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**PATTERNS AND MECHANISMS OF TICK-BORNE DISEASE EXPOSURE RISK IN  
ACADIA NATIONAL PARK**

By

Sara McBride

B.S. Indiana University of Pennsylvania, 2015

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

in Ecology and Environmental Sciences

The Graduate School

The University of Maine

May 2020

Advisory Committee:

Allison Gardner, Assistant Professor of Arthropod Vector Biology, Advisor

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**PATTERNS AND MECHANISMS OF TICK-BORNE DISEASE EXPOSURE RISK IN  
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By Sara McBride

Thesis Advisor: Dr. Allison Gardner

An Abstract of the Thesis Presented  
in Partial Fulfillment of the Requirements for the  
Degree of Master of Science  
in Ecology and Environmental Sciences  
May 2020

National park areas represent unique disease transmission settings compared to other private and public lands, limiting our capacity to understand tick-borne disease exposure risk using traditional approaches. These challenges may be overcome by using ecological metrics to develop spatial models of disease vector density and to identify areas of highest vector exposure risk within a park. The blacklegged tick and the infectious diseases of humans and wildlife that it transmits, including Lyme disease, anaplasmosis and babesiosis, have been expanding in the eastern United States within recent decades. Acadia National Park (ANP) located on Mount Desert Island, ME is highly visited, with more than 3 million visitors per year, and largely has not been assessed for tick-borne disease exposure risk. To determine the broad scale patterns of blacklegged tick densities in ANP, field surveys of blacklegged ticks were conducted at a total of 114 sites across the park between the months of June and August during two consecutive years. Using field-observed nymphal tick densities and geospatial landscape feature data (i.e., land cover, elevation, forest fragmentation, aspect, and UTM coordinates) a random forest model was created to model nymphal tick density across Mount Desert Island. We found that nymphal tick densities vary significantly across the island and are particularly high in areas characterized by

deciduous forest cover, relatively low elevations and northerly and easterly locations. To investigate the causal mechanisms driving spatial patterns of tick density, a subset of 19 sites were assessed for microclimate conditions (relative humidity and temperature), host activity (small mammal trapping and deer scat surveys), and vegetation metrics (percent canopy cover and leaf litter depth). Multivariate analysis of variance indicated that there are significant differences in microclimate conditions across landscape features but not among host activity or vegetation metrics. Generalized linear models indicated that mean temperature and mean humidity are significantly correlated to nymphal densities and therefore may provide a mechanistic link between landscape features and blacklegged tick densities. This project emphasizes the importance of using ecological metrics to estimate risk of exposure to vector-borne diseases, provides new insight into habitat characteristics that may drive tick-borne disease exposure risk across spatial scales, and demonstrates the design and effort required to operationalize similar vector-borne disease risk assessment protocols in other National Parks.

## **DEDICATION**

This thesis is dedicated to my family: My parents, Daniel and Janine and my siblings Dan, Cassie and Angie for their endless support and encouragement.

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# **PATTERNS AND MECHANISMS OF TICK-BORNE DISEASE EXPOSURE RISK IN ACADIA NATIONAL PARK**

## **1.1 Introduction**

There are 419 National Park System areas in the United States, including National Parks, Preserves, Recreation Areas, Historic Sites, and Monuments (hereafter referred to collectively as ‘national parks’), together comprising more than 85 million acres of land (National Park Service [NPS] 2020). The recreational and economic benefits of these protected areas are significant: each year, 327 million visitors and over 600,000 National Park Service volunteers and employees spend time in national parks across the country. Over the past five years, visitor spending in national parks has increased by almost 40%, and in 2018, visitors supported \$40.1 billion in economic output in the form of lodging, transportation, recreation, retail, and food (NPS 2019). In addition, national parks are regions of great epidemiological significance. While hiking and camping, visitors may place themselves at risk of exposure to vector-borne diseases (i.e., pathogens transmitted via the bite of an infected arthropod), including mosquito-borne diseases such as West Nile Virus, tick-borne diseases such as Lyme disease (Eisen et al. 2013), and flea-borne diseases such as plague (Danforth et al. 2016). These are among the top zoonotic diseases of concern to the U.S. Centers for Disease Control and Prevention (CDC 2019). Numerous other vector-borne diseases also are on the rise, including tick-borne anaplasmosis, babesiosis, ehrlichiosis and Powassan virus, and these pathogens similarly are expected to pose a growing health threat to national park visitors in the coming decades.

While vector-borne diseases are widespread throughout North America (CDC 2019), national parks represent unique disease transmission settings compared to other private and

public lands in several aspects, limiting our capacity to understand and manage disease exposure risk using traditional approaches. First, because national park lands are preserved and protected under several federal laws (NPS 2014), they have areas of relatively undisturbed land, resulting in ecological differences from neighboring areas outside of park lands. Greater proportions of late-successional forest, larger tree basal area and higher densities of both live and dead trees have been found in park forests compared to surrounding lands (Miller et al. 2016), and this may have consequences for transmission dynamics because undisturbed land can alter habitat quality for key hosts for vector-borne pathogens and habitat conditions for vectors. Second, because national parks across the country have geographically diverse visitor profiles and human cases of vector-borne disease typically are reported based on state of residence rather than presumptive location of exposure, traditional methods of human case surveillance to assess risk of exposure to vector-borne diseases (CDC 2019) prove unreliable in these areas. Human behavior related to and knowledge of vector-borne diseases also may be significantly different for park visitors than residents in surrounding areas (De Urioste-Stone, personal communication). Finally, because National Parks practice integrated pest management, which aims to minimize the environmental impact of pest control interventions, many typical management strategies for vector-borne disease (e.g., insecticide use for mosquito control or deer culls for tick control) cannot be easily applied (NPS 2019). Thus, there is a critical need to use alternative strategies to assess and inhibit risk of exposure to vector-borne diseases in national parks.

A recent review paper of vector-borne diseases in national parks suggests that an appropriate and feasible way to overcome these challenges is to assess vector-borne disease exposure risk using ecological metrics (Eisen et al. 2013). In particular, spatial models of disease vector occurrence or density can be developed by combining vector surveys with ecological

habitat feature data (e.g. microclimate, land cover, elevation) across spatial scales. Acadia National Park (ANP; Mount Desert Island, Maine) provides a representative example of a highly-visited park with potential risk of vector-borne disease transmission; here, the most important vector-borne diseases of concern are pathogens transmitted by the blacklegged tick, *Ixodes scapularis*, including *Borrelia burgdorferi* (the causative agent of Lyme disease), *Anaplasma phagocytophilum* (human granulocytic anaplasmosis) and *Babesia microti* (human babesiosis). Over the past 20 years, incidence of all three diseases reported to the Maine CDC has increased (CDC 2019) and an expansion in blacklegged tick distribution has been documented via passive surveillance through public tick submissions (EPHT 2018). It is likely that ANP has experienced a similar rise in density of blacklegged ticks and the pathogens it transmits within recent decades, yet the most recent large-scale tick-borne disease assessment of the park was published almost 30 years ago (Connery et al. 1992).

ANP has great epidemiological and economic significance to the state of Maine. In 2019 it was ranked the seventh most visited national park in the United States, with 3.4 million annual visitors, and generated \$284 million in tourism spending to the region (National Park Service [NPS] 2020). Most visitors arrive between the months of June and October (~488,000 - 767,000 visitors per month; National Park Service [NPS] 2015), coinciding with periods of high blacklegged tick activity, and thus place themselves at high risk of exposure to tick-borne pathogens. In a recent survey about the future consequences of climate change in ANP, visitors reported that they are concerned about increased risk of exposure to tick-borne disease (De Urioste-Stone et al. 2016). Therefore, there is a critical need to develop spatial models of tick densities in ANP, assess tick pathogen infection prevalence in the park, and understand the

causal ecological mechanisms underlying observed patterns to inform public health messaging and management efforts.

Tick-borne diseases are embedded within forest wildlife communities due to ticks' complex, two-year multi-host life cycles (CDC 2011). Blacklegged ticks generally acquire larval and nymphal blood meals from small mammals including several efficient hosts for disease causing pathogens such as the white-footed mouse (*Peromyscus leucopus*) and Eastern chipmunk (*Tamias striatus*) (LoGiudice et al. 2003, Hersh et al. 2012, Keesing et al. 2012). In the adult stage, ticks typically attach to large mammals such as white-tailed deer (*Odocoileus virginianus*), which serve as important reproductive hosts for ticks (Levi et al. 2012). Much of tick-borne disease ecology research has focused on the on-host part of the tick life cycle due to the hosts' importance to pathogen transmission, yet ticks spend more than 95% of their life cycles off-host on the ground, where environmental conditions (e.g., microclimate and leaf litter depth) regulate tick longevity and questing behavior (Lubelczyk et al 2004; Stafford 1994; Ogden et al. 2004; Vail and Smith 2002; Berger et al. 2014). Thus, a range of biotic and abiotic mechanisms may contribute to the distribution of blacklegged ticks and the pathogens they transmit in the landscape.

Large-scale landscape features such as land cover type, elevation, landscape fragmentation, slope and aspect may dramatically alter habitat conditions for off-host blacklegged ticks. Type of forest stand (i.e., coniferous, deciduous, or mixed) has been strongly correlated to tick presence and density. Specifically, studies have found a positive relationship between tick density and deciduous forest (Guerra et al. 2002, ECDC 2014, Madder et al. 2012). Elevation, slope (i.e., degree of change in elevation) and aspect (i.e., direction of slope) have also been hypothesized as important factors of habitat suitability for blacklegged ticks but remain

understudied (Eisen et al. 2016, Medlock et al. 2008). Fragmentation of forests has also been associated as an important factor to tick habitat suitability; a study in Hudson Valley, New York determined that tick density and pathogen prevalence increased as forest patch size decreased (Allan et al. 2003).

