INVESTIGATING THE IMPORTANCE OF VERTEBRATE HOSTS FOR LYME DISEASE ECOLOGY: A NATURAL EXPERIMENT PRESENTED BY LAKE MICHIGAN ISLANDS AT SLEEPING BEAR DUNES NATIONAL LAKESHORE

By

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ABSTRACT

INVESTIGATING THE IMPORTANCE OF VERTEBRATE HOSTS FOR LYME DISEASE ECOLOGY: A NATURAL EXPERIMENT PRESENTED BY LAKE MICHIGAN ISLANDS AT SLEEPING BEAR DUNES NATIONAL LAKESHORE

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In eastern North America, Lyme disease is caused by the bacterium, *Borrelia burgdorferi*, and is transmitted by the blacklegged tick, *Ixodes scapularis*. In Michigan's Lower Peninsula, the blacklegged tick and the bacterium are invading from the southwest corner, northward along the Lake Michigan coast with the presumed leading edge at Sleeping Bear Dunes National Lakeshore (SLBE). How the tick spreads and becomes established is of great public health importance. White-tailed deer (*Odocoileus virginianus*) are believed to be the most important hosts for adult *I. scapularis* and critical for its spread and maintenance, but few opportunities exist to investigate tick and pathogen dynamics in their absence. Two Lake Michigan islands, at SLBE, one with deer and one without, presented this opportunity.

The overall objective of this dissertation was to establish a baseline of abundance for the tick and pathogen on both islands and compare it to the ecologically diverse mainland, and assess the role of other mammals as alternative hosts for the adult stage of the tick in areas absent of deer. My hypothesis was that in locations devoid of deer, the blacklegged tick would not be established and/or would exist at much lower densities in comparison to areas with resident deer populations. Also, other medium-sized mammals would serve as hosts for the adult ticks that theoretically could support a tick population.

In Chapter 1, I continued to track the invasion of the Lyme disease pathogen and vector at SLBE over a nine-year period. I found that there was a four year delay between the first detection of blacklegged ticks and the presence of *B. burgdorferi* and there was a trend illustrating an increase in *I. scapularis* and *B. burgdorferi* over time. At an additional site on SLBE's mainland, the tick and the pathogen were detected at the same time supporting the "dual-invasion" scenario of invasion, yet, this may have been a consequence of when sampling began. At recently-invaded Lyme disease areas, such as

these two SLBE mainland locations, I found eastern chipmunks (*Tamias striatus*) to be an earlier indicator of the pathogen's presence in comparison to white-footed mice (*Peromyscus leucopus*).

SLBE's two offshore islands, one with white-tailed deer and the other devoid, were the focus of Chapter 2 as a means to evaluate the success of mammalian hosts for maintaining *I. scapularis* in the absence of deer. I found that although the island with the deer had a greater density of ticks and greater *B. burgdorferi* infection prevalence, the island that was deer-free had all three life stages of the blacklegged tick and *B. burgdorferi* was present. Thus, alternative hosts for the adult tick, including snowshoe hares (*Lepus americanus*), passerine birds, and coyotes (*Canis latrans*), were captured and it was determined that coyotes were maintaining the established tick population on the island. Eastern chipmunks played a crucial role with maintaining the juvenile stages of the tick on the islands.

Chapter 3 then compared SLBE's host-diverse mainland to the host-limited islands, testing the dilution and multiple niche polymorphism hypotheses. In order to test the dilution effect, the larval *I. scapularis* prevalence on white-footed mice between the two locations was compared. The proportion of mice infested with at least one *I. scapularis* larvae nor the larval burden on the mice supported the dilution hypothesis. However, on the islands and the mainland, more eastern chipmunks were captured than anticipated and this consequently reduced the larval burden on the mice in each community, thus, supporting the dilution effect at the location level. Host-seeking *I. scapularis* adult/nymphal infection prevalence and adult/nymphal density of infected ticks were greater on the host-limited islands, supporting the dilution hypothesis. However, *B. burgdorferi* IGS strain diversity was greater on the islands in comparison to the host-diverse mainland, which was unlike what was predicted by the multiple niche polymorphism hypothesis.

Future studies to better estimate the island mammalian population sizes and diversity, in addition to comparing the *B. burgdorferi* island diversity to an area with a known long-established population of ticks would be advantageous to further our Lake Michigan island Lyme disease ecology knowledge. Also, given that SLBE is now most likely not at the leading edge of the Lyme disease invasion, future work should evaluate if the tick and bacterium have spread into neighboring counties.

This dissertation is dedicated to my family.
Thank you for all of your love, support, and encouragement.

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KEY TO ABBREVIATIONS

EACH-Eastern chipmunk (*Tamias striatus*)

MDHHS-Michigan Department of Health and Human Services

MI-Michigan

MSU-Michigan State University

NMI-North Manitou Island

NPS-National Park Service

SLBE-Sleeping Bear Dunes National Lakeshore

SMI-South Manitou Island

US-United States

WFMO-White-footed mouse (*Peromyscus leucopus*)

INTRODUCTION

Lyme disease is the most commonly reported vector-borne disease in Michigan and the U.S. (MDHHS 2016; Mead 2015). Lyme disease is a significant public health problem as the number of human cases continues to increase nationally (CDC 2015-a). The disease is caused by the bacterium *Borrelia burgdorferi*, with the blacklegged tick, *Ixodes scapularis*, as the vector in eastern North America (Mead 2015). The majority of human cases in the United States are reported from two endemic areas, the Northeast and the upper Midwest (CDC 2015-a). In Michigan, the area of focus for this dissertation, the Lyme disease tick and bacterium are considered both endemic and invading, depending on the location. Habitat suitability models predicted that Michigan provided highly suitable habitats for the blacklegged tick (Guerra et al. 2002). One county in the upper peninsula, Menominee County which is adjacent to the endemic foci in Wisconsin, was determined to have an established tick population as of 1998 (Walker et al. 1998). By 2002, it appeared that the blacklegged tick had become established in a focal area in Michigan's southwest Lower Peninsula, near Lake Michigan (Foster 2004). From this southwestern corner, the tick began to invade northward along the lake with the presumed leading edge reaching the northwest portion of the Lower Peninsula by 2009 (Hamer et al. 2010).

Approximately three to thirty days after an infected tick bite, humans may develop general flu-like signs and symptoms including fever, chills, headache, fatigue, muscle and joint aches, and swollen lymph nodes, additionally approximately 70-80% of individuals develop an erythema migrans rash that gradually expands (CDC 2015-b). As time progresses and if treatment was not started after the onset of the initial symptoms, days to months after the infected tick bite, the individual may develop severe headaches and neck stiffness, arthritis with joint pain and swelling, facial palsy, heart palpitations, nerve or muscle pain, and/or inflammation of the brain (CDC 2015-b). Due to the generality of these symptoms, individuals not aware that they had a tick attached, and/or the continual invasion of the tick and bacterium into areas previously uninhabited by this vector and pathogen, Lyme disease may present as a challenging disease to diagnose. Laboratory diagnosis is recommended in a two-step process in order

to test the blood for the presence of antibodies. Typically an enzyme immunoassay or an indirect immunofluorescence assay is performed first; if this first test is positive or indeterminate then a Western blot is run as the follow-up or secondary test (CDC 2015-c). For surveillance purposes, the CDC defines a confirmed case of Lyme disease as the presence of an erythema migrans rash with a known tick exposure or the presence of an erythema migrans rash with laboratory evidence of infection and without a known exposure or a case with at least one late manifestation that has laboratory evidence of infection (CDC 2011). Treatment typically consists of antibiotics, such as doxycycline, and if started promptly, rapid and complete recovery may be achieved (CDC 2015-d).

