ABSTRACT

Title of Thesis: SMALL MAMMAL POPULATIONS, TICKS, AND TICK-BORNE DISEASES IN URBAN PARKS

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Master of Science 2024

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Environmental Science and Technology

As urban environments expand, the distribution of wildlife, particularly white-footed mice, influences public health through zoonotic pathogen transmission, such as Lyme disease. This study examined pathogen risk in urban green spaces, focusing on interactions among small mammal communities, black-legged ticks, and the bacterium *Borreliella (Borrelia) burgdorferi*. Over two years, small mammal trapping was conducted across six urban park sites in Maryland to 1) quantify small mammal densities in six unique urban sites and 2) identify correlations between small mammal community and habitat structure related to pathogen prevalence. Findings revealed significant differences in pathogen risk between parks, driven by elevation and landscape features, with open shrub-scrub, and upland habitats, such as powerline corridors, linked to increased transmission risk. These results underscore the importance of habitat-level management strategies for urban green spaces to mitigate pathogen risk, rather than focusing solely on white-footed mice.

SMALL MAMMAL POPULATIONS, TICKS, AND TICK-BORNE DISEASES IN URBAN PARKS

by

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2024

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Dedication

This thesis is dedicated to the memory of my parents, whose love, guidance, and encouragement have been the foundation of my life. Their profound loss during my graduate school years has been a source of both sorrow and strength. I carry their memories with me every day, and I hope to honor their legacy through my work.

Acknowledgments

A special thank you to Dr. Jennifer Mullinax, Dr. Matthew Gonnerman, and Dr. Grace Hummell for going above and beyond when it comes to the incredible levels of support that I have received over the years on this project.

I would like to thank the numerous technicians, volunteers, and fellow graduate students from the Applied, Spatial Ecology Lab at the University of Maryland. Those people, in no particular order, are Trinadee Jenkins, Virginia Borda, Cayla Mahon, Trinity Schindler, Taylor Sinclair, Maddie Jamsa, Matt Payne, Kai Lambert, Danilo Azevedo, Amanda Lindsay, Molly Stamets, Jack Ingrassia, Victoria Liu, Spencer Goldberg, Victor Salaverria, Isabella Tonti, Elizabeth MacNeice, Samantha Pounsberry, and Victoria Liu.

I also want to express my heartfelt gratitude to my family for their unwavering support throughout my academic journey. To my sister Kaitlyn Coriell and husband, Josh House, thank you for being my pillars of support during this challenging time. Your love and companionship have helped me navigate the highs and lows of this journey.

To my partner, Annie Sines, words cannot fully express my gratitude for your unwavering love and support over the past 10 years. Throughout the challenges and triumphs of graduate school, you have been my constant source of strength, encouragement, and understanding. Your patience and belief in me have carried me through the toughest moments, and I am endlessly grateful for your presence in my life. This journey would not have been possible without you by my side.

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CHAPTER 1: URBAN SMALL MAMMALS IN THE FIGHT AGAINST TICKS AND LYME DISEASE

INTRODUCTION

Lyme disease (causative agent: *Borreliella Borrelia burgdorferi*), hereafter referred to as *B. burgdorferi,* is the most common vector-borne disease in the United States. Since 2012, Lyme disease has been recognized as one of the top ten notifiable diseases by the CDC (CDC, 2024). Lyme disease in humans can cause a range of symptoms, including a characteristic "bull's-eye" rash, flu-like symptoms, joint pain, neurological issues, heart problems, and if left untreated, can lead to chronic conditions such as persistent fatigue, cognitive difficulties, and ongoing musculoskeletal pain, significantly impacting an individual's quality of life (CDC, 2024). The Centers for Disease Control and Prevention reported that, on average, 30,000 cases of Lyme disease are reported each year; however, the true number may be over 400,000 cases (CDC, 2024). Lyme disease is diagnosed based on symptoms, physical findings (e.g., erythema migrans rash), and possible exposure to infected ticks (CDC, 2024). Hard ticks like the blacklegged tick (*Ixodes scapularis*) are the vector of the pathogens that cause Lyme disease in humans and other tick-borne zoonotic diseases such as Anaplasmosis (Stone et al., 2017).

Blacklegged ticks have a complex life cycle, relying on host species to feed, reproduce, and disperse throughout an ecosystem. In natural systems, small mammals play a significant role in the tick life cycle. Many small mammals serve as reservoirs for tick-borne pathogens, with white-footed mice (*Peromyscus leucopus*), eastern chipmunk (*Tamias striatus*), and northern short-tailed shrew (*Blarina brevicauda*) being some of the most successful reservoir species for the Lyme disease-causing bacteria, *B. burgdorferi* (Gage et al. 1995).

Using spatially explicit capture-recapture methods and general linear regression models, I investigated the relationship between hard ticks, small mammals, and select tick-borne pathogen prevalence to gain a better understanding of the urban tick-borne pathogens in the ecosystem. Then, I suggest what host-based steps can be taken to mitigate the impacts of these pathogens, especially those responsible for Lyme disease. Specifically, I found that small mammals' tick loads varied across two urban parks based on land cover type, site history and use, and small mammal density.

Seukep et al. (2015) found that the spatial overlap between hosts and ticks was a key requirement in spreading vector-borne pathogens. Previous studies have focused on white-footed mice and white-tailed deer as the primary hosts and drivers of Lyme disease (Halsey & Miller, 2018; Ostfeld et al., 2018). Ticks are distributed to new areas via attachment of hosts moving large distances, such as white-tailed deer, as well as small, localized movements on small mammals, increasing the potential spread of Lyme (Pruitt 2022). Despite this knowledge, there is still little understanding of how small mammal community diversity, density, and abundance may alter their infection probability within urban ecosystems in the United States (Barko et al., 2003; Diuk-Wasser et al., 2021; Persons & Eason, 2017).

Urban sprawl and rapid urban growth contribute to the spread of zoonotic diseases by fragmenting natural habitats and increasing human-wildlife contact, intensifying the risk of disease transmission. Urbanization drivers such as higher human densities, increased animal movement, complex value chains for animal products, rural-to-urban migration, intra-city inequalities, and land-use changes further exacerbate these risks, creating conditions that facilitate pathogen spillover into human populations (Ahmed et al. 2019). Suburban and urban areas have co-occurring high densities of humans and peridomestic wildlife, which can increase

the likelihood of humans becoming hosts for ticks (Ogden et al., 2007). Furthermore, urban-suburban green spaces are typically covered with dense vegetation, providing good habitat for ticks and foraging options for terrestrial mammals without many predators. These mammals, including mammal hosts, typically survive longer and in higher densities (Daniels & Fish, 1995; DeNicola et al., 2000). Humans who frequent urban green spaces tend to have a higher chance of encountering a tick and receiving a tick bite due to the high densities of ticks (Keesing et al., 2010). To gain insight into how tick-borne pathogens are spread throughout urban and suburban communities, I quantified how mammal community density, focused on white-footed mice, related to the prevalence of ticks on these hosts and the pathogens that both attached ticks and the rodents carried. Better understanding these systems will enable managers to find better spatially explicit species-targeted solutions to address tick-borne disease treatments.

OBJECTIVES

In this study, I conducted a mark-recapture experiment to assess the density and infection rates of the overall small mammal population within two unique urban green spaces in Montgomery County, Maryland, USA. My primary study objectives were to:

1. *Quantify small mammal densities across six unique urban sites*- Literature on the relative densities of small mammals in urban-suburban areas is limited. Providing an analysis of their populations unique urban green spaces could aid park planning and management by better understanding small mammal densities as they relate to park characteristics.
2. *Assess how the small mammal community and habitat structure are related to host tick load and pathogen prevalence of attached ticks and small mammals in two*

unique urban parks- To address small mammal habitats as they relate to attached tick populations and pathogen presence, I collected tissue samples from small mammals for pathogen testing, as well as any attached ticks. Then, I compared the pathogen prevalence of mice and attached ticks to the densities of mice and habitat structure to understand any existing relationships. My study focused on *B. burgdorferi*, but many other diseases of concern exist including *Anaplasma phagocytophilum, Borrelia miyamotoi, Babesia microti,* Powassan virus, *Rickettsia parkeri,* and *Rickettsia rickettsii* (Rodino et al. 2020).

STUDY AREA

I conducted research within Montgomery County, Maryland, USA (MoCo), which is the most populated county in Maryland (U.S. Census Bureau, 2024) and is directly adjacent to Washington, D.C. (hereafter, 'DC'). Land use ranged from rural farmland in the northern areas to dense urban sprawl radiating from the DC metropolitan area into the southern section of MoCo.

Parks were selected based on their level of urbanization and urban sprawl, using the U.S. Census 2020 classification of metropolitan areas, which defines urban areas as having at least 2,000 housing units or 5,000 people. Urban areas are further classified based on housing unit density measured at the census block level. The initial urban core is defined as areas with at least 425 housing units per square mile, corresponding to approximately 1,000 people per square mile based on the national average of 2.6 people per housing unit. The remainder of an urban area includes regions with at least 200 housing units per square mile, which is similar to a population density of 500 people per square mile. To qualify as an urban area, there must also be at least one

high-density nucleus containing 1,275 housing units per square mile, reflecting the dense core typically found in urban environments.

Using these classifications, county parks were categorized into rural, moderately suburban, and highly urban areas. To evaluate landscape variables within highly populated regions, we focused on two highly urban parks: Rock Creek Regional Park (hereafter, 'RC'), a linear park surrounded by city centers, and Cabin John Regional Park (hereafter, 'CJ'), a suburban park characterized by a large single-family home population and significant human activity (Table 1). RC stretches across approximately 1,800 acres, has over 21 km of trails through riparian woodlands, and is located at the Maryland-DC border (Figure 1). CJ system consists of a 14.2 km trail system that follows Cabin John Creek in a wooded suburban park. Each park included 3 randomly selected study sites that could contain a trapping array, providing a total of 6 study sites for this project. This sample size allowed for comparisons between parks while controlling for site-level variation within each park.

CJ is closer to major highways and contains numerous athletic facilities, including manicured baseball fields, powerline corridors, an active train line, and various open green spaces. RC, in contrast, is narrower and more space-constrained, winding through high-density housing areas, active construction sites, and multiple stream systems that act as barriers to small mammal movement (Figure 2, Table 1). Though proportions differed, vegetation types in both parks were comparable, with dominant tree species including oaks (*Quercus spp.*), maples (*Acer spp.*), hickories (*Carya spp*.), American beech (*Fagus grandifolia*), and tulip tree (*Liriodendron tulipifera*).

Prior to European colonization in the 1600s, this region of Maryland that included CJ and RC was home to numerous Indigenous tribes, including the Piscataway-Conoy and Nacotchtank

nations. In the 18th and 19th centuries, much of what is now CJ was owned by Samuel Wade Magruder, who used the land for tobacco farming, with enslaved people laboring on the property (Locust Grove Nature Center, 2024). By 1912, the American Land Company purchased large tracts of this area and began dividing it into residential lots. Since then, community amenities have developed, especially following the establishment of the Cabin John Park Citizens Association in 1919, leading to the park structure with its trail system and ball parks as it exists today (Community of Cabin John, n.d.).

In the 1690's, ranging soldiers in Maryland used the Rock Creek area as a fort, and archaeologists have uncovered residential sites dating back to the 1700s (NPS, 2024). Later, in 1890, Rock Creek Park was established following the creation of the National Zoo. It became the third national park in the U.S., designated for the "benefit and enjoyment of the people of the United States" (NPS, n.d.), and is recognized as the oldest natural urban park in the National Park System (Sellers & Sander, 2014). The Maryland section of the park is currently managed by the Maryland-National Capital Park and Planning Commission, while the D.C. section is managed by the National Park Service. My study focused on the Maryland portion, known as Rock Creek Regional Park, which is a narrower green space that follows the primary waterway of Rock Creek.

METHODS

Trapping and Animal Handling

I trapped small mammals in 2022 and 2023 from June through August. To evaluate the relationship of attached larval and nymphal ticks to rodent densities, I focused trapping efforts during the summer months (June-August) to align with the peak activity period of larval and

most of nymphal blacklegged ticks (*Ixodes scapularis*) (C. S. Anderson et al., 2003; J. M. Anderson & Norris, 2006; Byman et al., 2013; Card et al., 2019; Kellner & Swihart, 2014; Ostfeld et al., 2006, Ostfeld et al., 2018). In each park, three trapping arrays were set 50 Sherman live traps (3 x 3.5 x 9": 5515AHD Non-Folding Live Capture Humane Squirrel Trap) in a 100 x100 meter grid, with five rows consisting of 10 traps and each trap placed 10 meters apart (Figure 3) (Jones & Lindquist, 2012; Perez et al., 2016; Card et al., 2019; Ostfeld et al., 2018; Parsons et al., 2021; Levi et al., 2016). Additionally, during the second year, each transect contained two larger Sherman live traps (5 x 5 x 15": 5515AHD Non-Folding Live Capture Humane Squirrel Trap) that were assigned locations using a random number generator to assist in targeting eastern gray squirrels (n = 10 per trapping array) for a total of 450 unique trap sites across the study. A minimum of three consecutive nights of trapping occurred once a month at each study site for mark-recapture density estimates (Byman et al., 2013; Card et al., 2019; Jones & Lindquist, 2012; Levi et al., 2016; Parsons et al., 2021). This design targeted white-footed mice but several non-target small mammal species were also captured, including eastern chipmunks, northern short-tailed shrews, southern flying squirrels (*Glaucomys volans*), and eastern gray squirrels (*Sciurus carolinensis*). It is important to note that both white-footed mice and deer mice (*Peromyscus maniculatus*) are native to Maryland and are difficult to reliably distinguish in the field. Therefore, mice captured during this study will be referred to as *Peromyscus spp.*, and henceforth, all references to mice will use this term to reflect this limitation (Machtinger and Williams 2020). Each trap was baited with peanut butter, oats, raisins, mealworms for food/attractant, apple slices for moisture, and cotton as nesting material (Ostfeld et al., 2018; Parsons et al., 2021). Field teams checked traps at 0600 and then re-baited and reset them in the late afternoon at 1800 to ensure a minimum of 50 traps being open for at

least 12 hours to maximize catchability (Byman et al., 2013; Hofmeester et al., 2017; Levi et al., 2016; Ostfeld et al., 2018; Parsons et al., 2021).

Upon capture, small mammals were gently shaken into a plastic 1-gallon bag at one end of the trap and secured. Animals were weighed with a Pesola® LightLine Spring Scale [Schindellegi, Switzerland] before administering anesthesia. Isoflurane was placed on a cotton ball and put in the bottom of a 5-liter polycarbonate desiccator with a 0.32 cm thick perforated plate separator to keep the animal away from the anesthetic-soaked cotton. The processor continuously monitored the animals for depth of anesthesia (Hummell et al., 2022; Milholland et al., 2021; Parker et al., 2008; Previtali et al., 2012). After anesthesia, each animal was given a distinct Stoelting® ear tag [Wood Dale, Illinois] with a color and number for individual identification in the right ear. Then, a 2mm ear punch was taken from the left ear with a Scientific Labwares Plier Ear Punch [Lorton, Virginia] for pathogen testing. Tissue was collected for systemic pathogen testing and put in a small vial with RNA*later*. While handling each animal, field teams recorded biological details such as an individual's age, body length, foot length, ear length, sex, and reproductive status. Reproductive status was determined by descended testes in males or pronounced nipples indicating a lactating female (Anderson et al., 2003; Daniels & Fish, 1995; Levi et al., 2016; Ostfeld et al., 2018).

In past research, most visible ticks were found on the ears and faces of captured mice (Ostfeld et al., 1993). Animals were thoroughly searched for ticks for at least one full minute after all other data collection was completed, with half of the time focused on ears and face. The total number of ticks per individual was recorded. Any ticks found on the animals were collected for pathogen testing via the Department of Entomology at the University of Maryland’s Fritz Lab. After processing, all animals were monitored for any negative reactions to handling, such as

lethargic behavior or strained breathing. All animals were returned to their exact locations to minimize disruptions to their natural movements. All animal handling was approved by the University of Maryland College Park (UMCP) IACUC under the project title [1916037-1] "Assessment of terrestrial mammal community structure as it relates to ticks and tick-borne disease prevalence."

