Forest and ecotone habitat impacts on *Ixodes scapularis* and *Amblyomma americanum* populations within New York City and Long Island parks.

Jessie Merrifield

First Reader: Christopher Morrison, (PhD, MPH)

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Abstract

Public parks provide many public health benefits to communities, but they can also be an area where humans are exposed to ticks and tick-borne pathogens. As tick abundance and distribution is highly dependent on the availability of suitable habitat, it is important to understand what features within parks are the greatest determinants of risk. In this study, we collected two medically important tick species, *Ixodes scapularis* and *Amblyomma americanum*, from three different types of habitats within parks across New York City and Long Island. The habitats of interest were forest/open field ecotones (transitional regions), forest/trail ecotones, and interior forests. The life stages of *I. scapularis* and *A. americanum* that were found in the greatest abundance across all parks were nymphal *I. scapularis* and nymphal and adult *A. americanum*. There was not a significant difference in the rates of *I. scapularis* and *A. americanum* nymphs collected between the interior forest habitat and the forest/trail ecotone, while nymphs of both species were collected in significantly lower rates in the forest/open field ecotone. *A. americanum* adults however were collected in statistically lower rates in interior forest habitats but similar rates between forest/trail ecotones and forest/open field ecotones. With the observed relationships between these tick species and ecotones, these habitats should be considered for tick control methods within parks.

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Introduction

Tick-borne diseases are a consistent and growing public health threat within the United States. Facilitated by factors such as climate change, host availability and movement, and anthropogenic land changes, the distributions of medically important tick species and their associated pathogens continue to expand across North America (Eisen et al. 2017; Sonenshine 2018; Urcuqui-Bustamante et al. 2023). Lyme disease is the most common vector-borne disease in the United States, with most cases in the Northeast, following the expanding geographic range of the primary vector, *Ixodes scapularis*, the blacklegged tick (VanAcker et al. 2019.). *I. scapularis* is also the primary vector associated with cases of human granulocytic anaplasmosis and human babesiosis (VanAcker et al. 2019). Another medically important tick with an increasing geographic range and abundance is the lone star tick, *Amblyomma americanum*. An aggressive tick species that has spread from its original range in the southern United States, *A. americanum* has become prevalent in the Northeast where it has sympatric populations with *I. scapularis*. The lone star tick is the vector for pathogens such as the agents of human granulocytic ehrlichiosis, *Ehrlichia chafeensis* and *E. ewingii*, and *Francisella tularensis*, the causative agent of tularemia (Gregory et al. 2022; Harris et al. 2016).

Human risk for tick-borne diseases is impacted by a variety of factors including human behavior, knowledge of preventative measures, and interactions with tick habitat (Hassett et al. 2022). Public green spaces such as parks are important for public health and have many documented benefits for mental health and wellness; however, they also present an area where humans can directly encounter environments that support tick populations, especially within urban areas (Hassett et al. 2022). The localized tick hazard within parks has the capacity to vary greatly across different habitats and associated biotic and abiotic features such as vegetation composition and cover, humidity, and temperature (Gregory et al. 2022). Ecotones are transitional regions between two environments such as where the forest meets maintained grass such as recreational areas or where the forest meets walking paths and trails (González et al. 2023). Within parks, ecotones are important to consider for human risk and environment interactions. It is important to consider how habitat requirements of different tick species could modify acarological risk in parks.

A. americanum of all life stages have been shown to have a higher tolerance to drier and warmer conditions compared to *I. scapularis* (Schulze and Jordan 2005). With greater sensitivity to desiccation, *I. scapularis* density and abundance has been positively associated with leaf litter depth, deciduous forests compared to coniferous forests, and overall forested areas versus grassland or open canopy areas. Across the range of *A. americanum*, there is not a consensus within the literature regarding the associations between tick abundance and habitat. Studies have found associations between open-canopy and low-density vegetation habitats with higher *A. americanum* populations, the opposite association, as well as no association (Mathisson et al. 2021). The questing behavior also differs between *I. scapularis* and *A. americanum*. While *A. americanum* has been documented to actively "hunt" or search for host signals such as carbon dioxide or vibrations, *I. scapularis* relies on an ambush host-seeking strategy in which the tick climbs nearby vegetation and passively waits for a passing host (Mathisson et al. 2021; Parola and Raoult 2001; Sonenshine 2018).

