TICK, HOST, AND PATHOGEN ECOLOGY IN URBAN FOREST FRAGMENTS

by

Solný Arnardóttir Adalsteinsson

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology and Wildlife Ecology

Summer 2016

TICK, HOST, AND PATHOGEN ECOLOGY IN

URBAN FOREST FRAGMENTS

by

Solný Arnardóttir Adalsteinsson

Approved: _____
Jacob L. Bowman, Ph.D.
Chair of the Department of Entomology and Wildlife Ecology

Approved: _____
Mark Rieger, Ph.D.
Dean of the College of Agriculture and Natural Resources

Approved: _____
Ann L. Ardis, Ph.D.
Senior Vice Provost for Graduate and Professional Education

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

W. Gregory Shriver, Ph.D.
Co-Professor in charge of dissertation

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Jeffrey J. Buler, Ph.D.
Co-Professor in charge of dissertation

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Jacob L. Bowman, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Vincent D'Amico, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Dustin Brisson, Ph.D.
Member of dissertation committee

ACKNOWLEDGMENTS

There are so many people I wish to thank, both professionally and personally, for their contributions toward this endeavor. I would like to start with my advisors and dissertation committee for their unwavering support and guidance. I will be forever appreciative of my co-advisors, Greg Shriver and Jeff Buler, for adopting me into their lab groups, for supporting my crazy ideas, and for being exemplary scientists and mentors; it has been a privilege to work under their guidance. I will always be grateful to Vince D'Amico, my unofficial third advisor, for his consistent support and many great conversations about ticks, beer, and philosophy. I am very appreciative of Jake Bowman for lending his mammal expertise to this work, and for helping me become a better mentor to undergraduate researchers. I thank Dustin Brisson for his guidance and enthusiasm for these projects; his input improved many aspects of this work. I feel honored to have worked with everyone on my committee, and I hope we will continue to collaborate in the future!

At University of Delaware, I would also like to thank all of the graduate students, post-docs, faculty, and staff in the department of Entomology & Wildlife Ecology. In particular, I want to acknowledge all members of the Buler and Shriver Labs and the following individuals for their camaraderie on and off campus: Jake Haus, Zach Ladin, Jackie Marchese, Kaity Handley, Shannon Kachel, Kyle Horton, Mason Sieges, Whitney Wiest, Becky Kern, Jaci Smolinsky, Sam Roberts, Tim Freiday, Conor Higgins, Desiree Narango, Liz Tymkiw, Scott Berg, Orrin Jones, and Jeremiah Heise. I also want to thank the many wonderful undergraduate students and

technicians (especially Joe Bondi, Rebecca Deegan, Matthias Sirch, Denise Ecker, Kim Serno, and Sean O'Connor) who made these field projects possible; I thank everyone individually in the acknowledgments of each chapter. I also appreciate the guidance from Brooke Uhde, Shirley Morton, Lizette Camacho, and Tina Kosinski, who made navigating grant applications and grad school requirements much easier.

I am grateful to past supervisors who were outstanding mentors and inspired me to continue with research: Blair Hedges, Ævar Petersen, Nick Holmes, and Angie Merritt. I also wish to thank Tom Smith and his lab group at UCLA for providing workspace and many great discussions during my time in California.

On a personal note, I am extremely thankful for the support of my family, my partner, and my friends. I owe everything to my parents, Eve and Örn Adalsteinsson, and my grandparents, Beverly and Mort Shafter, for their unconditional love and encouragement, no matter where (literally) my interests have taken me. For their consistent love, support, and positive influences on my life, I would also like to thank my aunts and uncles, my siblings, and my extended family and friends including: Viktor and Betsy Adalsteinsson; Bryndís, Brian, Khlöe, and Landon Davis; Evelyn and Larry Nicholson; the Berman family; Alexandra Malagón and Régulo Plazas; Yoko Arakawa; Deb Stuebing; and the Tuckers. In particular, I wish to acknowledge Evelyn and Larry Nicholson for teaching me many valuable lessons while always celebrating my educational pursuits. I am so grateful to Andrés Alejandro Plazas Malagón for sticking with me for all these years, believing in me, and brightening up my days; his love and support have made this endeavor possible. And finally, I must acknowledge Katla, my loyal sidekick who waits patiently for breaks in my workdays so we can wander around the mountains together.

DEDICATION

I dedicate this work to the memory of my mother, Eve Adalsteinsson, who always encouraged me to have curiosity, strength, and an appreciation for absurdity – three prerequisites for pursuing a doctoral degree.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Urbanization and human development are accelerating globally, rapidly changing surrounding ecosystems. Urbanization-induced changes to biotic and abiotic processes affect wildlife populations and the dynamics of associated infectious diseases. In this dissertation, I explore how features of urban forest fragments in the mid-Atlantic region of the United States impact the ecology of zoonotic tick-borne disease systems. In Chapter 1, I describe the relationship between nonnative woody plant invasion and the abundance of host-seeking nymphal blacklegged ticks (*Ixodes scapularis*) at two spatial scales. Within forest fragments with significant nonnative plant invasion in the understory, *I. scapularis* are aggregated within patches of invasive plants. At the forest fragment scale, however, invasion by nonnative plants is associated with loss of leaf litter, which reduces habitat quality for *I. scapularis*. Thus, at the broader, forest fragment scale, *I. scapularis* are found in higher abundance in forests without nonnative plant invasion. In Chapter 2, I characterize prevalence of four zoonotic tick-borne pathogens in northern Delaware, and I study the vegetation and landscape factors that determine prevalence of *Borrelia burgdorferi*, the causative agent of Lyme disease, among host-seeking *I. scapularis.* Nonnative plant invasion and several habitat features specific to small mammal and bird host niches increase the prevalence of *B. burgdorferi.* In a comparison of invaded and uninvaded forest fragments, *B. burgdorferi* prevalence is nearly twice as high among ticks from invaded forests compared to ticks from uninvaded forests. In Chapter 3, I investigate urban forest fragment characteristics that influence the distribution and abundance of an

important reservoir host for *B. burgdorferi*, the white-footed mouse (*Peromyscus leucopus*). I also model habitat and landscape features that promote interactions between *P. leucopus* and immature stages of *I. scapularis*, and ultimately could amplify infection prevalence. In spring, landscape variables have the greatest influence on *P. leucopus* distributions, but in fall a combination of landscape and habitat variables explain *P. leucopus* abundance. Both intrinsic *P. leucopus* traits and extrinsic habitat features predict the tick burdens found on *P. leucopus* individuals in spring and summer. In Chapter 4, I focus on population vital rates in urban forest fragments of another important group of tick hosts, ground-foraging passerine birds. Specifically, I estimate cumulative survival rates of Gray Catbirds (*Dumetella carolinensis*) and Wood Thrushes (*Hylocichla mustelina*) during the post-fledging stage, and I model how behavior and anthropogenic hazards in an urban landscape affect mortality risk. Gray Catbird fledglings have approximately half the survival rate of Wood Thrushes; but for both species, mortality risk increases as individuals cross more roads and become exposed to more anthropogenic hazards. Overall, urbanization has complex, cascading effects on wildlife populations and tick-borne disease dynamics. Given the heterogeneity of urban forest fragments, both topics warrant further study with careful attention to the issue of scale.

Chapter 1

SCALE-DEPENDENT EFFECTS OF NONNATIVE PLANT INVASION ON HOST-SEEKING TICK ABUNDANCE

Introduction

Altered ecosystems can produce cascading effects. For example, nonnative, invasive species impact a range of abiotic and biotic ecosystem components from soil chemistry (Howard et al. 2004) to biodiversity (Wilcove et al. 1986, Coblentz 1990), and even human health (Juliano and Lounibos 2005, Allan et al. 2010, Morlando et al. 2012). On the east coast of the United States, multiflora rose (*Rosa multiflora*) is an ubiquitous invader in forest understories (Huebner et al. 2014), with documented ecosystem effects including accelerated litter nitrogen loss (Ashton et al. 2005) and reduced avian nest success (Borgmann and Rodewald 2004). Herein, we investigate whether *R. multiflora* may also affect human health by increasing tick-borne disease risk.

Blacklegged ticks (*Ixodes scapularis* Say) are an important vector of zoonotic pathogens, including *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia miyamotoi*, and Powassan virus (Centers for Disease Control and Prevention 2015). Borrelia burgdorferi causes Lyme disease, the most commonly reported vector-borne disease in North America (Bacon et al. 2008). Recent work in forest ecosystems suggests that the abundance of host-seeking *I. scapularis* (nymphs and adults) is positively associated with invasion by nonnative shrubs (Lubelczyk et al. 2004, Elias et al. 2006, Williams et al. 2009, Williams and Ward 2010). *Ixodes*

scapularis abundance is a key factor in predicting human Lyme disease cases (Mather et al. 1996, Khatchikian et al. 2012); thus, nonnative plant invasion potentially increases human disease risk.

The dense understory structure created by nonnative plants can increase humidity and protection from extreme temperature fluctuations, thereby creating a more favorable microclimate for ticks (Williams et al. 2009). Dense understory structure is also used preferentially by host animals (e.g. white-tailed deer, *Odocoileus virginianus*) that are important to tick reproduction and survival (Allan et al. 2010). Both of these mechanisms (improved microclimate and increased host use) support a potential positive association between *I. scapularis* abundance and the presence of nonnative, invasive shrubs.

There is a critical need to determine whether fine-scale habitat associations between invasive shrubs and ticks create larger vector populations at broad enough scales to increase human disease risk (Allan et al. 2010). Ecological processes are often scale-dependent (Wiens 1989). Many biotic and abiotic factors affect *I. scapularis* density and distribution (Ostfeld et al. 1995) and likely operate at different scales. Nonnative plant invasion may increase tick abundance at a fine scale within a given forest patch, but it is unclear whether this would produce a larger vector population at a landscape scale relevant to human disease risk.

We assessed the effects of *R. multiflora* invasion at two spatial scales: at a fine scale within forest fragments and at a patch scale among different forest fragments. We tested the following: 1. whether *I. scapularis* abundance differs under *R. multiflora* compared to other shrub species or open understory within the same forest

patch; and 2. whether *I. scapularis* abundance differs among *R. multiflora*-invaded forest fragments compared to forest fragments without *R. multiflora* invasion.

Methods

Study area

Delaware has one of the highest per capita rates of human Lyme disease cases in the United States (Bacon et al. 2008). This, combined with fragmented forests containing significant nonnative plant invasion, makes Delaware an ideal location to examine the effects of nonnative plants on *I. scapularis* populations. We trapped ticks in forest fragments (Figure 1) composed of mixed deciduous hardwood stands in New Castle County, DE (Rega 2012).

Tick trapping

In 2013 and 2014, we selected eight forest fragments ranging from 6 to 16 ha for trapping–four "uninvaded fragments" with <1% *R. multiflora* cover in the understory and four "invaded fragments" with >10% *R. multiflora* cover in the understory. We used dry-ice (CO2) baited traps (Kensinger and Allan 2011) to target host-seeking *I. scapularis* nymphs because they pose the greatest disease risk to humans (Centers for Disease Control and Prevention 2015). Although dragging or flagging methods are often preferred for capturing *I. scapularis* nymphs (Falco and Fish 1992), CO2 traps avoid sampling bias due to vegetation structure (Falco and Fish 1992, Schulze et al. 1995, 1997, Daniels et al. 2000, Kensinger and Allan 2011). Drag cloths become snagged on *R. multiflora* thorns, making tick habitat beneath it inaccessible for dragging or flagging.

Within invaded fragments, we deployed traps in a paired design, with each pair consisting of one trap under *R. multiflora* and one trap 25 meters away, not under *R. multiflora*. We chose four pairs (8 traps total) in each invaded fragment. Trap locations beneath *R. multiflora* were randomly selected within previously mapped and digitized patches of *R. multiflora* in ArcGIS v10 (ESRI 2012); paired trap locations not under *R. multiflora* were designated 25 meters away in a random cardinal direction, with the only selection criterion being that they could not be under *R. multiflora*. As a result, we set up these paired traps beneath a variety of understory structures and plant species. Within each uninvaded forest fragment, we randomly chose four points as trap locations using ArcGIS. Each year, we deployed 16 pairs of traps (32 total) across four *R. multiflora*-invaded fragments and 16 traps across four uninvaded fragments. Over the two years of the study, we used a total of 64 trap locations (Figure 1). On any given trap night, half of the traps were active. To eliminate biases associated with weather, we always deployed trap pairs together and in equal numbers of invaded and uninvaded fragments on the same nights. Each trap was baited with 1.4 kg of pelleted dry ice, and we lined the edges of the plywood base with double-sided carpet tape (3M brand). We checked traps after 24h and collected all ticks with forceps and deposited them into individual microcentrifuge tubes. Ticks were transported live, frozen at -80°C, and later identified to species and life stage using dichotomous keys (Keirans and Litwak 1989, Durden and Keirans 1996, Keirans and Durden 1998). Between April and July of 2013 and 2014, we trapped up to two nights per week, avoiding rain, for a total of 671 trap nights.

Vegetation surveys

We measured understory vegetation at several scales surrounding each trap during June and July. Within a 12.5 m radius surrounding the trap, we identified the dominant four understory plant species and estimated the percent of the ground that each covered, the percent of ground covered by *R. multiflora*, the percent of ground covered by coarse woody debris, and we measured understory vegetation density using 2.0 m high Nudds boards divided into four 0.5 m sections (Nudds 1977). The 12.5 m radius area was chosen to represent the approximate size of *Peromyscus leucopus* (an important larval tick host) home ranges (Wolff 1985) that could influence immature tick abundance, while avoiding overlap with paired traps. Within a 2.5 m radius around the trap, we estimated the percent of ground covered by *R. multiflora* to more closely represent the effective trapping area (Falco and Fish 1991). We identified the plant species immediately over the trap, and we quantified leaf litter volume by gathering up all litter within a 0.5m2 quadrat next to the trap and measuring its volume in a 19L bucket.

We calculated landscape variables for each trap location in ArcGIS using 2007 Delaware Land Use Land Cover layer (State of Delaware 2008). We quantified landscape variables that might influence habitat suitability for ticks and/or host animals and that represent the human-dominated landscape context of the study sites (Nicholson and Mather 1996, Bunnell et al. 2003). These variables included distance to nearest stream, distance to nearest road, distance to nearest agriculture, distance to nearest human development, distance to nearest impervious surface, distance to nearest residential development, and distance to nearest forest edge from each trap location.

We included vegetation variables that characterized vegetation at the patch scale (Rega 2012). These variables included proportion of *Fagus grandifolia*, *Acer spp*, *Quercus spp*, *Liriodendron tulipifera*, or *Liquidambar styraciflua* as dominant canopy trees, percent of ground covered by *R. multiflora*, average leaf litter volume, tree basal area, average diameter at breast height (dbh) of trees, percent of ground covered by understory plants (all spp.), proportion of understory woody stems that were nonnative, year of canopy closure.

Statistical analyses

We standardized total *I. scapularis* nymphs captured at each trap by effort (number of trap nights) to get a daily mean tick capture rate, which we used as an index of host-seeking *I. scapularis* abundance (hereafter: tick abundance) at each location. Prior to analyses, we log-transformed the response, tick abundance, to satisfy parametric test assumptions. At the fine scale, we used a paired t-test to determine if tick abundance differed between paired traps under *R. multiflora* and not under *R. multiflora*. At the patch scale we used an analysis of variance (ANOVA), blocking on site, to test for a difference in tick abundance between invaded and uninvaded forest patches.

Tick abundance models

Because forest fragments differed in additional characteristics beyond presence/absence of *R. multiflora* invasion, we assessed which vegetation and landscape variables influenced tick abundance at the landscape scale. We used boosted regression trees (BRT) to assess relative variable importance (Friedman and Meulman 2003, Elith et al. 2008) using packages gbm (Ridgeway 2015) and dismo (Hijmans et

al. 2015) to run BRTs in R v 3.1.1 (R Development Core Team 2014); we set the error distribution for the response variable as gaussian, and specified the learning rate as 0.005, bag fraction as 0.5, and tree complexity at 2. Using the top four predictor variables according to BRT rankings, we built several generalized additive models (GAM) to compare for the best fit using package mgcv (Wood 2015) in R.

Results

Tick captures

We captured 282 total *I. scapularis* individuals (275 nymphs, 7 adults) over 671 trap nights. At traps deployed in *R. multiflora*-invaded fragments, we captured a total of 79 and 33 *I. scapularis* nymphs in *R. multiflora* and not in *R. multiflora*, respectively. We caught a total of 163 *I. scapularis* nymphs in uninvaded forest fragments. Forty-seven of the 64 traps (73%) captured at least one *I. scapularis* nymph, and tick abundance ranged from 0 to 2.33 (nymphs/day). At the fine scale within invaded forest fragments, tick abundance under *R. multiflora* was 2.3 times greater than not under *R. multiflora* (t_{19}=2.37, P = 0.03) (Figure 2). At the patch scale, we detected the opposite pattern; tick abundance in uninvaded forest fragments was 3.2 times greater than in *R. multiflora*-invaded fragments ($F_{(1,53)}$=30.27, P < 0.01) (Figure 2). To verify that patch-level differences were not driven by the low capture rates in the invaded patches at the traps not under *R. multiflora*, we confirmed that tick abundance at traps in uninvaded patches was greater than at traps under *R. multiflora* (t_{42}=2.75, P < 0.01).

Tick models

Several vegetation variables were correlated (coefficients ≥ 0.6), so we reduced the candidate set of local vegetation and landscape predictor variables to include only those listed in Table 1. Boosted regression trees (BRT) achieved a cross validation correlation 0.731 after fitting 1,350 trees. Of all potential predictor variables, leaf litter volume at the trap location had the highest relative influence (17% of total deviance explained) on tick abundance, followed by several variables with similar relative influence (11.7-11.9%): distance to nearest road, average tree dbh, and distance to nearest stream.

We log-transformed certain predictor variables to improve their distributions along the x-axis. Our best GAM (61% deviance explained) based on Akaike's information criterion adjusted for small sample size (Burnham and Anderson 2002) included log-transformed litter volume, distance to nearest road, log-transformed distance to nearest stream, and average tree dbh (Figure 3, Table 2). Tick abundance responded to leaf litter volume nonlinearly. Increase in leaf litter volume at low levels (<10 L/m2) had little effect on tick abundance. Between 12 L/m2 and 20 L/m2 there was a strong positive change in tick abundance with increasing leaf litter volume, which then leveled out around 30 L/m2. We saw a similar non-linear response with the distance to stream. Close to a stream, there were very few ticks captured, but this response increased rapidly up to a certain distance from the stream, beyond which there is little effect of the stream on tick abundance. Distance to nearest road had a positive effect on tick abundance at low and high values, and average tree dbh was inversely related to tick abundance.

Discussion

At a fine scale, we trapped 2.3 times more nymphal blacklegged ticks under *R. multiflora* compared to areas without *R. multiflora* within the same forest fragments. However, we trapped over 3 times as many ticks in uninvaded forest fragments compared to *R. multiflora*-invaded forest fragments. Host-seeking ticks were concentrated beneath *R. multiflora* within invaded forests, but they were more abundant in uninvaded forests. Thus, although at fine scales *R. multiflora* appears to increase tick abundance, *R. multiflora* invasion actually suppresses tick abundance at the patch scale. Our results suggest that reduced leaf litter volume constrains tick abundance (Schulze et al. 1995, Burtis et al. 2014). The lack of leaf litter in *R. multiflora*-invaded forest fragments creates an inhospitable environment for ticks, from which they are rescued by invasive shrubs with dense growth forms (e.g. Williams et al. 2009).

Within invaded forests, our results are consistent with prior studies that show a positive relationship between nonnative, invasive shrubs (e.g. *B. thunbergii*) and questing nymphal and adult *I. scapularis* abundance (Williams et al. 2009, Lubelczyk et al. 2004, Elias et al. 2006). Densities of nymphal and adult lone star ticks (*Amblyomma americanum*) were also higher in invasive honeysuckle (*Lonicera maackii*)-dominated plots compared to uninvaded, adjacent plots (Allan et al. 2010). At a landscape scale, however, we found ticks were more abundant in uninvaded forest fragments than invaded fragments. We captured more than three times as many *I. scapularis* nymphs in uninvaded forest fragments compared to *R. multiflora*-invaded forest fragments. *Rosa multiflora* invasion was associated with habitat changes at the forest-fragment scale that create an aggregation of ticks around *R. multiflora* within invaded forests. However, uninvaded habitats appear to be more suitable for ticks than

invaded habitats, seemingly due to the greater volume of leaf litter in uninvaded forest fragments.

Of all vegetation and landscape covariates tested, leaf litter volume was the best predictor of tick abundance. Leaf litter is an important component of *I. scapularis* habitat (Schulze et al. 1995, 2002, Lubelczyk et al. 2004, Elias et al. 2006). In the forests investigated, leaf litter volume was negatively correlated with invasion by nonnative plants at the patch scale. We found more than four times the volume of leaf litter in *R. multiflora*-free forest fragments compared to *R. multiflora*-invaded forest fragments ($P < 0.01$), and there was no difference in leaf litter volume between paired points within invaded forests. The widespread relationship between leaf litter and invasive plants is ultimately driven by nonnative, invasive earthworms (Nuzzo et al. 2009). These earthworms lead the nonnative plant invasion front by devouring leaf litter and disrupting mycorrhizal networks, inhibiting native plant growth and regeneration (Lawrence et al. 2003, Suárez et al. 2006, Hale et al. 2006). This reduction in habitat quality for native plants, combined with high densities of white-tailed deer over-browsing native plants, helps nonnative plants to invade. Therefore, at a patch scale, the forest fragments with significant nonnative plant invasion are lacking the layer of leaf litter that comprises favorable tick habitat.

The presence or absence of earthworm invasion and subsequent nonnative plant invasion are determined by numerous factors including soil chemistry (Moore et al. 2013), litter composition (Belote and Jones 2009), and historical land use patterns (Beauséjour et al. 2015). With increasing disturbance and propagule pressure in human-dominated landscapes, currently uninvaded forest fragments may eventually host high densities of earthworms and nonnative plants, with implications for tick and

litter arthropod survival. On the other hand, uninvaded forest fragments may possess certain characteristics, such as high soil acidity (Bernard et al. 2009), that make them more resistant to earthworm invasion. In either scenario, we suggest continued monitoring at multiple spatial scales to understand the impacts of invasion on tick population dynamics.

Conclusions and Future Directions

Our results suggest that while *R. multiflora* invasion concentrates host-seeking *I. scapularis* nymphs at a fine scale, the loss of leaf litter associated with *R. multiflora* invasion reduces tick habitat quality at the patch scale. We found the greatest abundance of host-seeking *I. scapularis* nymphs in uninvaded forests with deep litter layers. Thus, while removal of invasive plants may reduce tick abundance within certain forests, management actions toward reducing host-seeking tick abundance may have a greater impact in forests with deep litter layers.

Future work should test potential mechanisms influencing tick abundance in invaded and uninvaded forest fragments. Furthermore, we should implement broader scale studies using multiple landscapes within the temperate deciduous forest biome to test our hypotheses about invasive earthworms, leaf litter, and invasive shrub effects on host-seeking *I. scapularis* abundance.

Acknowledgments

SA was supported by a University of Delaware Graduate Research Fellowship. Other financial support was provided by the USDA Forest Service NRS-16 (VD), USDA McIntire Stennis (WGS), and University of Delaware (JJB). DB was supported by National Institutes of Health (AI076342 and AI097137), National Science

Foundation (DEB-1354184), and the Burroughs Wellcome Fund (1012376). We thank the following individuals who helped collect field data: Z. Ladin, K. Handley, J. Nimmerichter, A. Lutto, K. Serno, J. Bondi, C. Piazza, L. Newton, and J. Curry. We thank R. Falco and B. Kensinger for valuable input on CO2 trap design and acknowledge the cooperation by land managers of sites where we conducted field work: Newark City Parks, New Castle County Parks, University of Delaware, Mt. Cuba Center, and Delaware State Parks.

Table 1 Summary of vegetation and landscape covariates measured at the trap and patch scale. Covariates are summarized as mean ± standard error. Letters (A,B,C) denote significant differences among groups ($P < 0.05$) detected using analysis of variance (ANOVA), blocking on site, followed up with Tukey post-hoc comparison when there were more than two groups. "Nudds" refers to Nudds board (Nudds 1977) measurements, and "dbh" stands for diameter at breast height.

Trap and patch level covariates	**Uninvaded forests**	**Invaded forests**	
Trap-level covariates	**traps**	**traps in *R. multiflora***	**traps not in *R. multiflora***
Nudds at 0.5-1.0 m (%)	**18.0**^{A} ± 3.9	**73.9**^{B} ± 4.5	**53.3**^{C} ± 5.9
Leaf litter volume (L/m^2)	**28.0**^{A} ± 2.8	**6.1**^{B} ± 1.4	**6.7**^{B} ± 1.2
Coarse woody debris (%)	**6.5**^{A} ± 0.9	**3.4**^{B} ± 0.7	**4.2**^{B} ± 0.7
Distance to agriculture (m)	**288.3**^{A} ± 54.7	**156.7**^{B} ± 24.6	**159.6**^{B} ± 24.6
Distance to edge (m)	**67.8**^{A} ± 9.1	**39.8**^{B} ± 8.8	**41.9**^{B} ± 8.4
Distance to road (m)	**154.7** ± 16.7	**135** ± 18.1	**133.4** ± 15.4
Distance to residential (m)	**716.9** ± 377.6	**186.2** ± 31.5	**174.4** ± 32.9
Distance to stream (m)	**371.8**^{A} ± 62.7	**148.4**^{B} ± 35.7	**134.1**^{B} ± 35.6
Patch-level covariates			
***Fagus grandifolia* (%)**	**8.5**^{A} ± 2.8	**0.7**^{B} ± 0.2	
***Acer spp.* (%)**	**0.7**^{A} ± 0.1	**21.2**^{B} ± 1.2	
Year of canopy closure	**1916.7**^{A} ± 4.9	**1963**^{B} ± 5.1	
Nonnative stems (%)	**9.1**^{A} ± 2.7	**40.0**^{B} ± 3.3	
***Liriodendron tulipifera* (%)**	**21.2** ± 2.4	**22.9** ± 2.8	
Average tree dbh (m)	**0.6** ± 0.0	**0.6** ± 0.0	

Table 2 Summary of candidate generalized additive models including number of parameters (K), AIC_c, differences (ΔAIC_c), and Akaike weights (w_i). The variable "litter" is the volume of leaf litter (L/m^2) at each trap. The variables "road," and "stream" refer to the distance between these features and the trap; "dbh" is the average diameter at breast height of dominant canopy trees in each forest fragment. The response (tick abundance) and two predictors (litter and stream) were log-transformed prior to analysis.

