URBAN TICK ECOLOGY IN OKLAHOMA CITY: TICK DISTRIBUTION, PATHOGEN PREVALENCE AND AVIAN INFESTATION ACROSS AN URBANIZATION GRADIENT

By

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Abstract: Urbanization has been linked to the emergence and increased prevalence of many vector-borne diseases, including tick-borne diseases. Despite the growing public health importance of tick-borne diseases, little is known about how they are influenced by urbanization in North America, especially in the central U.S. where several pathogens occur at or near their highest levels of incidence nationally. To understand how urbanization effects tick-borne disease, we investigated tick distribution, tick-bone pathogen prevalence, and tick-host interactions across a gradient of urbanization in Oklahoma City, Oklahoma, USA. At 16 parks and greenspaces we collected ticks using CO_2 traps and flagging, measured temperature and humidity during tick sampling, conducted vegetation surveys, and used trail cameras to estimate an index of deer abundance. Adult ticks were tested for Rickettsia sp., Ehrlichia sp., Anaplasma sp., Borrelia sp., and *Francisella tularensis*. Additionally, we mist-netted birds during the breeding season, sampled them for ticks. Our results indicate there is a risk of encountering ticks and pathogens across the entire urbanization gradient from exurban areas to the urban core, although some tick species and pathogens (*Dermacentor variabilis* and *Ehrlichia ewingii*) may be less common in intensely urbanized areas. We also found that vegetation, temperature, and moisture variables predict tick abundance and that these correlates vary among species. The index of deer abundance was positively correlated with *A. maculatum* and *D. variabilis* abundance but unrelated to *A. americanum* abundance, but urbanization intensity did not influence either the proportion of birds with ticks or the tick load of infested birds. Our results suggest that urban residents are at risk for encountering a tick-borne disease, and it's unlikely that deer by themselves are driving tick abundance in Oklahoma City since such a large proportion of birds were infested with ticks. Public health officials and land managers can use such information about parks and their surroundings to focus public education and land management efforts designed to reduce tick-borne disease prevalence.

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CHAPTER I

MULTI SCALE DRIVERS OF TICK DISTRIBUTION AND TICK-BORNE PATHOGEN PREVALENCE ACROSS AN URBANIZATION GRADIENT IN A MAJOR METROPOLITAN AREA IN THE U.S. GREAT PLAINS

Abstract

Urbanization alters natural processes and spatial patterns that affect tick abundance and tick-borne disease prevalence. Likely due to these changes, tick abundance and tick-borne pathogen prevalence have increased in many U.S. urban areas. Despite the growing public health importance of tick-borne diseases, little is known about how they are influenced by urbanization in North America, especially in the central U.S. where several pathogens occur at or near their highest levels of incidence nationally. To identify fine-scale and landscape-related factors influencing tick abundance and prevalence of tick-borne pathogens in urban areas, we sampled ticks at 16 parks capturing a gradient of urbanization intensity in Oklahoma City, Oklahoma, USA. We used CO_2 traps and flagging to collect ticks, measured temperature and humidity during tick sampling, conducted vegetation surveys, and used trail cameras to estimate an index of deer abundance. Adult ticks were tested for Rickettsia sp., Ehrlichia sp., Anaplasma sp., Borrelia sp., and *Francisella tularensis*. Our results indicate there is a risk of

encountering ticks and pathogens across the entire urbanization gradient from exurban areas to the urban core, although some tick species and pathogens (*Dermacentor variabilis* and *Ehrlichia ewingii*) may be less common in intensely urbanized areas. We also found that vegetation, temperature, and moisture variables predict tick abundance and that these correlates vary among species. For example, *Amblyomma maculatum* decreased with increasing woody plant and leaf litter cover while *Amblyomma americanum* and *D. variabilis* were sampled in greater numbers on hot, dry days. The index of deer abundance was positively correlated with *A. maculatum* and *D. variabilis* abundance but unrelated to *A. americanum* abundance. Public health officials and land managers can use such information about parks and their surroundings to focus public education and land management efforts designed to reduce tick-borne disease prevalence.

Introduction

Urban intensification and encroachment into surrounding rural areas cause substantial land-use and land-cover changes that are strongly connected to the prevalence and emergence of infectious diseases worldwide (Patz et al. 2000; Foley et al. 2005; Bradley and Altizer 2007). In fact, urbanization has been identified as the second most important land-use process affecting infectious disease occurrence globally, behind only agricultural development (Patz et al. 2004). Tick-borne diseases, in particular, have been increasing in prevalence in many urban areas of the United States, where 95% of all cases of vector-borne disease are caused by ticks, and over 81% of the human population lives in urban areas (Maupin et al. 1991; Steere 1994; Jobe et al. 2007; Schwan et al. 2009; Rydzewski et al. 2012; Blanton et al. 2014; United Nations 2014; Paddock et al. 2016; Rosenberg et al. 2018). Despite the increasing importance and emergence of tick-borne

diseases, they remain poorly understood across much of the world, especially in urban settings (LaDeau et al. 2015).

Landscape epidemiology describes how pathogens, hosts, and vectors interact temporally and spatially to facilitate disease transmission in a suitable environment (Reisen et al. 2010), and our ability to predict tick-borne disease emergence requires an understanding of how environmental changes like urbanization affect the pathogen-host-vector relationship (LaDeau et al. 2015). Urbanization changes almost all components of natural ecosystems including abiotic conditions (e.g., humidity, temperature, light), vegetation (e.g., structural and spatial heterogeneity, plant diversity and community composition), and wildlife populations and communities (e.g., density, diversity, species composition, spatiotemporal patterns of activity; Aronson et al. 2014; McDonnell and MacGregor-Fors 2016; Lepczyk et. al 2017). Several studies also show that populations of arthropods (e.g., bees, butterflies, spiders) respond to urbanization and/or vary in abundance and diversity in relation to urban-to-rural gradients (Shochat et al. 2004; Clark et al. 2007; Ahrne et al. 2009). Therefore, patterns of urbanization are also likely to influence tick populations, and potentially, patterns of tick-borne disease transmission and prevalence (Patz et al. 2004; Bradley and Altizer 2007).

Despite a likely effect of urbanization on ticks and tick-borne diseases, almost no U.S. studies have systematically investigated this topic. Even basic ecological information about ticks (e.g., species composition, habitat preferences, and questing schedules) and the abiotic and biotic factors contributing to tick abundance are virtually unknown in urban areas. Lyme disease, a tick-borne disease caused by the spirochete bacteria *Borrelia burgdorferi* and vectored by *Ixodes scapularis*, has been studied

extensively in many rural areas of the U.S. but is comparatively understudied in urban and suburban areas (Maupin et al. 1991; Daniels et al. 1999; Allan et al. 2003; Jobe et al. 2007; Rydzewski et al. 2012; Levi et al. 2014). Even fewer studies have focused on non-Lyme pathogens in U.S. urban areas. The studies that have been conducted detected the presence of *Rickettsia parkeri*, '*Candidatus* Rickettsia andeanae', *Ehrlichia chaffeensis*, and/or *Ehrlichia ewingii* in urban and peri-urban greenspaces (Fornadale et al 2011; Hamer et al. 2012; Blanton et al. 2014; Raghavan et al. 2016; Noden et al. 2017). However, only one study (Noden et al. 2017) described a relationship between tick abundance and large-scale patterns of urbanization—specifically the amount of undeveloped land surrounding parks where ticks were sampled—and no studies have formally analyzed such relationships or simultaneously assessed other finer-scale factors known to influence tick populations and pathogen prevalence in non-urban areas (e.g., temperature, moisture, vegetation, host abundance). Such investigations are crucial to understanding the rise of tick-borne diseases in urban areas, particularly for poorly studied diseases other than Lyme disease, and especially given future projections of human population growth in North American urban areas.

To address these research gaps, we investigated how both landscape- and finer-scale abiotic and biotic factors influence tick abundance and tick-borne pathogen prevalence over an urbanization gradient. Specifically, we sampled ticks, vegetation, and deer hosts at 16 parks spanning a gradient of urban development intensity in Oklahoma City, Oklahoma, USA. We tested the following three hypotheses: 1) At the landscape scale, abundance of ticks and prevalence of tick-borne pathogens varies predictably across an urban landscape, with higher tick abundance and pathogen prevalence in areas

with extensive surrounding green space and undeveloped land, 2) At the local scale, both abiotic factors (e.g., temperature, humidity) and vegetation influence tick abundance, and 3) Abundance of deer is positively correlated with abundance of ticks across the urban landscape. Results of this study will increase ecological understanding of tick-borne disease transmission in urban areas and will also be useful to public health officials and land managers seeking to use information about parks and their surrounding urban landscape to focus public education and/or land management efforts designed to reduce tick-borne disease prevalence.

Methods

Study Design and Site Selection

Oklahoma City is the largest city in Oklahoma, in terms of both population size and land area (Figure 1), with 643,648 people residing in an area of 1605 km^2 (City of Oklahoma, 2015; US Census Bureau, 2011), and 1.2 million residents in the seven-county Oklahoma City metropolitan area. Oklahoma City is located in the US Great Plains ecoregion (EPA, 2016), and the surrounding land-use is primarily grasslands and cultivated crops west of the city and grasslands and deciduous forest with interspersed patches of pasture east of the city. With a mild climate and average annual temperature of 15.6℃ (Greater Oklahoma City, 2016), tick activity occurs at least ten months out of the year (Talley et al. 2014). Oklahoma is home to some of the highest rates of Spotted Fever Group rickettsiosis and ehrlichiosis in the U.S. (Biggs et al. 2016; Springer and Johnson, 2018), as well as high rates of tularemia (CDC 2015), which makes Oklahoma City an ideal study area for the purposes of our research objectives. Other less-common tick

borne diseases found in Oklahoma include anaplasmosis, Heartland virus, and Bourbon virus (Dahlgren et al. 2015; OSHD 2017); these pathogens could become more prevalent in urban areas in the future.

In the Oklahoma City metropolitan area, we used Google Earth and Google Street View to identify candidate study sites for tick sampling. We first identified all potential large areas (>2 hectares) of tick habitat, including parks, green spaces, and waste spaces with un-manicured understory vegetation, shrubs, savanna and woodland, but excluding undeveloped areas dominated by open manicured grass. We then manually digitized a polygon representing the boundaries of each candidate site and used ArcGIS 10.1 (ESRI 2011) to calculate percentages of developed land and impervious surface in a 1,000m buffer of each site's outer edge, with all land cover data from the national land cover database (NLCD; Homer et al. 2015). For developed land cover, we combined all NLCD cover classes representing human development (developed, open space; developed, low intensity, developed, medium intensity, and developed, high intensity) and excluded all other cover types (e.g., water, forest, and cultivated classes). Because percent surrounding impervious surface and percent surrounding developed land were strongly correlated (Pearson's $r = 0.74$), only percent developed land was used for the following stratified site selection approach. To capture a gradient of urban development intensity, we grouped sites into four categories based on percent developed land—17-40%, 40-60%, 60-80%, 80-100% (17% was the minimum observed value across all candidate sites)—and randomly chose four sites from each category (n=16). We ground-truthed and assessed all sites for safety and accessibility issues, and based on these logistical constraints, three sites had to be replaced with other randomly selected sites from the

same land cover category. The final sites selected are shown in Figure 1, and detailed information about site locations, stratification categories, and the percentage surrounding developed land is found in supplementary materials, Table 1.

We located ten 50 m-long transects at each site using Google Earth prior to site visits. We selected transects to representatively sample different vegetation types and for spatial coverage of sites. We ground-truthed specific transect locations during the first site visit, and due to accessibility or safety issues had to move some transects. After ground-truthing, we determined that two sites did not have enough accessible area to fit ten transects, so eight transects were instead located in each of these sites. Sampling over fixed-length transects allowed co-referencing of tick samples to fine-scale ecological characteristics.

Tick Collection

In both 2017 and 2018, we collected adult and nymphal ticks at each site from mid-May to early-August, which is the period of peak seasonal abundance for many tick species in our study area. We sampled 10 total times at each site, with 5 sampling events each year and approximately 2 weeks between each subsequent visit to a site. At each site, we placed one CO_2 trap at a random point along each transect (i.e., 8 or 10 traps/site via random number generator, where output=distance, in meters, from start of transect) along with a HOBO data logger (Oneset, Bourne, MA) that measured temperature and humidity every minute while traps were open. We placed the HOBO data loggers on the ground to measure fine-scale differences in temperature and humidity most likely to influence tick behavior. Traps consisted of a container of dry ice (solid CO_2) in the center

of a wooden board lined with wide masking tape (Noden et al. 2017). We left CO_2 traps open for approximately one hour, and recorded open and close times for all traps. After the trapping period, we removed ticks found on the board and stuck to the tape and placed the ticks into a 50 ml Eppendorf tube (VWR, Radnor, PA) containing 10mls of 70% ethanol.

While CO_2 traps were open, we flagged along each transect by sweeping a piece of fabric (Summer Infant waterproof multi L pad 27" x 36"; Walmart, Bentonville, AK) attached to a wooden pole across vegetation, and collecting any ticks that attached to the fabric. Flags were checked at least every 30 seconds, and we placed all ticks from each transect in an Eppendorf tube containing 70% ethanol. We identified all ticks collected using both methods to species, life stage, and sex using a dissecting microscope and established pictorial keys (Keirans and Litwak, 1989; Keirans and Durden, 1998; Dubie et al. 2017). Because *A. maculatum* in the United States is indistinguishable from *Amblyomma triste* (Lado et al. 2018), all references to *A. maculatum* in this manuscript actually refer to the *A. maculatum-A. triste* complex.

Vegetation Sampling

For vegetation sampling, we randomly chose four points along each transect using the same random number generation approach as described for tick sampling. At each point, we measured canopy density, leaf litter depth, and percent ground cover. Ticks spend most off-host time in leaf litter or questing on low vegetation (Needham et al. 1991), thus other than the canopy density measurement, we focused vegetation sampling on ground cover measurements. We used a spherical densiometer to measure canopy cover at each point as described in Strickler et al. (1959). We quantified leaf litter depth

by pushing a solid ruler into the litter until it met with resistance from soil. We determined percent ground cover by using a 1x1 meter square sampling frame (a modified Daubenmire cover scale) and visually estimating the percent ground cover of: bare ground, leaf litter, coarse woody debris (i.e., fallen dead trees and branches), and graminaceous, herbaceous, and woody vegetation. Additionally, we counted snags (standing dead trees) and estimated percent cover of eastern redcedar, *Juniperus virginiana*, in 5 m of the entire length of each transect. For eastern redcedar, we separately estimated cover in the understory (smaller trees or shrub-like trees, not tall enough to reach the canopy in wooded areas) and overstory (tall trees, included in the canopy of woody areas, or trees encroaching into grasslands).