Tick behavior and habitat preferences have also been noted to differ between regions. In southern populations, *I. scapularis* nymphs do not quest as high on vegetation, staying lower within the duff (high humidity region of soil and leaf litter) (Tietjen et al., 2020). *A. americanum* habitat preferences have shown variation across geographic regions, and the abundance of this species in the northeastern U.S has increased significantly in recent years (Mathisson et al. 2021; Sonenshine 2018). It is important to have risk assessments in various regions to understand the best practices for implementing local public health interventions. In the Northeast, previous research has predominantly focused on tick assessments in forest habitats and ecotones separately (González et al., 2023; Hassett et al., 2022). In this study, we aim to understand how the rates of questing *I. scapularis* and *A. americanum* change across three specific park habitats: interior forest, forest trails, and edge environments at the ecotone of forest and meadow or open grassland to better understand risk within public parks and inform public health interventions. We theorize that *A. americanum* as a "hunter" tick with greater movement capacity could travel towards forest trails and areas of higher host usage while *I. scapularis* as an ambush tick will remain in protective environments with higher humidity and shade such as the interior forest areas.

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Commented [MCN5]: Final paragraph of the Introduction should explain the study aims and hypotheses, and perhaps briefly mention strengths of this study and how it will advance the field, compared to the prior studies described above. The main argument here is around spatial variation within and between locations — this should be made very clear and strongly justified.

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Methods

Site selection:

Ninety-five public parks were selected from across Staten Island, Queens, Brooklyn, and Long Island in New York (Figure 1.a). Parks were selected based on having at least two of three habitats: forest, recreational trail or path within forest, or open field with maintained grass or meadow with forest ecotone. Tick collection took place in the summer of 2023. Each park was visited once during the 1 June to 23 June interval (labeled drag 1) and revisited once during the 21 June to 18 July interval (labeled drag 2). The goal for each park was to sample 800 m per sampling iteration with a total of 1600 meters between drag 1 and drag 2.

Transect protocol:

The treatment in this study was the habitat within the park that was sampled for tick collections (interior, edge, and trail) (Figure 1b). Interior was determined as within woods at a minimum of 10 m inward from any trail or edge to provide a barrier from other sampling regions. Trails were walking and biking paths through the woods. Edge was defined as the ecotone region or border between the woods and open field areas such as meadows or maintained grass. The total 800 m per sampling iteration at each park was broken into 100-m sections split amongst the three transect types by the availability and quantity of environments at each park.

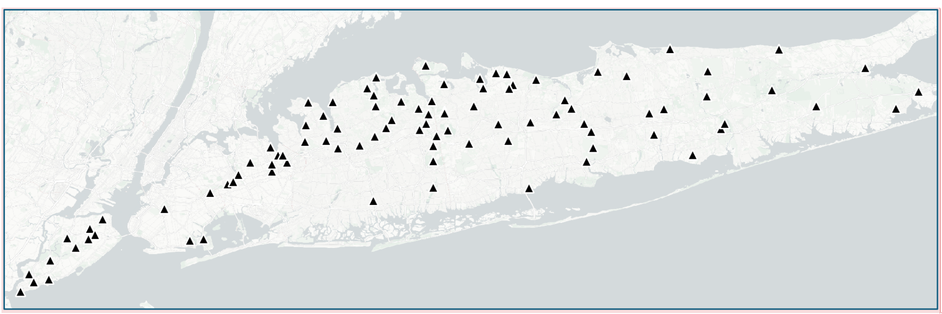
Tick collection protocol:

Questing ticks were collected with a 1 m^2 white corduroy drag cloth. The drags were checked every 10 m within each 100 m transect. A 10 m buffer was used in between transects to reduce overlap and resampling. Any attached ticks were removed from the cloth and placed into a vial of 80% ethanol to be identified in the lab with a dissecting microscope and taxonomic keys, and the field identifications of tick species, life stage, and counts were recorded for each transect (Keirans J. E. and Litwak T. R. 1989). Field identifications relied on site identification, which is a less reliable method of identification as it is based on fewer physiological features than are visible with a dissecting microscope.

For edge habitat, drags were done along the connection point of maintained or mowed lawn and forest unmaintained herbaceous layer. Trails were sampled by dragging into the vegetation along the sides of the trail. Interior forest transects were sampled by dragging into any vegetation and attempting to keep the drag where it could access leaf litter and lower levels of vegetation.