Predictor variable(s)	***K***	**AIC_c**	**ΔAIC_c**	**w_i**
litter, road, stream, dbh	6	209.47	0	0.59
litter, dbh, stream	5	211.65	2.18	0.20
litter, road, stream	5	212.66	3.19	0.12
litter, stream	4	213.26	3.79	0.09
litter, road, dbh	5	224.19	14.72	0.00
litter, road	4	231.72	22.25	<0.001
stream, road, dbh	5	232.41	22.93	<0.001
litter, dbh	4	233.07	23.60	<0.001
road, stream	4	233.74	24.27	<0.001
stream, dbh	4	237.33	27.86	<0.001
stream	3	237.84	28.37	<0.001
litter	3	238.47	28.99	<0.001
dbh	3	239.15	29.68	<0.001
road, dbh	4	239.64	30.17	<0.001
road	3	249.95	40.48	<0.001
null	2	258.45	48.98	<0.001

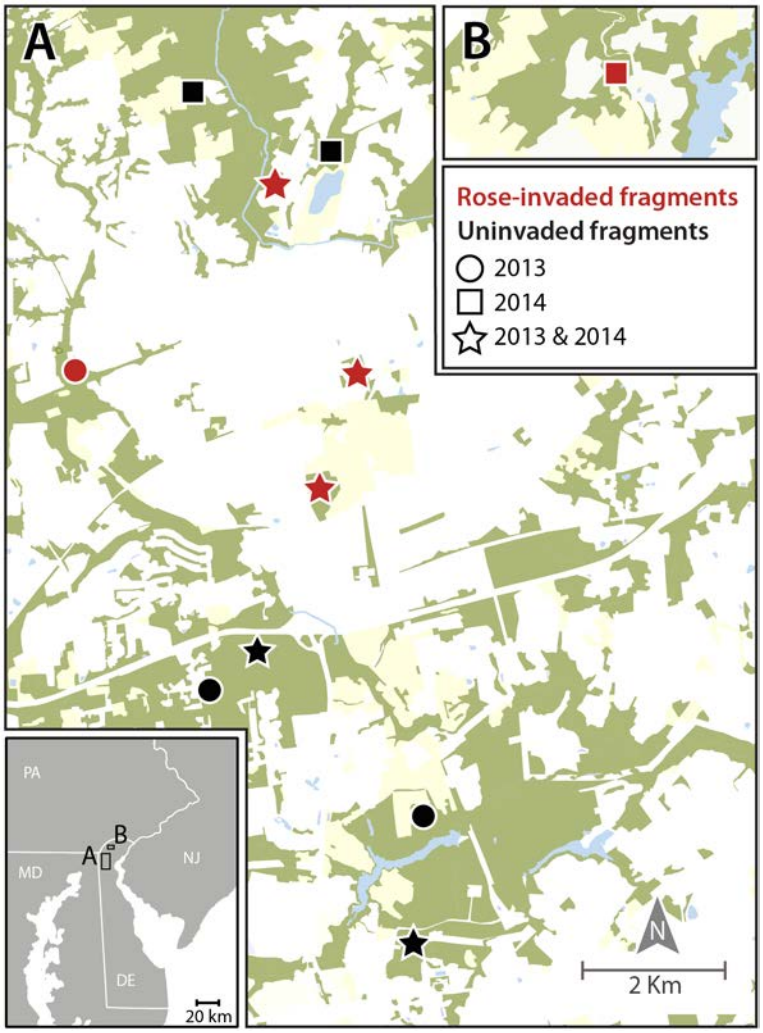

Figure 1 Study area in New Castle County, Delaware. Green represents forest cover; pale yellow is agriculture; blue is water; and white is any type of human development. "Rose-invaded" and "uninvaded" fragments refer to forest fragments with and without *R. multiflora* invasion.

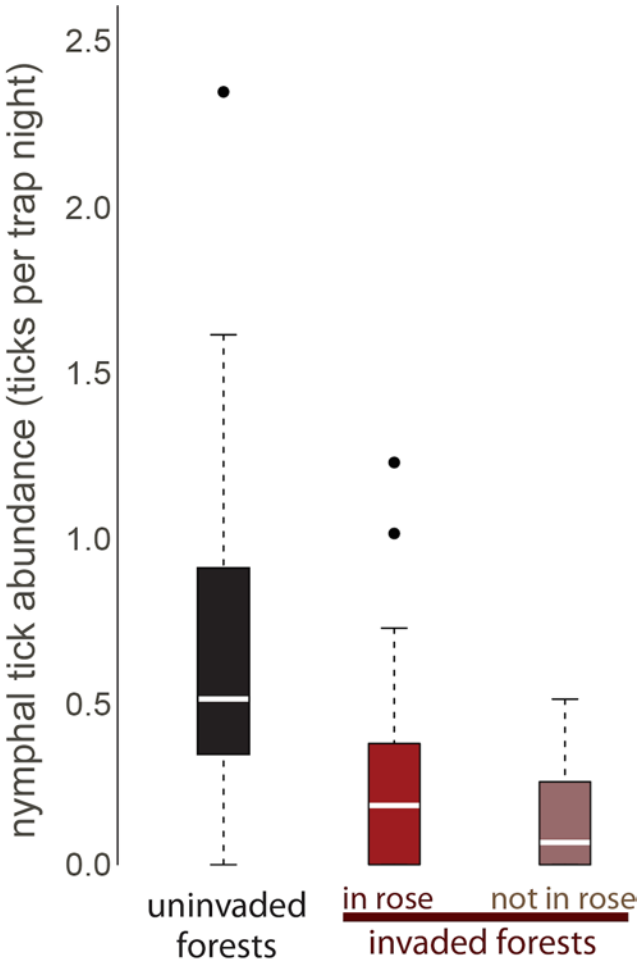

Figure 2 Boxplot of tick abundance measured at traps in uninvaded forests, at traps under *R. multiflora* in invaded forests ("in rose"), and at traps not under *R. multiflora* ("not in rose") in invaded forests. Horizontal white lines represent medians and boxes demonstrate the interquartile range.

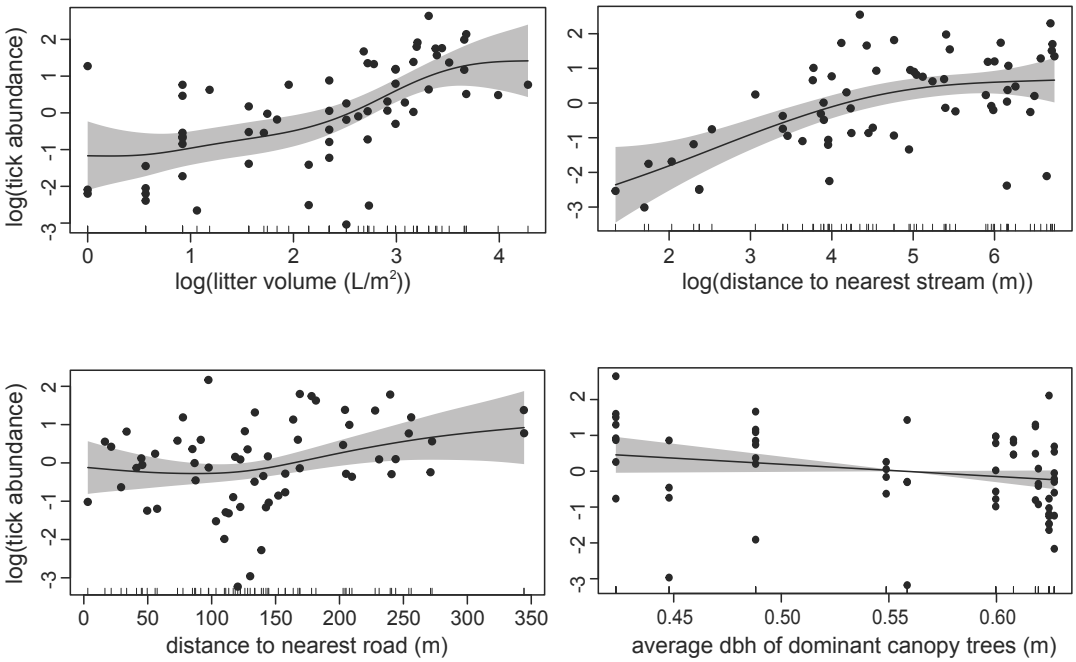

Figure 3 Generalized additive model (GAM) partial dependence plot of top-ranked model. Lines display the best fit for the relationship between log-transformed tick abundance and predictor variables. Gray bars represent 95% confidence interval and black dots show actual data points.

REFERENCES

Allan, B. F., H. P. Dutra, L. S. Goessling, K. Barnett, J. M. Chase, R. J. Marquis, G. Pang, G. A. Storch, R. E. Thach, and J. L. Orrock. 2010. Invasive honeysuckle eradication reduces tick-borne disease risk by altering host dynamics. Proceedings of the National Academy of Sciences 107:18523–18527.

Ashton, I. W., L. A. Hyatt, K. M. Howe, J. Gurevitch, and M. T. Lerdau. 2005. Invasive species accelerate decomposition and litter nitrogen loss in a mixed deciduous forest. Ecological Applications 15:1263–1272.

Bacon, R., K. Kugeler, and P. Mead. 2008. Surveillance for Lyme disease--United States, 1992-2006. Centers for Disease Control and Prevention, USA. http://www.cdc.gov/MMWR/PREVIEW/MMWRHTML/ss5710a1.htm

Beauséjour, R., I.T. Handa, M.J. Lechowicz, B. Gilbert, and M. Vellend. 2015. Historical anthropogenic disturbances influence patterns of non-native earthworm and plant invasions in a temperate primary forest. Biological Invasions 17:1267–1281.

Belote, R.T. and R.H. Jones. 2009. Tree leaf litter composition and nonnative earthworms influence plant invasion in experimental forest floor mesocosms. Biological Invasions 11:1045–1052.

Bernard, M.J., M.A. Neatrour, and T.S. McCay. 2009. Influence of soil buffering capacity on earthworm growth, survival, and community composition in the western Adirondacks and central New York. Northeastern Naturalist 16:269–284.

Borgmann, K. L., and A. D. Rodewald. 2004. Nest predation in an urbanizing landscape: the role of exotic shrubs. Ecological Applications 14:1757–1765.

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

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science & Business Media, New York, NY, USA.

Burtis, J. C., T. J. Fahey, and J. B. Yavitt. 2014. Impact of invasive earthworms on *Ixodes scapularis* and other litter-dwelling arthropods in hardwood forests, central New York state, USA. Applied Soil Ecology 84:148–157.

Centers for Disease Control and Prevention. 2015. Tickborne diseases of the United States. http://www.cdc.gov/ticks/diseases

Coblentz, B.E. 1990. Exotic organisms: a dilemma for conservation biology. Conservation Biology 4: 261–265.

Daniels, T. J., R. C. Falco, and D. Fish. 2000. Estimating population size and drag sampling efficiency for the blacklegged tick (Acari: Ixodidae). Journal of Medical Entomology 37:357–363.

Durden, L. A., and J. E. Keirans. 1996. Nymphs of the Genus *Ixodes* (Acari: Ixodidae) of the United States: Taxonomy, identification key, distribution, hosts, and medical/veterinary importance. Thomas Say Publications in Entomology: Monographs:1–50.

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

Elith, J., Leathwick, J.R., and T. Hastie. 2008. A working guide to boosted regression trees. Journal of Animal Ecology 77:802–813.

ESRI. 2012. ArcMap v 10.1. ESRI, Redlands, CA.

Falco, R. C., and D. Fish. 1991. Horizontal movement of adult *Ixodes dammini* (Acari: Ixodidae) attracted to CO2-baited traps. Journal of Medical Entomology 28:726–729.

Falco, R. C., and D. Fish. 1992. A comparison of methods for sampling the deer tick, *Ixodes dammini*, in a Lyme disease endemic area. Experimental & Applied Acarology 14:165–173.

Friedman, J. H., and J. J. Meulman. 2003. Multiple additive regression trees with application in epidemiology. Statistics in Medicine 22:1365–1381.

Hale, C. M., L. E. Frelich, and P. B. Reich. 2006. Changes in hardwood forest understory plant communities in response to european earthworm invasions. Ecology 87:1637–1649.

Hijmans, R. J., S. Phillips, J. Leathwick, and J. Elith. 2015. Species distribution modeling: package "dismo." CRAN.

Howard, T. G., J. Gurevitch, L. Hyatt, M. Carreiro, and M. Lerdau. 2004. Forest invasibility in communities in southeastern New York. Biological Invasions 6:393–410.

Huebner, C. D., J. Steinman, T. F. Hutchinson, T. E. Ristau, and A. A. Royo. 2014. The distribution of a non-native (*Rosa multiflora*) and native (*Kalmia latifolia*) shrub in mature closed-canopy forests across soil fertility gradients. Plant and Soil 377:259–276.

Juliano, S. A., and L. P. Lounibos. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health: Invasive mosquitoes. Ecology Letters 8:558–574.

Keirans, J. E., and L. A. Durden. 1998. Illustrated key to nymphs of the tick genus Amblyomma (Acari: Ixodidae) found in the United States. Journal of Medical Entomology 35:489–495.

Keirans, J. E., and T. R. Litwak. 1989. Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi River. Journal of Medical Entomology 26:435–448.

Kensinger, B. J., and B. F. Allan. 2011. Efficacy of dry ice-baited traps for sampling *Amblyomma americanum* (Acari: Ixodidae) varies with life stage but not habitat. Journal of Medical Entomology 48:708–711.

Khatchikian, C. E., M. Prusinski, M. Stone, P. B. Backenson, I.-N. Wang, M. Z. Levy, and D. Brisson. 2012. Geographical and environmental factors driving the increase in the Lyme disease vector *Ixodes scapularis*. Ecosphere 3:art85.

Lawrence, B., M. C. Fisk, T. J. Fahey, and E. R. Suárez. 2003. Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). New Phytologist 157:145–153.

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

Mather, T. N., M. C. Nicholson, E. F. Donnelly, and B. T. Matyas. 1996. Entomologic index for human risk of Lyme disease. American Journal of Epidemiology 144:1066–1069.

Moore, J.-D., R. Ouimet, and P.J. Bohlen. 2013. Effects of liming on survival and reproduction of two potentially invasive earthworm species in a northern forest Podzol. Soil Biology & Biogeochemistry 64:174–180.

Morlando, S., S. J. Schmidt, and K. LoGiudice. 2012. Reduction in Lyme disease risk as an economic benefit of habitat restoration. Restoration Ecology 20:498–504.

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

Nudds, T. D. 1977. Quantifying the vegetative structure of wildlife cover. Wildlife Society Bulletin 5:113–117.

Nuzzo, V. A., J. C. Maerz, and B. Blossey. 2009. Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. Conservation Biology 23:966–974.

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

R Development Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rega, C. 2012. Impacts of soil calcium availability and non-native plant invasions on an urban forest bird community. M.S. Thesis. University of Delaware, Newark, Delaware, USA.

Ridgeway, G. 2015. Generalized Boosted Regression Models: package "gbm." CRAN.

Schulze, T. L., R. A. Jordan, and R. W. Hung. 1995. Suppression of subadult *Ixodes scapularis* (Acari: Ixodidae) following removal of leaf litter. Journal of Medical Entomology 32:730–3.

Schulze, T. L., R. A. Jordan, and R. W. Hung. 1997. Biases associated with several sampling methods used to estimate abundance of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae). Journal of Medical Entomology 34:615–623.

Schulze, T.L., R.A. Jordan, and R.W. Hung. 2002. Effects of microscale habitat physiognomy on the focal distribution of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) nymphs. Environmental Entomology 31:1085–1090.

State of Delaware. 2008. May 19 2007 Delaware Land Use and Land Cover. Office of Management and Budget, Delaware Geographic Data Committee, Dover, Delaware, USA.

Suárez, E. R., T. J. Fahey, J. B. Yavitt, P. M. Groffman, and P. J. Bohlen. 2006. Patterns of litter disappearance in a northern hardwood forest invaded by exotic earthworms. Ecological Applications 16:154–165.

Wilcove, D.S., McLellan, C.H., and A.P. Dobson. 1986. Habitat fragmentation in the temperate zone. in Soule, M.E., editor. Conservation biology: The science of scarcity and diversity. Sinauer Associates, Sunderland, MA, USA.

Wiens, J. A. 1989. Spatial Scaling in Ecology. Functional Ecology 3:385.

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

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

Wolff, J. O. 1985. The effects of density, food, and interspecific inference on home range size in *Peromyscus leucopus* and *Peromyscus maniculatus*. Canadian Journal of Zoology 63:2657–2662.

Wood, S. 2015. Mixed GAM Computation Vehicle with GCV/AIC/REML Smoothness Estimation: Package “mgcv.” CRAN.

Chapter 2

MULTIFLORA ROSE INVASION AMPLIFIES PREVALENCE OF LYME DISEASE PATHOGEN AMONG BLACKLEGGED TICKS

Introduction

Urbanization affects all aspects of vector-borne disease ecology (Bradley and Altizer 2007). In the case of tick-borne disease systems such as Lyme disease (caused by *Borrelia burgdorferi*) in forested ecosystems, urbanization alters habitat suitability for vectors (i.e. ticks), hosts, and pathogens. Human development in the Lyme-disease endemic, mid-Atlantic region reduces overall forest cover and average patch size while increasing the area of edge and impervious surface. Reduced forest patch size, in particular, changes host community composition predictably to increase acarological risk in terms of nymphal infection prevalence and density of infected nymphs (Allan et al. 2003, LoGiudice et al. 2003, 2008, but see Zolnik et al. 2015). Yet in urban landscapes, patch size may have a smaller or perhaps unpredictable influence on host community relative to other effects of urbanization on forested ecosystems. How factors characteristic of urban forest fragments affect acarological risk has not been well explored.

Complex land use histories in human-dominated landscapes form networks of diverse, heterogeneous forest fragments. In the urban mid-Atlantic region, clear-cutting, intensive agriculture, and urban sprawl have created a variety of forest fragment types on a spectrum between two extremes: at one end are forest fragments that are true remnants of mature (>100 yr old) forests; at the opposite end are forest

fragments that have regrown from fallow agricultural land set aside while surrounding areas were developed. In the latter case, native tree species have competed with and grown up alongside nonnative species that were part of the agricultural landscape or subsequent development. As a result, regrown urban forest patches have closed canopies of mostly native trees with thick understories composed of nonnative, invasive species (Huebner et al. 2014).These two extremes of urban forest fragment types both face serious ecological problems, with implications for tick-borne disease risk.

Due to intensive browsing pressure imposed by high white-tailed deer (*Odocoileus virginianus*) densities (in Delaware, recent county surveys estimate between 18–52 deer/km^2 (Rogerson et al. 2010)), mature forests have sparse or no woody understories and cannot replace most species of dead or dying trees (Rossell et al. 2007). Although they maintain a thick litter layer and low soil pH, which may help buffer mature forests from invasion by nonnative plants (Bernard et al. 2009), most native woody plants cannot regenerate. The thick litter layer maintained in these forests provides suitable habitat for blacklegged ticks, which are found in greater abundance in mature forests relative to other urbanized forest fragment types (Adalsteinsson et al. 2016). In contrast, forest fragments with significant nonnative plant invasion in the understory have high densities of invasive earthworms and very little leaf litter (Lawrence et al. 2003, Suárez et al. 2006, Hale et al. 2006, Nuzzo et al. 2009), which constrains tick abundance (Schulze et al. 1995, Burtis et al. 2014, Adalsteinsson et al. 2016). However, the dense understory structure provided by invasive plants may aggregate immature ticks and infective hosts, potentially

amplifying acarological risk in invaded forest fragments (Lubelczyk et al. 2004, Elias et al. 2006, Williams et al. 2009, Allan et al. 2010).

Recent studies have identified higher pathogen prevalence in ticks and reservoir hosts associated with invasive shrubs (Lubelczyk et al. 2004, Prusinski et al. 2006, Elias et al. 2006, Williams et al. 2009). However, because leaf litter loss, which constrains tick abundance, is also associated with nonnative plant invasion, it is unclear how tick-borne disease risk differs in regrown, invaded compared to mature, uninvaded forest fragments. To contribute to our understanding of tick-borne disease ecology in urbanized landscapes, we designed a study in urban forest fragments with three main objectives: 1. Characterize *B. burgdorferi* and emerging tick-borne pathogen prevalence among questing ticks; 2. Test for differences in pathogen prevalence between forests invaded by nonnative understory plants and uninvaded forests; and 3. Determine which habitat and landscape features influence pathogen prevalence.

Methods

Study area and tick collection

We collected nymphal *I. scapularis* from April–July, 2013 and 2014, using CO_2-baited traps in 11 forest fragments around New Castle County, Delaware; see Adalsteinsson et al. 2016 for details on trap design, spatial arrangement, and tick capture rates. Forest fragments (6–16 ha) consisted of mixed deciduous hardwood stands and varied in understory woody species composition, particularly in the extent of nonnative *Rosa multiflora* invasion. Five of the 11 forest fragment understories had between 10% and 58% of total area covered by *R. multiflora* invasion (hereafter:

invaded), and six fragments lacked *R. multiflora* invasion (<1%), (hereafter: uninvaded). Within invaded sites, we captured ticks at paired traps – one trap within *R. multiflora* cover and its pair 25 m away, not in *R. multiflora*. In uninvaded sites, we deployed traps at random points. We transported all captured ticks to the laboratory live, in individual microcentrifuge tubes, and froze them at -80°C.

Pathogen testing

We used a modified version of the DNeasy Blood & Tissue Kit protocol (Qiagen) to extract DNA from ticks. Here, we explain the steps in which we deviated from the standard protocol (Qiagen). First, we used sterile pipette tips to manually crush each *I. scapularis* nymph individually in 20µL of Hyclone Dulbecco's Phosphate Buffer Saline Solution (Fisher). Next, we incubated samples with lysis buffer ATL and proteinase K in a 56°C hot water bath for three hours. We performed an extra spin step at 13,000 rpm to remove trace ethanol after the Buffer AW2 wash. Finally, we modified the last step by eluting our samples twice (50µL each time), for a final product of 100µL. We checked concentrations of a subset of our samples using a NanoDrop UV-Vis Spectrophotometer (Thermo Scientific) to confirm successful DNA extractions. We tested ticks for four pathogens; 1) *Borrelia burgdorferi*, 2) *Anaplasma phagocytophilum*, 3) *Babesia microti*, and 4) *Borrelia miyamotoi,* using two multiplex quantitative real-time polymerase chain reactions designed for *I. scapularis* (Hojgaard et al. 2014).

Data analysis

Enough ticks tested positive for *Borrelia burgdorferi* to allow statistical analyses; however infection prevalence was too low for the remaining pathogens to determine patterns related to invasion and other habitat and landscape features.

Sample sizes were uneven because of differences in tick abundance in invaded and uninvaded sites (Adalsteinsson et al. 2016) so we used logistic regression models in a Bayesian framework to compare pathogen (i.e. *B. burgdorferi*) prevalence among ticks collected from invaded and uninvaded forest fragments. This approach allowed us to include uncertainty in pathogen prevalence due to varying sample sizes rather than comparing raw proportions. We first tested whether pathogen prevalence differed among treatments (in rose vs. not in rose) within invaded forest fragments. Next, we tested whether infection prevalence differed between invaded and uninvaded forests. For both of these questions, we used the following model structure (McElreath 2016b):

$$Y_i \sim \text{Binomial}(1, p_i),$$

$$\text{logit}(p_i) = \alpha + \beta * X_i,$$

where Y is infection status of each individual tick (0 or 1), which has a binomial distribution with probability p. With the logit link, the probability of infection (p) was estimated as a linear function of baseline infection probability (α) plus the effect of being from one of two treatment groups ($\beta * X$), where the variable X indicates whether the sampling site was either in rose or not in rose, or in an invaded forest or not (0 or 1), depending on the question. We specified vague priors for the parameters α and β including a normal distribution, and we used a Hamiltonian Monte Carlo sampler to run the analysis with STAN (Stan Development Team 2015) using packages rstan (Guo et al. 2016) and rethinking (McElreath 2016a) in program R (R

Development Core Team 2014). Using the difference between posterior predictions of the two groups (in rose or not in rose, and invaded or uninvaded forest), we calculated the probability of infection prevalence in one treatment group being larger than the other, given our data and the specified model (McElreath 2016b).

We used aggregated logistic regression models in a Bayesian framework (McElreath 2016b) to understand the influence of habitat and landscape factors on *B. burgdorferi* prevalence,

$$Y_i \sim \text{Binomial}(n, p_i),$$

$$\text{logit}(p_i) = \alpha + \beta_1 * X_{1i} + \beta_2 * X_{2i} + \ldots + \beta_z * X_{zi}.$$

Counts *of B. burgdorferi* positive ticks (Y_i) were aggregated by trap (i), and the number of trials (n) was the number of ticks tested from each trap. We identified a set of variables that we expected would influence *B. burgdorferi* infection rates by increasing or decreasing interactions between larval ticks and competent reservoir hosts (Table 3). We developed multivariate model sets that grouped variables according to their classification (vegetation, habitat, landscape) and spatial scale (trap or forest fragment). To select between models, we used Watanabe-Akaike Information Criterion (WAIC) (Watanabe 2010, Gelman et al. 2014, McElreath 2016b) and converged on several "best models" that included similar combinations of variables (Table 4). We compared the relative influence of individual variables on the performance of the model by systematically dropping variables and comparing changes in WAIC scores and their standard errors (Yamashita et al. 2007).