Blacklegged ticks typically complete their life cycle over a two-year period of time, constituting four developmental stages-egg, larva, nymph, and adult (Van Buskirk and Ostfeld 1995). Approximately 1000 to 2000 eggs are laid in a single cluster in the spring and then hatch in August or early September (Anderson and Magnarelli 2008). The larvae are born uninfected and must feed upon an infected vertebrate host in order to acquire the bacterium (Piesman et al. 1986). Larvae typically feed in the summer or early fall with feeding lasting an average of three to five days. After which time, the engorged larvae fall off of the host and molt into nymphs by the following spring and begin host-seeking in the spring and early summer months (Anderson and Magnarelli 2008). Susceptible hosts then become infected by the bite of an infected nymph. In the Midwest, larval and nymphal activity typically peaks in a synchronous fashion during the month of June (Hamer et al. 2012, Gatewood et al. 2009). After the nymphs feed, they molt into adults in mid-October and early November. Mating typically occurs on the host, primarily white-tailed deer (*Odocoileus virginianus*). After they feed on the white-tailed deer or other large mammalian host, in the fall or the following spring, the engorged female will detach and then develop and lay eggs (Anderson and Magnarelli 2008).

Blacklegged ticks are generalist ticks, as this tick has been recorded feeding on more species of hosts in comparison to other North American ticks (Anderson et al. 1999). However, not all hosts are considered reservoir competent. In general, reservoir competence is characterized by the susceptibility of

the host to infection when bitten by an infected vector, the ability of the pathogen to magnify and persist in the host, and the efficiency of the host at transmitting the spirochete to feeding vectors (Richter et al. 2000). Based on modeling efforts, the most reservoir competent species include the white-footed mouse (*Peromyscus leucopus*), eastern chipmunk (*Tamias striatus*), Sorex shrews, and the short-tailed shrew (*Blarina brevicauda*) (LoGiudice et al. 2003). The primary reservoir for juvenile blacklegged ticks is white-footed mice (Levine et al. 1985, Donahue et al. 1987). White-footed mice typically have a greater *B. burgdorferi* infection prevalence, density, and infestation prevalence with larval *I. scapularis* in comparison to other potential hosts, thus, making mice one of the most important reservoirs for *B. burgdorferi* (Mather et al. 1989, LoGiudice et al. 2003). On the other hand, white-tailed deer, which the adult stage of the tick typically feed upon, are considered to be reservoir incompetent species (Telford et al. 1988).

Lyme disease incidence is continuing to escalade due to the increase in abundance and range expansion of wildlife species, human behavior and subsequent encroachment into tick habitat, the creation of peridomestic habitats that attract wildlife hosts of ticks and pathogens, and increased surveillance and awareness within the medical profession (Hamer et al. 2010). Thus, increasing our understanding regarding the method of Lyme disease invasion into an area is important as this knowledge can assist with the enactment of proper and effective precautionary measures. From 2004-2008, our lab tracked the invasion of the blacklegged tick and Lyme disease pathogen within Michigan's Lower Peninsula along two sampling transects, a coastal and an inland (Hamer et al. 2010). During this study, three hypotheses were tested to determine how the invasion of the Lyme disease vector and/or pathogen may be occurring in the Lower Peninsula: 1) The "tick-first" scenario in which the tick enters into the system first as a consequence of introduction by reservoir incompetent white-tailed deer and the pathogen follows later; 2) The "dual-invasion" scenario in which the tick and the pathogen enter into the system concurrently as a consequence of introduction by mammalian and/or avian reservoir hosts; 3) The "spirochete-first" scenario in which the pathogen is introduced into the system first and is maintained by

cryptic vectors and reservoir hosts until the blacklegged tick establishes at a later time. Further efforts geared toward testing these hypotheses would aid in better understanding how Lyme disease may be invading into additional locations throughout the nation.

How the tick spreads and becomes established is of great public health importance. White-tailed deer are believed to be the most important hosts for adult *I. scapularis* and critical for its spread and maintenance, but few opportunities exist to investigate tick and pathogen dynamics in their absence. Furthermore, evaluating the role of hosts can not only assist from an invasion perspective but also, from a disease reduction standpoint. The Lyme disease 'dilution effect' (Keesing et al. 2006) describes the reduction in pathogen abundance as biodiversity of the host community increases. If a given community is composed of multiple potential vertebrate hosts as opposed to a white-footed mouse dominated area, the proportion of ticks infected with the Lyme disease bacterium would decrease. Continued testing of this hypothesis, using field investigations, may aid in decreasing disease risk within locations after the tick and bacterium arrive.

One of the most visited landmarks in Michigan is Sleeping Bear Dunes National Lakeshore (SLBE) with more than 1.5 million annual visitors (NPS stats). Based on the tick invasion surveys that our lab previously performed, SLBE was determined to be at the presumed leading edge of the invasion by 2009 (Hamer et al. 2010). In 2015, more than 70% of the total visitors came during the months of June, July, and August (NPS stats), which overlaps with peak activity of the immature stages of the blacklegged tick (Hamer et al. 2012). Infected nymphs are responsible for the majority of human Lyme disease cases, and thus, have the greatest epidemiological significance (Barbour et al. 1993). Therefore, tracking the spread of the blacklegged tick, potential Lyme disease risk, and further understanding Lyme disease ecology would inform public health activities affecting this highly touristic location for both local and national visitors.

SLBE includes two large offshore islands-North Manitou Island (NMI) and South Manitou Island (SMI). The known wildlife on both islands includes small rodents such as white-footed mice and eastern chipmunks, as well as snowshoe hares (*Lepus americanus*), coyotes (*Canis latrans*), and white-tailed deer (NMI only). With the absence of white-tailed deer on SMI, it was assumed that the tick population was less able to reproduce, unless other medium-sized mammal species were serving as alternative hosts for the adult stage. Therefore, given the different host communities and how they are a primary tourist destination, the Manitou Islands presented a valuable opportunity for studying key aspects about Lyme disease ecology that also had great public health relevance.

The Manitou Islands are approximately 12 miles from Leland, MI, which is a small town on the mainland. As opposed to the islands, the wildlife community on SLBE's mainland is more extensive, consisting of, but not limited to, white-footed mice, eastern chipmunks, gray and fox squirrels (*Sciurus carolinensis*, *Sciurus niger*), red fox (*vulpes vulpes*), white-tailed deer, raccoons (*Procyon lotor*), opossums (*Didelphimorphia*), skunks (*Mephitidae*), porcupines (*Hystricidae*), flying squirrels (*Pteromyini*), cottontail rabbits (*Sylvilagus floridanus*), and coyotes (National Park Service 2016). Given the varying mammalian compositions between the Manitou Islands and SLBE's mainland, these two locations presented as a means to also test the dilution hypothesis.

The objectives and hypotheses of this dissertation were to:

1) Track the invasion of the Lyme disease pathogen and vector at SLBE, over a nine-year period. At SLBE, from 2007-2008, no *B. burgdorferi* nor questing *I. scapularis* were detected, although blacklegged ticks were found attached to a low proportion of mammalian hosts. This sequence of detection supported the "tick-first" process of invasion at SLBE (Hamer et al. 2010). We were interested in determining the time delay between tick and pathogen invasion; thus we continued surveillance at SLBE until 2015. Furthermore, to see how much further north the blacklegged tick may have invaded along the Lake Michigan coast, an additional coastal site northeast of the initial SLBE site was surveyed

beginning in 2011. I hypothesized given the ongoing invasion within Michigan's Lower Peninsula that the tick and pathogen would continue to invade northward along the Lake Michigan shoreline (Hamer et al. 2010).

2) *Evaluate the success of mammalian hosts for maintaining the Lyme disease vector, I. scapularis, in the absence of white-tailed deer.* I hypothesized in the absence of deer and given a depauperate community of medium/adult mammals, there would not be an established blacklegged tick population on the deer-free island. Since birds still played a role introducing ticks, I predicted that nymphal and adult ticks would be present, albeit in low numbers, on the deer-less island. Yet, no larval ticks would be detected (Elias et al. 2011), unless other hosts served as alternatives for the adult tick.

3) *Test the Lyme disease dilution effect and the multiple niche polymorphism hypothesis using an isolated, less host-diverse island and compare it to a mammalian-diverse mainland.* In nature, Lyme disease presents as an optimal example to test the dilution hypothesis given the wide variety of potential hosts that blacklegged ticks can feed upon and their varying *B. burgdorferi* reservoir competence (Ostfeld and Keesing 2000, LoGiudice et al. 2003). If a given community is composed of multiple potential vertebrate hosts as opposed to a white-footed mouse dominated area, the ticks would be diverted away from the mice consequently obtaining a blood meal from one of the alternative non-mouse hosts, thus, the proportion of ticks infected with the Lyme disease bacterium would decrease.