Figure 1: Methods overview

a: Park site locations



b: Transect protocol

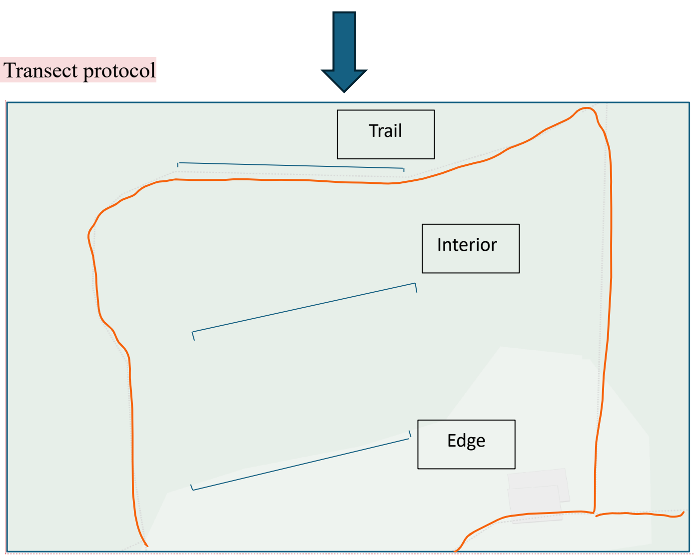


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Figure 1a shows the locations of the park sampling sites (black triangles) across New York City boroughs and Long Island.
Figure 1b describes the dragging structure of treatment groups (trail, interior, edge) within each park site.

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Data analysis:

All statistical analyses were conducted with R Version 4.3.1. As the laboratory tick identifications were not available for this thesis, the field identifications were used to approximate the laboratory results. Tick counts by treatment for *I. scapularis* nymphs, *A. americanum* adults, and *A. americanum* nymphs were modeled with three separate mixed effect negative binomial regression, using the function glmer.nb() from the package lme4 (v. 1.1-35.1). For each model, treatment (habitat transect type) and sampling iteration (drag 1 and 2) were fixed effects, site was a random effect, and the offset was the number of meters by park site and transect habitat type.

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Results

Tick collection

Nymphs were the predominant life stage collected for both *A. americanum* and *I. scapularis*. A total of 12,056 *A. americanum* were collected with 369 larvae, 9,933 nymphs, and 1,754 adults. We collected 8,609 *I. scapularis*, of which 197 were larvae, 8,349 were nymphs, and 63 were adults. Only the *A. americanum* nymphal and adult life stages and the *I. scapularis* nymphal life stage were considered for further analysis.

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Per sampling unit of sampling iteration (drag 1 and drag 2), site (park), and transect type (trail, interior, edge), the count data separately for *I. scapularis* nymphs, *A. americanum* adults, and *A. americanum* nymphs was skewed by a high frequency of zeros (Figure 2). *I. scapularis* nymphs were collected with a range of 0-515 ticks or 0-172 ticks/100 m sampled per sampling unit. *A. americanum* adults ranged from 0-173 ticks or 0-43 ticks/100 m sampled per sampling unit. The nymphal counts for *A. americanum* ranged from 0-465 ticks or 0-155 ticks/100 m sampled per sampling unit.

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From 127,830 meters sampled overall, there was large variation in the number of meters sampled between the treatment environments with 41% from trail, 39% from interior, and 19% from edge (Table 1). The total number of ticks collected was greater from drag 1 compared to drag 2 for all considered life stages of both tick species. For both sampling iterations, the percentage of ticks collected from edge transects comprised the smallest portion of ticks collected by species and life stage. The greatest percentage of ticks collected for *A. americanum* adults and nymphs came from trail transects for both drag 1 and drag 2. However, *I. scapularis* nymphs were collected in the greatest proportion from interior transects during drag 1 but then from trail transects during drag 2 (Table 1).

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Figure 2: Frequency of tick counts per transect and park for *I. scapularis* nymphs and *A. americanum* adults and nymphs.