Results

We tested 258 *I. scapularis* nymphs, from which we successfully extracted DNA. Twenty-nine ticks (11.2%) were positive for *Borrelia burgdorferi,* five (1.9%)

were positive for *Anaplasma phagocytophilum,* and two (0.8%) were positive for *Borrelia miyamotoi.* Only one tick was coinfected with *B. burgdorferi* and *A. phagocytophilum.* We did not find any *Babesia microti*-positive ticks. Within invaded forests, 11 of 75 ticks (14.6%) captured within *R. multiflora* and 6 of 32 ticks (18.7%) captured outside of *R. multiflora* were *B. burgdorferi*-positive. Of 151 ticks tested from uninvaded sites, 12 (7.9%) were positive for *B. burgdorferi*. Based on differences between posterior distributions, we estimated that there was a 64% probability that infection prevalence was higher outside of *R. multiflora* patches within invaded sites. However, there was a 97% probability that infection prevalence was higher among nymphs from invaded sites compared to nymphs from uninvaded sites (Figure 4).

Our best models for *B. burgdorferi* infection prevalence (Table 4) included woody debris, leaf litter, distance to road, mouse abundance, tick abundance (all at trap-scale), and total cover (fragment-scale). Woody debris, distance to road, mouse abundance, and total cover were positively related to infection prevalence. Leaf litter and tick abundance were negatively related to infection prevalence (Figure 5).

Discussion

In our comparison of invaded and uninvaded forest fragments, we found that *B. burgdorferi* prevalence among questing ticks did not differ within invaded forests, but that ticks from invaded forests had almost double the mean infection prevalence compared to ticks from uninvaded forests (Figure 4). *Borrelia burgdorferi* was the most common pathogen detected in nymphal *I. scapularis* from our study sites, followed by *A. phagocytophilum*, and *B. miyamotoi*. Only one *I. scapularis* nymph

was coinfected with *B. burgdorferi* and *A. phagocytophilum*, and we did not detect *Ba. microti* in any of the ticks tested. At finer scales within both invaded and uninvaded sites, infection prevalence was positively related to coarse woody debris, distance to the nearest road, mouse abundance, and extent of understory cover within the forest fragment (Figure 5). We found a negative relationship between infection prevalence and both leaf litter and tick abundance (Figure 5). *Rosa multiflora* invasion and the additional factors positively influencing pathogen prevalence point to suitable habitat characteristics for small mammal and bird hosts that are competent pathogen reservoirs.

Invaded and uninvaded fragments represent two extremes of different, degraded habitat fragment types that can be separated by the presence/absence of *R. multiflora* invasion in our landscape. Uninvaded sites have deep litter layers, no understory, lots of questing nymphs, and relatively lower infection prevalence (mean = 0.079). Invaded sites have very little leaf litter, dense understory structure, relatively fewer questing nymphs, and roughly double the infection prevalence (mean = 0.159). Our modeling results showed that the total understory cover in a forest fragment positively influenced pathogen prevalence. Understory structure, which is provided almost exclusively by invasive plants, may aggregate immature ticks and infective hosts, resulting in increased pathogen prevalence among ticks in invaded forest fragments (Elias et al. 2006, Williams et al. 2009). Because *B. burgdorferi* is not transmitted transovarially (Rollend et al. 2013), infected free-living nymphs have acquired it by feeding on infected hosts during their larval stage. Similarly, potential pathogen reservoirs must acquire *B. burgdorferi* by being fed upon by an infected

nymph. Therefore, both immature stages of ticks must interact with infective hosts to elevate pathogen prevalence among nymphs (Buskirk and Ostfeld 1995).

Previously, the prevalence of *B. burgdorferi* and *A. phagocytophilum* were reported from ticks collected from hunter-harvested deer in Delaware's three counties in 1988 and 1998 (Wolfe et al. 1994, Curran et al. 2000). In New Castle County, where we conducted our study, Curran et al. (2000) found that 6 of 50 ticks (12%) were positive for *B. burgdorferi,* 2 of 50 were positive for *A. phagocytophilum,* and no ticks were co-infected with both pathogens. Ticks in New Castle County had the highest *B. burgdorferi* prevalence of all three counties (12% vs. 8%), and no ticks from the other two Delaware counties tested positive for *A. phagocytophilum*. The age class of ticks was not reported, but because collections were made in November, these were most likely adult *I. scapularis* (Piesman et al. 1979, Carey et al. 1980, Daniels et al. 1989, Simmons et al. 2015). We found similar *B. burgdorferi* prevalence among nymphs, which typically have lower (~50%) infection prevalence than adults (Piesman et al. 1986, Schulze et al. 1986, Falco and Fish 1988, Lane et al. 1991). Therefore, if ticks in Curran et al. (2000) were adults, this suggests that *B. burgdorferi* prevalence among New Castle County *I. scapularis* has potentially doubled since 1998.

Understory structure facilitates interactions between immature ticks and competent *B. burgdorferi* reservoir hosts (Adler et al. 1992, Willson and Comet 1996, Prusinski et al. 2006). White-footed mouse and passerine bird territory densities are positively related to understory structure (Adler and Wilson 1987, Willson and Comet 1996, Leston and Rodewald 2006, Croci et al. 2008), i.e. invasive plants (*unpublished data*) and within invaded forests, immature ticks are aggregated in stands of invasive shrubs (Williams et al. 2009, Allan et al. 2010, Adalsteinsson et al. 2016). We

hypothesize that larval ticks in uninvaded sites derive a greater proportion of bloodmeals from larger-bodied hosts that are less-competent *B. burgdorferi* reservoirs (Cagnacci et al. 2012, Barbour et al. 2015). We expect that this is in contrast to larval tick bloodmeals in invaded sites, which we predict are composed of a greater proportion of small-bodied hosts that are positively affected by understory structure (Adler et al. 1992) and are competent *B. burgdorferi* reservoirs (Donahue et al. 1987, Ginsberg et al. 2005, Barbour et al. 2015). Future work should use bloodmeal analysis or identification of *ospC* types in *B. burgdorferi*-positive ticks to understand how nonnative plant invasion affects the interaction between specific hosts and ticks, and the resulting implications for transmission of human-invasive *B. burgdorferi* strains (Brisson and Dykhuizen 2004, Önder et al. 2013, Vuong et al. 2014).

An additional hypothesis for the higher nymphal infection prevalence in invaded sites concerns tick overwinter survival. Invaded habitats lack the litter layer that comprises suitable off-host tick habitat (Schulze et al. 1995, Burtis et al. 2014, Adalsteinsson et al. 2016). Ticks depend on the high humidity microclimate within the litter to conserve moisture and to buffer themselves from environmental fluctuations (Stafford 1994). Recent studies show that ixodid ticks infected with *B. burgdorferi* have greater energy reserves and are more robust to desiccation (Naumov 2003, Herrmann and Gern 2010, 2015, Herrmann et al. 2013). Therefore, the harsh litter-free environment of invaded forests may exert stronger pressure against over-winter survival of uninfected ticks, thus increasing overall infection prevalence.

The negative relationships of nymphal infection prevalence with leaf litter and tick abundance raise questions about our understanding of Lyme disease ecology in over-browsed, mature forest fragments. Uninvaded, mature forest fragments that lack

understory structure have higher litter volumes and questing tick abundance than invaded forests. We hypothesize that the lack of understory structure in uninvaded fragments shifts the composition of bloodmeal hosts toward reservoir-incompetent species such as white-tailed deer or other large-bodied hosts (Telford III et al. 1988, Barbour et al. 2015). Talleklint and Jaenson (1996) also detected a negative relationship between tick density and infection prevalence at high tick densities (>20 nymphs/m^2), which they attributed to higher roe deer (*Capreolus capreolus*) densities in those sites. Heightened deer densities could account for both higher tick density and lower infection prevalence if deer act as both reproductive hosts and the dominant bloodmeal source (Gray et al. 1995, Talleklint and Jaenson 1996). The close proximity of our study sites and high deer densities throughout our landscape (18–52 deer/km^2, Rogerson 2010) suggest that deer do not account for differences in tick abundance. However, deer may lower infection prevalence by shifting bloodmeals away from reservoir competent hosts that do not find suitable understory cover in over-browsed, uninvaded fragments.

The importance of invasion, habitat, and landscape variables from our models support that understory structure and woody debris aggregate infectious hosts and larval ticks, increasing pathogen transmission. Coarse woody debris, total understory cover, distance to road, and *P. leucopus* abundance, variables that directly or indirectly represent the distribution of reservoir hosts, were positively related to infection prevalence. Coarse woody debris provides cover, nest sites, movement corridors, and foraging opportunities for immature tick hosts such as white-footed mice (*P. leucopus*), *Sorex* and *Blarina* shrews, and ground-foraging birds (Roche et al. 1999, Greenberg 2002, Lohr et al. 2002, McCay and Komoroski 2004, Jones and Lindquist

2012). Shrews, in particular, are often overlooked in terms of their importance in the Lyme disease system, despite evidence that they can feed and infect more ticks than white-footed mice (Brisson et al. 2008). Outside of the Pacific Northwest and southern Appalachian regions of the U.S., there is a dearth of studies on habitat associations of shrews, especially in small forest fragments (Getz 1961, 1989, Miller and Getz 1977, McCay and Komoroski 2004). In regions where shrews have been well studied, coarse woody debris appears to be an important habitat component (Carey and Johnson 1995, McCay et al. 1998, Butts and McComb 2000, but see Brannon 2000). Similarly, total understory cover represents the structure available to white-footed mice and shrub-nesting birds (Adler and Wilson 1987, Willson and Comet 1996, Leston and Rodewald 2006). The importance of distance to road suggests that perhaps small mammals and birds avoid hard edges near roads in our landscape, or at least that larval ticks encounter infectious hosts farther from roads.

Conclusions

Although nymphal infection prevalence was higher in invaded forests, acarological risk in terms of density of infected nymphs may be higher in uninvaded sites; the uninvaded sites examined in this study supported ~3 times as many questing nymphs compared to invaded sites (Adalsteinsson et al. 2016). Although uninvaded sites lack understory structure and therefore support lower densities of immature tick hosts, their relatively intact litter layers may allow nymphal ticks to survive longer (Yuval and Spielman 1990) and quest more often (Randolph and Storey 1999), creating more opportunities to attach to humans than in invaded forests. Perhaps in uninvaded fragments, restoration of native understory structure (Morlando et al. 2012)

that promotes greater host diversity and abundance could reduce densities of questing infected nymphs.

Acknowledgments

We thank the University of Delaware, USDA McIntire Stennis, and the Aeroecology Program at University of Delaware for support of this work. We are grateful for the cooperation of land agencies involved in this study: Newark City Parks, New Castle County Parks, Delaware State Parks, and Mt. Cuba Center. We are also grateful for advice on DNA extraction protocols from S. Seifert and E. Stromdahl, and for laboratory assistance from M. Brown. We thank the following individuals who helped collect field data: Z. Ladin, K. Handley, J. Nimmerichter, A. Lutto, K. Serno, J. Bondi, C. Piazza, L. Newton, and J. Curry.

Table 3 Modified from Adalsteinsson et al. 2016. Summary of vegetation and landscape covariates measured at the trap and patch scale. Covariates are summarized as mean ± standard error. Letters (A,B,C) denote significant differences among groups ($P < 0.05$) detected using analysis of variance (ANOVA), blocking on site, followed up with Tukey post-hoc comparison when there were more than two groups. "Nudds" refers to Nudds board (Nudds 1977) measurements, "dbh" stands for diameter at breast height, and "rose" means *Rosa multiflora*.

Trap-level covariates	Uninvaded forests	Invaded: in rose	Invaded: not in rose
Nudds at 0.5-1.0 m (%)	$18.0^{A} \pm 3.9$	$73.9^{B} \pm 4.5$	$53.3^{C} \pm 5.9$
Rose cover, 12.5 m radius (%)	$2.5^{A} \pm 0.2$	$11.2^{B} \pm 0.6$	$7.0^{C} \pm 0.6$
Leaf litter volume (L/m^2)	$28.0^{A} \pm 2.8$	$6.1^{B} \pm 1.4$	$6.7^{B} \pm 1.2$
Coarse woody debris (%)	$6.5^{A} \pm 0.9$	$3.4^{B} \pm 0.7$	$4.2^{B} \pm 0.7$
Rose cover, 2.5 m radius (%)	$0.0^{A} \pm 0.0$	$67.1^{B} \pm 2.2$	$3.5^{A} \pm 0.7$
Distance to agriculture (m)	$288.3^{A} \pm 54.7$	$156.7^{B} \pm 24.6$	$159.6^{B} \pm 24.6$
Distance to edge (m)	$67.8^{A} \pm 9.1$	$39.8^{B} \pm 8.8$	$41.9^{B} \pm 8.4$
Distance to road (m)	154.7 ± 16.7	135 ± 18.1	133.4 ± 15.4
Distance to residential (m)	716.9 ± 377.6	186.2 ± 31.5	174.4 ± 32.9
Distance to stream (m)	$371.8^{A} \pm 62.7$	$148.4^{B} \pm 35.7$	$134.1^{B} \pm 35.6$
Tick abundance[1]	$0.8^{A} \pm 0.1$	$0.4^{B} \pm 0.1$	$0.2^{B} \pm 0.0$
Mouse abundance[2]	2.7±0.5	4.5±0.9	2.1±0.4
Mean larvae per mouse[2]	0.4±0.1	0.5±0.1	0.7±0.2

Patch-level covariates	Uninvaded forests	Invaded forests
Rose cover (%)	$0.8^{A} \pm 0.5$	$36.9^{B} \pm 7.7$
Total understory cover (%)	$19.6^{A} \pm 4.4$	$41.6^{B} \pm 6.1$
Leaf litter volume (L/m2)	$13.9^{A} \pm 1.1$	$6.8^{B} \pm 0.9$
Fagus grandifolia (%)	$8.5^{A} \pm 2.8$	$0.7^{B} \pm 0.2$
Acer spp. (%)	$0.7^{A} \pm 0.1$	$21.2^{B} \pm 1.2$
Year of canopy closure	$1916.7^{A} \pm 4.9$	$1963^{B} \pm 5.1$
Nonnative stems (%)	$9.1^{A} \pm 2.7$	$40.0^{B} \pm 3.3$
Average tree dbh (m)	0.6 ± 0.0	0.6 ± 0.0
Quercus spp. (%)	$42.0^{A} \pm 6.4$	$11.0^{B} \pm 5.8$
Mean mice per nest box[2]	0.4 ±0.1	0.5 ±0.2
Mean larvae per mouse[2]	0.7 ± 0.2	0.9 ± 0.3
Bird territory density[3]	3.6 ± 0.5	5.3 ±0.8

[1] Tick abundance values from traps that caught ticks which could be screened for pathogens (Adalsteinsson et al. 2016)

[2] Mouse abundance and larval burdens on mice from nest box study (Adalsteinsson et al., *in prep*). For trap-level estimates, we calculated the mean of the mice caught during fall at two nest boxes in closest proximity to the tick trap. For patch-level estimates, total mouse captures during fall were averaged across all 15 nest boxes in the site. Larval burdens are the average number of larvae per mice at either the two closest nest boxes (trap level) or across 15 nest boxes (patch level).

[3] Spot mapping data for all ground-foraging bird species, collected during 2010 and 2011 breeding seasons (Rega 2012).

Table 4 WAIC table of best models. Variables in the model set are coarse woody debris (CWD), leaf litter volume (litter), distance to the nearest road (dist. road), mouse abundance in fall (mice), total understory cover (total cover), and nymphal tick capture rate (tick abundance). Field headings refer to the effective number of parameters (pWAIC), the difference between WAIC estimates for each model and the top-ranked model (ΔWAIC), the Akaike weight (Weight), the standard error of the WAIC estimate (SE), and the standard error of ΔWAIC value (ΔSE).

Model structure	pWAIC	ΔWAIC	Weight	SE	ΔSE
CWD + litter + dist. road + mice	5.10	0.00	0.37	20.80	NA
CWD + litter + dist. road	3.80	0.80	0.25	26.70	3.78
CWD + litter + dist. road + mice + total cover	5.80	0.90	0.24	21.00	28.10
CWD + litter + dist. road + mice + tick abundance	6.40	2.00	0.14	21.10	1.98
NULL	1.00	13.60	0.00	21.30	8.36

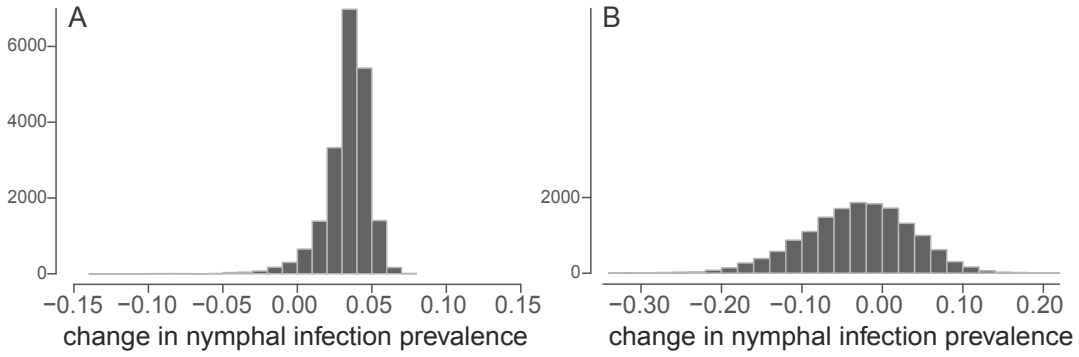

Figure 4 Differences in 89% posterior probabilities of *Borrelia burgdorferi* infection prevalence in nymphs between invaded and uninvaded forest fragments (A) and within invaded forests, between ticks captured within *Rosa multiflora* and outside of it (B).

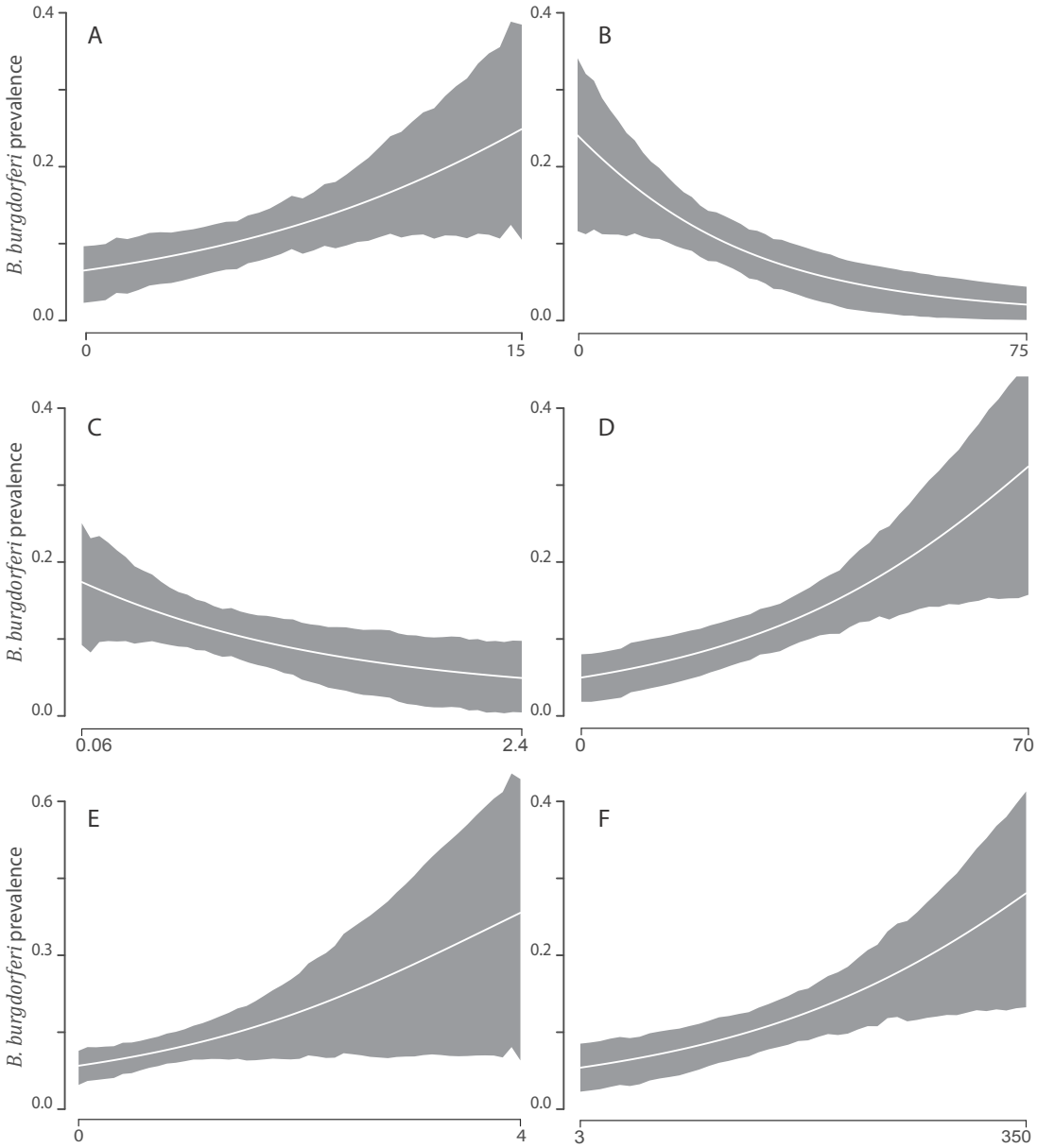

Figure 5 Mean partial predicted responses (with 89% posterior probability intervals) of *Borrelia burgdorferi* prevalence among ticks to six different variables: A) woody debris (%); B) leaf litter volume (L/m^2); C) tick abundance (nymphs/24 h); D) understory cover (%); E) mouse abundance (mice per nest box per check); F) distance to nearest road (m)

REFERENCES

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

Adler, G. H., S. R. Telford, M. L. Wilson, and A. Spielman. 1992. Vegetation structure influences the burden of immature Ixodes dammini on its main host, Peromyscus leucopus. Parasitology 105:105–110.

Adler, G. H., and M. L. Wilson. 1987. Demography of a Habitat Generalist, The White-Footed Mouse, in a Heterogeneous Environment. Ecology 68:1785.

Allan, B. F., H. P. Dutra, L. S. Goessling, K. Barnett, J. M. Chase, R. J. Marquis, G. Pang, G. A. Storch, R. E. Thach, and J. L. Orrock. 2010. Invasive honeysuckle eradication reduces tick-borne disease risk by altering host dynamics. Proceedings of the National Academy of Sciences 107:18523–18527.

Allan, B. F., F. Keesing, and R. S. Ostfeld. 2003. Effect of forest fragmentation on Lyme disease risk. Conservation Biology 17:267–272.

Barbour, A. G., J. Bunikis, D. Fish, and K. Hanincová. 2015. Association between body size and reservoir competence of mammals bearing Borrelia burgdorferi at an endemic site in the northeastern United States. Parasites & Vectors 8.

Bernard, M. J., M. A. Neatrour, and T. S. McCay. 2009. Influence of Soil Buffering Capacity on Earthworm Growth, Survival, and Community Composition in the Western Adirondacks and Central New York. Northeastern Naturalist 16:269–284.

Bradley, C. A., and S. Altizer. 2007. Urbanization and the ecology of wildlife diseases. Trends in Ecology & Evolution 22:95–102.

Brannon, M. P. 2000. Niche relationships of two syntopic species of shrews, Sorex fumeus and S. cinereus, in the southern Appalachian Mountains. Journal of Mammalogy 81:1053–1061.

Brisson, D., and D. E. Dykhuizen. 2004. ospC Diversity in Borrelia burgdorferi: Different Hosts Are Different Niches. Genetics 168:713–722.

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

Burtis, J. C., T. J. Fahey, and J. B. Yavitt. 2014. Impact of invasive earthworms on Ixodes scapularis and other litter-dwelling arthropods in hardwood forests, central New York state, USA. Applied Soil Ecology 84:148–157.

Buskirk, J. V., and R. S. Ostfeld. 1995. Controlling Lyme Disease by Modifying the Density and Species Composition of Tick Hosts. Ecological Applications 5:1133.

Butts, S. R., and W. C. McComb. 2000. Associations of Forest-Floor Vertebrates with Coarse Woody Debris in Managed Forests of Western Oregon. The Journal of Wildlife Management 64:95.

Cagnacci, F., L. Bolzoni, R. Rosà, G. Carpi, H. C. Hauffe, M. Valent, V. Tagliapietra, M. Kazimirova, J. Koci, M. Stanko, M. Lukan, H. Henttonen, and A. Rizzoli. 2012. Effects of deer density on tick infestation of rodents and the hazard of tick-borne encephalitis. I: Empirical assessment. International Journal for Parasitology 42:365–372.

Carey, A. B., and M. L. Johnson. 1995. Small mammals in managed, naturally young, and old-growth forests. Ecological Applications 5:336–352.

Carey, A. B., W. L. Krinsky, and A. J. Main. 1980. Ixodes dammini (Acari: Ixodidae) and associated ixodid ticks in south-central Connecticut, USA. Journal of Medical Entomology 17:89–99.

Croci, S., A. Butet, A. Georges, R. Aguejdad, and P. Clergeau. 2008. Small urban woodlands as biodiversity conservation hot-spot: a multi-taxon approach. Landscape Ecology 23:1171–1186.

Curran, K. L., J. B. Kidd, J. Vassallo, and V. L. Van Meter. 2000. Borrelia burgdorferi and the causative agent of human granulocytic ehrlichiosis in deer ticks, Delaware. Emerging infectious diseases 6:408.

Daniels, T. J., D. Fish, and R. C. Falco. 1989. Seasonal activity and survival of adult Ixodes dammini (Acari: Ixodidae) in southern New York State. Journal of medical entomology 26:610–614.