Deer Camera Trapping

White-tailed deer, *Odocoileus virginianus*, abundance is linked to abundance of several species of ticks (Wilson et al. 1985, Stafford et al. 2003, Allan et al. 2010), and additionally, deer can serve as reservoir hosts for *Ehrlichia* (Lockhart et al. 1997), Lyme disease (Magnarelli et al. 1986), and STARI (Moore et al. 2003). To generate an index of deer abundance at each site, we used Browning range ops btc-1xr trail cameras (Browning, Morgan, UT) that were deployed at the end of October 2017. This timing corresponds with the seasonal period during which deer antlers are fully developed but not yet shed (Masters et al. 2017), thus facilitating identification of individual male deer, and therefore, abundance estimation. One trail camera was placed at each site for two 14-day periods, a sampling intensity demonstrated to capture 88-100% of deer present (Jacobson et al. 1997). Further, since no site was larger than 40.5 hectares, the home range size of deer in Oklahoma (Masters et al. 2017), one camera per site was adequate

for capturing the majority of individuals and therefore provided a reasonable approximation of deer abundance at each site. Each trail camera was placed near the center of each site (taking into account accessibility, possibility for human disturbance/theft, and proximity to game trails or scrapes) and without bait to avoid potential biases associated with using baited traps (McCoy et al. 2011). Cameras were set to take bursts of three photos with each trigger, with a five second photo delay between each photo in the set (to avoid multiple photos of the same individual) and a time out period of one minute between triggers. We visually examined all photos, identified and counted unique individual deer based on antler size and branching, and used the proportion of unique antlered deer to total antlered deer captured on camera to index the abundance of all deer at each site using established equations in Jacobson et al. (1997).

Molecular testing

We tested adult field-collected ticks for DNA from a variety of bacteria species using modified PCR protocols **(**Supplementary materials, Table 2**)**. Only adult ticks were tested because they have fed on hosts twice, as opposed to nymphs that have fed only once, which results in a higher cumulative likelihood of adults carrying pathogens. To limit DNA contamination, all tick DNA extractions were conducted using site-specific reagents in a biosafety cabinet that was located in a different laboratory than where Polymerase chain reaction (PCR) assays were run. We washed individual adult ticks in de-ionized water and 70% ethanol, bisected each tick, and used one half used for DNA extraction and the other half was stored at -80°C. After bisection, we placed individual adult ticks in 2mL vials (Sarstedt) (Biospec, Bartlesville, OK) containing 100µL of DNAzol® Direct sample processing reagent (Molecular Research Center, Cincinnati,

OH) (Noden et al. 2017). After heating at 80-90°C for fifteen minutes, we added zirconia/silica beads (BioSpec Products) to each vial, and placed the vial in a Mini-Beadbeater-16 (BioSpec Products) for three minutes. After bead-beating, resulting supernatant was collected and stored at -20°C until DNA testing.

Prior to testing, we created pools of 1-10 ticks of the same species collected on the same date in the same location by pooling 5µl of extracted DNA into a 1.5ml tube (VWR, Radnor, PA). These tick pools were initially screened by end-point PCR using previously published PCR protocols (supplemental table, Table 2)—which targeted Rickettsia *sp.* (*gltA* (Labruna et al. 2004), *ompA* (Eremeeva et al. 1994) and *ompB* genes (Roux & Raoult 2000)) and the *ISFtu2* gene of *Francisella tularensis* (Versage et al. 2003)—and by nested PCR assays targeting the *groEL* gene of Ehrlichia and Anaplasma *sp.* (Tabara et al., 2007; Takano et al. 2009), and the flagellin (*flaB*) gene of *Borrelia burgdorferi* (Barbour et al. 1996; Gleim et al. 2016). Positive controls consisted of *R. rickettsii* DNA provided by Dr. William Nicholson (Ricketsial Zoonoses Branch, Centers for Disease Control and Prevention), *E. chaffeensis* MO strain DNA provided by Dr. Susan Little (OSU School of Veterinary Science), *Borrelia burgdorferi* strain B31 DNA acquired from American Type Culture Collection (Manassas, VA), and *F. tularensis* DNA provided by Dr. Ramachandran (Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University).

We initially screened all pools with the Rickettsia *gltA, Francisella tularensis,* Ehrlichia and Anaplasma sp, and *Borrelia burgdorferi* assays, since these assays target different pathogens. We identified positive pools and tested the individual ticks from each pool to obtain a true prevalence rate of infection. Because of their importance as

vectors for Rocky Mountain spotted fever (*R. rickettsii*) and *R. parkeri*, two important human pathogens, individual positive *D. variabilis* and *A. maculatum*, were respectively screened for these pathogens using PCR assays targeting the ompA and gltA genes and positive tick samples were retested for the ompB gene, which provided ~800bp sequences for effective sequence confirmation. All positive amplicons were bidirectionally sequenced at the Oklahoma State University Core Facility to confirm positivity and identify bacterial species. We verified each resulting sequence using BioEdit (Ibis Therapeutics, Carlsbad, CA), aligned to create consensus sequences using Clustal Omega (EMBL-EBI, Cambridgeshire, UK) and divided into different groups based on sequence differences. We compared resulting consensus sequences with GenBank submissions using default conditions on NCBI BLAST (highly similar sequences (megablast)) where the highest % sequence identity was used to determine species similarity. We sequenced all PCR *groEL* and *flaB* and all *ompA* and *ompB* positive *D. variabilis* and *A. maculatum*. However, due to the high number of positive *A. americanum* sampled in 2017, a random sample of 125 positive pools were selected for sequencing. A few noisy sequences from the first *Rickettsia* sp. *gltA A. americanum* screening were retested using both *ompA* and *ompB* assays, and all positive amplicons were sequenced and assessed as detailed above (Dubie et al. 2018).

Statistical Analyses

We performed all analyses in R 3.2.2 (R Core Team, 2016). To identify factors associated with tick abundance, we conducted three different model selection exercises for each species of tick—a site-scale analysis of urbanization and park size effects and transect-scale analyses of both biotic and abiotic correlates. Although flagging and

trapping targeted ticks during different stages of questing (active vs. passive, respectively), model selection results were unchanged between analyses combining flagged and trapped ticks or only including flagged ticks; therefore, we present analyses based on all ticks caught using both methods.

For the site-scale analysis, two land-use variables were assessed: surrounding developed land in a 1000-m buffer of the boundary of each site (a proxy for urbanization) and park size because we expected larger parks to have a higher diversity and density of hosts, and thus contain more ticks. For each site, all ticks of each species were summed across all sampling events in both years. We used a GLM with a negative binomial distribution to account for over dispersion, site as the unit of replication (n=16), tick abundance as the dependent variable, and urbanization and park size as additive fixed effects.

For the transect scale (i.e., 50 m segments) analysis of biotic correlates, we analyzed the association between tick abundance and vegetation variables. For this analysis, all ticks of each species were summed at the transect-level across all sampling events in both years. We used a GLMM with a negative binomial distribution, transect as the unit of replication (n=156), tick abundance as the dependent variable, vegetation variables as fixed effects, and site as a random effect to account for non-independence of multiple transects at each site. Vegetation variables were only included in the formal model selection analysis when they had a preliminary correlation coefficient with tick abundance of $r>0.15$. Thus, specific included variables were: for *A. americanum*, percent cover of graminaceous, herbaceous, and woody plants, leaf litter depth, and percent cover of overstory redcedar; for *D. variabilis*, percent cover of graminaceous and woody plants,

percent cover of understory redcedar and leaf litter; and for *A. maculatum*, percent cover of graminaceous and woody plants, percent cover of leaf litter and overstory redcedar.

For the transect scale analysis of abiotic correlates, we evaluated the association between tick abundance and temperature and humidity at the time of tick collections. For this analysis, all ticks of each species were summed for each individual sampling event at each transect. GLMMs were again used with a negative binomial distribution, sampling event as the unit of replication (n=1,560), tick abundance for each sampling event as the dependent variable, weather variables as fixed effects (average temperature, humidity, and dew point), and transect nested in site as a random effect to account for non-independence of sampling events on the same transect and non-independence of transects within sites. We considered dew point in addition to relative humidity because dew point provides a more accurate representation of the total amount of moisture in the air, whereas any given value of relative humidity represents a different amount of moisture depending on the air temperature. The average dew point during each tick sampling event was calculated using the formula:

$$T_{dp} \sim T - ((100 - RH)/5)$$

where T_{dp} is the temperature at dew point, T is the average temperature in degrees Celsius and RH is the average relative humidity during tick collection as measured by the HOBO data logger.

For all of the above tick abundance analyses, we conducted a model selection exercise for each dependent variable using Akaike's Information Criterion, corrected for small sample sizes (AIC_c; Burnham and Anderson 2002). AIC is an information-theoretic

approach that ranks a set of candidate models based on the tradeoff between model fit and parsimony; each additional parameter added to a model receives a penalty of +2 AIC units, but improved model fit associated with adding a parameter reduces the AIC value. Thus, models receive strong support when the improvement to model fit associated with the included variables outweighs the penalty associated with adding these variables. The group of models we considered for each analysis included a null (i.e., intercept-only) model, all possible single-variable models for each respective set of analyses, and all additive combinations of the predictor variables in each analysis. Models were considered competitive if they had $\Delta AIC \leq 2$ and at least 2 greater than the null model and if they did not contain uninformative parameters (Arnold 2010); for competitive models, we also assessed coefficients and only considered variables to be supported as meaningful predictors of the response variable when the 95% confidence interval (CI) of their coefficient estimates did not overlap zero.

To determine if tick abundance was associated with the index of white-tailed deer abundance, we used a GLM with a negative binomial distribution, site as a replicate (N=15, as one site had to be excluded due to camera failure), tick abundance at each site as the dependent variable, and the deer abundance index as a fixed effect. We assessed coefficients and only considered the deer-index to be meaningfully associated with the response variable when the 95% confidence interval (CI) of the coefficient estimate did not overlap zero.

Finally, to evaluate the association between urbanization and pathogen prevalence, we used a linear model (i.e., assuming a normal distribution, as pathogen prevalence data were normally distributed) with site as a replicate, pathogen prevalence

in adult ticks as the dependent variable, and percent surrounding developed land as the independent variable. There was not enough data to run an analysis for *R. rhiphicephali* because *D. variabilis*, the vector of this pathogen, was not present at enough sites (N=11), and *R. rhiphicephali* itself was only detected at 2 sites. Likewise, we did not conduct an analysis for *R. parkeri* because this pathogen was only detected at 1 site. For the pathogen analyses, we used p-values ($p<0.05$) to determine statistical significance of the land cover variable and multiple R^2 values to assess biological trends.

Results

Factors influencing tick abundance

A total of 10,299 ticks were collected over the course of the study, and at least one tick was collected at each of the 16 sites sampled (Supplementary materials, Table 3). More ticks were collected in 2017 (N=6,421 total ticks, 62.3%) than 2018 (N=3,878 total ticks, 37.7%). The vast majority of ticks collected were *Amblyomma americanum* (N=9,856 total ticks, 95.7%), followed by *Dermacentor variabilis* (N=255 total ticks, 2.48%) and *Amblyomma maculatum* (N=188 total ticks, 1.82%) (Supplementary materials, Table 3). All of the *D. variabilis* and *A. maculatum* ticks collected were adults, but of the 9,856 *A. americanum*, 4,688 (47.6%) were adults and 5,168 (52.4%) were nymphs (Supplementary materials, Table 3). At least two individual ticks and two different species of tick were found at every site. *A. americanum* was found at every site, *D. variabilis* was found at 11/16 (69%) sites, and *A. maculatum* was found at all but one (15/16; 94%) of the sites (Supplementary materials, Table 3). The highest count of both *A. americanum* (4,108 total, including 1,113 nymphs and 2,995 adults) and *D. variabilis*

(124 total, all adults) were from a site with extensive woodland and shrub land, and very little surrounding developed land. However, the highest count of *A. maculatum* (112, all adults) was at a park surrounded by intense urbanization and containing a relatively limited area of shrubby and wooded vegetation. The number of ticks collected for each species and life stage varied by sampling date, and this seasonal variation is illustrated in Supplementary materials, Table 4.

Results of the site-scale analyses are displayed in Supplementary material; Table 5. For both *A. americanum* and *A. maculatum*, the null model ranked highest, which indicates there is no support for either park size or urbanization as predictors of abundance for these species. For *D. variabilis*, the top model contained percent surrounding developed land and ranked 3 ΔAIC units higher than the null model (Supplementary material; Table 5); assessment of model coefficients illustrated a negative relationship between *D. variabilis* abundance and percent surrounding developed land (β= -0.03570 ± 0.01444; CI: -0.06285156; -0.01067718); in other words, areas with more surrounding developed land contained fewer *D. variabilis* (Figure 2).

Results of the transect-scale biotic analyses are displayed in Supplementary material; Table 6. For *A. americanum*, the null model ranked within 2 ΔAIC units of the top model, which indicates there is no support for vegetation variables as predictors of abundance for this species. For *D. variabilis*, the top model contained percent cover of understory redcedar and ranked 21.3 ΔAIC units above the next best model (the single-variable leaf litter depth model) and 22.8 ΔAIC units above the null model; however, assessment of model coefficients suggested little effect of understory red cedar because the 95% CI of the coefficient estimate overlapped zero (β= 0.005107 ± 0.012048; CI: -

0.01708267, 0.03082715). For *A. maculatum*, only one model (woody plant cover + leaf litter cover) had ranked above the null model and had ΔAIC less than 2. Assessment of model coefficients illustrated that *A. maculatum* abundance was negatively associated with both woody plant cover (β= -0.033566 ± 0.013685; CI:-0.06088842, -0.006559659) and leaf litter depth (β= -0.025241 ± 0.008826; CI:-0.04278456 -0.007823258; Figure 3).

Results of the transect-scale abiotic analyses are displayed in Supplementary material; Table 7. For both *A. americanum* and *D. variabilis*, the top model contained additive effects of temperature and humidity, ranked ≥10 ΔAIC units above the next best model, and ranked ≥108.8 ΔAIC units above the null model. Assessment of model coefficients showed that abundance of both *A. americanum* and *D. variabilis* were negatively associated with dew point (*A. americanum*: β= -0.13060 ± 0.02316; CI:-0.17635681, -0.08550119; *D. variabilis*: β=-0.15331 ± 0.04396; CI:-0.19210336, -0.05034296) (Figure 4a) and positively associated with temperature (*A. americanum*: β= 0.06166 ± 0.01554; CI: 0.03133414, 0.09230976; *D. variabilis*: β=0.10367 ± 0.02947; CI: 0.02983217, 0.12756497) (Figure 4a,b). For *A. maculatum*, only the single-variable dew point model was strongly supported, ranking 4.3 ΔAIC units above the next best model that did not contain any uninformative parameters (the single variable temperature model) and 114.9 ΔAIC units above the null model. Assessment of model coefficients illustrated a negative association between *A. maculatum* abundance and dew point (β= -0.08646 ± 0.03484; CI:-0.09930794, -0.005857594). (Figure 4c).

Association between deer abundance and tick abundance

Based on trail camera sampling, we identified 23 individual male deer across our entire study area (range: 0-81 photos of deer per site), which translated to an abundance index that ranged from 0 to 9.8 deer per site. As determined by assessing the 95% CIs for the abundance index coefficient in GLM models, deer abundance was positively associated with abundance of both *A. maculatum* (β=0.07467 ± 0.02868; CI: 0.0400, 0.1384) and *D. variabilis* (β=0.3764 ± 0.1048; CI: 0.1303, 0.5477) (Figure 5a,b), but not associated with *A. americanum* abundance (β=0.08135 ± 0.11834; CI: -0.1882, 0.3848).

Factors influencing pathogen prevalence

We tested all 5,131 adult ticks for pathogens. Although we did not find any *A. phagocytophilum* or *F. tularensis*, at least one pathogen or group of pathogens (Rickettsia spp., Ehrlichia spp., and/or *Borrelia lonestari*) was detected at 12 out of 16 sites (Supplementary materials, Table 8). Of the *A. americanum* ticks collected, *E. chaffeensis* was detected at 50% of sites (8/16), *E. ewingii* at 62.5% of sites (10/16), and *B. lonestari* at 62.5% of sites (10/16) (Table 5a), while Spotted fever group rickettsiosis was found at 81.25% of sites (13/16; Appendix Table 9). Of the *D. variabilis* ticks collected, *R. rhipicephali* was detected at 37.5% of sites (6/16), and *C. R. andeanae* at 12.5% of sites (2/16) (Table 5b). Of the *A. maculatum* ticks collected, *C. R. andeanae* was detected at 12.5% of sites (2/16) and *R. parkeri* was detected at one site (Table 1).

