5	25.7	11
Mendon	1	SW	1.4	79.0	0.35	20.7	98.4	0.50	1.10	4.25	61.0	53.8	4
Mendon	1	BARE	3.2	0.21	0.86	98.9	92.9	4.20	2.90	3.60	68.3	137.8	5
Mendon	2	SW	2.7	61.5	0.32	38.2	90.4	5.30	4.30	2.55	66.7	35.8	7
Mendon	2	BARE	1.3	0.00	0.33	99.7	96.1	2.40	1.60	3.30	80.2	42.2	6
Mendon	3	SW	2.1	40.0	0.96	59.0	95.9	4.10	0.00	3.04	58.9	69.2	5
Mendon	3	BARE	0.5	0.00	0.06	99.9	97.6	2.80	0.00	3.57	63.1	68.3	3
Powder Mills		SW	1.4	87.0	0.50	11.6	79.8	20.1	0.20	1.93	69.8	48.8	1
Powder Mills		VEG	1.9	0.00	39.2	60.8	77.8	9.50	12.7	0.91	59.4	64.3	1

Powder Mills	BARE	1.0	3.31	6.70	90.0	84.0	13.7	2.20	1.66	59.44	100.12	3
Horizon Hill	SW	2.7	30.3	7.04	62.7	91.2	6.60	2.10	1.28	82.32	59.0	8
Horizon Hill	BARE	3.9	0.20	5.95	93.9	62.0	10.9	27.2	0.82	80.24	42.1	8
Durand Eastman	SW	1.8	59.2	0.85	40.0	96.3	1.00	2.70	2.90	77.64	78.6	3
Durand Eastman	BARE	2.0	0.01	0.46	99.5	87.0	11.8	1.20	2.83	78.68	119.6	4
Genesee County	SW	3.0	62.8	7.55	29.7	85.8	5.50	2.40	1.83	53.72	84.7	4
Genesee County	VEG	6.3	0.00	31.0	69.0	96.5	2.30	1.20	1.53	61.52	0.00	0

Table 3. Mean values and standard deviations for several microclimatic variables in three plot types for blocks 1 and 2. Z- and p-values are from paired Wilcoxon tests to determine differences between swallowwort and bare control plots in each block and category. Veg controls were not included in statistical analyses, but mean values and standard deviations are given for comparison.

	Block	**Swallowwort Plots**	**Bare Control**	**Veg Control**	**Z-value**	**p-value**
Relative	1	85.97 (3.95)	83.25 (4.10)	89.62 (10.2)	-1.483	0.138
Humidity	2	99.46 (3.61)	94.14 (1.27)	99.24 (5.37)	-2.023	**0.043**
RH	1	32.83 (12.5)	32.34 (9.93)	26.59 (16.4)	-0.674	0.500
Range	2	5.86 (1.92)	13.76 (7.25)	7.227 (3.79)	-2.023	**0.043**
VPD	1	0.612 (0.20)	0.611 (0.13)	0.435 (0.31)	-0.135	0.893
	2	0.192 (0.88)	0.340 (0.03)	0.195 (0.13)	-2.023	**0.043**
VPD	1	1.746 (1.35)	1.369 (0.49)	0.930 (0.95)	-0.944	0.345
Range	2	0.172 (0.061)	0.498 (0.24)	0.209 (0.12)	-2.023	**0.043**

Table 4. Number of unique white-footed mice individuals and population estimates at each plot in Oatka Creek Park and Mendon Ponds Park using the modified Petersen estimate from 2017 trapping data.

Site	Plot	Unique Individuals	Petersen N Mice	CI Lower	CI Upper
Oatka 1	SW	9	23	-3.03	49.03
	VEG	7	19	-4.55	42.55
	BARE	3	5	-0.66	10.66
Oatka 2	SW	2	7	1.4	12.6
	VEG	5	9	-2.16	20.16
	BARE	0	15	-3	33
Oatka 3	SW	5	2	-0.26	4.26
	VEG	5	11	-2.2	24.2
	BARE	5	0	0	0
Oatka 4	SW	3	3	-0.6	6.6
	VEG	0	0	0	0
	BARE	1	1	0.02	1.98
MP 1	SW	7	17	-4.51	38.51
	BARE	1	1	1	1
MP 2	SW	6	14	-3.35	31.35
	BARE	4	7	-1.4	15.4
MP 3	SW	5	5	5	5
	BARE	4	8	-1.05	17.05

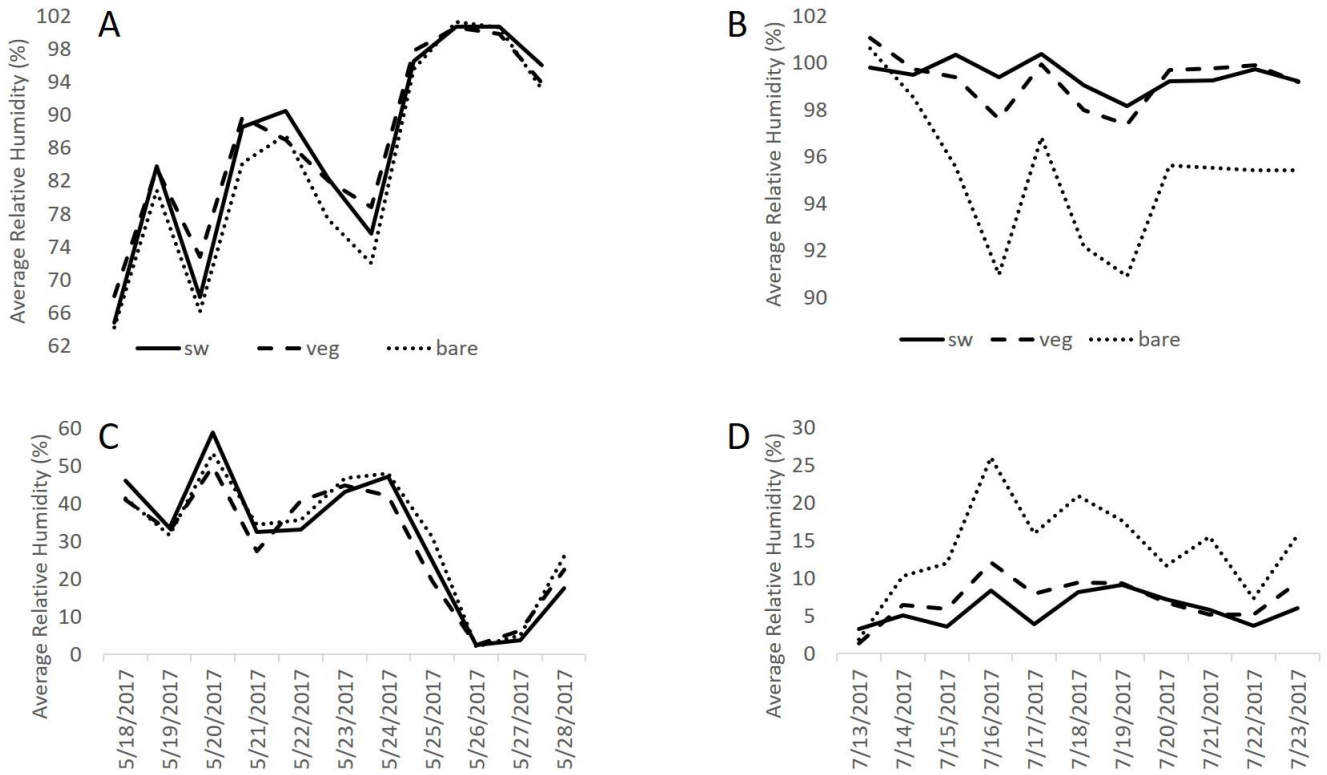

Figure 1. Relative humidity data from Oatka Creek Park and Mendon Ponds Park. Panels A and B are average relative humidity data and panels C and D are average relative humidity ranges. Panels A and C represent block 1, while panels B and D represent block 2.