CHAPTER 2: DENSITY PATTERNS OF WHITE-FOOTED MICE IN URBAN GREEN SPACES

INTRODUCTION

Small mammals play a significant role in spreading zoonotic diseases such as Lyme disease from the bacterial agent *B. burgdorferi*. Specifically, White-footed mice (*Peromyscus leucopus*) have the highest level of reservoir competence for blacklegged tick (*Ixodes scapularis*) cases of *B. burgdorferi* in the northeastern United States (Diuk-Wasser et al., 2021; Donahue et al., 1987; LoGiudice et al., 2003). Other small mammals play an understudied role as various levels of tick-borne disease reservoirs, such as eastern chipmunk, northern short-tailed shrew, northern flying squirrels, and eastern gray squirrel. LoGiudice et al. (2003) found that these alternative tick hosts are relatively ineffective reservoirs for the spirochete causing Lyme disease and instead may act as "dilution hosts," which are associated with high tick burdens, low reservoir competence, and dense populations. So, while more work is needed to better understand those relationships, I focused on *Peromyscus spp.* during this study.

While relationships between ticks, mice, and human infection is commonly acknowledged in the literature, there is a significant gap in understanding of how these interactions occur in urban green spaces. Managers and epidemiologists "lack an understanding of how urban structure influences functional connectivity to humans in relation to their risk of exposure to tick-borne pathogens" (Diuk-Wasser et al., 2021). Specifically, how urban configurations, such as forest fragmentation, disrupt natural habitats and affect movement patterns and interactions among wildlife hosts, like *Peromyscus spp.*, and humans has been understudied. These altered landscapes can create isolated pockets of high densities of ticks and

wildlife that may inadvertently amplify the risk of pathogen spillover. Understanding how these fragmented urban spaces impact functional connectivity is essential to assessing Lyme disease exposure risks across different urbanization gradients and spatial scales. Without these insights, it is challenging to predict disease dynamics in urban areas where both human activity and ecological fragmentation are high.

Peromyscus spp. are influenced by many landscape variables from elevation to specific vegetation in rural and urban areas. Higher elevations can change vegetation types, temperature, moisture levels, food availability, and overall habitat quality for *Peromyscus spp.* (Brooks et al. 1998). For example, lower elevations may offer more diverse vegetation and warmer temperatures, fostering greater insect activity and plant growth and supporting food resources for the mice. Conversely, higher elevations can present harsher weather conditions and reduced food sources, potentially limiting their distribution and density. Additionally, elevation can influence the presence of predators and competitors, further shaping *Peromyscus spp.* populations. One study by Li et al. (2003), suggested that there is a peak activity at mid elevations, where maximum richness and diversity of small mammals was observed. This study also found that plant diversity and primary productivity were also at their peak, alongside the highest levels of rainfall and humidity at these mid-elevations.

Slope ratio can influence the distribution and behavior of mice by affecting habitat structure and resource availability (Berl et al. 2018). Steeper slopes may offer greater cover from predators by providing more opportunities for shelter and nesting. However, very steep slopes can also limit the movement and foraging efficiency of the mice, reducing their access to food resources (Brooks et al. 1998). Slope can also influence microclimates, as steeper areas drain faster and retain less moisture, impacting vegetation growth and food availability. Additionally,

slope orientation (aspect) can affect temperature and sunlight exposure, further shaping the habitat's suitability for mice.

Riparian habitats can provide essential resources like water and food that support diverse wildlife, including small mammals such as mice (Brinson et al., 1981; O'Donnell, 2011). Proximity to water is particularly crucial during warmer months or in dry habitats, as it offers hydration and supports lush vegetation and abundant insect populations, enhancing foraging opportunities for small mammals. So, while mice can survive in drier upland areas, riparian zones attract a rich variety of species, creating a dynamic food web (Getz, 1968). These areas support seed-eating and insect-eating small mammals like shrews (O'Donnell, 2011). However, this proximity to water also brings additional risks, as it attracts both predators and competitors, shaping the behavior and distribution of mice and other small mammals in these habitats.

Forest habitats provide crucial cover and shelter for mice, helping them evade predators while offering ample nesting opportunities. A study by Stark (2014) found that *Peromyscus leucopus* foraged more frequently at trees with owl pellets and at trees that had coyote scent, showing a preference for foraging near the base rather than higher up in the trees. Foraging near trees may offer a safer environment for these mice, as their climbing abilities and capacity to hide can help them evade predators (Stark, 2014). Forest density influences understory vegetation and may differentially affect the distribution of *Peromyscus spp.* between edge and interior habitats in forest patches of varying sizes by providing a greater variety of food sources and enhancing foraging success (Anderson et al. 2003). Additionally, the complexity of dense forests supports various stages of plant and insect life, contributing to a richer ecosystem. Studies such as Bohlman (1995) provide evidence that forests help maintain higher humidity and moisture levels, fostering vegetation growth that benefits small mammals. Moreover, areas with

greater forest density are typically less accessible to human disturbances, which enhances habitat quality. Many studies have indicated that higher densities of mice occur in areas with a large forest edge, known as the "edge effect," which states that there is a change in the plant and animal populations that occur where two different habitats meet. For example, Berl et al. (2018) found a greater density of mice in the edges between forest and agricultural fields. However, Wolf and Batzli (2002) reported a greater abundance of mice in the forest interior than near the edge.

Dense shrub cover protects from predators, allowing mice to hide and move more safely within their environment (Powell & Banks, 2004). Shrubs also offer nesting sites, giving mice secure places to raise their young. Many shrubs produce seeds and fruits and support insect populations, which are important components of mice diets. Shrub density can also create a favorable microclimate by maintaining moisture and moderating temperatures, which further supports the growth of vegetation and food sources that benefit mice. One study found that, as opposed to only the existence of edge influencing mouse density, that "understory vegetation may differentially influence the distribution of *P. leucopus* between edge and interior habitats" (Anderson et al. 2003).

There are often more trails in urban green spaces, and human-made trails can impact the habitat and behavior of mice in several ways. Trails lead to habitat fragmentation, altering the structure and availability of resources. Areas closer to trails may experience higher levels of human disturbance, increasing stress, reducing foraging efficiency, and exposing mice to predators. Wolf and Batzli (2004) identified forest edges as lower-quality habitats for white-footed mice compared to forest interiors due to heightened predation risk. However, trails can also serve as corridors for movement or access to new habitats. Similarly, Hummell et al. (2023)

observed that mice utilized open areas, forest edges, and forests but showed a strong preference for forested habitats. The edge effects along trails can alter vegetation patterns, microclimates, and predator-prey dynamics.

Additionally, invasive plant species are often more prevalent along trails, with numerous studies documenting elevated levels of plant invasion near roads and trails (Ballantyne and Pickering, 2015). The clearing of forests for trail creation disrupts the natural canopy, allowing more light to reach the understory, a condition frequently exploited by invasive plants to establish and thrive (Peters, 2019). Trails can also act as corridors for the introduction and spread of invasive species, as seeds and propagules are transported by human activity, wildlife, and environmental factors such as wind and water flow. Buckley et al. (2003) investigated increased invasion along haul roads in Michigan forests and suggested that variations in soil moisture, light availability, and species richness near trails are significant contributors to this phenomenon. These factors create microhabitats along trail edges that are particularly conducive to the establishment of invasive plant species, further altering the ecological balance of forested areas. Pearson & Fletcher (2008), found instances of mice utilizing invasive shrubs, commonly found in urban parks, to reduce predation risk, which may enhance survivability within urban green spaces. Specific use of trails depends on species and, one study found that "after trail building, eastern gray squirrels showed decreased use of the trail area" (Miller et al. 2020).

Roads can fragment habitats, creating isolated patches and barriers that hinder the dispersal of mice, particularly in urban areas. Oxley et al. (1974) demonstrated this effect, reporting that only 8 of 254 marked individuals crossed roads, indicating a strong reluctance to travel onto road surfaces. Similarly, Merriam et al. (1989) found that while *P. leucopus* frequently moved along roads, their crossings were rare, highlighting the significant role roads

play in restricting movement and connectivity for these mice. The environment near roads often differs from surrounding areas due to vegetation clearance, changes in soil composition, increased edge effects, etc. (Godefroid & Koedam, 2004). Additionally, roads typically experience higher human activity, which generates noise and pollution. These disturbances could attract mice to road edges or deter mice from crossing roads. One study that focused specifically on mouse densities around roads found there was no effect of roads on mouse densities in Spring and a positive effect in Summer, and it concluded that the negative impacts of roads on mouse movement were outweighed by the positive effects of road edges or some other unknown positive resource roads provide (Rytwinski & Fahrig 2007).

When considering urban and highly suburban systems, a deep understanding of these ecological relationships does not exist. Yet, to develop species-targeted strategies for addressing tick-borne diseases in urban areas with high human usage is needed, especially as shrinking barriers between humans and wildlife increases tick bite risks. By analyzing the relationship between small mammals and their habitats, I hope to identify high-density areas where disease transmission is likely to occur and where interventions may be most effective. Ultimately, this nuanced approach can lead to more sustainable outcomes in controlling tick-borne diseases while mitigating the risks posed by closer human-wildlife contact.

The primary objectives of this chapter were to assess *Peromyscus spp.* densities across six unique urban sites and examine their relationship with environmental covariates that may influence these densities. Current literature on mice densities in urban-suburban areas is limited; therefore, analyzing their populations in these urban green spaces could aid park managers in planning that considers small mammal habitats and densities. I hypothesized that mouse densities would be higher in the more space-limited areas of RC, where dense vegetation and canopy

cover create smaller, more fragmented habitats. These conditions may offer more shelter and food sources, as well as limit dispersal, which can create higher mouse densities (Berl et al. 2018). In contrast, I expected mouse densities to be lower in the larger, more exposed spaces of CJ, where more space could create lower densities or less forest cover might make it harder for mice to thrive despite the space. This hypothesis was based on the idea that *Peromyscus spp.* may occur at higher densities in urban parks with a greater proportion of forest edge habitat and fewer open spaces, as these conditions confine them to the limited suitable habitat available.

MATERIALS AND METHODS

Study Area

For a detailed overview of my study sites, please refer to Chapter 1. In the following sections, the three distinct sites at Cabin John will be designated as CJ1, CJ2, and CJ3, while the sites at Rock Creek will be labeled as RC1, RC2, and RC3 (Figure 1).

Trapping and Animal Handling

Please see Chapter 1.

Spatial Analysis

I analyzed land cover characteristics immediately surrounding each of the 6 trap sites. Home range size of *Peromyscus spp.* can vary between 156 and 20,730 m^2 (Wilder and Meikle 2006, Marrotte et al. 2017, Hummell et al. 2023). Given this broad range of home range sizes and the limited space in which traps were distributed, I created variables across multiple scales to represents fine-scale and coarser-scale landscape variables at each trapping location. I chose

landscape characteristics based on the previously discussed literature regarding habitat used by mice, including elevation; slope; density of trails, roads and water; distance to trails and water; forest density; and shrub densities.

The spatial data used for land cover was obtained from the Chesapeake Conservancy High-Resolution Land Cover dataset, which had a resolution of 1 meter for Montgomery County, Maryland (CBPO 2022). Road data for the area was sourced from the U.S. Census Bureau's Tiger/Line shapefiles (U.S Census Bureau 2019, TIGER/Line Shapefiles: Maryland Roads). Elevation and water data were retrieved from the MD iMAP Data Catalog, utilizing Maryland's statewide Lidar data (Eastern Shore Regional GIS Cooperative 2018, Maryland LiDAR Statewide-DEM, MD iMAP Data). Trail data was obtained from the MNCPPC Montgomery County Data Catalog.

All land cover manipulations were done in ArcPro© in the Spatial Analyst toolbox. First, landcover classes were grouped using the *reclassify* tool. The forest metrics considered included: forest, riverine wetlands forest, riverine wetlands tree canopy, tree canopy over impervious surfaces, tree canopy over roads, tree canopy over structures, and tree canopy over turf grass. These metrics were selected because mice, a semiarboreal species, rely on trees for 15% to 50% of their movements (Klein & Cameron, 2012). The shrub category included natural succession herbaceous, natural succession scrub/shrub, pasture/hay herbaceous, riverine wetlands herbaceous, suspended succession herbaceous, transitional barren, and turf grass. These classifications were selected due to GIS categorizations that often label powerline rights-of-way as pasture/hay, barren, or turf grass. Elevation was taken directly from the LiDAR DEM and the slope was calculated using the *Slope* function. Distance to trail, water, and roads were calculated using the *Euclidean Distance* tool. To assess spatial variation and landscape composition around

each sampling location, I conducted moving window analyses on various environmental layers within ArcPro© using the *Focal Statistics* tool. The moving window analysis used a specified statistical operation (e.g., mean) to calculate values across a defined neighborhood around each cell in a raster layer. This process summarized landscape characteristics within the chosen spatial scale, capturing patterns and variations relevant to the study.

For this study, I selected the following layers and scales, where each scale represents the buffered radius around the trapping grid, to capture potentially relevant ecological patterns: forest layer (50 m, 75 m, 150 m), shrub layer (50 m, 75 m, and 150 m), water layer (150 and 300 m), trail layer (150 and 300 m), and road layer (150 and 300 m). Each window size represented a specific spatial scale, allowing for the examination of how landscape features influenced the study species across different distances. For example, smaller windows (e.g., 50 m) captured fine-scale patterns directly adjacent to sampling locations, while larger windows (e.g., 300 m) accounted for broader landscape contexts. Using the *Focal Statistics* tool, I calculated metrics such as the mean proportion of a specific cover type (e.g., forest or shrub cover) and density (e.g., trail or road density) within each window. The results generated new raster layers for each combination of variable and window size. Then, all layers were averaged across the trapping grid plus the corresponding buffer size via the *Summarize by Zone* command. These methods created a comprehensive framework for exploring the home ranges of *Peromyscus spp.*, allowing for a detailed understanding of how they interact with and utilized their surrounding landscape. The integration of both moving window and zonal analyses enabled the consideration of both fine-scale habitat features (captured by the moving window) and broader landscape patterns (represented by the zonal averages), offering a more complete picture of how environmental variables influence the distribution and behavior of the mice across different spatial scales.

Density Estimation

The density of small mammal populations was modeled using maximum-likelihood estimation with spatially explicit capture-recapture (ML-SECR) using the DENSITY program [Efford MG 2012. Density 5.0]. I constructed a closed population model assuming density followed the Poisson distribution (Berl et al. 2018). Estimates for mouse densities were obtained for each specific capture period, ensuring density was derived for each trapping session and environmental variability and success were incorporated. I assigned a half-normal distribution as the SECR detection function, as it appropriately described the declining probability of detection as distance from the traps increased (Efford & Fewster, 2013). I used a Poisson distribution, because I assumed all animals had an equal, independent opportunity to be captured. I assumed this was appropriate because of the size of the study area relative to animal movements and spacing of traps relative to the average home range sizes of *Peromyscus*, which provide ample overlap and consistent catchability.

The SECR model is defined as

$$D = \frac{n}{A \cdot \hat{p}}$$

where n represented the total number of unique individuals captured, A was the effective area of the trapping grid (e.g., approximately 1 ha), and $\hat{p}$ was the estimated capture probability based on distance from the home range center. These home range centers were calculated using an exponential decay function,

$$\hat{p} = p0 \cdot exp(\frac{-d^2}{2\sigma^2})$$

where $p0$ represented the capture probability when the trap was located at the center of the home range (i.e., at zero distance), d denoted the distance from the trap to the center of the home range, and σ represented the scaling parameter for home range size. The primary reason for using an exponential decay function was it reflected the biological reality that the likelihood of capturing an animal decreased as the distance from the center of its activity area increases (Thompson, 2023).

I chose this method because it allowed us to obtain density estimates, detection functions, and home ranges for each of the two years of sampling (Freeman et al., 2022). Additionally, this model allowed population densities to be estimated without bias from edge effects and incomplete detections. I was able to create capture histories for each individual; all trap encounters were converted into frequencies of encounters of when and how often individuals were captured (0, 1, 2, 3, etc.). Trap locations were set as the detector locations (x/y). The final data organization included session ID (week of trapping event), individual ID (unique identifiers for each small mammal), occasion ID (date of capture), and trap ID (unique trap locations).