Host diversity within a community may also influence the genetic diversity of the Lyme disease pathogen. Host association has been suggested at certain *B. burgdorferi* loci (Brisson and Dykhuizen 2004). Varying host community composition may increase the pathogen diversity by increasing the ecological 'niches' for the pathogen, which would result in the maintenance of polymorphisms within the pathogen population, known as the 'multiple niche polymorphism' hypothesis (Levene 1953, Kurtenbach et al. 2006. States et al. 2014). Thus, in communities composed of a high diversity of potential hosts, *B.*

burgdorferi diversity would be greater based on this hypothesis (Brisson and Dykhuizen 2006; States et al. 2014).

I hypothesized that if the dilution effect and multiple niche polymorphism hypothesis held true, that locations with low species diversity (i.e. the islands) would have a greater larval infestation prevalence on white-footed mice, greater host-seeking *I. scapularis* adult/nymphal infection prevalences and greater density of infected nymphs/adults, in addition to a lower *B. burgdorferi* strain diversity in comparison to locations with high species diversity (i.e. the mainland) (LoGiudice et al. 2003, States et al. 2014).

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CHAPTER 1

Invasion of the Lyme disease pathogen and vector at Sleeping Bear Dunes National Lakeshore, Michigan over a nine-year period

Abstract:

Lyme disease is invading within Michigan's Lower Peninsula, with the leading edge, as determined previously by our lab, at Sleeping Bear Dunes National Lakeshore (SLBE). During these previous efforts, scenarios of invasion were tested and it was determined that SLBE supported the "tick-first" process of invasion. We were interested in determining the time delay between tick and pathogen invasion; thus we continued surveillance by small mammal trapping and drag sampling for questing ticks. We found a four year delay between the first detection of blacklegged ticks and the presence of *B. burgdorferi* and there was a trend illustrating an increase in *I. scapularis* and *B. burgdorferi* over time. At an additional site within SLBE, the tick and pathogen arrived at approximately the same time; yet, this may have been a consequence of when sampling began. This work was compared to a Lyme disease endemic site and as expected, the endemic site had a significantly greater abundance of blacklegged ticks and greater *B. burgdorferi* infection prevalence than both SLBE sites, supporting the recent emergence at SLBE. At recently-invaded Lyme disease areas, such as SLBE, we found eastern chipmunks (*Tamias striatus*) to be an earlier indicator of the pathogen's presence in comparison to white-footed mice (*Peromyscus leucopus*). Given the level of infestation and infection with *B. burgdorferi* at SLBE, it is now likely that this area is no longer at the leading edge of the invasion, thus, future work should evaluate if the tick and bacterium have spread into neighboring counties.

Introduction:

Lyme disease is the most commonly reported vector-borne disease in Michigan and the U.S. (MDHHS 2016-a; Mead 2015). The disease is caused by the bacterium *Borrelia burgdorferi*, with the

blacklegged tick, *Ixodes scapularis*, as the vector in eastern North America (Mead 2015). *Ixodes scapularis* also is the vector for several other zoonotic pathogens, including the agents of human anaplasmosis, babesiosis, and Powassan encephalitis. Lyme disease is a significant public health problem as the number of human cases continues to increase nationally (CDC 2015). The geographic distribution of human cases reported matches the distribution of infected disease vectors, which occur primarily in the northeastern and north central United States (Mead 2015).

In Michigan, previous surveillance in the 1990's showed that the distribution of the blacklegged tick was limited mainly to one county in the Upper Peninsula (Walker et al. 1998). However, by 2002, it appeared that the blacklegged tick had become established in focal areas in Michigan's Lower Peninsula, in the southwestern region, near the coast of Lake Michigan (Foster 2004). Blacklegged ticks then began to invade northward along the lake with the presumed leading edge reaching Sleeping Bear Dunes National Lakeshore (SLBE) by 2009 (Hamer et al. 2010, Figure1).

In 2015, there were more than1.5 million visitors at SLBE, and more than 70% of the total visitors came during the months of June, July, and August (NPS stats). The peak visitation period overlaps when the immature stages of the blacklegged tick are host-seeking. Infected nymphs are responsible for the majority of human Lyme disease cases, and thus, have the greatest epidemiological significance (Barbour et al. 1993). Therefore, tracking the spread of the blacklegged tick and potential Lyme disease risk will inform public health activities affecting this highly touristic location for both local and national visitors.

From 2004-2008, our lab tracked the invasion of the blacklegged tick and Lyme disease pathogen within Michigan's Lower Peninsula along a coastal and an inland transect (Hamer et al. 2010). SLBE, Benzie County, was the furthest north site along the coastal transect. At SLBE, from 2007-2008, no *B. burgdorferi* nor questing *I. scapularis* were detected, although blacklegged ticks were found attached to a low proportion (13%) of mammalian hosts. This sequence of detection supported the "tick-first" process

of invasion at SLBE (Hamer et al. 2010), which hypothesizes that the blacklegged tick is introduced first (e.g., by reservoir incompetent white-tailed deer), and then the Lyme disease agent is introduced second (e.g., by reservoir competent white-footed mice). We were interested in determining the time delay between tick and pathogen invasion; thus we continued surveillance at SLBE until 2015. Furthermore, to see how much further north the blacklegged tick may have invaded along the Lake Michigan coast, an additional coastal site northeast of the initial SLBE site was surveyed beginning in 2011.

The objectives of this study were: 1) to determine if/when *B. burgdorferi* would appear in ticks/wildlife at the initial SLBE site, and how long it is delayed from the initial detection of *I. scapularis*; 2) to determine if the Lyme disease tick and pathogen are present at the second, further north, SLBE site; and 3) to compare *I. scapularis* infestation and infection prevalence over time at both SLBE sites to the endemic reference site from 2007-2011.

Materials and Methods:

Site Selection and Sampling Regime:

SLBE Sites-Two mainland SLBE sites located in Benzie and Leelanau Counties were sampled-Platte River Campground area (SLBEs) and DH Day Campground area (SLBEn) respectively (Figure 1.1). SLBEs was surveyed previously by our lab during a multi-year study investigating the invasion of the blacklegged tick and was referred to as "C4" at that time (Hamer et al. 2010). The new, more northern SLBE site, SLBEn, was located 30 km and 33 degrees northeast from SLBEs.

Reference Site-Van Buren State Park, Van Buren County, served as a reference site from 2007-2011 (Figure 1.1). Previous surveys from our lab found that this site was endemic for the Lyme disease tick and bacterium (Hamer et al. 2010). A reference site was used as a measure of comparison for sites that were newly invaded by the tick and pathogen. If blacklegged ticks were not collected during a sampling period at an invasion site, we wanted a means to ensure that it was not due to the technique(s)

we were using or time periods in which we were sampling, but rather it was due to lack of tick establishment at that site. Therefore, the Van Buren reference site was visited approximately the same time within the calendar year as the SLBE sites and identical field procedures were performed at all sites. Van Buren was sampled until *I. scapularis* were detected at both SLBE sites.

Sample sites were chosen based on several factors including habitat suitability for the blacklegged tick and proximity to recreational areas. Appropriate *I. scapularis* habitat included: deciduous/mixed forests, sandy soils, leaf litter layer, and an abundance of small mammals (e.g., white-footed mice and eastern chipmunks) and white-tailed deer (Guerra et al. 2002). To assess the risk of tick-borne disease, attempts were made to sample areas near where visitors may come into contact with potential ticks (e.g., campgrounds and often used trails).

Sampling Regime-All sites were sampled in late May/June (indicated as "*late spring*" throughout the remainder of the paper) when all three post-egg life stages of *I. scapularis* would be active. If a site was visited in both May and June, the June effort was used for the "*late spring*" comparisons and then the May effort was grouped within the additional sampling efforts (i.e. "*post-spring*"). Sampling took place from 2007-2015, with the exception of: Van Buren State Park (2007-2011 only), and SLBEn (2011-2015 only). In addition to the *late spring* sampling, additional sampling took place at SLBEs and SLBEn as listed in Table 1.1 (indicated as "*post-spring*" throughout the remainder of the paper).