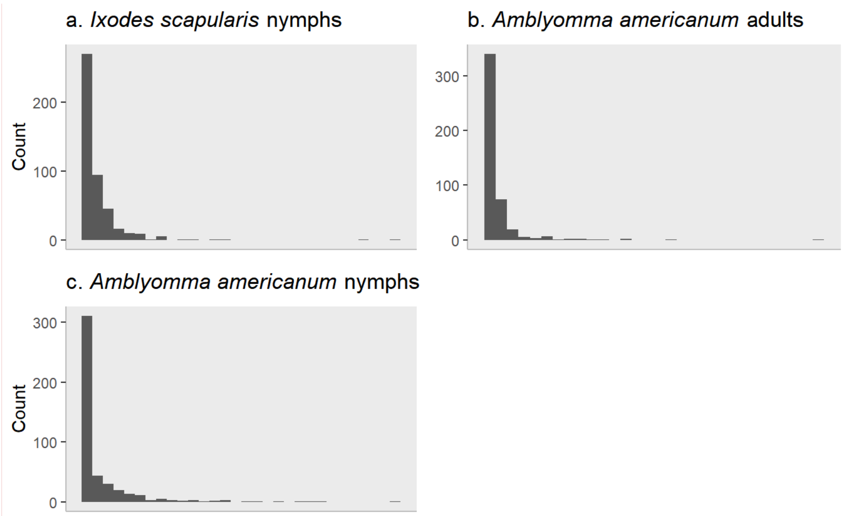

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Table 1: Descriptive statistics for *Ixodes scapularis* nymphs and *Amblyomma americanum* nymphs and adults by transect habitat type and sampling iteration (drag 1 and drag 2).

		Treatment: Transect Type			
Drag 1					
Species	**Life stage**	**Trail**	**Interior**	**Edge**	Totals:
Ixodes scapularis	nymph	2300 (45.545%)	2445 (48.416%)	305 (6.040%)	5050
Amblyomma americanum	adult	657 (55.443%)	350 (29.536%)	178 (15.021%)	1185
Amblyomma americanum	nymph	2923 (48.218%)	2377 (39.211%)	762 (12.570%)	6062
Drag 2					
Ixodes scapularis	nymph	1586 (48.075%)	1436 (43.528%)	277 (8.396%)	3299
Amblyomma americanum	adult	315 (55.360%)	136 (23.902%)	118 (20.738%)	569
Amblyomma americanum	nymph	1701 (43.942%)	1643 (42.444)	527 (13.614%)	3871

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Meters:	52860 (41.352%)	50060 (39.161%)	24910 (19.487%)	127830

Relationship between tick counts and habitat:

The three final regression models for the counts of each tick species and life stage were mixed-effects negative binomial models with treatment environment and sampling iteration as predictors (Tables 2-4). Site was included as a random effect and the difference in meters sampled by treatment was corrected by adding the log transformed meters per sampling unit by sampling iteration, site, and treatment as an offset.

The rate of *I. scapularis* nymphs collected was lower for the second sampling iteration with an incidence rate ratio of 0.714 (CI 95%: 0.593-0.860). There was not a significant difference between the rate of I. scapularis nymphs collected between the interior woods and trail transect types, but *I. scapularis* nymphs were collected at a 65% (CI 95%: 55%-73%) lower rate in edge transects compared to trail (Table 2).

A. americanum adult ticks were collected at a 24% (CI 95%: 2%-41%) lower rate in the second sampling iteration. The rate of adult *A. americanum* ticks collected did not significantly differ between edge and trail transects. However, there were 43% (CI 95%: 25%-57%) fewer *A. americanum* adults collected from interior compared to trail (Table 3).

For *A. americanum* nymphs, there was not a statistically significant difference between the rate of ticks collected between the first and second sampling. Similar to *I. scapularis* nymphs, there was a lower rate of *A. americanum* nymphs collected from edge compared to trail with an incidence rate ratio of 0.657 (CI 95%: 0.490-0.882), but there was not a significant difference between the rate of ticks collected from interior and trail transects (Table 4).

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Table 2: Incidence rate ratios for *Ixodes scapularis* nymphs by treatment (trail, interior, edge). Mixed-effects negative binomial regression model with site as a random effect and log transformed sampling distance as an offset.

Table 2: Model 1: *Ixodes scapularis* nymphs

	IRR	95% CI
Intercept	0.022	(0.014, 0.035)
Treatment		
Edge vs. Trail	0.349	(0.270, 0.451)
Interior vs. Trail	1.006	(0.824, 1.228)
Drag 2	0.714	(0.593, 0.860)

Note:
Random Effect: Site. Offset: log(meters)

Table 3: Incidence rate ratios for *Amblyomma americanum* adults by treatment (trail, interior, edge). Mixed effect negative binomial regression model with site as a random effect and log transformed sampling distance as an offset.