Assumptions of ML-SECR were that 1) the population was closed (i.e. no births/deaths or dispersal events during the trapping session), 2) capture did not affect the pattern of movement of an animal within a trapping session, 3) tags were not lost, and the identity and location of each recaptured individual was recorded accurately, 4) traps were set at known locations for a fixed time, 5) true trap placement was random with respect to the location of animal ranges, and ranges were oriented at random, 6) animals occupied home ranges that did not change during a trapping session, 7) home-range centers followed a Poisson distribution within the area sampled, and 8) detections happened independently for each animal.

General Linear Mixed Model

I compared models of mouse densities using a lognormal regression framework, specifically a generalized linear mixed model (GLMM) to accommodate random effects and address violations of the assumption of unrestrained, continuous response variables. GLMMs, an extension of Generalized Linear Models (GLM), can include both fixed and random effects. Random effects represent processes that were expected to impact the system but don't require directly estimate. These are often nuisance parameters which, for example, affect capture variability but not the ecological process of interest, such as time or spatial differences—like the unique conditions of different parks—without needing to model these factors as refined predictors (i.e., fixed effects). My GLMM accounted for random effects across different parks and months while addressing both between-group and within-group differences (Breslow and Clayton 1993).

I chose to run a GLMM in R (version 4.4.1) using the "GLMMtmb" package (version 1.1.10) to analyze the relationship between the dependent variable (e.g., mice per hectare) and the relevant landscape covariates. The general GLMM equation is:

$$\log(y_i) = X_i\beta + Z_i u_i$$

Here, y_i, represented the dependent variable (e.g., expected mouse density). I applied a log-link function to ensure that the predicted values of y_i remain positive, and not violate regression assumptions. The term β indicates the fixed effects, which estimate variation associate with covariates (X_i) that are expected to influence y in a consistent manner across all observations. The term u_i indicates the random effects, accounting for variability associated with groups (Z_i). The base model structure used to compare covariates was implemented in R as:

$$\text{Density} \sim \text{Environmental Covariates} + (1 \mid \text{Park}) + (1 \mid \text{Month})$$

This formulation included fixed effects for environmental covariates hypothesized to influence mouse density and random intercepts for park to account for spatial variability between study sites and month to account for temporal variability.

This mixed-effects model provided a comprehensive understanding of how fixed environmental effects, along with site and time specific random effects, contributed to the observed patterns in mouse density. To account for the relative difference in sample size between trap arrays, I incorporated the inverse of the standard error for each SECR density estimate as a weight for each data point. This weighting accounted for the varying precision of the density estimates, giving greater influence to data points with lower uncertainty. By integrating this approach, the GLMM provided a more robust framework for understanding how fixed environmental effects, along with random effects for site and month, contributed to the observed patterns in mouse density. To test statistical significance between parks and their respective density estimates, I calculated a non-parametric Wilcoxon rank-sum test to compare the independent groups and determine whether their population distributions differed.

Landscape Covariates

Before conducting GLMMs using environmental covariates, I z-standardized independent covariate data so each had a mean of zero and one unit of change was equal to one standard deviation in the original dataset. Such z-standardization enables comparisons on a common scale and clearer interpretation of their relative influence, while also allowing for easier identification of outlier values, but results were presented with the original untransformed values for easier interpretation. I used GLMMs to analyze these relationships and calculated Akaike information criterion (AIC) values using Maximum Likelihood (ML) to assess model performance and guide

model selection. ML is particularly suited for comparing models with different fixed effects structures, as it provides a consistent framework for evaluating model fit. Once the optimal model was identified based on AIC, I refitted the final model using Restricted Maximum Likelihood (REML) to obtain more reliable estimates (Bolker, 2015).

My null model included no environmental relationships but incorporated random effects for park and month to account for spatiotemporal variation. I evaluated the suitability of these random effects in my models using the intraclass correlation coefficient (ICC). An ICC score close to zero would have indicated minimal variability, suggesting that the random effect was not a significant factor in these models. Conversely, an ICC score closer to one would have indicated the random effect contributed substantially to model variability.

I analyzed all covariates for correlation strength via the Pearson correlation coefficient. For any pair of similar variables (e.g., distance to trails and trail density) with a correlation above 60%. For pairs of variables that had a high correlation, I ranked covariates based on their lowest AIC values. This ranking allowed us to contextualize relative support for each environmental covariate (Barnett et al., 2010). I retained only the variable with the lower AIC value and removed the other (Table 2). I selected eight covariates based on their AIC scores and the strength of their correlations with similar covariates, eliminating those with high similarity, as summarized in Table 3. I attempted all-possible-combinations model building but had insufficient data with only six total study sites to appropriately describe such complex relationships. Given my limited sample size, I opted not to conduct a comprehensive model-building process. I focused on comparing single-covariate models to evaluate their relative strength in estimating mouse densities. This approach allowed me to identify key relationships between specific landscape covariates and mouse densities.

RESULTS

Descriptive Statistics

I had a total of 5,940 trap nights across both trapping seasons (June-August 2022 and 2023), resulting in 770 captures of 352 unique individuals. Of those 352 animals caught, 325 were mice (*Peromyscus spp.*), 15 were eastern chipmunks (*Tamias spp.*), 4 were eastern gray squirrels (*Sciurus spp.*), 3 were southern flying squirrels (*Glaucomys spp.*), 2 were southern short-tailed shrews (*Blarina spp.*), 2 were Carolina wrens (*Thryothorus spp.*), and 1 was an American toad (*Anaxyrus spp.*). The sex ratio of captured mice, as presented in Table 4, was approximately 1:1 (177 males to 144 females), with no significant difference between the sexes (*p-value = 0.75*). This balanced ratio aligns with typical patterns observed in *Peromyscus spp.* populations. In contrast, the ratio of adults to juveniles/subadults was significantly skewed *(p-value < 0.05*), with 300 adults/subadults to 18 juveniles. This result is consistent with findings in the literature, as adults typically exhibit greater mobility, increasing their likelihood of capture.

Mouse Densities

The differences in mouse density between CJ and RC were found to be statistically significant (*W = 251, p-value < 0.001).* SECR maximum-likelihood estimation provided density estimates ranging from 0.44 to 224.06 mice per hectare across study sites within a given month (Table 5). The highest densities were recorded in June 2022 at RC2, while site CJ1 in July of 2022 exhibited the lowest density. It is important to note that the high-density estimate (224 mice/ha ± 255.61) observed in June 2022 at site RC2 may be influenced by the limited number of recaptures, which included only 18 total captures and 17 unique individuals. This low

recapture rate reduces the statistical robustness of the density estimate, potentially skewing the data representation (Table 5). Similarly, there were two instances where insufficient recapture data prevented us from producing valid density estimates. Specifically, at site CJ1 in August 2022, only 3 captures from 3 individuals were recorded, and at CJ2 in July 2023, only 5 captures from 5 individuals were observed. As such, it was more appropriate to compare median values (Table 6).

GLMM Model

The ICC analysis of my null models yielded a score of 0.995, indicating that the random effects were substantial and justified their inclusion in further model development. Evaluation of my single-covariate GLMMs identified three significant landscape covariates, each showing a negative relationship with mouse densities: elevation at a scale of 300m ($\beta = -0.730 \pm 0.110$, $p\text{-value} < 0.001$, $r^2 = 0.017$), trail density at a scale of 150m ($\beta = -0.685 \pm 0.117$, $p\text{-value} < 0.001$, $r^2 = 0.014$), and forest density at a scale of 150m ($\beta = -0.673 \pm 0.177$, $p\text{-value} < 0.001$, $r^2 = 0.016$) (Table 3, Figures 4-6). Mouse densities decreased with 1 standard deviation increases of 0.48 m (± 0.11) for elevation, 0.50 m^2 (± 0.117) for trail density, and by 0.51 m^2 (± 0.177) for forest density.

DISCUSSION

Density Estimates

My findings supported my hypothesis that mouse densities would be higher in the space-limited areas of RC compared to the more open canopy and larger spaces of CJ. Consistent with my predictions, RC's dense vegetation and limited open space provided conditions conducive to higher mouse densities. This was likely due to the greater availability of food resources and

protective cover from predators allowing for generally higher density and space-limitations and hard forest edges constraining the population.

My SECR model yielded density estimates ranging from 0.44 to 224.06 mice per hectare across the study sites (Table 5), with median densities spanning from 0.66 to 31.00 (Table 6). In comparison to the more agricultural study conducted by Berl et al. (2018), which had average ranges from 9.87 to 19.58, my urban study revealed significantly greater variation in densities. The statistical difference in mouse densities suggested that these two parks varied enough to create distinct impacts on mouse populations. These differences were likely driven by a combination of resource availability, predation pressures, human disturbances, and other ecological factors that collectively influenced mouse density. Hannebaum et al. (2017) found that *P. leucopus* generally select habitat types that maximize their fitness, suggesting that these environmental variables play a critical role in determining habitat preference and population dynamics. Consequently, the disparities in environmental conditions between these two parks were likely key drivers of the statistically significant differences in mouse densities observed. However, it is worth noting that there was extremely high levels of variability across these urban sites. Such diverse land cover and use across such small spaces likely created substantial variability in resource availability and disturbance levels, which affected population estimates. Thus, urban park heterogeneity likely affected the generalizability of my findings and emphasized the need to replicate these kinds of studies.

Interestingly, mouse populations showed spatial variation in density along a gradient defined by several environmental covariates. Elevation, trail density, and forest density were related to mouse densities (Table 3, Figures 4-6). All variables exhibited a statistically significant and strongly negative relationship with mouse densities. Yet, as one of the most widespread and

abundant small mammals in the region, *Peromyscus spp.* were widely regarded as habitat generalists, capable of thriving in a broad range of environments (Adler & Wilson, 1987). However, specific environmental features appeared to influence their habitat preferences, particularly at lower elevations. My findings did support previous observations that small mammal diversity is often greater in low-lying riparian areas compared to upland regions (Doyle, 1990). That relationship may have stemmed from the influence of elevation on vegetation types within urban green spaces, potentially creating higher-quality habitat at lower elevations. Greater food availability and enhanced overall habitat quality at lower elevations may play a key role in the higher mouse densities (Brooks et al., 1998). For instance, mice tend to favor areas with hardwood forests, large volumes of downed woody debris, abundant stumps and logs, and dense ground cover (Brannon, 2005). Webster and Jenkins (2005) observed the highest levels of downed woody debris at mesic sites, often associated with streamside areas, similar to those in RC study sites. However, their study was conducted in a rural environment, which may not reflect the same conditions present in my urban study area. In RC, anthropogenic disturbances have historically been high and continue to impact the site, likely contributing to the significant reduction in downed woody debris observed here.

Mouse densities decreased with increasing trail densities within these parks. Trails were likely fragmenting existing habitats, altering vegetation structure, impacting predator-prey dynamics, or mouse immigration and emigration. My findings contrast with those of Berl et al. (2018), who found that mouse densities declined with increasing distance from habitat edges, however the habitat patch sizes in my project are much smaller than those of Berl et al. (2018). Additionally, I was analyzing trail density, which likely had more compounding impacts on mice that the simple straight distance to trail, and Berl et al. (2018) was look at agricultural-forest

edges. My findings differ from those of Rytwinski (2006), who reported a significant positive effect of road density on mouse relative abundance in the urban landscapes of Ontario. However, my study area was considerably more urbanized, with over 80% of the general area covered by urban development. Additionally, while the population of Montgomery County is comparable to that of the Ottawa region studied by Rytwinski, Montgomery County occupies only half the area, highlighting differences in urban density and landscape composition. These discrepancies suggests that the highly urban environment of my study areas may be influencing the results of my study, revealing uniquely urban relationships. For instance, high trail densities in my study areas could indirectly impact mouse populations by creating favorable conditions for predators, thereby increasing predation pressure. A predation study by Miller & Hobbs (2000) found that mice appeared to avoid artificial nest sites located near trails, suggesting that recreational trails and human activity may disrupt predator-prey dynamics. These findings underscore the importance of considering the ecological consequences of habitat alterations in human-dominated landscapes, especially at rates seen in highly urban areas.

Lastly, forest density was shown to have a significant negative effect on mouse densities, indicating large areas with closed canopies have less mice. This aligned with findings from Buckner and Shure (1985), who documented *Peromyscus spp.* occurred at high densities in forest openings of all sizes throughout the southern Appalachians. Their study also noted that larger forest openings promoted vegetation growth due to greater resource availability. They observed that *P. leucopus* readily inhabited openings of all sizes, while colonization by other *Peromyscus spp.* increased as openings expanded. The negative forest density relationship I documented strengthens the argument found in literature that edge habitat may be driving higher species densities not only in rural areas but also highly urban areas. So, while a minimum threshold of

forest is needed, the edges likely increased the local carrying capacity. My study highlighted some unique and some consistent dynamics of mouse densities in urban environments, revealing how various landscape covariates impact small mammal populations.

The highest densities of *Peromyscus spp.* were recorded in RC2, an area characterized by low elevation, dense shrubs, complex forest openings, and abundant nearby water sources, which may indicate greater availability of food resources. Located within a wetland ecosystem, RC2 featured an intricate vegetative structure that supported ample habitat for mice, providing not only shelter but also an extensive range of foraging opportunities. The high vegetation density and structural complexity contributed to the availability of essential resources, which were crucial for sustaining elevated populations. This relationship between vegetation density and small mammal density was identified as critically important in a study conducted by Monamy and Fox (2000). Additionally, RC2 was largely undisturbed, with minimal human interference apart from a few infrequently used trails, which minimized disruptions to the habitat and allowed for more stable and suitable conditions. Those conditions likely supported higher mouse densities compared to more frequently trafficked areas. The convergence of these characteristics—abundant resources, dense cover with forest openings, and minimal human presence—made RC2 an ideal environment for *Peromyscus spp.* (Monamy and Fox, 2000).

Conversely, the lowest mouse density was observed at site CJ1, an area that presents challenging conditions for supporting substantial rodent populations but was a common urban park set-up. Characterized by limited shrub density, CJ1 likely offered sparse shelter and reduced food sources, both of which were essential for mice to thrive. This site's proximity to major highways and numerous human trails contributed to frequent disturbances that further disrupted habitat suitability. Regular human traffic and noise, coupled with the fragmentation of

vegetation, likely made this area less hospitable for mice compared to more secluded sites. Additionally, CJ1 was adjacent to a campground known for hosting events, such as Girl Scout camping and similar group activities, which could increase disturbance from visitor presence, temporary structures, and nighttime activity. These combined factors—scarcity of resources, lack of dense cover, and high levels of human interaction—likely created an environment where mice faced challenges in establishing stable populations, resulting in notably lower densities compared to other, less-disturbed sites.

This study emphasized the need for further investigation using landscape metrics, such as those provided by Fragstats, to assess landscape connectivity, edge habitat, and clumpiness of the landscape (McGarigal 1995). These metrics can offer deeper insights into how spatial arrangements of habitats influence mouse densities and movement patterns. Future research that incorporates these landscape metrics will likely capture the unique characteristics of urban settings.

Note, in areas with low recapture counts, such as those observed at site RC2 in June 2022, CJ1 in August 2022, and CJ2 in July 2023, the challenges of estimating accurate population densities become evident. Limited recapture data in these instances can lead to underestimations or skewed density figures, underscoring the importance of larger sample sizes and consistent recapture rates for reliable density estimation. To address these gaps, future research could increase the number of trap nights to obtain more recapture data or explore alternative methods for estimating densities with limited recaptures. Approaches such as occupancy models or Bayesian methods could be used to account for detection probabilities and variability in recapture rates. This approach would also enable a more nuanced understanding of population distributions, critical for management and conservation efforts, especially in complex

urban environments where populations are subject to fluctuating conditions. With this in mind, it is worth considering whether some of the observed results were influenced by how I calculated the moving windows. An alternative approach, such as conducting analyses by trap location with more site-specific information, could provide a finer-scale understanding of population dynamics. Future studies might explore different methods for calculating moving windows to account for localized habitat features and urban heterogeneity, potentially leading to more precise and actionable insights.

