ENVIRONMENTAL FACTORS AFFECTING THE RANGE EXPANSION OF *IXODES SCAPULARIS*, THE BLACKLEGGED TICK, (ACARI:IXODIDAE) IN ILLINOIS

BY

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THESIS

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Abstract

Tick-borne pathogens are zoonoses whose transmission ecology involves complex interactions among the environment, competent reservoir or reproductive hosts, ticks acting as vectors, humans and their companion animals. *Ixodes scapularis* (Acari:Ixodidae) Say, 1821, the blacklegged tick, is an important vector of the disease agents of Anaplasmosis, Babesiosis, Powassan virus disease, Ehrlichiosis (*Ehrlichia muris eauclairensis*), *Borrelia miyamotoi* disease, and several strains of spirochetal bacteria which can cause Lyme disease. Both the human prevalence of Lyme disease and distribution of *I. scapularis* have been increasing in Illinois. Acquisition of pathogens by *I. scapularis* ticks is highly dependent on a variety of biotic and abiotic factors affecting both their life history traits and that of their hosts. Small, fragmented forested patches are less likely to sustain extensive species diversity and may host large populations of pathogen reservoir hosts such as the white-footed mouse (*Peromyscus leucopus*) and eastern chipmunk (*Tamias striatus*) while providing abundant edge habitat for large mammal *I. scapularis* reproductive hosts, such as white-tailed deer (*Odocoileus virginianus*). Dominant tree species may also influence the presence and success of various animal hosts. Soil composition, elevation, and drainage may impact the survival and overwintering success of ticks.

As part of the statewide surveillance program of ticks and their pathogens in Illinois during the autumn seasons of 2019 and 2020, a total of 283 adult female *I. scapularis* were collected via drag sampling. At least five 150 meter transects were sampled per site. The abundance of *I. scapularis* was established in 28 of 44 sampled counties across Illinois. To assess the effect of forest fragmentation, soil composition, elevation, proximity to water bodies, climate, natural divisions and dominant tree species on the abundance of *I. scapularis* in Illinois, I developed a

General Additive Model that accounted for spatial structure, using remote sensing data at scales most pertinent to two hosts important to the life cycle of *I. scapularis*, a buffer with a radii of 100 m to examine activity of the white-footed mouse, and a buffer with a radii of 900 m for the white-tailed deer. At the scale most relevant to the activity of the white-tailed deer, metrics related to habitat fragmentation such as number of patches (NP) ($p=<0.001$) and mean area (AREA_MN) ($p=<0.001$) were predictive of adult female *I. scapularis* abundance, meaning as patch number increased, and mean area decreased, adult female *I. scapularis* abundance increased. At the scale most relevant to the activity of the white-footed mouse, distance further from a body of water was positively correlated with *I. scapularis* tick abundance ($p= 0.006$). Latitude and longitude (spatial coordinates) were found to be significantly associated with tick abundance indicating that the density of *I. scapularis* varies across the state, with *I. scapularis* abundance higher in the northern portion of the state, rather than central and southern Illinois. The results of this study indicate that habitat fragmentation metrics including patchiness of the landscape and patch size influenced tick abundance more than other environmental factors like soil textures and proximity to water bodies.

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Introduction

Zoonotic illnesses with vector-borne transmission cycles like Lyme disease and West Nile virus are the end result of a long chain of complex interactions between humans, animals, and arthropod vectors. While we know much about how to treat the pathogens infecting the human body, we are learning more about the paths they take to get there and the factors affecting their transmission. Understanding the complex dynamics involved in the invasion, establishment, amplification, maintenance and transmission of pathogens is important in preventing their spread. In the eastern United States, Lyme disease is caused by the bacteria *Borrelia burgdorferi* s.s. and *B. mayonii*, which are transmitted through the bite of *Ixodes scapularis* Say, 1821, the blacklegged tick. A 2021 analysis by Kugeler et al. of health insurance claims over the 2010-2018 interval estimates an average of ≈476,000 people were diagnosed with Lyme disease each year in the contiguous United States (Kugeler et al. 2021).

As of 2017 surveillance efforts have established the presence of *I. scapularis* in 1,420 of the 3,110 counties in the continental United States, an increase from the 1,058 counties previously reported in 1998 (Eisen et al. 2017, Dennis et al. 1998) likely reflecting both an increase in detection effort and tick range expansion. Two previously distinct foci of *I. scapularis* in the Northeast and Midwest states are converging in the Ohio River Valley, while populations in the far South and South-Central states have remained distinct (Eisen et al. 2017). Population distributions in the Midwestern states of Minnesota and Wisconsin have broadened in all cardinal directions and Northeastern populations have radiated inland (Eisen et al. 2017). Additionally, surveillance in Canada has documented the northward spread of *I. scapularis* across 36 sites in eastern Ontario at a rate of 46 km per year (Clow et al. 2017). Humphrey et al.

(2010) postulated that the range of *I. scapularis* once covered the entire Northeastern and North-Central United States following the recession of the Pleistocene ice sheet, and the current range expansion of *I. scapularis* is a recolonization of the species' historical range. Both *I. scapularis* and *B. burgdorferi* occur in the Southeastern and Southwestern United States, but not at the same prevalence as is encountered in the Midwest and the Northeast (Halsey et al. 2018). This difference is hypothesized to be due to higher temperatures in the South in the summer months which result in increased larval mortality as a result of desiccation as well as increased of less competent reservoir hosts of *B. burgdorferi* in the South (Tietjen et al. 2019).

The life cycle of *I. scapularis* typically lasts two years and involves bloodmeals from three different hosts – one at each life stage (larva, nymph, adult) (Anderson 1989). Adult male *I. scapularis* may feed intermittently on the host prior to mating, but do not always take a third blood meal (Kocan et al. 2015). *Ixodes scapularis* presence and abundance may be impacted by the range and distribution of their animal hosts, and this tick species has been found on more than 123 host species in the Midwest, and 167 species across the United States (Halsey et al. 2018). Important hosts for the larval and nymphal stages of *I. scapularis* includes rodents, lagomorphs, ungulates, and birds (Anderson and Magnarelli 1980). The white-footed mouse (*Peromyscus leucopus*) is one of the most common mammal hosts of *I. scapularis* juveniles and is an extremely competent reservoir host of *B. burgdorferi* (Anderson 1989). Adult *I. scapularis* may also commonly be found on large to medium sized mammals such as white-tailed deer (*Odocoileus virginianus*), which serve as a tick reproductive host and provide an important blood meal for adults (Anderson and Magnarelli 1980).

Increased biodiversity may decrease disease transmission risk as diverse host communities dilute the prevalence of a pathogen (Ostfeld and Keesing 2000). When one or more animal hosts in a community are inefficient reservoir hosts for a pathogen, the presence of the pathogen can be "diluted" via infected ticks feeding on hosts that will not maintain the pathogen and thereby will not subsequently transmit pathogens to another tick (Ostfeld and Keesing 2000, Ogden and Tsao, 2009). As host diversity decreases, when suitable reservoir hosts remain, the likelihood of a tick encountering an infected host increases (Allan et al. 2003).

Anthropogenic habitat fragmentation is a process in which large contiguous habitat is divided into smaller isolated patches as a result of human development (Harris 1984). Small fragmented patches of forest often are unable to sustain diverse host communities (Wilcox and Murphy 1985). Habitat fragmentation may impact pathogen presence and prevalence in ticks by affecting what host species can thrive in fragmented patches, and therefore what hosts are present for ticks to feed on and at what level of abundance. Habitat fragmentation additionally impacts ticks by affecting host movement and range. The mean home range area of a white-tailed deer is highly dependent on life stage, seasonality, and sex and is highly uneven in distribution across the landscape but is estimated to be on average 2.31 km^2 (Quinn et al. 2013). The home range of a white-footed mouse may vary in size from 0.1 to 1.6 ha, with a population density of up to 37 mice per hectare (Timm and Howard 1994, Beer 1961). An analysis by Wolff (1985) gave a smaller estimate of the average home range at 590 m^2. Fragmentation of habitat may result in an uneven spatial distribution of reservoir hosts and the pathogens they carry (Diuk-Wasser et al. 2021). Hosts of tick-borne pathogens, such as the white-footed mouse, and the eastern chipmunk (*Tamias striatus)* are well suited for survival in small patches of fragmented forest where predator diversity and competition for resources is low, and typically reach high population densities in these fragments (Nupp & Swihart 2000). Forest edge is thought to provide an ideal habitat for deer foraging behavior as forest edge is composed of a greater variety of plants that deer feed on than plants found in forest interior (Alverson et al. 1988). Previous research found that smaller wooded areas had an increase in density of infected *I. scapularis* nymphs, but the same relationship was not seen between density of larval ticks and patch size (Allan et al. 2003).