While associations with landscape features can help explain broad patterns of blacklegged tick abundance, understanding variation in fine-scale habitat features (e.g., microclimate, host activity, canopy cover, leaf litter depth) that directly influence off-host tick survival and tick-host encounter frequency across the landscape is critical to informing tick-borne disease risk management strategies. One hypothesized mechanism to explain variation of tick abundance across landscape features is differences in microclimate conditions (i.e., temperature and humidity). Due to their large surface to volume ratio, microclimate conditions can influence tick survival and development as well as distance of movement and questing behaviors (Stafford 1994; Ogden et al. 2004; Vail and Smith 2002; Berger et al. 2014). Tick density may also be affected by fine-scale vegetation characteristics that vary across landscape feature classes. For instance, a study of habitat associations of blacklegged ticks in Maine found that the probability of tick abundance was greater in the presence of a shrub layer, >50% canopy cover, deciduous leaf litter, forest grasses and moist soil ferns (Lubelczyk et al. 2004). Richness and abundance of herbaceous plants in forest understory is higher in forests with deciduous canopy while mosses and lichens are more diverse and abundant in coniferous canopies (Fourrier et al. 2015). Because both tick abundance and deciduous forest types have been positively correlated to shrub cover, canopy cover and deciduous leaf litter, these factors may best explain why ticks have been found in higher abundances in deciduous over coniferous forests.

Another proposed mechanism of variation in tick densities across the landscape is that landscape features may influence habitat suitability for important tick host species. Differences in forest type can influence the presence of important blacklegged ticks host species. Basal area of deciduous forests is positively associated with occupancy of white-footed mice, a common host for immature ticks in the northeast (Stephens and Anderson 2014). In addition, activity of white-tailed deer is positively associated with the percentage of deciduous shrubs and negatively with the density of coniferous shrubs (Massé and Côté 2009). Finally, the activity, abundance and diversity of available hosts may be mechanistic drivers of tick pathogen infection prevalence (LoGiudice et al. 2003; Ostfeld and Keesing 2012).

The main objectives of this project were 1) to develop a predictive spatial model of blacklegged tick densities in ANP, integrating field-collected tick density data and geospatial landscape features data (i.e., land cover type, elevation, aspect and habitat fragmentation level), and 2) to determine the causal ecological mechanisms underlying these observed tick density patterns. In the first phase of our analysis, we developed a random forest spatial model to identify the landscape features most strongly correlated to tick densities across ANP. In the second phase, we characterized potential mechanistic drivers of tick densities (i.e., temperature, humidity, small mammal capture rates, deer scat counts, percent canopy cover, ground cover and leaf litter depth) across broad-scale landscape feature classes. Finally, in the third phase, we tested the hypothesis that these habitat characteristics are correlated to tick densities at a fine spatial scale. We also present new descriptive data concerning the seasonality and pathogen infection prevalence of blacklegged ticks in ANP. Together, these findings can be used by the National Park Service to inform protective measures and management strategies within the park.



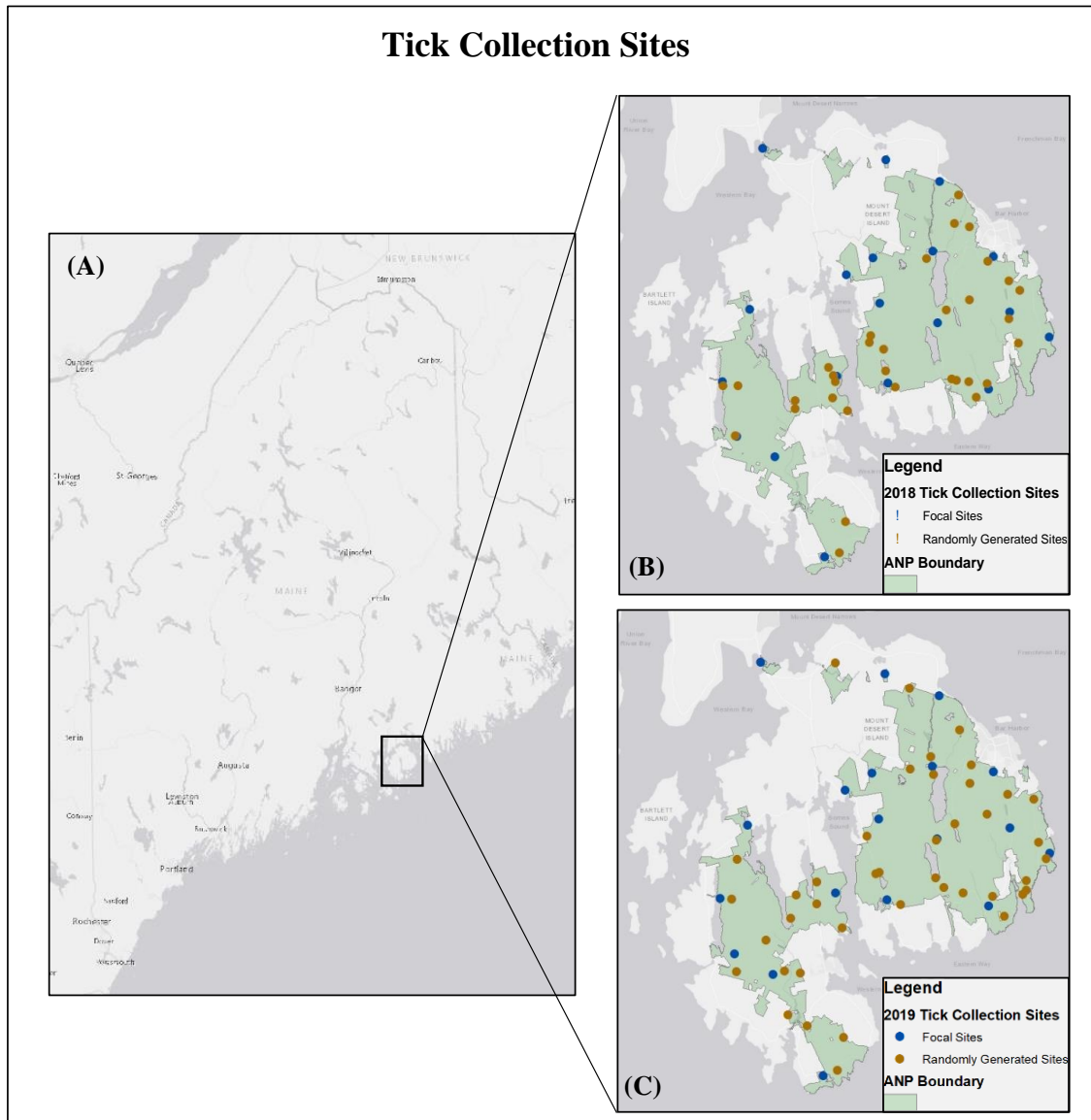
## 1.2 Methods

### 1.2.1 Study Site Selection

All study sites were located within the ANP boundary, encompassing over 47,000 acres of land. To test the hypothesis that blacklegged tick densities vary with broad-scale landscape features (i.e., land cover type, elevation, aspect, slope, fragmentation of land by roads), we visited 53 sites in 2018 and 61 sites in 2019 (Figure 1). Nineteen of these sites were visited during both years of the study (see below); the remainder (i.e., 34 sites in 2018 and 42 in 2019) were randomly selected using the “create random points” tool in ArcMap 10.7 (ESRI, Redlands, CA). For ease of access, in 2018, these randomly sites were constrained within 500 m of a road or trail. In 2019, to better represent areas used by visitors, sites were constrained within 500 m of a road and within 200 m of a trail, including some sites directly on trails (De Urioste-Stone pers. comm.). If this randomization procedure failed to provide adequate coverage of certain areas of the park, additional sites were selected in underrepresented areas. This site selection procedure ensured sufficient spatial representation of the entire park, including trails where visitors often spend time.

To identify mechanisms that explain variation in tick density across landscape feature classes and to assess changes in tick density over time, 19 sites (hereafter referred to as “focal sites”) were selected non-randomly and visited multiple times during each year of the study. Focal sites were the same as those surveyed during the most recent large-scale tick-borne disease study in the park almost 30 years ago (Connery et al. 1992), allowing for direct comparisons of tick densities over time. Because these sites were originally identified in 1990, in some locations, the landscape had changed, making them unsuitable for tick collections today. For example, flooding caused unsuitable conditions because tick collection methods require dry conditions

while areas infested by the European fire ant (*Myrmica rubra*) were not suitable for the small mammal trapping because the ants can cause injury to rodents while in traps. If a site from the 1992 study was unsuitable, we moved in a randomly selected direction until we found suitable conditions. New locations were within 200 m of the original coordinates, except one instance in which a site had to be moved more than 300 m away.



**Figure 1.** A map of the state of Maine (A) and inset maps of Acadia National Park and sites surveyed for ticks in (A) 2018 (C) 2019

### **1.2.2 Off-host Tick Collections**

To determine observed patterns of nymphal blacklegged tick densities across the park, surveying for off-host blacklegged ticks was conducted at one time point at all sites between 13 June and 19 August 2018 and between 16 June and 16 August 2019, coinciding with the peak activity period of nymphal blacklegged ticks in Maine. To investigate nymphal blacklegged tick seasonality, the 19 focal sites were visited multiple times: two times in 2018 (13-29 June and 14-19 August) and four times in 2019 (16-19 June, 15-23 July, 31 July to 7 August, and 12-16 August). Tick collections were conducted using drag sampling, a standard collection method wherein a 1m<sup>2</sup> corduroy cloth is pulled across vegetation or leaf litter and inspected every 10 m for ticks (Daniels et al. 2000). Although nymphal blacklegged ticks were the focus of this study, all life stages and other tick species were collected and identified to species using morphological keys (Keirans and Clifford 1978; Keirans and Litwak 1989; Durden and Keirans 1996). Ticks were preserved in vials of 70% ethanol in a -20°C freezer for subsequent species identification and pathogen testing.

To reduce bias, all sites were sampled when environmental conditions were favorable (i.e., no ongoing rain or soaked ground vegetation). At each site, we dragged 500 m for ticks. At most sites, sampling was conducted along four 125 m transects radiating from a center point, but if obstacles (e.g., roads, steep rock, swamp) precluded this design, additional shorter transects were selected, again radiating from the center point for a total of 500 m dragged per site.

### **1.2.3 Fine-scale Habitat Characterization**

To investigate the potential causal mechanisms of tick abundance and pathogen infection prevalence, additional fine-scale measurements were conducted at the 19 focal sites, including

small mammal trapping, deer scat surveys, pathogen testing of small mammals and ticks, microclimate measurements and vegetation surveys.