The relationship between *Peromyscus spp.* densities and urban environments remains poorly understood, with limited exploration in the current literature. I sought to address this gap by investigating how various urban landscape features influence mouse densities via statistical models to identify areas that may contribute to higher densities. I found that the structure of urban landscapes involved intricate interactions among environmental factors, each of which may significantly impact small mammal populations. These results underscored the need to further examine these multifaceted relationships in urban ecosystems, as they likely play a critical role in shaping the distribution and density of *Peromyscus spp*. Understanding these dynamics is essential for informing urban wildlife management and zoonotic disease mitigation strategies.

Management Implications

These models indicated several key management implications for park managers developing integrated pest management (IPM) strategies to control mouse populations. Effective mouse management is essential for ecological balance, for reducing health risks, and reducing nuisance behaviors of mice located near suburban-urban housing. More specifically, mouse

densities—both at the small trapping scale and across entire parks—carry significant implications for public health, as mice are well-known reservoirs of zoonotic diseases, including *B. burgdorferi* pathogen, hantavirus, and Powassan virus. My findings underscore the importance of adopting a holistic IPM approach that considers the full range of landscape and environmental factors influencing mouse densities. Fragmented trail areas warrant close examination, as these zones may create unique ecological dynamics, including altered predator-prey interactions and resource availability, which can significantly affect mouse populations. Forest openings, both large and small, should also be a focal point, given their potential to support high densities of *Peromyscus spp.* due to enhanced vegetation growth and resource release. By integrating these specific habitat features into monitoring and management strategies, IPM efforts can more effectively mitigate the risks associated with mouse populations, such as zoonotic disease transmission, while promoting healthier ecosystem dynamics in urban green spaces.

Urban parks are inherently diverse landscapes, featuring everything from dense forest patches to open fields and human trails. This diversity requires managers to avoid narrowly focused strategies, such as concentrating solely on one factor, overlooking other key drivers of mouse populations. Furthermore, my research highlighted the importance of accounting for resource availability across different park habitats to develop balanced, adaptive management plans. Effective IPM may involve a combination of strategies, from targeted treatments such as rodenticides or tick tubes, and habitat modifications to public awareness initiatives aimed at minimizing human-wildlife interactions.

Finally, the consistency of field data collection is essential for successful management, and this relies on thorough training and clear protocols for field staff and volunteers. Fieldwork

challenges—like early mornings, inclement weather, or lengthy field days—can introduce data inconsistencies, which underscores the need for robust training and supervision to ensure data accuracy. In summary, an integrated IPM approach that respects all relevant landscape variables will not only enhance mouse population control but also help protect public health and sustain the ecological integrity of urban park environments for the long term.

These findings have important management implications, suggesting that urban planners and wildlife managers should consider the multifaceted relationships among these variables when developing strategies for urban wildlife conservation. Given the highly stochastic nature of urban environments, it is crucial to adopt a holistic approach that addresses not just individual habitat features but also their interactions.

CHAPTER 3: WHITE-FOOTED MICE DENSITIES, HOST TICK LOADS, AND TICK-BORNE PATHOGENS ACROSS TWO DISTINCT URBAN GREEN SPACES

INTRODUCTION

Lyme disease is now widespread across the Northern Hemisphere and continues to increase globally, driven by multiple environmental factors and greater human incursion into habitats that harbor the causative spirochete bacterium (Radolf et al. 2021). Tick-borne diseases are on the rise in the United States, with Lyme disease accounting for the majority of reported cases (Rosenberg et al., 2018). In Maryland, where this research was conducted, Lyme disease was first identified in 1979 and designated a reportable zoonotic disease in 1989 (Strickland et al., 1994). Currently, Maryland ranks among the top 10 states for annual Lyme disease cases (CDC, 2020). Lyme disease is caused by *B. burgdorferi*, a spirochete bacterium maintained through a host-parasite transmission cycle involving the blacklegged tick (*Ixodes scapularis*) and several vertebrate hosts. Ticks are the primary vectors for the Lyme disease pathogen. When these ticks feed on infected animals, such as *Peromyscus spp.* or white-tailed deer (*Odocoileus virginianus*), they acquire the bacteria. When the infected tick bites a human, it can transmit the bacteria, leading to illness. In this system, ticks' primary role is transmission, where ticks serve as carriers of the Lyme disease pathogen (Radolf et al. 2021). In addition to Lyme disease, several other tick-borne pathogens in the environment can be transmitted to humans through tick bites. These include *Babesia microti* and *Anaplasma phagocytophilum*. *Babesia microti*, a blood-borne parasitic protozoan, is transmitted by blacklegged ticks and causes babesiosis—a malaria-like illness characterized by fever and hemolysis (CDC, 2024). *Anaplasma phagocytophilum*, the bacterium responsible for anaplasmosis, is also spread by blacklegged ticks and can lead to

severe symptoms, including headache, muscle aches, respiratory failure, and, in some cases, death (CDC, 2024).

Peromyscus spp. are a common host for ticks during their immature stages. Mice are one of the most competent reservoirs for several tick-borne pathogens (LoGiudice et al., 2003). When ticks feed on infected mice, they acquire the bacteria and can later transmit it to humans and other animals through subsequent bites (Radolf et al. 2021). One study found that, on average, *Peromyscus spp.* carried approximately 23 ticks, although there was considerable variability in tick loads among individuals. Some mice hosted over 200 ticks, underscoring significant differences in ectoparasite, organisms that live externally on another animal, burdens within the population (Ferro, 2014). This variation may be influenced by several factors, including habitat conditions, host behavior, and the availability of suitable tick hosts, indicating that certain individuals may be more susceptible to tick infestations than others. According to Ostfeld, a leading researcher in mouse and tick ecology, "[mice] are by far the most important host in determining our risk of exposure to Lyme and other tick-borne diseases" (Ferro, 2014).

Given the overlap between *P. leucopus* habitats and human-modified landscapes, coupled with the presence of ectoparasites associated with these mice, the potential for *B. burgdorferi* transmission in urban settings is substantial. Urbanization can modify the biology and population densities of both ticks and their hosts, potentially resulting in heightened transmission of pathogens between vectors and urban-adapted hosts (Rizzoli et al. 2014). The construction of roads, buildings, and other anthropogenic modifications often leads to habitat fragmentation and spatial limitations for various species. However, some species, such as the *Peromyscus spp.*, can adapt to these changes, and some studies even suggest a positive correlation between road density and *P. leucopus* abundance (Rytwinski & Fahrig, 2007). Although *P. leucopus* may

reduce foraging activity in areas with high human presence, they do not avoid these areas entirely, and human presence does not always directly alter foraging behavior (Persons & Eason, 2017).

This study aims to investigate the relationship between *Peromyscus spp.* densities and the presence of tick-borne diseases, including Lyme disease, babesiosis, and anaplasmosis, in urban green spaces. By identifying relationships across these shared environments, I seek to enhance the understanding of the spatial distribution of these diseases in areas frequented by human visitors. My primary objectives were to assess how the small mammal community and habitat structure related to prevalence of ticks on hosts and the prevalence of pathogens in attached ticks and small mammals across two unique urban parks. I hypothesized that mouse densities would have a direct positive relationship with the proportion of infections observed in the landscape.

MATERIALS AND METHODS

Study Area

Please see Chapter 1 and 2.

Trapping Methods

Please see Chapter 1 and 2.

Tick Collection and Disease Analysis

Upon concluding animal processing during captures, field teams conducted a meticulous examination for ticks on each animal for a minimum of one minute. Searches concentrated around the ears and faces of the captured mice, as ectoparasites frequently inhabit such areas

(Ostfeld et al., 1993). Ticks were carefully collected for pathogen analysis at the University of Maryland's Fritz Lab within the Department of Entomology, and the total count of ticks per animal was documented. After processing, each animal was observed for any signs of negative reactions to handling, such as lethargy or difficulty breathing. Ultimately, all animals were released back into their original habitats to reduce disruption to their established territories.

Field teams conducted tick collection using a comb and tweezers to gently detach ticks from the animals, which were primarily located around their ears and faces (Card et al., 2019; Kwak et al., 2021; Milholland et al., 2021; Ostfeld et al., 2018). Using the latest data from the CDC (2024), I identified each tick based on species, life stage, sex, and level of engorgement. Engorgement was determined by a visual inspection for the presence of a blood meal. I labeled all collected ticks with the unique identification number of the small mammal they were removed from and subsequently stored in a freezer at -80°C (Najjar et al., 2020). A 2-mm ear punch was taken from each individual mouse for tissue analysis, preserved in RNAlater and placed in a freezer set at -10°C (J.M. Anderson & Norris, 2006; Milholland et al., 2021; Owen M.B. et al., 2019). At the conclusion of each field season, all tissue samples and ticks were sent to either the State University of New York (SUNY) Upstate Medical University or the Fritz Lab at the University of Maryland for testing of various tick-borne pathogens, with particular emphasis on the Lyme disease spirochete, *B. burgdorferi*.

DNA Extraction and qPCR

Ear punches were collected in the field, stored in *RNAlater* in a -80 freezer, and samples were processed in a BSL2 environment at the University of Maryland. Testing was conducted by Dr. Fritz and Thea Bliss. Based on standard protocols for Qiagen DNeasy® Blood and Tissue kit

[Hilden, Germany], samples were then placed in individual lysis tubes with a metal bead and vortexed for 90 seconds to break down tissue. Each tube received 180µL of lysis buffer (ATL) and 20µL of proteinase K, then was vortexed and incubated at 56°C for 48 hours. After incubation, 200µL of a lysis buffer (AL) and 200µL of 100% ethanol were added. The samples were vortexed and transferred to Qiagen spin columns, where they were spun at 8000 rpm for 1 minute. After discarding the flow-through, 500µL of AW1 wash buffer was added, spun again, and the flow-through discarded. The same process was repeated with AW2 wash buffer, spun at 14000 rpm. To dry the matrix, tubes were spun an additional minute. The columns were then transferred to 1.7mL microtubes, and 25µL of PCR-grade H2O warmed to 37°C was added, incubated for a minute, then spun at 8000 rpm. Another 25µL of warmed water was added, incubated, and spun again. A total of 50µL of purified DNA was collected from each sample and stored at -20°C for qPCR (Stellar Scientific (plates) and Bio-Rad (iTaq mix). Whole body ticks were extracted similarly, except incubation lasted 24 hours and DNA was eluted in two rounds of 20uL for a total of 40uL. Samples were thawed and vortexed before adding 5µL of DNA to each well in a 96-well plate, followed by 15µL of mastermix, making a total reaction volume of 20µL. Each sample was run in duplicate alongside a standard curve and both PCR and extract negative controls. A Roche LightCycler 480 was set to 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. A melt curve was generated between 65°C and 97°C, followed by cooling to 40°C for 30 seconds. Samples that produced results were saved for Sanger sequencing to confirm their identity.

In addition to testing for *B. burgdorferi* infection, *Ixodes scapularis* ticks were also screened for *Anaplasma phagocytophilum* and *Babesia microti* infections. Primers for *Anaplasma* were obtained from Kawahara et al. (2006), while primers for *Babesia* were sourced

from Stensvold et al. (2015). Samples that tested positive were submitted to Azenta Life Sciences for Sanger sequencing. For each sample, 2µL of mastermix was added to 6µL of PCR product and incubated at 37°C for 15 minutes, followed by 80°C for 15 minutes, and held at 15°C using the Bio-Rad thermocycler. Then, 2.5µL of forward primer and 4.5µL of PCR-grade water were added, bringing the total volume to 15µL. After submission, data files were analyzed using NCBI BLAST to confirm the identity of the amplicon. Based on the qPCR results, a sample was considered positive if at least one of the two replicates had a Ct value of 40 cycles or less. To minimize the risk of reporting non-target amplification, the melt temperatures of the samples were compared to those of the controls, which ranged from 81°C to 83°C. Samples outside this range were excluded. Additionally, samples that produced double-peaked melt curves were also omitted from the results. To confirm the identity of the samples, the lower Ct value from the two replicates was sent for Sanger sequencing. A total of 53 samples were submitted to Azenta Life Sciences, with 40 identified as *B. burgdorferi* upon searching NCBI. Six samples returned as rodent species, while seven were deemed inconclusive.

General Linear Mixed Model

I used a beta regression framework with a logit link to model variation in the proportion of mice with infection across sampling sites. To investigate the relationship between infection proportion, mouse density estimates, and 17 environmental covariates, I employed GLMMs. I assessed model performance and selection using Maximum Likelihood-AIC values. After identifying the optimal model, I refitted it using Restricted Maximum Likelihood, which provides unbiased estimates of variance components, ensuring more reliable inferences about random effects (Table 7; Bolker, 2015). My null model excluded environmental covariates but

incorporated random effects for park and month to account for spatiotemporal autocorrelation in my dataset. To evaluate the contribution of these random effects to describing total variability, I used the intraclass correlation coefficient (ICC). An ICC analysis of the null models yielded a score of 1.00, indicating a substantial contribution of random effects and justifying their inclusion in further modeling efforts.

I assessed all landscape covariates from Chapter 2, along with mouse densities as an additional covariate, for statistical significance to identify the best model for predicting disease proportion. Because mouse densities had an associated error that could not easily be incorporated into the model, I also ran the GLMMs using a count index (the number of unique individuals captured/total number of captured animals per site). To address multicollinearity, I calculated Pearson correlation coefficients for all pairs of covariates. For highly correlated pairs (e.g., forest density and shrub density) with correlation coefficients exceeding 60%, I retained only the covariate associated with the lower AIC value, as detailed in Table 7. This approach minimized redundancy while maintaining the simplicity and efficiency of the model. Ultimately, I retained seven covariates based on their AIC scores and the strength of their correlations with infection proportion.

As in Chapter 2, prior to conducting GLMMs with environmental covariates, I z-standardized the independent covariate data. This process ensured that each covariate had a mean of zero and a standard deviation of one, enabling comparisons on a common scale and facilitating clear interpretation of their effects. Standardization also allowed the coefficients to reflect the influence of a one-standard-deviation change in the original dataset. We present results using the original untransformed values for easier interpretation.

RESULTS

Tissue Samples

Over the course of the two-year study, *B. burgdorferi* was detected in 113 out of 504 tested samples. These samples included ear tissue from *Peromyscus spp.*, chipmunks, short-tailed shrews, flying squirrels, and gray squirrels. After excluding recapture events, 348 unique individuals were tested, of which 84 were found to be infected with *B. burgdorferi* at some point during the sampling period (Figure 8). Of those infected individuals, 72 (85.7%) were from CJ. At the CJ sites CJ1, CJ2 and CJ3, 7/13 (53.85%), 20/30 (66.67%), and 45/68 (66.18%), respectively, individuals were infected with *B. burgdorferi*. At the Rock Creek sites RC1, RC2, and RC3, 10/103 (9.71%), 2/97 (2.06%), and 0/37 (0%), respectively, of individuals were infected with *B. burgdorferi.* A detailed visual representation of these infection rates is provided in Table 8 and Figure 9. A Wilcoxon rank sum test supported a statistically significant difference in infection prevalence between CJ and RC (*p-value* < *0.001*). Across 2022 and 2023, there was no significant difference in infection rates between the years. CJ had infection rates of 47/69 (68.12%) in 2022 and 27/45 (60.00%) in 2023, while RC showed rates of 11/122 (9.02%) in 2022 and 1/123 (0.81%) in 2023 Figure 10. Lastly, there was a single detection of *Anaplasma phagocytophilum* in the 2022 tissue samples from CJ3, indicating a coinfection in one individual mouse.

Host Ticks

During my study, I collected 125 larval ticks on 56 small mammal hosts across all my trapping efforts (Table 9). The number of ticks collected from hosts differed significantly between the two parks as indicated by a Wilcoxon rank-sum test (*p-value* < *0.05*). Notably, CJ

had a much higher tick count (116) compared to RC (9), suggesting a significantly greater tick abundance or higher levels of host infestation in that area. Most ticks were collected from *Peromyscus spp.*, except for 5 found on a single chipmunk and 13 from a single eastern gray squirrel. In 2022, 87 ticks were collected from 39 individuals, while in 2023, 38 ticks were collected from 17 individuals. Of the 125 ticks, 6 tested positive for *B. burgdorferi*, 3 for *Babesia microti*, and 2 for *Anaplasma phagocytophilum.* Additionally, 2 ticks showed coinfections with *B. burgdorferi* and *Babesia microti* (Table 9).