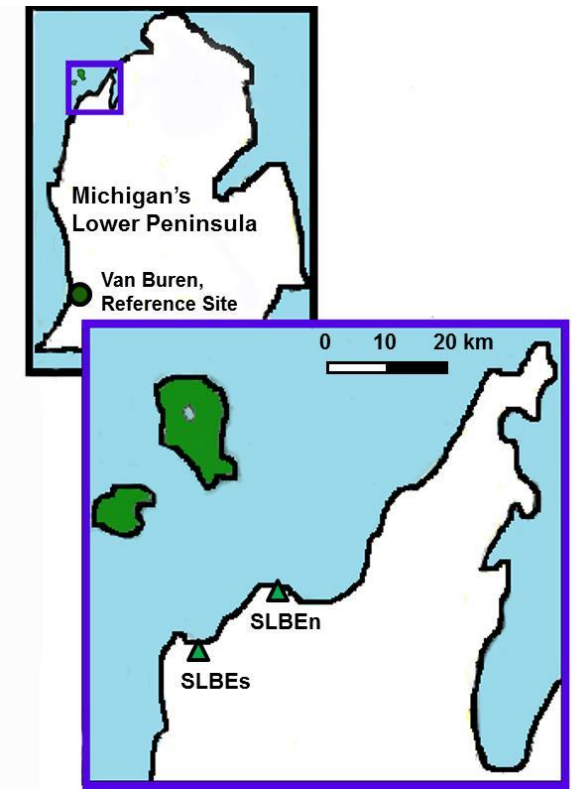

Figure 1.1 Map of Michigan's Lower Peninsula with the Van Buren reference site depicted by a dot along the southwestern coast and the two Sleeping Bear National Lakeshore (SLBE) sites, SLBEs and SLBEn, illustrated by triangles along the northwestern coast.

Table 1.1 Type of sampling (drag; mammal) that occurred during each month and year at SLBEs and SLBEn. The black shading illustrates drag sampling and the gray indicates mammal trapping. When sampling was performed twice within a given month, "2" is written on that month/year.

SLBEs

	April		May		June		July		August		September		October		November	
2007																
2008																
2009																
2010																
2011																
2012					2	2	2	2								
2013																
2014																
2015																

SLBEn

	April		May		June		July		August		September		October		November	
2011																
2012					2	2	2	2								
2013																
2014																
2015																

Drag Sampling **Mammal Trapping**

Small Mammal Trapping:

Small mammals were trapped following the protocol used in Hamer et al. 2010. Briefly, at each site, small mammals were trapped along 6 transects of 25 live traps (H. B. Sherman Traps, Tallahassee, FL) spaced 10 m apart and baited with crimped oats for two consecutive nights. Trapping took place at least once per month when immature stages of the tick were active. Traps were set in the early evening and checked the following morning. All captured mammals were identified to species and sex, examined for ticks, biopsied in both ears using a 2-mm biopsy punch (Miltex Instruments, York, PA), and an unique ear tag was placed in one ear (National Band and Tag, Newport, KY). Ticks and ear biopsies were stored separately in 70% ethanol. Recaptured animals that were caught the previous day were strictly examined for ticks with no additional ear biopsies taken; animals recaptured from a previous trapping period, however, were biopsied again. All captured mammals were released back at the point of capture. The number of trap nights per trapping period was adjusted for tripped traps as follows: number of traps set - (0.5*no. of tripped traps). Animal handling procedures were approved by Michigan State University's Institutional Animal Care and Use Committee Animal Use Form #: 06/12-103-00.

Questing Tick Sampling:

Each site was sampled for questing ticks by dragging a 1 m^2 corduroy cloth along the forest floor (Hamer et al. 2010). This was performed along the same transects (5-10 m on either side of the transect) that were used for small mammal trapping. A minimum of 1000 m^2 (i.e., 4 transects that were randomly chosen) was dragged during each site visit. Both sides of the cloth were examined every 20 m and any ticks that were found attached to the cloth or to the individual performing the sampling were collected and stored in 70% ethanol.

Pathogen Detection:

All ticks were identified morphologically to species and life stage using dichotomous keys (Keirans et al. 1978; Sonenshine 1979; Durden et al. 1996). Total DNA from ticks and ear biopsies, with the exception of tick samples from 2014, were extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's animal tissue protocol with the modifications described previously (Hamer et al. 2010). Only *I. scapularis* ticks were assayed; any other species found was strictly recorded and stored for potential future analysis. In situations where more than three adult or nymphal ticks of the same species, life stage, and sex were removed from an individual animal, three were randomly selected for testing (Hamer et al. 2010). *Borrelia burgdorferi* was detected using a quantitative polymerase chain reaction (qPCR) targeting a fragment of the 16S rDNA gene (Tsao et al. 2004). With the exception of the 2014 tick samples, the qPCR protocol was performed as previously described (Hamer et al. 2010) and reactions were performed with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) at the Michigan State University Research Technology Support Facility. The 2014 tick samples were extracted and tested by collaborators at the Centers for Disease Control and Prevention, Division of Vector Borne-Infectious Diseases. Total DNA from the 2014 ticks were extracted and *B. burgdorferi*, *Anaplasma phagocytophilum*, and *Babesia microti* were detected following procedures as described in Hojgaard et al. 2014. The consistency between the two assays was compared prior to screening the 2014 samples. Of 304 ticks and tissue biopsies that were tested by both labs, there was 97.4% agreement; there was no significant difference in pathogen detection between the two assays (Fisher's exact test: $p = 0.798$).

Statistics:

Linear regression was used to assess trends in nymphal tick densities within the sites over time. Fisher's exact test was used to assess differences in capture success between sites and also the pathogen testing procedures used at Michigan State University versus what was used at the CDC. This was

performed using GraphPad Software, Inc. (La Jolla, CA), with an alpha level of 0.05. In order to assess differences in infestation and infection prevalence between sites and also within the same site over time, the z-score and associated two-tailed probabilities were calculated. This was performed using a web-based calculator, Social Science Statistics (Stangroom 2016), with the assumption of normality and equal variance and using an alpha level of 0.05. Error bars correspond to 95% binomial confidence intervals for proportions (Agresti and Coull 1998) and were created using GraphPad Software, Inc. (La Jolla, CA).

Results:

Small Mammal Captures:

Among all of the years, in the *late spring* sampling periods, we captured a total of 603 white-footed mice (*Peromyscus leucopus*) (70.3%) and eastern chipmunks (*Tamias striatus*) (29.7%) from the three sites, of which 15.4% were captured the previous day. These mammals were captured in a total of 4,540.5 adjusted trap nights. There was no difference in capture success within species among field sites.

Across the study period, during the *post-spring* sampling periods at SLBEs, a total of 469 white-footed mice (69.7%) and eastern chipmunks (30.3%) were captured, of which 22.2% were captured the previous day. These mammals were captured in a total of 3,373 adjusted trap nights. At SLBEn, a total of 496 white-footed mice (68.9%) and eastern chipmunks (31.1%) were captured, of which 16.1% were captured the previous day. These mammals were captured in a total of 3,113 adjusted trap nights. White-footed mice capture rates did not differ between SLBEs and SLBEn during the *post-spring* trapping efforts ($p=1.000$) nor did eastern chipmunk capture rates ($p=1.000$).

Additional, non-mice and non-chipmunk captures at the three sites (SLBEs, SLBEn, and the Van Buren reference site) can be found in Appendix 1.1.