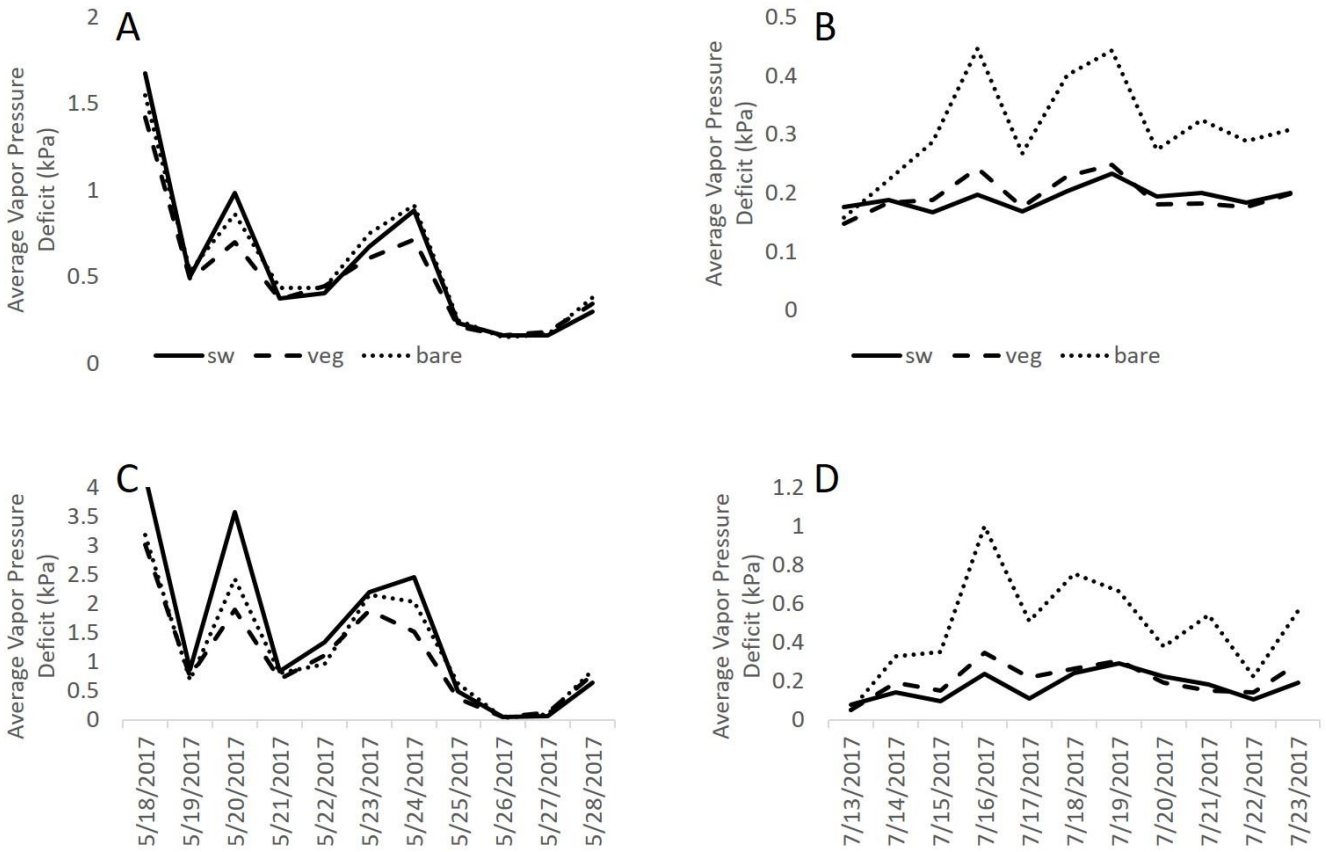

Figure 2. Vapor pressure deficit data from Oatka Creek Park and Mendon Ponds Park. Panels A and B are average vapor pressure deficit data and panels C and D are average vapor pressure deficit ranges. Panels A and C represent block 1, while panels B and D represent block 2.

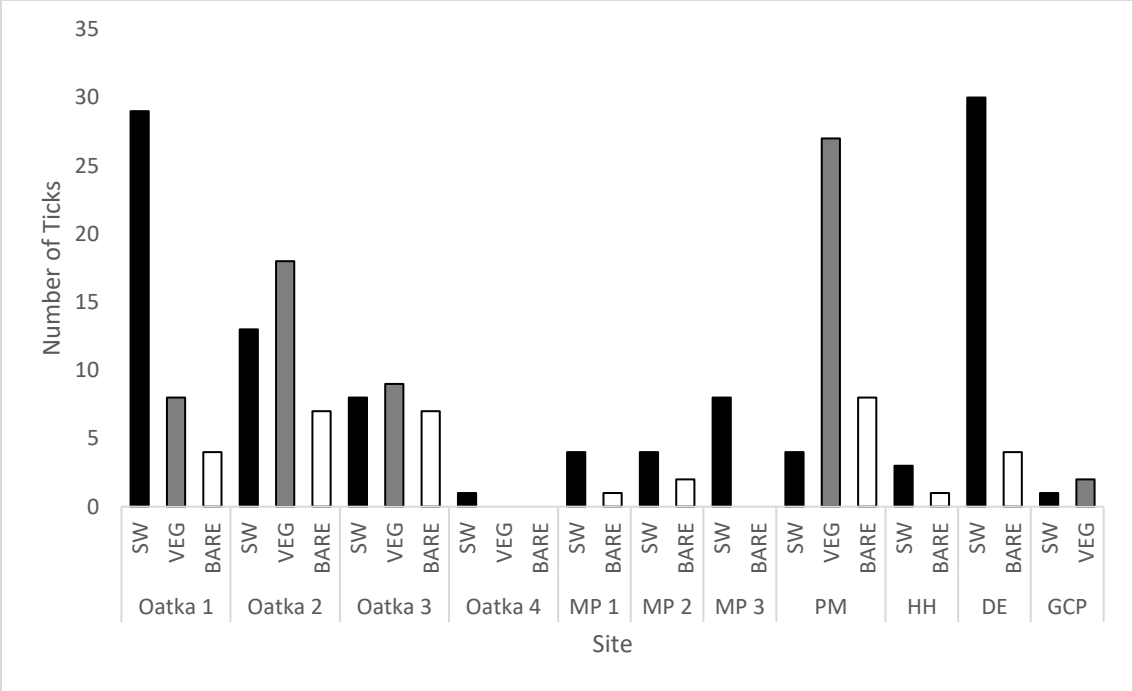

Figure 3. Total number of adult ticks collected under different vegetative conditions (swallowwort, other vegetation, bare) at field sites in 2016 and 2017.

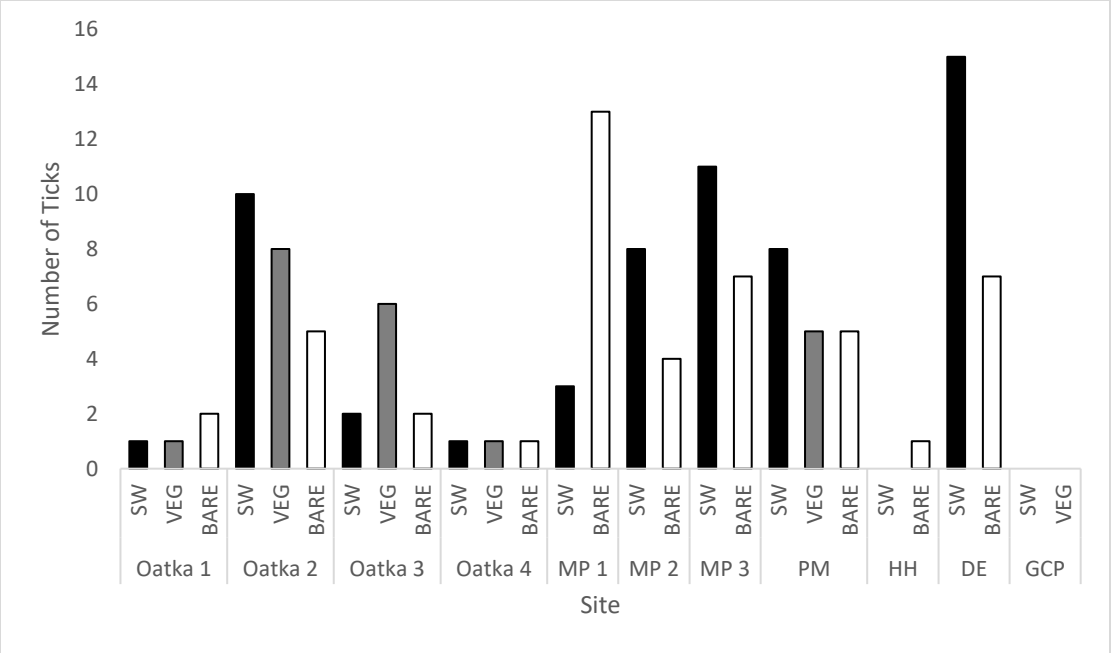

Figure 4. Total number of nymphal ticks collected under different vegetative conditions (swallowwort, other vegetation, bare) in 2016 and 2017 at all field sites.

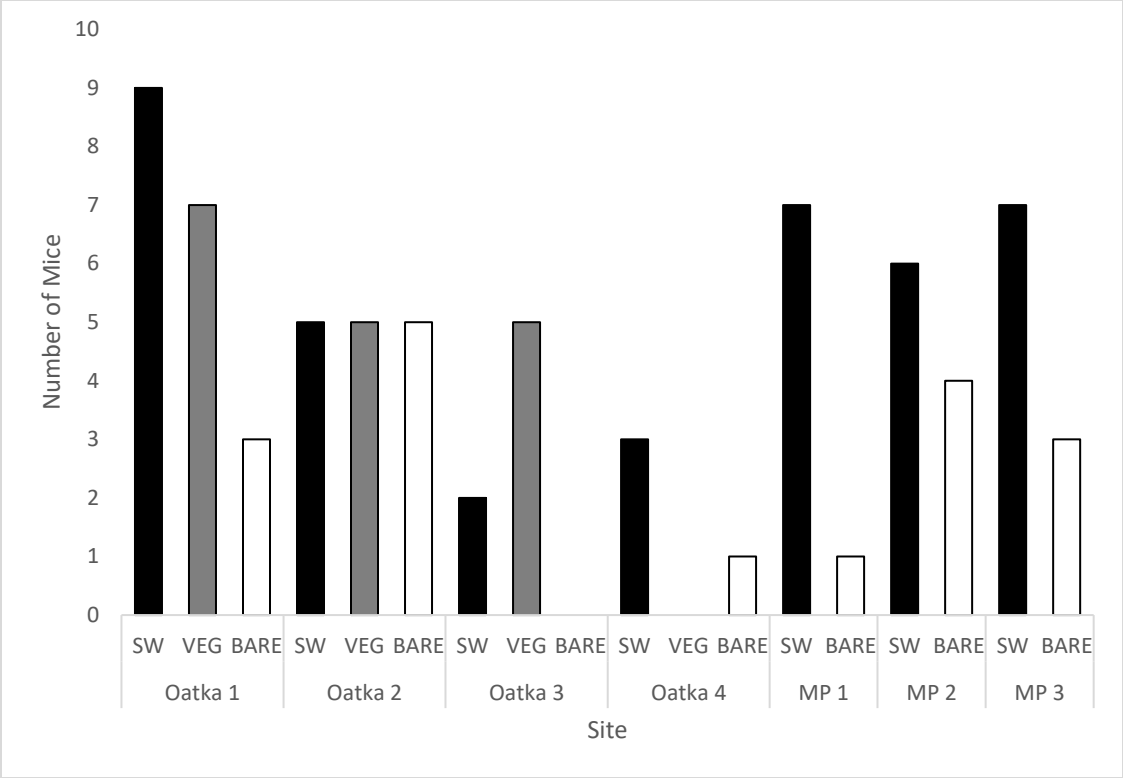

Figure 5. Total number of individual mice collected under different vegetative conditions (swallowwort, other vegetation, bare) during the 2 trapping periods of 2017 at Oatka Creek Park and Mendon Ponds Park.