Although pathogen prevalence differed by site (Appendix Tables 8-9), the overall prevalence rates for each pathogen did not vary much by year (Appendix Table 10). Among *A. americanum* (n=4,706) tested, the overall prevalence of *Ehrlichia chaffeensis* was 1.0% (1.1% (2017) and 0.8% (2018)) while *E. ewingii* was 2.4% (2.6% (2017) and

2.1% 2018)) and Panola Mountain Ehrlichia was 0.4% (both years). The prevalence of *Borrelia lonestari* in *A. americanum* was 0.6% (0.8% (2017) and 0.4% (2018)). *Rickettsia rhipicephali* in *D. variabilis* (n=254) was 7.5% (7.9% (2017) and 6.5% (2018)) while '*Candidatus* R. andeanae' in *A. maculatum* (n=197) was 60.9% (69.1% (2017) and 47.3% (2018)). The prevalence of 183 randomly selected pools of *A. americanum* ((2017, 133 pools, 678 ticks) and (2018, 50 pools, 446 ticks) for Rickettsia sp. was 35.0% (37.3% (2017) and 31.4% (2018)). Of the 5,159 ticks tested in this study, we only detected in 54 *A. americanum* (2017– 33 ticks; 2018– 21 ticks) individual ticks had two different pathogens: Rickettsia sp./*E. ewingii* (n=31 ticks) (19 (2017) and 12 (2018))(Votec (n=16), Trosper (n=5), Arcadia (n=6), Scissor (n=2), Lacy (n=1), Ingels (n=1)), Rickettsia sp./*E. chaffeensis* (n=11) (5 (2017) and 6 (2018)) (Votec (n=7), Scissor (n=2) and Ray (n=2)), Rickettsia sp./PME (n=6) (5 (2017) and 1 (2018)) (Scissor (n=3), Ruby (n=2) and Votec (n=1), and Rickettsia sp./*B. lonestari* (n=6) (4 (2017) and 2 (2018)) (Hafer (n=2), Arcadia, Scissor, Ingels and Votec (n=1). Ticks of the other species were only infected with one pathogen.

Of the 74 positive Rickettsia samples that were chosen for sequencing, 70 *A. americanum* samples (70/74), one *D. variabilis* sample (1/22), and one *A. maculatum* sample (1/46) were 100% identical to known *Rickettsia amblyommatis* sequences (Genbank sequence numbers: MH425445.1, MG674587.1, and MF188914.1, and CP015012.1). Three Rickettsia-positive *D. variabilis* samples (3/22) were also 100% identical to *R. andeanae* (Genbank sequence no. GU395297.1), and eighteen samples (18/22) were 100% identical to *R. rhipicephali* (Genbank sequence no. CP003342.1). Forty-two Rickettsia-positive *A. maculatum* samples (42/46) were 100% identical to

known sequences of *C. R. andeanae* (Genbank sequence nos. GU395297.1 and KY402179.1), and three samples (3/46) were 100% identical to *R. parkeri* (Genbank sequence no. CP003341.1) (Table 5c). Of the 47 positive *E. chaffeensis* samples, all forty-seven *A. americanum* samples (47/177) were 100% identical to known sequences of *E. chaffeensis* (Genbank sequence no. KJ907753.1). Of the 111 positive *E. ewingii* samples, 111 *A. americanum* samples (111/177) were 100% identical to known sequences of *E. ewingii* (Genbank sequence no. KJ907744.1). Nineteen Panola Mountain Ehrlichia-positive *A. americanum* samples (19/177) were 100% identical to known sequences of this pathogen (Genbank sequence no. HQ658904.1) (Table 5a). All thirty of the Borrelia-positive *A. americanum* were 100% identical to known sequences of *B. lonestari* (Genbank sequence no. AY850063.1) (Table 1).

For the site-level analysis of pathogen prevalence, *E. ewingii* prevalence had a significant inverse relationship with surrounding land cover (Figure 3a; $p = 0.0415$; multiple $r^2 = 0.2823$) (Figure 6). However, we found no significant associations with surrounding land cover for *E. chaffeensis* ($p = 0.1573$; multiple $r^2 = 0.1477$), Panola Mountain Ehrlichia ($p = 0.2026$; multiple $r^2 = 0.1217$), *C. R. andeanae* ($p = 0.8018$; multiple $r^2 = 0.0050$), or *B. lonestari* ($p = 0.9191$; multiple $r^2 = 0.0008$).

Discussion

In a major urban area in the U.S. Great Plains, we found that there is a risk of encountering ticks and tick-borne pathogens across the entire urbanization gradient from outlying exurban areas to the city's urban core. We also found that the abundance of some tick species (*D. variabilis*) and prevalence of some tick-borne pathogens (*E.*

ewingii) decrease with increasing urbanization intensity. Further, vegetation, temperature, and moisture variables predicted tick abundance and these correlates varied among species. For example, *A. maculatum* decreased with increasing woody plant and leaf litter cover while *A. americanum* and *D. variabilis* were sampled in greater numbers on hot, dry days. This study is one of the first to formally investigate tick abundance and tick-borne pathogen prevalence in relation to patterns of urbanization, and to also simultaneously consider finer-scale effects of vegetation and weather conditions in urban parks.

Factors influencing tick abundance

Ticks were ubiquitous across our study area; at least two individual ticks and two tick species were collected at every site, and our formal analysis illustrated that abundance of two of the three tick species sampled *(A. americanum* and *A. maculatum*) did not vary in relation to urbanization. However, urbanization intensity was negatively associated with *D. variabilis* abundance. Past research on *I. scapularis* in urban areas suggests that abundance of this vector tick species may be more likely to decrease with increasing urbanization (Jobe et al. 2006; Rydowski et al. 2012), while other studies of *A. americanum*, and *A. maculatum* also show the same lack of association with urbanization that were found for these species (Fornadale et al. 2011; Blanton et al. 2014; Noden et al. 2017). These species-varying patterns may reflect varying levels of urban-adaptability, or alternatively, differences in sampling effort and intensity among studies or differences in detectability of ticks in relation to the gradient of urbanization intensity (i.e., ticks harder to detect when they occur in highly urbanized areas). For *D. variabilis*, populations in rural areas are known to be highly clustered and most commonly found in agricultural

areas (Trout-Fryxell et al. 2015); therefore, we hypothesize that the negative association with urbanization in our study could reflect a lack of suitable, agricultural habitat in highly urbanized landscapes. The lack of relationship with urbanization for *A. americanum* and *A. maculatum* suggests factors other than surrounding undeveloped land influence abundance of these species. For example, other large-scale factors like landscape heterogeneity and habitat connectivity could be important, as these factors are linked to abundance, density, and movements of bird and mammal hosts (Buskirk et al. 1998; Estrada Pena et al. 2003).

For the transect-scale analysis of vegetation, abundance of *A. maculatum* was negatively associated with percent cover of leaf litter and woody plants, but abundance of *A. americanum* and *D. variabilis* was not associated with any vegetation variables. *A. maculatum* is known as a grassland specialist in rural contexts (Fryxell et al. 2015), and our results support that this species may also select relatively open habitats in urban areas (i.e., locations with limited woody vegetation and leaf litter). However, these areas may not always be grasslands, as indicated by the lack of an effect of percent graminaceous cover in our analysis. Our results for *A. americanum* and *D. variabilis* are consistent with studies in rural areas, which generally show either no association between vegetation and abundance of these species (Soneshine and Levy 1971, Trout-Fryxell et al. 2015; Gilliam et al. 2018) or a greater correlation with weather-related variables (Davidson et al. 1994), the latter of which we also found. Alternatively, the lack of relationship with vegetation and *A. americanum* and *D. variabilis* could be because of the way we classified our vegetation categories. For example, we grouped all grassy, herbaceous, and woody plants and leaf litter into the same category. We hypothesize by further differentiating plants

between native and invasive and leaf litter into type of litter (i.e. oak, sumac, juniper) could be important as these factors have been linked to tick abundance previously (Lindström et al. 2003; Civitello et al. 2008; Allan et al. 2010).

For the transect-scale analysis of abiotic conditions, abundance of both *A. americanum* and *D. variabilis* was negatively associated with dew point and positively associated with temperature, while abundance of *A. maculatum* was negatively associated with dew point. The importance of temperature for *A. americanum* and *D. variabilis* matches past research showing that questing activity for many tick species increases with temperature (Clark 1995; Perret et al. 2000). However, because ticks are sensitive to desiccation (Estrada-Pena 2015), the negative association with dew point for all three tick species is difficult to explain. Further, we found no support for relative humidity in predicting abundance of any tick species despite this variable predicting tick activity in some past studies (Vail and Smith, 2002; Perret et al. 2003). Dew point provides an absolute measure of the total amount of moisture in the air, independent of air temperature, and it is often used in weather forecasts to represent how uncomfortable the air feels. Relative humidity represents a ratio of the amount of moisture in the air to the total amount of moisture air can hold, the latter of which is positively associated with temperature, making interpretation of relative humidity dependent on temperature. We suggest that future studies of tick ecology could consider dew point as a potential predictor of tick activity (Stafford 1994; Vail and Smith 1998; Estrada-Pena 2001).

Association between deer abundance and tick abundance

White-tailed deer abundance was positively associated with *A. maculatum* and *D. variabilis* abundance but not associated with *A. americanum* abundance. The latter finding was unexpected because white-tailed deer are widely found in urban areas of the U.S. (Grund et al. 2002; Walter et al. 2011), and have been linked to *A. americanum* abundance and prevalence of the pathogens carried by this species (Paddock et al. 2007; Yabsley et al. 2010). This result suggests that other hosts, such as birds or small mammals, may be just as important to the distribution and abundance of *A. americanum* in urban areas. Support for this hypothesis is provided by a companion study conducted in the same 16 sites that found a high percentage of resident birds parasitized by *A. americanum* ticks (Roselli, Chapter 2). The positive association between deer abundance and abundance of *A. maculatum* and *D. variabilis*, tick species that are poorly studied in urban areas, suggests that deer may be an important host for these species in urban areas and/or that *A. maculatum*, *D. variabilis*, and white-tailed deer all select similar habitats, such that their abundance levels vary in similar ways in relation to urbanization and park-level vegetation characteristics.

Factors influencing pathogen prevalence

At least one pathogen or group of pathogens (Rickettsia spp., Ehrlichia spp., and/or *Borrelia lonestari*) was detected in adult ticks from 75% of the sites visited, and we documented several novel vector-pathogen associations. All of the Ehrlichia positive individuals, including *E. chaffeensis*, *E. ewingii*, and Panola Mountain ehrlichia, were from *A. americanum*, which is known as the main vector of Ehrlichia spp., in the U.S. (Paddock and Yabsley 2007). Most of the Rickettsia positive *A. americanum* ticks aligned most closely with *R. amblyommatis*, which is common in Oklahoma (Heise et al.,

2010; Barrett et al., 2014), and may cause a mild form of spotted fever rickettsiosis in humans (Apperson et al., 2008, Dahlgren et al., 2016). One single *A. maculatum* and *D. variabilis* individual aligned with *R. amblyommatis*, which has been reported previously (Smith et al., 2010; Trout et al. 2010; Fritzen et al., 2011). *R. rhipicephali* was identified in three *A. americanum* ticks, although it is normally associated with *D. variabilis* ticks (Wikswo et al. 2014), the species in which the majority (18/22) of *R. rhipicephali* positives was found. Most of the Rickettsia positive *A. maculatum* ticks aligned most closely with *C. R. andeanae*, which is not known to cause disease in humans (Paddock et al., 2015). Notably, three *A. maculatum* aligned with high homology with *R. parkeri*, a known human pathogen which is not thought to be found in Oklahoma (Paddock et al. 2015). Positive amplicons for *B. lonestari* were only found in *A. americanum* ticks, and although the relationship between this bacteria and human disease is still unclear (Masters et al. 2008), the bacteria is commonly found in *A. americanum* ticks (Burkot et al. 2001).

The pathogens we detected have also been reported in other urban or peri-urban areas in the U.S. (Burkot et al. 2001; Fornadel et al. 2011; Nadolny et al. 2014; Raghavan et al. 2016; Noden et al. 2017), but few studies have formally analyzed pathogen prevalence in relation to patterns of urban development. We found that prevalence of *E. ewingii* in adult *A. americanum* ticks was significantly lower in urban areas than peri-urban areas, and non-significant negative trends were observed for prevalence of *E. chaffeensis*, Panola Mountain Ehrlichia, and *C. R. andeanae*, but urbanization intensity had no effect on prevalence of *B. lonestari*. Our results suggest that although *A. americanum* (the main vector of Ehrlichia spp. in the U.S. (Paddock and Yabsley 2007))

abundance is relatively equal in urban and peri-urban areas, ticks of this species collected in more urbanized areas may be less likely to harbor *E. ewingii* pathogens; this same pattern may occur for other species, but the strength of the relationship is likely pathogen-specific. Although similar patterns have been found in Europe with Babesia spp. and Rickettsia spp. (Overzier 2013), one possible reason that urbanization was not associated with abundance of the other two tick species or prevalence of the other four pathogen species is that they may be more strongly affected by variation in host communities associated with urbanization. Since wildlife diversity and species composition impacts spatio-temporal patterns of tick-borne disease (Schmidt and Ostfeld 2001; Allan et al. 2010; Hamer et al. 2012; Keesing et al. 2012; Pfaffle et al. 2013) and urbanization causes changes in wildlife communities (McKinney et al. 2008), future studies should also investigate the role of wildlife reservoir hosts in tick-borne disease transmission and how this role varies in relation to patterns of urbanization.

An additional foundational area of research that must be addressed to understand how urbanization affects tick abundance and pathogen prevalence is a basic understanding of tick host-preferences and reservoir host capacity for tick-borne pathogens in urban areas. Although white-tailed deer have been linked to expanding *A. americanum* populations and increasing Ehrlichia spp. prevalence in the U.S. (Paddock et al. 2007; Yabsley et al. 2010), little is known about the potential importance of small mammals, coyotes, and foxes as hosts for this pathogen in both urban and rural areas (Davidson et al. 1999; Comer et al. 2000; Kocan et al. 2000; Yabsley et al. 2010). Even less is known about the reservoir hosts of Rickettsia spp. throughout the U. S. Cottontail rabbits (Sylvilagus spp.) may be important reservoir hosts (Shirai et al. 1962; Burgdorfer

et al. 1980), as may birds, as suggested by research in Europe (Elfving et al. 2010; Hildebrandt et al. 2010). Once important host species are identified, future studies can assess how host habitat requirements vary in relation to urbanization and other factors, and how these variations influence tick populations and tick-borne pathogens.

In addition to urbanization intensity and other large-scale characteristics of urban areas likely to influence pathogen transmission via effects on wildlife reservoir hosts (e.g., land cover heterogeneity and habitat connectivity), vector-borne diseases including tick-borne diseases can be associated with a variety of socioeconomic factors that vary across urban areas (Randolph et. al 2010; Lohmus and Balbus 2015; Springer and Johnson 2018). For example, incidence of *E. chaffeensis* is greater in urban areas with high rates of unemployment, and comparably affected by vacant housing and ecological factors (e.g. deer density, forest cover) (Springer and Johnson 2018). These types of effects likely arise because socioeconomic factors affect vegetation cover at scales ranging from the plants in individual yards to the green spaces across entire municipalities (Hope et al. 2008; Strohbach et al. 2009; Dai et al. 2011; Wolch et al. 2014). Such vegetation variation leads to variation in wildlife communities and tick abundance at a similar range of spatial scales (Grund et al. 2002; Allan et al. 2010; Hornok et al. 2014; Narango et al. 2018), with associated implications for tick-borne pathogen prevalence.