Ixodes scapularis on Small Mammals:

To make sure counts were independent and not affected by tick removal the prior day, we estimated tick infestation prevalence using data from the first time an animal was captured, regardless if it were captured on the first day or second day of the trapping period. Counts from individuals captured in multiple trapping periods were considered independent. In the *late spring*, at least one white-footed mouse was infested with *I. scapularis* every year at SLBEs and SLBEn and with mammals infested with larvae found by the second year of sampling at each site (Figure 1.2). At SLBEs, chipmunks were infested with *I. scapularis* all years except in 2009, and at SLBEn, chipmunks were only infested in 2013 and 2014 (Figure 1.3). In general, there was a trend showing that white-footed mice were infested with more larvae than nymphs and the reverse pattern for eastern chipmunks (Figures 3, 4). The average proportion of white-footed mice and eastern chipmunks infested with at least one *I. scapularis* tick at the reference site (mice: 0.867, n=118; chipmunks: 0.875, n=27) was approximately 5 and 3 times greater for the mice and chipmunks, respectively, in comparison to SLBEs (2007-2011) (mice: 0.170, n=23, $p < 0.001$; chipmunks: 0.291, n=13, $p = 0.004$). However, from 2011-2014, the average proportion of white-footed mice and eastern chipmunks infested with at least one *I. scapularis* tick at SLBEs (mice: 0.427, n=19; chipmunks: 0.596, n=28) versus SLBEn (mice: 0.485, n=11; chipmunks: 0.336, n=20) was not significantly different (mice: $p = 0.835$; chipmunk: $p = 0.308$).

During the *post-spring* sampling performed at SLBEs, 73 of the 247 (29.6%) and 28 of the 118 (23.7%) white-footed mouse and eastern chipmunk first-time captures respectively were infested with at least one *I. scapularis*. During the *post-spring* trapping at SLBEn, 21 of the 289 (7.3%) and 15 of the 127 (11.8%) white-footed mouse and eastern chipmunk first-time captures were infested with at least one *I. scapularis*, respectively. The average proportion of white-footed mice infested at SLBEs was significantly greater than the average proportion infested at SLBEn during these *post-spring* trapping efforts ($p = 0.033$). Yet, the average proportion of eastern chipmunks infested at SLBEs versus SLBEn during the *post-spring* trapping efforts was not considered significantly different ($p = 0.477$).

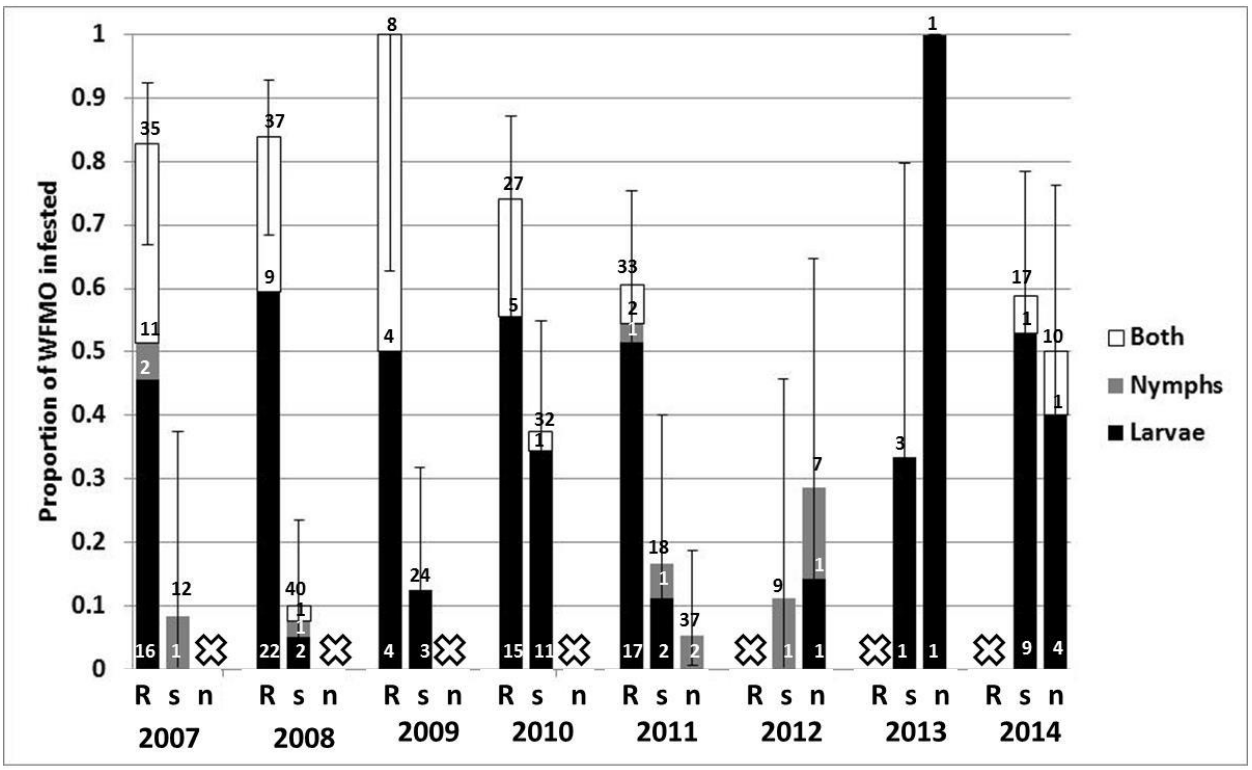

Figure 1.2 Proportion of captured white-footed mice (WFMO) infested with *I. scapularis* larvae (black), nymphs (gray), and/or both larvae and nymphs (white) in late spring at the Van Buren reference site (2007-2011; "R"), SLBEs (2007-2014; "s"), and SLBEn (2011-2014; "n"). This is a non-cumulative stacked graph. The (95% binomial confidence interval) error bars correspond to the overall proportion of mice infested with at least one *I. scapularis* tick. The "x" represents when sampling did not take place at SLBEn or the Reference site. The number within the bars indicates the number of mice infested with that specific tick life stage; the number above all of the bars indicates the total number of mice captured.