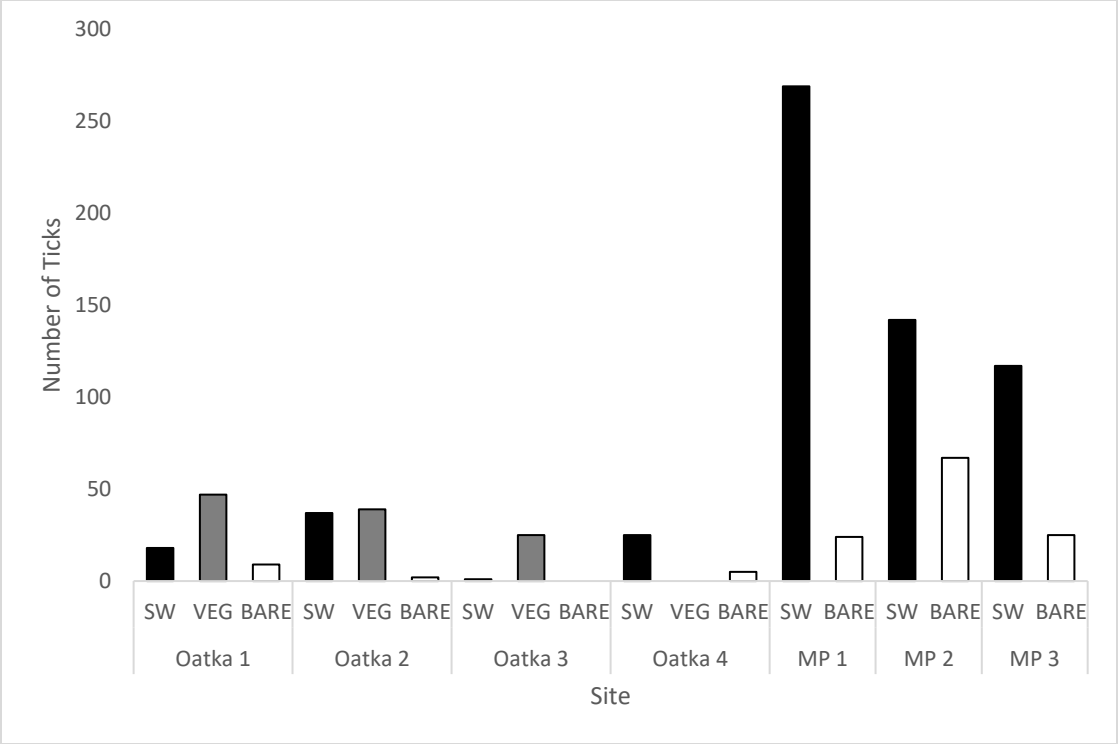

Figure 6. Total number of embedded ticks counted on captured mice under different vegetative conditions (swallowwort, other vegetation, bare) at each plot in the 2017 field season at Oatka Creek Park and Mendon Ponds Park.

Part Two: Landscape-level distribution of blacklegged ticks in public parks in the Rochester, NY area

Introduction

Ticks and the associated diseases that they transmit are of growing concern in the northeastern United States. The blacklegged tick (*Ixodes scapularis*) is the primary vector for the etiological agent of several diseases, including Lyme disease (*Borrelia burgdorferi*), human granulocytic anaplasmosis (*Anaplasma phagocytophilum*), and human babesiosis (*Babesia microti*), as well as the newly emerging diseases tick-borne relapsing fever (*Borrelia miyamotoi*) and Powassan encephalitis (Deer Tick Virus/Powassan Virus). Historically in New York State, ticks and tick-borne diseases have been limited to the southeastern area of the state. The Hudson Valley region has been the hotbed of Lyme disease incidences, while babesiosis and anaplasmosis cases have mostly been limited to Long Island and the surrounding downstate counties (NYSDOH 2015a-c). Much of the research on ticks and tick-borne diseases in New York has focused on this hyperendemic area.

Researchers have noted that ticks and their diseases have been expanding their range northwest into new parts of the state, becoming prevalent in areas previously unoccupied just a few decades ago (Prusinski *et al.* 2014, Khatchikian *et al.* 2015). The range of blacklegged ticks has expanded northward due to climate change and the utilization of migrating songbirds as hosts (Dantas-Torres 2015, Ostfeld and Brunner 2015). In addition to expanding their range, blacklegged ticks are moving back into ranges that they once inhabited before European settlers converted forested

areas into farmland (Simmons *et al*. 2015). Abandoned fields subject to reforestation and habitat engineering by overabundant deer have recreated favorable conditions for blacklegged ticks (Wood and Lafferty 2013, Simmons *et al*. 2015).

The leading edge of blacklegged tick range expansion to the northwest is currently somewhere between Syracuse and Rochester (Piedmonte *et al.* 2018). In western New York, the incidence rate of Lyme disease more than tripled from 2011 to 2015. In 1994, there were three reported cases of Lyme disease in Monroe County, while the most recent data from 2017 showed 184 reported cases. Anaplasmosis and babesiosis were historically absent in Monroe County, but a case of babesiosis was reported in 2006, followed by another in 2014, with at least a couple of cases reported every year since. Anaplasmosis was reported for the first time in 2016 in Monroe County (NYSDOH 1994-2017, 2015a, 2015b, 2015c).

The transmission of these diseases to humans in western New York indicates not only the presence of ticks, but also the presence of infected hosts. Ticks acquire transmissible diseases by feeding on an infected host (Barbour and Fish 1993), but hosts vary in their capacity to serve as a reservoir for pathogens (Keesing *et al.* 2009). White-footed mice (*Peromyscus leucopus*), the main host for juvenile ticks, have an 85% reservoir competence for *B. burgdorferi*, while white-tailed deer (*Odocoileus virginianus*) are poor reservoirs of *B. burgdorferi* but contribute to Lyme disease cycles by serving as the primary host for reproductive adults (Brisson *et al.* 2008). As ectoparasites, tick distribution is therefore mainly based on distribution of their hosts and their ability to survive where they detach from their host after feeding (Bunnell *et*

al. 2003). The landscape therefore structures host-vector interactions by determining the availability of host habitat. Crops in agricultural lands provide food for deer, while forested areas provide cover and additional food sources (Augustine and Jordan 1998). White-footed mice are considered habitat generalists and can be found in areas ranging from pristine forests to highly disturbed areas with low plant diversity (LoGiudice *et al*. 2003).

Not only the proportion of host habitats, but also their arrangement on the landscape may play a role in tick infection prevalence and disease transmission. Some studies have found that more fragmented landscapes with smaller forest patches have an increased prevalence of infected ticks and that efforts to decrease fragmentation may decrease Lyme disease risk (Allan *et al* 2003, Brownstein *et al*. 2005). Conversely, a more recent study found that highly fragmented forests were negatively associated with blacklegged tick abundance, and the lack of forest fragmentation was the most important predictor of blacklegged tick density (Ferrell and Brinkerhoff 2018). Other studies have found no effect of forest fragmentation on tick density or pathogen infection prevalence (Diuk-Wasser *et al*. 2010, Zolnik *et al*. 2015). Historically, Lyme disease has been associated with suburban residential areas adjacent to neighboring woodlots (Maupin *et al*. 1991), but both rural and urban areas support populations of ticks (Ostfeld *et al*. 1995, Rydzewski *et al*. 2012, Noden *et al*. 2017). In urban areas, ticks can be introduced into parklands that serve as wooded habitat islands (Daniels *et al.* 1997). The presence of ticks in an area is not an automatic public health threat, as the existence of a pathogen and contact with

humans is necessary for disease transmission. Metropolitan areas are important to study for public risk, as they are often characterized by the juxtaposition of residential areas with a mosaic of forest fragments and woodlots (Rydzewski *et al.* 2012).

Monroe County is a good study area since it is on the edge of tick expansion and is characterized by a diverse land-use matrix with highly developed urban areas, surrounding residential suburbs, and rural agricultural areas, with several parks located in each setting. Monroe County and the surrounding western New York areas also have some of the greatest deer densities in the state (NYSDEC 2017). As ticks expand their ranges into new areas, it is important that the public be aware of the associated risks so they can best protect themselves against tick-borne diseases. Although one of the main preventative methods against tick-borne diseases is quickly removing any embedded ticks before disease transmission occurs (Maupin *et al.* 1991), this can be an issue in areas where residents are unfamiliar with ticks. Such areas include urban environments or places where ticks have recently arrived, since people may not know to check themselves for ticks after being outside (Daniels *et al.* 1997). The risk of acquiring a tick-borne disease is high in highly populated areas that abut forested areas that serve as habitat for ticks and their hosts (Barbour and Fish 1993, Allan *et al*. 2003). Public parks are areas where the public and ticks often come into contact with one another, which can represent a serious public health threat when those ticks are infected with disease (Falco and Fish 1989, Paskewitz *et al*. 2001). Park visits also spike in the spring and summer months, when nymphal ticks (the lifestage most likely to transmit disease) are most active (Paskewitz *et al*. 2001).

Many residents visit parks wearing shorts and sandals, which can expose them to a tick bite, or with their dogs, which can pick up ticks, and thus pose an additional threat to humans (Paskewitz *et al.* 2001, Noden *et al.* 2017). Sampling for ticks along public hiking trails is one means that has been used to estimate human risk of encountering a tick (Siegel *et al.* 1991).

My study aimed to understand the spatial distribution of ticks in the Monroe County metropolitan area by surveying trails in public parks. The goals were to quantify tick density to see where the public was likely to encounter a tick and analyze larger scale land-use data to see if tick density could be explained by surrounding land-use patterns. I expected that the surrounding land-use matrix would affect tick density for adults and nymphs in similar ways. Due to deer habitat preferences, I hypothesized that ticks would be more abundant in forested and agricultural areas and less abundant in developed areas. I also thought that older forest stands or larger parks would help buffer against large tick populations. Due to the current distribution of ticks in New York, I expected to find more ticks at eastern sites than western sites.