When ticks aren't questing, they can be found burrowed into moist leaf litter where they can escape drying solar radiation, unfavorable weather conditions, and predators (Eisen et al. 2016). Unlike mosquitoes which are able to travel relatively long distances and lay eggs where ever they find standing water and a favorable microclimate, ticks are reliant on the movement of their hosts and limited by many biotic and abiotic factors including temperature, humidity, host presence, and the quality of the soil and duff layer (Eisen et al. 2016). Microhabitat refers to the small-scale environment and landscape features that ticks most directly interact with such as leaf litter and soil, while macrohabitat encompasses the larger scale features that are more likely to affect hosts such as habitat fragmentation (Randolph and Storey 1999, Diuk-Wasser et al. 2021). Optimal temperature and humidity levels for tick survival range between -10 and 35 °C and a minimum humidity level of 80% with increased mortality observed outside of these parameters (Linske et al. 2019). Prolonged exposure to suboptimal atmospheric moisture has been demonstrated to have a negative effect on the survival of *I. scapularis* nymphs and larvae (Rogers 2007). Desiccation and moisture uptake in ticks has a pronounced effect on their spatial distribution pattern and seasonal population dynamics (Hay et al. 2000). *Ixodes scapularis* colonization of a geographic area is closely associated with dense woody vegetation and amount of forest cover (Eisen et al. 2016). A thick layer of duff and fine sandy or loamy-sand textured soil provides *I. scapularis* with an ideal environment in which to survive the colder months and enables them to maintain internal water balance (Eisen et al. 2016).

Plant species composition may impact tick density and survival (Lauterbach et al 2013). For instance, when acorns are present in large crops, white-tailed deer and white-footed mouse populations increase as a result of increased access to food, which in turn results in an increase in *I. scapularis* nymph populations 1.75 years after a large acorn crop (Ostfeld et al. 2001). Oak dominated forests have a dense canopy layer that provides additional protection for the underlying vegetation as well as for ticks that would otherwise be vulnerable to the drying effects of solar radiation and the elements when questing (Guerra et al. 2002).

Ixodes scapularis ticks spend much of their time questing for hosts or avoiding desiccation and excessive solar radiation by seeking refuge in the leaf litter, or further down in the soil in the duff layer below leaf litter (Burtis et al. 2019). Soil texture affects drainage, aeration and water content (USDA, 1993). *Ixodes scapularis* ticks have previously been associated with alfisol soils and sandy or loam-sand textured soils overlying sedimentary rock (Guerra et al. 2002).

Harsh weather conditions contribute to tick mortality across all life stages and tick species (Needham and Teel 1991). Elevation may impact the long-term establishment of *I. scapularis* as elevation and flooding are closely linked due to soil at a higher elevation draining more efficiently than soil at a lower elevation (Ferrell and Brinkerhoff 2018). Ticks can survive submersion under laboratory conditions for several weeks, but their eggs cannot tolerate periodic flooding events lasting longer than 1-2 weeks and residual sediment left behind by receding flood water may bury ticks (Weiler et al. 2017).

Proximity to a river or other body of water may also influence tick abundance as animal hosts are more likely to congregate near bodies of water (Gardner et al. 2020). Migration is energetically costly, and birds commonly use stopover sites along flight paths which follow riparian corridors during spring and autumn migrations (Talbot et al. 2019). Water bodies at least 5 hectares in size are important as stopover points for bird migration and their proximity to sampling sites may be correlated to tick abundance (Talbot et al. 2019).

The continued geographic expansion of *I. scapularis* across Illinois and the greater Midwest region will likely bring with it an increase in prevalence of *B. burgdorferi s.s.* and other pathogenic agents carried by this species. Identifying habitat factors associated with *I. scapularis* abundance may be useful to identify current and future areas of increased risk for human exposure to Lyme disease and other tick-borne pathogens (Gardner et al. 2020). My analysis will closely examine the hypotheses that various aspects of habitat fragmentation (at the scale of activity for two important animal hosts, the white-footed mouse and white-tailed deer), proximity to and area of bodies of water, the proportion of soil composed of sand or loamy-sand, proportion of the sampled area composed of oak-dominated forest, climate (mean annual temperature and precipitation levels), and measures of slope and elevation are positively correlated with the abundance of adult female *I. scapularis* in Illinois during the autumn peak in tick questing activity.

Materials and Methods

Site Selection

Dragging locations on public and private land were chosen based on patient exposure interviews conducted by the Illinois Department of Public Health. For counties that reported cases of Lyme disease or other tick-borne pathogens but had no exposure data available, local parks, natural areas and other areas of outdoor recreation were chosen for sampling by examining forest cover and soil maps of Illinois.

Field Collections

Active tick collections were accomplished by drag sampling. This involves dragging a $1m^2$ of white flannel or canvas cloth attached to a wooden dowel and pulled behind the collector by a rope as they walk through appropriate forested habitat (Barbour & Fish 1993). A minimum of five transects measuring 150 meters each were dragged at each site. Occasionally, collections were extended at individual sites, with additional 150 m transects, in order to reach a minimum sample size of 25 adult female ticks per site to estimate infected tick density. Ticks were removed from the drag every 10 m and placed into a vial containing 85% ethanol every 10 m and were later identified to species by light microscopy using standard dichotomous keys (Brinton et al. 1965, Keirans and Litwak 1989, Keirans and Durden 1998, Durden and Keirans 1996). In total, from 2019 to 2021 61 sites across 44 counties were sampled for *I. scapularis* (Table 5).

Ixodes scapularis adults exhibit two peaks in activity, one each in early spring and late autumn, with nymph activity peaking from May to June (Ogden et al. 2018). Collection during these peaks is crucial to developing a relevant sample size for pathogen testing and collection efforts took place during these critical windows (Kollars et al 1999).

Spatial Analysis

A database of all autumn collection sites for female *I. scapularis* during the autumn peak in tick questing activity was compiled and loaded into ArcGIS. Moran's Index was run in ArcGIS to measure spatial-autocorrelation between sampling points to examine how clustered data points were. Each site had a minimum of five transects but could include as many as ten. Buffers with radii of 300 m and 900 m were created around the GPS sampling point for each transect. The smallest buffer used was 300 m as this represented the best minimum fit of the transect area sampled by collectors. Dragging was typically conducted by at least two collectors spread out from a central point and minimum fit was determined by the distance covered by two collectors.

For the habitat fragmentation analyses, two buffer sizes were used as an approximation of smaller- and larger-sized hosts. A buffer with a radius of 100 m was chosen as this was the smallest possible buffer that could be used to obtain useful spatial data in Fragstats. Fragstats is a software program developed to quantify landscape structure, areal extent, spatial distribution and size of patches within a landscape by examining pixels of forested versus non-forested habitat within a user defined buffer (McGarigal and Marks 1994). A buffer with a radius of 900 m was chosen to correspond to the average home range of the white-tailed deer, a common host species of *I. scapularis* adults (Timm and Howard 1994, Quinn et al. 2013). Both of the habitat fragmentation analysis buffers used may overestimate home range size of the respective species as the habitats in which these species are found can be irregular in shape and distribution across the landscape (Timm and Howard 1994, Quinn et al. 2013). All datasets were clipped to the political boundary of Illinois and projected to Albers Equal Area North American Datum 1983.