Donahue, J. G., J. Piesman, and A. Spielman. 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes. The American Journal of Tropical Medicine and Hygeine 36:92–96.

Elias, S. P., C. B. Lubelczyk, P. W. Rand, E. H. Lacombe, M. S. Holman, and R. P. Smith. 2006. Deer Browse Resistant Exotic-Invasive Understory: An Indicator of Elevated Human Risk of Exposure to <I>Ixodes scapularis</I> (Acari: Ixodidae) in Southern Coastal Maine Woodlands. Journal of Medical Entomology 43:1142–1152.

Falco, R. C., and D. Fish. 1988. Prevalence of Ixodes dammini near the homes of Lyme disease patients in West Chester County, New York. American Journal of Epidemiology 127:826–830.

Gelman, A., J. Hwang, and A. Vehtari. 2014. Understanding predictive information criteria for Bayesian models. Statistics and Computing 24:997–1016.

Getz, L.L. 1961. Factors influencing the local distribution of shrews. *American Midland Naturalist* 65:67-68.

Getz, L.L. 1989. A 14-year study of *Blarina brevicauda* populations in East-Central Illinois. *Journal of Mammalogy,* 70(1).

Ginsberg, H. S., P. A. Buckley, M. G. Balmforth, E. Zhioua, S. Mitra, and F. G. Buckley. 2005. Reservoir Competence of Native North American Birds for the Lyme Disease Spirochete, *Borrelia burgdorferi*. Journal of Medical Entomology 42:445–449.

Gray, J. S., O. Kahl, C. Janetzki, J. Stein, and E. Guy. 1995. The spatial distribution of Borrelia burgdorferi-infected Ixodes ricinus in the Connemara region of County Galway, Ireland. Experimental & applied acarology 19:163–172.

Greenberg, C. H. 2002. Response of white-footed mice (Peromyscus leucopus) to coarse woody debris and microsite use in southern Appalachian treefall gaps. Forest Ecology and Management 164:57–66.

Guo, J., D. Lee, K. Sakrejda, J. Gabry, B. Goodrich, J. de Guzman, E. Niebler, T. Heller, and J. Fletcher. 2016. rstan: R Interface to Stan.

Hale, C. M., L. E. Frelich, and P. B. Reich. 2006. Changes in hardwood forest understory plant communities in response to european earthworm invasions. Ecology 87:1637–1649.

Herrmann, C., and L. Gern. 2010. Survival of Ixodes ricinus (Acari: Ixodidae) Under Challenging Conditions of Temperature and Humidity Is Influenced by Borrelia burgdorferi sensu lato Infection. Journal of Medical Entomology 47:1196–1204.

Herrmann, C., and L. Gern. 2015. Search for blood or water is influenced by Borrelia burgdorferi in Ixodes ricinus. Parasites & Vectors 8:6.

Herrmann, C., M. J. Voordouw, and L. Gern. 2013. Ixodes ricinus ticks infected with the causative agent of Lyme disease, Borrelia burgdorferi sensu lato, have higher energy reserves. International Journal for Parasitology 43:477–483.

Hojgaard, A., G. Lukacik, and J. Piesman. 2014. Detection of Borrelia burgdorferi, Anaplasma phagocytophilum and Babesia microti, with two different multiplex PCR assays. Ticks and Tick-borne Diseases 5:349–351.

Huebner, C. D., J. Steinman, T. F. Hutchinson, T. E. Ristau, and A. A. Royo. 2014. The distribution of a non-native (Rosa multiflora) and native (Kalmia latifolia) shrub in mature closed-canopy forests across soil fertility gradients. Plant and Soil 377:259–276.

Jones, C. G., and E. S. Lindquist. 2012. Utilization of Woody Debris by *Peromyscus leucopus* in a Fragmented Urban Forest. Southeastern Naturalist 11:689–698.

Lane, R. S., J. Piesman, and W. Burgdorfer. 1991. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annual review of entomology 36:587–609.

Lawrence, B., M. C. Fisk, T. J. Fahey, and E. R. Suárez. 2003. Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (Acer saccharum). New Phytologist 157:145–153.

Leston, L. F. V., and A. D. Rodewald. 2006. Are urban forests ecological traps for understory birds? An examination using Northern cardinals. Biological Conservation 131:566–574.

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

LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences 100:567–571.

Lohr, S. M., S. A. Gauthreaux, and J. C. Kilgo. 2002. Importance of coarse woody debris to avian communities in loblolly pine forests. Conservation Biology 16:767–777.

Lubelczyk, C. B., S. P. Elias, P. W. Rand, M. S. Holman, E. H. Lacombe, and R. P. Smith. 2004. Habitat Associations of <I>Ixodes scapularis</I> (Acari: Ixodidae) in Maine. Environmental Entomology 33:900–906.

McCay, T. S., and M. J. Komoroski. 2004. Demographic responses of shrews to removal of coarse woody debris in a managed pine forest. Forest Ecology and Management 189:387–395.

McCay, T. S., J. Laerm, M. A. Menzel, and W. M. Ford. 1998. Methods used to survey shrews (Insectivora: Soricidae) and the importance of forest-floor structure. Brimleyana:110–119.

McElreath, R. 2016a. Rethinking: An R package for fitting and manipulating Bayesian models.

McElreath, R. 2016b. Statistical Rethinking. First edition. CRC Press.

Miller, H. and Getz, L.L. 1977. Factors affecting the local distribution and species diversity of forest small mammals in New England. *Canadian Journal of Zoology*, 55:806-814.

Morlando, S., S. J. Schmidt, and K. LoGiudice. 2012. Reduction in Lyme Disease Risk as an Economic Benefit of Habitat Restoration. Restoration Ecology 20:498–504.

Naumov, R. L. 2003. Longevity of forest and taiga ticks (Ixodidae) infected and non-infected with Borrelia burgdorferi groups. Parazitologiia 37:527–532.

Nuzzo, V. A., J. C. Maerz, and B. Blossey. 2009. Earthworm Invasion as the Driving Force Behind Plant Invasion and Community Change in Northeastern North American Forests. Conservation Biology 23:966–974.

Önder, Ö., W. Shao, B. D. Kemps, H. Lam, and D. Brisson. 2013. Identifying sources of tick blood meals using unidentified tandem mass spectral libraries. Nature Communications 4:1746.

Piesman, J., T. N. Mather, J. G. Donahue, J. Levine, J. D. Campbell, S. J. Karakashian, and A. Spielman. 1986. Comparative prevalence of Babesia microti and Borrelia burgdorferi in four populations of Ixodes dammini in eastern Massachusetts. Acta Tropica 43:263–270.

Piesman, J., A. Spielman, P. Etkind, T. K. Ruebush, and D. D. Juranek. 1979. Role of deer in the epizootiology of Babesia microti in Massachusetts, USA. Journal of Medical Entomology 15:537–540.

Prusinski, M. A., H. Chen, J. M. Drobnack, S. J. Kogut, R. G. Means, J. J. Howard, J. Oliver, G. Lukacik, P. B. Backenson, and D. J. White. 2006. Habitat Structure Associated with <I>Borrelia burgdorferi</I> Prevalence in Small Mammals in New York State. Environmental Entomology 35:308–319.

Qiagen. (n.d.). DNeasy Blood & Tissue Kit. Venlo.

R Development Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Randolph, S. E., and K. Storey. 1999. Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. Journal of Medical Entomology 36:741–748.

Rega, C. 2012. Impacts of soil calcium availability and non-native plant invasions on an urban forest bird community. University of Delaware.

Roche, B. E., A. I. Schulte-Hostedde, and R. J. Brooks. 1999. Route Choice by Deer Mice (Peromyscus maniculatus): Reducing the Risk of Auditory Detection by Predators. The American Midland Naturalist 142:194–197.

Rogerson, J., M. Globetti, R. Hossler, E. Moore, K. Reynolds, D. Hotton, M. Pepper, and A. E. Shovers. 2010. Delaware Deer Management Plan. Delaware Department of Natural Resources and Environmental Control.

Rollend, L., D. Fish, and J. E. Childs. 2013. Transovarial transmission of Borrelia spirochetes by Ixodes scapularis: A summary of the literature and recent observations. Ticks and Tick-borne Diseases 4:46–51.

Rossell, C. R., S. Patch, and S. Salmons. 2007. Effects of Deer Browsing on Native and Non-native Vegetation in a Mixed Oak-Beech Forest on the Atlantic Coastal Plain. Northeastern Naturalist 14:61–72.

Schulze, T. L., R. A. Jordan, and R. W. Hung. 1995. Suppression of Subadult Ixodes scapularis (Acari: Ixodidae) Following Removal of Leaf Litter. Journal of medical entomology 32:730–3.

Schulze, T. L., M. K. Lakat, W. E. Parkin, J. K. Shisler, D. J. Charette, and E. M. Bosler. 1986. Comparison of rates of infection by the Lyme disease spirochete in selected populations of Ixodes dammini and Amblyomma americanum (Acari: Ixodidae). Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Series A: Medical Microbiology, Infectious Diseases, Virology, Parasitology 263:72–78.

Simmons, T. W., J. Shea, M. A. Myers-Claypole, R. Kruise, and M. L. Hutchinson. 2015. Seasonal Activity, Density, and Collection Efficiency of the Blacklegged Tick (*Ixodes scapularis*) (Acari: Ixodidae) in Mid-Western Pennsylvania. Journal of Medical Entomology 52:1260–1269.

Stafford, K. C. 1994. Survival of immature Ixodes scapularis (Acari: Ixodidae) at different relative humidities. Journal of medical entomology 31:310–314.

Stan Development Team. 2015. Stan: A C++ Library for Probability and Sampling.

Suárez, E. R., T. J. Fahey, J. B. Yavitt, P. M. Groffman, and P. J. Bohlen. 2006. Patterns of litter disappearance in a northern hardwood forest invaded by exotic earthworms. Ecological Applications 16:154–165.

Talleklint, L., and T. G. T. Jaenson. 1996. Relationship between Ixodes ricinus density and prevalence of infection with Borrelia-like spirochetes and density of infected ticks. Journal of Medical Entomology 33:805–811.

Telford III, S. R., T. N. Mather, S. I. MOORE, M. L. Wilson, and A. Spielman. 1988. Incompetence of deer as reservoirs of the Lyme disease spirochete. The American Journal of Tropical Medicine and Hygeine 39:105–109.

Vuong, H., C. D. Canham, D. M. Fonseca, D. Brisson, P. J. Morin, P. E. Smouse, and R. S. Ostfeld. 2014. Occurrence and transmission efficiencies of Borrelia burgdorferi ospC types in avian and mammalian wildlife. Infection, Genetics and Evolution 27:594–600.

Watanabe, S. 2010. Asymptotic equivalence of Bayes cross validation and widely applicable information criterion in singular learning theory. The Journal of Machine Learning Research 11:3571–3594.

Williams, S. C., J. S. Ward, T. E. Worthley, and K. C. Stafford. 2009. Managing Japanese Barberry (Ranunculales: Berberidaceae) Infestations Reduces Blacklegged Tick (Acari: Ixodidae) Abundance and Infection Prevalence With <I>Borrelia burgdorferi</I> (Spirochaetales: Spirochaetaceae). Environmental Entomology 38:977–984.

Willson, M. F., and T. A. Comet. 1996. Bird Communities of Northern Forests: Ecological Correlates of Diversity and Abundance in the Understory. The Condor 98:350–362.

Wolfe, D., C. Fries, K. Reynolds, and L. Hatchcock. 1994. The epidemiology of Lyme disease in Delaware 1989-1992. Delaware Medical Journal 66:603–613.

Yamashita, T., K. Yamashita, and R. Kamimura. 2007. A Stepwise AIC Method for Variable Selection in Linear Regression. Communications in Statistics - Theory and Methods 36:2395–2403.

Yuval, B., and A. Spielman. 1990. Duration and regulation of the developmental cycle of Ixodes dammini (Acari: Ixodidae). Journal of Medical Entomology 27:196–201.

Zolnik, C. P., R. C. Falco, S.-O. Kolokotronis, and T. J. Daniels. 2015. No Observed Effect of Landscape Fragmentation on Pathogen Infection Prevalence in Blacklegged Ticks (Ixodes scapularis) in the Northeastern United States. PLOS ONE 10:e0139473.

Chapter 3

DISTRIBUTIONS OF RESERVOIR HOSTS AND ZOONOTIC DISEASE VECTORS IN FRAGMENTED FORESTS

Introduction

The white-footed mouse (*Peromyscus leucopus*) is a habitat generalist and an important reservoir host for zoonotic pathogens within eastern deciduous forests (Levine et al. 1985, Adler and Wilson 1987, Mather et al. 1989). Not only is *P. leucopus* an efficient host for immature stages of the blacklegged tick (*Ixodes scapularis*), but it is also a competent reservoir for multiple zoonotic pathogens vectored by blacklegged ticks including *Borrelia burgdorferi* (causative agent of Lyme disease), *Babesia microti*, and *Anaplasma phagocytophilum* (Donahue et al. 1987, Lane et al. 1991, Telford and Spielman 1993, Telford et al. 1996, LoGiudice et al. 2003, Brisson and Dykhuizen 2004). Prevalence of *B. burgdorferi* among ticks is amplified when immature ticks take a larger proportion of bloodmeals from *P. leucopus* relative to less competent reservoir hosts (Ostfeld and Keesing 2000, Schmidt and Ostfeld 2001, LoGiudice et al. 2003). Although it is a habitat generalist, *P. leucopus* densities vary across habitat types and seasons (e.g., Merriam and Wegner 1992, Nupp and Swihart 1996, Barko et al. 2003). How urbanization affects the distributions of *P. leucopus* and *I. scapularis,* and their resulting interactions, is not well understood.

In the absence of transovarial transmission, ticks acquire pathogens from their hosts inter-stadially when taking larval bloodmeals from hosts infected during

nymphal bloodmeals (Randolph and Storey 1999). Therefore, the extent of interactions between reservoir hosts (e.g. *P. leucopus*) and potentially infectious vectors (nymphal *I. scapularis*) is crucial in determining the rate of pathogen transmission to larval ticks. It also follows that interactions between infectious mice and larval ticks will influence the pathogen prevalence among questing nymphs the following year. Thus, features of the urban landscape that increase interactions between *P. leucopus* and larval or nymphal *I. scapularis* may amplify acarological risk. Identifying such features will contribute to our understanding of vector-borne disease systems in urban ecosystems and ideally will guide targeted disease risk mitigation strategies.

Invasive species are a common feature in urban landscapes of Eastern North America (Kowarik 2008, Trentanovi et al. 2013); where White-tailed Deer (*Odocoileus virginianus*) densities are high, invasive shrubs may comprise the dominant form of understory cover within urban forest fragments (Ehrenfeld 1997, Elias et al. 2006, Vidra et al. 2007, Williams et al. 2009). *Peromyscus leucopus* prefer complex understory habitat structure for increased protection and sometimes food resources (Myton 1974, M'Closkey and Lajoie 1975, Kaufman et al. 1983, Seagle 1985, Adler and Wilson 1987, Drickamer 1990, Seamon and Adler 1996, Prusinski et al. 2006, Mattos and Orrock 2010). Within forests with invasive understories, ixodid tick distributions are clumped within the invasive plants that provide structure (Lubelczyk et al. 2004, Williams et al. 2009, Allan et al. 2010, Williams and Ward 2010, Adalsteinsson et al. 2016). Therefore, invasive plants seem to facilitate interactions between *P. leucopus* and *I. scapularis*, resulting in higher tick burdens where invasives are present (Prusinski et al. 2006, Williams et al. 2009).

In addition to invasive plants, other factors in urban landscapes may influence interactions between *P. leucopus* and *I. scapularis*. Specifically, leaf litter, ambient temperature, humidity, and extent of edge habitat are all affected by the urban environment (Kim 1992, Pouyat et al. 1997, Kostel-Hughes et al. 1998). Ticks maintain water balance using leaf litter, and their survival and questing behavior is determined largely by temperature and humidity (Stafford 1994, Lindsay et al. 1995, 1999, Schulze et al. 1995, Randolph and Storey 1999, Rodgers et al. 2007). Urban forests are fragmented, with extensive edge habitat that is typically warmer and drier than forest interiors. Although Bertrand and Wilson (1997) did not detect a difference in nymphal tick survival between forest edge and interior habitats, a more recent study found *I. scapularis* nymphs were in greater abundance and with higher *B. burgdorferi* infection prevalence within forests compared to forest/old field edges (Horobik et al. 2007). Similarly, habitat edges have varying effects on *P. leucopus* populations, depending on surrounding land uses (Cummings and Vessey 1994, Barko et al. 2003, Rytwinski and Fahrig 2007), predation pressure (Wolf and Batzli 2004, Eagan et al. 2011), food availability (Morris and Davidson 2000, Anderson et al. 2003), and seasonality (Wilder et al. 2005, Wilder and Meikle 2006). Thus, these habitat and landscape factors influence the distributions of ticks and hosts and may determine the potential for pathogen transmission.

Intrinsic characteristics of hosts also affect host-parasite interactions (Perkins et al. 2003, Brunner and Ostfeld 2008, Calabrese et al. 2011, Devevey and Brisson 2012). Ticks are often aggregated on hosts, such that a small subset of individuals feed the majority of ticks (Schmidt 1999, Perkins et al. 2003, Brunner and Ostfeld 2008, Kiffner et al. 2011, Devevey and Brisson 2012). Large aggregations of ticks on a

minority of *P. leucopus* may be determined by individuals' condition or behavioral traits (e.g., Shaw et al. 2003) instead of habitat or landscape factors mediating exposure to ticks. Age, sex, mass, and home range size have all been significant predictors of nymphal or larval tick burdens on mice (Ostfeld et al. 1996, Schmidt 1999, Perkins et al. 2003, Brunner and Ostfeld 2008, Kiffner et al. 2011, Bouchard et al. 2011, Devevey and Brisson 2012).

To characterize factors affecting tick-host interactions in urban forest fragments, we designed a study with two main objectives: 1. Determine factors influencing *P. leucopus* abundance during nymphal and larval tick seasons, and 2. Describe which intrinsic and extrinsic variables impact larval and nymphal tick burdens. In both cases, we were interested in identifying the important habitat, vegetation, landscape, phenological, or individual-specific variables and assessing their relative importance for predicting either mouse abundance or tick burdens. Overall, we expected invasive shrubs (high vegetation density) to aggregate *P. leucopus* and ticks during both larval and nymphal seasons by providing cover for *P. leucopus* and questing habitat for ticks. We predicted that *P. leucopus* abundance would be positively related to edge habitat, and that tick burdens in both seasons would be influenced by both intrinsic and extrinsic factors.

Methods

Study area

We captured mice in 22 forest fragments (5-163 ha) in New Castle County, Delaware. Forest fragments consisted of mixed deciduous hardwood stands with variation in age, dominant canopy species, and understory species composition.

Canopy trees included *Fagus grandifolia, Quercus spp., Liriodendron tulipifera, Liquidambar styraciflua, Acer rubrum.* The most common native woody shrub was *Lindera benzoin,* but some fragments also had stands of *Viburnum spp. Vaccinium,* and *Clethra alnifolia.* Forest fragments varied in the extent of nonnative shrubs in the understory; *Rosa multiflora* understory cover ranged from 0 to 58%. Other common nonnative woody species were *Eleagnus umbellata, Rubus spp., Celastrus orbiculatus*. Additional descriptions of forest fragments and their soil and vegetation characteristics can be found in Rega (2012).

Mouse captures

We used nest boxes to estimate mouse abundance. Mouse density estimates derived from nest boxes censuses are correlated with those from Sherman trap data (Lewellen and Vessey 1999). Therefore, we assumed that mice per nest box counts were correlated with mouse abundance within our study sites. We constructed cubic nest boxes from 2 cm thick cypress with dimensions measuring 15 cm x 15 cm x 15 cm. Nest box lids were constructed at a slight angle to allow water drainage. Two 3.8 cm diameter entrance holes were on either side of the box, placed toward the back. We stuffed all nest boxes with polyester fiber for bedding and tied them to tree trunks using heavy gauge wire, approximately 1.5 m above the ground. Nest box design and placement was based on prior studies of *P. leucopus* in deciduous forests (e.g. Anderson et al. 2003). We deployed nest boxes at each of 10-17 random locations within each forest fragment where monitoring stations exist for intensive vegetation and landscape data collection. All nest box locations were at least 50 m apart. We deployed 327 nest boxes across 22 forest fragments. To complement an existing dataset at each nest box location, we also conducted vegetation surveys within a 12.5

m radius surrounding each nest box to account for habitat characteristics within an area roughly the size of one *P. leucopus* home range (Wolff 1985). We quantified landscape variables by overlaying nest box locations on Delaware Land Use Land Cover 2007 (State of Delaware 2008) in ArcGIS v10 (ESRI 2012) (Table 1).

We checked every nest box monthly from June through September during 2013 and April through September in 2014, for a total of 3,270 individual nest box checks. During each nest box check, we plugged the entrance holes with rubber stoppers and emptied the nest box contents into a plastic container with steep ~1 m sides to prevent mice from escaping. We counted, identified, aged, and sexed all individuals. With the exception of nursing females (which were returned immediately to the nest box with their pups), we also weighed (g) and quantified tick burdens on all individual mice. In spring 2014, we initiated ear biopsy punches for a concurrent study, which allowed us to determine if individuals had been previously captured/sampled. In addition to quantifying tick burdens, in 2014 we also removed ticks from mice using forceps and collected them in microcentrifuge tubes, which were frozen in the laboratory at -80C until they could be identified using taxonomic keys (Keirans and Litwak 1989, Durden and Keirans 1996, Keirans and Durden 1998). After data collection, we returned mice to the nest box and re-mounted it to the tree.

All capture and handling procedures were carried out with approval from University of Delaware Institutional Animal Care and Use Committee under Animal Use Protocol #1249 and with permit #2013-007W from the State of Delaware Department of Natural Resources and Environmental Control to J.L.B.

Analyses

Mouse occupancy and abundance

We used zero-inflated Poisson models in a Bayesian framework to simultaneously assess which variables influenced mouse occupancy and abundance. Zero-inflated Poisson models use a logistic function to account for separate processes that produce zeros in addition to zeros in the count data modeled by the Poisson process, with the following basic structure (McElreath 2016a):

$$y_i \sim \text{Zero-Inflated Poisson}(p_i, \lambda_i)$$

$$\text{logit}(p_i) = \alpha_p + (\beta_{p1}x_{i1} + \ldots + \beta_{pn}x_{in}),$$

$$\log(\lambda_i) = \alpha_\lambda + (\beta_{\lambda 1}x_{i1} + \ldots + \beta_{\lambda n}x_{in}),$$

where p is the probability of producing zeros (unoccupied nest box), and λ is the mean count of mice in a nest box, given that the logistic function does not produce a zero. Therefore, we make predictions using this model structure by multiplying $(1-p) * \lambda$.

Our response variable was the number of mice in a nest box at each check. Mouse populations may respond differently to the same habitat features during different seasons, and this has implications for the enzootic pathogen transmission cycle (Brunner et al. 2008). Therefore, we estimated the main effects of covariates on mouse abundance during two seasons: 1) Nymphal tick season, "Spring," (April – June) and 2) During larval tick season, "Summer" (July – September). Recaptured individuals were excluded from mouse abundance analyses. We used the same covariates and model selection procedures for both seasons in order to make comparisons about factors affecting mouse distribution between nymphal and larval tick seasons.

We used the "rethinking" package (McElreath 2016b) in Program R (R Development Core Team 2014) to compile models using rstan (Guo et al. 2016). We used an iterative process to construct model sets for comparison using Watanabe Akaike Information Criterion (WAIC) (Watanabe 2010, Gelman et al. 2014). First, we grouped variables into four categories that could influence mouse occupancy or abundance: visit (ordinal day, temperature), understory vegetation (descriptors of understory structure and nonnative plant invasion surrounding nest boxes and within the whole forest fragment), habitat (Woody debris, leaf litter, basal area, mean diameter-at-breast-height of dominant canopy trees), and landscape variables (distance to nearest stream, distance to nearest edge, proportion of agriculture surrounding the forest fragment, road density surrounding the fragment) (Table 5). We then selected the best model from within each category, and compare the relative influence of different variable types. Next, we created an additive model by combining variables from the best model of each category into one global model. Because relative contributions of parameters may change when combined with new parameters, we used a reverse stepwise procedure to eliminate variables that were not contributing to the global model; we systematically dropped each variable from both the logistic and Poisson parts of the model and compared WAIC scores. If dropping a particular variable either did not affect or improved the WAIC score, then we removed it from subsequent iterations until we arrived at a best model according to WAIC (Table 6).

Tick burdens

In analyses of tick burdens, we treated recaptured individuals as independent samples because all ticks were removed and any repeat samples occurred one month apart. However, we excluded individuals from whom we were not able to obtain tick

counts: individuals that escaped during handling, nursing females, and pups not yet weaned. We used the same model structure and selection procedure described above to describe factors influencing tick burdens on mice. However, in this case, our response variable was the number of ticks (either nymphs or larvae) counted on each individual mouse. As above, we conducted two separate analyses for nymphal and larval tick seasons. Because we were interested in which habitat and landscape factors facilitated interactions between mice and ticks, we used the same extrinsic covariates in this analysis. We also added a few intrinsic covariates (total number of mice in a nest box, age, gender, mass) to control for variation from differences among individual *P. leucopus*.

Results

Spring mouse abundance and nymphal tick burdens

In June 2013 and April – June 2014, we had 265 *P. leucopus* captures (55 in 2013 and 210 in 2014) and counted 92 nymphal ticks (17 in 2013 and 75 in 2014) on those mice. In 2014, we only recaptured one individual during spring. After removing individuals from whom we could not assess tick counts (see Methods), we analyzed tick burden data from 185 mice in the spring. Of nymphal ticks that have been identified so far, 25 of 26 were *I. scapularis* (*unpublished data*). Forty-two of 185 mice (23%) hosted at least 1 nymphal tick, with nymphal burdens ranging from 1–8 nymphs per mouse. Twenty-seven mice hosted both nymphs and larvae, and an additional 14 mice hosted only larvae in spring. Spring larval burdens ranged from 1–8 larvae per mouse. Among mice that were analyzed for nymphal tick burdens, we

captured 63 females, 87 males, and 35 of unknown sex. Of those, 103, 16, and 66 were adults, sub-adults, and juveniles, respectively.