Conclusions

In conclusion, this study illustrates that, although abundance of *D. variabilis* and prevalence of *E. ewingii* decrease with increasing urbanization, urban residents in the

Great Plains region are at risk of encountering ticks and tick-borne pathogens in parks and greenspaces across the entire urbanization gradient. Although tick-borne diseases are of substantial public health concern in the U.S., a large percentage of the urban population is unaware they should be taking steps to reduce encounters with ticks and the risk of transmitting tick-borne diseases in urban areas where Lyme Disease is uncommon (Bayles et al. 2013; Hook et al. 2015), such as in our study area, Oklahoma City, which is characterized by a high prevalence of several non-Lyme pathogens. Although further similar research is needed in other urban areas and for other tick-borne pathogens, our results broadly suggest that urban residents across the U.S. should familiarize themselves with tick-borne pathogens commonly diagnosed in their region, and that natural resource managers and public health officials could consider integrating tick-borne disease education and management programs into urban areas in addition to rural areas.

Urbanization is a fast-growing phenomenon that shows no signs of stopping, especially in the U.S. where 81% of people can be found on roughly 3% of the nation's land area (US Census Bureau, 2015). Our results serve as a stepping stone for understanding tick populations and tick-borne diseases across gradients of urbanization in the U.S. Great Plains, and more broadly, in North American urban areas. These findings highlight that additional research is needed to understand the increasing prevalence and emergence of tick-borne diseases and other vector-borne diseases in urban areas of the U.S. (LaDeau et al. 2015), as well as the risk these diseases pose to humans, domestic animals, and wildlife.

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Tables and Figures

Table 1: Ehrlichia, Rickettsia, and Borrelia genotypes identified from field-collected adult ticks from 16 sites in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018.

Year	Genotype	Primer	Frequency	*A. americanum*	*D. variabilis*	*A. maculatum*	% identity (GenBank)
				Ehrlichia groEL amplicons			
2017	Ehrlichia OKC-1	groEL	47	31			100% Ehrlichia chaffeensis (KJ907753.1)
2018		groEL		16			100% Ehrlichia chaffeensis (KJ907753.1)
2017	Ehrlichia OKC-2	groEL	111	69			100% Ehrlichia ewingii (KJ907744.1)
2018		groEL		42			100% Ehrlichia ewingii (KJ907744.1)
2017	Ehrlichia OKC-3	groEL	19	10			100% Ehrlichia Panola Mtn (HQ658904.1)
2018		groEL		9			100% Ehrlichia Panola Mtn (HQ658904.1)
				Rickettsia amplicons			
2017	Rickettsia OKC-1	gltA	62	61			100% R. amblyommatis (MH425445.1)
2018		gltA			1		100% R. amblyommatis (MH425445.1)
2017	Rickettsia OKC-2	gltA	2	2			100% R. amblyommatis (MG674587.1)
2017	Rickettsia OKC-3	ompA	7	7			100% R. amblyommatis (MF188914.1)
2018	Rickettsia OKC-4	ompB	1			1	100% R. amblyommatis (CP015012.1)

2017	Rickettsia OKC-5	gltA	5			3	100% R. andeanae (KY402179.1)
2018		gltA				2	100% R. andeanae (KY402179.1)
2017	Rickettsia OKC-6	ompB	40		1	3	100% R. andeanae (GU395297.1)
2018		ompB			2	34	100% R. andeanae (GU395297.1)
2017	Rickettsia OKC-7	gltA	18	2	12		100% R. rhipicephali (CP003342.1)
2018		gltA		1	3		100% R. rhipicephali (CP003342.1)
2017	Rickettsia OKC-8	ompA	2	1	1		100% R. rhipicephali (CP003342.1)
2018	Rickettsia OKC-9	ompB	2		2		100% R. rhipicephali (CP003342.1)
2017	Rickettsia OKC-10	ompB	3			2	100% R. parkeri (CP003341.1)
2018		ompB				1	100% R. parkeri (CP003341.1)
				Borrelia amplicons			
2017	BorreliaOKC-1	FLA		21			100% Borrelia lonestari (AY850063.1)
2018		FLA		9			99% Borrelia lonestari (AY850063.1)

Figure 1: Sixteen field sites used for sampling birds in Oklahoma City, OK, USA, 2017-2018. Inset map indicates the location of the sampling area. Main map shows major highways (ESRI 2019, Redlands CA, USA) in white and land cover categories (National Land Cover Database 2001, US Geological Survey, Sioux Falls, SD, USA): light gray is human-developed land cover (developed, open space; developed, low intensity; developed, medium intensity; and developed, high intensity) dark gray is all other land-cover categories. Size of site labels indicate percent surrounding developed land in 1,000 m radius.

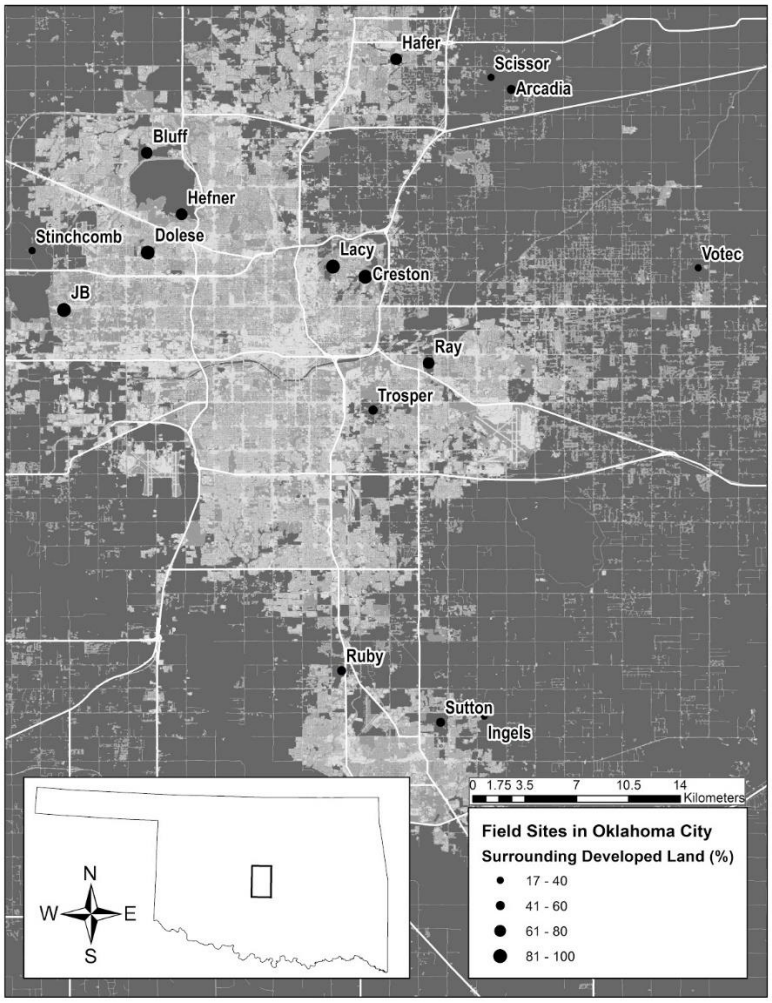


Figure 2: Relationship between percentage of developed land in a 1000 m radius of study sites and *D. variabilis* tick abundance in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018 (Points indicate observed values, line indicates fitted model, and shading indicates 95% confidence interval for the fitted model).

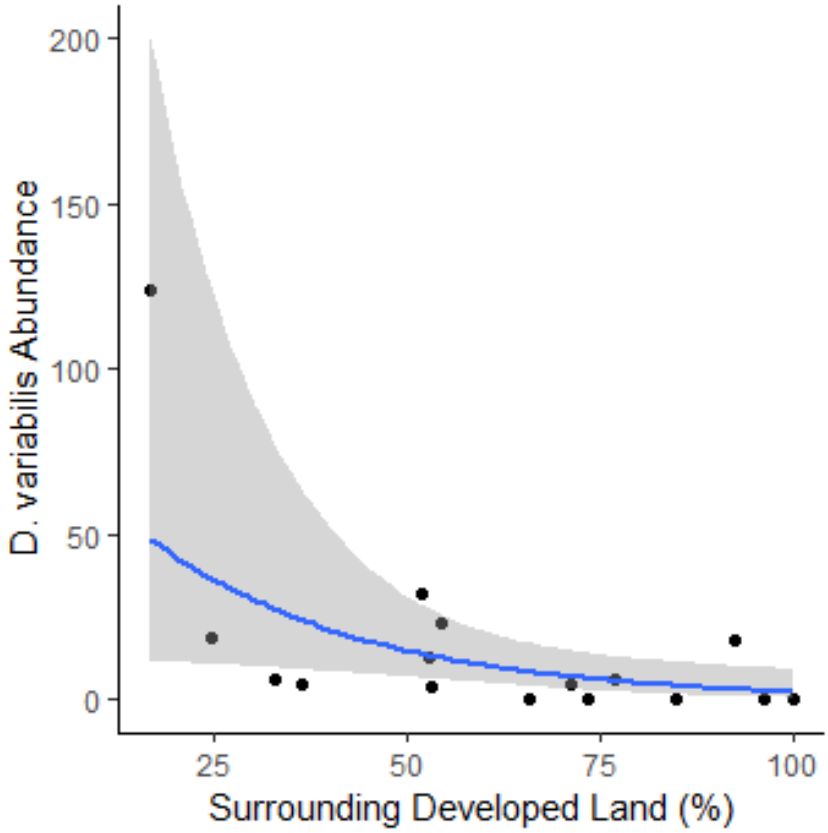


Figure 3: Relationship between percentage woody plant and leaf litter cover at a 50 m long transect and *A. maculatum* tick abundance in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018 (line indicates egression line from a GLMM, shading indicates 95% confidence interval for the fitted model, point color indicates percent woody plant cover).

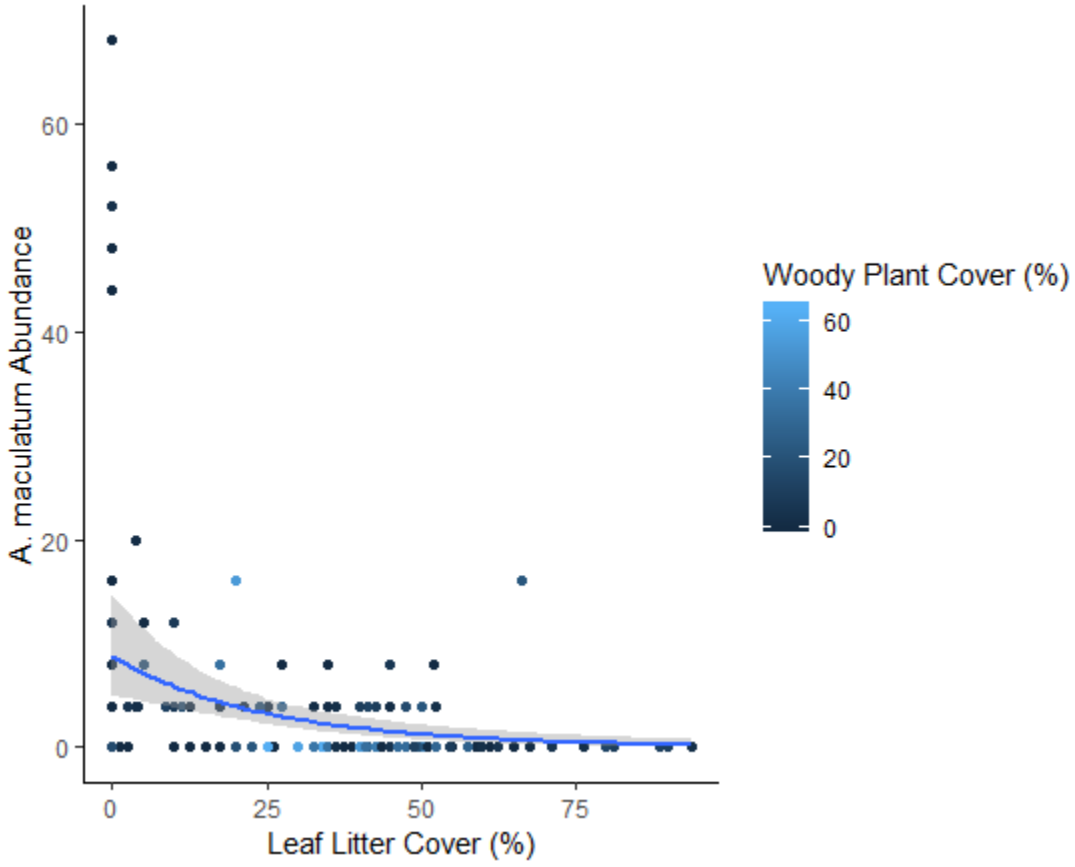


Figure 4: Relationships between a) *A. americanum* tick abundance, dew point, and temperature b) *D. variabilis* tick abundance, dew point, and temperature and c) *A. maculatum* tick abundance and dew point in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018 (line indicates regression line from a GLMM, shading indicates 95% confidence interval for the fitted model, and point colors in 4a and 4b indicate temperature).

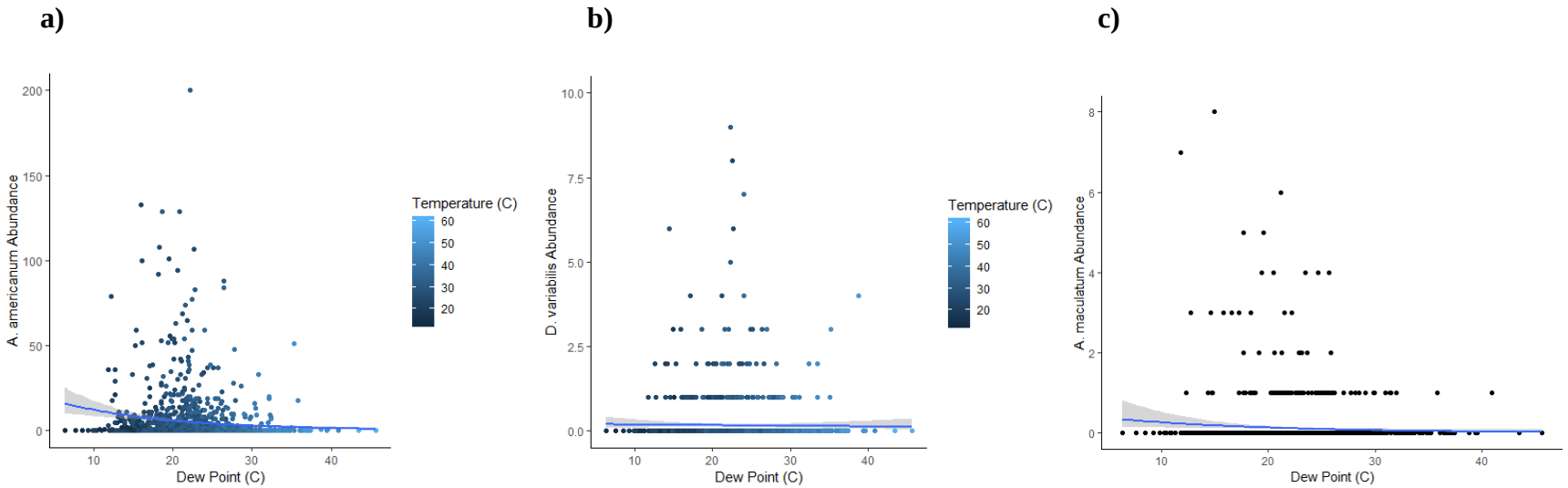


Figure 5: Relationships between estimated deer visitors at each study sites and abundance of a) *A. maculatum ticks* and b) *D. variabilis* ticks using a GLM with negative binomial error distribution for field-sampled ticks at 15 sites in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018 (Points indicate observed values, line indicates fitted model, and shading indicates 95% confidence interval for the fitted model).