The National Land Cover Database (2016) was used to examine forest fragmentation. The database was clipped to the political boundary of the state of Illinois and was reclassified into a binary raster of forest versus non-forested area. The NLCD2016 land cover classification legend classifies 41, 42 and 43 as Deciduous Forest, Evergreen Forest, and Mixed Forest respectively. Classes 41, 42 and 43 were assigned a value of 1, and all other classes were assigned a value of 0. Fragstats was used to calculate the following metrics for forest land cover: number of patches (NP), patch density (PD), largest patch index (LPI), total edge (TE), Euclidean nearest neighbor mean, Shannon's diversity index (SHDI), Simpson diversity index (SIDI), modified Simpson diversity index, Shannon's evenness index, Simpson evenness index, modified Simpson's evenness index, core area, percentage of landscape (PLAND), and area mean (AREA_MN) (Table 1) (McGarigal and Marks 1994). The habitat fragmentation metrics chosen for this analysis correspond to metrics chosen in a previous analysis by Brinkerhoff and Ferrell (2018).

The POLARIS Soil Properties data set was chosen to examine the sand content of the soil at a resolution of 30 m (Table 2). To examine soil class, the SSURGO data set was chosen as this provides the most detailed data regarding soil class and texture at the county level. To examine how distance to a body of water might affect tick abundance, the Illinois Streams and Shorelines dataset was downloaded from the Illinois Geospatial Data Clearinghouse and the Illinois Wetlands Geodatabase was downloaded from the National Wetlands Inventory (Table 2). These datasets were combined and run simultaneously in ArcGIS. To examine whether areas consisted mostly of oak-dominated forest types, the LANDFIRE (Fuel Vegetation Cover 2021) database was converted to forest, non-forest, and oak dominated forest classes (Table 2). Jon Schwegman's Natural Divisions of Illinois delineates geographic regions of the state containing similar landscapes and habitats based on soil, glacial history, bedrock, plants, and topography (Schwegman et al. 1973). To determine in which of Schwegman's Natural Divisions of Illinois a transect was located, transects were plotted in a county line political map of Illinois in ArcGIS and manually assigned to division. To examine variation in elevation and hillslope position, a topographic position index derived from a digital elevation model was used (Weiss 2001). Topographic diversity is a surrogate variable used to examine fluctuations in temperature (continuous heat-insolation load index) and local moisture conditions derived from a digital elevation model (Theobald et al. 2015). Annual mean temperature and precipitation layers were extracted from Google Earth Engine (Hijmans et al. 2005) (Table 2).

A topographic position index was used to examine elevation as this provides a more detailed analysis of slope and elevation within a specified cell or buffer by averaging elevation values derived from a digital elevation model (Weiss 2001). Topographic diversity provides information about temperature and moisture conditions as local habitats and higher values may indicate greater vegetational and environmental diversity (Theobald et al. 2015).

Statistical Analyses

A dataset at the transect level for collections performed during the autumn of 2019 and 2020 was created with abundance data on female *I. scapularis*, and values for the environmental variables. The choice was made to focus only on females collected during the autumn to prevent any confounding effects from varying environmental influences on other sexes or life stages during different seasons.

Explanatory variables were inspected for collinearity by inspecting pairplots and correlation coefficients for all combinations of variables. Variance inflation factors were then calculated and used to sequentially drop environmental variables that were too closely correlated based on the calculated variance inflation factors (VIF cutoff value > 5) (Zuur et al. 2009).

Environmental and habitat fragmentation variable datasets were inspected for outliers and outliers were dropped, and certain variables were log, square, or square root transformed to reduce skew (Table 3). All variables included in each model are reported in Table 4. Metrics relating to habitat fragmentation that were dropped due to collinearity in the 100 m model include number of patches (NP), patch density (PD), largest patch index (LPI), total edge (TE), Euclidean nearest neighbor mean, Shannon's diversity index (SHDI), Simpson diversity index (SIDI), modified Simpson diversity index, Shannon's evenness index, Simpson evenness index, modified Simpson's evenness index, core area, percentage of landscape (PLAND), and area mean (AREA_MN). Topographic diversity index (TDIV) and proportion of the soil composed of sand were also dropped from the 300 m model due to collinearity with other variables. Metrics relating to habitat fragmentation that were dropped due to collinearity in the 900 m model include patch density (PD), total edge (TE), edge density (ED), area weighted mean (AREA_AM), Shannon's Diversity Index (SHDI), percentage of landscape (PLAND), largest patch index (LPI) and Simpson's Diversity Index (SIDI). Environmental variables dropped from the 900 m model include percent sand mean, topographic position index (TPI), majority tree species and water distance variables due to collinearity with other variables in the model.

The Moran's spatial autocorrelation report returned a Moran's value of 0.890, a p-value of <0.001 and a z-score of 4.47, indicating the data was clustered and indicating the need to account for spatial autocorrelation in the analysis. Data was analyzed using a spatial Generalized Additive Model (GAM) with the 900 m spatial scale analyzed separately as its own model. A separate model was created using the smaller buffers (100 m for habitat fragmentation variables and 300 m for environmental variables). The smaller buffers were analyzed together to examine tick abundance at the scale most likely to correlate to habitat fragmentation effects on a white-footed mouse's average home range, and the environmental aspects of the area sampled by collectors. A negative binomial distribution was used to model the abundance of female *I. scapularis* as many sites were negative for their presence (i.e. a large proportion of zeroes in the dataset). Because abundance of ticks could be affected both by the location of a transect in relation to other collection sites, as well as a spatial invasion process (e.g., collections at northern latitudes being more productive due to a longer history of establishment), it was necessary to account for spatial structure in the model. In spatial GAMs this is accomplished by including an interaction term (or smooth) for latitude and longitude. Additionally, a GAM model will fit data using an REML (restricted maximum likelihood) algorithm to determine best fit of the line (Zuur et al. 2009). A full model consisting of all the variables left following the inspection for collinearity was then fit to the data, and non-significant terms removed one at a time, and models compared by their AIC score, to arrive at a best model. The final GAM at the 900 m spatial scale included number of patches (NP), area mean (AREA_MN), proportion of the soil composed of loamy-sand, proportion of the soil composed of sand, mean annual precipitation, mean annual temperature, topographic diversity index (TDIV), Schwegman's Natural Divisions, year, and latitude and longitude (Table 4). The final GAM of the 100 m habitat fragmentation analysis and 300 m spatial scale included topographic position index (TPI), proportion of the soil composed of loamy-sand, sand percent mean, majority tree species, distance to the nearest body of water, body of water area, mean annual precipitation, mean annual temperature, Schwegman's Natural Divisions, year, and latitude and longitude (Table 4).

Results

Over the course of the study period a total of 283 adult female *I. scapularis* were collected. The occurence of *I. scapularis* was established in 28 of 44 sampled counties across Illinois (Figures 7 and 8). The abundance of *I. scapularis* at a site was most strongly correlated with number of patches ($p=<0.001$) and mean area (AREA_MN) ($p=<0.001$) using a buffer with a radius of 900 m (Table 4, Figure 1). At 900 m, proportion of the soil composed of sand was found to be significant ($p= 0.009$) (Figure 2). Schwegman's Natural Divisions 1 (Wisconsin Driftless), and 8 (Middle Mississippi Border) were also significantly correlated with adult female *I. scapularis* abundance (Table 4). Topographic diversity index (TDIV) ($p= <0.002$) and mean annual precipitation (mm) ($p= 0.012$) were also significantly correlated with adult female *I. scapularis* abundance (Table 4, Figures 3 and 4). All fragmentation and environmental variables examined in the 900 m buffer along with their significance can be found in table 4. The r^2 value for the 900 m model was 0.455.

The smaller scale model showed that mean percent of the soil composed of sand, body of water area, and temperature were not significantly correlated with *I. scapularis* abundance at the this scale (100 m + 300 m) (Figures 5 and 6). Non-forest and non-oak dominated forest types (majority tree species) were found to be significantly correlated with *I. scapularis* abundance ($p= 0.030$). None of Schwegman's Natural Divisions were correlated with *I. scapularis* abundance in this model (Table 4). The r^2 value for this model was 0.588.