To test the hypothesis that tick abundance is correlated with small mammal activity, small mammals were live-trapped along transects during the peak activity period of larval blacklegged ticks between 1 August and 30 September 2018 (IACUC protocol numbers: NPS, NER\_ACAD\_Gardner\_SmMammal\_2019.A2 ; UMaine, A2018\_11\_07). In brief, H.B. Sherman traps (H.B. Sherman Traps, Inc., catalog #LFA, Tallahassee, FL) were baited with black oil sunflower seeds and rolled oats. At each site, 25 trap stations (2 traps per station) were spaced 10 m apart (under cover or near logs that small mammals may use for travel and foraging) along two 125 m transects (for a total of 50 traps per site). Upon capture, each mammal was removed from the trap and placed into a plastic bag for processing. Each animal received a unique numbered metal ear-tag for identification (National Band Company, catalog #1005-1, Newport, KY) and was identified to species, except in the case of *Peromyscus* mice (*P. leucopus*, *P. maniculatus*), which were identified to genus due to ambiguous diagnostic morphological features (Reid 2006). To assess pathogen prevalence in small mammal reservoir hosts, a 2 mm tissue sample was taken from the ear using a metal hole punch (Kent Scientific Corporation, catalog #INS750078-10, Torrington, CT).

To test the hypothesis that tick density is correlated with percent relative humidity and temperature, both were measured at focal sites using ibutton data loggers (DS1922L-F5 ThermoChron, Maxim Integrated, San Jose, CA). In 2018, temperature and relative humidity were measured once per hour between 13 June and 1 November. Means of weekly temperature, weekly minimum temperature, weekly maximum temperature, weekly humidity, weekly minimum humidity, and weekly maximum humidity were calculated for tick collection periods

(13-29 June and 14-19 August). To protect the ibutton loggers from exposure to rain and direct sunlight and to accurately reflect air measurements, each logger was attached to the inside of a plastic cup on a stake. One ibutton was placed near the center point at each site ensuring it was as representative of entire site as possible. For instance, if a site was mostly coniferous forest, the logger was placed in coniferous habitat, not in a small open area or deciduous patch within the site. If the ground cover was mostly moss, it was placed in a mossy area.

To test the hypothesis that nymphal tick abundance is correlated with canopy and ground vegetation cover, vegetation surveys were conducted at each site using multiple standard methods (Lubelczyk et al 2004). Vegetation measurements included canopy composition (i.e., deciduous, coniferous, mixed) assessment and percent canopy cover measurements using a PVC pipe densitometer. Percent ground cover (i.e., classified as deciduous leaf litter, coniferous leaf litter, vascular plants, moss/lichen, woody debris, bare ground/rock) was measured using a 1 m<sup>2</sup> quadrat and leaf litter depth was measured using a ruler. All measurements were taken at five equidistant points on a 125 m transect.

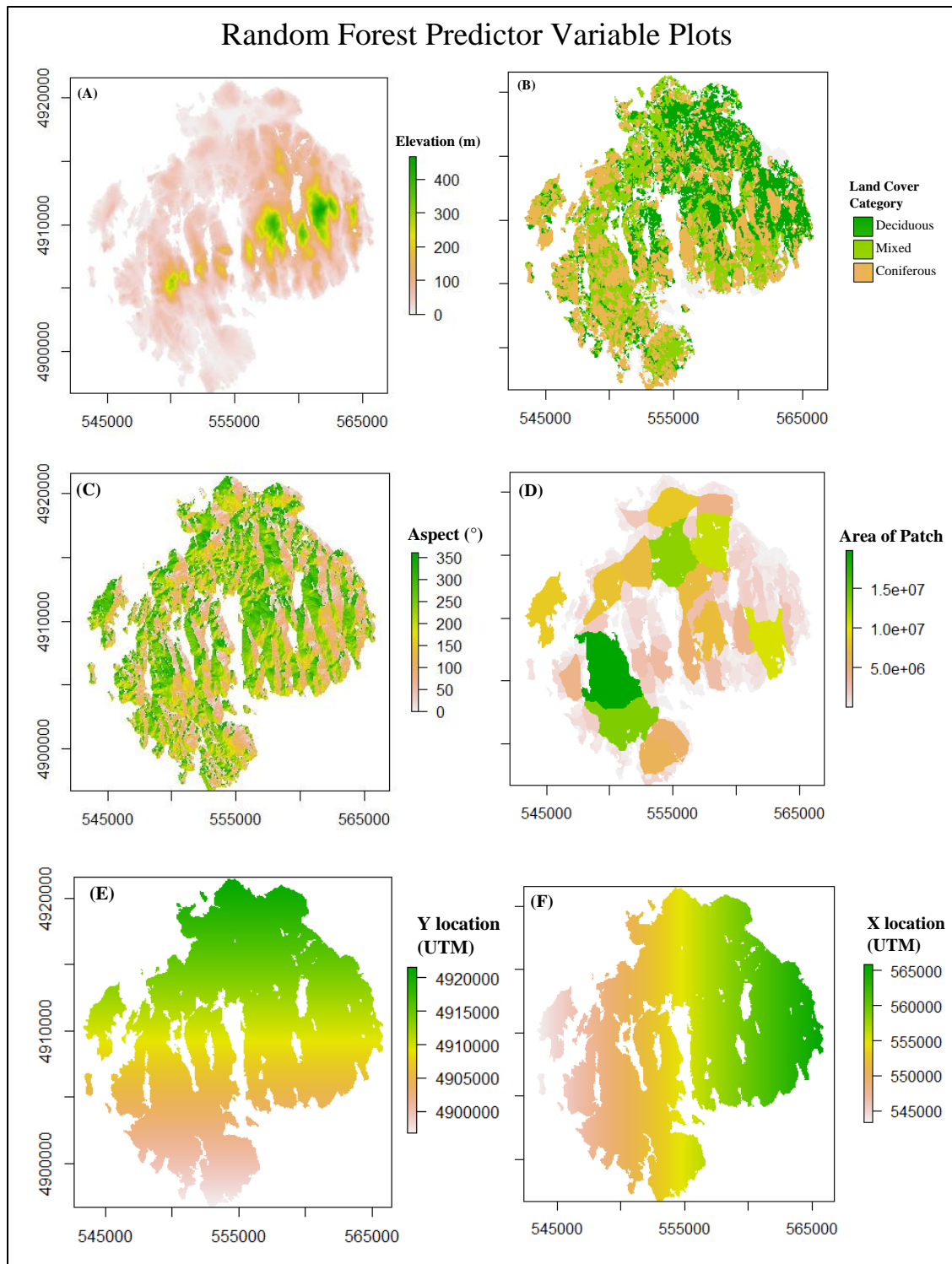
To test the hypothesis that tick abundance is correlated with deer activity, deer scat surveys were conducted for 250 m at each focal site. We walked along transects, stopping and scanning every meter to observe any deer scat present within one meter on either side of the transect. A one-meter pole was used to ensure any identified scat was within one meter of transect. We recorded each time a deer scat pile with greater than 10 pellets was observed and calculated a total number of deer scat piles per site.

#### **1.2.4 Spatial Modeling of Blacklegged Tick Densities**

We developed separate random forest spatial models of nymphal blacklegged tick densities in 2018 and 2019 using the randomForest package in RStudio (Breiman 2001). Random

forest is one of several widely-used species distribution modeling techniques; we chose this approach over other algorithms, e.g., MaxEnt, GARP, GLM, GAM (Philips et al. 2006; Stockwell 1999; Guisan et al. 2002), because random forest makes no assumptions about the distribution of the dependent variable, is appropriate for non-linear relationships between variables, and has the ability to predict continuous dependent variables (Breiman 2001). In this study, we used random forest regression in which decision trees are used to predict a continuous independent variable based on a set of permuted predictor variables (Breiman 2001). Each model included the square root of observed tick densities (transformed to create a more normal distribution) as the dependent variable and landscape features as predictor variables (i.e., land cover type, elevation, aspect, fragmentation of land by roads; Figure 2). We also included vertical and horizontal UTM coordinates as predictors (Figure 2) to account for any spatially autocorrelated unquantified variables (e.g., microclimate, soil type or host abundance). Data layers of land cover, elevation and aspect variables were obtained from the NPS data store (<https://irma.nps.gov/>), habitat fragmentation was downloaded from the ArcGIS directory (<https://www.arcgis.com/>), and UTM coordinates were derived from a location grid created in RStudio. Because the vast majority of ANP is forested and all sites were in forested areas, land cover type was aggregated into three categories: deciduous forest, coniferous forest and mixed forest. Therefore, any cells that could not be aggregated into these categories (e.g. developed land, pasture, open water) within the land cover spatial layer were excluded from our model. Elevation was represented in meters, and aspect is the direction of maximum slope represented by positive degree (0 to 360, clockwise starting North) measurements (Dieffenbach 2005). Habitat fragmentation was represented by the area of patch not divided by road. ArcMap was used to project layers into UTM Zone 19 and to restrict the extent of variable layers to the

boundaries of Mount Desert Island. All layers were imported into RStudio to create a random forest model. Model selection based on percent variance explained by the model was performed to identify landscape features that are correlated to tick density.



**Figure 2.** Predictor variables included in the random forest model: (A) Elevation (B) Land cover (C) Aspect (D) Fragmentation represented by patch size (E) UTM Y-axis location (South – North) and (F) UTM X-axis location (West – East)



### 1.2.5 Fine-Scale Tick Habitat Associations

To identify fine-scale habitat characteristics (i.e., temperature, humidity, small mammal capture rate, deer scat surveys, percent canopy cover, and deciduous leaf litter depth) that vary across broad-scale landscape feature classes (i.e., land cover type, elevation, aspect, fragmentation of land by roads, vertical UTM coordinate, horizontal UTM coordinate) two multivariate analyses of variance (MANOVA) were performed in R using the package *vegan* (Oksanen et al. 2019). First, to determine if abiotic factors vary across landscape features, a MANOVA was conducted with microclimate characteristics (i.e., mean of weekly temperature, minimum temperature, maximum temperature, humidity, minimum humidity, maximum humidity and) and month of collection as dependent variables and landscape feature classes (classified into three categories using percentiles) as predictors. Second, to determine if biotic factors vary across landscape features, the same analysis was conducted with small mammal capture rate, deer scat surveys, percent canopy cover, and leaf litter depth as dependent variables. If significant differences were detected in this global test, we conducted follow-up Analyses of Variance (ANOVA) tests and Tukey's honestly significant difference post-hoc tests in R using the package *vegan* (Oksanen et al. 2019) to infer which landscape feature classes differed.