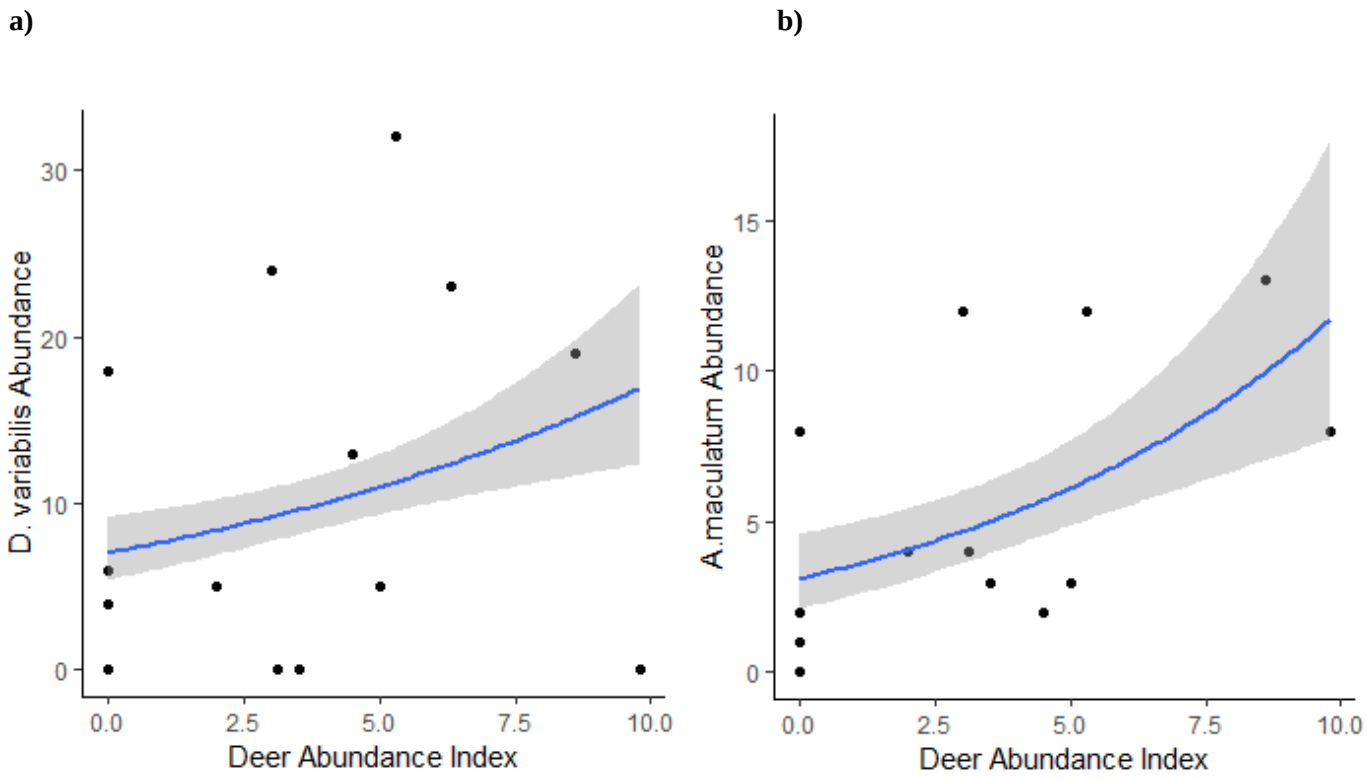
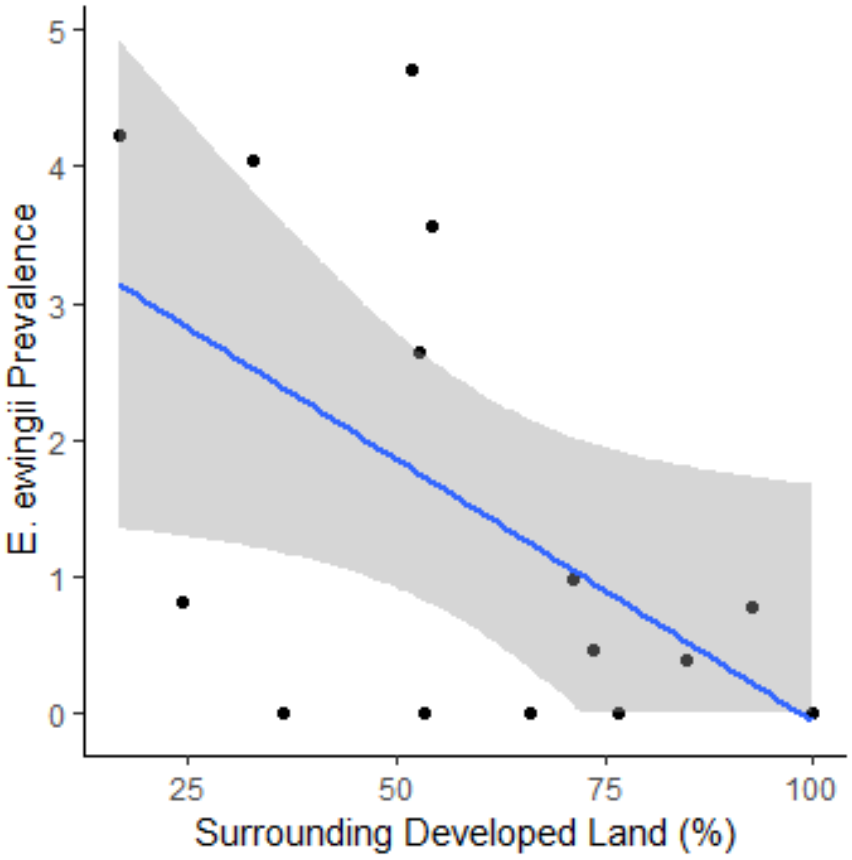


Figure 6- Relationship between *Ehrlichia ewingii* prevalence and percent surrounding developed land from a linear regression in *A. americanum* adult ticks collected across an urbanization gradient in Oklahoma City, Oklahoma, USA (Points indicate observed values, line indicates fitted model, and shading indicates 95% confidence interval for the fitted model).



Appendix

Table 1: Location of 16 sites for field sampling in Oklahoma City, OK from a stratified random sampling technique based on development category, ordered by percent surrounding developed land (most to least)

Site	Latitude	Longitude	Development Category	Surrounding Developed Land (%)
JB	35.49102	-97.6566	0-20%	100
Dolese	35.52573	-97.6058	0-20%	96.2
Creston	35.51112	-97.4737	0-20%	92.5
Lacy	35.51726	-97.4933	0-20%	84.9
Hafer	35.6427	-97.4551	20-40%	76.8
Ray	35.45886	-97.4354	20-40%	73.5
Hefner	35.54893	-97.5852	20-40%	71.1
Bluff	35.58603	-97.6063	20-40%	66
Ruby	35.27273	-97.4882	40-60%	54.4
Sutton	35.24153	-97.4281	40-60%	53.3
Trosper	35.4303	-97.4692	40-60%	52.8
Arcadia	35.62415	-97.3852	40-60%	52
Stinchcomb	35.52675	-97.676	60-83%	36.5
Scissor	35.6315	-97.3975	60-83%	33
Ingels	35.24533	-97.4016	60-83%	24.6
Votec	35.51635	-97.2718	60-83%	16.9

Table 2: Polymerase chain reaction protocols used for testing field-collected adult ticks from Oklahoma City, OK in May-August of 2017 and 2018 for selected pathogens

Pathogen	Gene Target/Reference	Primers	Annealing Temperature Primary/Secondary
Rickettsia spp.	gltA screening (Labruna et al. 2004)	gltACS78F: GCAAGTATCGGTGAGGATG TAAT	40 cycles (15 sec 95C, 30secs 48C, 30secs 72C), 7 min 72C
	~300bp	gltACS323R: GCTTCCTTAAAATTCAATA AATCAGGAT	
	ompA (Eremeeva et al. 1994)	Rr190.70p: ATGGCGAATATTTCTCCAA AA	35 cycles (20 sec 95C, 30secs 48C, 2min 60C), 7 min 72C
	~500bp	Rr190.602n: AGTGCAGCATTCGCTCCCC CT	
	ompB (Roux & Raoult 2000)	120-2788: AAACAATAATCAAGGTACT GT	40 cycles (30 sec 95C, 30secs 50C, 90sec 68C), 7 min 68C
	~800bp	120-3599: TACTTCCGGTTACAGCAAA GT	
Ehrlichia/ Anaplasma sp.	groEL (Tabara et al., 2007; Takano et al. 2009)	Primary: gro607F: GAA GAT GC(A/T) GT(A/T) GG(A/T) TGT AC(G/T) GC	40 cycles (30 sec 95C, 30secs 58C, 30s 72C), 7 min 72C
		Primary: gro1294R: AG(A/C) GCT TC(A/T) CCT TC(A/T) AC(A/G) TC(C/T) TC	
		Secondary: gro677F: ATT ACT CAG AGT GCT TCT CA(A/G) TG	35 cycles (30 sec 95C, 30secs 58C, 30s 72C), 7 min 72C
		Secondary: gro1121R: TGC ATA CC(A/G) TCA GT(C/T) TTT TCA AC	

Borrelia spp.	Flagellin (flaB) (Barbour et al. 1996; Gleim et al. 2016)	Primary: FLALL (5′-ACATATTCAGATGCAGACAGAGGT) Primary: FLARL (5′-GCAATCATAGCCATTGCAGATTGT) Secondary: FLALS (5′-AACAGCTGAAGAGCTTGGAATG) Secondary: FLARS (5′-CTTTGATCACTTATCATTCTAATAGC)	40 cycles (30 sec 95C, 30secs 55C, 45sec 68C), 5 min 72C
Francisella tularensis	ISFtu2 (Versage et al. 2003)	ISFtu2F 5'-TTGGTAGATCAGTTGGTGGGATAAC-3' ISFtu2R 5'-TGAGTTTTACCTTCTGACAACAATATTTC-3'	30 cycles (60s 95C, 60s 55C, 60s 72C), 5min 72C

Table 3: Total abundance of ticks by site, species, and life stage from field-collected ticks in at 16 sites in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018

				No. of ticks collected			
				A. americanum		*D. variabilis*	*A. maculatum*
Urbanization Category	Surrounding Developed Land (%)	Site	No. of ticks collected	adult	nymph	adult	adult
0-20%	100	JB	9	2	5	0	2
0-20%	96.2	Dolese	2	0	1	0	1
0-20%	92.5	Creston	830	647	157	18	8
0-20%	84.9	Lacy	866	262	601	0	3
20-40%	76.8	Hafer	784	453	323	6	2
20-40%	73.5	Ray	395	221	170	0	4
20-40%	71.1	Hefner	148	102	37	5	4
20-40%	66	Bluff	71	48	15	0	8
40-60%	54.4	Ruby	583	196	252	23	112
40-60%	53.3	Sutton	15	11	0	4	0
40-60%	52.8	Trosper	875	608	252	13	2
40-60%	52	Arcadia	856	445	367	32	12
60-83%	36.5	Stinchcomb	27	8	11	5	3
60-83%	33	Scissor	333	198	127	6	2
60-83%	24.6	Ingels	1550	374	1144	19	13
60-83%	16.9	Votec	2955	1113	1706	124	12
		Total	10299	4688	5168	255	188

Table 4: Total abundance of ticks by year, round, life stage, and species from field-collected ticks in at 16 sites in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018. In 2017, round 1 began on 15 May 2017 and in 2018 round 1 began on 14 May 2018. Each round lasted approximately two weeks.

		No. ticks collected			
Year	Round	*A. americanum* adults	*A. americanum* nymphs	*D. variabilis* adults	*A. maculatum* adults
2017	1	972	676	35	41
2017	2	706	530	51	37
2017	3	494	427	35	24
2017	4	469	1703	36	10
2017	5	87	69	10	9
2018	1	946	872	35	38
2018	2	541	550	39	11
2018	3	330	240	4	14
2018	4	115	64	8	3
2018	5	28	37	2	1

Table 5: Model selection results for analyses of large-scale characteristics affecting abundance of a) *A. americanum*, b) *D. variabilis*, and c) *A. maculatum* ticks, for field-sampled ticks in at 16 sites in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018.

Model[a]	ΔAIC[b]	df[c]	weight[d]
a) *A. americanum*			
Null	0	2	0.48
Surrounding developed land	0.6	3	0.35
Park size	2.9	3	0.11
Park size + Surrounding developed land	4.1	4	0.06
b) *D. variabilis*			
Surrounding developed land	0	3	0.69
Null	3	2	0.156
Park size + Surrounding developed land	3.5	4	0.12
Park size	6.1	3	0.034
c) *A. maculatum*			
Null	0	2	0.54
Surrounding developed land	1.2	3	0.293
Park size	3.1	3	0.117
Park size + Surrounding developed land	4.9	4	0.048

[a]Variables included in each candidate model
[b]Number of parameters in the generalized linear model (includes intercept parameter)
[c]Difference in AICc value between model and the most strongly supported model
[d]AIC weight–relative strength of support for the model

Table 6: Model selection results for analyses of vegetation characteristics affecting abundance of a) A. americanum, b) D. variabilis, and c) A. maculatum ticks, for field-sampled ticks at 16 sites, 156 transects, and 1,560 sampling events in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018.

Model[a]	ΔAIC[b]	df[c]	weight[d]
a) *A. americanum*			
Woody plants	0	4	0.128
Woody plants + leaf litter	0.1	5	0.125
Graminaceous plants	0.3	5	0.109
Graminaceous plants + Red cedar	0.7	5	0.09
Leaf litter + Red cedar	1.3	5	0.067
Red Cedar	1.4	4	0.063
Herbaceous + woody plants	1.4	5	0.062
Graminaceous + woody plants	1.5	5	0.062
Null	1.7	3	0.056
Leaf litter	1.8	4	0.053
Graminaceous plants	1.8	4	0.052
Herbaceous plants	2.2	4	0.043
Herbaceous plants + Red cedar	2.4	5	0.038
Herbaceous plants + Leaf litter	3.0	5	0.028
Graminaceous plants + Leaf litter	3.3	5	0.024
b) *D. variabilis*			
Understory red cedar	0	4	1
Leaf litter depth	21.3	4	<0.001
Null	22.8	3	<0.001
Leaf litter depth + Red Cedar	22.8	5	<0.001

Graminaceous plants + Leaf litter depth	22.9	5	<0.001
Woody plants + Leaf litter depth	23.3	5	<0.001
Graminaceous plants	23.8	4	<0.001
Woody plants	24.1	4	<0.001
Graminaceous plants + Red cedar	25.4	5	<0.001
Graminaceous + Woody plants	25.6	5	<0.001

c) *A. maculatum*

Woody plants + leaf litter	0	5	0.688
Leaf litter	3.5	4	0.1176
Leaf litter + Red cedar	5.4	5	0.046
Graminaceous plants + leaf litter	5.6	5	0.0411
Woody plants	5.7	4	0.0406
Red cedar	6.2	4	0.03113
Graminaceous + woody plants	7.2	5	0.0185
Graminaceous plants	9	4	0.0078
Null	9.4	3	0.0062
Graminaceous plants + Red cedar	11.0	5	0.0029

[a]Variables included in each candidate model
[b]Number of parameters in the generalized linear model (includes intercept parameter)
[c]Difference in AICc value between model and the most strongly supported model
[d]AIC weight–relative strength of support for the model

Table 7: Model selection results for analyses of meteorological characteristics affecting abundance of a) A. americanum, b) D. variabilis, and c) A. maculatum ticks, for field-sampled ticks at 16 sites and 156 transects in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018.