Several sites were negative for the presence of *I. scapularis* despite the presence of oak dominated forests, bodies of water, and soil of textures associated with *I. scapularis* abundance in prior analyses. Distance from the start of sampled transects to the nearest permanent body of water varied from a river in Henderson County located 1121m away from a transect, to another river in Lee County located 40 m away from a transect. Permanent bodies of water near a transect ranged in size from a lake measuring 4827 acres in DeWitt County, to a fresh water pond measuring 0.1708 acres in Putnam County. Elevation ranged in height from the highest at 292.5m at a site located in Stephenson County, to the lowest at 117.7m at a site in Wabash County. Rather than proximity to a body of water being associated with *I. scapularis* abundance, at a radii of 300 m, the inverse relationship was found, where *I. scapularis* were more likely to be found in abundance further from a body of water near a transect ($p = 0.006$) (Figure 5). Spatial coordinates (latitude x longitude) were found to be the most significant predictor of *I. scapularis* abundance in the 900 m model (Figures 7 and 8). Female *I. scapularis* were most abundant in northern counties in Illinois (Figure 7). All counties sampled during the autumn peak and the density of ticks per 1000 m^2 are presented in Table 5.

Discussion

As number of patches (NP) present increased and mean patch area (AREA_MN) decreased, *I. scapularis* abundance increased at a site at the 900 m buffer scale. Mean proportion of the soil comprised of sand and topographic diversity were also positively correlated with *I. scapularis* abundance at a site at the 900 m buffer scale. These findings are similar to a previous analysis by Allan et al. 2003 which found increased density of nymphal *I. scapularis* in smaller forest fragments compared to larger fragments. Sand influences soil drainage and sandy and loam-sand textured soils have previously been associated with *I. scapularis* abundance (Guerra et al. 2002). Annual mean precipitation on *I. scapularis* abundance was examined and determined to be significant only at the scale of the 900 m buffer. Tick abundance was found to be significantly lower in Schwegman's Natural Divisions 2 (Rock River Hill Country), 3 (Northeastern Morainal) and 4 (Grand Prairie) compared to Natural Division 1 (Wisconsin Driftless Area) and 8 (Middle Mississippi Border division) (Table 4).

Temperature and precipitation datasets were also added to the models and mean annual precipitation levels between 900-950 mm were found to influence tick abundance. These variables were examined as climate and precipitation have been tied to tick survival, but only mean annual precipitation (mm) and topographic diversity (a metric encompassing local temperature and moisture conditions) was found to be correlated with tick abundance at the 900 m scale. Optimal temperature and humidity levels are strongly correlated with tick survival, and prolonged exposure to low humidity conditions is linked to increased tick mortality (Linske et al. 2019, Rogers 2007).

Abundance of *I. scapularis* was also found to be affected by distance away from a body of water at a radius of 300 m (with higher tick numbers predicted with increasing distance), as well as with non-forest and non-oak dominated forest (with areas predominantly characterized as “non-forest” having a higher abundance). Previous studies have found a strong correlation between oak dominated forests and *I. scapularis* abundance (Ostfeld et al. 2001). As the landscape analysis in Fragstats found that forest habitat in Illinois was extremely patchy and patches tended to be small in size, it is possible this finding is the result of the buffer picking up non-forest cells.

White-footed mice occupy smaller habitat ranges than white-tailed deer and at a higher population density than white-tailed deer (Timm and Howard 1994, Quinn et al. 2013). Habitat fragmentation metrics that were included in the GAM were not included in the final model, indicating that at this scale, these metrics did not correspond to *I. scapularis* abundance due to the small size of the home range and habitat fragmentation effects impacting host movement on larger scales.

The 900 m model encompassed a broader area and provided more information about a larger swath of the sampled habitat than the smaller 100 m + 300 m model meant to examine tick abundance at the scale of the sampled area and the scale most relevant to a white-footed mouse. Environmental factors at both the micro and macro scale impact tick abundance. A buffer size corresponding to the habitat range of a white-tailed deer represents the macro-scale and my results indicate that at this scale the factors most strongly correlated with *I. scapularis* abundance include number of patches and mean patch size. Patches are dynamic environmental units quantified by the landscapes in which they are found, and landscapes themselves are units within larger landscapes (McGarigal and Marks 1995). Forest fragmentation reduces average patch size and the total area of habitat within a landscape (Murcia 1995). Both patch size and proximity to a body of water are macro scale factors which impact the tick reproductive host movement more than the movement and survival of the tick itself. In my analysis, forest area composed of "non-forest" (landcover classes not categorized as forest) was found to be more significantly correlated with *I. scapularis* abundance than oak-dominated forest. Previous analyses have found the inverse relationship, with oak-dominated forests strongly predicting the presence of *I. scapularis* due to the impact of acorn masting on deer activity (Ostfeld 1997). One reason for this could be that the area within the buffer encompassed a large portion of un-forested area as many of the transects occurred on or near the forest edge.

White-tailed deer and migrating birds carry ticks across long distances and have played important roles in the distribution of other invasive tick species such as *Haemaphysalis longicornis* in North America (Raghavan et al. 2019). Ticks rely on host movement to colonize new habitat and for transport across otherwise impassable landscape features such as mountains and bodies of water (Raghavan et al. 2019). Birds are important in dispersing *I. scapularis* across vast distances and on average infestation of northward migrating land birds from the United States was found to be 1.66 ticks per bird, or an estimated 50 to 175 million *I. scapularis* ticks dispersed across Canada each spring (Ogden et al. 2008). Migratory birds are more likely to host ticks than resident birds and are efficient reservoirs of *B. burgdorferi s.s.* (Rand et al. 1998). Human driven land use and land cover changes such as deforestation for agricultural purposes resulting in habitat fragmentation, influence host movement and are key drivers of the range expansion of *I. scapularis* (Diuk-Wasser et al. 2021). The longer birds spend at a migration stopover site and the more diverse species presence is, the more likely ticks are to be deposited in novel territory (Hoogstraal et al. 1963). Forest fragmentation and land use change have been well documented to reduce species diversity in both mammals and birds in forest patches, but experimental manipulation of water bodies and their impact on tick hosts and tick presence has not (Blake and Karr 1987).

The northern-most counties in Illinois reported the highest abundance of *I. scapularis* compared to sites sampled further south. Schwegman's Natural Divisions provide information about historical patterns of glaciation in Illinois and delineates geographic regions of the state containing similar landscapes and habitats based on soil, glacial history, bedrock, plants, and topography (Schwegman et al. 1973). The three divisions least correlated with abundance of *I. scapularis* were Rock River Hill Country, Northeastern Morainal and Grand Prairie compared to the Wisconsin Driftless area and the Middle Mississippi Border division which had a higher abundance of *I. scapularis* (Table 4). The Wisconsin Driftless region remained unglaciated during the Pleistocene glaciation and was historically covered by hardwood forests dominated by black, red and white oak, sugar maple, and basswood. The weakly to moderately developed soils of the Wisconsin Driftless region are composed of windblown loess, flood deposits and disintegrated rock and the bedrock is primarily Ordovician and Silurian limestone, dolomite and shale with lead deposits. The Middle Mississippi Border division has both glaciated and unglaciated sections and well developed and well drained loess derived soil covering limestone and sandstone bedrock. The vegetation in this region is mesic and dry with forests dominated by black and white oak. The abundance of *I. scapularis* was high in these regions of the state likely due to remnant populations preceding or remaining untouched by periods of historic glaciation, as well as increased presence of sandy and loamy-sand textured well drained soil compared to patterns of glaciation in other regions, and dominant soil types (Humphrey et al. 2010, Guerra et al. 2002).

As the range of *I. scapularis* is expected to continue expanding from the northern to southern counties of Illinois (Gardner et al. 2020), negative sites should be revisited and re-sampled to document the arrival and possible establishment of this species. Local abundance of *I. scapularis* is a measure of risk of exposure to Lyme disease and is dependent on the range expansion of this species and a variety of biotic and abiotic factors affecting life history traits of both vector and host (Eisen et al. 2018). The further distribution and establishment of *I. scapularis* across Illinois and the greater Midwest has the potential to increase incidence of Lyme disease and other emerging tick-borne diseases. Vector surveillance and pathogen testing and identification are important in preventing tick-borne disease, and awareness of where a pathogen is present in a geographic region enables healthcare providers to better diagnose patients who present with symptoms of tick-borne disease. Identifying key environmental features associated with *I. scapularis* can help target surveillance efforts.