Table 3: Model 2: *Amblyomma americanum* adults

	IRR	95% CI
Intercept	0.001	(0.001, 0.003)
Treatment		
Edge vs. Trail	0.727	(0.524, 1.009)
Interior vs. Trail	0.57	(0.429, 0.755)
Drag 2	0.76	(0.589, 0.980)

Note:
Random Effect: Site. Offset: log(meters)

Table 4: Incidence rate ratios for *Amblyomma americanum* nymphs by treatment (trail, interior, edge). Mixed effect negative binomial regression model with site as a random effect and log transformed sampling distance as an offset.

Table 4: Model 3: *Amblyomma americanum* nymphs

	IRR	95% CI
Intercept	0.003	(0.001, 0.007)
Treatment		
Edge vs. Trail	0.657	(0.490, 0.882)
Interior vs. Trail	1.045	(0.821, 1.330)
Drag 2	0.931	(0.743, 1.166)

Note:
Random Effect: Site. Offset: log(meters)

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Discussion

Based on *I. scapularis* questing behavior, there was expected to be a greater rate of *I. scapularis* nymphs collected from the interior forest environments compared to trails or edges. While there was not a statistical difference between the rate of *I. scapularis* nymphs collected from the interior forest and trails, there was a difference between the rate collected from trails and edge with a higher rate of ticks collected from trails. With low tolerance to desiccation and previously noted habitat preferences such as high leaf litter, it is interesting that *I. scapularis* nymphs were found in equal rates between the interior and trail environments (Mathisson et al. 2021). Alternatively, *A. americanum* is described with more aggressive questing behavior and generalist habitat preferences; we hypothesized that this tick species would have higher prevalence around trails due to higher possible host usage. The results for *A. americanum* nymphs were similar to *I. scapularis* nymphs in that there was not a significant difference in the rate of ticks collected between the interior forest and trail but there was a greater rate for trail compared to edge. Interestingly, *A. americanum* adults had the opposite results and there was not a significant difference in the rate of ticks collected from trail and edge but there was a significant difference in the rate between ticks collected from the interior forest and trail with a greater rate from trail.

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In previous work, *A. americanum* nymphs and males were found at greater rates in shade more than sunlit areas while *A. americanum* females were found at a greater rate in sunlight. It was hypothesized that the smaller nymphal and male *A. americanum* may be more susceptible to desiccation compared to the larger female *A. americanum* (Koch and Burg 2006). In this study, we did not identify adult ticks to sex in field identification. However, it could be considered that while the edge transect habitat did not support larger populations of *A. americanum* nymphs, the larger adult ticks overall were able to survive in both ecotone regions. It is not clear why *A. americanum* adults were found in lower rates in the interior forest transects. This result could reflect biological systems present across the habitats sampled, but sampling error should also be considered. During tick collection in interior forest transects, taller and denser vegetation could have impacted whether ticks were able to remain attached to the drag cloth without being brushed off.

In the interpretation of these results, it should also be noted that this data uses site identification from field notes for tick species and life stage rather than the preferred method of laboratory identification with a dissecting microscope and taxonomic keys (Keirans J. E. and Litwak T. R. 1989). In future exploration, the laboratory data for tick identification will be used to verify the results from the field tick sight identification. Following laboratory identification to species, life stage, and sex, the data will be more accurate and further analyses can be conducted. For example, determining the percentage of females among the adult *A. americanum* ticks could provide greater insight into the rates of *A. americanum* adults found in the edges.

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From the results, the risk of encountering a questing *I. scapularis* or *A. americanum* nymphs does not appear to be mitigated by remaining on trails and paths rather than interior woods. Although nymphs of these two species were found in much lower rates in the ecotone of open fields and forest, this was not the case for adult *A. americanum* ticks. Ecotones should be considered as an area of possible risk of exposure to *A. americanum* adults. Parks and public greenspaces provide essential public health benefits to communities, but it is important to understand how park environments can impact tick hazard. With a greater understanding of the predictive features of tick abundance within parks, the public can be educated on the areas of greatest hazard and how best to use park areas to prevent tick encounters.

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Acknowledgements

The author would like to thank Dr. Maria Diuk-Wasser and the Eco-Epidemiology lab at Columbia University and Dr. Christopher Morrison, Columbia University Mailman School of Public Health Department of Epidemiology, for their support and guidance through the research and manuscript development process.

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