Model[a]	ΔAIC[b]	df[c]	weight[d]
a) *A.americanum*			
Temperature + dew point	0	6	1
Dew point	13.9	5	<0.001
Relative humidity	22.2	5	<0.001
Temperature	30.3	5	<0.001
Null	834.9	4	<0.001
b) *D. variabilis*			
Temperature + dew point	0	6	0.9889
Temperature	10.4	5	0.0054
Dew point	10.6	5	0.005
Relative humidity	14.5	5	<0.001
Null	108.8	4	<0.001
c) *A. maculatum*			
Dew point	0	5	0.586
Temperature + dew point	1.1	6	0.346
Temperature	4.3	5	0.068
Relative humidity	47.4	5	<0.001
Null	114.9	4	<0.001

[a]Variables included in each candidate model
[b]Number of parameters in the generalized linear model (includes intercept parameter)
[c]Difference in AICc value between model and the most strongly supported model
[d]AIC weight–relative strength of support for the model

Table 8: Pathogen prevalence (%) in a) *A. americanum*, b) *D. variabilis*, and c) *A. maculatum* ticks, for field-sampled adult ticks at 16 sites in Oklahoma City, Oklahoma, USA, May-Aug 2017-2018.

a)

			Pathogen Prevalence in *A. americanum*											
			E. chaffeensis			*E. ewingii*			Panola Mnt Ehrlichia			*B. lonestari*		
Urbanization Category	Surrounding Developed Land (%)	Site	2017	2018	Total	2017	2018	Total	2017	2018	Total	2017	2018	Total
0-20%	100	JB	-	0	0	-	0	0	-	0	0	-	0	0
0-20%	96.2	dolese	-	-	-	-	-	-	-	-	-	-	-	-
0-20%	92.5	creston	0	0	0	0.46	1.40	0.77	0	0	0	0.46	0.46	0.46
0-20%	84.9	lacy	1.48	0.79	1.14	0.74	0	0.38	0	0	0	0.74	0.79	0.76
20-40%	76.8	hafer	0	0	0	0	0	0	0.32	0	0.22	1.29	0	0.88
20-40%	73.5	ray	2.68	0	1.81	0.67	0	0.45	0.67	0	0.45	0.67	2.78	1.36
20-40%	71.1	hefner	0	0	0	1.85	0	0.98	0	0	0	0	0	0
20-40%	66	bluff	0	0	0	0	0	0	0	0	0	0	0	0
40-60%	54.4	ruby	0.82	2.70	1.53	2.50	5.41	3.57	2.50	1.35	2.04	3.28	0	2.04
40-60%	53.3	sutton	0	0	0	0	0	0	0	0	0	0	0	0
40-60%	52.8	trosper	0	0.68	0.33	4.13	1.02	2.63	0	0.34	0.16	0.95	0.34	0.66

40-60%	52	arcadia	0.37	0	0.22	4.43	5.17	4.72	0.738	0	0.45	0	1.15	0.45
60-83%	36.5	stinchcomb	0	0	0	0	0	0	0	0	0	0	0	0
60-83%	33	scissor	5.77	1.37	2.53	3.85	4.11	4.04	3.85	1.37	2.02	1.92	0	0.51
60-83%	24.6	ingels	0	0.59	0.27	0.49	1.18	0.80	0	0	0	0.49	0.59	0.53
60-83%	16.9	votec	3.07	1.30	2.33	5.07	3.25	4.31	0.15	1.30	0.63	0.61	0	0.36

b)

			Pathogen Prevalence in *D. variabilis*					
			R. rhipicephali			*R. andeanae*		
Urbanization Category	Surrounding Developed Land (%)	Site	2017	2018	Total	2017	2018	Total
0-20%	100	JB	-	0	-	-	0	-
0-20%	96.2	dolese	-	-	-	-	-	-
0-20%	92.5	creston	0	0.46	5.56	0	0	0
0-20%	84.9	lacy	-	0	-	-	0	-
20-40%	76.8	hafer	0	0	0	0	0	0
20-40%	73.5	ray	-	0	-	-	0	-
20-40%	71.1	hefner	0	0	0	0	0	0
20-40%	66	bluff	-	0	-	-	0	-
40-60%	54.4	ruby	6.67	2.70	13.04	6.67	1.35	8.70
40-60%	53.3	sutton	0	0	0	0	0	0
40-60%	52.8	trosper	0	0	0	0	0	0
40-60%	52	arcadia	5.26	0	3.13	0	0	0
60-83%	36.5	stinchcomb	33.33	100	40	0	0	0
60-83%	33	scissor	0	0	0	0	0	0
60-83%	24.6	ingels	14.29	0	10.53	0	0.59	5.26
60-83%	16.9	votec	8.14	0	5.65	0	0	0

c)

			Pathogen Prevalence in *A. maculatum*					
			R. andeanae			*R. parkeri*		
Urbanization Category	Surrounding Developed Land (%)	Site	2017	2018	Total	2017	2018	Total
0-20%	100	JB	0	-	0	0	-	0
0-20%	96.2	dolese	0	-	0	0	-	0
0-20%	92.5	creston	0	50	12.5	0	0	0
0-20%	84.9	lacy	33.33	-	33.33	0	-	0
20-40%	76.8	hafer	0	-	0	0	-	0
20-40%	73.5	ray	0	100	25	0	0	0
20-40%	71.1	hefner	0	33.33	25	0	0	0
20-40%	66	bluff	0	80	50	0	0	0
40-60%	54.4	ruby	6.17	35.48	14.29	2.47	3.23	2.68
40-60%	53.3	sutton	-	-	-	-	-	-
40-60%	52.8	trosper	-	0	0	-	0	0
40-60%	52	arcadia	0	66.67	16.67	0	0	0
60-83%	36.5	stinchcomb	0	0	0	0	0	0
60-83%	33	scissor	0	-	0	0	-	0
60-83%	24.6	ingels	0	50	30.77	0	0	0
60-83%	16.9	votec	0	0	0	0	0	0

Table 9: Prevalence of SFGR in *A. americanum* collected in Oklahoma City, 2017-2018.

Urbanization Category	Surrounding Developed Land (%)	Site	*Rickettsia sp.*									
			Number pools		Number positive pools		Number of ticks tested		Positive pools (%)		MIR infection rate (%)	
			2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
0-20%	100	JB	2	2	0	0	2	4	0.0	0.0	N/A	N/A
0-20%	96.2	Dolese	1	0	0	0	1	0	0.0	0.0	N/A	N/A
0-20%	92.5	Creston	99	26	51	21	432	219	51.5	80.8	11.8	9.59
0-20%	84.9	Lacy	51	15	24	13	138	128	47.1	86.7	17.39	10.16
20-40%	76.8	Hafer	72	18	40	17	315	147	55.6	94.4	12.7	11.56
20-40%	73.5	Ray	49	10	27	10	154	75	55.1	100	17.53	13.33
20-40%	71.1	Hefner	27	7	12	6	56	48	44.4	85.7	21.43	12.5
20-40%	66	Bluff	13	7	4	6	21	30	30.8	85.7	19.05	20
40-60%	54.4	Ruby	85	19	22	6	217	76	25.9	31.6	10.14	7.89
40-60%	53.3	Sutton	2	1	0	0	10	1	0.0	0	N/A	N/A
40-60%	52.8	Trosper	75	34	40	29	309	306	53. 3	85.3	12.94	9.48
40-60%	52	Arcadia	75	24	39	21	299	176	52.0	87.5	13.04	11.93
60-83%	36.5	Stinchcomb	11	1	5	0	12	1	45.4	0.0	41.67	N/A
60-83%	33	Scissor	29	19	13	18	60	147	44.8	94.7	21.67	12.24
60-83%	24.6	Ingels	60	27	19	22	222	177	31.7	81.5	8.56	12.43
60-83%	16.9	Votec	139	59	79	51	749	472	56.8	86.4	10.55	10.8

Table 10: Yearly differences in pathogen prevalence from ticks collected in Oklahoma City, 2017-2018.

Prevalence rates

	E. chaffeensis	*E. ewingii*	PME	*B. lonestari*	Total *A. americanum* tested	*R amblyommatis*	Total *A. americanum* tested
2017	31	70	10	21	2701	253	678
2018	16	42	9	9	2005	140	446
Total	**47**	**112**	**19**	**30**	**4706**	**393**	**1124**

Prevalence rates

	R andeanae	Total *A. maculatum* tested	*R. rhiphacephali*	*R. amblyommmatis*	*R. andeanae*	Total *D. variabilis* tested
2017	85	123	14	2	0	177
2018	35	74	5	1	1	77
Total	**120**	**197**	**19**	**3**	**1**	**254**

CHAPTER II

VARIATION IN TICK INFESTATION OF BIRDS ACROSS A GRADIENT OF URBANIZATION INTENSIY IN A MAJOR METROPOLITAN AREA IN THE U.S. GREAT PLAINS

Abstract

Urbanization has been linked to the emergence and increased prevalence of many vector-borne diseases, including tick-borne diseases. Migratory birds play an important role in the large-scale dispersal of ticks and tick-borne pathogens; however, less is known about how birds interact with ticks during sedentary periods of avian annual cycles, especially in urban landscapes. At 16 green spaces capturing an urbanization gradient across the Oklahoma City metropolitan area in summer 2017 and 2018, we mist-netted birds during the breeding season, sampled them for ticks, and identified factors influencing the proportion of birds infested by ticks and tick load of infested birds. Of 459 birds searched, 111 (24.2%) were infested with one or more tick, a relatively high infestation rate compared to most previous North American studies. The most frequently infested species included Carolina Wren (52%), Brown Thrasher (41%), and Northern Cardinal (25%). Half of collected ticks (51%) were the Lone Star Tick (*Amblyomma americanum*), 35% were the Gulf Coast Tick (*A. maculatum*), and 16% were Rabbit Ticks (*Haemaphysalis leporispalustris*), and all of these tick species are known to harbor

multiple pathogens that infect humans. Urbanization intensity, as measured by the proportion of developed land surrounding sites, did not influence either the proportion of birds with ticks or the tick load of infested birds. Our results suggest that, in addition to being long-distance dispersal agents, birds are important dispersers of ticks within urban landscapes, and that summer resident birds and migrant birds during more sedentary periods of their annual cycle may be particularly vulnerable to tick infestations. Clarifying local contributions of urban birds to tick populations, as well as the role of urbanization in shaping bird-tick interactions, will provide increased understanding of transmission dynamics for pathogens that affect urban residents.

Introduction

Ticks are parasitic vectors of many pathogens that cause disease in humans, wildlife, and domestic animals worldwide. Wildlife reservoir hosts play an important role in the life cycles of tick-borne pathogens because tick populations, tick-host interactions, and thus spatiotemporal patterns of disease prevalence, are all influenced by the density, diversity, and species composition of wildlife host communities (Schmidt and Ostfeld 2001; Allan et al. 2010; Hamer et al. 2012a; Keesing et al. 2012; Pfaffle et al. 2013; Silaghi et al. 2015). Land-use and land cover changes can greatly alter habitats of pathogens, vectors, and hosts, and thus the nexus of all three transmission components (Patz et al. 2000; Patz et al. 2004; Foley et al. 2005; Hornok et al. 2014). In particular, urbanization is increasing globally and fundamentally changes almost all aspects of ecosystems (Grimm et al. 2008) with concomitant impacts on disease transmission due to alteration of microclimates that influence vectors and pathogens, reduction of host immune function via increased stress and pollutant exposure, and elevation of

transmission rates due to greater host densities (Bradley and Altizer 2007). Consequently, urbanization has been linked to the emergence and increased prevalence of many tick-borne diseases (Steere 1994; Maupin et al. 1991; Jobe et al. 2007; Rydzewski et al. 2012; Schwan et al. 2009; Blanton et al. 2014).

Understanding the emergence and increased prevalence of tick-borne diseases in urban areas requires research into the role of urban wildlife as carriers of ticks and pathogens. In Lyme Disease-endemic suburban areas of the U.S., the density of some wildlife reservoirs has been linked to increased disease exposure in humans. For example, increased forest fragmentation and biodiversity loss associated with urbanization allow increases in populations of white-footed mice (*Peromyscus leucopus*), the main reservoir host of Lyme Disease, and thus higher prevalence of *Borrelia burgdorferi* in both mice and ticks (Allen et al. 2003; LoGiudice et al. 2003). However, beyond Lyme disease, very little is known about the role of wildlife in tick-borne disease transmission cycles in urban areas (Paddock et al. 2003; Loss et al. 2016).

In addition to mammalian wildlife, accumulating evidence indicates that birds also play an important role in the transmission of tick-borne pathogens. Birds, especially those that undertake long-distance migrations, can disperse and establish populations of ticks in new areas (Ogden et al. 2008; Hamer et al. 2012a; Mukherjee et al. 2014; Cohen et al. 2015), and they may contribute to broad-scale expansion of tick-borne pathogens because they carry ticks harboring pathogens, such as *Anaplasma* spp., *Borrelia* spp., *Rickettsia* spp., and tick-borne encephalitis virus (Scott et al. 2012; Hasle et al. 2013; Hornock et al. 2013; Schneider et al. 2015). Moreover, both migratory and resident birds are capable of serving as reservoir hosts necessary for local amplification of tick-borne

pathogens (Comstedt et al. 2006). Despite the increasingly recognized importance of birds in the transmission of tick-borne diseases, very little is known about their role in carrying ticks and tick-borne pathogens in urban areas, especially in North America. The limited research on birds in urban areas suggests that migratory species likely supplement existing urban populations of *Ixodes scapularis*, the primary vector of Lyme disease throughout eastern North America, and may be capable of introducing neotropical tick species into temperate urban areas (Morshed et al. 2005; Hamer et al. 2012a; Cohen et al. 2015).

In addition to the above general research gap, little is known about how land cover and intensity of development influence the role of birds in carrying ticks in urban areas. One study in the eastern US (Norfolk, VA) found that birds in areas with extensive impervious surface (i.e. more urbanized areas) and water bodies were less likely to carry ticks, but percent imperviousness alone did not predict infestation (Heller et al. 2019). Rates of pathogen prevalence in ticks carried by birds may also vary in relation to urbanization, as suggested by a study showing that urban birds were less likely to carry *B. burgdorferi*-infected ticks (Hamer et al. 2012b). Notably, most of these urban studies, as well as most studies of the role of birds in carrying ticks, have focused on long distance dispersal by migrating birds, even though a recent meta-analysis found that non-migratory birds carry more ticks and a greater proportion of pathogen-infected ticks (Loss et al. 2016).