GLMM results

Evaluation of single covariate GLMMs identified four significant covariates related to infection proportion. Positive relationships were observed for mouse density ($\beta = 0.111 \pm 0.055$, $p\text{-value} < 0.05$, $r^2 = 0.001$), elevation ($\beta = 2.458 \pm 0.561$, $p\text{-value} < 0.001$, $r^2 = 0.709$), and distance to trails ($\beta = 2.379 \pm 0.682$, $p\text{-value} < 0.001$, $r^2 = 0.631$). However, when I substituted the ratio of unique individuals to total captures per site, the relationship remained positive but with a stronger effect size ($\beta = 0.415 \pm 0.078$, $p\text{-value} < 0.001$, $r^2 = 0.02$) (Figure 11). In contrast, water density ($\beta = -2.109 \pm 0.912$, $p\text{-value} < 0.05$, $r^2 = 0.456$) exhibited a negative relationship with infection proportion. Detailed p-values for each covariate are provided in Table 10 and illustrated in Figures 10-14. Proportion of infection increased with each 1 standard deviation increase in several covariates: by 1.076 individuals (± 0.055) for mouse densities, 2.858 m (± 0.561) for elevation, and 1.977 m (± 0.682) for distance to trail. Proportion of infection decreased with each 1 standard deviation increase by 0.166 m^2 (± 0.912) for water density. Given my sample sizes, models analyzing each individual landscape covariate are presented.

DISCUSSION

The results of this study, while generally supportive of a relationship between mouse densities and pathogens, still challenged my hypothesis that mouse densities would exhibit a direct and strong relationship with the proportion of pathogens in the environment. Although mouse density and the count index showed statistically significant effects on pathogen prevalence (*p-value < 0.05* and *p-value < 0.001*, respectively), the strength of these relationships was relatively weak. In contrast, environmental covariates, such as habitat characteristics and landscape features, demonstrated a much stronger influence on pathogen prevalence across the study sites (Table 10). These findings suggest that while host density plays a role, environmental factors may be more critical drivers of pathogen distribution in urban green spaces. While I cannot say for certain that my study area had high host diversity, my findings aligned with Brunner and Ostfeld (2008), who observed that in areas with abundant populations of alternate hosts (e.g., chipmunks), interactions between ticks and their primary hosts—mice for larvae and chipmunks for nymphs—could be reduced. This phenomenon, termed "encounter reduction" by Keesing et al. (2006), occurred when ticks were deflected away from hosts crucial to pathogen transmission. Keesing et al. further demonstrated that high host diversity tended to lower pathogen risk because the transmission of *B. burgdorferi* is frequency dependent. As such, that phenomenon is one potential explanation of the relationship I observed. Furthermore, by removing larval ticks from mice before completing their first blood meals, as expected, I observed a lower pathogen prevalence in those ticks. Ticks that feed on mice at later life stages, such as during the nymphal stage, may display significantly higher infection rates. Lastly, to get a full picture of pathogen presence, questing ticks must be collected and analyzed. My study was

part of a larger, comprehensive study examining questing ticks and their interactions with a wide range of animals across the ecosystem, extending beyond the scope of small mammals. In the future, questing tick data should be thoroughly analyzed, offering a more complete picture of the dynamics at play in this system.

While there were several limitations, my results did suggest that the individual park's landscape features drove the relationships seen in tick numbers and pathogen levels in mice. My study revealed statistically significant differences in tick counts on mice between the two parks (*p-value < 0.05*). This finding indicated that the habitat characteristics at CJ were critical to the drivers influencing tick populations when compared to RC. Specifically, the environmental conditions at CJ provided a more favorable habitat for ticks, possibly due to factors such as vegetation density, microclimate, and the availability of hosts (Estrada-PeñA, 2001). The green spaces at CJ, including powerline right-of-ways, had limited management and featured more extensive litter layers, which likely provided protection for ticks against dry weather conditions (Heylen et al., 2019). Those elements could significantly affect tick life stages and their interactions with hosts like *Peromyscus spp*. So, as opposed to mouse densities alone, areas with high elevations, reduced water density, and further distances from human trails in CJ seemed to influence pathogen prevalence in the mouse populations (Figures 10-14).

In contrast, the wetter and more diverse habitats of RC presented conditions less conducive to tick survival or reproduction (Estrada-PeñA, 2001). Concurrently, the results of my analysis revealed a negative relationship between the proportion of infection and water density in these parks (Figure 14, Table 10). As such, RC likely had less suitable tick habitats given the presence of waterlogged or wetland type areas. This relationship does not seem to be widely recognized in literature, with some studies finding no significant correlation between *B.*

burgdorferi prevalence and the presence of large water bodies (Raizman et al., 2010). However, a study by James et al. (2013) found a positive association between another *Ixodes* species, *I. ricinus*, and soil water capacity in woodland habitats; though, these factors were not linked to higher rates of *B. burgdorferi* infection. Additionally, my study revealed a positive relationship between distance from trails and the proportion of *B. burgdorferi* in hosts in the environment. These findings suggested that as the distance from a trail increases, typically happening in areas of lower trail density, so does the proportion of pathogen infection, potentially due to changes in host-vector interactions. Interestingly, RC was quite narrow, so most area was generally close to a trail, yet it had fewer ticks and almost no infection. This pattern may also be linked to greater tick exposure, as seen in my previous analysis (detailed in Chapter 2) which demonstrated a negative relationship between mouse densities and trail density.

Overall, the observations from my study showed areas with higher mouse densities, such as RC, exhibited lower levels of pathogens within the mouse populations. This finding contrasts with studies like Brunner and Ostfeld (2008), which showed that areas with higher small mammal densities often had higher tick burdens on hosts. While mice serve as competent reservoirs for zoonotic diseases, ticks are the vectors of pathogen transmission. My study suggested it would be more beneficial to model tick populations and their habitats rather than focusing solely or even heavily on mouse populations.

When analyzing differences in mouse densities, tick prevalence on hosts, and pathogen presence, the structural layout of the parks themselves was the key factor. CJ and RC differed notably in infrastructure and landscape features. CJ, situated near major roadways, offered extensive athletic facilities, well-maintained baseball fields, powerline corridors, an active train route, and large open green spaces that lacked connectivity to other green areas. Those open

areas, relatively isolated, likely created an environment conducive to higher concentrations of host-ticks and increased disease transmission. In fact, Heylen et al. (2019) found that landscape connectivity and urbanization significantly affected tick abundances, suggesting that the connectivity of CJ may foster tick aggregation and elevate disease spread risks.

In contrast, RC was a narrower, more constrained space threading through urban developments, ongoing construction, and a network of stream systems that act as natural barriers to small mammal movement (Figure 2, Table 1). The park's design, characterized by stream valleys and limited connectivity with neighboring green spaces, may have limited resource availability for mice and ticks alike, potentially reducing disease transmission from mice to ticks and, subsequently, to humans (Elmieh, 2022). The urban park configuration with limited connectivity of RC likely disrupts the habitat continuity needed to support high tick densities, contributing to lower zoonotic disease rates in this area. The distinction in tick populations underscored the importance of habitat management in understanding and mitigating the risks associated with tick-borne diseases. By identifying and addressing the specific habitat features that contributed to higher tick densities at CJ, park managers could implement targeted strategies to reduce tick populations and, consequently, likely reduce the risk of zoonotic diseases in both parks.

Primary hosts such as deer, birds, and rodents inadvertently carry ticks to new habitats as they move. However, as Madhav et al. (2004) found, hosts with small home ranges, like mice, may limit range expansion by diverting a significant proportion of ticks away from more mobile hosts. Consequently, habitat characteristics that create optimal conditions for tick survival and reproduction may have a stronger influence on pathogen transmission than mouse population density alone. These habitat characteristics could be critical to understanding and managing

zoonotic pathogen risk, as they directly affect tick distribution and survival, thereby enhancing transmission potential.

Management Implications

When managing pathogen risk, it is essential to look beyond simple metrics of mouse density and consider the broader landscape elements that shape vector ecology, particularly those that create conducive environments for ticks. This integrated perspective can support more effective and targeted management strategies that address the root ecological drivers of disease spread within urban parks and similar landscapes. Ultimately, if managers had to choose, they should prioritize managing ticks directly, as controlling tick populations can significantly reduce the risk of pathogen transmission to both wildlife and humans.

Overall, understanding how park structure influences the interconnected dynamics of mice, ticks, and infection ecology is crucial for developing effective management strategies to mitigate disease risks posed by rodent and tick populations. My study suggests that parks characterized by relatively high mouse densities, elevated terrain, and extensive dense trail systems—while having limited water sources—are associated with a heightened risk of *B. burgdorferi*. These factors could create an environment conducive to *B. burgdorferi* transmission, that may facilitate the interactions between mice, ticks, and their habitats. Consequently, such parks may present an increased risk for tick-borne pathogens underscoring the importance of targeted management strategies for ticks in these specific environments. To enhance confidence in these findings, future studies should replicate this research across diverse urban locations that encompass the full geographic range of small mammal hosts and tick populations. Replicating this study across diverse urban locations would help verify the

consistency of observed patterns, clarify the influence of local environmental factors on host and vector dynamics, and strengthen the generalizability of results for a more comprehensive understanding of disease dynamics in urban landscapes.

TABLES

Table 1. *Specific descriptions of each trap site and any relevant urban/suburban components in two parks in Montgomery County, Maryland, USA, 2022-2023.*

Site	Description
CJ1	Active campsite, proximity to interstate highway (I-270)
CJ2	Baseball field, hiking trails throughout
CJ3	Proximity to powerline flyway, active biking trail
RC1	Facilities mgmt. and storage area, active trail system, borders homes
RC2	Meadow/wetland area, active hiking, and youth camp center
RC3	Proximity to homes, active trail system

Table 2. *Correlation matrix of 17 environmental covariates and their corresponding Maximum Likelihood AIC (ML-AIC) values for selecting appropriate covariates for the study. The predictor variables included: 1) Elevation, 2) Slope, 3) Distance to trail, 4) Trail density (150m), 5) Trail density (300m), 6) Distance to water, 7) Water density (150m), 8) Water density (300m), 9) Distance to roads, 10) Road density (150m), 11) Road density (300m), 12) Forest density (50m), 13) Forest density (75m), 14) Forest density (150m), 15) Shrub density (50m), 16) Shrub density (75m), and 17) Shrub density (150m) in two parks in Montgomery County, Maryland, USA, 2022-2023.*

	AIC	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1	75	1.0																
2	84.9	0.7	1.0															
3	80.6	0.9	0.9	1.0														
4	76.4	0.9	0.7	0.9	1.0													
5	84	0.5	0.4	0.4	0.7	1.0												
6	84	0.7	0.6	0.6	0.8	0.9	1.0											
7	83.9	0.5	0.5	0.5	0.7	1.0	0.9	1.0										
8	83.4	0.8	0.7	0.8	0.9	0.8	1.0	0.9	1.0									
9	85.6	0.4	0.2	0.2	0.5	0.7	0.9	0.7	0.7	1.0								
10	83.8	0.4	0.3	0.4	0.3	0.2	0.3	0.1	0.1	0.6	1.0							
11	84.9	0.2	0.2	0.3	0.1	0.3	0.4	0.3	0.3	0.8	1.0	1.0						
12	80.8	0.5	0.4	0.3	0.6	0.6	0.5	0.7	0.6	0.4	0.2	0.1	1.0					
13	79.3	0.5	0.3	0.3	0.6	0.6	0.5	0.7	0.6	0.4	0.3	0.1	1.0	1.0				
14	78.2	0.3	0.2	0.0	0.4	0.5	0.3	0.5	0.3	0.3	0.2	0.1	0.8	0.9	1.0			
15	83.2	0.0	0.6	0.3	0.1	0.1	0.3	0.1	0.3	0.2	0.4	0.4	0.3	0.5	0.8	1.0		
16	83.6	0.1	0.6	0.4	0.1	0.2	0.3	0.2	0.4	0.2	0.4	0.4	0.3	0.4	0.8	1.0	1.0	
17	85.8	0.4	0.7	0.6	0.4	0.4	0.7	0.4	0.7	0.6	0.5	0.6	0.1	0.2	0.5	0.8	0.9	1.0

Table 3. *Model comparison of best performing landscape covariates as they related to mouse density in two parks in Montgomery County, Maryland, USA, 2022-2023*

Covariate	Scale (meters)	Estimate	Std. Error	z-value	R^2	p-value
Elevation	300	-0.730	0.110	-6.604	0.017	$p < 0.001$
Trail Density	150	-0.685	0.117	-5.850	0.014	$p < 0.001$
Water Density	300	0.445	0.278	1.597	0.006	0.11
Road Density	150	-0.389	0.277	-1.404	0.005	0.16
Forest Density	150	-0.673	0.177	-3.802	0.016	$p < 0.001$
Shrub Density	50	0.428	0.301	1.421	0.007	0.155

Table 4. *Comparison of sex and age ratios, average weight and length, of mice captured in two parks in Montgomery County, Maryland, USA (2022–2023).*

Site	Sex (% Male)	Age (% Adult)	Average Weight (g)	Average Length (mm)
CJ1	54	92	17.5	149.8
CJ2	60	100	19.9	148.5
CJ3	61	97	19.2	154.6
RC1	52	92	19.6	153.7
RC2	53	94	20.0	152.3
RC3	62	94	19.2	152.3

Table 5. *Density estimates for each month of sampling, organized by park in two parks in Montgomery County, Maryland, USA, (2022-2023).*

Density Estimates by Month										
Park	**Season**	**Captures**	**Individuals**	**Density**	**SE**	**Season**	**Captures**	**Individuals**	**Density**	**SE**
CJ1	June 2022	5	4	3.25	3.51	June 2023	3	2	0.66	0.76
CJ2		10	7	6.81	3.39		10	6	3.56	1.99
CJ3		30	22	15.49	5.73		23	15	9.17	3.30
RC1		34	21	16.53	4.70		38	22	14.95	3.85
RC2		18	17	224.06	255.61		43	35	37.35	11.97
RC3		10	7	7.90	4.42		14	10	13.83	6.99
CJ1	July 2022	9	5	0.44	0.26	July 2023	3	2	2.13	2.57
CJ2		24	10	5.70	1.95		5	5	NA	NA
CJ3		24	17	14.32	5.02		22	11	6.40	2.14
RC1		36	25	25.33	6.56		43	23	16.05	3.69
RC2		28	22	22.28	8.22		27	22	53.42	31.47
RC3		17	10	8.30	3.18		14	11	15.21	7.09
CJ1	August 2022	3	3	NA	NA	August 2023	5	3	0.54	0.51
CJ2		13	7	0.66	0.37		13	8	4.65	2.29
CJ3		34	19	15.13	3.87		22	13	6.31	2.38
RC1		33	20	13.32	3.59		36	20	12.31	3.31
RC2		29	22	24.64	8.93		30	19	6.76	2.96
RC3		22	11	3.70	2.93		12	9	13.69	6.62

Table 6. *Median density estimates with associated standard errors for each site within two parks in Montgomery County, Maryland, USA (2022–2023).*

Site	Density	Standard Error
CJ1	0.66	± 1.24
CJ2	4.65	± 2.35
CJ3	11.75	± 4.35
RC1	15.50	± 4.65
RC2	31.00	± 81.20
RC3	10.99	± 4.50

Table 7. *Correlation matrix of density and 17 environmental covariates and their corresponding Maximum Likelihood AIC (ML-AIC) values for selecting appropriate covariates for the study. The predictor variables included: D) Density, 1) Elevation, 2) Slope, 3) Distance to trail, 4) Trail density (150m), 5) Trail density (300m), 6) Distance to water, 7) Water density (150m), 8) Water density (300m), 9) Distance to roads, 10) Road density (150m), 11) Road density (300m), 12) Forest density (50m), 13) Forest density (75m), 14) Forest density (150m), 15) Shrub density (50m), 16) Shrub density (75m), and 17) Shrub density (150m) in two parks in Montgomery County, Maryland, USA, 2022-2023*