To test the hypothesis that habitat characteristics which are different among landscape features are also correlated with tick density at the 19 focal sites, a generalized linear model (GLM) was performed with microclimate variables (i.e. mean humidity, mean temperature, maximum humidity, minimum temperature) as predictors of the square root of nymphal density in R. To ensure that the data met the required assumptions for GLM analyses, nymphal abundance data were square root ( $x+0.1$ ) transformed. Because these data were only collected in 2018, a single model was developed using month as an independent variable. To identify the best

correlative model, the `glmulti` function (Calcagno and de Mazancourt 2010), an automated model selection command which generates all possible models and finds the best in terms of a given criteria (i.e. AIC value), was performed in R. to identify the most important predictors of nymphal abundance and to identify the best predictive model for each collection period.

### 1.2.6 Pathogen Analyses

To assess pathogen infection prevalence in nymphal blacklegged ticks in ANP, 174 ticks were tested for *B. burgdorferi*, *A. phagocytophilum*, and *B. microti*. To disrupt the exoskeleton and expose internal tissues, nymphal ticks were cut into halves. DNA was extracted and purified from tissues using Qiagen DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA) and modified extraction protocol. *Borrelia burgdorferi*, *A. phagocytophilum*, *B. microti*, and *Ixodes* spp. DNA were detected using qPCR quadruplex at the University of Maine Cooperative Extension Diagnostic and Research Laboratory (Orono, ME, USA) (Hojgaard 2014). Each PCR reaction was of 10µL reaction size containing 2µL of DNA template (extracted from ticks or control samples), 5µL of Bio-Rad (Hercules, CA, USA) iQ Multiplex Powermix and 3µL of premixed primers and probes (Table 1). The qPCR reactions were completed on a Bio-Rad CFX 96 with a 3 minute initial burn-in at 95°C and annealing-extension through 40 cycles of 95°C (15 seconds) and 60°C (45 seconds). Samples were considered true positives if the internal tick DNA control was amplified and if the target CQ(CT) value was less than 32. Due to concerns in testing methods of *B. microti*, samples that were initially positive for *B. microti* were tested in duplicate again with the same protocol but substituting Bio-Rad iQ Powermix for PowerUp SYBR Green Master Mix to ensure positives were in fact true positives.

To determine the proportion of small mammals infected with tick-borne pathogens, 125 small mammal ear biopsies were tested for 21 potential tick-borne pathogens (*Anaplasma*

*phagocytophilum*, *A. marginale*, *Babesia microti*, *Bartonella spp*, *Borrelia burgdorferi*, *B. mayonii*, *B. miyamotoi*, *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, *Mycoplasma haemocanis*, Powassan virus, *Rickettsia spp.*, *Rickettsia* spotted fever group, *Francisella genus*, SFTSV, *Theileria orientalis*) at Cornell University Animal Health Diagnostic Center (Ithaca, NY, USA) using nanoliter scale real-time PCR modified for tissue samples (Goodman et al. 2016). Tissue was disrupted through bead beating and DNA was extracted and purified using the MagMAX Total Nucleic Acid Isolation Kit (cat.#AM1840) and KingFisher Flex. Extracted DNA was analyzed for pathogens using real-time PCR on the QuantStudio 12k Flex OpenArray (OA) platform.

**Table 1.** Premixed primers and probes for tick pathogen analyses.

Target	Gene	Type	Sequence (5'-3')	Conc (nM)	Reference
<i>Borrelia spp.</i>	23S	FWD	CGAGTCTTAAAAGGGCGATTTAGT	500	Xu et al. 2016
		REV	GCTTCAGCCTGGCCATAAATAG	500	
		Probe	FAM –AGATGTGGTAGACCCGA AGCCGAGTG – BHQ 1	250	
Tick DNA Control	16S	FWD	AATACTCTAGGGATAACAGCGT AATAATTTT	500	Xu et al. 2016
		REV	CGGTCTGAACTCAGATCAAGTAGGA	500	
		Probe	Cy5–AAATAGTTTGCGACCTCGATGTT GGATTA GGAT – BHQ 1	250	
<i>Anaplasma phagocytophilum</i>	MSP2	FWD	ATGGAAGGTAGTGTTGGTTATGGTATT	500	Hojgaard et al. 2014
		REV	TTGGTCTTGAAGCGCTCGTA	500	
		Probe	HEX –TGGTGCCAGGGTTGAGCTTGA GATTG – BHQ1	250	
<i>Babesia microti</i>	18S	FWD	CGACTACGTCCCTGCCCTTTG	500	Hojgaard et al. 2014
		REV	ACGAAGGACGAATCCACGTTTC	500	
		Probe	Tex615 –ACACCGCCCGTCGCTCCT ACCG – BHQ2	250	
<i>Babesia microti</i>	Surface Antigen 1	FWD	ACAGAATGCAGTCGGTGAAG	1000	Hojgaard et al. 2014
		REV	ATCAAGGAGAGTGGATAGGTTTG	1000	

## 1.3 Results

### 1.3.1 Off-host tick collections

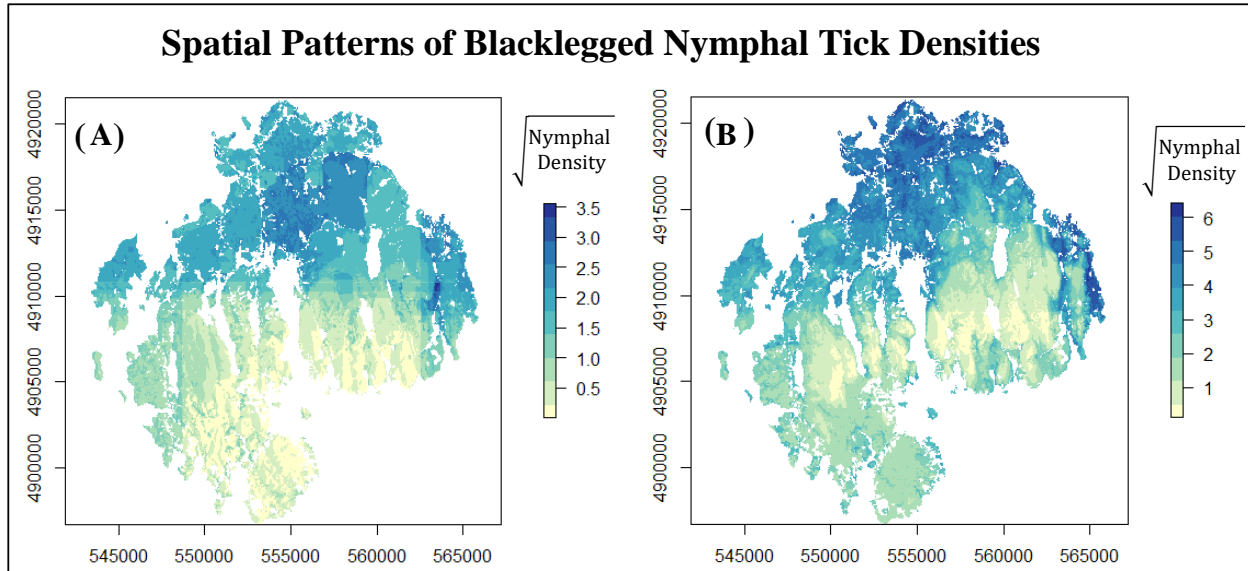
Overall, blacklegged tick densities were higher in 2019 than in 2018 at focal sites in June and August (Table 2). In 2018, 204 nymphal blacklegged ticks, 2,217 larval ticks, 7 adult female and 15 adult male ticks were collected from all sites. The mean number of nymphs collected across all sites was  $2.83 \pm 0.54$  nymphs per 500 m<sup>2</sup>. In 2019, 1,640 nymphal ticks, 1,837 larval ticks and 26 adult female and 29 adult male ticks were collected from all sites. The mean number of nymphs collected across all sites was  $15.2 \pm 1.87$  nymphs per 500 m<sup>2</sup>. In 2018, we also collected 119 larval and 12 nymphal *Haemaphysalis leporispalustris*, while in 2019, we collected 342 larval, 24 nymphal and 2 adult male *H. leporispalustris*.

**Table 2.** Comparison of densities of blacklegged ticks (per 500 m<sup>2</sup>) at 19 focal sites in Acadia National Park in 2018 and 2019. One collection in June and one collection in August for each year is shown to provide the most comparable densities.