In Spring, landscape variables (road density, agricultural land use, and distance to stream) were the most influential for describing mouse abundance. Road density had a negative relationship with mouse abundance, and agricultural land use had a positive relationship with mouse abundance. Distance to stream had a strong positive net effect on mouse abundance (Table 6). Nymphal tick burdens on mice in spring were influenced by: percent of multiflora rose (*Rosa multiflora*) cover (at the patch scale), mass, and coarse woody debris. Multiflora rose cover at the patch scale had a slight negative effect on the probability of nymphs parasitizing mice. Mouse mass and woody debris emerged in the log link function for estimating lambda, suggesting that given the presence of nymphs on mice, heavier mice host more ticks than lighter mice (Figure 7). Coarse woody debris had a slightly negative effect on nymphal tick counts (Table 6).

Summer mouse abundance and larval tick burdens

From July through September of 2013 and 2014, we had 481 mouse captures (250 in 2013 and 231 in 2014) with 1,352 larvae (541 in 2013 and 811 in 2014). Based on biopsy markings in 2014, 11 of the 231 mice captured during summer of 2014 were recaptures. After removing individuals from whom we could not assess tick counts (see Methods), we analyzed tick burden data 345 mice in the summer. Of 737 larvae that have been identified so far, 99% were *Ixodes spp.* and 1% were *Dermacentor variabilis.* In the summer, 248 of 345 mice (72%) hosted at least one larval tick; tick burdens ranged from 1 – 64 larvae per mouse. In summer, 98 of 345 mice (28%) hosted 1,023 of 1,352 larvae (76%), approaching the 80-20 description of parasite

aggregation on hosts (Perkins et al. 2003). Nineteen mice in the summer also hosted between 1–5 nymphs. Among summer mice that were analyzed for tick burdens, there were 124 females, 159 males, and 62 of unknown sex. By age classes, there were 201 adults, 51 sub-adults, and 91 juveniles.

In summer, the overall best model for mouse abundance included variables from all four categories (Table 6). Mouse abundance was positively related to ordinal day (Figure 9) and negatively related to temperature. The percent of nonnative stems contributed to both the logit and log link functions, suggesting that it positively contributed to both the probability of nest box occupancy and mouse abundance, given an occupied box. Leaf litter volume and road density contributed to both functions; however, their contributions had a net effect close to zero. The proportion of agricultural land use within a 250 m buffer surrounding a forest patch had a strong positive effect on mouse abundance. Distance to nearest edge and distance to nearest stream (consistent with spring mouse models) also had positive effects on mouse abundance (Figure 8).

Larval tick burdens on mice in summer were influenced by ordinal day, mouse sex, basal area, woody debris, leaf litter, and distance to nearest edge (Table 6, Figures 8, 9). Larval tick burdens were negatively related to increasing ordinal day, reflecting larval tick phenology during this time (Ostfeld et al. 1996, Brunner and Ostfeld 2008, Devevey and Brisson 2012). Female mice in reproductive condition had higher larval tick burdens than males and mice of unknown sex. Larval tick burdens were negatively related to basal area, coarse woody debris, and leaf litter volume, but positively related to distance to forest edge (Table 6).

Discussion

We used identical covariate sets and model structures to identify and compare which factors drive mouse and predict interactions with nymphal and larval ticks in heterogeneous urban forest fragments. Our analyses revealed differences in the variables driving mouse abundance by season and affecting tick burdens by life stage, thus underscoring the complexity of processes behind the seasonal distributions of reservoir hosts and pathogen vectors (Brunner and Ostfeld 2008, Calabrese et al. 2011). In the spring, landscape factors best predicted mouse abundance, while characteristics of individual mice most strongly influenced nymphal tick burdens (Devevey and Brisson 2012). Summer mouse abundance was driven by a combination of understory habitat characteristics and landscape variables. Larval tick burdens on summer mice were best predicted by a combination of phenology, habitat, and landscape factors, and characteristics of individual mice.

Spring mouse abundance and nymphal tick burdens

In spring, *P. leucopus* populations disband winter huddles, disperse, and initiate breeding once again (Wolff and Durr 1986). Simultaneously, fed larvae that have successfully molted and over-wintered emerge as questing nymphs (Carey et al. 1980, Daniels et al. 1989). Infected, questing nymphs transmit pathogens to *P. leucopus* individuals that subsequently can infect larval ticks. We found that spring mouse abundance was affected by two patch-level landscape variables, the proportion of agricultural land use and road density within a 250 m buffer surrounding the patch. *Peromyscus* populations experience high overwinter mortality rates that may cause local extinctions, especially in small habitat patches like those found in urban areas (Merriam and Wegner 1992, Rytwinski and Fahrig 2007). Therefore, landscape

features that promote or inhibit recolonization of patches from surrounding areas may have strong impacts on spring mouse abundance (Taylor 1978, Fahrig and Merriam 1985, Merriam and Wegner 1992, Krohne and Hoch 1999, Rytwinski and Fahrig 2007). The positive effect of agricultural land use on spring mouse abundance we found may indicate that agricultural land is relatively more permeable to mice than urban cover types (Whitaker 1967, Goundie and Vessey 1986, Wegner and Merriam 1990, Cummings and Vessey 1994, Krohne and Hoch 1999). The negative relationship between road density and mouse abundance is consistent with prior studies showing roads create barriers to small mammal movement (Oxley et al. 1974, Kozel and Fleharty 1979, Mader 1984, Garland and Bradley 1984, Swihart and Slade 1984, Merriam et al. 1989, Barko et al. 2003, Fahrig and Rytwinski 2009) and that wider roads are stronger barriers (McLaren et al. 2011). Spring mouse abundance increased with increasing distance from stream, reflecting known habitat preferences for drier and better-drained soils (Adler et al. 1992, Prusinski et al. 2006). The positive effect of stream distance was stronger for mouse abundance in spring compared to summer, perhaps because spring rains may raise water levels and saturate soil close to stream banks.

Overall, nymphal tick burdens were influenced by nonnative plant invasion, woody debris, and mass of individual mice (Table 6, Figure 7). We previously found a positive relationship between distance to stream and questing nymphal tick abundance (Adalsteinsson et al. 2016); however, this was not reflected in nymphal tick burdens on mice. Nymphal tick burdens were negatively related to *R. multiflora* cover at the patch scale, consistent with relative abundance of questing nymphs between invaded and uninvaded sites (Adalsteinsson et al. 2016), and therefore site-specific questing

nymph densities (Lyon et al. 1996, Ostfeld et al. 1996, Markowski et al. 1997). The lack of leaf litter in invaded sites aggregates ticks beneath densely growing invasive plants, but at the patch scale, the absence of litter reduces habitat suitability (Schulze et al. 1995, Burtis et al. 2014). Malo et al. (2013) reported lower densities of nymphal ticks in patches of invasive *Rhododendron,* while Adler et al. (1992) and Prusinski et al. (2006) found higher nymphal tick burdens on *P. leucopus* in plots with high shrub densities; however, none of these studies measured leaf litter. If the links among invasive shrubs, shrub densities, and reductions in leaf leaf litter are forest or landscape specific, there may be differential effects on nymphal tick burdens in invaded forests.

While nonnative understory plant invasion at the patch scale predicted the presence/absence of nymphs on mice, intrinsic mouse characters had the strongest effect on the aggregation of nymphs on mice (Devevey and Brisson 2012). In particular, mass had a positive relationship with nymphal tick burdens (Devevey and Brisson 2012). Mass is strongly correlated with age across juvenile, subadult, and adult age classes, so the positive relationship with mass also demonstrates that adult mice had higher nymphal tick burdens than juveniles (Bouchard et al. 2011). Although we included a categorical age class variable in our analysis, the continuous mass variable was a better predictor of nymphal tick burdens. In contrast to our results, Brunner and Ostfeld (2008) found that smaller, younger mice had higher nymphal tick burdens than larger, older mice, which is perhaps attributable to self-grooming and allogrooming practices (Shaw et al. 2003). It is conceivable that juveniles inhabiting nest boxes with their mothers and littermates might receive more allogrooming than

independent, trapped juveniles; however, we did not find a relationship between the total number of mice in a nest box and nymphal tick burdens.

Summer mouse abundance and larval tick burdens

In late summer, *P. leucopus* populations are still breeding and they begin to encounter questing larval ticks that emerge from egg masses laid by females earlier in the season. Given the high reservoir competency of *P. leucopus* for pathogens such as *B. burgdorferi*, larval ticks feeding on infected *P. leucopus* are likely to acquire pathogens (Lane et al. 1991, Telford and Spielman 1993, LoGiudice et al. 2003, Brisson and Dykhuizen 2004). Infected larvae that molt and survive winter may transmit those pathogens to future host animals. We found that in summer, timing and temperature had the strongest influence on mouse abundance, most likely due to population growth and behavioral shifts during this time period (Lewellen and Vessey 1999, Wilder and Meikle 2006). The relationship between mouse abundance and agricultural land use, road density, and distance to stream was consistent for both spring and summer, but landscape variables had a lesser influence in the summer relative to other variable types. Unexpectedly, distance to edge in summer had a positive correlation with mouse abundance. Edge effects on mammal populations may be complex and dependent on habitat type and surrounding landscapes (Yahner 1988, Lidicker Jr 1999, Kingston and Morris 2000). Forest habitat edges may increase mouse densities by providing greater structural complexity, protection from predators, and food resources relative to forest interiors (Nupp and Swihart 1996, Krohne and Hoch 1999, Anderson et al. 2003, Wilder et al. 2005). However, edges may also provide relatively poorer quality habitat resulting in lower mouse densities due to increased predation risk or reduced food availability (Morris and Davidson 2000, Wolf

and Batzli 2002, 2004, Eagan et al. 2011). Our data support the latter scenario, though we did not explicitly test the mechanism here.

As predicted, mouse abundance in summer was positively related to nonnative stems, suggesting that mice respond positively to increasing understory structure and complexity (Myton 1974, M'Closkey and Lajoie 1975, Kaufman et al. 1983, Seagle 1985, Adler and Wilson 1987, Drickamer 1990, Seamon and Adler 1996, Prusinski et al. 2006, Dutra et al. 2011, Malo et al. 2013).

Larval burdens on summer mice were best explained by habitat and understory variables, but our best model also included variables related to landscape, intrinsic characters, and phenology. Larval burdens were negatively correlated with basal area (Prusinski et al. 2006), coarse woody debris, and leaf litter volume. Understory vegetation density between 0 – 0.5 m was negatively correlated with leaf litter volume; models with leaf litter volume performed slightly better, so we retained litter volume in the final model sets when we combined variable groups. The negative association between litter volume and understory vegetation density is well-documented in landscapes like ours where nonnative, invasive plants comprise the majority of understory structure because of intense browsing pressure from white-tailed deer (*Odocoileus virginianus*) (Nuzzo et al. 2009). Most studies of larval tick burdens on hosts have not measured the correlation between litter volume and understory structure, but there is a positive link between larval tick burdens and understory vegetation structure (Adler et al. 1992, Lindsay et al. 1999, Prusinski et al. 2006, Williams et al. 2009, Kiffner et al. 2011).

Concordant with the summer mouse abundance response, larval burdens increased with distance from the forest edge (Figure 8). Although summer nymphal *I.*

scapularis survival does not differ between forest interior and edge (Bertrand and Wilson 1997), we are not aware of any studies examining egg mass survival and hatching success between interior and edge habitats. Given constant overwinter survival of fed larvae between interior and edge habitats, our results are consistent with greater abundance of - and *B. burgdorferi* prevalence among - questing *I. scapularis* nymphs found in forest interior relative to edge (Horobik et al. 2007).

We did not detect an influence of mouse density (within a nest box) on tick burdens, which has been shown to dilute the mean larval burden across individual hosts (Schmidt 1999, Brunner and Ostfeld 2008, Kiffner et al. 2011, but see Monello and Gompper 2010). Especially because multiple individuals inhabiting the same nest box share a nest site and likely at least some of the surrounding territory, we expected we would see reduced larval burdens among these mice.

In contrast to summer mouse abundance, larval burdens declined with ordinal day, most likely reflecting the seasonal peak and decline of questing larval ticks (Ostfeld et al. 1996, Markowski et al. 1997, Brunner and Ostfeld 2008, Kiffner et al. 2011, Devevey and Brisson 2012) (Figure 9). Unexpectedly, female mice carried higher larval tick burdens than males, which had higher burdens than mice of unknown sex. Adult males typically have higher larval tick loads than females (Ostfeld et al. 1996, Schmidt 1999, Perkins et al. 2003, Brunner and Ostfeld 2008, Kiffner et al. 2011, Bouchard et al. 2011, Devevey and Brisson 2012). Male mice defend larger territories than females; increased movement could lead to greater accumulation of parasites (Calabrese et al. 2011). Unlike males, female mice increase their territory size to compensate for reductions in habitat quality or foraging opportunities (Klein and Cameron 2012). If female mice in urban forest fragments

have expanded territories, this could help explain their high tick burdens relative to males.

Conclusions

Understory structure, largely attributed to invasive nonnative shrubs, increased summer mouse abundance and larval tick burdens, but not mouse abundance nor nymphal tick burdens during spring. This may be because questing nymph densities in invaded sites are relatively lower than in uninvaded sites at the patch scale, presumably due to poorer quality habitat (i.e. lack of leaf litter) (Schulze et al. 1995, Nuzzo et al. 2009, Burtis et al. 2014, Adalsteinsson et al. 2016). The loss of leaf litter associated with invasion potentially reduces overwinter survival rates for nymphs in these habitats. Spring mouse abundance was more influenced by landscape features than habitat structure. Therefore, if spring mice are not aggregated in forests with invasive understories then surviving nymphal ticks may exhaust energy reserves before finding hosts in invaded fragments. In a tick survival study, Carroll (2003) reported the rapid disappearance of leaf litter in deciduous forest sites during summer and posited that this contributed to the low survival rate unfed nymphal *I. scapularis*. In contrast, summer mouse abundance was positively related to nonnative stems and understory structure. Provided that egg masses can successfully develop and hatch, larvae may quickly find mouse hosts in invaded fragments (due to high mouse abundance and ample questing habitat), which could explain the increased larval tick burdens associated with invasion.

Our results demonstrate that host characteristics as well as understory, habitat, and landscape factors mediate interactions between reservoir hosts and vectors, with implications for pathogen transmission rates. We described certain trends consistent

with tick-mouse interactions found elsewhere such as the effects of mouse age and mass on tick burdens (Brunner and Ostfeld 2008, Kiffner et al. 2011, Bouchard et al. 2011). However, we also found new patterns that may be unique to urban landscapes including the relatively higher tick burdens on female mice compared to males and the differential effects of nonnative plant invasion on nymphal vs. larval tick burdens. Therefore, heterogeneous forest fragments in urban landscapes require special consideration as urbanization may alter interactions between hosts and vectors through changes in habitat and behavior.

Acknowledgments

Funding for this work was provided by the Aeroecology Program at University of Delaware, USDA McIntire Stennis, and a University of Delaware Graduate Research Fellowship. We are grateful to Doug Meikle for his input and advice on nest boxes, and we thank George Harrison for building the nest boxes used in this study. We appreciate the excellent field assistance received from Zach Ladin, Joe Bondi, Ashley Lutto, Abby Walter, Lily Newton, Emily Dunn, Kim Serno, and Jess Nimmerichter, and we thank Rebecca Deegan for lab assistance. We thank the following organizations for their cooperation: Mt. Cuba Center, Delaware State Parks, New Castle County Parks, Newark City Parks, University of Delaware.

Table 5 Summary (mean and standard error) of continuous covariates grouped according to variable classification. Measurements included in the name of the variable indicate the radius (or height, in the case of Nudds) around the nest box or the patch in which the variable was measured. "Nudds" refers to Nudds board measurements of vegetation density (Nudds 1977), sampled 12.5 m from the nest box at 0-0.5 m height stratum. "Mean DBH" is the mean diameter at breast height of the dominant canopy trees within an 11 m radius of the nest box. Categorical variables (year, mouse age, mouse sex) used in analyses are summarized in the Results section.

Covariates	Mean	SE
Visit		
Spring ordinal day	148.2	0.6
Summer ordinal day	228.2	0.6
Spring temperature (°C)	20.2	0.3
Summer temperature (°C)	22.2	0.2
Understory		
Nudds (% 0-0.5m)	50.8	1.6
Rose cover (% in 12.5 m)	10.4	0.9
Total stem count (2.5 m)	16.7	1.5
Nonnative stem count (2.5 m)	8.1	1.2
Nonnative stems (% in 2.5 m)	24.8	2.0
Rose cover (% in entire study plot)	13.6	3.5
Habitat		
Woody debris (% in 12.5 m)	6.6	0.4
Basal area (m^2/ha)	26.6	0.6
Mean DBH (cm)	53.8	0.9
Litter volume (L/m2)	10.4	0.2
Landscape		
Distance to stream (m)	256.6	14.2
Distance to edge (m)	57.6	2.6
Agriculture (% in 250 m)	10.7	3.1
Road density (250 m)	4.6	0.6
Intrinsic characters		
Spring total individuals	2.3	0.1
Summer total individuals	2.2	0.1
Spring mass (g)	19.2	0.5
Summer mass (g)	19.7	0.3

Table 6 Summary of parameter estimates (mean and standard deviation, SD) with 89% credibility intervals for best models of spring and summer mouse abundance and tick burdens. Model parameters in the logit link function for estimating the probability of a nest box (for mouse abundance models), or a mouse (for tick burden models), being unoccupied are denoted with "*p*." Parameters in the log link function for estimating counts of mice (for mouse abundance models), or ticks (for tick burden models), are indicated with "λ." Negative coefficients in the probability (*p*) models increase the probability of occupancy, while negative coefficients in the lamba (λ) models decrease the mean count of the response (mice or ticks).

Model parameters	Mean	SD	L 89%	U 89%
Spring mouse abundance				
α_p	1.53	0.13	1.32	1.73
β_p - road density	0.14	0.10	-0.03	0.31
β_p - agriculture	-0.17	0.10	-0.32	-0.01
β_p - distance to stream	-0.13	0.10	-0.28	0.02
α_λ	-0.02	0.11	-0.19	0.16
β_λ - distance to stream	0.35	0.07	0.24	0.46
Spring nymphal tick burdens				
α_p	0.53	0.27	0.07	0.95
β_p - percent rose (patch)	0.77	0.27	0.34	1.18
α_λ	-0.15	0.19	-0.44	0.16
β_λ - mass	0.66	0.15	0.44	0.91
β_λ - woody debris	-0.77	0.17	-1.03	-0.50
Summer mouse abundance				
α_p	1.05	0.12	0.85	1.23
β_p - ordinal day	0.32	0.11	0.14	0.50
β_p - temperature	0.43	0.09	0.28	0.57
β_p - percent nonnative stems	-0.22	0.10	-0.38	-0.06
β_p - litter volume	0.42	0.12	0.24	0.62
β_p - road density	0.32	0.10	0.15	0.49
β_p - distance to stream	-0.24	0.09	-0.38	-0.11
α_λ	-0.32	0.09	-0.47	-0.18
β_λ - ordinal day	0.48	0.06	0.39	0.59
β_λ - percent nonnative stems	0.26	0.06	0.15	0.36
β_λ - litter volume	0.30	0.09	0.16	0.44
β_λ - road density	0.26	0.08	0.13	0.38
β_λ - agriculture	0.34	0.05	0.25	0.42

β_λ - distance to edge	0.32	0.05	0.23	0.40
Summer larval tick burdens				
α_p	-0.10	0.63	-1.10	0.90
β_p - ordinal day	0.47	0.14	0.23	0.69
β_p - distance to edge	-0.79	0.17	-1.05	-0.50
β_p - sex	-0.36	0.20	-0.66	-0.03
α_λ	0.88	0.15	0.65	1.11
β_λ - ordinal day	-0.23	0.03	-0.28	-0.18
β_λ - basal area	-0.28	0.03	-0.33	-0.24
β_λ - woody debris	-0.12	0.03	-0.17	-0.07
β_λ - litter volume	-0.21	0.03	-0.26	-0.16
β_λ - distance to edge	0.22	0.03	0.18	0.27
β_λ - sex	0.19	0.04	0.13	0.26

Figure 6 Locations of 22 forest fragments where we trapped *Peromyscus leucopus* in New Castle County, Delaware, USA. Land cover types are color-coded: forest (green), urban (white), agriculture (beige), water (blue).

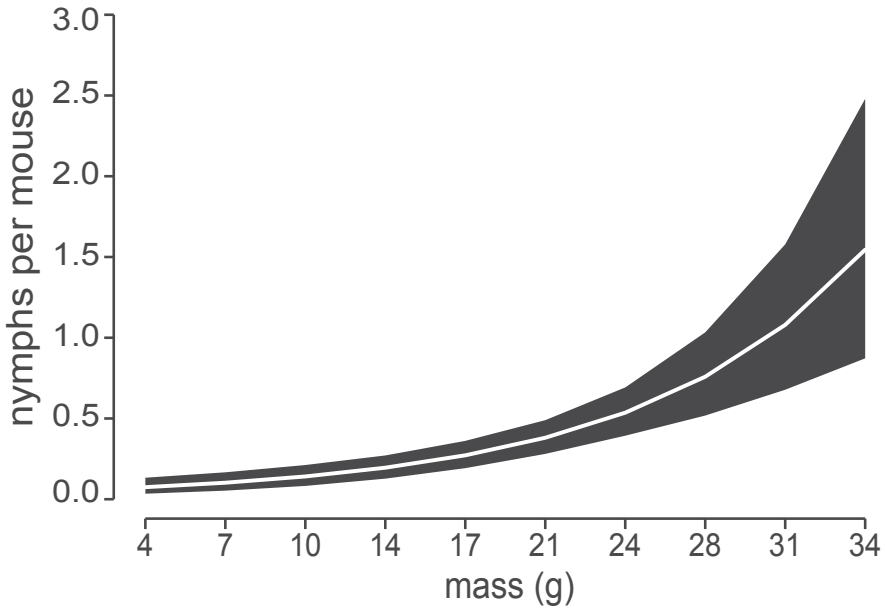

Figure 7 Partial response of nymphal tick burdens in spring to *P. leucopus* mass. Mean (white line) and 89% posterior distribution (shaded region) shown.

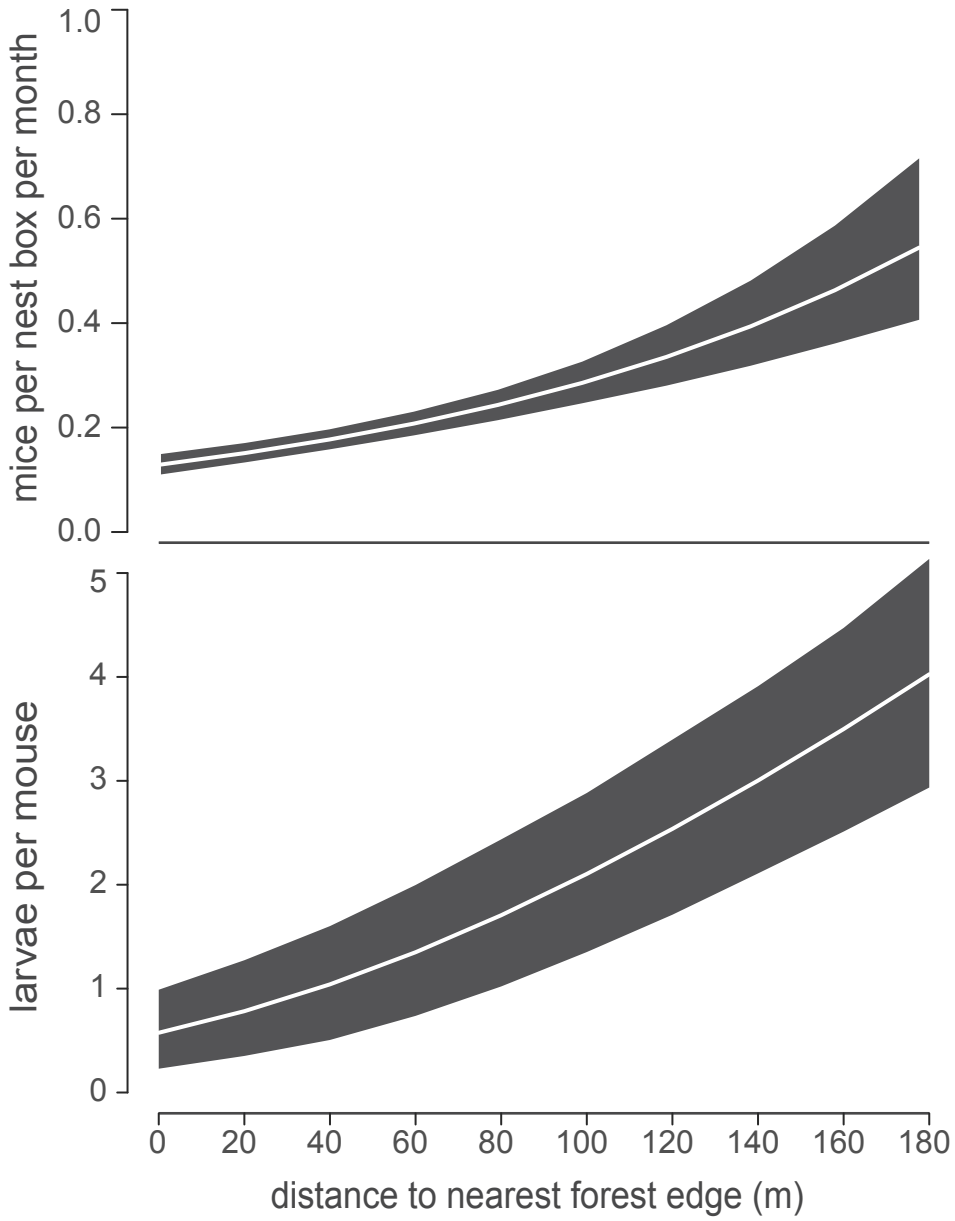

Figure 8 Response of summer mouse abundance and larval tick burdens on summer mice to distance to forest edge. Mean (white line) and 89% posterior distribution (shaded region) shown.