Other factors that have been previously linked to *I. scapularis* abundance but not examined in my analysis include distance to a road or major transportation route which been noted to have an impact on density of *I. scapularis* and prevalence of infection with *B. burgdorferi*. Roads may facilitate the dispersal of *I. scapularis* ticks as roads fragment habitat and create wildlife corridors and would be useful to incorporate into a future analysis (Talbot et al 2019). Snow can also act as insulation and the experimental removal of snow covering burrowed *I. scapularis* nymphs may result in a decreased survival rate (Linske et al. 2019). Additionally, Salkeld et al. 2021 in California encountered infected *Ixodes pacificus* ticks in unexpected habitats such as beach areas and coastal chaparral even in the absence of obvious reservoir hosts, indicating the possibility that other habitat types might be under sampled due to bias in site selection.

My analysis examined the impact of soil texture, elevation, tree species, climate, body of water area and proximity, natural divisions and habitat fragmentation metrics at two scales chosen to correspond to the average home ranges of two animal hosts pertinent to the life cycle of *I. scapularis*. Future analyses should include habitat features such as proximity to roads to create a more detailed analysis of the environment in which ticks are collected, as well as examining novel or under-sampled habitat. Inclusion of these abiotic factors in a future model may better predict areas of future *I. scapularis* establishment.

Tables and Figures

Landscape Metric	**Definition**	**Application**
Number of patches (NP)	equals the number of patches of the corresponding patch type.	Number of patches.
Patch density (PD)	equals the number of patches of the corresponding patch type (NP) divided by total landscape area, multiplied by 10,000 and 100 (to convert to 100 hectares).	Expresses number of patches on a per unit area basis.
Largest patch index (LPI)	equals the percentage of the landscape comprised by the largest patch.	Percentage of the landscape comprised by the largest patch.
Total edge (TE)	equals the sum of the lengths (m) of all edge segments in the landscape.	Represents the amount of border between patches.
Euclidean Nearest Neighbor Mean (ENN_MN)	ENN equals the mean distance (m) to the nearest neighboring patch of the same type, based on shortest edge-to-edge distance.	Measures the distance to the nearest neighboring patch of the same class from edge to edge.
Shannon Diversity Index (SHDI)	equals minus the sum, across all patch types, of the proportional abundance of each patch type multiplied by that proportion.	A measure of diversity present in the landscape.
Simpsons diversity index (SIDI)	equals 1 minus the sum, across all patch types, of the proportional abundance of each patch type squared.	Represents the probability that any two random patch types selected at random will be different types.
Percentage of landscape (PLAND)	equals the sum of the areas (m2) of all patches of the corresponding patch type, divided by total landscape area (m2), multiplied by 100 (to convert to a percentage); in other words, %LAND equals the percentage the landscape comprised of the corresponding patch type.	An area and edge metric that measures the percentage of landscape comprised of the corresponding patch type. A measure of the amount of landscape composed of a particular patch type and the proportion of pixels within the buffer composed of forest.
Area mean (AREA_MN)	equals the sum, across all patches in the landscape, of the corresponding patch metric values, divided by the total number of patches. MN is given in the same units as the corresponding patch metric.	describes the composition of the landscape and patch structure (many small patches or few large patches)

Table 1. FRAGSTATS metrics, their manual definitions and description of metric applications (MacGarigal and Marks 1995).

Environmental Factor	**Database**
Topographic Diversity Index (temperature and moisture)	U.S. Geological Survey
Topographic Position Index (Elevation)	Weiss, AD. 2001. Topographic position and landforms analysis.
Natural Divisions	Schwegman's Natural Divisions of Illinois – Illinois Natural History Survey
Distance to and body of water area	The Illinois Streams and Shorelines (1994), U.S. Fish & Wildlife Service (2019) National Wetlands Inventory Wetlands Mapper.
Dominant Tree Species	LANDFIRE Database (Fuel Vegetation Cover 2021)
Weather (Annual mean temperature and annual mean precipitation)	WorldClim V1 Bioclim (Google Earth Engine)
Habitat fragmentation	The National Land Cover Database (2016)
Soil (% sand, soil texture)	POLARIS Soil Properties, SSURGO

Table 2. Environmental factors examined in this analysis and data origin.

900 m Model	**Transformation**
Number of patches (NP)	sqrt
Area mean (AREA_MN)	log
Mean annual precipitation (mm)	none
Mean annual temperature (°C)	none
Proportion of loamy-sand	none
Proportion of sand	none
Topographic diversity index (TDIV)	none
Schwegman's Natural Divisions	none
300 m + 100 m Model	**Transformation**
Schwegman's Natural Divisions	none
Majority Tree	none
Sand % Mean	log
Water Distance	log
Water Body Size	log
Proportion of loamy-sand	none
Topographic point index (TPI)	log
Mean annual precipitation (mm)	none
Mean annual temperature (°C)	none

Table 3. Complete list of environmental and habitat fragmentation factors analyzed in both models with their respective transformations.

Variable - 300 m model	Estimate	z- value	p-value
Year (2020)	-0.43	-1.3	0.192
Swegman’s Natural Division 2	0.95	0.429	0.667
Swegman’s Natural Division 3	3.66	0.835	0.403
Swegman’s Natural Division 4	0.51	0.118	0.905
Swegman’s Natural Division 5	1.91	0.402	0.687
Swegman’s Natural Division 7	1.71	0.391	0.696
Swegman’s Natural Division 8	1.23	0.277	0.782
Swegman’s Natural Division 10	-0.40	0	1
Swegman’s Natural Division 11	-1.60	-0.288	0.773
Majority tree species	-0.36	-2.16	0.03

Variable - 300 m model	edf	chi-sq	p-value
Latitude x longitude (spatial coordinates)	15.062	25.275	0.105
Distance to a body of water (m)	1	7.393	0.006
Body of water size (acres)	3.757	8.83	0.092
Proportion of loamy-sand	1	1.131	0.287
Sand percent (mean)	2.405	3.266	0.347
Topographic point index (TPI)	1	2.506	0.113
Mean annual precipitation (mm)	4.36	5.443	0.399
Mean annual temperature (°C)	5.91	14.68	0.058

Variable - 900 m model	Estimate	z-value	p-value
Year (2020)	-0.51	-1.718	0.085
Swegman’s Natural Division 2	-3.3	-2.871	0.004
Swegman’s Natural Division 3	-5.64	-2.951	0.003
Swegman’s Natural Division 4	-3.7	-2.135	0.032
Swegman’s Natural Division 5	-1.84	-0.904	0.365
Swegman’s Natural Division 7	-2.16	-1.189	0.234
Swegman’s Natural Division 8	0.035	0.019	0.984
Swegman’s Natural Division 10	-0.52	0	1
Swegman’s Natural Division 11	-4.02	-1.868	0.061

Variable - 900 m model	edf	chi- sq	p-value
Latitude x longitude (spatial coordinates)	4.751	13.743	0.025
Number of patches (NP)	3.288	27.32	0.000
Mean area (AreaMN)	1	24.758	0.000
Proportion of loamy-sand	1	3.578	0.058
Proportion of sand	1	6.811	0.009
Topographic diversity index (TDIV)	4.785	30.805	0.000
Mean annual precipitation (mm)	1	6.223	0.012
Mean annual temperature (°C)	5.591	9.18	0.103

Table 4. All variables examined across both models and the associated p-values, z-values, chi-sq, and edf (effective degrees of freedom, a value of 1 indicating a linear relationship and greater values indicating a non-linear relationship) values of each variable in its relation to the abundance of adult female *I. scapularis.*

Collection County	**Total of adult *I. scapularis* females collected**	**Number of transects dragged**	**Estimated density per 1000 m^2**
Adams	0	10	0
Boone	3	5	4
Brown	2	10	1.333
Bureau	0	5	0
Carroll	26	10	17.333
Cass	7	10	4.667
Champaign	0	5	0
Clark	7	25	1.867
Coles	6	20	2
Crawford	0	5	0
DeKalb	0	10	0
DeWitt	4	10	2.667
Edgar	0	5	0
Ford	0	5	0
Fulton	1	5	1.333
Henderson	15	12	8.333
Jefferson	0	5	0
Jo Daviess	31	15	13.778
LaSalle	14	10	9.333
Lee	15	15	6.667
Logan	16	5	21.333
Marion	0	10	0
Morgan	0	6	0
Moultrie	0	10	0
Ogle	30	10	20
Peoria	14	30	3.111
Pike	0	5	0
Putnam	8	12	4.444
Rock Island	3	5	4
Sangamon	0	10	0
Stephenson	2	15	0.889
Wabash	0	5	0
Warren	2	5	2.667
Whiteside	2	15	0.889
Will	8	15	3.556
Winnebago	13	15	5.778
Woodford	4	10	2.667

Table 5. All counties sampled during the autumn collection peaks and tick density per 1000 m^2.