Methods

Site Selection

In 2016, I sampled (dragged) 10 different trails in nine different parks in the greater Rochester area: Oatka Creek Park, Horizon Hill Conservation Area, Ganondagan State Historic Site, Powder Mills Park, Greece Canal Park, Durand

Eastman Park, Genesee County Park, Abraham Lincoln Park, and two drags in Mendon Ponds Park (Figure 1). Each drag took place in a section of the park dominated by mature forest. These drags took place between 24 October and 4 December 2016. In 2017, I revisited the same parks from 2016 but sampled different trails. I also sampled trails at eight additional parks in 2017: Letchworth State Park, Northampton Park, Lucien Morin Park, Webster Park, Ellison Park, Tryon Park, Irondequoit Bay Park West, and Hamlin Beach State Park (Figure 1). Sampling in 2017 occurred between 23 April and 19 July. For each park, I recorded park size and forest age of the sampled area, which was determined using historical photos from Genesee County Web Mapping (Andre 2018) and Mapping Monroe (GIS Services Division 2018).

Dragging Method

I collected ticks using a standard tick drag consisting of a $1m^2$ piece of flannel (the "flag"), weighted at one end, and connected to an L-shaped PVC handle. All ticks collected in this study were blacklegged ticks, and the use of "ticks" throughout refers to this species. Tick sampling occurred only on days when the vegetation was dry enough not to dampen the flag significantly. All sampling occurred between 10:00 and 17:00. For each transect, I dragged the vegetation bordering the trail for 20 m and then inspected both sides of the flag for ticks. I did this 50 times, for a total drag length of 1000 m in each park. I removed ticks from the flag with tweezers and stored them in alcohol until they could be transferred to a 4ºC cold room. I recorded

the number of ticks, along with their life stage and sex (if adult). I also recorded the start time and coordinates for each drag.

Geographic Information Systems Analysis

I used ArcGIS 10.4.1 (Environmental Systems Research Institute, Redlands, CA) software to map and spatially analyze the landscape surrounding the trail drags. I calculated and plotted the midpoint of each drag to determine a single point to be used for latitude and longitude comparison between drags. For each trail drag, I created four buffers around the sampled area: 100 m, 500 m, 1000 m and 2000 m. I imported land-cover raster data of New York from the National Land Cover Database (Multi-Resolution Land Characteristics Consortium, Sioux Falls, SD) and used the Tabulate Area tool in ArcMap to calculate the percentage of different land-cover types in each buffer for all of the trail drags. Land-cover variables included open water, developed open space, low intensity development, medium intensity development, unconsolidated shore, deciduous forest, evergreen forest, mixed forest, pasture/hay, cultivated crops, urban/recreational grass, palustrine forested wetland, and palustrine scrub/shrub wetland.

To determine what landscape features were potentially driving tick densities at the different spatial scales, I looked at two different sets of the landscape variables: the original landscape variables and then a combined version of the landscape variables. I combined similar variables to create five general categories: open water and shore (open water and unconsolidated shore), open grass (developed open areas and urban/recreational grasses), development (low intensity development and

medium intensity development), agriculture (cultivated crops and pasture/hay), and woody (deciduous forest, mixed forest, evergreen forest, palustrine forested wetland, and palustrine scrub/shrub wetland). The combined variables allowed me to look at a broad overview of the effect of land-cover types, and then I used the original landscape variables to tease out the finer scale differences.

Statistical Analyses

I used three different metrics of blacklegged tick density as response variables: total number of adults, total number of nymphs, and total number of ticks, which included adults, nymphs, and larvae. I did not collect enough larvae to consider them independently. I used IBM SPSS Statistics 24 (IBM Corporation, Armonk, NY) for all statistical analyses.

I ran a total of eight principal component analyses (PCA) on my land-cover data to reduce the number of landscape variables. I used the original land-cover data to perform four PCAs, one for each of the four buffer zones. I also ran an additional PCA for each buffer zone using the combined landscape variables.

I used Spearman rank correlations to determine relationships between the three metrics of tick density and forest age, park size, start time, Julian day, latitude and longitude. I considered a significance level of 0.1 throughout analyses due to a small sample size. I also used Spearman rank correlations to create a correlation matrix between tick density (adult, nymphs, and total) and the percent cover of the original landscape variables, percent cover of the combined landscape variables, principal components of the original landscape variables, and principal components

of the combined landscape variables for each spatial buffer. There were no significant correlations between tick density of any life stage and any of the principal components at the 100 m, 500 m, or 1000 m spatial scales. The principal components at the 2000 m buffer were the only ones to show any correlation with tick density, so they were the only ones I used in subsequent analyses.

I created generalized linear models using backward model selection to see which variables were important in predicting tick density. In backwards model selection, all selected variables are entered into the model, the least significant variable is removed, and the model is run again until all variables left are significant. I included seven predictor variables in my models. Start time, Julian day, latitude, longitude, original principal component 1 and original principal component 2 were entered as covariates while year was entered as a factor. Additionally, I included interactions between original PC1*original PC2 and latitude*Julian day. In 2017, many of my sites around Lake Ontario were flooded; this prevented me from sampling them as early as I wanted to, which is why I included the latitude*Julian day interaction. I created three different models, one for each level of tick density (adult, nymphs, total tick). I used the finite sample corrected Akaike's Information Criterion (AIC_c) to select the most representative models and reported all models with a $\Delta AIC_c < 2.0$ (Burnham and Anderson 2002).

Results

Overall, I collected a total of 388 blacklegged ticks from 27 trail drags in 17 different parks (Table 1). Due to the timing of my sampling, the majority of ticks captured in 2016 were adults, while most captured in 2017 were nymphs.

There were no significant correlations between tick density at any stage (adult, nymph, total) and park size or forest age. Nymphal tick density increased with later sampling dates ($p=0.009$, Spearman's $\rho= 0.492$) while adult tick density ($p<0.0001$, Spearman's $\rho= -0.781$) and total tick density ($p=0.020$, Spearman's $\rho= -0.444$) decreased with later dates. Start time was positively correlated with both adult ticks ($p=0.044$, Spearman's $\rho=0.397$) and total ticks ($p=0.026$, Spearman's $\rho=0.436$). Total tick density increased with longitude, such that more ticks were found further east in my sampling area ($p=0.023$, Spearman's $\rho=0.435$). (It should be noted that while the absolute value of longitude increases from east to west, longitude in the western hemisphere is represented as a negative value, so the true value of longitude increases west to east (a longitude value of -78 is further west than a longitude value of -77)). Total tick density decreased with increasing latitude, revealing that more ticks were found further from Lake Ontario ($p=0.047$, Spearman's $\rho= -0.385$).

Significant correlations between tick density and individual landscape variables are shown in Table 2. Variables measured in the 2000 m buffer showed the most significant correlations out of the four buffer zones. Total tick density only showed two significant correlations with the landscape variables, while nymphs and adults both had ten significant correlations each. Adult tick populations were denser

in areas with more forest cover and less dense in developed areas. Total tick density also showed a significant positive correlation with deciduous forest at the 1000 m buffer. Nymphal ticks showed numerous significant correlations at the larger landscape scales; however, they were all negative. Fewer nymphs were found in areas characterized with more agricultural, forested, and developed lands.

For both the original and combined 2000 m principal components analyses, I retained the first two principal components. The principal component analysis for the original landscape analysis yielded two principal components, collectively explaining 54.7% of the variance (Table 3). The first principal component was positively associated with the three forest types, pasture/hay, and cultivated crops. The second principal component was positively associated with development. The principal component analysis for the combined landscape variables did a better job of explaining variance than the analysis with original variables, collectively explaining 73.1% of the variance (Table 4). Conversely, the principal component analysis on the combined landscape variables showed that the first principal component was associated with open grassy areas and development, while the second principal component was associated with agriculture and wooded areas. Adult ticks were positively correlated with original PC1 ($\rho = 0.347$, $p = 0.076$), meaning adult density was positively associated with amount of land in forest and agriculture. Conversely, nymphs were negatively correlated with original PC1 ($\rho = -0.384$, $p = 0.048$), meaning that nymphal density was negatively associated with forest and agriculture. Nymphal

ticks were also negatively correlated with combined PC2 (ρ= -0.364, p= 0.062), which had positive associations with forest and agriculture.

The predictive models for adult, nymphal, and total tick density are shown in Table 5. The best model for total tick density suggested a positive relationship with longitude and a negative relationship with latitude, meaning denser total tick populations in more eastern and southern sites. The model also suggests a positive relationship with forested and agricultural land (PC1) and a negative relationship with developed and open areas (PC2), as well as a positive interaction between PC1 and PC2. The model also included Julian day as a positive predictor. The second best model for total tick density did not include latitude as a significant predictor variable by itself but did include a negative interaction between latitude and Julian day. The relationship between total tick density and longitude, PC1, PC2, Julian day, and the interaction between PC1 and PC2, was the same in the second model as it was in the first model. The model for adult tick density showed a positive relationship with year, which was the most important predictor of adult ticks in my study. The adult tick density model also suggested positive relationships with increasing longitude and amount of forested and agricultural lands (PC1), as well as a positive interaction between PC1 and PC2. Year was also the most important predictor variable in my nymphal tick density model, showing a negative relationship. The nymphal model showed a positive relationship with longitude and negative relationship with latitude, again suggesting tick populations are denser in eastern and southern parts of my study area. The nymphal model also suggested a positive relationship with increased

forested and agricultural land use and a negative relationship with increasing development. In summary, adult ticks tended to be denser in the eastern portion of my study area while nymphs were denser in both the east and the south. The models also show that both adults and nymphs were more likely found in areas with increased forested and agricultural land and decreased development.