	AIC	**D**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
D	-2858	1																	
1	-2866.2	0.4	1																
2	-2862.9	0.4	0.7	1															
3	-2864	0.4	0.9	0.9	1														
4	-2869.2	0.2	0.9	0.7	0.9	1													
5	-2859.7	0.1	0.5	0.4	0.4	0.7	1												
6	-2863	0.1	0.7	0.6	0.6	0.8	0.9	1											
7	-2861.2	0.1	0.5	0.5	0.5	0.7	1.0	0.9	1										
8	-2867.9	0.1	0.8	0.7	0.8	0.9	0.8	1.0	0.9	1									
9	-2857.8	0.0	0.4	0.2	0.2	0.5	0.7	0.9	0.7	0.7	1								
10	-2856.4	0.2	0.4	0.3	0.4	0.3	0.2	0.3	0.1	0.1	0.6	1							
11	-2856	0.2	0.2	0.2	0.3	0.1	0.3	0.4	0.3	0.3	0.8	1.0	1						
12	-2860.5	0.0	0.5	0.4	0.3	0.6	0.6	0.5	0.7	0.6	0.4	0.2	0.1	1					
13	-2860	0.0	0.5	0.3	0.3	0.6	0.6	0.5	0.7	0.6	0.4	0.3	0.1	1.0	1				
14	-2857	0.2	0.3	0.2	0.0	0.4	0.5	0.3	0.5	0.3	0.3	0.2	0.1	0.8	0.9	1			
15	-2856	0.1	0.0	0.6	0.3	0.1	0.1	0.3	0.1	0.3	0.2	0.4	0.4	0.3	0.5	0.8	1		
16	-2856.1	0.1	0.1	0.6	0.4	0.1	0.2	0.3	0.2	0.4	0.2	0.4	0.4	0.3	0.4	0.8	1.0	1	
17	-2857.5	0.2	0.4	0.7	0.6	0.4	0.4	0.7	0.4	0.7	0.6	0.5	0.6	0.1	0.2	0.5	0.8	0.9	1

Table 8. *Showing the number of ticks and their associated infections (*Borrelia burgdorferi, Babesia microti, Anaplasma phagocytophilum*) across different parks and years in two parks in Montgomery County, Maryland, USA, 2022-2023.*

Site	**Season**	**Ticks Collected**	***B.b.***	***A.p.***	**Season**	**Ticks Collected**	***B.b.***	***B.m.***	***A.p.***
CJ1	2022	3	-	-	2023	7	1	1	
CJ2	2022	46	1	-	2023	5	1	-	-
CJ3	2022	34	-	1	2023	21	2	2	1
RC1	2022	3	-	-	2023	1	-	-	-
RC2	2022	1	-	-	2023	4	-	-	-
RC3	2022	-	-	-	2023	-	-	-	-

Table 9. *Proportion of infected individuals at each site during each trapping events in two parks in Montgomery County, Maryland, USA, 2022-2023.*

Infection Proportions				
Park	**Season**	**% of Infected Individuals**	**Season**	**% of Infected Individuals**
CJ1	June 2022	0.50	June 2023	1.00
CJ2		0.86		0.83
CJ3		0.74		0.80
RC1		0.19		0.00
RC2		0.06		0.03
RC3		0.00		0.00
CJ1	July 2022	0.60	July 2023	1.00
CJ2		0.80		0.80
CJ3		0.65		0.82
RC1		0.16		0.00
RC2		0.05		0.00
RC3		0.00		0.00
CJ1	August 2022	0.33	August 2023	0.67
CJ2		1.00		0.80
CJ3		0.79		0.50
RC1		0.15		0.05
RC2		0.05		0.00
RC3		0.00		0.00

Table 10. *Model comparison of best performing landscape covariates as they related to mouse infection in two parks in Montgomery County, Maryland, USA, 2022-2023.*

Covariate	Estimate	Std. Error	z-value	R^2	p-value
Density	0.111	0.055	2.006	0.001	$p < 0.05$
Count Index	0.415	0.078	5.302	0.020	$p < 0.001$
Elevation	2.458	0.561	4.38	0.709	$p < 0.001$
Distance to Trails	2.379	0.682	-3.489	0.631	$p < 0.001$
Trail Density	1.893	1.035	1.829	0.352	0.0673
Water Density	-2.109	0.912	-2.311	0.456	$p < 0.05$
Distance to Roads	1.285	1.179	1.09	0.154	0.276
Forest Density	1.102	1.259	0.875	0.110	0.381

FIGURES

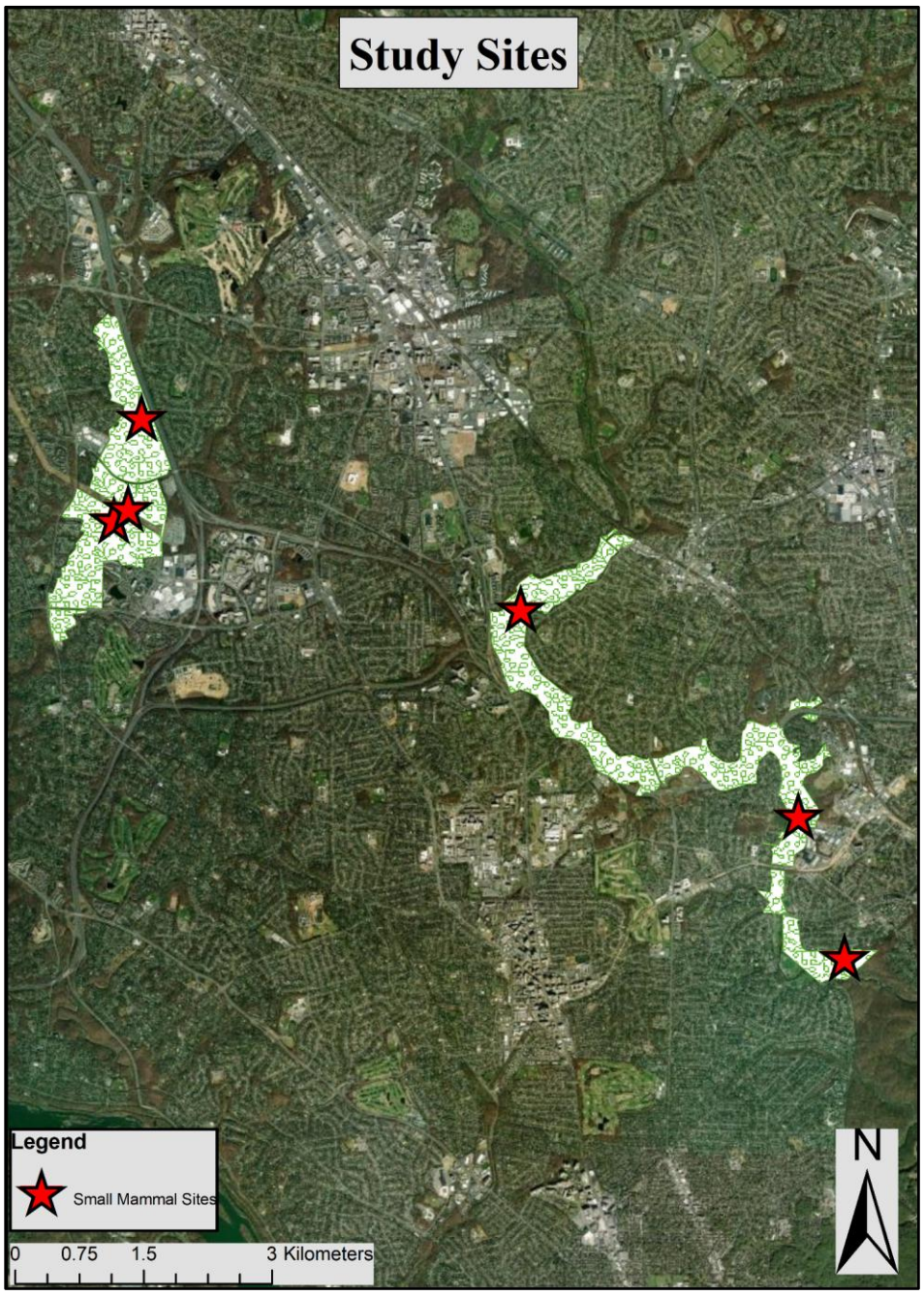

Figure 1. *Small mammal trapping sites across two parks; Cabin John Stream Valley Park and Rock Creek Regional Park, Montgomery County, Maryland, USA, 2022-2023.*

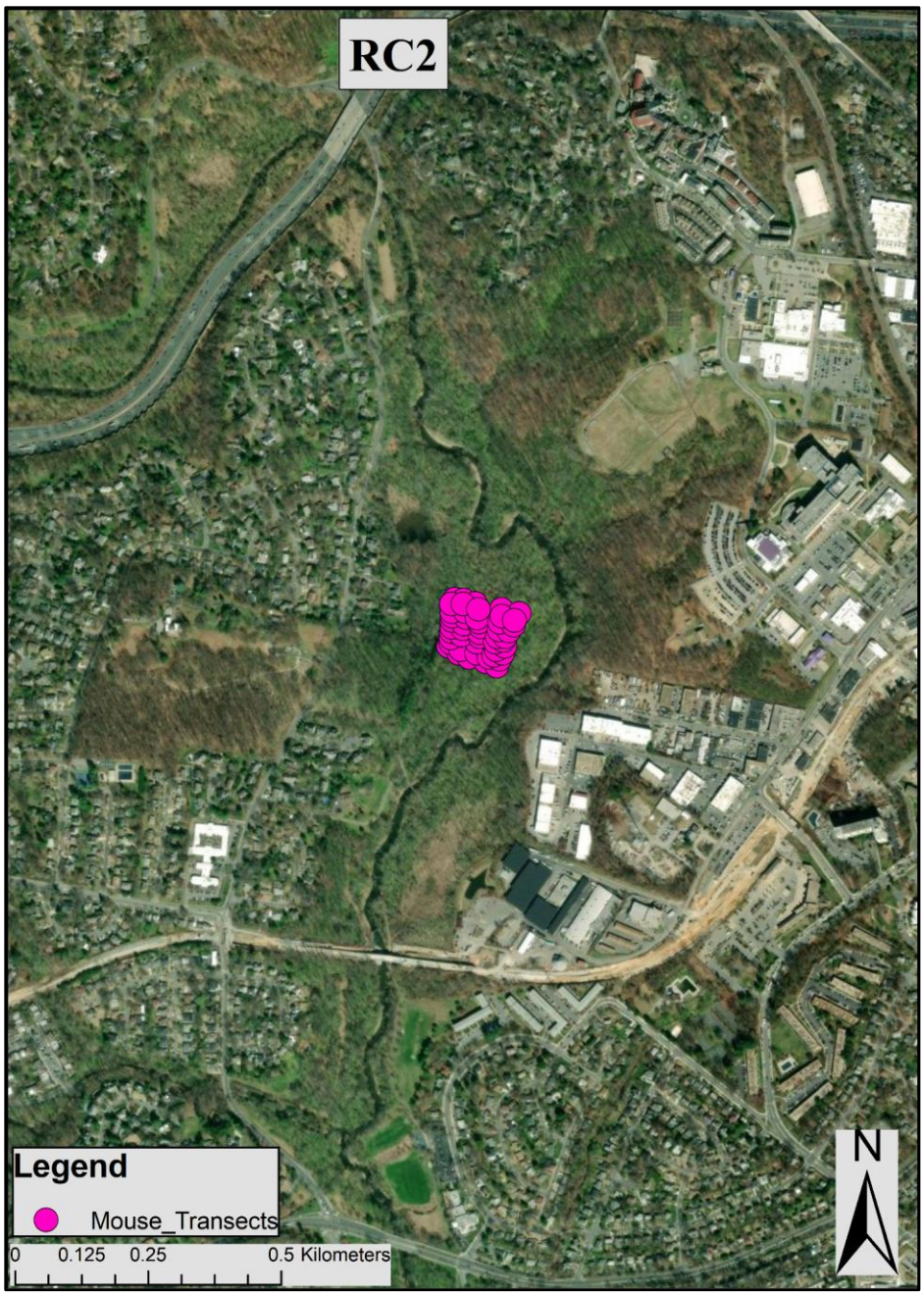

Figure 2. *Small mammal trapping site #2 in Rock Creek Regional Park, within the spatial context of the surrounding urban/suburban areas, Montgomery County, Maryland, USA, 2022-2023.*

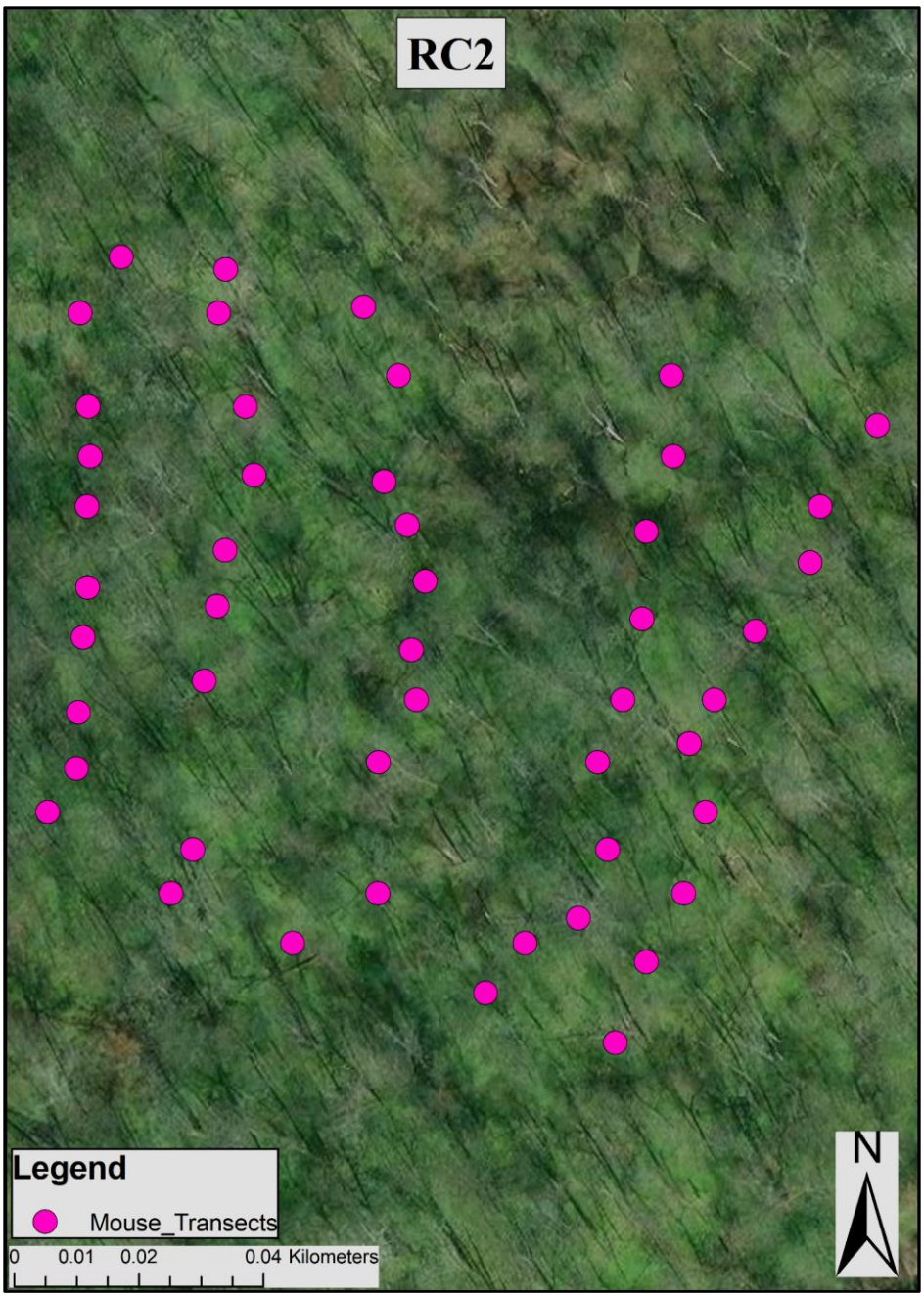

Figure 3. *Small mammal trapping transects from trap site #2 in Rock Creek Regional Park, Montgomery County, Maryland, USA, 2022-2023.*