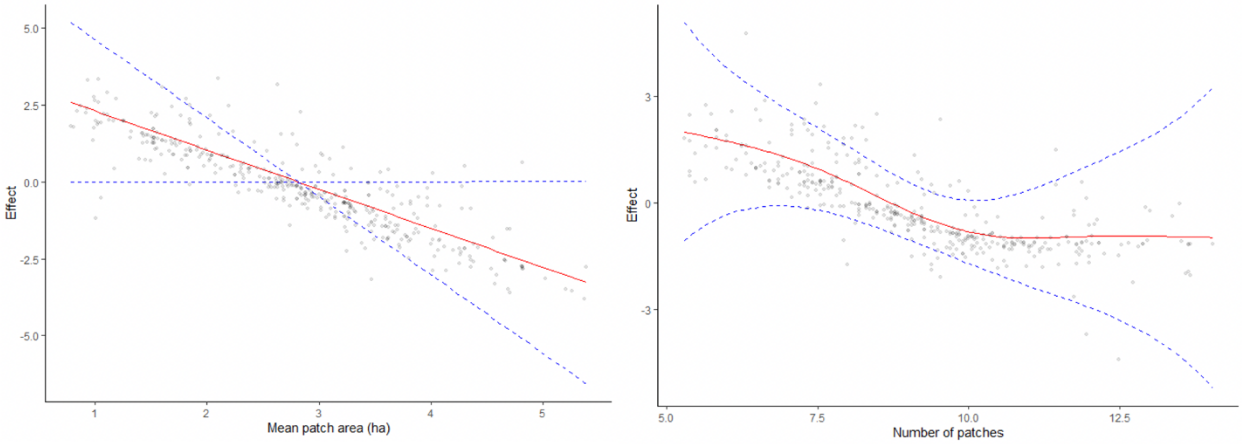

Figure 1. Smooth plots showing adult female *I. scapularis* abundance (y-axis) as a function of mean patch area (ha) and number of patches (5 – 20) per 150 m transect using a 900 m buffer. Blue lines represent confidence intervals (5 standard errors). Mean tick abundance centered at 0 on the y-axis.

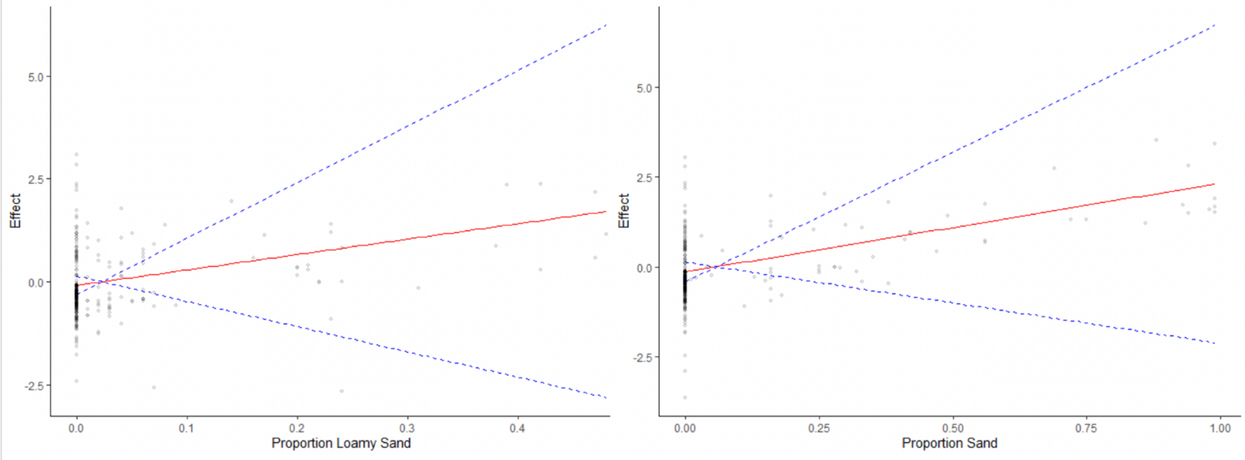

Figure 2. Examination of the relationship between proportion of the soil composed of loamy-sand and proportion of the soil composed of sand on adult female *I. scapularis* abundance per 150 m transect using a 900m buffer. Blue lines represent confidence intervals (5 standard errors).

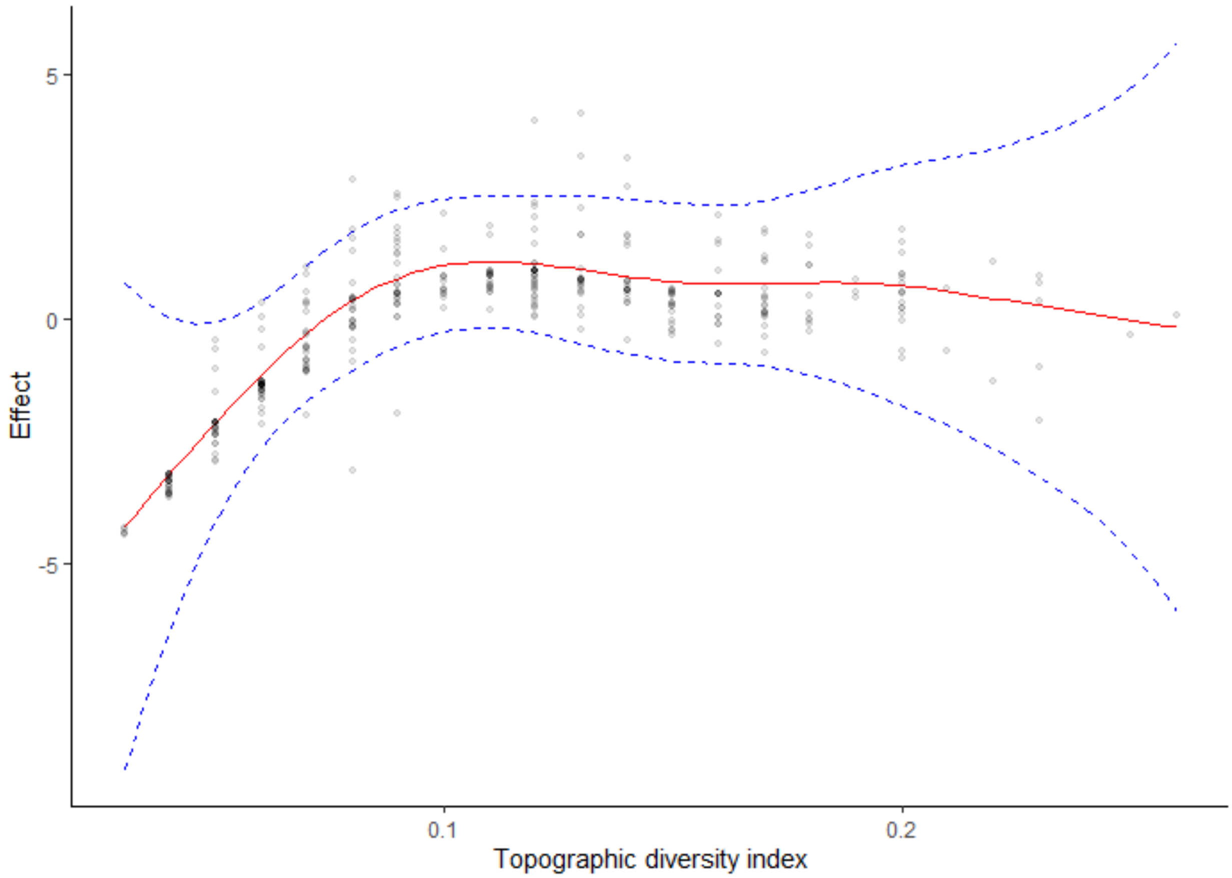

Figure 3. Topographic diversity index effect (mean centered at 0 on y-axis, and TDIV (predictor variable) on y-axis) on adult female *I. scapularis* abundance per 150 m transect using a 900m buffer. Blue lines represent confidence intervals (5 standard errors). Mean tick abundance centered at 0 on the y-axis.