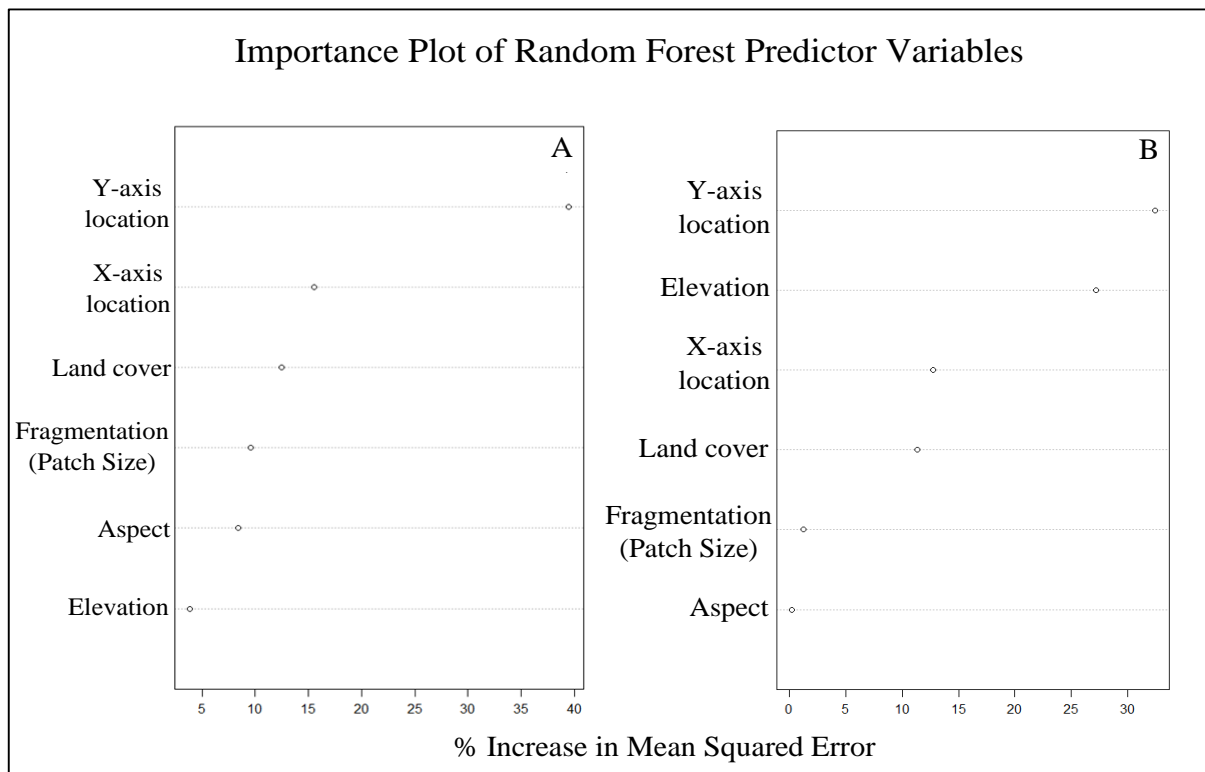
Blacklegged tick Life stage	2018				2019			
	June (6/13-6/29)		August (8/14-8/19)		June (6/12-6/19)		August (8/12-8/16)	
	Mean	Total	Mean	Total	Mean	Total	Mean	Total
<b>Larvae</b>	17.89	340	89.89	1,708	23.89	430	8.59	146
<b>Nymph</b>	4.58	87	3.26	62	19.33	348	11.59	197
<b>Adult Female</b>	0.26	5	0.05	1	0.44	8	0.18	3
<b>Adult Male</b>	0.53	10	0.05	1	0.61	11	0.12	2

### 1.3.2 Spatial Patterns of Nymphal Blacklegged Ticks

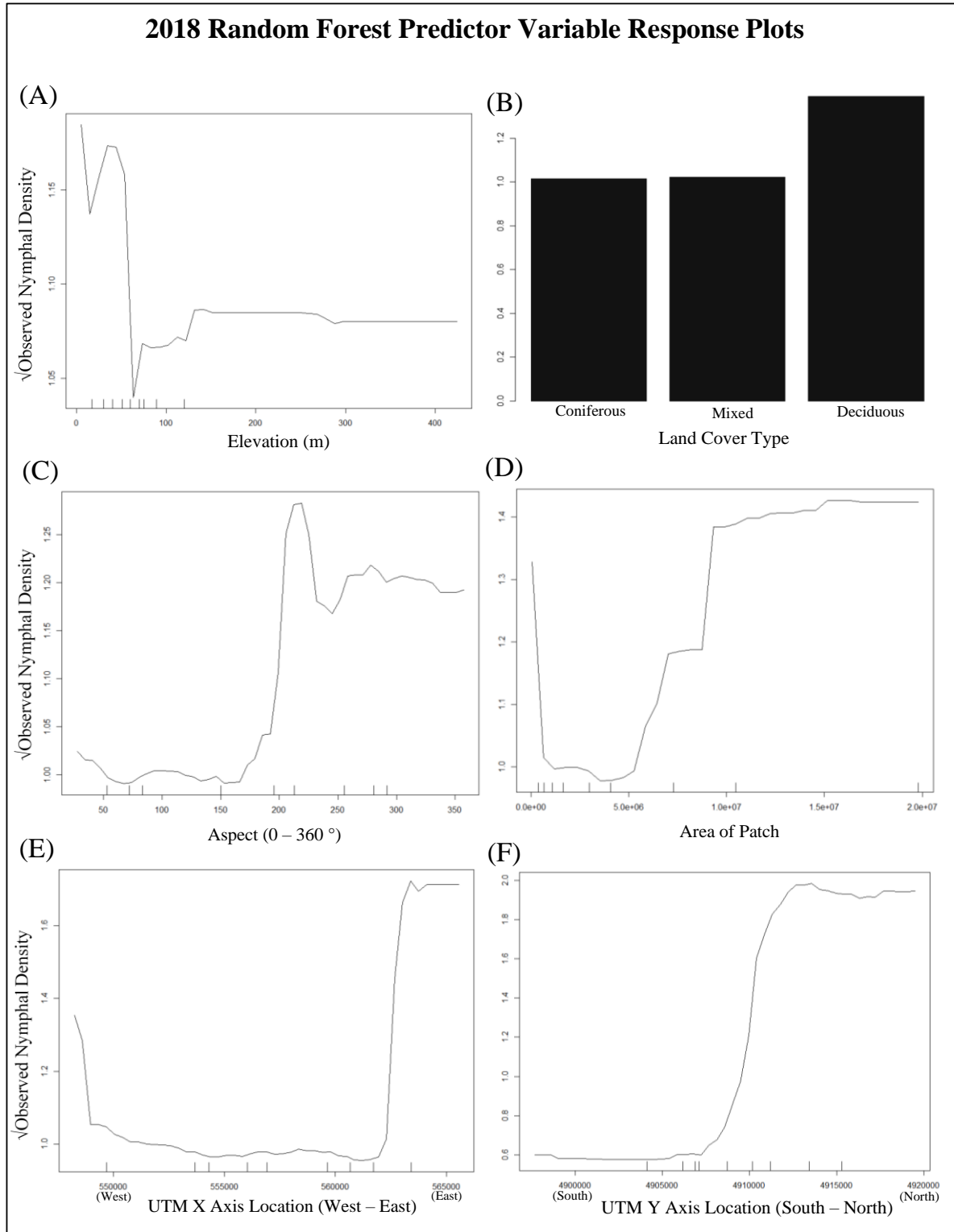
In both 2018 and 2019, random forest models demonstrated that blacklegged nymph densities varied significantly across MDI (Figure 3) and are correlated with land cover, elevation, fragmentation, aspect, X-axis location (UTM West – East) and Y-axis location (South – North) as predictors of nymphal tick density (Figure 4). Response curves indicate how tick densities varied across landscape features and similar patterns emerged for both years (Figure 5 ; Figure 6). There was a negative correlation between tick density and elevation with higher tick densities at lower elevations. In addition, higher tick densities were associated with deciduous forests over coniferous and mixed forests. There was a positive relationship between tick density and aspect, patch size, X-axis location and Y axis location. In, general there were higher densities in aspects with higher degrees (facing westerly between north and south) than in lower degrees (facing easterly between north and south) and in larger patch sizes than in smaller patch sizes. Finally, there were higher densities predicted at locations in northern locations over southern locations and in eastern locations over western locations. In 2018, the model explained 55.48% of the variance detected in nymphal densities and produced a mean squared error of 0.49. In 2019, the model explained 51.55% of the variance detected and produced a mean squared error of 2.58. For both years, predicted and observed values indicated a strong correlation (2018:  $R^2 = 0.5483$  and 2019:  $R^2 = 0.5107$ ) and no significant outliers appeared (Figure 7).



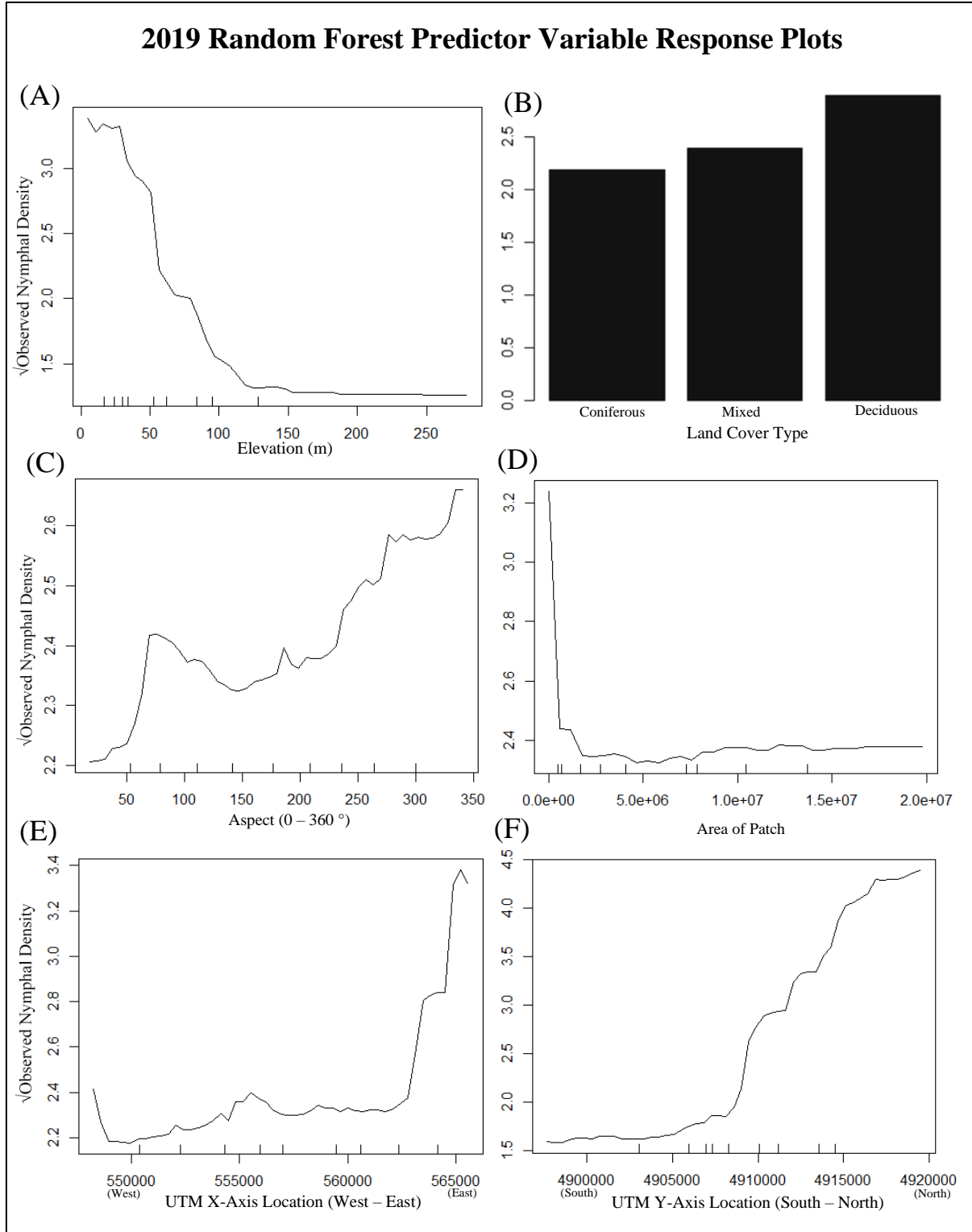
**Figure 3.** Random forest model predicting spatial patterns of blacklegged nymphal tick densities (square root of ticks per 500m<sup>2</sup>) in (A) 2018 and (B) 2019.