Discussion

The results of my study provide a preliminary look into the current spatial distribution of blacklegged ticks in the Monroe County area and can be used as a reference for future studies as the range of ticks and tick-borne diseases continues to expand farther across western New York. In this study, I found that geographic location (latitude and longitude) was the most important predictor of blacklegged tick density. The spatial distribution of blacklegged ticks showed that tick density was greatest in the southeastern area of my study site, which supports my hypothesis that ticks would be more prevalent in the east. A higher concentration of ticks in the southeastern portion of my study area is congruent with other studies that have found that ticks and tick-borne disease are migrating from the hyperendemic Hudson Valley region in a northwestern wave (Prusinski *et al*. 2014, Khatchikian *et al*. 2015). My model for total tick density showed that tick densities increased towards more southern latitudes, which is consistent with a similar study from the Hudson Valley region (Khatchikian *et al*. 2012). Increased tick density at more southerly sites also meant that I collected fewer ticks closer to Lake Ontario. Researchers from the State

University of New York at Oswego also found the same relationship of increased tick density with increased distance from Lake Ontario (Dr. Tim Braun, personal communication). However, a study of ticks in Chicago's metro area found that tick density increased as researchers got closer to Lake Michigan (Rydzewski *et al*. 2012). Another study in Rhode Island found a highly significant trend of decreasing tick density as latitude increased (Nicholson and Mather 1996). Further investigation is needed to determine if the relationship between tick density and distance to Lake Ontario is driven by a simple latitudinal gradient or ways in which the lake affects parameters such as onshore microclimate, prevailing wind patterns, and plant phenology.

The spatial distribution of ticks in this study shows that they are migrating into this region, and analyzing tick densities along with land-use data allowed me to see how landscape patterns were driving tick abundance. My results suggested that ticks were more abundant in forested and agricultural areas, and less abundant in open, developed areas, which supports my hypothesis. The original PC1, which is categorized by positive associations with forests and agricultural land, and negative associations with open, developed areas, was a significant predictor variable in the models for adult, nymphal, and total tick density. Studies have repeatedly shown that forested areas support greater numbers of ticks of all life stages than open areas (Maupin *et al*. 1991, Guerra *et al*. 2002, Killilea *et al*. 2008). Adult ticks were positively associated with forested areas in the landscape correlations, most significantly at the 2000 m buffer level. Normally, deciduous forests support greater

tick populations, but many studies have found negative correlations between evergreen forests and nymphal and adult tick density (Guerra *et al*. 2002, Bunnell *et al*. 2003, Lubelczyk *et al*. 2004). In my study, ticks were positively correlated with both deciduous and evergreen forests at the 2000 m buffer. Deer in this area are known to utilize coniferous forests in the winter which could explain this finding (Dechen Quinn 2010). It should also be noted that the percentage of evergreen forest in the 2000 m buffer zones was extremely low at all sites, between 0 and 2 %. Throughout the rest of the year, deer in New York tend to select for deciduous forests and agricultural areas, which provide both food and cover (Dechen Quinn 2010). Naturally, adult tick density in this study corresponds with preferred deer habitat, as white-tailed deer serve as the primary host for questing adult ticks. Adult tick density was also negatively associated with development. Few studies in the United States have focused on ticks in highly developed urban areas. In a study around Oklahoma City, Oklahoma, Noden *et al*. (2017) found a trend of increased tick density in areas surrounded by undeveloped land, which is consistent with my results. Development often leads to fragmentation of forested areas, which may affect the habitat of ticks and their hosts, although this is debated. Conflicting studies have found that forest fragmentation can either increase tick density and infection prevalence (Allan *et al*. 2003, Brownstein *et al*. 2005), have no effect at all (Diuk-Wasser *et al*. 2010, Zolnik *et al*. 2015), or can negatively affect blacklegged tick abundance (Ferrell and Brinkerhoff 2018). I hypothesized that there may be fewer ticks in large parks, but I did not find significant correlations to support this. Significant interactions between

PC1 and PC2 in my models for adult and total tick density suggest that the interspersion of different land-use types and their overall arrangement in the landscape matrix may significantly affect tick densities. While my study looked at the total cover of landscape variables, fragmentation and interspersion are likely also important variables and they should be considered as metrics in future studies.

Finally, I hypothesized that the surrounding land-use matrix would affect tick densities of adults and nymphs in similar ways, but results showed mixed support for this hypothesis. The nymphal density model showed that, like adults, nymphs were significantly associated with original PC1, and nymphal density increased with increasing cover of forests and agricultural areas which supports my hypothesis. However, the landscape correlations contradicted the model as nymphs were negatively correlated with original PC1, forested areas, as well as agricultural areas. This was surprising, as nymphs are known to be associated with wooded areas (Ginsberg and Ewing 1989, Maupin *et al*. 1991, Guerra *et al*. 2002, Ginsberg *et al*. 2004).

Since I found adult tick density was positively associated with white-tailed deer habitat in a large buffer zone (2000 m) consistent with their range size, I suspected that nymphal tick density may be correlated with landscape variables in the 100-m buffer zone, which represents the home range of the white-footed mouse, the main host for juvenile ticks (Zolnik *et al*. 2018). However, the majority of the correlations between nymphs and the landscape were found at the 2000 m buffer zone, all of which were negative. Nymphs were not strongly positively correlated

with any landscape metric, which suggests either a weaker effect of the landscape matrix on nymphal densities or may be an indication that different scales need to be investigated. Khatchikian *et al*. (2012) found that landscape covariates were more correlated with adult blacklegged ticks than nymphs, although only slightly. Another study of the entire eastern United States found that landscape variables were not as predictive as climatic data when modeling for nymphs; nymphal density was instead driven by altitude, seasonality of temperature, and vapor pressure deficit (Diuk-Wasser *et al*. 2010). Ferrell and Brinkerhoff (2018) used landscape analysis to study tick abundance in Virginia and detected correlations between nymphal density and land-use patterns at larger spatial scales. They used buffer zones of 1 km, 5 km, and 10 km and found that abundance of blacklegged nymphs was positively associated with the amount of forest cover in the 10 km buffer zone. Analyzing my land-use data at larger scales may therefore have yielded positive correlations between landscape variables and nymphal density.

Although I was unable to clearly determine if the land-use matrix affected nymphs and adults in similar ways, Khatchikian *et al*. (2012) found that models explaining adult and nymphal density estimates in the Hudson Valley region of New York were nearly identical and stated that climatic and landscape patterns at a broad-scale were sufficient to predict blacklegged tick densities. They found that variation in the density of nymphs could best be explained by year, location, season, proportion of forest cover, minimum winter temperature, and summer precipitation. The adult density model used winter precipitation instead of summer precipitation, and

additionally included urbanization, but was otherwise similar to the nymphal model (Khatchikian *et al*. 2012).

Results from my study suggest that while the land-use matrix may be an important driver of tick density, different types of data sets, mainly climatic data, should be utilized by future studies to more accurately model tick densities. Other studies that have modeled tick abundance have used variables such as elevation, soil texture, level of forest fragmentation, size of forest patches, leaf litter volume, abundance of tick hosts, and several different metrics of temperature and precipitation data (Guerra *et al*. 2002, Bunnell *et al*. 2003, Brownstein *et al*. 2005, Diuk-Wasser *et al*. 2010, Khatchikian *et al*. 2012, Ozdenerol 2015, Adalsteinsson *et al*. 2016). Including climatic data in my analyses likely would have yielded a stronger model for tick density and may have provided additional clarity for my analyses.

Analysis of my data was likely limited due to the fact that I only had one sampling session for each lifestage of tick and that adults and juvenile ticks were sampled in different years. Temporal aspects of my sampling strategy can explain several of my results for tick density. Nymphal tick density increased with later sampling dates while adult tick density decreased with Julian day, which is likely due to when I began sampling. Peak activity for ticks in this region is from June to early July for nymphs and from October to November for adults (Prusinski *et al*. 2014). I began to sample adult ticks right at their peak activity point in October 2016. As the season progressed, I collected fewer ticks since ticks had likely already found a host and the number of questing ticks had decreased. Conversely, I started sampling for

nymphs at the end of April 2017 and finished in July, so the later in the season I sampled, the closer it was to the nymphal activity peak, and therefore, the more nymphs I collected. The strongest variable by far in both the adult and nymphal models was year. Due to the timing of sampling, I collected mainly adult ticks in 2016 and nymphal ticks in 2017, which likely affected my results. Start time was positively correlated with both adult ticks and total tick density, meaning that more ticks were collected later in the day. This was a surprising results as more adult blacklegged ticks are usually found early in the morning, between 6:00 and 9:00, when temperatures are lower and relative humidity is around its daily maximum level (Schulze *et al*. 2001). Future studies should use a more robust sampling strategy with at least two years of data for each lifestage.

My study offers preliminary analyses depicting how land-cover data may be useful for understanding distribution and density of tick populations in western New York. As tick density and pathogen prevalence continues to increase in the Rochester, New York area, it is important for park officials and land managers to engage in outreach and education for the public so they can understand the risks posed by ticks and their associated diseases. Residents who live in areas with historically large tick populations and higher incidence of tick-borne diseases generally know to take precautions against ticks, such as hiking in long pants, checking themselves for ticks after being outside, quickly removing embedded ticks, using tick repellents, avoiding tick habitat during their peak activity periods, etc. Rochester is currently on the leading edge of tick expansion out of southeastern New York (Piedmonte *et al*. 2018),

and therefore, many residents likely either do not know how to protect themselves against ticks or do not think about it when they head outside. In areas where Lyme disease has been around longer, both public citizens and health care workers are better able to recognize and report cases of Lyme disease (Killilea *et al*. 2008). Prusinski *et al*. (2014) stated that preventative measures taken by the educated public may explain the lack of correlation between risk indices and actual numbers of tick-borne disease cases in New York State. Many of the parks that I visited had Lyme disease signage at the trailheads, but many visitors I encountered on the trail were unaware of the proper precautions to take against ticks and tick-borne diseases. Increased public awareness through outreach efforts and increased signage in park areas will help to better protect residents against ticks and tick-borne diseases.