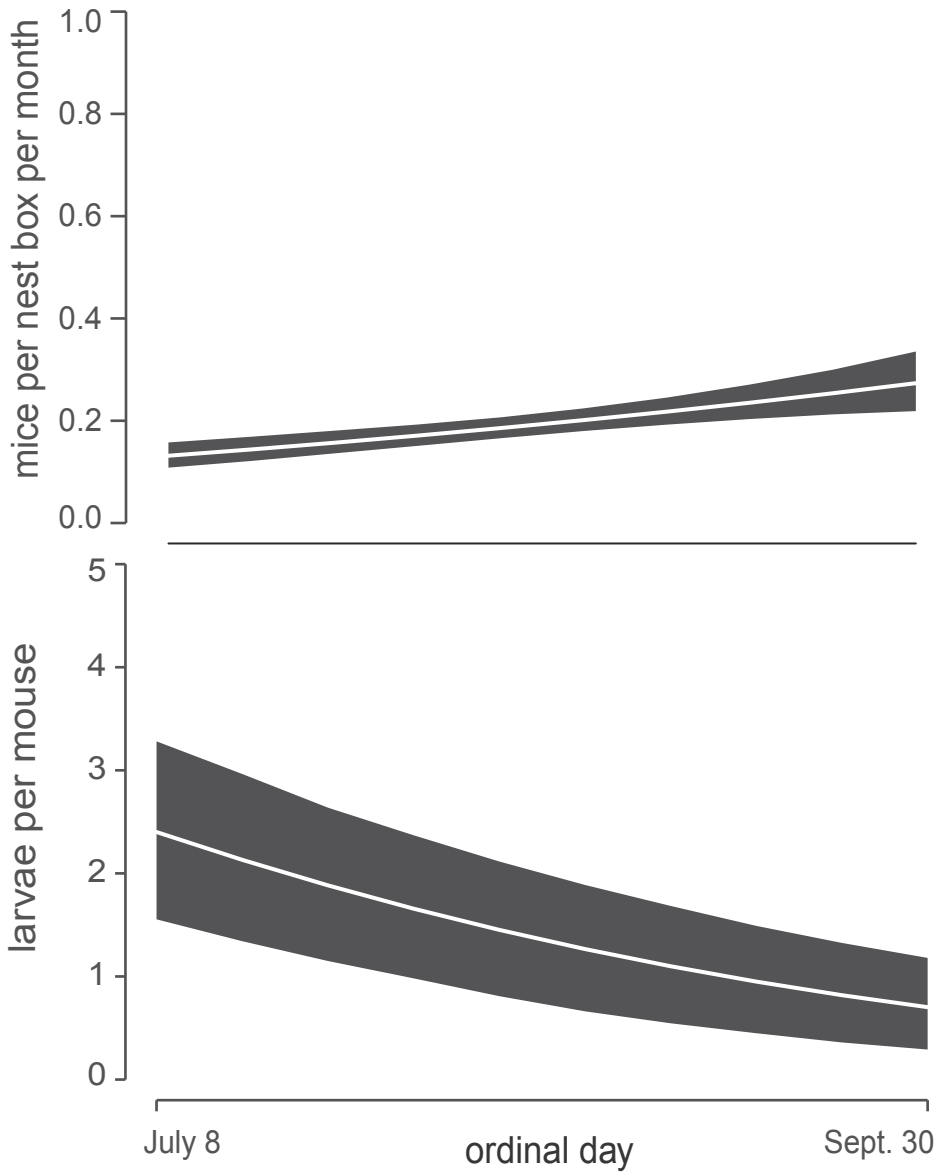

Figure 9 Response of summer mouse abundance and larval tick burdens on summer mice to ordinal day. Mean (white line) and 89% posterior distribution (shaded region) shown.

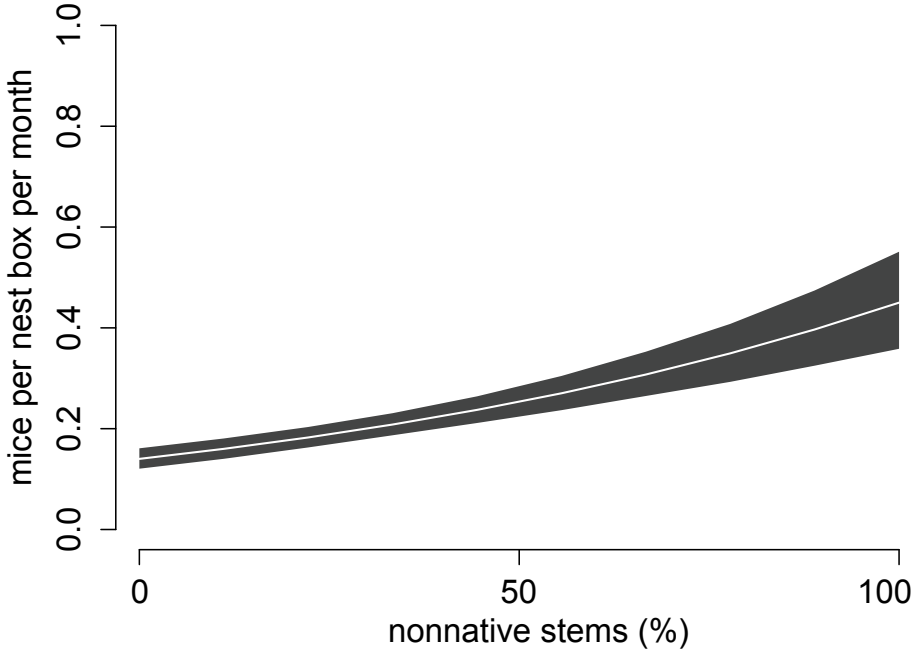

Figure 10 Response of summer *P. leucopus* abundance to percentage of nonnative stems surrounding the nest box. Mean (white line) and 89% posterior distribution (shaded region) shown.

REFERENCES

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

Adler, G. H., S. R. Telford, M. L. Wilson, and A. Spielman. 1992. Vegetation structure influences the burden of immature Ixodes dammini on its main host, Peromyscus leucopus. Parasitology 105:105–110.

Adler, G. H., and M. L. Wilson. 1987. Demography of a Habitat Generalist, The White-Footed Mouse, in a Heterogeneous Environment. Ecology 68:1785.

Allan, B. F., H. P. Dutra, L. S. Goessling, K. Barnett, J. M. Chase, R. J. Marquis, G. Pang, G. A. Storch, R. E. Thach, and J. L. Orrock. 2010. Invasive honeysuckle eradication reduces tick-borne disease risk by altering host dynamics. Proceedings of the National Academy of Sciences 107:18523–18527.

Anderson, C. S., A. B. Cady, and D. B. Meikle. 2003. Effects of vegetation structure and edge habitat on the density and distribution of white-footed mice (Peromyscus leucopus) in small and large forest patches. Canadian Journal of Zoology 81:897–904.

Barko, V. A., G. A. Feldhamer, M. C. Nicholson, and D. K. Davie. 2003. Urban habitat: a determinant of white-footed mouse (*Peromyscus leucopus*) abundance in southern Illinois. Southeastern Naturalist 2:369–376.

Bertrand, M. R., and M. L. Wilson. 1997. Microhabitat-independent regional differences in survival of unfed Ixodes scapularis nymphs (Acari: Ixodidae) in Connecticut. Journal of medical entomology 34:167–172.

Bouchard, C., G. Beauchamp, S. Nguon, L. Trudel, F. Milord, L. R. Lindsay, D. Bélanger, and N. H. Ogden. 2011. Associations between Ixodes scapularis ticks and small mammal hosts in a newly endemic zone in southeastern Canada: Implications for Borrelia burgdorferi transmission. Ticks and Tick-borne Diseases 2:183–190.

Brisson, D., and D. E. Dykhuizen. 2004. ospC Diversity in Borrelia burgdorferi: Different Hosts Are Different Niches. Genetics 168:713–722.

Brunner, J. L., and R. S. Ostfeld. 2008. Multiple causes of variable tick burdens on small-mammal hosts. Ecology 89:2259–2272.

Burtis, J. C., T. J. Fahey, and J. B. Yavitt. 2014. Impact of invasive earthworms on Ixodes scapularis and other litter-dwelling arthropods in hardwood forests, central New York state, USA. Applied Soil Ecology 84:148–157.

Calabrese, J. M., J. L. Brunner, and R. S. Ostfeld. 2011. Partitioning the Aggregation of Parasites on Hosts into Intrinsic and Extrinsic Components via an Extended Poisson-Gamma Mixture Model. PLoS ONE 6:e29215.

Carey, A. B., W. L. Krinsky, and A. J. Main. 1980. Ixodes dammini (Acari: Ixodidae) and associated ixodid ticks in south-central Connecticut, USA. Journal of Medical Entomology 17:89–99.

Carroll, J. F. 2003. Survival of larvae and nymphs of Ixodes scapularis Say (Acari: Ixodidae) in four habitats in Maryland. Proceedings of the Entomological Society of Washington 105:120–126.

Cummings, J. R., and S. H. Vessey. 1994. Agricultural Influences on Movement Patterns of White-Footed Mice (Peromyscus leucopus). American Midland Naturalist 132:209.

Daniels, T. J., D. Fish, and R. C. Falco. 1989. Seasonal activity and survival of adult Ixodes dammini (Acari: Ixodidae) in southern New York State. Journal of medical entomology 26:610–614.

Devevey, G., and D. Brisson. 2012. The effect of spatial heterogenity on the aggregation of ticks on white-footed mice. Parasitology 139:915–925.

Donahue, J. G., J. Piesman, and A. Spielman. 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes. The American Journal of Tropical Medicine and Hygeine 36:92–96.

Drickamer, L. C. 1990. Microhabitat preferences of two species of deermice Peromyscus in a northeastern United States deciduous hardwood forest. Acta Theriologica 35:241–252.

Durden, L. A., and J. E. Keirans. 1996. Nymphs of the Genus Ixodes (Acari: Ixodidae) of the United States: Taxonomy, Identification Key, Distribution, Hosts, and Medical/Veterinary Importance. Thomas Say Publications in Entomology: Monographs:1–50.

Dutra, H. P., K. Barnett, J. R. Reinhardt, R. J. Marquis, and J. L. Orrock. 2011. Invasive plant species alters consumer behavior by providing refuge from predation. Oecologia 166:649–657.

Eagan, T. S., J. C. Beasley, Z. H. Olson, and O. E. Rhodes. 2011. Impacts of generalist mesopredators on the demography of small-mammal populations in fragmented landscapes. Canadian Journal of Zoology 89:724–731.

Ehrenfeld, J. G. 1997. Invasion of Deciduous Forest Preserves in the New York Metropolitan Region by Japanese Barberry (Berberis thunbergii DC.). Journal of the Torrey Botanical Society 124:210.

Elias, S. P., C. B. Lubelczyk, P. W. Rand, E. H. Lacombe, M. S. Holman, and R. P. Smith. 2006. Deer Browse Resistant Exotic-Invasive Understory: An Indicator of Elevated Human Risk of Exposure to <I>Ixodes scapularis</I> (Acari: Ixodidae) in Southern Coastal Maine Woodlands. Journal of Medical Entomology 43:1142–1152.

ESRI. 2012. ArcMap v 10.1. ESRI, Redlands, CA.

Fahrig, L., and G. Merriam. 1985. Habitat Patch Connectivity and Population Survival. Ecology 66:1762–1768.

Fahrig, L., and T. Rytwinski. 2009. Effects of roads on animal abundance: an empirical review and synthesis. Ecology and society 14:21.

Garland, T., and W. G. Bradley. 1984. Effects of a Highway on Mojave Desert Rodent Populations. American Midland Naturalist 111:47.

Gelman, A., J. Hwang, and A. Vehtari. 2014. Understanding predictive information criteria for Bayesian models. Statistics and Computing 24:997–1016.

Goundie, T. R., and S. H. Vessey. 1986. Survival and Dispersal of Young White-Footed Mice Born in Nest Boxes. Journal of Mammalogy 67:53–60.

Guo, J., D. Lee, K. Sakrejda, J. Gabry, B. Goodrich, J. de Guzman, E. Niebler, T. Heller, and J. Fletcher. 2016. rstan: R Interface to Stan.

Horobik, V., F. Keesing, and R. S. Ostfeld. 2007. Abundance and Borrelia burgdorferi-infection Prevalence of Nymphal Ixodes scapularis Ticks along Forest–Field Edges. EcoHealth 3:262–268.

Kaufman, D. W., S. K. Peterson, R. Fristik, and G. A. Kaufman. 1983. Effect of Microhabitat Features on Habitat Use by Peromyscus leucopus. American Midland Naturalist 110:177.

Keirans, J. E., and L. A. Durden. 1998. Illustrated Key to Nymphs of the Tick Genus Amblyomma (Acari: Ixodidae) Found in the United States. Journal of Medical Entomology 35:489–495.

Keirans, J. E., and T. R. Litwak. 1989. Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi River. Journal of Medical Entomology 26:435–448.

Kiffner, C., T. Vor, P. Hagedorn, M. Niedrig, and F. Rühe. 2011. Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany. Parasitology Research 108:323–335.

Kim, H. H. 1992. Urban heat island. International Journal of Remote Sensing 13.

Kingston, S. R., and D. W. Morris. 2000. Voles looking for an edge: habitat selection across forest ecotones. Canadian Journal of Zoology 78:2174–2183.

Klein, G. P., and G. N. Cameron. 2012. Effect of habitat gradients on space use by white-footed mice (Peromyscus leucopus). Journal of Mammalogy 93:706–715.

Kostel-Hughes, F., T. P. Young, and M. M. Carreiro. 1998. Forest leaf litter quantity and seedling occurrence along an urban-rural gradient. Urban Ecosystems 2:263–278.

Kowarik, I. 2008. On the role of alien species in urban flora and vegetation. Pages 321–338*in* P. Pysek, K. Prach, M. Rejmanek, and P. M. Wade, editors.Plant invasions - General aspects and special problems. Springer, Amsterdam, Netherlands.

Kozel, R. M., and E. D. Fleharty. 1979. Movements of Rodents across Roads. The Southwestern Naturalist 24:239.

Krohne, D. T., and G. A. Hoch. 1999. Demography of Peromyscus leucopus populations in habitat patches: the role of dispersal. Canadian Journal of Zoology 77:1247–1253.

Lane, R. S., J. Piesman, and W. Burgdorfer. 1991. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annual review of entomology 36:587–609.

Levine, J. F., M. L. Wilson, and A. Spielman. 1985. Mice as reservoirs of the Lyme disease spirochete. The American Journal of Tropical Medicine and Hygeine 34:355–360.

Lewellen, R. H., and S. H. Vessey. 1999. Estimating Densities of Peromyscus leucopus Using Live-Trap and Nestbox Censuses. Journal of Mammalogy 80:400–409.

Lidicker Jr, W. Z. 1999. Responses of mammals to habitat edges: an overview. Landscape Ecology 14:333–343.

Lindsay, L. R., I. K. Barker, G. A. Surgeoner, S. A. McEwen, T. J. Gillespie, and J. T. Robinson. 1995. Survival and development of Ixodes scapularis (Acari: Ixodidae) under various climatic conditions in Ontario, Canada. Journal of Medical Entomology 32:143–152.

Lindsay, L. R., S. W. Mathison, I. K. Barker, S. A. McEwen, T. J. Gillespie, and G. A. Surgeoner. 1999. Microclimate and habitat in relation to Ixodes scapularis (Acari: Ixodidae) populations on Long Point, Ontario, Canada. Journal of Medical Entomology 36:255–262.

LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences 100:567–571.

Lubelczyk, C. B., S. P. Elias, P. W. Rand, M. S. Holman, E. H. Lacombe, and R. P. Smith. 2004. Habitat Associations of <I>Ixodes scapularis</I> (Acari: Ixodidae) in Maine. Environmental Entomology 33:900–906.

Lyon, S. M., J. D. Edman, and R. G. Van Driesche. 1996. Field estimates of numbers of Ixodes scapularis (Acari: Ixodidae) larvae and nymphs per hectare successfully feeding on Peromyscus leucopus in Massachusetts. Journal of Medical Entomology 33:812–818.

Mader, H.-J. 1984. Animal habitat isolation by roads and agricultural fields. Biological Conservation 29:81–96.

Malo, A. F., B. Godsall, C. Prebble, Z. Grange, S. McCandless, A. Taylor, and T. Coulson. 2013. Positive effects of an invasive shrub on aggregation and abundance of a native small rodent. Behavioral Ecology 24:759–767.

Markowski, D., K. E. Hyland, H. S. Ginsberg, and R. Hu. 1997. Spatial Distribution of Larval Ixodes scapularis (Acari: Ixodidae) on Peromyscus leucopus and Microtus pennsylvanicus at Two Island Sites. The Journal of Parasitology 83:207.

Mather, T. N., M. L. Wilson, S. I. Moore, J. M. C. Ribeiro, and A. Spielman. 1989. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (Borrelia burgdorferi). American Journal of Epidemiology 130:143–150.

Mattos, K. J., and J. L. Orrock. 2010. Behavioral consequences of plant invasion: an invasive plant alters rodent antipredator behavior. Behavioral Ecology 21:556–561.

McElreath, R. 2016a. Statistical Rethinking. First edition. CRC Press.

McElreath, R. 2016b. Rethinking: An R package for fitting and manipulating Bayesian models.

McLaren, A. A. D., L. Fahrig, and N. Waltho. 2011. Movement of small mammals across divided highways with vegetated medians. Canadian Journal of Zoology 89:1214–1222.

M'Closkey, R. T., and D. T. Lajoie. 1975. Determinants of Local Distribution and Abundance in White-Footed Mice. Ecology 56:467–472.

Merriam, G., M. Kozakiewicz, E. Tsuchiya, and K. Hawley. 1989. Barriers as boundaries for metapopulations and demes of Peromyscus leucopus in farm landscapes. Landscape Ecology 2:227–235.

Merriam, G., and J. Wegner. 1992. Local extinctions, habitat fragmentation, and ecotones. Pages 150–159*in* A. Hansen and F. di Castri, editors.Landscape boundaries: consequences for biotic diversity and ecological flows. Springer-Verlag, New York, N.Y.

Monello, R. J., and M. E. Gompper. 2010. Differential effects of experimental increases in sociality on ectoparasites of free-ranging raccoons. Journal of Animal Ecology 79:602–609.

Morris, D. W., and D. L. Davidson. 2000. Optimally foraging mice match patch use with habitat differences in fitness. Ecology 81:2061–2066.

Myton, B. 1974. Utilization of Space by Peromyscus Leucopus and Other Small Mammals. Ecology 55:277.

Nudds, T. D. 1977. Quantifying the Vegetative Structure of Wildlife Cover. Wildlife Society Bulletin 5:113–117.

Nupp, T. E., and R. K. Swihart. 1996. Effect of forest patch area on population attributes of white-footed mice (Peromyscus leucopus) in fragmented landscapes. Canadian Journal of Zoology 74:467–472.

Nuzzo, V. A., J. C. Maerz, and B. Blossey. 2009. Earthworm Invasion as the Driving Force Behind Plant Invasion and Community Change in Northeastern North American Forests. Conservation Biology 23:966–974.

Ostfeld, R. S., and F. Keesing. 2000. Biodiversity series: the function of biodiversity in the ecology of vector-borne zoonotic diseases. Canadian Journal of Zoology 78:2061–2078.

Ostfeld, R. S., M. C. Miller, and K. R. Hazler. 1996. Causes and Consequences of Tick (Ixodes scapularis) Burdens on White-Footed Mice (Peromyscus leucopus). Journal of Mammalogy 77:266–273.

Oxley, D. J., M. B. Fenton, and G. R. Carmody. 1974. The Effects of Roads on Populations of Small Mammals. The Journal of Applied Ecology 11:51.

Perkins, S. E., I. M. Cattadori, V. Tagliapietra, A. P. Rizzoli, and P. J. Hudson. 2003. Empirical evidence for key hosts in persistence of a tick-borne disease. International Journal for Parasitology 33:909–917.

Pouyat, R. V., M. J. McDonnell, and S. T. Pickett. 1997. Litter decomposition and nitrogen mineralization in oak stands along an urban-rural land use gradient. Urban Ecosystems 1:117–131.

Prusinski, M. A., H. Chen, J. M. Drobnack, S. J. Kogut, R. G. Means, J. J. Howard, J. Oliver, G. Lukacik, P. B. Backenson, and D. J. White. 2006. Habitat Structure Associated with <I>Borrelia burgdorferi</I> Prevalence in Small Mammals in New York State. Environmental Entomology 35:308–319.

R Development Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Randolph, S. E., and K. Storey. 1999. Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. Journal of Medical Entomology 36:741–748.

Rega, C. 2012. Impacts of soil calcium availability and non-native plant invasions on an urban forest bird community. University of Delaware.

Rodgers, S. E., C. P. Zolnik, and T. N. Mather. 2007. Duration of exposure to suboptimal atmospheric moisture affects nymphal blacklegged tick survival. Journal of medical entomology 44:372–375.

Rytwinski, T., and L. Fahrig. 2007. Effect of road density on abundance of white-footed mice. Landscape Ecology 22:1501–1512.

Schmidt, C. A. 1999. Variation and Congruence of Microsatellite Markers for Peromyscus leucopus. Journal of Mammalogy 80:522–529.

Schmidt, K. A., and R. S. Ostfeld. 2001. Biodiversity and the Dilution Effect in Disease Ecology. Ecology 82:609.

Schulze, T. L., R. A. Jordan, and R. W. Hung. 1995. Suppression of Subadult Ixodes scapularis (Acari: Ixodidae) Following Removal of Leaf Litter. Journal of medical entomology 32:730–3.

Seagle, S. W. 1985. Patterns of Small Mammal Microhabitat Utilization in Cedar Glade and Deciduous Forest Habitats. Journal of Mammalogy 66:22–35.

Seamon, J. O., and G. H. Adler. 1996. Population performance of generalist and specialist rodents along habitat gradients. Canadian Journal of Zoology 74:1130–1139.

Shaw, M. T., F. Keesing, R. McGrail, and R. S. Ostfeld. 2003. Factors influencing the distribution of larval blacklegged ticks on rodent hosts. The American journal of tropical medicine and hygiene 68:447–452.

Stafford, K. C. 1994. Survival of immature Ixodes scapularis (Acari: Ixodidae) at different relative humidities. Journal of medical entomology 31:310–314.

State of Delaware. 2008, May 19. 2007 Delaware Land Use and Land Cover. State of Delaware, Office of Management and Budget, Delaware Geographic Data Committee, Dover, Delaware.

Swihart, R. K., and N. A. Slade. 1984. Road Crossing in Sigmodon hispidus and Microtus ochrogaster. Journal of Mammalogy 65:357–360.

Taylor, D. G. 1978. The population biology of white-footed mice in an isolated and a non-isolated woodlot in southeastern Ontario. Carleton University.

Telford, S. R., J. E. Dawson, P. Katavolos, C. K. Warner, C. P. Kolbert, and D. H. Persing. 1996. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. Proceedings of the National Academy of Sciences 93:6209–6214.

Telford, S. R., and A. Spielman. 1993. Reservoir competence of white-footed mice for Babesia microti. Journal of Medical Entomology 30.

Trentanovi, G., M. von der Lippe, T. Sitzia, U. Ziechmann, I. Kowarik, and A. Cierjacks. 2013. Biotic homogenization at the community scale: disentangling the roles of urbanization and plant invasion. Diversity and Distributions 19:738–748.

Vidra, R. L., T. H. Shear, and J. M. Stucky. 2007. Effects of vegetation removal on native understory recovery in an exotic-rich urban forest [1]. The Journal of the Torrey Botanical Society 134:410–419.

Watanabe, S. 2010. Asymptotic equivalence of Bayes cross validation and widely applicable information criterion in singular learning theory. The Journal of Machine Learning Research 11:3571–3594.

Wegner, J., and G. Merriam. 1990. Use of spatial elements in a farmland mosaic by a woodland rodent. Biological Conservation 54:263–276.

Whitaker, J. O. 1967. Habitat Relationships of Four Species of Mice in Vigo County, Indiana. Ecology 48:867–872.

Wilder, S. M., A. M. Abtahi, and D. B. Meikle. 2005. The effects of forest fragmentation on densities of white-footed mice (Peromyscus leucopus) during the winter. The American midland naturalist 153:71–79.

Wilder, S. M., and D. B. Meikle. 2006. Variation in the effects of fragmentation on the white-footed mouse (Peromyscus leucopus) during the breeding season. Journal of Mammalogy 87:117–123.

Williams, S. C., and J. S. Ward. 2010. Effects of Japanese Barberry (Ranunculales: Berberidaceae) Removal and Resulting Microclimatic Changes on Ixodes scapularis (Acari: Ixodidae) Abundances in Connecticut, USA. Environmental Entomology 39:1911–1921.

Williams, S. C., J. S. Ward, T. E. Worthley, and K. C. Stafford. 2009. Managing Japanese Barberry (Ranunculales: Berberidaceae) Infestations Reduces Blacklegged Tick (Acari: Ixodidae) Abundance and Infection Prevalence With <I>Borrelia burgdorferi</I> (Spirochaetales: Spirochaetaceae). Environmental Entomology 38:977–984.

Wolf, M., and G. Batzli. 2004. Forest edge-high or low quality habitat for white-footed mice (Peromyscus leucopus)? Ecology 85:756–769.

Wolf, M., and G. O. Batzli. 2002. Effects of forest edge on populations of white-footed mice Peromyscus leucopus. Ecography 25:193–199.

Wolff, J. O. 1985. The effects of density, food, and interspecific inference on home range size in Peromyscus leucopus and Peromyscus maniculatus. Canadian Journal of Zoology 63:2657–2662.