**Figure 4.** Importance plots derived from the random forest model show the % increase in mean squared error (MSE) of each predictor variable when randomly permuted. Variables with higher %MSE are more important for predicting  $\sqrt{(\text{nymphal tick densities})}$  and are listed in order of importance from top to bottom in (A) 2018 and (B) 2019 random forest models.

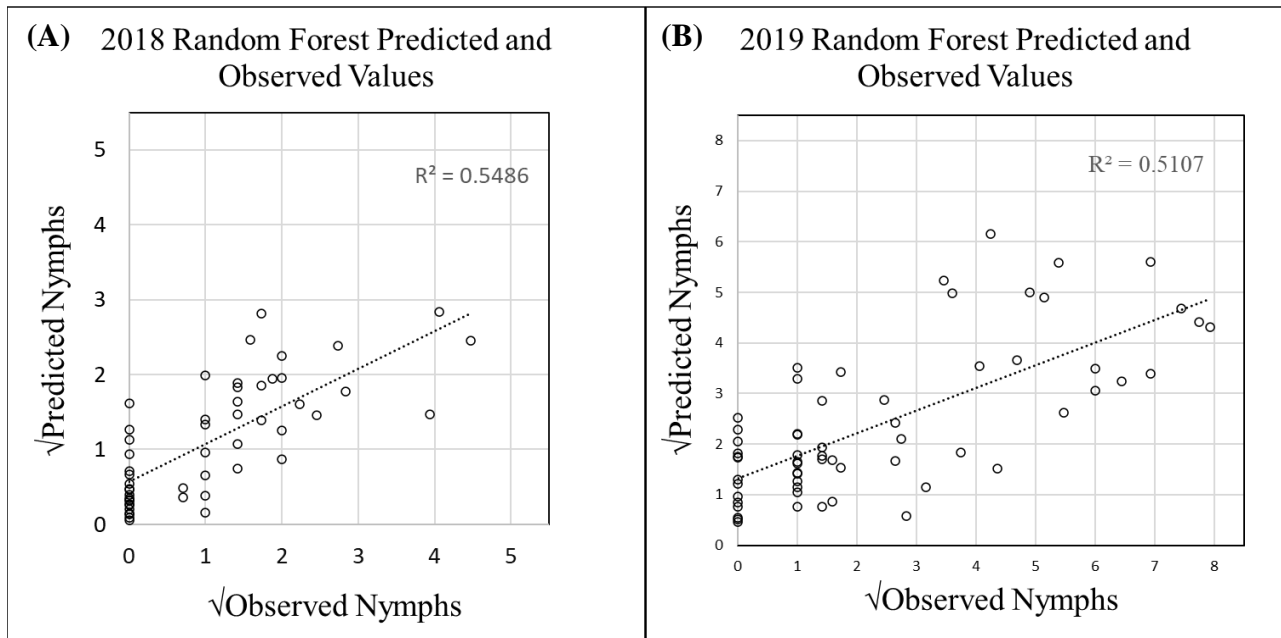


**Figure 5.** Response curves of  $\sqrt{(\text{observed nymphal tick densities})}$  for each landscape feature predictor variables included in the 2018 random forest model: (A) Elevation (B) Land cover (C) Aspect (D) Fragmentation represented by patch size, (E) UTM X-axis location (West – East) and (F) UTM Y-axis location (South – North).



**Figure 6.** Response curves of  $\sqrt{\text{(observed nymphal tick densities)}}$  for each landscape feature predictor variables included in the 2019 random forest model: (A) Elevation (B) Land cover (C) Aspect (D) Fragmentation represented by patch size, (E) UTM X-axis location (West – East) and (F) UTM Y-axis location (South – North).





**Figure 7.** Predicted and Observed values or the square root of blacklegged nymphal tick densities from the (A) 2018 and (B) 2019 random forest models.

### 1.3.3 Characterization of Fine-scale Habitat features Across Landscape Feature Classes

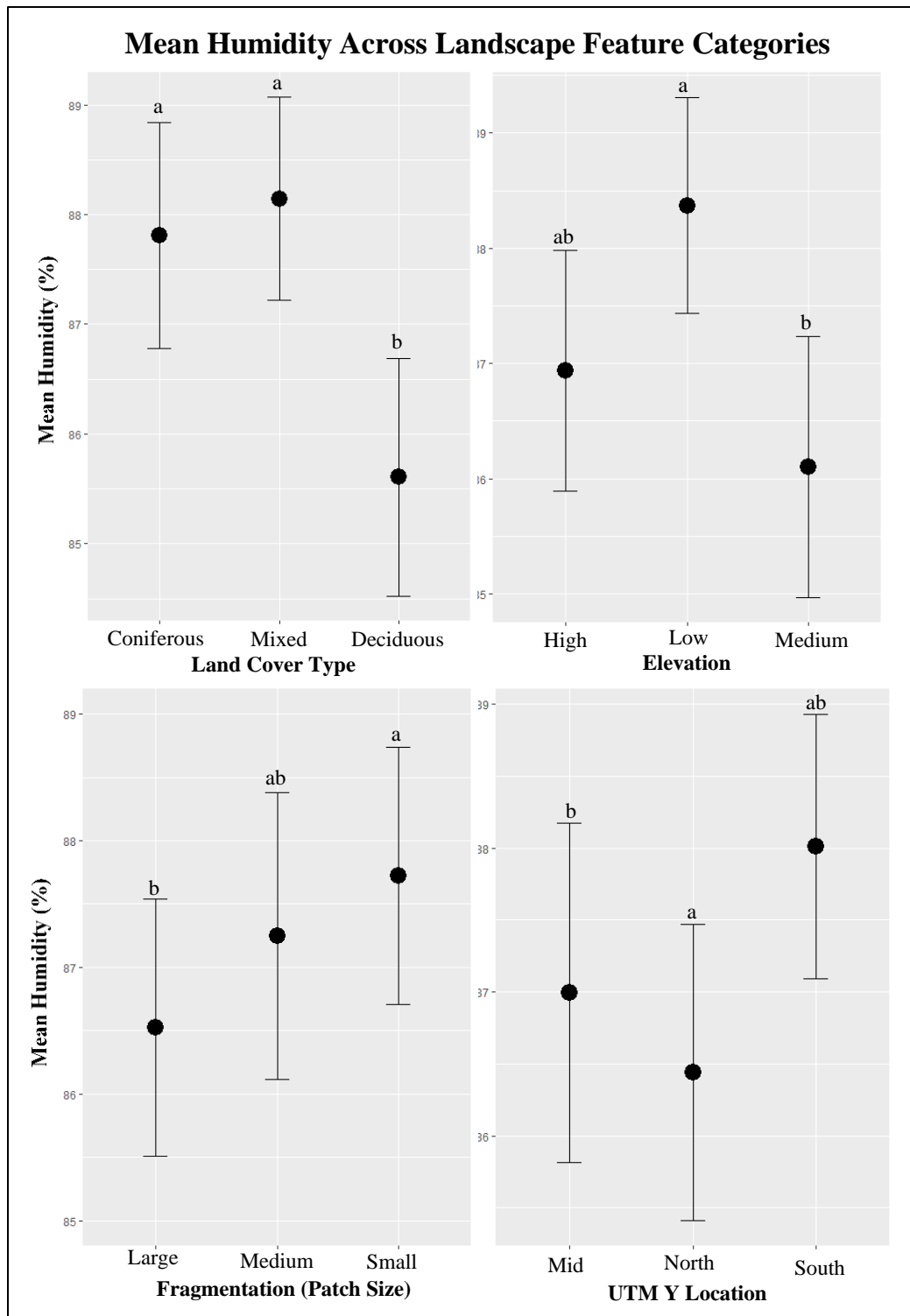
A multivariate analyses of variance (MANOVA) indicated differences in microclimate measurements (i.e. weekly means of temperature, minimum temperature, maximum temperature, humidity, minimum humidity, and maximum humidity) among land cover type, elevation, forest fragmentation, aspect, Y-location and X-location categories and month during collection periods in June and August (Table 3). Mean humidity was significantly different among all land cover type, elevation, fragmentation (patch size) and Y-location (north – south), mean temperature was different among land cover type and Y-location (north – south) and mean minimum temperature was different across elevation and landcover (Figure 8, Figure 9, Figure 10). Biotic fine-scale measurements (i.e. small mammal capture rate, percent canopy cover, percent deciduous leaf litter, percent coniferous leaf litter, deciduous leaf litter depth and deer scat counts) did not differ

among land cover type, elevation, forest fragmentation, aspect Y-location and X-location categories.