Although I sampled some parks only one time, it seemed that Horizon Hill Conservation Area, Powder Mills Park, and Mendon Ponds Park were the parks that I was most likely to encounter a tick at. Due to the extreme popularity of Mendon Ponds Park, this may be a site that park officials would want to target to increase awareness. As tick abundance and tick-borne diseases are likely to increase in this area, park officials should continue to monitor for ticks in the future. My study provides a look at the current spatial distribution of ticks in public parks in the Rochester, NY, which can serve as a reference for future studies as ticks continue to expand into the area and provide park officials with valuable data so they can better address the public health risk imposed by increased tick density.

Literature Cited

Adalsteinsson, S. A., V. D'Amico, W. G. Shriver, D, Brisson, and J. J. Buler. 2016. Scale-dependent effects of nonnative plant invasion on host-seeking tick density. Ecosphere. 7: 1-9.

Allan, B. F., H. P. Dutra, L. S. Goessling, K. Barnett, J. M. Chase, R. J. Marquis, G. Pang, G. A. Storch, R. E. Thatch, and J. L. Orrock. 2010. Invasive honeysuckle eradication reduces tick-borne disease risk by altering host dynamics. Proceedings of the National Academy of Sciences. 107: 18523-185-27.

Andre, C. 2018. Genesee County Web Mapping. [Online]. Genesee County Department of Planning, Batavia, NY. Available at: http://cogeneseeny.maps.arcgis.com/apps/webappviewer/index.html?id=0600d51c145b44c1b13dfb773063af03

Augustine, D. J. and P. A. Jordan. 1998. Predictors of white-tailed deer grazing intensity in fragmented deciduous forests. The Journal of Wildlife Management. 62: 1076-1085.

Barbour, A. G., and D. Fish. 1993. The biological and social phenomenon of Lyme disease. Science. 260: 1610-1616.

Brisson, D., D. E. Dykhuizen, and R. S. Ostfeld. 2008. Conspicuous impacts of inconspicuous hosts on the Lyme disease epidemic. Proceedings of the Royal Society B. 275: 227-235.

Brownstein, J. S., D. K. Skelly, T. R. Holford and D. Fish. 2005. Forest fragmentation predicts local scale heterogeneity of Lyme disease risk. Oecologia. 146: 469-475.

Bunnell, J. E., S. D. Price, A. Das, T. M. Shields and G. E. Glass. 2003. Geographic information systems and spatial analysis of adult *Ixodes scapularis* (Acari:Ixodidae) in the Middle Atlantic region of the U.S.A. Journal of Medical Entomology. 40: 570-576.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Second edition. Springer-Verlag, New York, New York.

Daniels, T. J., R. C. Falco, I. Schwartz, S. Varde and R. G. Robbins. 1997. Deer ticks (*Ixodes scapularis*) and the agents of Lyme disease and human granulocytic ehrlichiosis in a New York City park. Emerging Infectious Diseases. 3: 353-354.

Dantas-Torres, F. 2015. Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. International Journal for Parasitology: Parasites and Wildlife. 4: 452-461.

Dechen Quinn, A. 2010. Influences of movement behavior and space use in evaluating disease risk among white-tailed deer in central New York. PhD dissertation, State University of New York College of Environmental Science and Forestry.

Diuk-Wasser, M. A., G. Vourc'h, P. Cislo, A. G. Hoen, F. Melton, S. A. Hamer, M. Rowland, R. Cortinas, G. T. Hickling, J. I. Tsao, A. G. Barbour, U. Kitron, J. Piesman and D. Fish. 2010. Field and climate-based model for predicting the density of host-seeking nymphal *Ixodes scapularis*, an important vector of tick-borne disease agents in the eastern United States. Global Ecology and Biogeography. 19: 504-514.

Eisen, R. J. and L. Eisen. 2018. The blacklegged tick, *Ixodes scapularis*: an increasing public health concern. Trends in Parasitology. 34: 295-309.

Falco, R. C. and D. Fish. 1989. Potential for exposure to tick bites in recreational parks in a Lyme disease endemic area. American Journal of Public Health. 79: 12-15.

Ferrell, A. M., and R. J Brinkerhoff. 2018. Using landscape analysis to test hypotheses about drivers of tick density and infection prevalence with *Borrelia burgdorferi*. International Journal of Environmental Research and Public Health. 15: 737.

Ginsberg, H. S. and C. P. Ewing. 1989. Habitat distribution of *Ixodes dammini* (Acari: Ixodidae) and Lyme disease spirochetes on Fire Island, New York. Journal of Medical Entomology. 26: 183-189.

Ginsberg, H. S., E. Zhioua, S. Mitra, J. Fischer, P. A. Buckley, F. Verret, H. B. Underwood, and F. G. Buckley. 2004. Woodland type and spatial distribution of nymphal *Ixodes scapularis* (Acari: Ixodidae). Environmental Entomology. 33: 1266-1273.

GIS Services Division. 2018. Interactive historic photo map. [Online]. Monroe County Department of Environmental Services, Rochester, NY. Available at: https://mappingmonroe.monroecounty.gov/Html5Viewer2/index.html?viewer=Historic

Keesing, F., J. Brunner, S. Duerr, M. Killilea, K. LoGiudice, K. Schmidt, H. Vuong, and R. S. Ostfeld. 2009. Hosts as ecological traps for the vector of Lyme disease. Proceedings of the Royal Society B. Doi:10.1098/rspb.2009.1159.

Khatchikian, C. E., M. A. Prusinski, M. Stone, P. B. Backenson, I. Wang, E. Foley, S. N. Seifert, M. Z. Levy, and D. Brisson. 2015. Recent and rapid population growth and range expansion of the Lyme disease tick vector, *Ixodes scapularis*, in North America. Evolution. 69: 1678-1689.

Khatchikian, C. E., M. A. Prusinski, M. Stone, P. B. Backenson, I. Wang, M. Levy and D. Brisson. 2012. Geographical and environmental factors driving the increase in the Lyme disease vector *Ixodes scapularis*. Ecosphere. 3: 85.

Killilea, M. E., A. Swei, R. S. Lane, C. J. Briggs and R. S. Ostfeld. 2008. Spatial dynamics of Lyme disease: a review. EcoHealth. 5: 167-195.

LoGiudice, K., S. T. K. Duerr, M. J. Newhouse, K. A. Schmidt, M. E. Killilea, and R. S. Ostfeld. 2008. Impact of host community composition on Lyme disease risk. Ecology. 89: 2841-2849.

Lubelczyk, C. B., S. P. Elias, P. W. Rand, M. S. Holman, E. H. Lacombe, and R. P. Smith, Jr. 2004. Habitat associations of *Ixodes scapularis* (Acari:Ixodidae) in Maine. Environmental Entomology. 33: 900-906.

Maupin, G. O., D. Fish, J. Zultowsky, E. O. Campos and J. Piesman. 1991. Landscape ecology of Lyme disease in a residential area of Westchester County, New York. American Journal of Epidemiology. 133: 1105-1113.

Nicholson, M. C. and T. N. Mather. 1996. Methods for evaluating Lyme disease risks using geographic information systems and geospatial analysis. Journal of Medical Entomology. 33: 711-720.

Noden, B. H., S. R. Loss, C. Maichak and F. Williams. 2017. Risk of encountering ticks and tick-borne pathogens in a rapidly growing metropolitan area in the U. S. Great Plains. Ticks and Tick-borne Diseases. 8: 119-124.

NY State Department of Health (NYSDOH). 1994-2017. Communicable disease annual reports. New York State Department of Health, Albany, NY. Available at: https://www.health.ny.gov/statistics/diseases/communicable/.

NY State Department of Environmental Conservation (NYSDEC). 2017. White-tailed deer harvest summary. New York State Department of Environmental Conservation, Albany, NY. Available at: https://www.dec.ny.gov/docs/wildlife_pdf/2017deerrpt.pdf

NY State Department of Health (NYSDOH). 2015a. Anaplasmosis/ehrlichiosis. New York State Department of Health, Albany, NY. Available at: https://www.health.ny.gov/statistics/diseases/communicable/surveillance_summaries/docs/anaplasmosis_and_ehrlichiosis_2015.pdf.

NY State Department of Health (NYSDOH). 2015b. Babesiosis. New York State Department of Health, Albany, NY. Available at: https://www.health.ny.gov/statistics/diseases/communicable/surveillance_summaries/docs/babesiosis_2015.pdf.

NY State Department of Health (NYSDOH). 2015c. Lyme disease. New York State Department of Health, Albany, NY. Available at: https://www.health.ny.gov/statistics/diseases/communicable/surveillance_summaries/docs/lyme_2015.pdf.

Ostfeld, R. S., and J. L. Brunner. 2015. Climate change and *Ixodes* tick-borne disease of humans. Philosophical Transactions of Royal Society B. 370: 20140051.

Ostfeld, R. S., O. M. Cepeda, K. R. Hazler, and M. C. Miller. 1995. Ecology of Lyme disease: habitat association of ticks (*Ixodes scapularis*) in a rural landscape. Ecological Applications. 5: 353-361.

Ozdenerol, E. 2015. GIS and remote sensing use in the exploration of Lyme disease epidemiology. International Journal of Environmental Research and Public Health.12: 15182-15203.

Paskewitz, S. M., M. Vandermause, E. A. Belongia and J. J. Kazmierczak. 2001. *Ixodes scapularis* (Acari:Ixodidae): Density and rate of infection with *Borrelia burgdorferi* in four state parks in Wisconsin. Journal of Medical Entomology. 38: 33-38.

Piedmonte, N. P., S. B. Shaw, M. A. Prusinski and M. K. Fierke. 2018. Landscape features associated with blacklegged tick (Acari:Ixodidae) density and tick-borne pathogen prevalence at multiple spatial scales in central New York State. Journal of Medical Entomology. doi: 10.1093/jme/tjy111.

Prusinski, M. E., H. Chen, J. M. Drobnack, S. J. Kogut, R. G. Means, J. J. Howard, J. Oliver, G. Lukacik, P. B. Backenson and D. J. White. 2006. Habitat structure associated with *Borrelia burgdorferi* prevalence in small mammals in New York State. Community and Ecosystem Ecology. 35: 308-319.