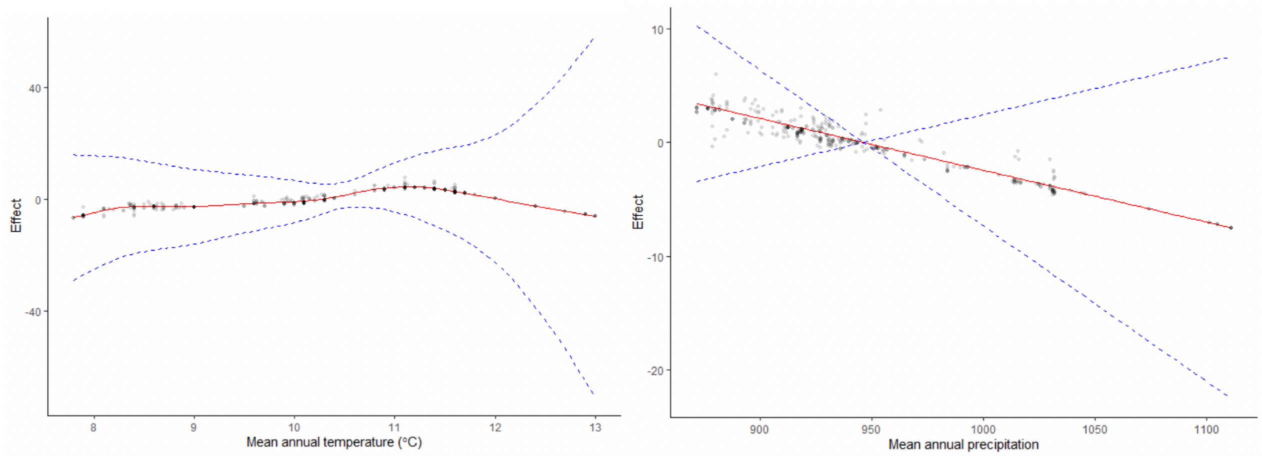

Figure 4. Effect of mean annual temperature (°C) and mean annual precipitation (mm) (x-axes) on *I. scapularis* abundance per 150 m transect using a 900m buffer. Blue lines represent confidence intervals (5 standard errors).

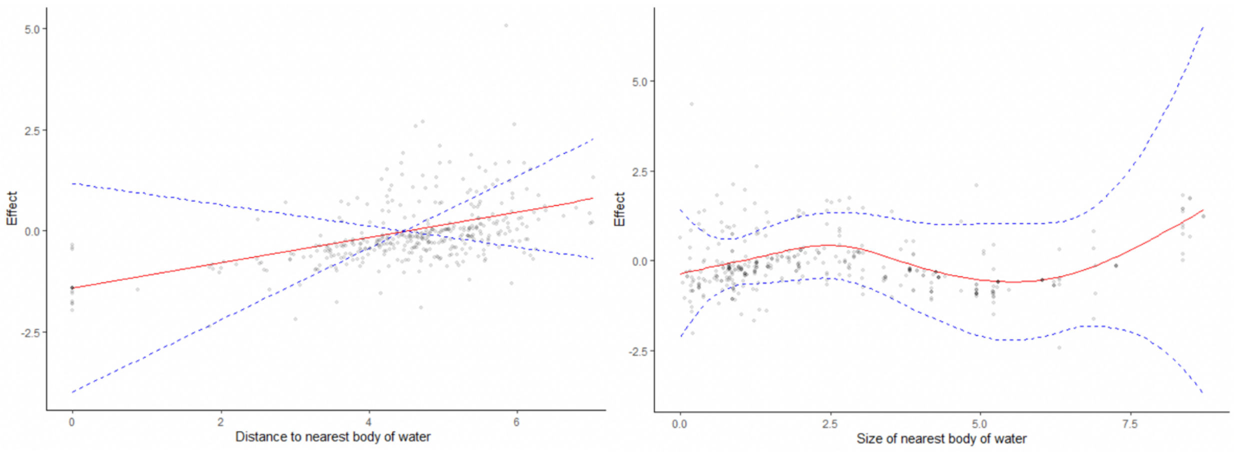

Figure 5. Distance from a transect to the nearest body of water (m) and area of the nearest body of water (acres) on adult female *I. scapularis* abundance per 150 m transect using a 300 m buffer. Blue lines represent confidence intervals (5 standard errors). Mean tick abundance centered at 0 on the y-axis.

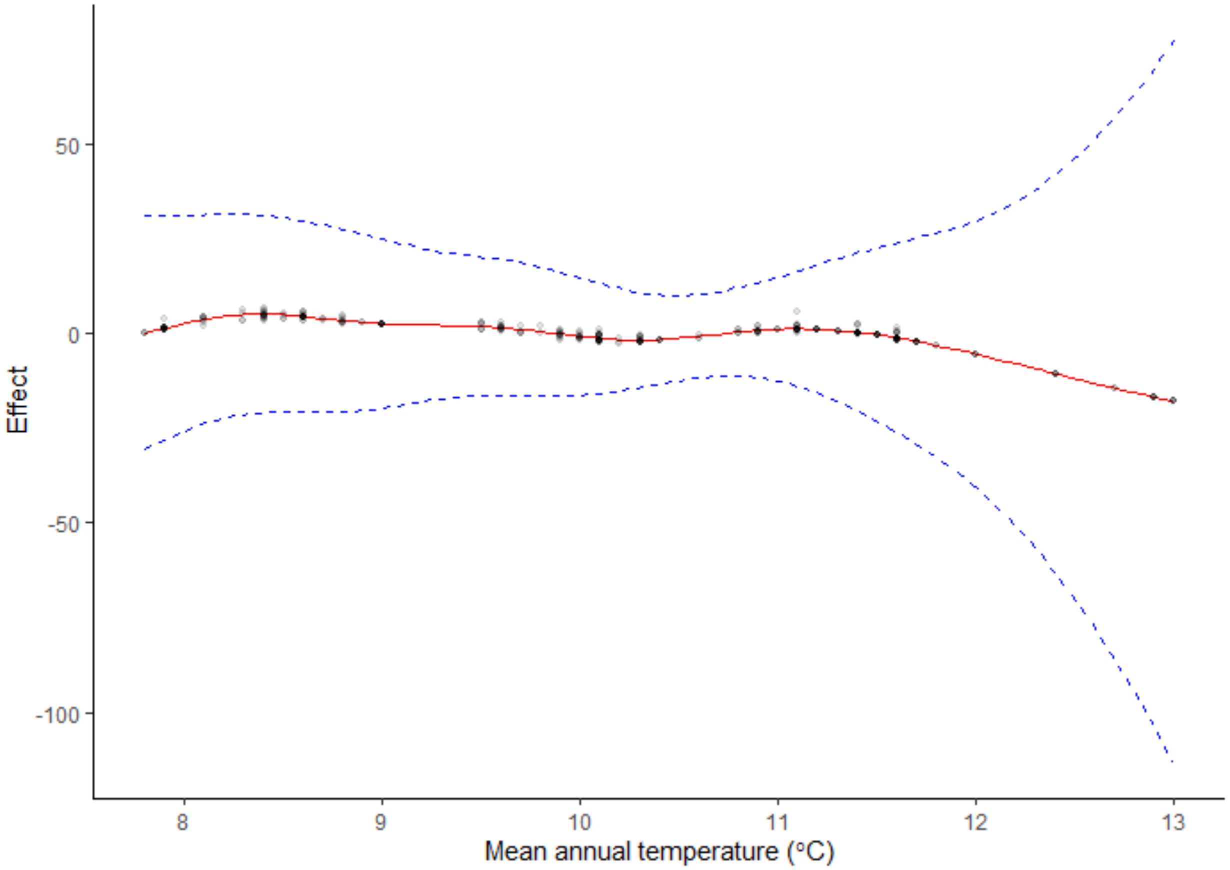

Figure 6. Influence of mean annual temperature in °C on adult female *I. scapularis* abundance per 150 m transect using a 300 m buffer. Mean tick abundance centered at 0 on y-axis, and predictor variable temperature range on x-axis. Blue lines represent confidence intervals (5 standard errors).