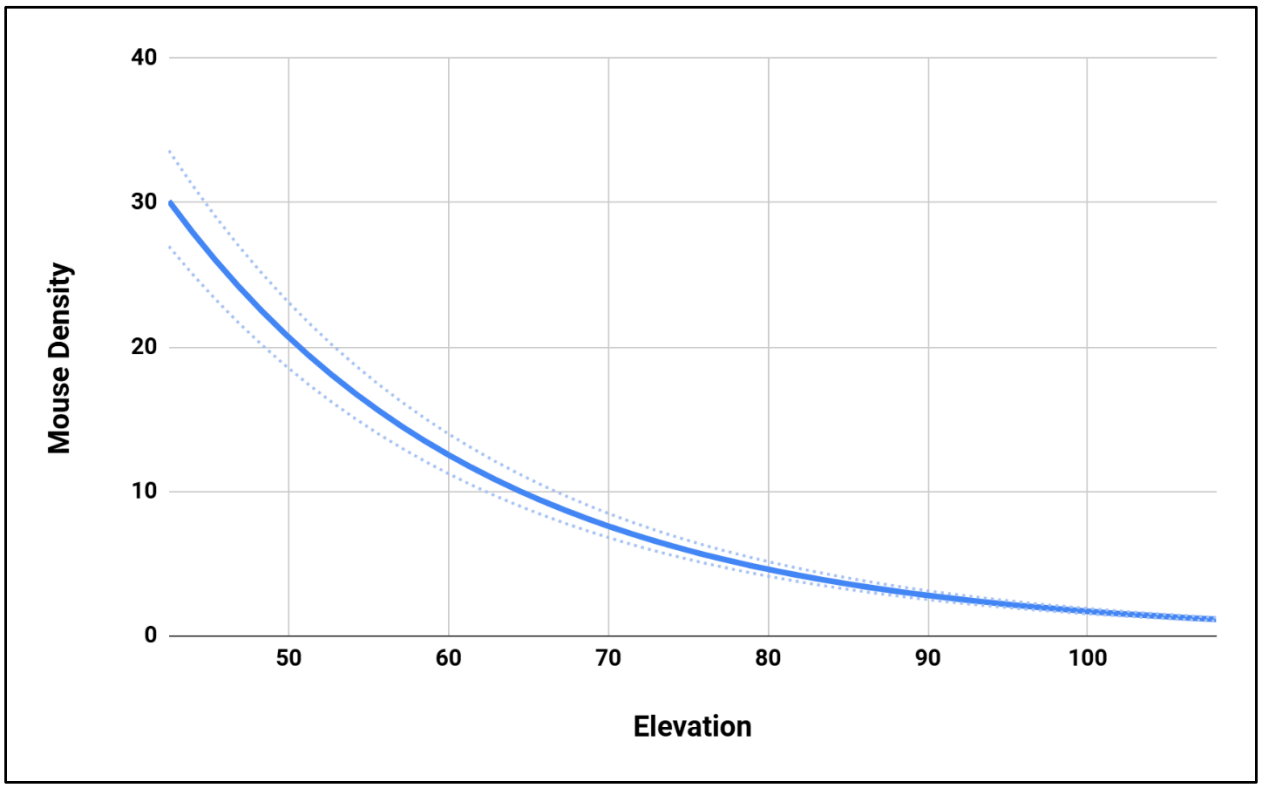

Figure 4. *Relationship between mouse density and elevation. The y-axis represents mouse density (individuals per hectare), while the x-axis indicates elevation (meters above sea level). This figure illustrates how variations in elevation correlate with the density of the mouse population, providing insights into habitat preferences and ecological dynamics in two parks in Montgomery County, Maryland, USA, 2022-2023.*

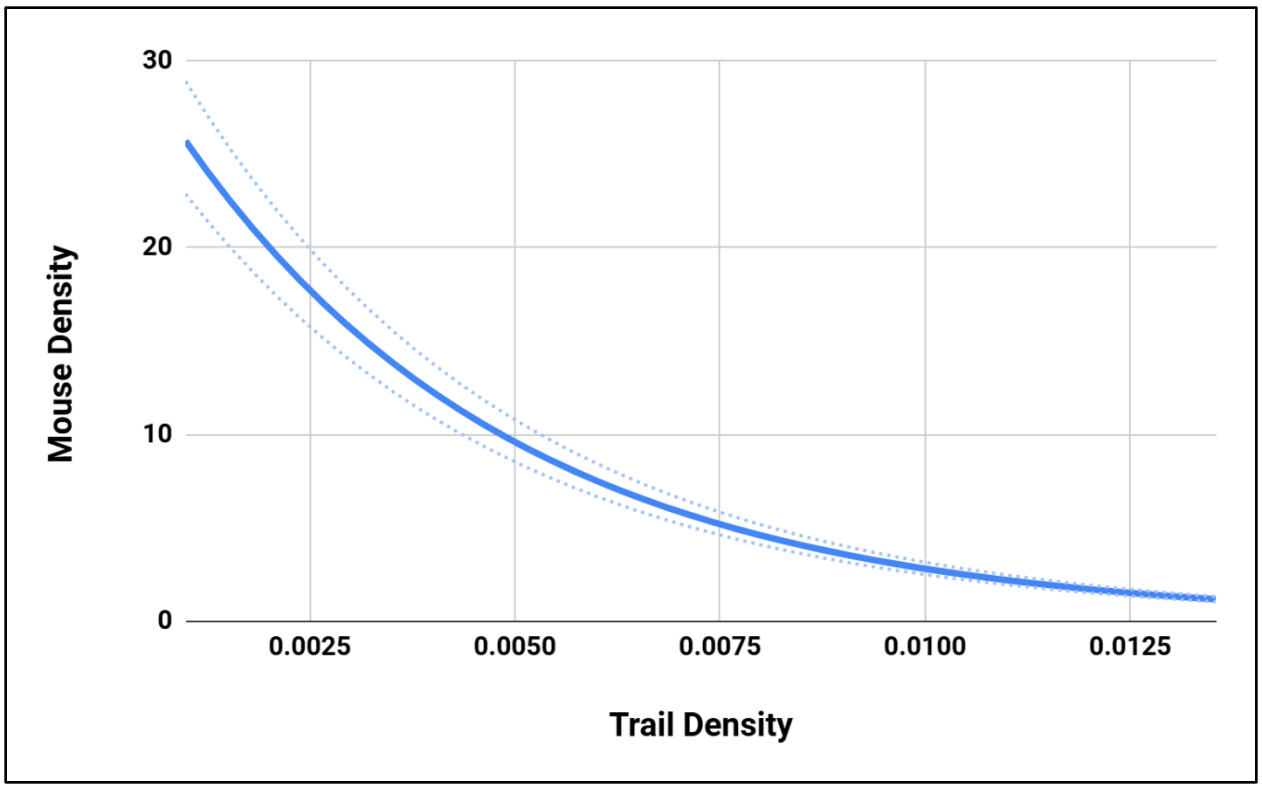

Figure 5. *Relationship between mouse density and trail density. The y-axis represented mouse density (individuals per hectare), while the x-axis indicated trail density (meters of trail per 150 square meters). This figure illustrated the correlation between the density of mouse populations and the availability of trails in two parks in Montgomery County, Maryland, USA, 2022-2023.*

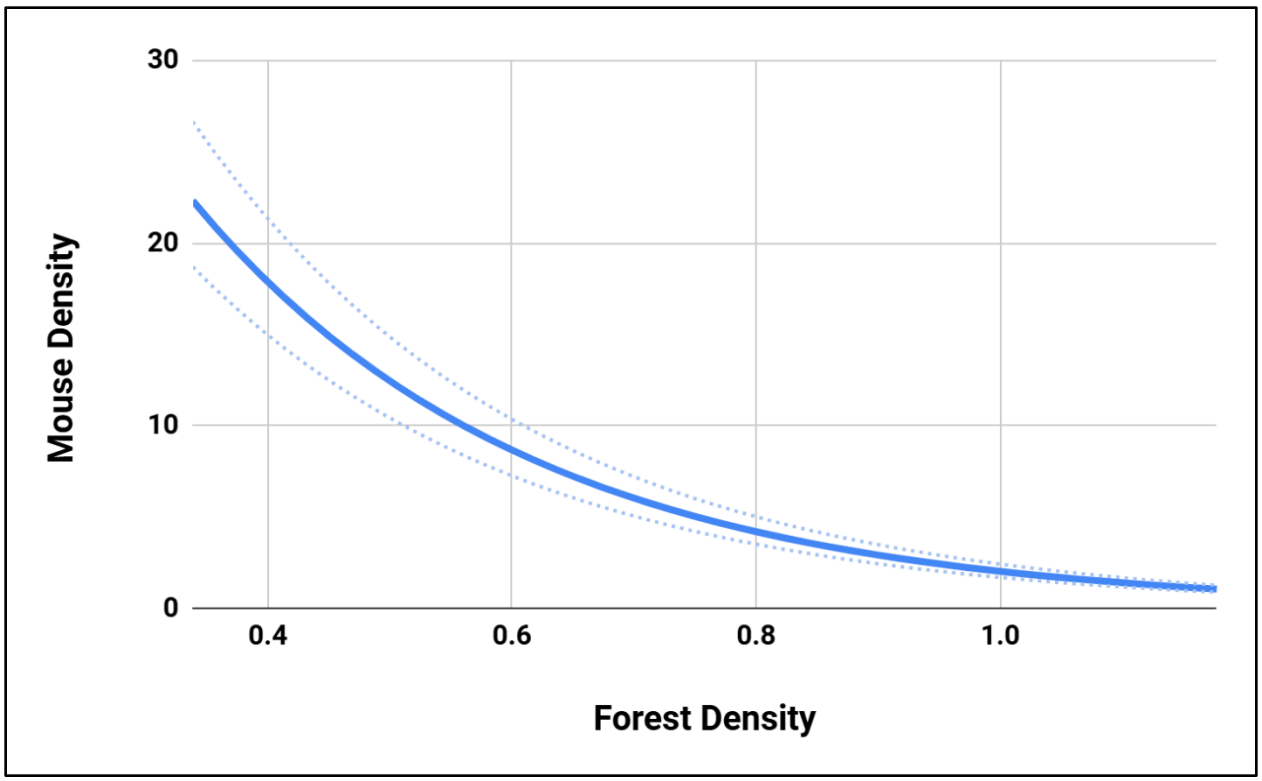

Figure 6. *Relationship between mouse density and forest density. The y-axis represented mouse density (individuals per hectare), while the x-axis indicated forest density (percentage cover as a decimal per 0.283 square kilometers). This figure illustrated the correlation between mouse populations and forest density in two parks in Montgomery County, Maryland, USA, 2022-2023.*

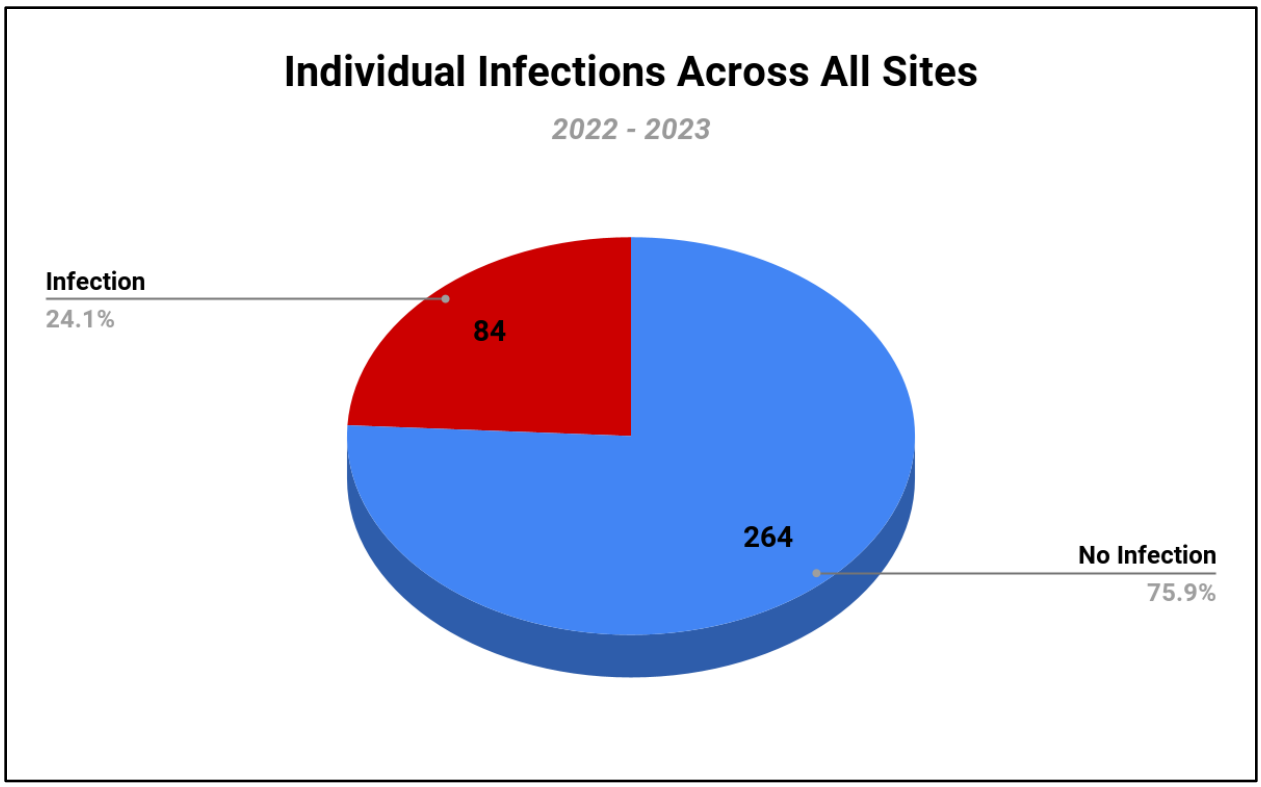

Figure 7. *Number of infected samples across all study sites for unique individuals in two parks in Montgomery County, Maryland, USA, 2022-2023.*

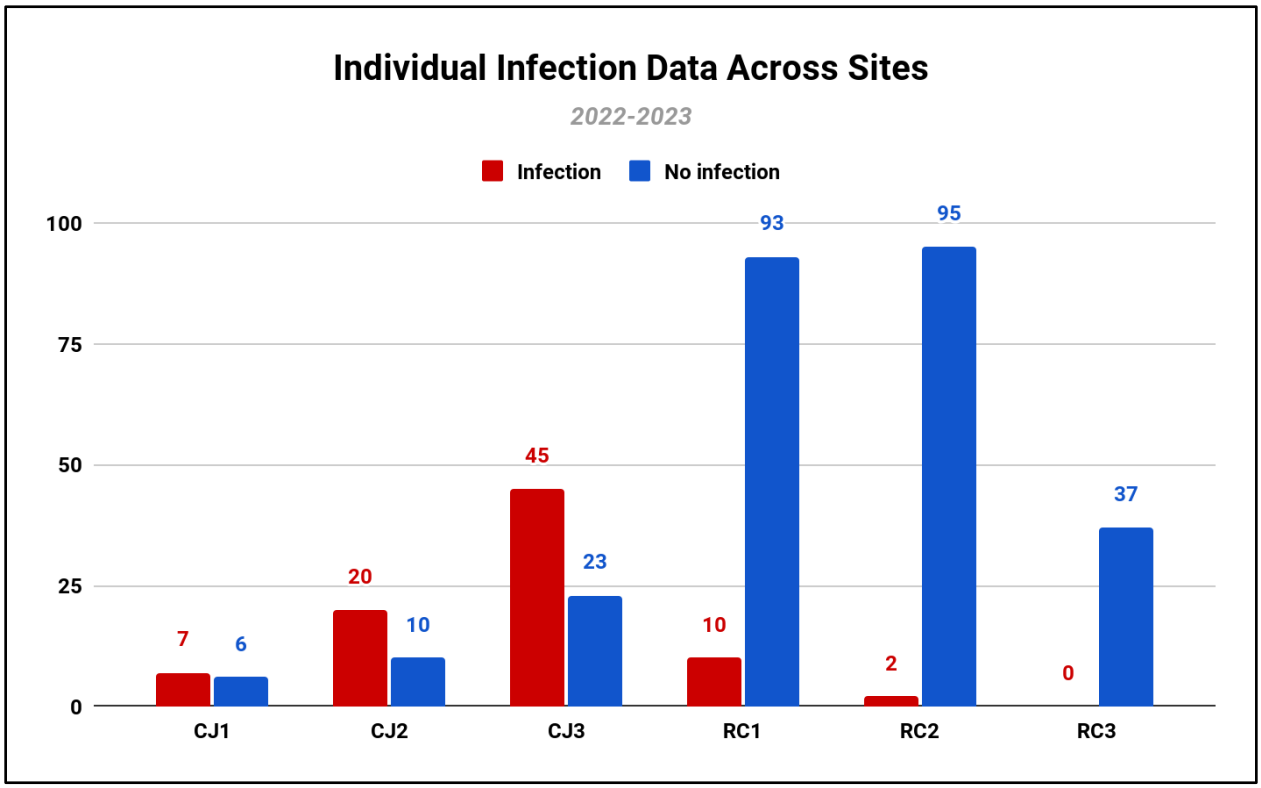

Figure 8. *Number of infected individuals across all study sites in two parks in Montgomery County, Maryland, USA, 2022-2023.*