Prusinski, M. A., J. E. Kokas, K. T. Hukey, S. J. Kogut, J. Lee and P. B. Backenson. 2014. Prevalence of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae), *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae), and *Babesia*

microti (Piroplasmida: Babesiidae) in *Ixodes scapularis* (Acari:Ixodidae) collected from recreational lands in the Hudson Valley Region, New York State. Journal of Medical Entomology. 51: 226-236.

Rydzewski, J., N. Mateus-Pinilla, R. E. Warner, J. A. Nelson and T. C. Velat. 2012. *Ixodes scapularis* (Acari: Ixodidae) distribution surveys in the Chicago metropolitan region. Journal of Medical Entomology. 49: 955-959.

Schulze, T. L., R. A. Jordan, and R. W. Hung. 2001. Effects of selected meteorological factors on diurnal questing of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae). Journal of Medical Entomology. 38: 318-324.

Siegel, J. P., U. Kitron and J. K. Bouseman. 1991. Spatial and temporal distribution of *Ixodes dammini* (Acari: Ixodidae) in a northwestern Illinois State Park. Journal of Medical Entomology. 28: 101-104.

Simmons, T. W., J. Shea, M. A. Myers-Claypole, R. Kruise, and M. L. Hutchinson. 2015. Seasonal activity, density, and collection efficiency of the blacklegged tick (*Ixodes scapularis*) (Acari: Ixodidae) in mid-western Pennsylvania. Journal of Medical Entomology. 52: 1260-1269.

Stafford III, K. C. 2007. Ticks of the Northeastern United States [Online.] Connecticut Agricultural Experiment Station, New Haven, Connecticut. Available at http://www.ct.gov/caes/lib/caes/documents/publications/bulletins/b1010.pdf.

Vail, S. G., and G. Smith. 1998. Air temperature and relative humidity effects on behavioral activity of blacklegged tick (Acari:Ixodidae) nymphs in New Jersey. Journal of Medical Entomology. 35: 1025-1028.

Williams, S. C. and J. S. Ward. 2010. Effects of Japanese barberry (Ranculales:Berberidaceae) removal and resulting microclimate changes on Ixodes scapularis (Acari:Ixodidae) density in Connecticut, USA. Environmental Entomology. 39: 1911-1921.

Wood, C. L. and K. D. Lafferty. 2013. Biodiversity and disease: a synthesis of ecological perspectives on Lyme disease transmission. Trends in Ecology and Evolution. 28: 239-247.

Zolnik, C. P., R. C. Falco, S. Kolokotronis, and T. J. Daniels. 2015. No observed effect of landscape fragmentation on pathogen infection prevalence in blacklegged ticks (*Ixodes scapularis*) in the northeastern United States. PLOS One. Doi: 10.1371/journal.pone.0139473.

Tables and Figures

Table 1. Date and number of ticks collected for all 27 trails drags in 2016 and 2017.

Park	Day	Adult	Nymphal	TickTotal
Horizon Hill	26 Oct 2016	37	0	37
Ganondagan	31 Oct 2016	11	0	11
Powder Mills	7 Nov 2016	46	0	46
Oatka Creek	24 Oct 2016	22	1	24
Mendon Ponds	7 Nov 2016	32	0	32
Mendon Ponds	7 Nov 2016	20	0	20
Greece Canal	19 Nov 2016	0	0	0
Genesee County	4 Dec 2016	3	0	3
Durand Eastman	4 Dec 2016	16	0	16
Abraham Lincoln	12 Dec 2016	2	0	2
Powder Mills	23 Apr 2017	4	4	9
Horizon Hill	27 Apr 2017	21	0	21
Mendon Ponds	17 May 2017	4	24	29
Genesee County	24 May 2017	0	1	1
Ganondagan	1 June 2017	0	34	36
Letchworth	3 June 2017	2	13	15
Greece Canal	9 June 2017	0	0	0
Northampton	9 June 2017	0	0	0
Lucien Morin	10 June 2017	1	16	17
Webster Park	10 June 2017	1	10	11
Abraham Lincoln	10 June 2017	3	27	30
Ellison	14 June 2017	0	1	1
Tryon	14 June 2017	0	7	7
Oatka Creek	6 July 2017	0	17	17
Durand Eastman	18 July 2017	0	3	3
Irondequoit Bay Park West	18 July 2017	0	1	1
Hamlin Beach	19 July 2017	0	0	0

Table 2. Matrix of significant ($p<0.1$) Spearman's rank correlations between tick density and landscape variables at each of the buffer distances. ρ = Spearman's rho.

Lifestage	Buffer Distance (m)			
	100	**500**	**1000**	**2000**
Adult	**Developed Low** (ρ= -0.375, p=0.054) **Deciduous Forest** (ρ= -0.330, p=0.092)	**Developed Low** (ρ= -0.414, p=0.032) **Cultivated Crops** (ρ= -0.332, p=0.091)	**Developed Low** (ρ= -0.388, p=0.045) **Deciduous Forest** (ρ= 0.333, p=0.090)	**Deciduous Forest** (ρ= 0.358, p=0.067) **Evergreen Forest** (ρ= 0.492, p=0.009) **Woody** (ρ= 0.349, p=0.074) **OriginalPC1_2000m** (ρ= 0.347, p=0.076)
Nymph			**Pasture/Hay** (ρ= -0.383, p=.049)	**Developed Medium** (ρ= -0.366, p=0.060) **Deciduous Forest** (ρ= -0.371, p=0.057) **Evergreen Forest** (ρ= -0.356, p=0.069) **Mixed Forest** (ρ= -0.357, p=0.067) **Pasture/Hay** (ρ= -0.378, p=0.052) **Cultivated Crops** (ρ= -0.398, p=0.040) **Agriculture** (ρ= -0.391, p=0.043) **OriginalPC1_2000m** (ρ= -0.384, p=0.048) **CombinedPC2_2000m** (ρ= -0.364, p=0.062)
Tick Total	**Open Water** (ρ= -0.378, p=.052)		**Deciduous Forest** (ρ= 0.397, p=.040)	

Table 3. Component score values from the principal components analysis of original landscape variables.

	PC1	PC2
Eigenvalue	3.617	3.492
Total variance explained (%)	27.827	26.859
Cumulative variance (%)	27.827	54.686
Variable		
Open water	0.242	0.383
Developed open	-0.110	0.859
Developed low	-0.208	0.836
Developed medium	0.360	0.732
Unconsolidated shore	0.176	-0.209
Deciduous forest	0.887	0.230
Evergreen forest	0.666	-0.235
Mixed forest	0.761	0.076
Pasture/hay	0.744	-0.362
Cultivated crops	0.769	-0.341
Urban/recreational grasses	0.088	0.892
Palustrine forested wetland	0.486	0.264
Palustrine scrub/shrub wetland	0.377	0.461

Table 4. Component score values from the principal components analysis of the combined landscape variables.

	PC1	PC2
Eigenvalue	2.073	1.581
Total variance explained (%)	41.451	31.615
Cumulative variance (%)	41.451	73.066
Variable		
Open water & shore	0.411	0.340
Open grass	0.907	0.025
Developed	0.925	0.093
Agriculture	-0.457	0.794
Woody	0.127	0.908

Table 5. Best models ($\Delta AIC_c < 2$) for tick density at each lifestage. AIC_c is Akaike's Information Criterion corrected for sample size, w_i is the weight of the variables in the model and B is represents the strength and direction of the interaction.

Lifestage	Rank	AICc	ΔAICc	wi	Variables	B
Total Tick	1	290.423	0	0.483144	Longitude	3.55
					Latitude	-2.663
					PC1	0.281
					PC2	-0.566
					JulianDay	0.039
					PC1*PC2	0.324
Total Tick	2	290.563	0.14	0.450481	Longitude	2.966
					PC1	0.264
					PC2	-0.43
					JulianDay	1.161
					PC1*PC2	0.37
					Latitude*JulianDay	-0.026
Adult	1	171.745	0	0.90051	Year	11.894
					Longitude	1.257
					PC1	0.582
					PC1*PC2	0.911
Nymph	1	159.182	0	0.840353	Year	-16.521
					Longitude	7.973
					Latitude	-3.447
					PC1	0.384
					PC2	-1.398

Figure 1. Map of all 17 parks where trail drags took place. IBPW stands for Irondequoit Bay Park West.

Appendices

Three appendices are included. The first is a list of the site coordinates from all of the trail drags in the second chapter of my thesis. The second is the disease testing results from ticks collected on the trail drags. The third includes detailed information on how to create a tick sampling drag and discusses other forms of drags that I tested.

Appendix 1. Coordinates of all trail drag sampling sites.