To begin to more formally assess the role of resident bird species as carriers of ticks and tick-borne pathogens in U.S. urban areas, as well as the effect of urban development intensity on tick infestation of birds, we captured birds and sampled them

for ticks at 16 green spaces surrounded by varying levels of urbanization in Oklahoma City, Oklahoma, USA. Despite high prevalence of several tick-borne diseases, including Spotted Fever Group rickettsiosis, ehrlichiosis, and tularemia (CDC 2015; Biggs et al. 2016; Drexler et al. 2016; Heitmann et al. 2016), tick populations and tick-borne pathogen dynamics in this region are poorly studied (Paddock and Childs 2003; Loss et al. 2016; Springer & Johnson 2018). We hypothesized that both the proportion of birds that harbor ticks and the intensity of tick infestation (i.e., numbers of ticks carried or "tick burden") would vary over a gradient of urbanization intensity, and that different bird species would show different patterns of infestation relative to urbanization intensity. The results of this study will contribute to an increased understanding of the ecology of tick-borne pathogens, and thus of the factors influencing human disease risk in urban areas.

Methods

Site Selection

Oklahoma City is the largest city in both population size and land area in Oklahoma (Figure 1), with 643,648 people residing in 620 mi^2 (City of Oklahoma, 2015; US Census Bureau, 2011). The Oklahoma City metropolitan area consists of over 1.2 million residents in seven counties. Oklahoma City is located in the US Great Plains ecoregion (EPA, 2016), and the surrounding land-use is primarily grasslands and cultivated crops west of the city, and grasslands and deciduous forest with interspersed patches of pasture east of the city. With a mild climate and average annual temperature of 15.6℃ (Greater Oklahoma City, 2016), tick activity occurs at least ten months out of the year (Talley et al. 2014). Oklahoma is home to some of the highest rates of Spotted Fever

Group rickettsiosis and ehrlichiosis in the U.S. (Biggs et al. 2016; Springer and Johnson, 2018), and high rates of tularemia (CDC 2015), which make Oklahoma City an ideal study area for the purposes of our research objectives. Other less-common tick borne diseases found in Oklahoma include: Heartland and Bourbon virus (Dahlgren et al. 2015; OSHD 2017).

In the Oklahoma City metropolitan area, we used Google Earth and Google Street View to identify candidate study sites for bird and tick sampling. We first identified all potential large areas (>2 hectares) of tick habitat, including parks, green spaces, and waste spaces with un-manicured understory vegetation, shrubs, savanna and woodland, but excluding undeveloped areas dominated by open manicured grass. We then manually digitized a polygon representing the boundaries of each candidate site and used ArcGIS 10.1 to calculate percentages of developed land and impervious surfaces in a 1,000m buffer of each site's outer edge, with all land cover data from the national land cover database (NLCD; Homer et al. 2015). For developed land cover, we combined all NLCD cover classes representing human development (developed, open space; developed, low intensity, developed, medium intensity, and developed, high intensity) and excluded all other cover types (e.g., water, forest, and cultivated classes). Because percent surrounding impervious surface and percent surrounding developed land were strongly correlated (Pearson's r = -0.74), only percent developed land was used for the following stratified site selection approach. To capture a gradient of urban development intensity, we grouped sites into four categories based on percent developed land—17-40%, 40-60%, 60-80%, 80-100% (17% was the minimum observed value across all candidate sites)—and randomly chose four sites from each category (n=16). All sites were ground-

truthed and assessed for safety and accessibility issues, and based on these logistical constraints, three sites had to be replaced with other randomly selected sites from the same land cover category. Final sites selected for inclusion in the study are shown in Figure 1.

Bird Capture

At all 16 sites, we captured birds using mist nets, with sampling occurring twice between June and August in both 2017 and 2018 (i.e., 4 total site visits), with the exception of one site that was visited only once in 2018 due to safety reasons. These months were chosen to focus sampling on species that are non-migratory year-round residents, as well as migratory species during their summer residency period (i.e., excluding in-transit migratory birds). Within each year, site visits were roughly one month apart. For each site visit, and at approximately sunrise, we set and opened 5-6 mist-nets (2.6m in height, 12m in length, 36mm mesh, Avinet Inc., Dryden, NY) and captured birds until 11am, or earlier if temperatures became too warm to safely restrain birds in nets. We also attached alpha-numeric aluminum bands to each bird (US Department of Interior, bird banding laboratory) and recorded species, sex, weight, and age class according to Pyle (1997). All bird handling was permitted under a U.S. federal bird banding permit (#23929) and a State of Oklahoma wildlife collection permit (#6963); bird handling was also approved by the Institutional Animal Care and Use Committee at Oklahoma State University (protocol #AG-14-6).

Tick Searches

Before release, each bird was visually searched for ticks by blowing apart feathers to see all skin surfaces. The whole body of each bird was searched, and we took special care to thoroughly search around the thighs and wings due to the difficulty of viewing the folds of skin, bones, and hollows in these locations. When a tick was found, it was removed with fine-tip tweezers, except when doing so posed a potential harm to the bird's safety (e.g. if the tick was inside the ear canal or close to the eye and/or if the bird showed signs of physical stress that required us to release it before tick removal). In all cases, even when ticks could not be removed, we recorded data on numbers and locations of ticks encountered on each sampled bird. All extracted ticks were immediately placed in 70% alcohol before later identifying them to species using pictorial keys (Keirans and Litwak, 1989; Keirans and Durden, 1998; Coley, 2015; Dubie et al. 2017). Because *A. maculatum* in the United States is indistinguishable from *Amblyomma triste* (Lado et al. 2018), all references to *A. maculatum* in this manuscript actually refer to the *A. maculatum-A. triste* complex.

Statistical Analyses

All recaptures of individual birds were treated as separate events. Although recaptures of a previously sampled bird are not truly independent samples, a recent meta-analysis found that most studies treat recaptures in this manner (Loss et al. 2016). All tick species were analyzed together due to limitations in sample size when considering the 16 sites as replicates; specifically, although *A. americanum* were found on birds at all 16 sites, *A. maculatum* and *H. leporispalustris* were only found at 50% (8 of 16) and 43.7%

(7 of 16) of sites respectively. We performed all analyses using R 3.2.2 (R Core Team 2016). We used R^2 and p-values to determine statistical or biological significance in the models described below.

To address our first hypothesis (variation in the proportion of birds that harbor ticks relative to urbanization intensity), the proportion of birds infested with ticks at each site was calculated by dividing the number of birds infested with at least one tick by the total number of birds captured. To determine if the proportion of birds infested varied with urban development intensity, we used a linear model (LM) with site as the unit of replication (n=16), total prevalence of infestation (across all bird species at each site) as the dependent variable, and percent surrounding developed land as a fixed effect. To address hypothesis 2 (regarding variation in the intensity of tick infestation relative to urbanization intensity), intensity of tick infestation was calculated for each site by dividing the total number of ticks observed on all birds by the total number of birds infested with ticks. To determine if intensity of tick infestation varied with urban development intensity, we used an LM, with site as the replicate, total intensity of infestation (i.e., across all bird species at a site) as the dependent variable, and percent surrounding developed land as a fixed effect.

To address hypothesis 3 (regarding species-specific associations between urbanization intensity and infestation prevalence and intensity), we calculated both proportion infested and intensity of infestation at each site (as described above) for the two most commonly captured bird species: Northern Cardinal (*Cardinalis cardinalis*) and Carolina Wren (*Thryothorus ludovicianus*). For each species, we used an LM with site as

the replicate, proportion infested or intensity of infestation as the dependent variable, and percent surrounding developed land as a fixed effect.

Results

Descriptive summary of bird and tick sampling

We conducted 459 tick searches on 432 individual birds (27 re-captures) representing 31 species (Table 1). Northern Cardinal (*Cardinalis cardinalis*) and Carolina Wren (*Thryothorus ludovicianus*) comprised 56.6% (n=260) of total captures; American Robin (*Turdus migratorius)* and Painted Bunting (*Passerina ciris*) were also caught more than 30 times. More tick searches were conducted in 2017 (n=282, 61.4%) than 2018 (n=177, 38.6%), despite approximately equal mist-netting effort at all sites in both years (except for the single site sampled once in 2018 due to safety concerns). The most commonly sampled bird age class was after hatch year (71.2% of capture events/tick searches), followed by hatch year (i.e., hatched the same year; 22.7%), and unknown (i.e., age could not be determined or aging was not attempted; 6.1%). Most hatch-year individuals cannot be reliably sexed; however, of after hatch year birds, 39.8% were females, 37.9% were males, and 22.3% were of unknown sex (i.e., individuals of species with sexually monomorphic feather plumages and no sex-specific anatomical characteristics discernible).

A total of 111 birds representing ten species were infested with 495 total ticks. The prevalence of infestation (the percentage of birds carrying at least one tick) was 24.2%, and the mean intensity of infestation (the average number of ticks on infested birds) was 4.46 ticks per bird. Carolina Wren was the most likely species to be infested

(55.9%), followed by Brown Thrasher (*Toxostoma rufum*, 37.5%) and Northern Cardinal (27.1%). Out of the 495 ticks observed, 322 were removed for identification, and among this sample, we found three species of ticks—*Amblyomma americanum* (51%), *Amblyomma maculatum* (36%), *and Haemaphysalis leporispalustris* (13%)—with all individuals representing either larval (69%) or nymphal (31%) life-stages (Table 1).

Patterns of Tick Infestation Relative to Urbanization Intensity

There was no statistically significant association between the percent of surrounding developed land and prevalence of tick infestation (the proportion of birds infested) across all birds species (Figure 1a) or for Northern Cardinal ($p=0.1692$, multiple $r^2=0.1305$; Figure 1b) or Carolina Wren ($p=0.06758$, multiple $r^2=0.2342$; Figure 1c). However, there was a non-significant overall negative trend for all three models (i.e., decreasing infestation with increasing developed land), especially when considering the relatively high r^2 values based on inclusion of a single predictor variable. For intensity of infestation (i.e., tick load per infested bird), there was no significant influence of developed land when considering all bird species ($p=0.1984$, multiple $r^2=0.1152$; Figure 2a), Northern Cardinal ($p=0.5346$, multiple $r^2=0.03601$; Figure 2b), or Carolina Wren ($p=0.08817$, multiple $r^2=0.2889$; Figure 2c). However, there was again a non-significant overall negative trend in all three models, especially both the overall and Carolina Wren models, which had relatively high r^2 values.

Discussion

We documented a high rate of tick infestation in birds in a large metropolitan area of the U.S. Great Plains, a region where little is known about the importance of birds as

carriers of ticks and tick-borne pathogens. About one-quarter of all birds carried one or more ticks, suggesting that year-round resident species, as well as migratory birds during summer residency periods, are important for localized dispersal of ticks, even in urban areas. We also conducted one of the first analyses of the prevalence and intensity of tick infestation of birds across an urbanization gradient. When considering all birds combined, we found no significant difference in either prevalence or intensity of infestation relative to urban development, although individual bird species, particularly Carolina Wren, may have lower prevalence and intensity of infestation in more intensely developed areas. Our results suggest that birds may be important as dispersers of ticks across the entire urban to peri-urban gradient, and that they may be contributing to the increased prevalence of tick-borne diseases in urban areas.

The U.S. Great Plains is characterized by a high prevalence of several tick-borne diseases, including Spotted Fever Group rickettsiosis, ehrlichiosis, and tularemia (Biggs et al. 2016; CDC 2015; Drexler et al. 2016; Heitmann et al. 2016); however, the role of birds in carrying ticks was heretofore unknown in this region (Loss et al. 2016). We found that in Oklahoma City, birds were parasitized by *A. maculatum*, *H. leporispalustris*, and most commonly, *A. americanum*. The greater rate of parasitism by *A. americanum* could be a result of the aggressive feeding habits of this species, which may enhance its host-preference for ground-foraging birds (Goddard and Varela-Stokes, 2009), or its status as the most abundant tick in our study area (Roselli, Chapter 1). *H. leporispalustris*, the least commonly observed tick, was only found on four bird species: American Robin, Brown Thrasher, Carolina Wren, and Northern Cardinal. This finding could reflect that these bird species were sampled most frequently—and a larger sample

of other species would result in observations of *H. leporispalustris* on additional bird species—or that *H. leporispalustris* are more likely to parasitize these species due to their life history traits and/or habitat associations (e.g., all four species forage on or near the ground, a trait that has repeatedly been shown to increase tick infestation (Comstedt et al 2006; Cohen et al. 2015; Loss et al. 2016). Support for the explanation regarding limited sample sizes is provided by a study in Chicago, USA, that sampled >6,000 birds and found *H. leporispalustris* on 12 different bird species), and 5/12 were also captured during our study (Hamer et al. 2012a). We also observed species-specific differences in which tick life stages were most likely to parasitize birds. A roughly equal number of *A. americanum* larvae and nymphs parasitized birds whereas the majority of sampled *A. maculatum* and *H. leporispalustris* were larvae. This finding confirms the observations that *A. maculatum* (Barker et al. 2004) and *H. leporispalustri*s (Kollars & Oliver 2003) nymphs have less host-preference for smaller animals like birds, whereas *A. americanum* nymphs may not have such differential preferences. These descriptive results provide important foundational information for understanding tick host preferences in the U.S. Great Plains.

Most previous studies have focused on migratory birds as long distance transporters of ticks and pathogens (e.g., Kinsey and Durden 2000; Morshed et al. 2005; Hamer et al. 2012a; Mukherjee et al. 2014; Cohen et al. 2015) despite a recent meta-analysis showing infestation to be greatest for non-migratory bird species (Loss et al. 2016). Our study, which was designed to focus on bird species that are permanent residents, as well as migratory species during their more sedentary summer resident period, further supports the importance of stationary birds in influencing tick populations,

and potentially, transmission of tick-borne pathogens. The earlier meta-analysis, which included many studies that sampled birds during migration periods, found an overall prevalence of infestation of 5.1% based on 38,929 total birds across 11 studies. Our study found a much higher overall infestation rate of 24.2%, suggesting that birds may be more important for localized transportation of ticks than previously thought, even in urban areas. Additional support for this statement can be found in a complimentary study, where only *A. americanum* were collected from extensive field sampling at a highly urbanized site, but one *A. maculatum* tick was found on a bird at that same site *(*Roselli et al. *unpublished data*). This suggests that birds are transporting ticks within urban areas, and potentially depositing them into previously uncolonized areas where they may become established.

Besides the purposeful exclusion of migrating birds, the location of our study in the U.S. Great Plains, where no studies had previously been conducted, could contribute to the exceptionally high infestation observed. Specifically, the high infestation rate appears to be driven by *A. americanum*, which is the dominant tick species in the region but is less commonly found and studied in the north-eastern and western U.S despite recent abundance increases in parts of the eastern U.S. (Jordan et al 2019; Raghavan et al. 2019). Notably, results of one study in the south-eastern U.S. are contrary to this explanation, as overall tick infestation rates on birds were found to be similar in areas with and without *A. americanum* (Kinsey et al. 2000). Another explanation for the high rates of infestation is related to methodology; specifically, while many previous studies focused searching effort only on the head and neck of birds, we searched the entire body, and this approach results in more ticks being found (Roselli et al, *unpublished data*).

More research is needed to elucidate the role of non-migratory and summer-resident birds in the emergence and increased prevalence of disease in urban areas, especially in understudied regions like ours.