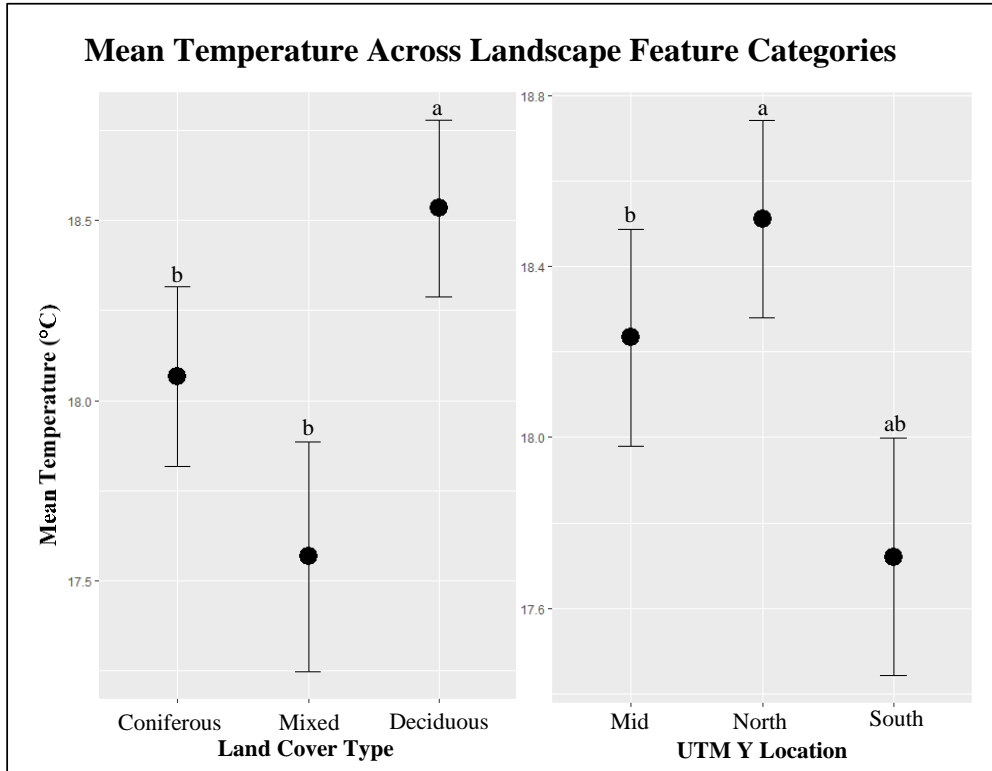
**Table 3.** Summary of MANOVA on microclimate conditions\* across categories in each landscape feature in 2018.

<b>Independent Variable</b>	<b>Df</b>	<b>Pillai</b>	<b>F</b>	<b>Num df</b>	<b>Den df</b>	<b>Sig</b>
Land cover	2	1.13	4.53	12	42	<0.001
Elevation	2	1.05	3.89	12	42	<0.001
Patch Size	2	0.96	3.20	12	42	0.002
UTM Location (North – South)	2	0.79	2.27	12	42	0.03
UTM location (West – East)	2	1.19	5.14	12	42	<0.001
Month	1	0.99	622.92	6	20	<0.001

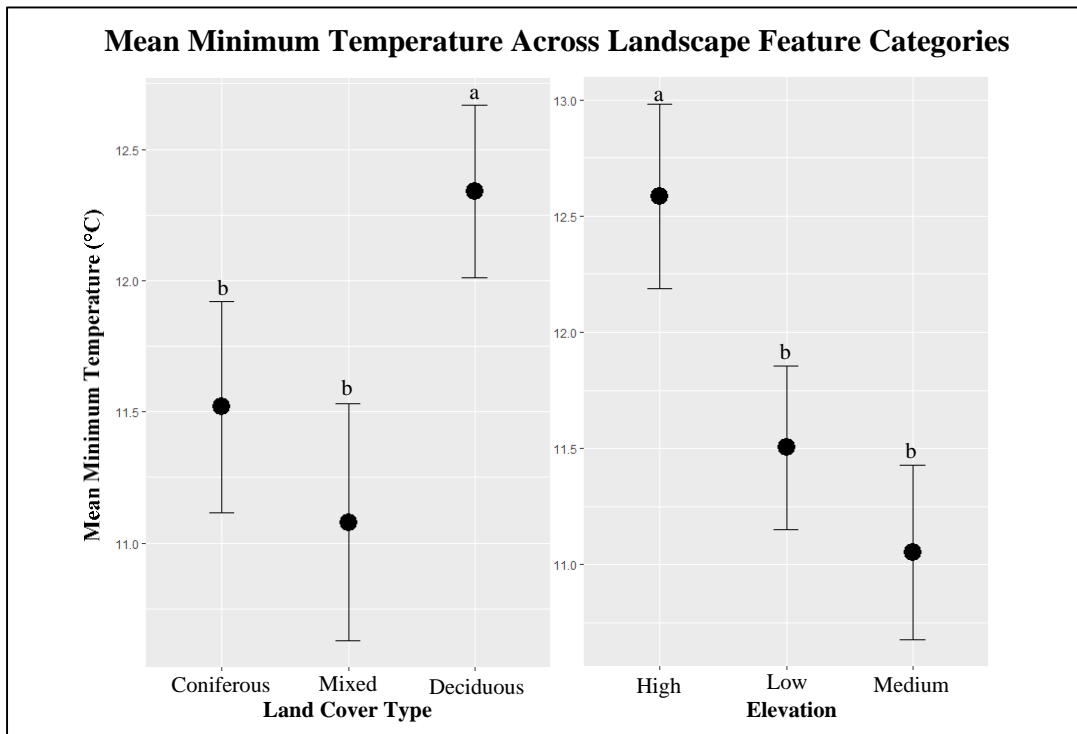
\*Dependent Variable – Microclimate condition (Mean of weekly: Temperature, minimum temperature, maximum temperature, humidity, minimum humidity and maximum humidity)



**Figure 8.** Tukey's significant difference post-hoc test on weekly mean humidity across landscape features at the 19 focal sites



**Figure 9.** Tukey’s significant difference post-hoc test on mean temperature across landscape features at the 19 focal sites



**Figure 10.** Tukey’s significant difference post-hoc test on mean minimum temperature across landscape features at the 19 focal sites

### 1.3.4 Associations Between Habitat Characteristics and Tick Densities

Model selection indicated that the best model to explain nymphal density included mean temperature and mean humidity (Table 4). There was a positive relationship between nymphal density and mean temperature and a negative relationship between nymphal density and mean humidity.

**Table 4.** Summary of generalized linear model with microclimate measurements as predictive factors of nymphal density at the 19 focal sites in 2018.

Factor	Estimate	Std. Error	t-value	p- value
Mean Humidity	-0.1688	0.0419	-4.0290	0.0003
Mean Temperature	0.5900	0.1696	3.4790	0.0014

### 1.3.5 Pathogen Analyses

Pathogen analyses indicated that both blacklegged nymphal ticks (174 tested) and small mammals (115 *Peromyscus* mice, six *T. striatus* and two *T. hudsonicus*) were infected with tick-borne pathogens (Table 5). The highest proportion of blacklegged ticks were infected with *B. burgdorferi* (28.7%) followed by *B. microti* (5.7%) and *A. phagocytophilum* (2.3%). The mean density of infected nymphs (nymphs/500m<sup>2</sup>) across all sites in 2018 was 0.81 for *B. burgdorferi*, 0.07 for *A. phagocytophilum* and 0.16 for *B. microti*. Small mammals were most highly infected with *B. burgdorferi* (46.3%) followed by *A. phagocytophilum* (10.6%) and *B. microti* (5.7%). Two more potential tick-borne pathogens species of the genus *Bartonella* (3.3%) and *Rickettsia* (1.6%) were found in small mammals but were not tested for in ticks.

**Table 5.** Infection prevalence of tick-borne pathogens in blacklegged nymphal ticks (from all sites) and small mammal ear tissue samples (from 19 focal sites) in 2018.

Sample Analyzed	Number Analyzed	Infection Prevalence (%)				
		<i>B. burgdorferi</i>	<i>A. phagocytophilum</i>	<i>B. microti</i>	<i>Bartonella</i> spp.	<i>Rickettsia</i> spp.
Blacklegged Nymphal Ticks	174	28.7	2.3	5.7	NA	NA
Small Mammal Ear Tissue	123	46.3	10.6	5.7	3.3	1.6

#### 1.4 Discussion

The main objectives of this project were 1) to develop a predictive spatial model of blacklegged tick densities in ANP, integrating field-collected tick density data and geospatial landscape features data (e.g. land cover type, elevation and habitat fragmentation level) and 2) to determine the causal ecological mechanisms underlying these observed tick density patterns. We also present new descriptive data concerning of pathogen infection prevalence of blacklegged ticks and small mammals in ANP. This study found that spatial patterns of tick densities vary across the landscape in ANP and are influenced by elevation, land cover type, aspect, fragmentation, X-location and Y-location. Based on analyses of data from our 19 focal sites, the causal mechanism underlying these observed patterns of tick densities appears to be largely be variation in microclimate conditions (i.e. mean humidity and mean temperature) across landscape features. In addition, blacklegged ticks and small mammals were found to be infected with tick-borne pathogens including *B. burgdorferi*, *A. phagocytophilum* and *B. microti*. Together, these findings provide new insights into patterns and mechanisms of tick-habitat associations and can be used by the National Park Service to inform protective measures and management strategies within the park.

Similar spatial patterns of blacklegged nymphal tick densities emerged in 2018 and 2019, with land cover and location (X and Y coordinates) as the most important predictors of tick density in 2018 and elevation and location in 2019. There were higher densities of ticks in deciduous forests compared to coniferous and mixed forests and at low-medium elevations compared to higher elevations. This is consistent with the results of previous studies. For example, research on habitat suitability of blacklegged ticks in North Central US found a positive association between tick presence and deciduous forest and a negative association in coniferous forests (Guerra et al. 2002), while a study of *Ixodes ricinus*, an important vector of Lyme disease in Europe (ECDC 2014), found that across all life stages, ticks were more abundant in oak stands compared to pine stands and with increasing shrub cover (Madder et al. 2012). A study in woodlands of California found that several other *Ixodes* tick species (*Ixodes angustus*, *Ixodes auritulus*, or *Ixodes spinipalpis*) were negatively associated with elevation and *I. angustus* was additionally negatively associated with slope (Eisen et al. 2016). Also, there were higher densities of ticks between westerly and northerly aspects in both years in ANP. However, research conducted in South Wales found that presence of questing *I. ricinus* were higher in easterly and westerly aspects (Medlock et al. 2008). In 2019, elevation was more important as a predictor than in 2018. Therefore, it is possible that in years of high tick densities (2019), elevation becomes more evident as a limiting factor for blacklegged tick habitat suitability and range. In particular, densities may increase at lower elevations where habitat is suitable but not at high elevations, creating a clear difference in tick density. There were higher tick densities associated with northern and eastern locations of the island. Location was included to be used as a proxy for potential drivers of tick densities, that could not be included as

variables in the random forest model, such as human activity, mammal host abundance, soil type or microclimate conditions.