Park	Year	Latitude	Longitude
Ganondagan State Historic Site	2016	42.964	-77.4179
Abraham Lincoln Park	2016	43.18076	-77.5132
Greece Canal Park	2016	43.1948	-77.7419
Mendon Ponds Park - WW	2016	43.01946	-77.5798
Mendon Ponds Park - DB	2016	43.0245	-77.5773
Powder Mills Park	2016	43.04654	-77.4755
Horizon Hill Conservation Area	2016	43.04874	-77.4571
Oatka Creek Park	2016	43.00417	-77.8026
Durand Eastman Park	2016	43.23175	-77.5609
Genesee County Park	2016	42.87472	-78.1239
Hamlin Beach State Park	2017	43.3592	-77.9407
Irondequoit Bay Park West	2017	43.18394	-77.5293
Oatka Creek Park	2017	43.00988	-77.7968
Durand Eastman Park	2017	43.23242	-77.5739
Northampton Park	2017	43.18771	-77.8823
Greece Canal Park	2017	43.18975	-77.7394
Webster Park	2017	43.25319	-77.4624
Abraham Lincoln Park	2017	43.19024	-77.514
Lucien Morin Park	2017	43.17302	-77.5238
Ellison Park	2017	43.15183	-77.5205
Tryon Park	2017	43.16817	-77.5357
Genesee County Park	2017	42.87689	-78.1214
Powder Mills Park	2017	43.04542	-77.4755
Horizon Hill Conservation Area	2017	43.05169	-77.4616
Mendon Ponds Park	2017	43.01664	-77.579
Letchworth State Park	2017	42.5908	-78.0441
Ganondagan State Historic Site	2017	42.97144	-77.4186

Appendix 2. Infection testing results from blacklegged ticks collected on trail drags

			B. burgdorferi		*A. phagocytophilum*	
Park	Year	Total ticks collected	# Infected	% Infected	# Infected	% Infected
Mendon Ponds Park - 1	2016	32	23	71.9%	2	6.3%
Mendon Ponds Park - 2	2016	20	14	70.0%	0	0.0%
Abraham Lincoln Park	2016	2	0	0.0%	0	0.0%
Ganondagan State Historic Site	2016	11	6	54.5%	0	0.0%
Powder Mills Park	2016	46	26	56.5%	2	4.3%
Durand Eastman Park	2016	16	13	81.3%	0	0.0%
Horizon Hill Conservation Area	2016	37	28	75.7%	0	0.0%
Oatka Creek Park	2016	22	10	45.5%	1	4.5%
2016 Total		**186**	**120**	**64.5%**	**5**	**2.7%**
Powder Mills Park	2017	3	0	0.0%	0	0.0%
Mendon Ponds Park	2017	24	3	12.5%	0	0.0%
Genesee County Park	2017	1	0	0.0%	0	0.0%
Ganondagan State Historic Site	2017	33	13	39.4%	0	0.0%
Letchworth State Park	2017	13	1	7.7%	0	0.0%
Abraham Lincoln Park	2017	31	6	19.4%	1	3.2%
Lucien Morin Park	2017	14	2	14.3%	0	0.0%
Webster Park	2017	13	4	30.8%	0	0.0%
Ellison Park	2017	1	0	0.0%	0	0.0%
Tryon Park	2017	7	1	14.3%	0	0.0%
Oatka Creek Park	2017	18	9	50.0%	0	0.0%
Durand Eastman Park	2017	3	0	0.0%	0	0.0%
Irondequoit Bay Park West	2017	1	0	0.0%	0	0.0%
2017 Total		**162**	**39**	**24.1%**	**1**	**0.6%**

Ticks collected in 2016 were adults and while all ticks in 2017 were nymphs. Two additional adult ticks were collected in 2017 – one female from Lucien Morin Park and one male from Webster Park. Neither were infected with disease. Four ticks from 2016 were co-infected with more than one disease. One tick from Powder Mills Park and two ticks from the first trail drag at Mendon Ponds Park were co-infected

with *B. burgdorferi* and *A. phagocytophilum*. The fourth co-infected tick was also found at Mendon Ponds Park -1 and was co-infected with *B. burgdorferi* and *Borrelia miyamotoi*. This was the only case of *B. miyamotoi* found in any tick in either year. The disease testing also screens for *Babesia microti*, but none of the collected ticks tested positive. Ticks were tested for disease by the New York State Department of Health Bureau of Communicable Disease Control Vector Ecology Laboratory following the methodology in Prusinski *et al*. (2014). Elyse Banker, Alexis Russell, Michael Suatoni, Kaitlin Driesse, and Margaret Mahoney assisted with molecular testing. Funding was provided by New York State Department of Health and the U.S. National Institutes of Health (grant no. AI097137). Elyse Banker, Alexis Russell, Michael Suatoni, Kaitlin Driesse, and Margaret Mahoney assisted with molecular testing.

Appendix 3. Details on making and using a tick drag

In 2016, I created a tick sampling flag by attaching a $1m^2$ canvas drop cloth to a 1.375in x 96in round poplar dowel with 2 hose clamps. I sewed fishing weights to the bottom of the drag to help weigh it down. I had issues with using this to sample for ticks. The fishing weights also weren't heavy enough to sufficiently weigh down the canvas. The canvas material I used was tan with small brown flecks in it which made it difficult to find ticks on the fabric. You could see adult ticks, and you could find nymphs with some difficulty, but larval ticks were almost impossible to see on that fabric. The tick flag worked okay on taller vegetation, but in areas with low vegetation or just leaf litter on the ground, I had to drag while bent over, to make contact with the ground which was very uncomfortable.

In 2017, I switched from a tick flag to a tick drag which is the same concept, except the tick drag was attached to the handle at a 90º angle, so I could sample while standing upright. To make this tick drag, I connected a 1.05 m ¾" PVC base to a 1.45 m ¾" PVC handle with a 90º PVC elbow, so it formed an L shape. I based the handle length on what was comfortable for me to hold, but could be made longer or shorter based on the height of the person sampling. I attached a 1m x 1m piece of white flannel to the PVC base to use as the flag. I switched from tan canvas to white flannel to allow me to see collected ticks better. The finished product for the flag should be 1m x 1m, but I actually used a 2m long piece of fabric folded in half to create a 2-ply $1m^2$ flag so it would be more sturdy. I allowed for extra fabric at each end to be folded over to create roughly a 4 cm space at each end of the flag. The space should

be enough for the PVC base to be able to slide in with ease to attach the drag. The PVC base should stick out of the flag enough to allow for the PVC elbow to be attached. Before putting on the elbow, I secured the flag to the PVC base with a hose clamp. The flag was sewed shut on the other end of the PVC pipe so that the PVC would not slide out the end. At the other end of the flag, I slid a 1m long piece of chain in the flannel fold to weight down the flag. The flap holding the chain should be large enough to easily remove the chain and put it back in, as the chain needs to be removed before being washed. I secured the chain at each end with two safety pins. To prevent the chain from tearing the fabric, I sewed the fold onto the flag several times to reinforce the fabric. I did not attach a chain to the other end with the PVC base, although I think this might have been helpful to keep the flag on the ground more. The other two sides of the flag (without the PVC base or chain) were sewed so there was not an opening in the flag. All cuts I did on the flag were done with pinking shears to prevent as much unraveling as possible of the flannel. The sides were sewed as close to the edge as possible to prevent little flaps from forming (sometimes ticks were in here and it was difficult to check).

When I used the drag, I always made sure to have an extra ¾" PVC 90º elbow socket, as sometimes the stress of the drag caused it to break. I also kept a ¾" PVC 45º elbow socket on hand because it made it easier to drag under shrubs. The ends of both the PVC handle and the PVC base should be cut with an electric saw to ensure they are as straight and smooth as possible to attach to the PVC elbow. Once I used a hand saw to cut the PVC, which left a rough angle to the cut, and made it difficult for

the PVC to stay in the elbow socket. When sampling, I always had extra hose clamps (sometimes they would fall off while sampling), a flathead screwdriver (to tighten the hose clamps), and extra safety pins (to secure the chain if one fell off). After using the trail drag for a while, it would sometimes become too dirty to be able to easily see the ticks. When this happened, I would take the flannel off of the PVC and remove all of the metal components, and then wash it alone in the washing machine using All Free and Clear detergent so there was not a scent to deter ticks.

Ticks were stored in 100% ethanol in 1.5 mL microcentrifuge tubes with caps. To label the tubes, I used post-it note page markers, with the label written in pencil, taped to the tube. Using pen or marker should be avoided as the alcohol that spills out of the tubes will erase the ink. It is a good idea to have a vial of extra alcohol too, as you lose alcohol from the vials from repeatedly putting your tweezers in and out, and leaving the cap open. I stored several of those tubes together in 50 ml Falcon conical centrifuge tubes to keep them together and avoid them getting lost. The Falcon tubes are also good to store the tweezers to avoid being stabbed. Tweezers with the finest point possible should be used to easily remove the ticks from the flag. Ticks should be stored in a cold room at 4º C until they are ready to be analyzed.

Sampling for ticks using the tick drag worked well in most places, but the drag had difficulty making contact with the ground under swallowwort. I tried many different sampling methods to find a solution to this problem. First, I tried to use a weed cutter to remove swallowwort so that I could drag underneath. The swallowwort was too viney and kept getting caught in the weed cutter. It took too much time to

constantly have to stop and remove the swallowwort from the weed cutter, so I decided against that method. Next, I tried to drag over the sampling area with a large wooden board, similar to a makeshift plow, to flatten the vegetation, and then use the tick drag to sample over the flattened vegetation. This method was very cumbersome, time consuming, and still not really effective at making contact with the ground. Melissa Prusinski from the Department of Health mentioned that in vegetation that is difficult to sample, some people use a white diabetic sock filled with sand and attached to a rope which can be thrown into vegetation, pulled out, and inspected for ticks. I tried this but the sock got very dirty and it would have been difficult to find any ticks that were on there. The sock method also did not cover a lot of area, and it was difficult to control where it went. Then I tried using a normal 1 m^2 piece of white flannel weighted at one end with chain and attached at the other end with a rope used to pull the drag along the ground. The flannel part worked fine, but attaching it to a rope like that caused the front part of the drag to pull up off the ground so much that only the part with the chain was making contact with the ground. I then tried using the same setup, but instead I cut the flannel into 10 strips and weighted each strip down with fishing weights, hoping that the strips would be able to make contact with the ground between the swallowwort plants. In the end, the fishing weights weren't heavy enough, the strips kept twisting around which made the surface areas much too small, and it was extremely time consuming to check for ticks. The next tick sampling device I made consisted of $1m^2$ white flannel cut in various strips and attached vertically to a PVC handle, resembling a feather duster. This also took too long to

check for ticks and it was hard to cover a large area. I ended up using the L-shaped PVC piece attached to the weighted $1m^2$ white flannel. This was the best option, and allowed me to pretty easily sample for nymphs, before the swallowwort came up, and adults, when much of the swallowwort died back. Larval ticks were most active during swallowwort's growing season which made it extremely difficult to sample in those plots. Future studies may want to utilize carbon dioxide tick traps in difficult vegetation such as this.