Wolff, J. O., and D. S. Durr. 1986. Winter Nesting Behavior of Peromyscus leucopus and Peromyscus maniculatus. Journal of Mammalogy 67:409–412.

Yahner, R. H. 1988. Changes in wildlife communities near edges. Conservation biology 2:333–339.

Chapter 4

ANTHROPOGENIC HAZARDS IN URBAN LANDSCAPES REDUCE JUVENILE SONGBIRD SURVIVAL AFTER INDEPENDENCE FROM PARENTAL CARE

Introduction

Urbanization is increasing globally; from 1950–2014 the world's urban population increased from 746 million to 3.9 billion people (United Nations 2014). The effects of urbanization on bird populations occur through changes in habitat quality, increases in predator densities, introductions of invasive species, exposure to pollutants and pathogens, or other novel threats such as collisions with windows or cars (Rodewald and Gehrt 2014, Loss et al. 2014). For forest-dwelling birds, urbanization reduces overall habitat area and creates habitat fragments, which affects survival during the post-fledging stage. In urban environments, fledglings from nests closer to forest edges have greater mortality risk than fledglings from nests farther from the edge (Shipley et al. 2013). Fledgling predation risk varies with proximity to urban land cover, depending on the species and timing during the post-fledging stage (Ausprey and Rodewald 2011). Proximity to urban cover and matrix habitat use (Whittaker and Marzluff 2009) may also determine risks from collisions with human structures and vehicles. Although, forest birds are generally hesitant to cross habitat gaps (Bélisle et al. 2001), in urban landscapes where habitat patches are small and isolated, fledglings may have no other dispersal options. Dispersal in urban

environments requires crossing roads, and juvenile/naïve birds may be more vulnerable than adults to road hazards (Mumme et al. 2000).

With ~58% of migratory bird species in decline across North America (Sauer et al. 2013), effective conservation measures require an understanding of population-limiting processes affecting all aspects of the annual cycle for all life stages (Marra et al. 2015). Annual breeding productivity of migratory passerine birds is best defined as the number of offspring that survive until recruitment into the breeding population the following year as adults. However, estimates of annual productivity often rely solely on evidence of nesting success (Anders and Marshall 2005, Streby et al. 2014a). Other life stages of juvenile birds, including the period of time between leaving the nest until initiating first migration (hereafter referred to as the "post-fledging stage"), are poorly understood (Cox et al. 2014). Mortality may be high during the post-fledging stage, potentially exhibiting evolutionary tradeoffs with nestling survival or limiting population growth (Streby et al. 2014a, b; Cox et al. 2014). Post-fledging survival may vary significantly among species and populations, but only a few studies have measured post-fledging survival of passerines in urban landscapes (Whittaker and Marzluff 2009, Ausprey and Rodewald 2011, Balogh et al. 2011, Shipley et al. 2013) or other human-dominated environments (Jackson et al. 2011).

Urbanization has differential effects on bird species depending on life history traits and habitat requirements. For example, we consider two migratory passerine species of eastern deciduous forest, Gray Catbird (*Dumetella carolinensis*) and Wood Thrush (*Hylocichla mustelina*); the Gray Catbird is considered synanthropic, while the Wood Thrush is considered an urban-avoider. Gray Catbirds readily nest in small forest fragments and in backyard shrubs within the urban/suburban matrix (Ryder et

al. 2010, Balogh et al. 2011, Evans et al. 2015) while Wood Thrushes nest exclusively in forest habitat (James et al. 1984, Robbins et al. 1989). In the mid-Atlantic U.S., Gray Catbird populations are increasing while Wood Thrush populations have declined 2.33% annually since 1966 (Sauer et al. 2015). Loss of breeding habitat and climate change are limiting Wood Thrush populations (Rushing et al. 2016), and additional hazards in urban landscapes during the post-fledging period may further reduce annual productivity.

Given the importance of the post-fledging period in the overall annual population growth rate (Cox et al. 2014, Streby et al. 2014a), we sought to contribute to our understanding of this period for urban birds using Gray Catbird and Wood Thrush as models for synanthropic and urban-avoider species, respectively. Specifically, we estimated Gray Catbird and Wood Thrush post-fledging survival rates, and modeled factors that influence fledgling mortality risk. We predicted that the urban-avoider would have a higher mortality rate than the synanthropic species if the post-fledging period were more limiting in these populations. We also predicted that increased movement and crossing habitat gaps would increase mortality risk for both species.

Methods

Study Area

We used VHF radio transmitters to track catbird and wood thrush fledglings in 13 urban and suburban forest fragments around Newark, Delaware, and Landenberg, Pennsylvania, USA. Forest fragments consisted of mixed deciduous hardwood stands and ranged in size from 5 to 163 ha. Common canopy tree species included *Fagus*

grandifolia, *Acer rubrum*, *Quercus spp.*, *Liriodendron tulipifera*, and *Liquidambar styraciflua*. A mix of native and nonnative woody species such as *Lindera benzoin*, *Viburnum spp*., *Clethra alnifolia*, *Rosa multiflora*, *Eleagnus umbellata*, and *Rubus spp.* comprised forest fragment understories. Detailed descriptions of forest fragments and their breeding bird communities can be found in Rega (2012).

Transmitter Attachment

From May–August, 2012–2014, we searched for and monitored Gray Catbird and Wood Thrush nests in 23 forest fragments, of which 13 fragments produced fledglings that we were able to radio-tag. We checked nests at 3-4 day intervals (Martin and Geupel 1993), and we banded and radio-tagged birds when nestlings were between 1-3 days before fledging: day 7-9 for Gray Catbirds and day 9-11 for Wood Thrushes (Evans et al. 2011, Smith et al. 2011). We radio-tagged 1-2 nestlings selected randomly per nest from all nests in which nestlings were available. We attached <1 g VHF radio-transmitters that ranged from ~150–151 MHz (Blackburn transmitters, Nacogdoches, Texas) using 1 mm elastic thread in a figure 8 loop harness (Rappole and Tipton 1991). Nestlings were replaced in nests after tagging. All handling, marking, and tagging procedures were carried out in accordance with University of Delaware Institutional Animal Care and Use and Committee approval under protocol #1129 and USFWS permit #23475 to W.G. Shriver.

Tracking Data

We used programmable receivers and hand-held three-element Yagi antennas (Advanced Telemetry Systems, Isanti, MN) to locate all individuals once a day, seven days per week until one of three outcomes occurred: mortality, transmitter battery

failure, or dispersal from study area. Each time we located a bird, we recorded the precise location (latitude, longitude). We defined mortalities when a transmitter was recovered either with part or all of a carcass, blood, or broken feathers. When a bird was not in range of its last known location, we searched for it using an omnidirectional antenna mounted on the roof of a vehicle. We drove a progressively wider search grid starting with the bird's last known location. We continued to search for all missing birds each day until five days beyond the estimated date of transmitter battery failure.

We collected sets of time-independent and time-dependent variables that we expected may impact survival probability. Time-independent covariates were specific to each individual bird and remained static over the study duration: species, year, ordinal day when fledged, and nest distance to forest edge (m). Time-dependent covariates changed with each bird's daily movements and habitat use: daily movement distance between one day's location and the previous one (m), displacement distance from the nest (m), cumulative number of roads crossed, distance to urban land use (m), and a binary variable to represent whether the bird was in a forest each day. We used ArcGIS (ESRI 2012) and Geospatial Modelling Environment (Beyer 2012) to quantify time-dependent movement and habitat variables based on daily locations (Table 7).

Analysis

Because we sometimes tagged two individuals from the same nest, we tested whether siblings could be treated as independent samples. We used an exact binomial test for goodness of fit to determine whether fledglings from the same nest were more likely to have the same fate than randomly paired birds. To assess survival, we first fit Kaplan-Meier curves to calculate species-specific cumulative survival rates using the 'survival' package (Therneau 2015) in program R (R Development Core Team 2014).

We then used Cox's proportional hazard models to assess which time-independent and time-dependent covariates had the greatest impact on mortality risk. We performed model selection in a two step process using Akaike's Information Criterion (Burnham and Anderson 2002). First, we compared models with only time-independent covariates. Then we used the variable(s) from the best time-independent model in all subsequent models with time-dependent covariates.

Finally, because we were interested in urban effects on post-fledging survival, we also implemented models with competing risks under the Cox's proportional hazard framework. We compared the relative risk of the most influential covariates on two possible outcomes: anthropogenic mortality, defined as mortalities directly related to human activities (included car, window, and building strikes), and non-anthropogenic mortality (i.e., predation or drowning). We recognize that predator communities, for example, may be artificially high in urban areas (Prange et al. 2004); however, as a relatively indirect effect, we treated all predation mortalities as non-anthropogenic.

Results

From 2012–2014, we searched for and monitored nests in 23 forest fragments and radio-tagged 158 fledglings, 83 catbirds and 75 wood thrushes from 93 nests in 13 fragments. Based on transmitter battery life constraints and duration of time spent in the study area, we tracked surviving birds from 45 to 85 days, yielding ~4,500 total locations for all individuals. We reduced the dataset to 55 days to limit the number of right-censored individuals due to battery failure. To satisfy assumptions of known-fate models, we removed individuals from the dataset for which we could not be certain of outcomes. For example, if we recovered a transmitter but could not be certain whether

a bird was depredated or slipped out of its harness we removed these records (n = 31 individuals) from the dataset. Our analyses were based on 127 fledglings: 60 Gray Catbirds and 67 Wood Thrushes (3,583 total locations over 55 days). Sibling fates were not more likely to be similar than those of randomly paired birds (catbirds: P = 0.40; wood thrush: P = 0.20); therefore, we treated all 127 birds as independent individuals. We observed 64 mortalities: 40 Gray Catbirds and 24 Wood Thrushes. The majority of mortalities were due to predation (86%, 55 fledglings). We also classified 6 mortalities as anthropogenic, caused by window/building strikes (3), cars (2), and an open pipe (1) in the forest in which one of the birds became trapped. Three birds apparently drowned due to flash flooding. The overall majority of mortalities happened within fledglings' earliest days out of the nest; fifty percent of mortalities occurred on days 1 and 2 post-fledging, and 75% of mortalities occurred on day 9 or earlier.

The cumulative survival rate for Gray Catbirds (0.32, 95% CI: 0.22–0.47) was half that of Wood Thrushes (0.63, 95% CI: 0.52–0.75) (Figure 11). Among time-independent variables in Cox models, the categorical species variable had the strongest effect, with catbirds experiencing nearly twice the mortality risk compared to wood thrushes (Table 8). We did not detect an effect of timing (ordinal day when fledged) or year on mortality risk. In a univariate model, nest distance to edge was significant, but its effect was reduced when combined with species in a multivariate model. Nest distance to edge was a bimodally distributed variable, driven by species – catbirds typically nested closer to the edge than wood thrush. Because of this potential confound and the fact that the univariate species model performed similarly to the

model with species and nest distance to edge (Table 8), subsequent time-dependent models included species as the only time-independent variable.

Our best time-dependent model included species, step length, and road crossings (Table 9). Relative mortality risk increased by 41% (95% CI: 16–69%) with an increase of ~2.5 road crossings (Figure 12), but decreased by 33% (10–50%) with a 200 m increase in daily movement distance. We estimated 337 total road crossings over all individuals of both species, ranging from 0 to 31 and 0 to 16 for individual Gray Catbirds and Wood Thrushes, respectively. Urban land use was the only habitat variable that influenced mortality, suggesting that mortality risk declined as distance to urban land use increased.

All anthropogenic mortalities occurred after birds reached independence, >20 days post-fledging (Evans et al. 2011, Smith et al. 2011), while the majority of non-anthropogenic mortalities occurred before independence (Figure 13). Competing risks analysis indicated that road crossings increased the risk of anthropogenic mortality relative to non-anthropogenic mortality by 59%. For natural mortalities, daily movement distance reduced the risk of non-anthropogenic mortality by 27% with increasing daily movement distance (Table 10).

Discussion

Contrary to our predictions about synanthropic vs. urban-avoider species, catbird post-fledging survival was half that of Wood Thrushes in our urbanized landscape. Mortality risk for both species increased with the number of road crossings, but decreased with longer daily movements. While the majority of mortality events were due to predation, nearly ten percent of all mortalities resulted from direct human impacts after fledglings reached independence from parental care. Our results

demonstrate the hazards faced by fledgling birds in built-up environments and the need to estimate species-specific survival rates in urban landscapes.

Despite being considered a synanthropic species (Evans et al. 2015), the catbird fledglings in our study had a lower cumulative survival probability compared to Wood Thrushes. We suspect that the 31% discrepancy in post-fledging survival rates between the two species results from life history differences. Ausprey and Rodewald (2011) found a similar pattern when comparing fledgling northern cardinals (*Cardinalis cardinalis*) and Acadian flycatchers (*Empidonax virescens*), urban-adapters and urban-avoiders, respectively. Wood Thrushes, like Acadian Flycatchers, are older and more developed when they fledge compared to catbirds and cardinals. This developmental difference may make Wood Thrushes better able to avoid predators immediately after fledging; on a broad scale across species, nestling period duration is positively associated with post-fledging survival (Cox et al. 2014, Lloyd and Martin 2015). To better understand limiting factors for these types of species in urban environments, future work should consider post-fledging survival rates within the context of overall breeding season productivity and annual survival and recruitment rates.

Compared to prior studies of catbird post-fledging survival (Maxted 2001, Balogh et al. 2011), catbirds in our study generally had a lower cumulative survival rate. In contrast, Wood Thrush fledglings in our study had a higher cumulative survival rate than previous wood thrush post-fledging survival studies elsewhere (Anders et al. 1997, Fink 2003, Schmidt et al. 2008). Catbirds in our study had lower cumulative survival than catbird fledglings tracked over 12 weeks (0.58) in shrubland habitat in Indiana (Maxted 2001). However, we observed comparable catbird fledgling

survival to that in urban sites around Washington D.C. (Balogh et al. 2001); see further discussion below. During the parental-dependence period, wood thrush survival in our study was slightly higher than that of wood thrush fledglings in a 325 ha forest in Dutchess County, NY (mean cumulative survival = 0.65, Schmidt et al. 2008), but lower than observed in Missouri forests (0.83, Fink 2003). Wood Thrushes in our study also had higher cumulative survival over ~8 weeks than wood thrush fledglings in contiguous forested habitat in the Missouri Ozarks (0.42, Anders et al. 1997).

Among other time-independent variables we tested, nest distance to forest edge was the only other factor that had a marginal influence on mortality risk. Its effect could not be separated from species-specific differences, however, as catbirds tended to nest closer to the forest edges. Species also appeared to be driving this relationship because of the independence among fates of birds from the same nests. Although there is support for developmental differences driving variation in survival between species (Cox et al. 2014, Lloyd and Martin 2015), we cannot rule out negative edge-effects on catbird post-fledging survival. Shipley et al. (2010) found that spotted towhee (*Pipilo maculatus*) nests closer to the forest edge tended to be more successful, but that fledglings near the edge were more likely to be depredated. Among golden-winged warblers (*Vermivora chrysoptera*), Streby et al. (2014b) identified tradeoffs in survival for nestling and post-fledging periods with respect to nest placement distance from forest edge. Future work should incorporate nest data to examine potential tradeoffs for Gray Catbirds' and Wood Thrushes' stage-specific survival related to nest distance to edge.

Anthropogenic Mortality

Our data raise the question of whether anthropogenic mortality is additive or compensatory to non-anthropogenic mortality rates. We observed 6 mortalities that were a direct result of anthropogenic hazards in built-up urban environments: nearly 10% of the mortality in our study. Since in all cases anthropogenic mortalities occurred after fledglings reached independence, when fledglings are expected to be at lower risk of mortality (Ricklefs 1968, Cox et al. 2014, Anders et al. 1997, Berkeley et al. 2007), this highlights the value of monitoring fledglings beyond the dependence period.

We found that causes of mortality after juveniles become independent from parental care can differ from those while they are dependent on their parents. Thus, our general understanding of juvenile mortality in birds, especially after independence from parents, may be biased since many post-fledging studies do not follow birds beyond ~20–30 days (reviewed in Cox et al. 2014). The novel threats imposed by urbanized landscapes may be underestimated if we do not have adequate survival estimates for is a very vulnerable period after independence from parental care but before dispersal and migration. Juvenile and naïve birds are more vulnerable to anthropogenic hazards than adults (Mumme et al. 2000), and the post-independence part of the post-fledging period is a bird's first attempt to disperse and navigate its landscape independently. Improvements in transmitter technology will make feasible tracking studies of longer duration with a greater variety of small passerine species (Bridge et al. 2011). Emphasis should be placed on studying the post-independence period of the post-fledging stage to fully characterize anthropogenic mortality rates.

Cox et al. (2014) identified 0.4 as a threshold for post-fledging survival, below which juvenile overwinter survival and/or adult survival and fecundity would need to

be unusually high to prevent population decline. The fledgling catbirds in our study had a cumulative survival rate of 0.32 over the study period (55 days). When catbirds reached the end of the parental-dependence period, cumulative survival was already down to 0.40; subsequent anthropogenic mortality reduced this to 0.32 during the post-independence period. We only sampled birds from nests within forest fragments, so perhaps natural forested areas are ecological sinks for catbirds in urban landscapes. Alternatively, elevated nest success in urban forests could potentially compensate for low fledgling survival (Streby et al. 2014b). Post-fledging survival rates for catbirds in urban/suburban matrix habitat and shrubland were 0.10–0.45 (depending on site, Balogh et al. 2011), and 0.60 (Maxted 2001), respectively. Follow up work should focus on catbird productivity in backyards within the same landscape to determine whether this may offset the low survival rates within forests.

Our analyses showed that crossing roads, which is unavoidable in many urbanized areas, significantly increased the risk of mortality by anthropogenic causes. Although not all of the anthropogenic mortalities were due to cars, road crossings likely also represented individual birds' behavioral responses to urban habitat features. Rather than remain in small habitat fragments, the birds that succumbed to anthropogenic causes repeatedly crossed roads and ventured into built up areas. Road crossings did not have a significant impact on the likelihood of depredation, in contrast to Streby and Andersen (2013). We expected that proximity to urban land cover would have a stronger effect on mortality risk; the trend indicates a negative relationship, but we did not have sufficient statistical power to detect it. In contrast to the trend we observed, Ausprey and Rodewald (2011) found that degree of

surrounding urbanization had a positive effect on northern cardinal survival during the first few days after fledging.

Other studies on fledgling songbirds in built-up environments have observed road mortality of focal birds (Mumme et al. 2000, Maxted 2001) and their siblings (Ausprey and Rodewald 2011). Roads pose fatal risks to wildlife (Gibbs and Shriver 2002, Erickson et al. 2005, Row et al. 2007, Hovick et al. 2014), but the extent of their impact on bird population dynamics is not well known (Loss et al. 2014). Given the naturally high background mortality rates among fledgling passerines, anthropogenic hazards in urban environments potentially cause additive mortality as inexperienced birds attempt to navigate both natural and anthropogenic risks.

When considering natural and anthropogenic risks, we point out that our definitions here do not fully characterize urban effects on risk. Anthropogenic mortality, in our study, is used to describe only the deaths that could be directly attributed to human activity: e.g. car and building strikes. We were not able to quantify indirect effects such as potentially high mesopredator densities in urban areas (Prange et al. 2004) or altered hydrology (Hollis 1975) that may have contributed to several drownings. As a result, the actual number of anthropogenic mortalities was most likely underestimated in our study. To improve on our understanding of urban risks during the post-fledging period, we suggest that future work compare populations in both urban and more natural landscapes.

Contrary to our prediction, we found that longer daily movements decreased mortality risk, especially for non-anthropogenic mortality. We expected that increased movement would make fledglings more conspicuous and therefore vulnerable to predation. Especially because the majority of non-anthropogenic occurred during the

early days after fledging, we predicted that more mobile birds would be at greater risk than well-hidden birds (King et al. 2006). We consider two interpretations of this finding: 1. Body condition/development, and 2. Measurement ambiguity. In the way that longer nestling developmental periods are correlated with higher post-fledging survival among species (Cox et al. 2014, Lloyd and Martin 2015), the magnitude of net movement distance over 24 h may serve as a proxy for individual developmental status and body condition. More developed birds in better condition may have improved flight capabilities, move farther, and be better able to evade predators. We did not directly test for an effect of nestling condition on fledgling survival because of the recognized unreliability of body mass measures as indicators of condition (Streby et al. 2014). Furthermore, our daily movement distance measure is simply the Euclidian distance between locations taken ~24 h apart that represent the net displacement distance a bird moved from day to day. This measure may not accurately reflect the gross (i.e., cumulative) distance moved or time spent in motion during the intervening 24 h.

We did not observe an influence from either our forest variable or displacement distance from nest. In agreement with prior studies, we also did not detect an effect of fledging date on mortality risk (Maxted et al. 2001, Balogh et al. 2011, Anders et al. 1997). We expected that nest displacement distance might be related to mortality risk at the beginning of the fledgling period, either negatively if birds that stay near their nest are more vulnerable to predators that find the nest location or positively by increased exposure to predators for birds that move farther from the nest. Our variable reflecting proportion of locations in forest was likely too coarse to describe the fine-scale behavioral habitat use patterns of the fledglings in our

study. While wood thrush certainly cross and move through the urban matrix, they were nearly always found in forests. Although catbirds spent less time in forests, other variables (distance to urban and number of road crossings) likely were better representations of their habitat preferences and use.

Conclusions and Future Directions

Wood Thrush fledglings had a higher cumulative survival rate than gray catbirds, presumably due to life history differences. Mortality risk for both species was driven by net daily movement distance and road crossings. We found that anthropogenic causes of mortality were restricted to the period after fledglings become independent from their parents (after ~20 days), and that anthropogenic mortality in urban environments could have an additive effect on mortality. Future work should prioritize investigation into whether anthropogenic mortality is compensatory or additive in urban landscapes. By comparing urban and rural populations, future studies should elucidate the full impacts (direct and indirect) of urbanization on the post-fledging period. Both of our focal species are migratory, and it is likely they will not escape the effects of roads and urbanization during migration or other life stages. Therefore, we recommend full life cycle studies of these same populations (Marra et al. 2015) in order to understand the context for mortality during the post-fledging period and whether it may have limiting effects on urban bird populations.

Acknowledgments

We thank the following individuals for assistance in the field nest-searching, monitoring, and tracking fledglings: M. Sirch, D. Ecker, J. Harmon, D. Knauss, J. Curry, N. Hengst, A. Walter, J. Richardson, K. Serno, M. Walker, M. Gartland, S.

O'Connor, Z. Rachell, and K. Pastirik. We are grateful for the data-processing help from S. O'Connor, and the code for the competing risks analysis from J. Benson. We thank land managers for their cooperation at Newark City Parks, New Castle County and Delaware State Parks, and Natural Lands Trust's Peacedale Preserve. Our research was funded by University of Delaware Research Foundation (13A00832), University of Delaware Aeroecology Lab, USDA McIntire Stennis, and a University of Delaware Graduate Research Fellowship.

Table 7 Summary of data used in Cox proportional hazards models. Continuous variables are shown as mean (standard error) and categorical variables are represented as proportions to describe groups within the data. For time-independent variables (nest distance to edge, ordinal day when fledged, and year) there is one value for each individual in the dataset (60 for catbirds and 67 for wood thrushes). The sample size for time-dependent variables (daily step length, daily displacement from nest, distance to urban cover, roads crossed, forest) is the sum of all locations for all individuals (1,359 for catbirds and 2,224 for wood thrushes).

Variable	Gray Catbird	Wood Thrush
nest distance to edge (m)	77.8 (6.3)	115.2 (7.0)
ordinal day when fledged	191.1 (2.7)	185.7 (3.0)
daily step length (m)	80.0 (4.0)	102.6 (4.8)
daily displacement from nest (m)	291.7 (10.1)	350.7 (11.9)
distance to urban cover (m)	131.9 (3.4)	180.3 (4.0)
number of roads crossed	1.5 (0.1)	0.9 (0.1)
proportion locations in forest	930/1,359	2,092/2,224
proportion survived 2012	5/14	11/19
proportion survived 2013	8/20	16/19
proportion survived 2014	7/26	16/29

Table 8 Results of comparisons among Cox models using only time-independent predictor variables: species (binary variable); distance between nest location and forest edge; ordinal day when individual fledged; and year (1, 2, or 3).

Model	edf	ΔAICc	Weight
species	1	0	0.49
species + nest distance to edge	2	0.92	0.31
species + ordinal day + year	3	3.17	0.10
species + nest distance to edge + year + ordinal day	4	4.15	0.06
nest distance to edge	1	6.70	0.01
null	0	8.43	0.01
nest distance to edge + ordinal day + year	3	10.07	0.00
ordinal day + year	2	11.48	0.00

Table 9 Results of comparisons among Cox models using species and time-dependent covariates: cumulative number of roads crossed (roads); straight line distance moved between one location and the next (step length); displacement from nest location (displacement); distance to urban land use (urban); whether bird was using forest habitat or not (forest).

Model	edf	ΔAICc	Weight
species + roads + step length	3	0	0.82
global	6	4.39	0.09
species	1	6.39	0.03
species + urban	2	7.22	0.02
species + roads + displacement	3	7.42	0.02
species + forest + urban	3	9.42	0.01
null	0	14.86	0.00

Table 10 Beta estimates for Cox model with competing risks demonstrate the relative importance of road crossings for anthropogenic mortality risk and movement rate for natural mortality risk.