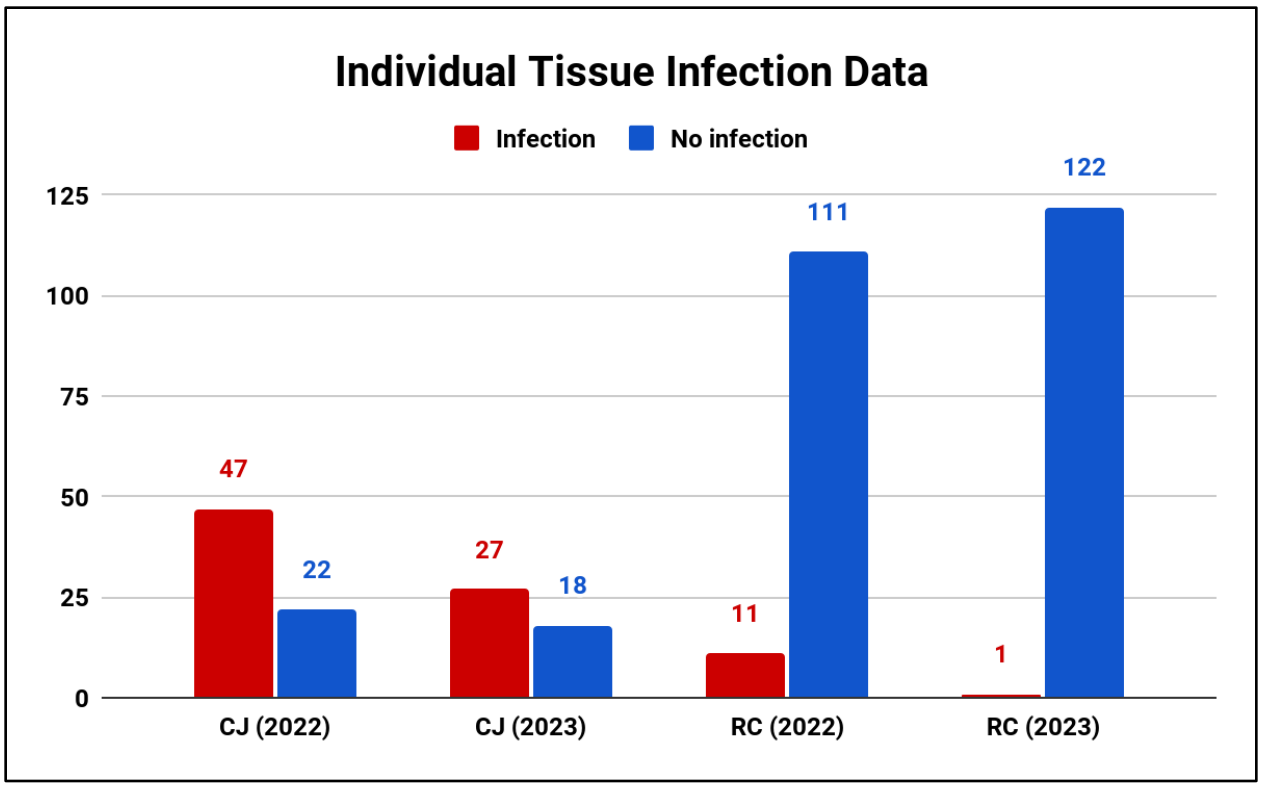

Figure 9. *Number of infected individuals by park per year across the study in two parks in Montgomery County, Maryland, USA, 2022-2023.*

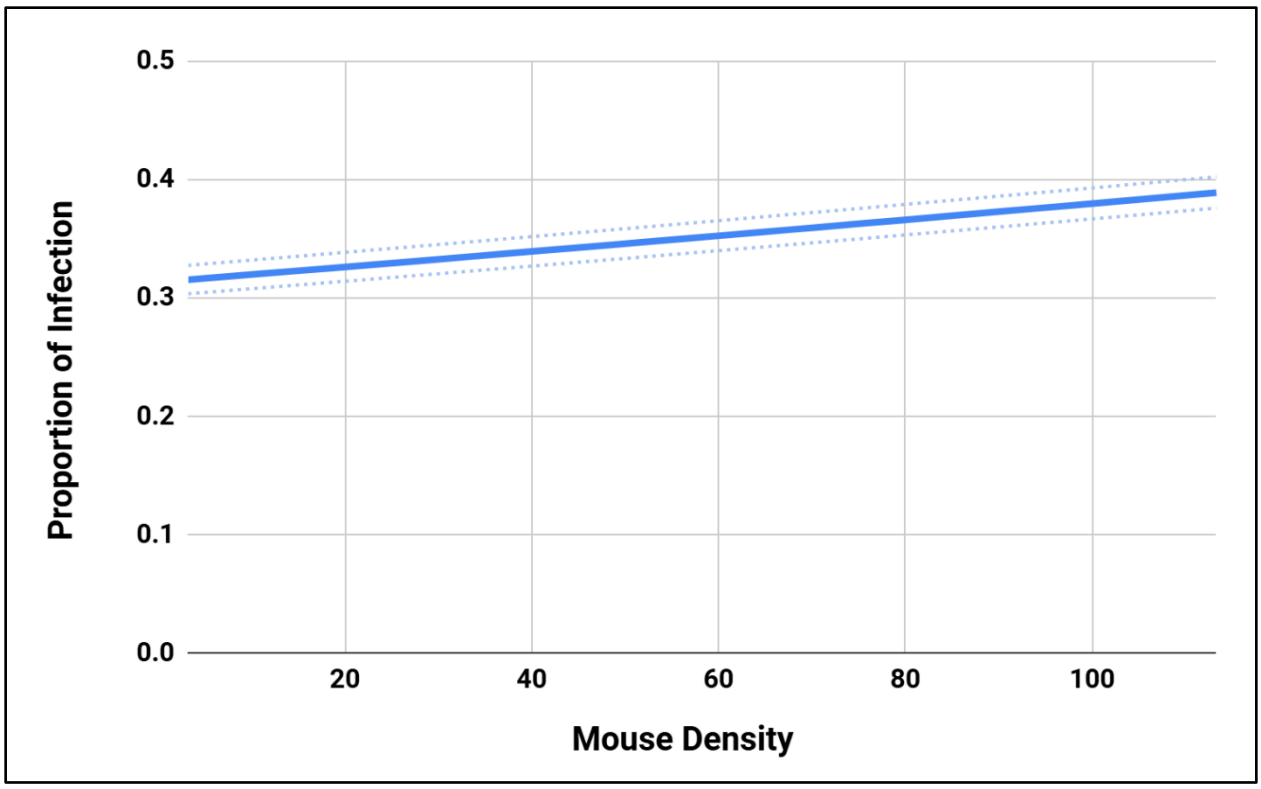

Figure 10. *Relationship between the proportion of infected individuals and relative mouse densities. The y-axis represents the proportion of infected individuals, while the x-axis indicates mouse density (individuals per hectare). This figure explores the correlation between mouse populations and the proportion of infected individuals in two parks in Montgomery County, Maryland, USA, 2022-2023.*

Figure 11. *Relationship between the proportion of infected individuals and mouse index of captures. The y-axis represents the proportion of infected individuals, while the x-axis indicates index of captures (individuals per hectare). This figure explores the correlation between mouse populations and the proportion of infected individuals, highlighting how variations in mouse populations may influence disease prevalence in the population in two parks in Montgomery County, Maryland, USA, 2022-2023.*

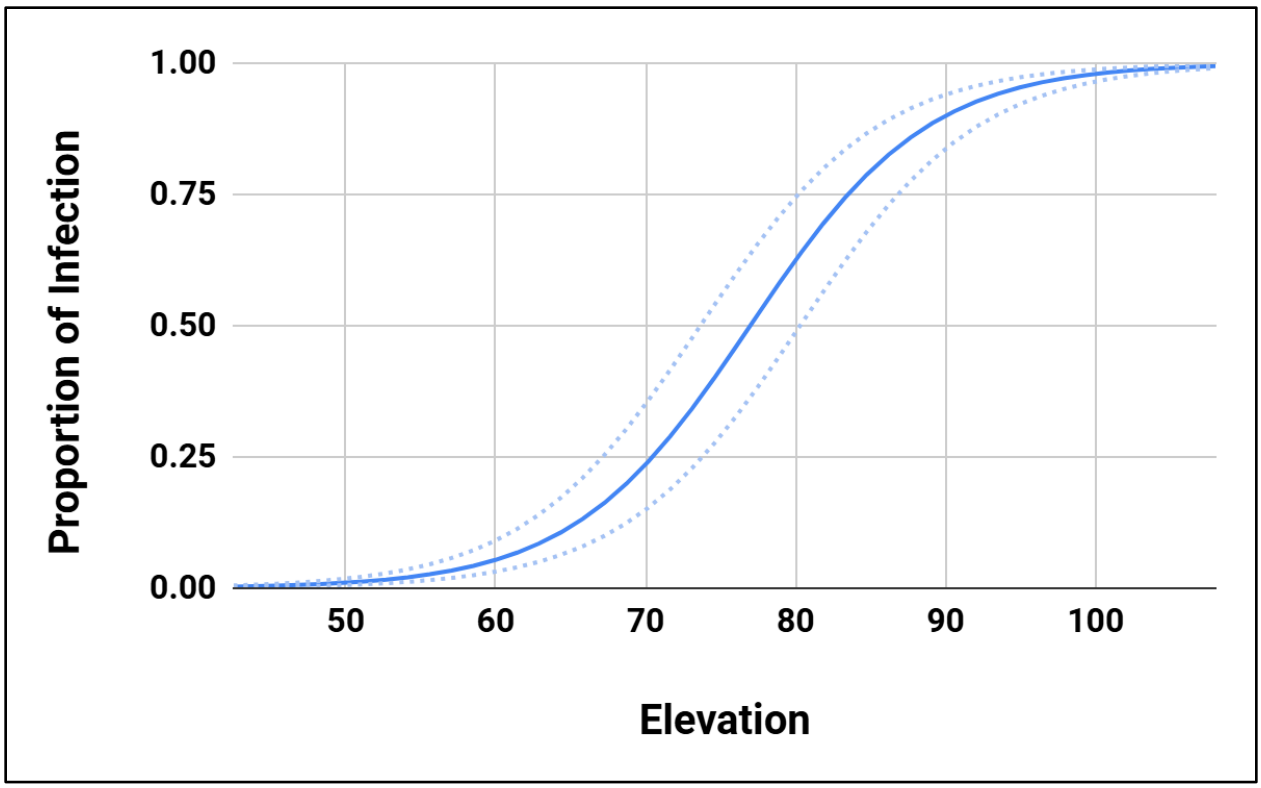

Figure 12. *Relationship between the proportion of infected mice and elevation. The y-axis represents the proportion of infected mice, while the x-axis indicates elevation (meters above sea level). This figure examines the correlation between elevation and the prevalence of infected mice in two parks in Montgomery County, Maryland, USA, 2022-2023.*

Figure 13. *Relationship between distance to trails and the proportion of infected mice. The y-axis represents the proportion of infected mice, while the x-axis indicates the distance to trails (meters of trail per 300 square meters). This figure explores the correlation between proximity to trails and the prevalence of infected mice in two parks in Montgomery County, Maryland, USA, 2022-2023.*

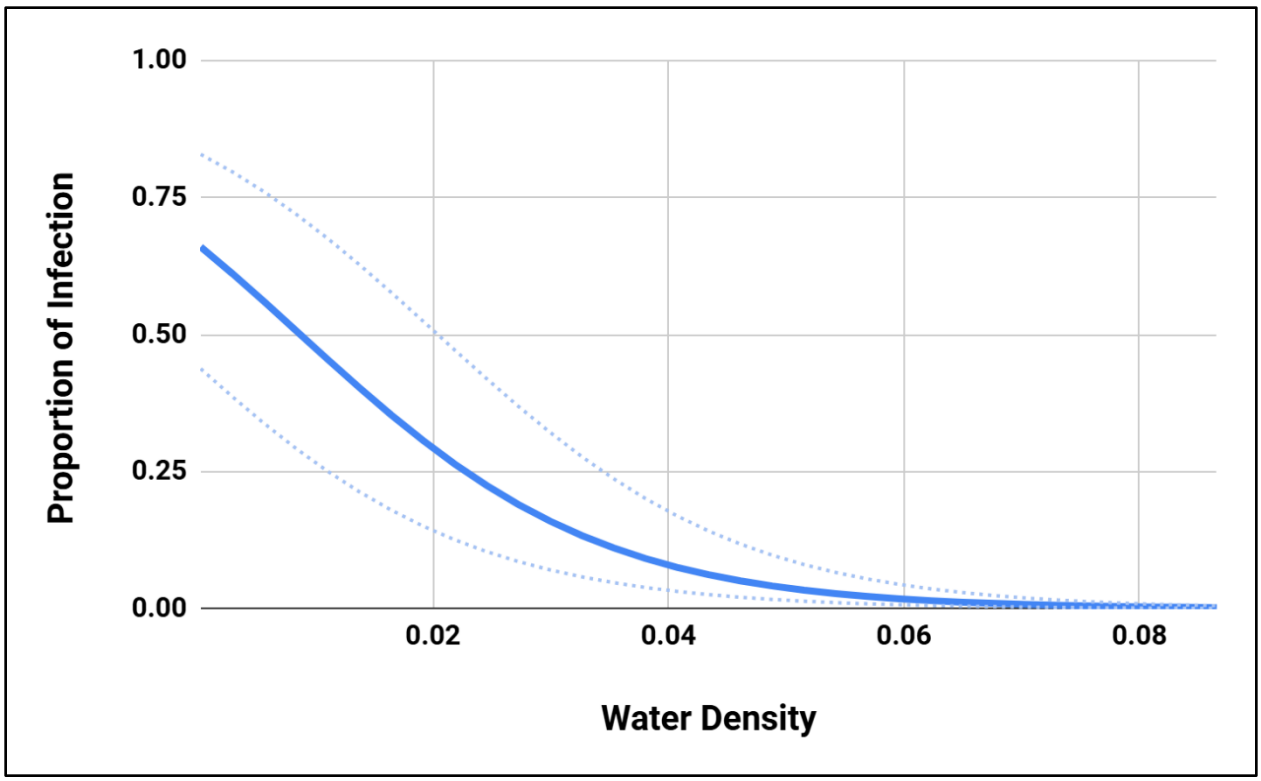

Figure 14. *Relationship between water density and the proportion of infected mice in the landscape. The y-axis represents the proportion of infected mice, while the x-axis indicates water density (liters of water per 0.0707 square kilometers.). This figure investigates the correlation between water density and the prevalence of infected mice in two parks in Montgomery County, Maryland, USA, 2022-2023.*

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