Overall, we found no association between urban development intensity and prevalence or intensity of tick infestation in birds, despite previous studies showing a link between large-scale land use and ticks, hosts, and pathogens (Allen et al. 2003; LoGiudice et al. 2003; Heller et al. 2019). This result suggests birds may carry a high number of ticks despite the level of urban development or that other factors influence tick abundance, host populations, and/or tick-host interactions. For example, in the Lyme disease transmission system, tick infestation of white-footed mice depends on many factors, including presence of top-level and meso-predators (Levi et al. 2012), acorn mast (Ostfeld et al. 2006), forest fragmentation (Allen et al. 2003), and host diversity (Keesing and Ostfeld, 2001). Likewise, because urbanization changes almost all aspects of ecosystems from large to small spatial scales—including temperature, humidity, vegetation structure/composition, wildlife communities, and landscape-scale habitat fragmentation, heterogeneity, and connectivity (Hage, 1975; Ackerman, 1987; Savard et al. 2000; Arnfield 2003; Kim 2007; Bradley and Altizer 2007; Kowarik et al. 2008; Chaves et al. 2011; Ramalho and Hobbs 2012)—tick infestation of birds in our urban study system is likely driven by a similarly complex suite of factors. In particular, habitat connectivity and heterogeneity at the landscape scale have been shown to influence both host populations (e.g., deer and birds; Walter et al 2009; Kang et al. 2015) and vector populations, including ticks (Estrada-Peña 2003; Chaves et al. 2011; Uspensky 2014). Because urbanization is a major driver of these landscape characteristics, urbanization-

associated decreases in habitat connectivity and increases in heterogeneity may affect host movements, populations, and/or communities in ways that influence infestation rates for birds and other hosts. We encourage future urban studies to consider the entire community of potential wildlife hosts for ticks and tick-borne pathogens, as well as additional abiotic and biotic factors that operate across multiple scales to influence various facets of tick-borne pathogen transmission.

Although we found no overall relationship between tick infestation of birds and urbanization intensity, individual bird species, particularly Carolina Wren, appear to have lower prevalence and intensity of infestation in parks and greenspaces within highly developed landscapes. This pattern could reflect behavioral changes in birds caused by urbanization (Jokimaki et al. 2011), and this may explain the documented infestation pattern for Carolina Wren, which appears highly sensitive to urban development (Evans et al. 2015). Specifically, this species may experience a behavioral change in highly developed landscapes that could make them less susceptible to tick infestations—for example, a change in preening behavior, the amount of time spent foraging in vegetation types that contain large numbers of ticks, and/or the amount of time foraging near the ground. A complementary explanation is that tick community changes may influence variation in Carolina wren infestation; specifically, this species was found to be infested by relatively high numbers of all three tick species, and an urbanization-related change in the abundance of one or more of these species could result in an overall change in tick infestation prevalence and/or intensity. Future research should explore how human land-uses, including urbanization, affect species-specific avian traits that influence tick infestation.

Our study suggests that birds are important as dispersers of ticks, and potentially in transmission systems for tick-borne pathogens, in urban and suburban areas. However, it remains unclear whether this result is broadly applicable as our study was heavily focused on passerines (i.e., perching birds/songbirds) and fairly limited in sample size (n=432 total bird searches), seasonal coverage (June-August), and geographic area (one city in the central U.S). Despite this, most of the bird and tick species sampled have relatively large geographic distributions, potentially making these results widely generalizable. For example, most of the bird species captured, including the two most commonly sampled species (Northern Cardinal and Carolina Wren) have geographic distributions that span at least the eastern half of the U.S. Two of the three tick species collected (*A. americanum* and *H. leporispalustris)* also range across the eastern half of the U.S., as well as much of Mexico and southern Canada (Brown et al. 2005, Springer et al. 2015). Although our results are likely applicable across at least much of North America, additional research is needed in other regions, during seasons other than summer, and with a variety of tick species and non-passerine birds, to elucidate whether exceptions exist to the patterns documented.

The percentage of earth's human population living in urban areas is expected to continue increasing (United Nations, 2014) and tick-borne diseases are continuing to emerge and increase in prevalence in urban areas in the United States (Steere 1994; Maupin et al. 1991; Jobe et al. 2007; Rydzewski et al. 2012; Schwan et al. 2009; Blanton et al. 2014). Further, due to the effects of climate change and other major global changes like habitat conversion to urban and agricultural uses (Brownstein 2005; Ogden et al. 2006; Porretta et al. 2013), previously inhospitable areas are likely to be increasingly

suitable for tick colonization. As a result of these factors, more of the human population than ever before will likely be at risk for contracting a tick-borne disease in or around the places they live. Thus, it will be essential to understand the role of birds and other highly-mobile animals as dispersers of ticks in urban areas to understand and better predict tick-borne disease transmission and emergence.

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Tables and Figures

Table 1- Numbers and species of birds sampled for ticks, and prevalence of infestation for each species (i.e., proportion of captures with at least one tick found), based on field sampling in Oklahoma City, Oklahoma, USA, Jun-Aug 2017-2018. Counts of each tick species are based only on the 322 ticks that could be removed for identification. Species common names follow the American Ornithologists Society 2018 (Chesser et al. 2015); recaptures of the same individual bird (n=27) were counted as unique capture events.

				No. birds infested with each tick species/stage					
				A. americanum		*A. maculatum*		*H. leporispalustris*	
Species	No. of searches	No. ticks observed	Proportion infested	Larvae	Nymphs	Larvae	Nymphs	Larvae	Nymphs
American robin	34	13	0.12	1	8			2	2
Bewick's wren	4	15	0.5	2	2	7			
Blue jay	3	0							

Brown thrasher	16	10	0.38		3			1	2
Carolina chickadee	17	0							
Carolina wren	68	143	0.56	40	22	14	2	18	2
Common grackle	3	0							
Downy woodpecker	4	0							
Eastern bluebird	1	1	1		1				
European starling	4	0							
Great-crested flycatcher	5	0							
Gray catbird	4	0							
House sparrow	7	0							
Indigo bunting	15	37	0.2	1		44			
Least flycatcher	4	0							

Northern Cardinal	192	257	0.27	34	47	31	2	13	2
Northern mockingbird	5	0							
Painted bunting	32	16	0.09	2		13	1		
Tufted titmouse	12	1	0.08		1				
White-eyed vireo	13	0							
Yellow-billed cuckoo	3	2	0.33				2		
Total	459*	495	0.25	80	84	109	7	34	8

*Total includes 9 unlisted bird species with a sample size of captures <3 (13 total individuals across the 9 species) and no ticks found. Species excluded are: American goldfinch, Bell's vireo, Blue-gray gnatcatcher, Brown-headed cowbird, Eastern phoebe, Eastern wood-pewee, Louisiana waterthrush, Red-eyed vireo, Summer tanager, *Empidonax* spp. (unidentified flycatchers in the *Empidonax* genus).

Figure 1: Sixteen field sites used for sampling birds in Oklahoma City, OK, USA, 2017-2018. Inset map indicates the location of the sampling area. Main map shows major highways (ESRI 2019, Redlands CA, USA) in white and land cover categories (National Land Cover Database 2001, US Geological Survey, Sioux Falls, SD, USA): light gray is human-developed land cover (developed, open space; developed, low intensity; developed, medium intensity; and developed, high intensity) dark gray is all other land-cover categories. Size of site labels indicate percent surrounding developed land in a 1,000 m radius.

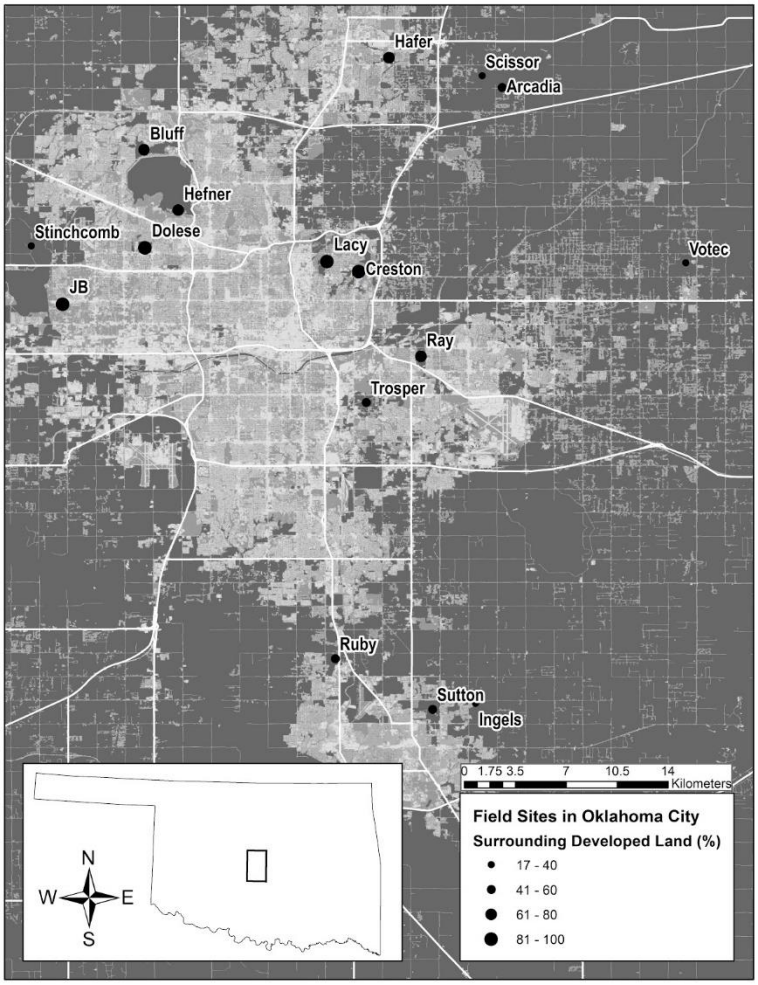


Figure 2: Relationships between percentage of developed land in a 1,000 m radius surrounding study sites and prevalence of infestation (i.e., proportion of birds carrying at least one tick) for a) all bird species combined, b) Northern Cardinal, and c) Carolina Wren in Oklahoma City, Oklahoma, USA, Jun-Aug 2017-2018 (Points indicate observed values, line indicates fitted model, and shading indicates 95% confidence interval for the fitted model)

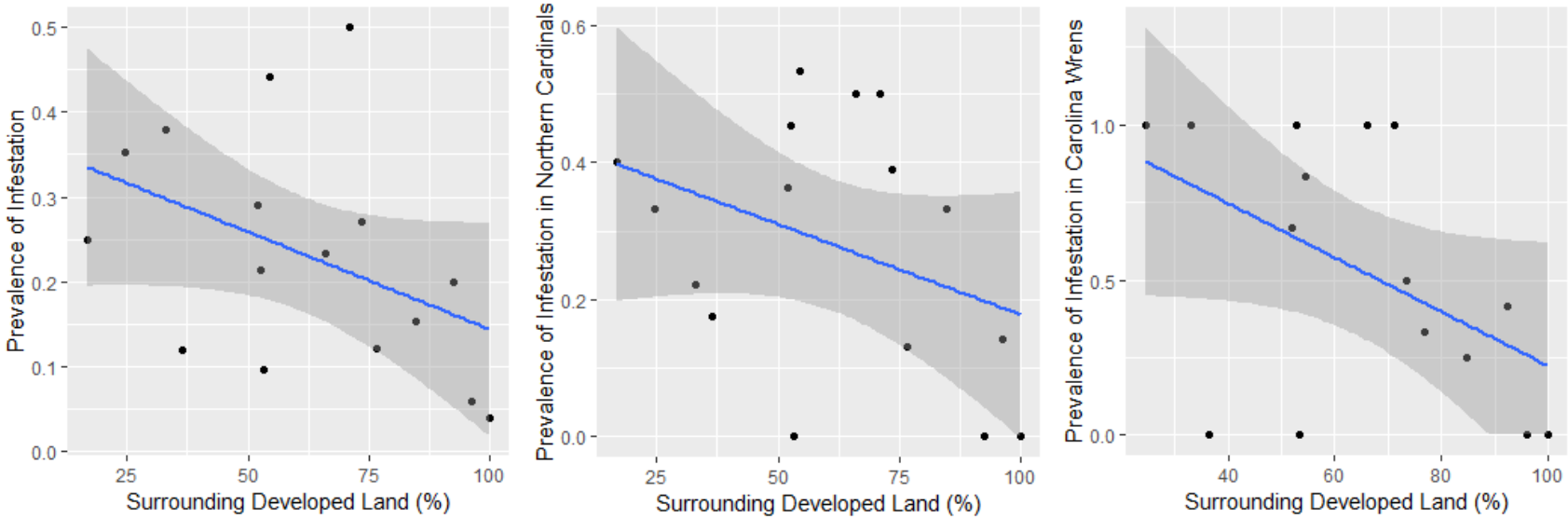
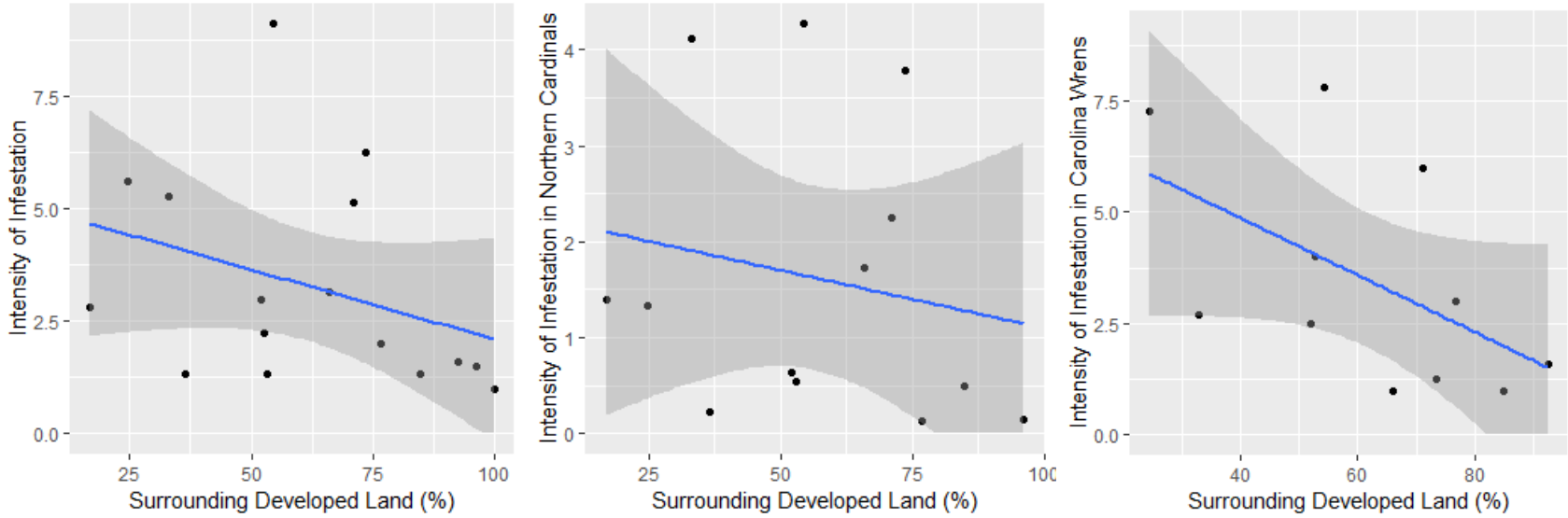


Figure 3: Relationships between percentage of developed land in a 1,000 m radius surrounding study sites and intensity of infestation (i.e., average number of ticks on each infested bird) for a) all bird species combined, b) Northern Cardinal, and c) Carolina Wren in Oklahoma City, Oklahoma, USA, Jun-Aug 2017-2018. (Points indicate observed values, line indicates fitted model, and shading indicates 95% confidence interval for the fitted model)



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