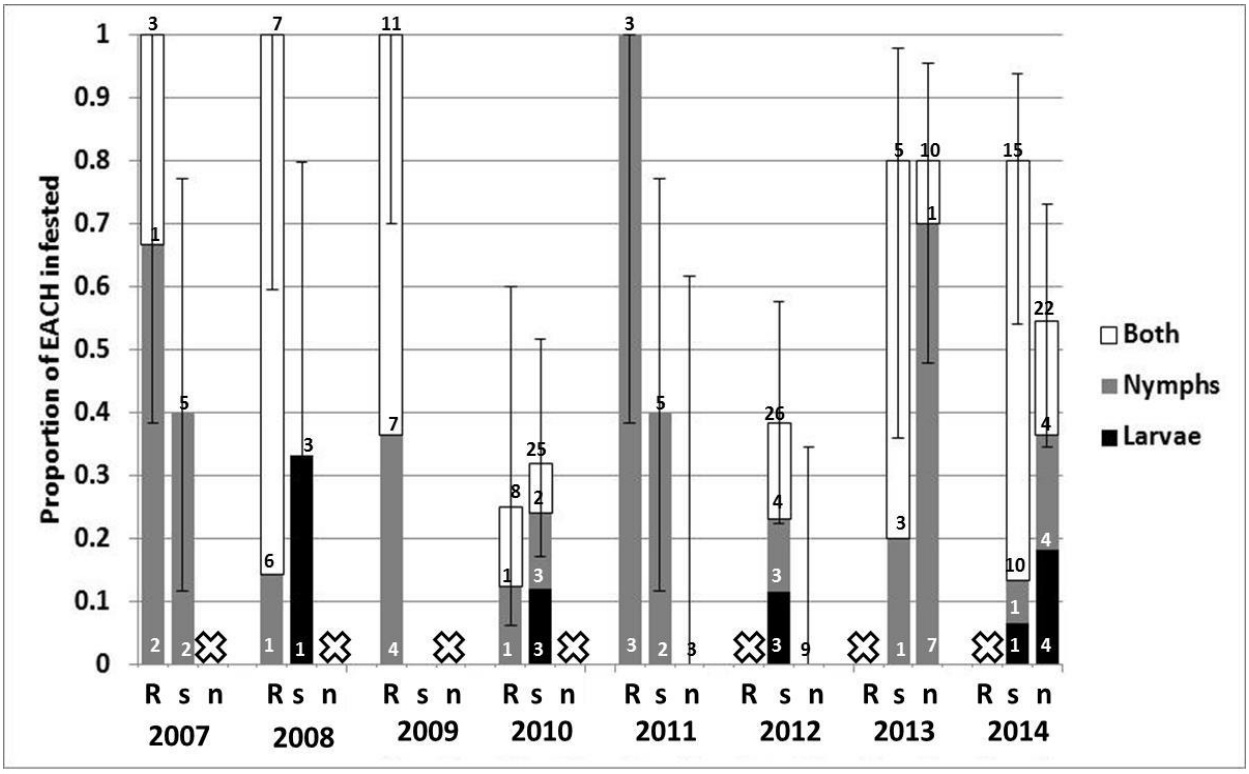

Figure 1.3 Proportion of captured eastern chipmunks (EACH) infested with *I. scapularis* larvae (black), nymphs (gray), and/or both larvae and nymphs (white) in late spring at the Van Buren reference site (2007-2011; "R"), SLBEs (2007-2014; "s"), and SLBEn (2011-2014; "n"). This is a non-cumulative stacked graph. The (95% binomial confidence interval) error bars correspond to the overall proportion of chipmunks infested with at least one *I. scapularis* tick. The "x" represents when sampling did not take place at SLBEn or the Reference site. The number within the bars indicates the number of chipmunks infested with that specific tick life stage; the number above all of the bars indicates the total number of chipmunks captured.

Borrelia burgdorferi Infection in Small Mammals:

During the *late spring* sampling efforts at the Van Buren reference site and the two SLBE sites, a total of 339 white-footed mouse and 155 eastern chipmunk tissue biopsies were tested of which 6.2% and 21.9% were infected with *B. burgdorferi*, respectively (Figure 1.4). Of the total white-footed mice infected, 95.2% (n = 20), 4.8% (n = 1), and 0% (n = 0) originated from the Van Buren reference site, SLBEs, and SLBEn mice, respectively. Of the total infected eastern chipmunks, 41.2% (n = 14), 26.5% (n = 9), and 32.4% (n = 11) were from chipmunks at the Van Buren reference site, SLBEs, and SLBEn, respectively. Overall, eastern chipmunks had significantly greater infection prevalence than white-footed

mice ($p < 0.001$). The combined small animal infection prevalence at SLBEs remained relatively consistent over time and did not differ significantly from SLBEn (2013: $p = 0.242$; 2014: $p = 0.285$).

During the *post-spring* small mammal trapping at SLBEs, 228 and 108 white-footed mouse and eastern chipmunk tissue biopsies were tested respectively, of which 0.9% (n = 2) and 3.7% (n = 4) were infected with *B. burgdorferi*, respectively. The two infected white-footed mice were trapped in July 2011 and July 2014; the infection prevalence during each of those trapping efforts were 2.5% and 3.2%, respectively. Of the infected eastern chipmunks, 3 were trapped in May 2014 (infection prevalence: 12%) and one was trapped in July 2014 (infection prevalence: 16.7%). The difference between the white-footed mouse and the eastern chipmunk infection prevalences was marginally significant ($p = 0.067$).

During the *post-spring* small mammal trapping performed at SLBEn, 248 and 107 white-footed mouse and eastern chipmunk tissue biopsies were tested respectively, of which 0.4% (n = 1) and 7.5% (n = 8) were infected with *B. burgdorferi*, respectively. The one infected white-footed mouse was trapped in September 2014, making 3% of the tested white-footed mice (n = 33) during that month/year infected. Of the infected eastern chipmunks, 6 were trapped in July 2014 (32% prevalence), one was from late June 2012 (5% prevalence), and one was from May 2014 (9% prevalence). Overall, eastern chipmunks had higher infection prevalence than white-footed mice ($p < 0.001$).

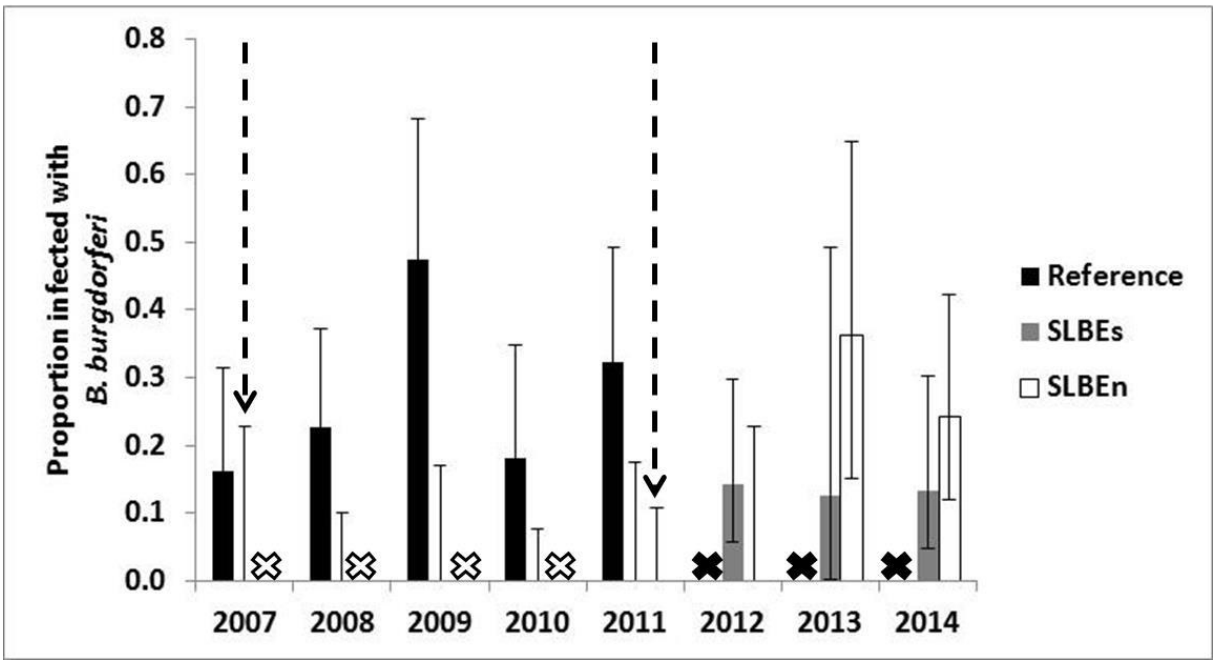

Figure 1.4 Proportion of white-footed mice and eastern chipmunk tissue biopsies infected with *B. burgdorferi* at the Van Buren reference site (2007-2011; black), SLBEs (gray), and SLBEn (white) during the *late spring* sampling periods. Mammals were captured every year at SLBEs (2007-2014) and each year at SLBEn (2011-2014), thus, when zeroes are depicted it indicates lack of infection. The black arrows indicate when immature stages of the tick were first found on mammals at SLBEs and SLBEn. The "x" represents when sampling did not take place at a given site (white "x": SLBEn; black "x": Reference). Error bars correspond to 95% binomial confidence intervals.

Borrelia burgdorferi Infection in *I. scapularis* removed from Small Mammals:

During the *late spring* sampling efforts at the Van Buren reference site and the two SLBE sites, a total of 77 *I. scapularis* nymphs and 158 *I. scapularis* larval pools were tested from white-footed mice and 159 *I. scapularis* nymphs and 67 *I. scapularis* larval pools were tested from eastern chipmunks (Table 1.2). At SLBEs, infected ticks were first detected in 2012 from eastern chipmunks, which was prior to the detection from white-footed mice (2014). From 2012-2014, both eastern chipmunk larvae and nymphs were infected at SLBEs; 2014 was the only year that both stages were infected at SLBEn. No infected ticks have yet been detected from white-footed mice trapped at SLBEn. The larval infection prevalence significantly increased at SLBEs from 2012 to 2014 from 20% to 60% ($p = 0.038$). Although the nymphal infection prevalence did not significantly increase ($p = 0.271$) from the first year infected eastern chipmunk nymphs were detected to the last year of mammal trapping, there was a positive trend.