	β	exp(β)	lower 95%	upper 95%	*p*
Roads model					
species	0.80	2.22	1.33	3.7	0.002
roads: anthro	0.46	1.59	1.31	1.93	<0.001
roads: natural	-1.56	0.21	0.02	2.29	0.20
Movement model					
species	0.64	1.89	1.11	3.20	0.02
move: natural	-0.32	0.73	0.54	0.99	0.04
move: anthro	-0.69	0.50	0.20	1.24	0.13

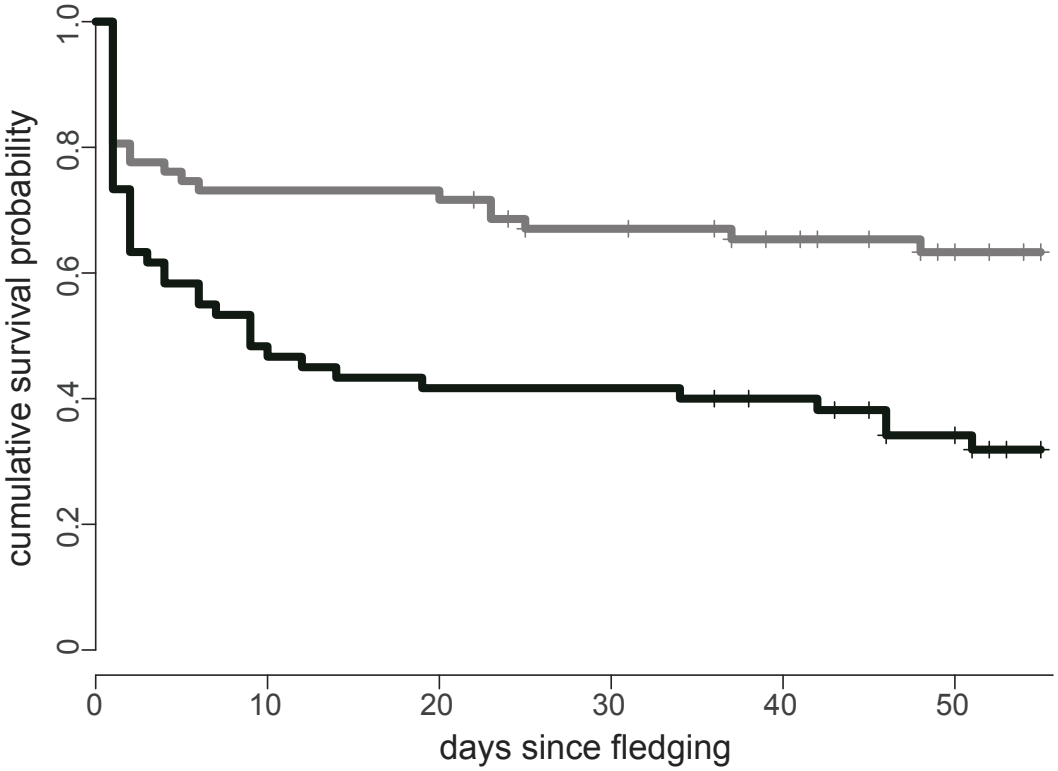

Figure 11 Kaplan-Meier survival curves for Wood Thrushes (gray) and Gray Catbirds (black) through time during the post-fledging period. Vertical lines indicate right-censored individuals.

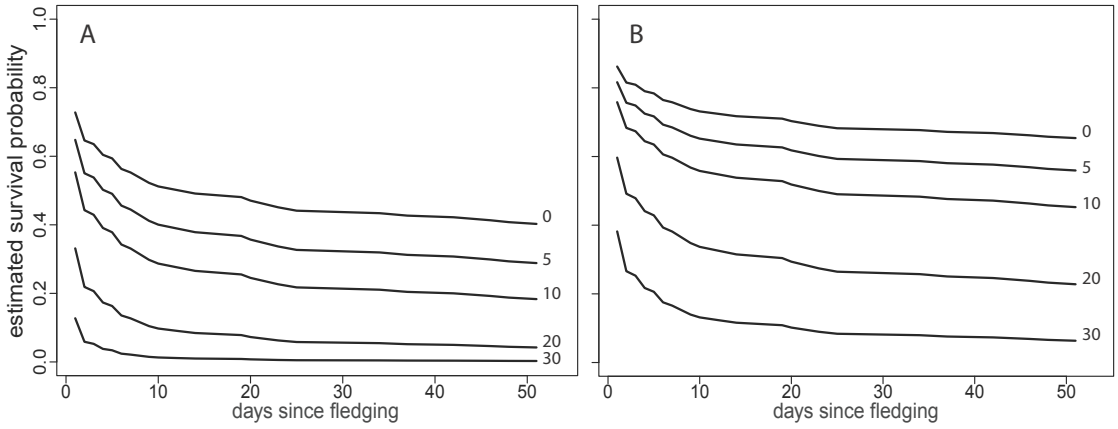

Figure 12 Estimated survival probabilities of Gray Catbirds (A) and Wood Thrushes (B) given varying numbers of road crossings (0,5,10,20,30, indicated on the right of each plot), with step length held constant.

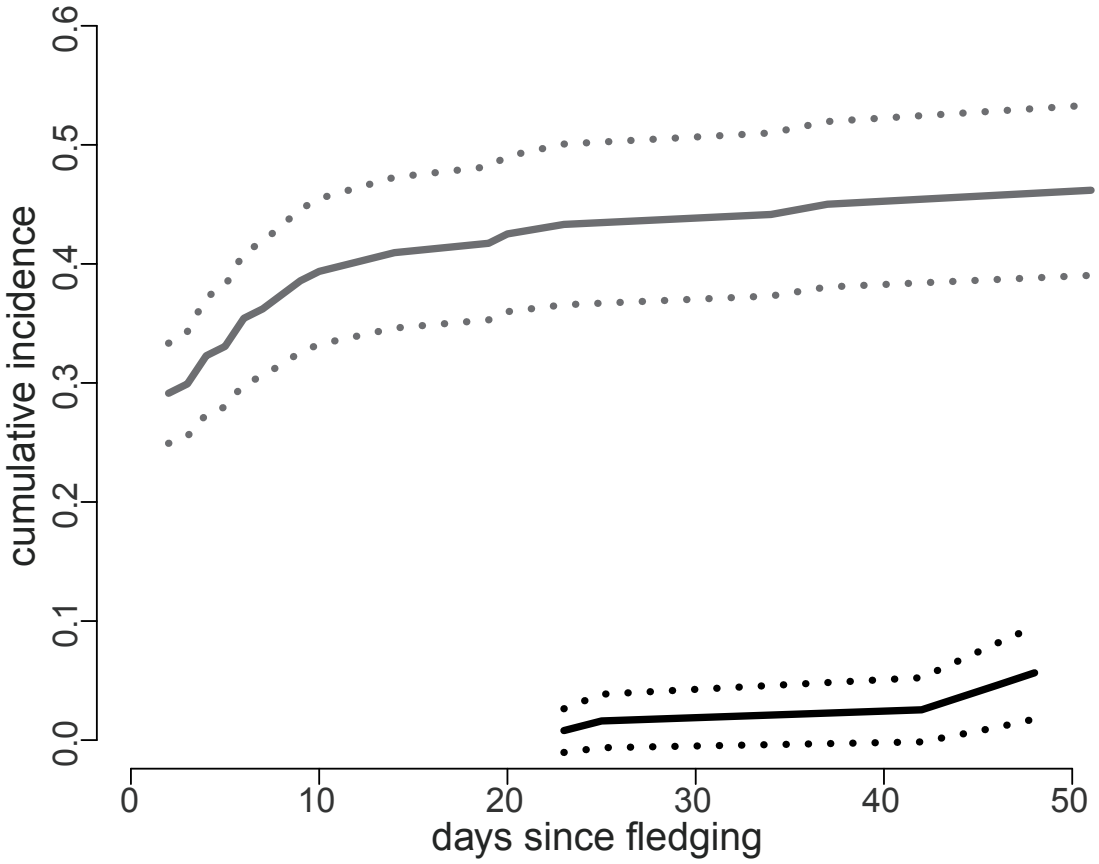

Figure 13 Cumulative incidence of competing risks, anthropogenic (black) vs. non-anthropogenic mortality (gray), over time for fledgling Gray Catbirds and Wood Thrushes.

REFERENCES

Anders, A. D., D. C. Dearborn, J. Faaborg, and F. R. Thompson III (1997). Juvenile survival in a population of Neotropical migratory birds. Conservation Biology 11:698–707.

Anders, A. D., and M. R. Marshall (2005). Increasing the accuracy of productivity and survival estimates in assessing landbird population status. Conservation Biology 19:66–74.

Ausprey, I. J., and A. D. Rodewald (2011). Postfledging Survivorship and Habitat Selection Across a Rural-to-Urban Landscape Gradient. The Auk 128:293–302.

Balogh, A. L., T. B. Ryder, and P. P. Marra (2011). Population demography of Gray Catbirds in the suburban matrix: sources, sinks and domestic cats. Journal of Ornithology 152:717–726. doi: 10.1007/s10336-011-0648-7

Bélisle, M., A. Desrochers, and M.-J. Fortin (2001). Influence of forest cover on the movements of forest birds: a homing experiment. Ecology 82:1893–1904.

Beyer, H. L. (2012). Geospatial Modelling Environment. In.

Bridge, E. S., K. Thorup, M. S. Bowlin, P. B. Chilson, R. H. Diehl, R. W. Fléron, P. Hartl, R. Kays, J. F. Kelly, W. D. Robinson, and M. Wikelski (2011). Technology on the Move: Recent and Forthcoming Innovations for Tracking Migratory Birds. BioScience 61:689–698. doi: 10.1525/bio.2011.61.9.7

Burnham, K. P., and D. R. Anderson (2002). Model selection and multimodel inference: a practical information-theoretic approach. In. Springer Science & Business Media.

Cox, W. A., F. R. Thompson, A. S. Cox, and J. Faaborg (2014). Post-fledging survival in passerine birds and the value of post-fledging studies to conservation: Post-Fledging Survival in Passerines. The Journal of Wildlife Management 78:183–193. doi: 10.1002/jwmg.670

Erickson, W. P., G. D. Johnson, P. David Jr, and others (2005). A summary and comparison of bird mortality from anthropogenic causes with an emphasis on collisions.

ESRI (2012). ArcMap v 10.1. In. ESRI, Redlands, CA.

Evans, B. S., T. B. Ryder, R. Reitsma, A. H. Hurlbert, and P. P. Marra (2015). Characterizing avian survival along a rural-to-urban land use gradient. Ecology 96:1631–1640.

Evans, M., E. Gow, R. R. Roth, M. S. Johnson, and T. J. Underwood (2011). Wood Thrush (Hylocichla mustelina). *The Birds of North America Online*. [Online.] Available at http://bna.birds.cornell.edu/bna/species/246.

Fink, M. L. (2003). Post-fledging survival of juvenile wood thrush in fragmented and contiguous landscapes.

Gibbs, J. P., and W. G. Shriver (2002). Estimating the effects of road mortality on turtle populations. Conservation Biology 16:1647–1652.

Hollis, D. E. (1975). The effect of urbanization on floods of different recurrence interval. Water Resources Research 11:431–435.

Hovick, T. J., R. D. Elmore, D. K. Dahlgren, S. D. Fuhlendorf, and D. M. Engle (2014). REVIEW: Evidence of negative effects of anthropogenic structures on wildlife: a review of grouse survival and behaviour. Journal of Applied Ecology 51:1680–1689. doi: 10.1111/1365-2664.12331

Jackson, A. K., J. P. Froneberger, and D. A. Cristol (2011). Postfledging survival of eastern bluebirds in an urbanized landscape. The Journal of Wildlife Management 75:1082–1093. doi: 10.1002/jwmg.154

James, F. C., R. F. Johnston, N. O. Wamer, G. J. Niemi, and W. J. Boecklen (1984). The Grinnellian niche of the wood thrush. American Naturalist:17–47.

King, D. I., R. M. Degraaf, M.-L. Smith, and J. P. Buonaccorsi (2006). Habitat selection and habitat-specific survival of fledgling ovenbirds (Seiurus aurocapilla). Journal of Zoology 269:414–421. doi: 10.1111/j.1469-7998.2006.00158.x

Loss, S. R., T. Will, and P. P. Marra (2014). Estimation of bird-vehicle collision mortality on U.S. roads: Bird-Vehicle Collisions on U.S. Roads. The Journal of Wildlife Management 78:763–771. doi: 10.1002/jwmg.721

Marra, P. P., E. B. Cohen, S. R. Loss, J. E. Rutter, and C. M. Tonra (2015). A call for full annual cycle research in animal ecology. Biology Letters 11:20150552. doi: 10.1098/rsbl.2015.0552

Martin, T. E., and G. R. Geupel (1993). Nest-monitoring plots: methods for locating nests and monitoring success. Journal of Field Ornithology 64:507–519.

Maxted, A. M. (2001). Post-fledging survival, dispersal, and habitat use in two migratory shrubland bird species.

Mumme, R. L., S. J. Schoech, G. E. Woolfenden, and J. W. Fitzpatrick (2000). Life and death in the fast lane: demographic consequences of road mortality in the Florida Scrub-jay. Conservation Biology 14:501–512.

Prange, S., S. D. Gehrt, and E. P. Wiggers (2004). Influences of anthropogenic resources on raccoon (Procyon lotor) movements and spatial distribution. Journal of Mammalogy 85:483–490. doi: 10.1644/BOS-121

R Development Core Team (2014). R: A Language and Environment for Statistical Computing. In. R Foundation for Statistical Computing, Vienna, Austria.

Rappole, J. H., and A. R. Tipton (1991). New harness design for attachment of radio transmitters to small passerines. Journal of Field Ornithology 62:335–337.

Rega, C. (2012). Impacts of soil calcium availability and non-native plant invasions on an urban forest bird community. [Online.] Available at http://udspace.udel.edu/handle/19716/11728.

Ricklefs, R. E. (1968). The survival rate of juvenile Cactus Wrens. Condor 70:388–389.

Robbins, C. S., D. K. Dawson, and B. A. Dowell (1989). Habitat area requirements of breeding forest birds of the middle Atlantic states. Wildlife Monographs:3–34.

Rodewald, A. D., and S. D. Gehrt (2014). Wildlife population dynamics in urban landscapes. In Urban Wildlife Conservation (R. A. McCleery, C. E. Moorman and M. N. Peterson, Editors). Springer, New York, N.Y., pp. 117–148.

Row, J. R., G. Blouin-Demers, and P. J. Weatherhead (2007). Demographic effects of road mortality in black ratsnakes (Elaphe obsoleta). Biological Conservation 137:117–124. doi: 10.1016/j.biocon.2007.01.020

Rushing, C. S., T. B. Ryder, and P. P. Marra (2016). Quantifying drivers of population dynamics for a migratory bird throughout the annual cycle. Proceedings of the Royal Society B: Biological Sciences 283:20152846. doi: 10.1098/rspb.2015.2846

Ryder, T. B., R. Reitsma, B. Evans, and P. P. Marra (2010). Quantifying avian nest survival along an urbanization gradient using citizen-and scientist-generated data. Ecological Applications 20:419–426.

Sauer, J. R., J. E. Hines, J. E. Fallon, K. L. Pardieck, D. J. Ziolkowski Jr., and W. A. Link (2015). The North American Breeding Bird Survey, Results and Analysis 1966 - 2013. *USGS Patuxent Wildlife Research Center, Laurel, MD*. [Online.] Available at http://www.mbr-pwrc.usgs.gov/bbs/.

Sauer, J. R., W. A. Link, J. E. Fallon, K. L. Pardieck, and D. J. Ziolkowski (2013). The North American Breeding Bird Survey 1966–2011: Summary Analysis and Species Accounts. North American Fauna 79:1–32. doi: 10.3996/nafa.79.0001

Schmidt, K. A., S. A. Rush, and R. S. Ostfeld (2008). Wood thrush nest success and post-fledging survival across a temporal pulse of small mammal abundance in an oak forest. Journal of Animal Ecology 77:830–837. doi: 10.1111/j.1365-2656.2008.01378.x

Shipley, A. A., M. T. Murphy, and A. H. Elzinga (2013). Residential edges as ecological traps: Postfledging survival of a ground-nesting passerine in a forested urban park. The Auk 130:501–511. doi: 10.1525/auk.2013.12139

Smith, R., M. I. Hatch, D. A. Cimprich, and F. Moore (2011). Gray Catbird (Dumetella carolinensis). *The Birds of North America*. [Online.] Available at http://bna.birds.cornell.edu/bna/species/167.

Streby, H. M., and D. E. Andersen (2013). Survival of Fledgling Ovenbirds: Influences of Habitat Characteristics at Multiple Spatial Scales. The Condor 115:403–410. doi: 10.1525/cond.2013.110178

Streby, H. M., J. M. Refsnider, and D. E. Andersen (2014a). Redefining reproductive success in songbirds: Moving beyond the nest success paradigm. The Auk 131:718–726. doi: 10.1642/AUK-14-69.1

Streby, H. M., J. M. Refsnider, S. M. Peterson, and D. E. Andersen (2014b). Retirement investment theory explains patterns in songbird nest-site choice. Proceedings of the Royal Society B: Biological Sciences 281:20131834–20131834. doi: 10.1098/rspb.2013.1834

Therneau, T. (2015). A package for survival analysis in S. In.

Whittaker, K. A., and J. M. Marzluff (2009). Species-specific Survival and Relative Habitat Use in an Urban Landscape during the Postfledging Period. The Auk 126:288–299. doi: 10.1525/auk.2009.07136

Appendix A

TICKS PARASITIZING BIRDS IN 13 URBAN FOREST FRAGMENTS DURING MAY – AUGUST, 2013 AND 2014

FRAME Site	Species	Tick hosts	*Ixodes spp.*		*Ixodes scapularis*		*Ixodes minor*	U	Totals	
			N	L	N	L	L	L	N	L
Cover-dale	*Site totals*	*13/52*	*3*	*9*	*6*	*30*		*11*	*9*	*50*
	Blue Jay	1/1		1		1		1	0	3
	Carolina Wren	1/3		2	2	4		3	2	8
	Eastern Towhee	½		2		2			0	4
	House Wren	½			1				1	0
	Northern Cardinal	6/15		4	1	24		5	1	33
	Red-eyed Vireo	1/7						2	0	2
	Wood Thrush	2/6	3		2				5	0
	Acadian Flycatcher	0/5							0	0
	American Robin	0/1							0	0
	Carolina Chickadee	0/6							0	0
	Ovenbird	0/1							0	0
	Tufted Titmouse	0/2							0	0
	White-breasted Nuthatch	0/1							0	0
Ecology Woods	*Site totals*	*10/54*	*1*	*5*	*8*	*14*		*3*	*9*	*22*
	American Robin	2/6	1	1	1				2	1
	Carolina Wren	2/4		2		3		1	0	6
	Gray Catbird	3/20			2	3		1	2	4
	Northern Cardinal	1/5				1			0	1
	Ovenbird	1/1		2		1		1	0	4
	Wood Thrush	1/7			7	6			7	6
	Carolina Chickadee	0/3							0	0

	Common Yellowthroat	0/3						0	0	
	Downy Woodpecker	0/1						0	0	
	Red-eyed Vireo	0/4						0	0	
Folk	*Site totals*	*7/21*	*2*	*4*	*8*	*9*		*3*	*10*	*16*
	American Robin	5/5	1	1	7	3		2	8	6
	Carolina Wren	2/2	1	3	1	6		1	2	10
	Carolina Chickadee	0/1							0	0
	Common Yellowthroat	0/1							0	0
	Red-eyed Vireo	0/1							0	0
	Swainson's Thrush	0/1							0	0
	Wood Thrush	0/7							0	0
	White-throated Sparrow	0/3							0	0
Iron Hill 2	*Site totals*	*½*			*3*				*3*	*0*
	Carolina Wren	1/1			3				3	0
	Wood Thrush	0/1							0	0
Motor-pool	*Site totals*	*1/14*				*1*			*0*	*1*
	American Robin	½				1			0	1
	Acadian Flycatcher	0/2							0	0
	Blue-gray Gnatcatcher	0/1							0	0
	Carolina Wren	0/5							0	0
	Indigo Bunting	0/1							0	0
	Magnolia Warbler	0/1							0	0
	Northern Cardinal	0/2							0	0
Mt. Cuba Rose	*Site totals*	*27/43*		*32*		*214*	*2*	*34*	*0*	*282*
	American Robin	1/1				2			0	2
	Carolina Wren	3/3		12		47		9	0	68
	Eastern Towhee	1/1		3		18	1	1	0	23
	Gray Catbird	15/23		9		65		10	0	84
	House Wren	1/1		1		14		1	0	16
	Northern Cardinal	2/5				17	1	5	0	23
	Tufted Titmouse	2/4				2			0	2
	Veery	2/2		7		49		8	0	64

	Carolina Chickadee	0/2							0	0
	Red-eyed Vireo	0/1							0	0
Reservoir	*Site totals*	*16/44*	*3*	*5*	*7*	*22*		*10*	*10*	*37*
	American Robin	9/11	1	2	3	8		2	4	12
	Carolina Wren	4/5	2	1	3	6		3	5	10
	Ovenbird	½		2		6		5	0	13
	Red-eyed Vireo	1/5			1				1	0
	Wood Thrush	1/3				2			0	2
	Blue Jay	0/1							0	0
	Downy Woodpecker	0/1							0	0
	Eastern Wood-Peewee	0/1							0	0
	Gray Catbird	0/4							0	0
	Northern Cardinal	0/3							0	0
	Scarlet Tanager	0/1							0	0
	Tufted Titmouse	0/5							0	0
	White-breasted Nuthatch	0/2							0	0
Ritten-house	*Site totals*	*3/25*		*1*		*11*		*1*	*0*	*13*
	American Robin	½		1		11		1	0	13
	Carolina Chickadee	1/9						1	0	1
	Carolina Wren	1/3						1	0	1
	Downy Woodpecker	0/5							0	0
	Northern Cardinal	0/1							0	0
	Red-eyed Vireo	0/1							0	0
	Tufted Titmouse	0/2							0	0
	White-breasted Nuthatch	0/2							0	0
Webb Farm	*Site totals*	*3/14*		*5*	*2*	*17*		*6*	*2*	*28*
	Carolina Wren	3/8		5	2	17		6	2	28
	Carolina Chickadee	0/2							0	0
	Gray Catbird	0/2							0	0
	Northern Cardinal	0/1							0	0
	Tufted Titmouse	0/1							0	0

White Clay 1	*Site totals*	*3/9*			*1*	*10*			*1*	*10*
	Red-eyed Vireo	1/3				1			0	1
	Wood Thrush	2/5			1	9			1	9
	Ovenbird	0/1							0	0
Christina Creek 1	*Site totals*	*0/6*							*0*	*0*
	Carolina Chickadee	0/2							0	0
	Least Flycatcher	0/1							0	0
	Northern Cardinal	0/2							0	0
	Tufted Titmouse	0/1							0	0
Christina Creek 2	*Site totals*	*0/27*							*0*	*0*
	American Redstart	0/1							0	0
	American Robin	0/2							0	0
	Carolina Chickadee	0/5							0	0
	Carolina Wren	0/4							0	0
	Northern Cardinal	0/9							0	0
	Northern Parula	0/1							0	0
	Red-eyed Vireo	0/3							0	0
	Tufted Titmouse	0/1							0	0
	Wood Thrush	0/1							0	0
Glasgow 2	*Site totals*	*0/15*							*0*	*0*
	Blue Jay	0/1							0	0
	Carolina Chickadee	0/5							0	0
	Downy Woodpecker	0/1							0	0
	Eastern Wood-Peewee	0/1							0	0
	Northern Cardinal	0/1							0	0
	Ovenbird	0/1							0	0
	Tufted Titmouse	0/3							0	0
	Wood Thrush	0/1							0	0
Grand Total	*All species, all sites*	*84/326*	*9*	*61*	*37*	*329*	*2*	*67*	*46*	*459*

Appendix B

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE RESEARCH PERMIT

RECEIVED
APR 12 2013
IACUC

University of Delaware
Institutional Animal Care and Use Committee
Application to Use Animals in Research and Teaching

(Please complete below using Arial, size 12 Font.)

Title of Protocol: **Estimating mouse densities in urban forest fragments**	
AUP Number: 1249-2013-0	**← (4 digits only — if new, leave blank)**
Principal Investigator: Dr. Jacob L. Bowman	
Common Name: White-footed mouse, Deer mouse **Genus Species:** *Peromyscus leucopus, Peromyscus maniculatus*	

Pain Category: ***(please mark one)***

USDA PAIN CATEGORY: ***(Note change of categories from previous form)***

Category	Description
☐ B	Breeding or holding where NO research is conducted
☒ C	Procedure involving momentary or no pain or distress
☐ D	Procedure where pain or distress is alleviated by appropriate means (analgesics, tranquilizers, euthanasia etc.)
☐ E	Procedure where pain or distress cannot be alleviated, as this would adversely affect the procedures, results or interpretation

Official Use Only

IACUC Approval Signature: Glenn Tallman DVM

Date of Approval: 4/17/2013

Appendix C

RIGHTS LINK FROM ECOSPHERE

Copyright Clearance Center RightsLink®

Home | Create Account | Help

WILEY

Title: Scale-dependent effects of nonnative plant invasion on host-seeking tick abundance

Author: Solny A. Adalsteinsson,Vincent D'Amico,W. Gregory Shriver,Dustin Brisson,Jeffrey J. Buler

Publication: Ecosphere

Publisher: John Wiley and Sons

Date: Mar 25, 2016

LOGIN

If you're a copyright.com user, you can login to RightsLink using your copyright.com credentials. Already **a RightsLink user** or want to learn more?

Welcome to RightsLink

This article is available under the terms of the Creative Commons Attribution License (CC BY) (which may be updated from time to time) and permits use, distribution and reproduction in any medium, provided that the Contribution is properly cited.

For an understanding of what is meant by the terms of the Creative Commons License, please refer to Wiley's Open Access Terms and Conditions.

Permission is not required for this type of reuse.

Wiley offers a professional reprint service for high quality reproduction of articles from over 1400 scientific and medical journals. Wiley's reprint service offers:

- Peer reviewed research or reviews
- Tailored collections of articles
- A professional high quality finish
- Glossy journal style color covers
- Company or brand customisation
- Language translations
- Prompt turnaround times and delivery directly to your office, warehouse or congress.

Please contact our Reprints department for a quotation. Email corporatesaleseurope@wiley.com or corporatesalesusa@wiley.com or corporatesalesDE@wiley.com .

CLOSE WINDOW