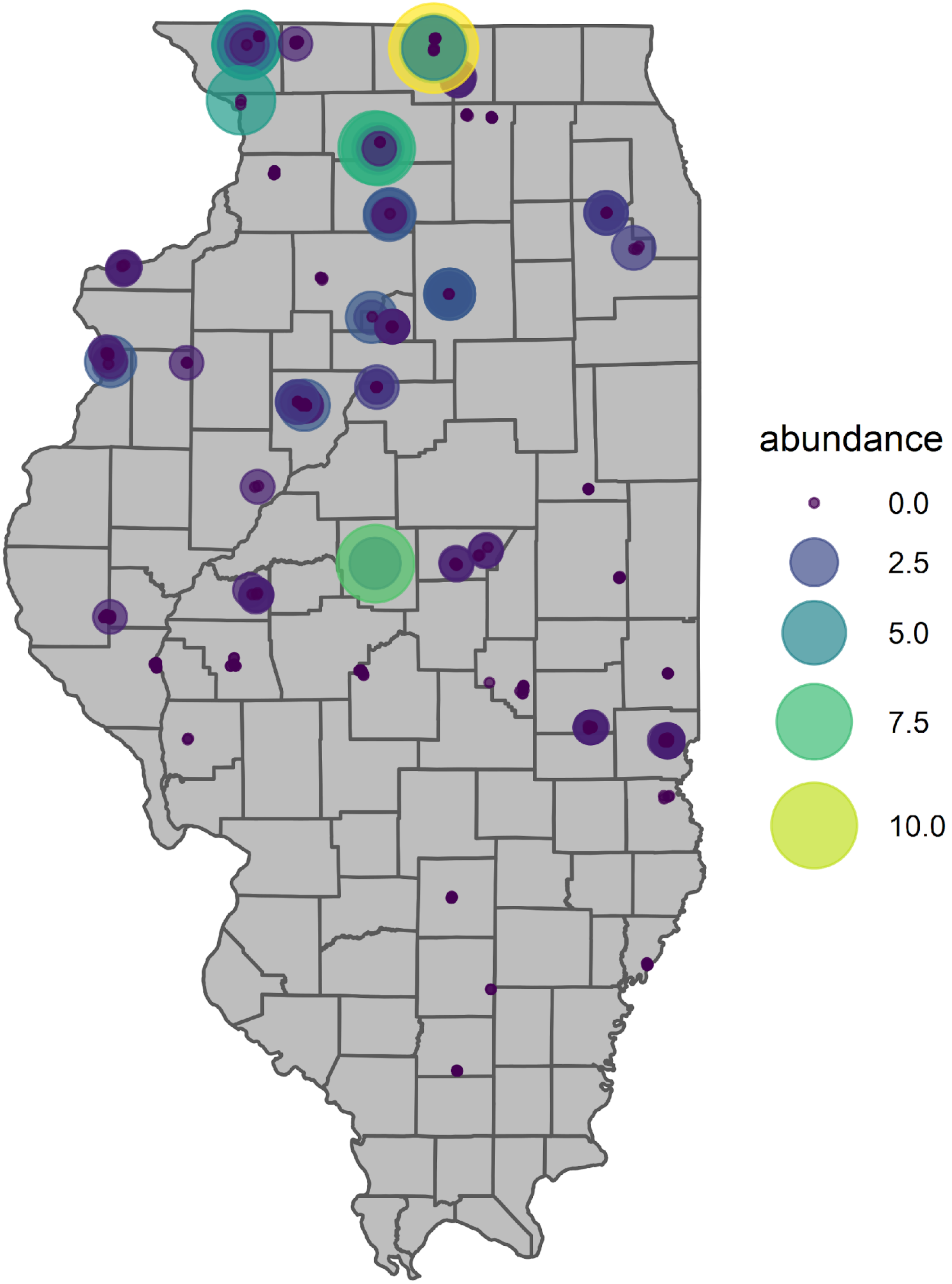

Figure 7. Tick collection locations sampled across 2019-2020 study period showing the absence (0 ticks) or abundance (>10 ticks) of adult female *I. scapularis* collected overall in each county.

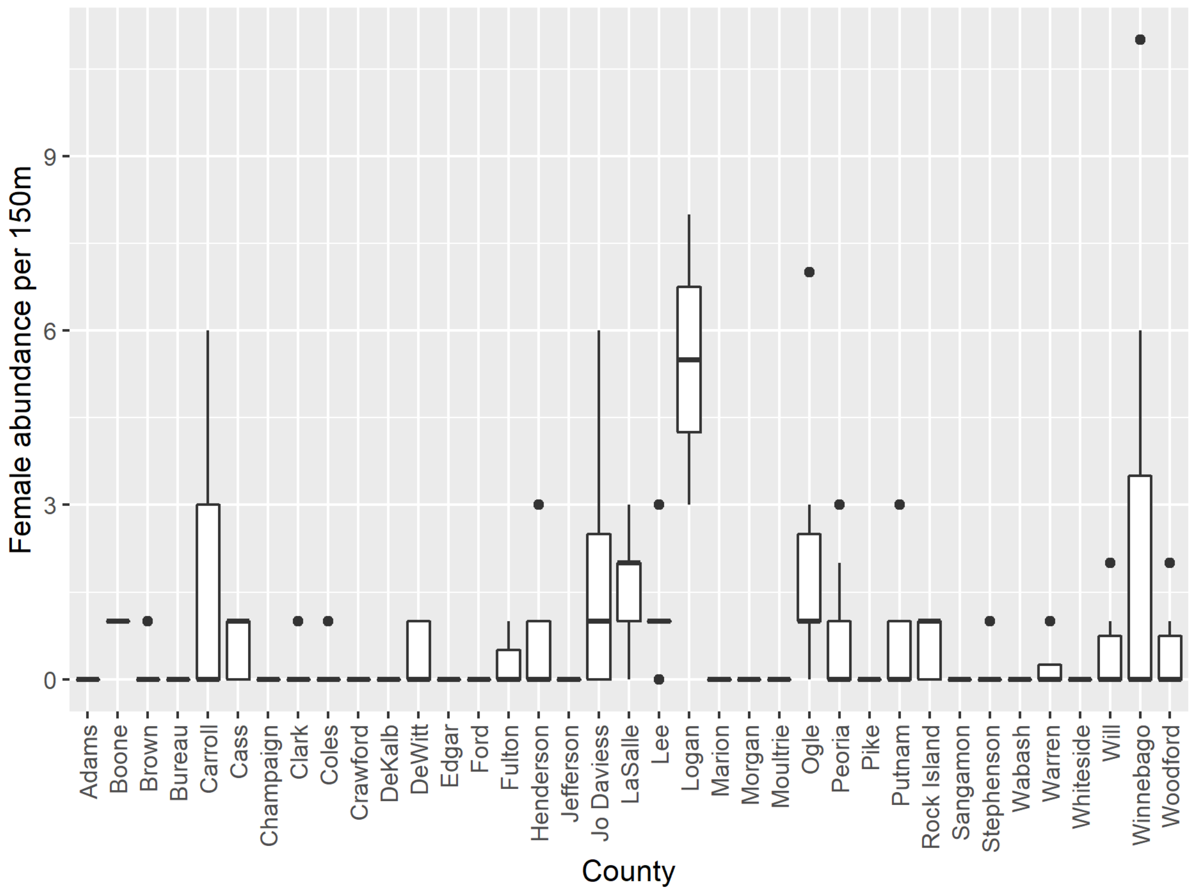

Figure 8. Abundance of adult female *I. scapularis* per sampled transect (150m) by county in Illinois.

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