During the *post-spring* small mammal trapping at SLBEs, a total of 15 nymphs and 69 larval pools were tested from white-footed mice, of which the only infected tick was a nymph in July 2011 (nymphal infection prevalence of 11.1%). A total of 57 *I. scapularis* nymphs and 12 *I. scapularis* larval pools were tested from eastern chipmunks, of which the only infected ticks were in 2012 (25.0% nymphal infection prevalence) and 2014 (9.3% nymphal infection prevalence). No larvae were infected.

During the *post-spring* small mammal trapping performed at SLBEn, a total of 21 *I. scapularis* larval pools and 2 *I. scapularis* nymphs removed from white-footed mice were tested, of which none were infected with *B. burgdorferi*. A total of 10 *I. scapularis* larval pools and 18 *I. scapularis* nymphs removed from eastern chipmunks were tested, of which 6 nymphs (33% nymphal infection prevalence) were infected (one in July 2011, five in May 2014). No larvae were infected.

When all of the tested ticks were combined (larvae plus nymphs) from SLBEs and also from SLBEn, during both the *late-spring* and the *post-spring* efforts, there was not a significant difference between the infection prevalence of the ticks removed from mice between the two sites ($p = 0.465$) nor was there a significant difference between the infection prevalence of the ticks removed from chipmunks between the two sites ($p = 0.093$).

Table 1.2 Infection prevalence of *I. scapularis* larval pools and nymphs removed from white-footed mice (WFMO) and eastern chipmunks (EACH) at the Van Buren reference site (2007-2011;"R"), SLBEs (2007-2014; "s"), and SLBEn (2011-2014; "n") during the late spring sampling efforts. The number in the parentheses indicates the number of ticks tested. "NS" indicates that the site was Not Sampled.

	2007		2008		2009		2010		2011		2012		2013		2014	
	Larval	Nymphal	Larval	Nymphal	Larval	Nymphal	Larval	Nymphal	Larval	Nymphal	Larval	Nymphal	Larval	Nymphal	Larval	Nymphal
WFMO	**R:** 48.5% (33)	**R:** 69.2% (26)	**R:** 14.7% (34)	**R:** 12.5% (16)	**R:** 55.6% (9)	**R:** 50.0% (10)	**R:** 24.0% (25)	**R:** 20.0% (10)	**R:** 10.5% (19)	**R:** 60.0% (5)	**R:** NS	**R:** NS	**R:** NS	**R:** NS	**R:** NS	**R:** NS
	s: N/A (0)	**s:** 0.0% (1)	**s:** 0.0% (3)	**s:** 0.0% (2)	**s:** 0.0% (3)	**s:** N/A (0)	**s:** 0.0% (9)	**s:** 0.0% (1)	**s:** 0.0% (1)	**s:** N/A (0)	**s:** N/A (0)	**s:** 0.0% (3)	**s:** 0.0% (2)	**s:** N/A (0)	**s:** 7.7% (13)	**s:** 0.0% (1)
	n: NS	**n:** NS	**n:** NS	**n:** NS	**n:** NS	**n:** NS	**n:** NS	**n:** NS	**n:** N/A (0)	**n:** N/A (0)	**n:** N/A (0)	**n:** 0.0% (1)	**n:** 0.0% (1)	**n:** N/A (0)	**n:** 0.0% (6)	**n:** 0.0% (1)
EACH	**R:** 100% (1)	**R:** 40.0% (5)	**R:** 50.0% (6)	**R:** 46.2% (13)	**R:** 37.5% (8)	**R:** 41.7% (48)	**R:** 100% (2)	**R:** 100% (3)	**R:** N/A (0)	**R:** 57.1% (7)	**R:** NS	**R:** NS	**R:** NS	**R:** NS	**R:** NS	**R:** NS
	s: N/A (0)	**s:** 0.0% (3)	**s:** 0.0% (1)	**s:** N/A (0)	**s:** N/A (0)	**s:** N/A (0)	**s:** 0.0% (4)	**s:** 0.0% (7)	**s:** N/A (0)	**s:** 0.0% (2)	**s:** 20.0% (10)	**s:** 10.0% (10)	**s:** 25.0% (4)	**s:** 40.0% (15)	**s:** 60.0% (20)	**s:** 28.6% (14)
	n: NS	**n:** NS	**n:** NS	**n:** NS	**n:** NS	**n:** NS	**n:** NS	**n:** NS	**n:** N/A (0)	**n:** N/A (0)	**n:** N/A (0)	**n:** N/A (0)	**n:** 0.0% (1)	**n:** 28.6% (21)	**n:** 40.0% (10)	**n:** 54.5% (11)

Questing Ticks:

A total of 53,900 m^2 were drag sampled for questing ticks during the *late spring*, with 11,450 m^2 at the Van Buren reference site (2007-2011), 24,950 m^2 at SLBEs (2007-2015), and 17,500 m^2 at SLBEn (2011-2015). A total of 603 *I. scapularis* ticks were dragged during the *late spring* at the three sites, with 94.2% of these ticks coming from the reference site. All three life stages of the blacklegged tick were collected at the reference site throughout the time period it was sampled (2007-2011; Figure 1.5). Only considering the *late spring* drag efforts, at SLBEs the first adult *I. scapularis* was collected in 2009 and the first nymph in 2011; no questing larvae were collected. There was a significant increase in nymphal density detected over time at SLBEs ($R^2 = 0.6763$, $P = 0.007$; Figure 1.5). Considering only the *late spring*, the first adult and nymph were collected at SLBEn in June 2013, and no questing larvae were detected. Questing nymphs were only detected in 2013 and 2014 at SLBEn (n=2), thus, no statistics could be done given the small sample size (Figure 1.5). Two other tick species were collected via drag cloth: *Dermacentor variabilis* (at all three sites) and *Haemaphysalis leporispalustris* (the reference site and SLBEs).

A total of 62,590 m^2 were drag sampled at SLBEs during the *post-spring* sampling efforts. A total of 432 ticks were collected, of which 113 were adults, 49 nymphs, and 255 larvae. Questing larvae were first detected during the *post-spring* sampling (September 2012). The relative nymphal activity in 2012 and 2014 across the calendar year for SLBEs peaked in the month of July (Figure 1.6). Additional tick species that were collected during the *post-spring* sampling included: *Dermacentor variabilis* and *Haemaphysalis leporispalustris*.

A total of 49,600 m^2 were drag sampled at SLBEn during the *post-spring* sampling efforts. A total of 37 ticks were collected, of which 31 were adults and 6 were nymphs. The first questing adult was detected outside of the *late spring* efforts, April 2012. Two other additional tick species were collected: *Dermacentor variabilis* and *Haemaphysalis leporispalustris*.

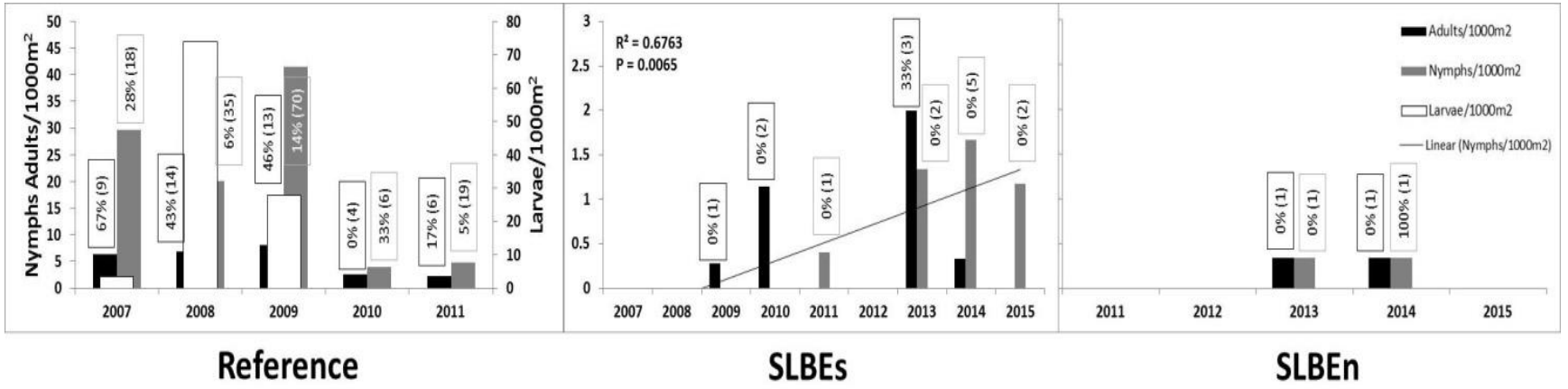

Figure 1.5 Density of questing *I. scapularis* adults (black), nymphs (gray), and larvae (white) over time at the Van Buren reference site (2007-2011), SLBEs (2007-2015), and SLBEn (2011-2015) during the *late spring*. The secondary y-axis (Larvae/1000 m^2) from the reference site is repeated for SLBEs and SLBEn. The primary y-axis (Nymphs Adults/1000 m^2) from SLBEs is repeated for SLBEn. Regression lines and coefficients for nymphal densities are shown, if statistically significant. The infection prevalence of *I. scapularis* adults (outlined in black) and nymphs (outlined in gray) are indicated in rectangles above the density bars with the total number of ticks tested indicated in parentheses.