While similar tick density patterns emerged for both years, tick densities were generally higher in 2019 than in 2018. We hypothesize that this occurred due to a relatively high small mammal population in 2018 providing ample host for 2018 larvae to feed and molt into a high population of nymphs in 2019 (Ostfeld et al. 2001 ; Ostfeld et al. 2006 ; Ostfeld et al. 2018). In addition, it is possible that the high small mammal population in 2018 provided ample hosts for nymphal ticks and thus removed many host-seeking ticks, resulting in lower observed densities while performing off- host tick collections (Ostfeld et al. 2018). These results exemplify the importance of studying tick-borne disease for multiple years. In summary, spatial patterns of tick densities are correlated with land cover, elevation aspect, fragmentation and location (X and Y coordinates) in ANP and natural yearly variations in tick densities may influence which features are most important.

In ANP, a major driver of the current distribution of habitat types is the Fire of 1947, a large-scale forest fire that spread almost half of the park, significantly changing the landscape. Burned areas, previously occupied by coniferous forests stands, are now dominated by deciduous forest stands (U.S. National Park Service 2015) and consequently are correlated with higher densities of ticks. This study joins a growing body of work that suggests that landscape change can have long-term implications for vector-borne disease transmission. Changes in the structure of ecosystems can influence biodiversity and change how hosts and pathogens interact (Keesing et al. 2010). For example, areas with high diversity of birds are correlated with a decline in West Nile virus prevalence in humans (Enzenwa 2006). Another study found that forest fragmentation is correlated with Lyme disease risk, where smaller patches of forest have higher densities of



ticks and higher proportion infected with *B. burgdorferi* (Allan et al. 2003). There is also a growing interest in prospects for mitigating tick-borne disease risk via habitat management, including the use of landscape management practices such as fire in the form of controlled burns. Fire has been shown to reduce tick populations immediately following a burn. However, ticks occupy burned areas soon after, suggesting that the reduction is temporary (Stafford et al. 1998, Allan et al. 2009). Longer term effects of fire on tick populations and entomological disease risk remain largely uninvestigated in scientific literature. This study provides insight into long term effects following a large-scale human caused fire. Blacklegged tick nymphs were relatively higher in areas of deciduous forest, suggesting that fire may not be a sustainable tick management strategy and may in fact produce areas more suitable as tick habitat.

We chose to conduct our study at the same locations as the most recent published tick survey in ANP to provide understanding of changes in the tick distribution in the park over the past 30 years. In 1990, blacklegged ticks appeared to be isolated to the northeast side of the island (Connery et al. 1992). This project revealed that since then the blacklegged tick has since spread across the island and was found present at all focal sites in 2018 and 18 of 19 focal sites in 2019. This result is unsurprising based on passive surveillance which suggests a dramatic geographic range shifts of the blacklegged tick in southern and coastal Maine (EPHT 2018). We hypothesize that the distribution shift is due to climate change providing more suitable habitat farther north up the coast of Maine (now reaching ANP). Previous studies have identified climate change as an important determinant of suitable habitat for the blacklegged tick. For example, one study identified increase in winter temperatures was a key factor of unsuitable habitat changing to suitable habitat for blacklegged ticks (Estrada-Peña 2002). Another study of *Ixodes ricinus*

ticks found that the northern shift in range was related to fewer winter days with low minimum temperatures (Lindgren et al. 2000).

Analyses of fine-scale measurements at focal sites revealed that mean humidity and mean temperature were not only significantly different among landscape features that are predictive of broad-scale patterns of tick density, but also were significantly correlated to tick density. Due to their large surface to volume ratio, microclimate conditions can greatly affect tick survival, behavior and development and have been well documented in the scientific literature. When exposed to various temperatures, nymphs have been found to travel further and remain in questing posture for longer at 25°C than at lower or higher temperatures (Vail and Smith 2002). In addition, the developmental rate of ticks can be influenced by temperature. In laboratory settings, molting success was greatest for larvae at 28°C and 24°C for nymphs (Ogden et al. 2004). Mean temperatures for both June and August were below 25°C for all sites, however there was a positive relationship between nymphal density and mean temperature, indicating that densities are increasing as it approaches optimal degrees for activity and molting success. A laboratory study of immature blacklegged ticks under exposure to constant relative humidity (RH) found that RH can significantly influence tick survival. Nymphs exposed to 100% and 93% RH survived significantly longer than nymphs exposed to 85%, 75% and 65% RH (Stafford 1994). In addition, RH can influence nymphal questing height, being significantly greater at 100% RH than below 75% RH (Vail and Smith 2002). A field study of blacklegged ticks echoed these findings, showing cumulative hours at >82% RH threshold had a significant negative correlation to the number of ticks collected (Berger et al. 2014). In contrast, results from this study indicate a negative relationship between humidity and nymphal tick density.

Because both tick abundance and deciduous forest types have both been positively correlated to shrub cover, canopy cover and deciduous leaf litter (Lubelczyk et al. 2004; Fourrier et al. 2015), these factors may help explain why ticks have been found in higher abundances in deciduous over coniferous forests. Deciduous forests may have higher humidity and relatively milder temperatures on the ground due to increased canopy cover, shrub cover and leaf litter depth. When ticks are exposed to suboptimal microclimate conditions in natural environments, tick may hide under leaf litter to avoid desiccation (Ostfeld and Brunner 2015) which reinforces the hypothesis that increased deciduous leaf litter would be optimal for tick habitat suitability. In addition, areas of higher elevations may be colder and less humid.

Because nymphal ticks are the most epidemiological significant life stage, nymphal blacklegged ticks were the only life stage tested for pathogens. The highest proportion of ticks were infected with *B. burgdorferi* (28.7%), followed by *B. microti* (5.7%) and *A. phagocytophilum* (2.3%). The infection prevalence of *B. burgdorferi* and *A. phagocytophilum* appears to be higher than estimates of infection prevalence in ANP from a study of northeastern national parks in 2016 (Johnson et al. 2016) which found the proportion of infected nymphs was: *B. burgdorferi* (18%), *B. microti* (4%). However, we found lower infection prevalence of *A. phagocytophilum* (2.3%) than found in this study (3%). In addition to these three pathogens, small mammals were found to be infected with two potential tick-borne pathogens of the genus *Bartonella* (3.3%) and *Rickettsia* (1.6%), but these pathogens were not tested for in ticks. Our results demonstrate that pathogen transmission is likely occurring between blacklegged ticks and small mammal reservoir hosts and presents the potential for pathogen spillover to humans in ANP.

While this study provides significant insight into the broad scale patterns of tick abundance in ANP, the fine-scale mechanisms driving these patterns have been investigated to a lesser extent. As is common with many ecological field studies, there are limitations to field collected data. For example, due to weather and time-constraints, sites were only sampled for small mammals for one trap night in 2018. In 2019, due to low region-wide capture rates, sites were not trapped. One trap night does not provide enough data for an accurate or precise estimate of small mammal abundance. In addition, deer scat surveys may be problematic due to bias in detection probabilities which may vary across different habitat types. For instance, deciduous forests with more broad-leaf litter and ground vegetation may cause low detection probabilities, even in areas where there is deer scat, simply because it is difficult to see. In addition, vegetation surveys were only performed on one occasion. However, because they were conducted in August, prior to leaf fall, we believe it is a suitable relative measurement of vegetation during nymphal blacklegged tick activity. We recommend that future research addressing tick-borne disease risk consider these limitations. It would be beneficial to investigate the spatial patterns of tick densities as well as the possible underlying mechanisms for several years with multiple measurements each year, to account for yearly and seasonal variations in ticks and their hosts. A review paper of vector-borne diseases in National Parks suggests that vector field collection studies are not routinely performed in National Parks and that possible differences in human demographics and behaviors presents an issue when estimating risk based on human case data alone (Eisen et al. 2013). It suggests that spatial models be developed to overcome these confounding issues and this study provides evidence of the validity and feasibility of such studies being performed in other national parks.

The spatial model that resulted from this project may be used to inform future park management and infrastructure. For instance, park officials may choose to produce tick-borne disease awareness documents informing visitors that ticks are present across the island and may be denser in areas with deciduous forest cover. In addition, signage informing visitors of possible tick-borne disease risk can be prioritized in areas where visitors frequent and where ticks are denser. If a new trail, shop or visitors center is to be built, this spatial model can provide insight into the tick-borne disease exposure risk-associated with the area it is to be built. We anticipate that the spatial model resulting from this study will be utilized by ANP officials to reduce the risk of tick-borne disease exposure risk for visitors, employees and volunteers.

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Sara was born and raised in White Oak, Pennsylvania and graduated from McKeesport High School in 2011. She continued her education and conducted tick-borne disease research at Indiana University of Pennsylvania where she obtained her Bachelor of Science in Biology in 2015. Upon graduation, she worked at the Cary Institute of Ecosystem Studies in Millbrook, New York on a project investigating neighborhood-based management strategies to reduce human cases of tick-borne disease. To pursue her professional interest in working at the interface of disease ecology and public health, she applied to the Ecology and Environmental Sciences Masters program at the University of Maine in 2017. There, she worked under Dr. Allison Gardner to develop a research project, suited to her interests, investigating tick-borne disease exposure risk in Acadia National Park, Maine. Upon receiving her degree, Sara will pursue a career in vector-borne diseases and Public Health. She is a candidate for the Master of Science degree in Ecology and Environmental Sciences from the University of Maine in May 2020.