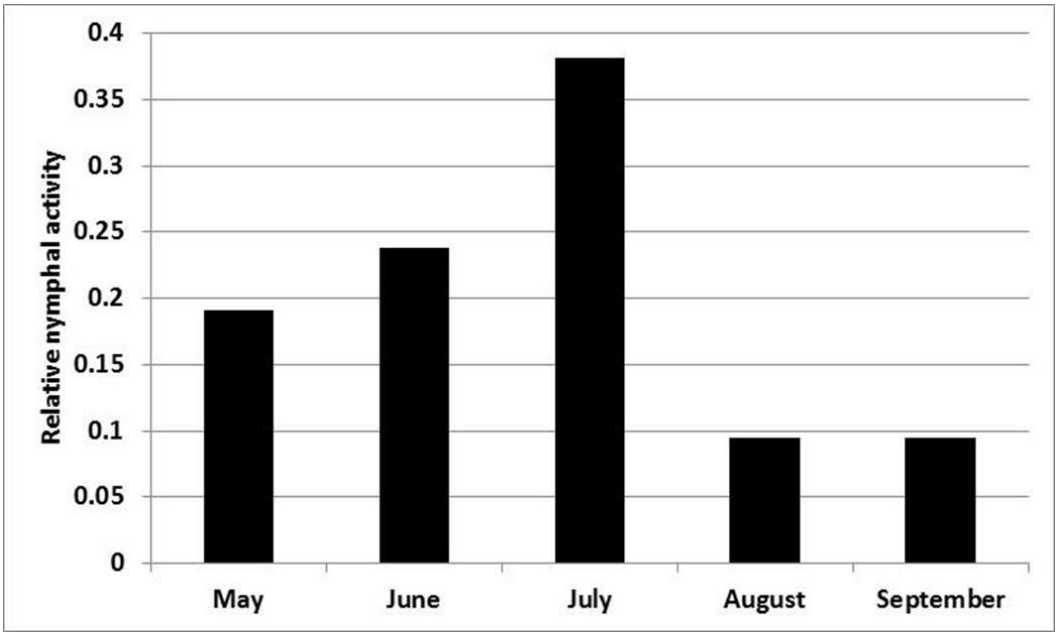

Figure 1.6 Relative *I. scapularis* nymphal activity (proportion of all nymphs active) at SLBEs in 2012 and 2014 by month. All other months that are not depicted on the graph did not have nymphs collected.

Borrelia burgdorferi Infection in Questing Ticks:

During the *late spring* sampling at the Van Buren reference site and the two SLBE sites, overall a total of 37.0%, 13.1%, and 0% of the tested questing *I. scapularis* adults (N = 54), nymphs (N = 160), and larvae (N = 182 total, divided into 3 pools) were infected with *B. burgdorferi* (Figure 1.5). Of these infected ticks, 95% of the positive adults and nymphs were from the reference site. Although both infected nymphs and adults were collected from the reference site, only infected adults were collected from SLBEs, and only infected nymphs were collected from SLBEn.

At SLBEs, during the *post-spring* sampling efforts, overall 22.9%, 6.1%, and 0% of the tested questing *I. scapularis* adults (N = 105), nymphs (N = 49), and larvae (N = 62 total, divided into two pools) were infected with *B. burgdorferi*. Of the infected adults, 92% were from the fall, which also was when 80% of the total number of adults were collected. The first fall in which infected adults were collected was in 2012, with an infection prevalence of 1.2%. In 2014, the adult infection prevalence increased to 6.1% and by 2015, it was 19.5%; this increase in infection prevalence (2012 to 2015) was significant ($p < 0.001$). Of the three infected questing nymphs, the first was collected in July 2013 (3.2%) then the remaining two in May 2014 (25%) and September 2014 (50%); the infection prevalence for each of these months is indicated in the parenthesis.

At SLBEn, during the *post-spring* sampling efforts, overall 20% of the questing adults that were tested (N = 25) were infected with *B. burgdorferi*. None of the questing nymphs were infected during the *post-spring* efforts. The first infected questing adults were collected in October 2014 (25% infection prevalence) and this infection prevalence remained relatively consistent the following fall (27%).

Overall, when all of the tested adult ticks were combined from both sampling efforts (*late-spring* plus *post-spring*) at each of the SLBE sites, there was not a significant difference between the adult infection prevalence from SLBEs versus SLBEn ($p = 0.653$). The same pattern held true with the nymphal infection prevalence, as there was not a significant difference between the two sites ($p = 0.407$).

Additional Pathogen Detection:

In 2014, all of the mammal and questing ticks at SLBEs and SLBEn were tested by collaborators at CDC, at which time they not only tested the ticks for *B. burgdorferi*, but also for *Anaplasma phagocytophilum* and *Babesia microti*. Fifteen of the mammal ticks/pools at SLBEn (28.3%) were infected with *A. phagocytophilum*, one of the mammal tick pools at SLBEs (0.85%) was infected with *A. phagocytophilum*, and none of the mammal ticks were infected with *Ba. microti*. Of the 15 *A. phagocytophilum* mammal ticks at SLBEn, all were removed from eastern chipmunks (12 nymphs and 3 larval pools from four different chipmunks). The one *A. phagocytophilum* infected mammal tick pool at SLBEs was collected from a white-footed mouse (2 larvae). Sixty-six percent and 100% of the *A. phagocytophilum* infected ticks at SLBEn and SLBEs, respectively, were also co-infected with *B. burgdorferi*.

Two questing adult male *I. scapularis* ticks from SLBEs were infected with *A. phagocytophilum*. None of the questing ticks at SLBEs were infected with *Ba. microti* and none of the questing ticks collected at SLBEn were infected with either *A. phagocytophilum* or *Ba. microti*.

Discussion:

Lyme disease is the most commonly reported vector-borne disease in Michigan and it is continuing to emerge across the state (MDHHS 2016-a). By 2009, it was determined that the leading edge of the blacklegged tick invasion along Lake Michigan was at SLBE (Hamer et al. 2010), which is one of the most popular tourist destinations in the state, attracting >1.5 million visitors in a year (NPS stats). From 2004-2008, our lab tracked the invasion of the blacklegged tick and Lyme disease pathogen within Michigan's Lower Peninsula along two sampling transects, a coastal and an inland, testing the process of invasion of the Lyme disease vector and pathogen using three hypotheses (Hamer et al. 2010). At SLBE, from 2007-2008, no *B. burgdorferi* had been detected, although blacklegged ticks were found attached to a low proportion of mammalian hosts, thus, supporting the "tick-first" scenario of invasion at SLBE (Hamer et al. 2010). We continued tick and pathogen surveillance at SLBE until 2015 to further monitor the invasion and establishment of the tick population, to quantify the duration of time until establishment of the pathogen population, and to provide data on Lyme disease risk to SLBE park administrators.

Following similar field and laboratory protocols previously performed by our lab, small mammal trapping targeting white-footed mice and eastern chipmunks and drag sampling for questing ticks were carried out during the *late spring*, when all three life stages of *I. scapularis* would be active, from 2007-2015, with additional ("*post-spring*") fieldwork performed in the summer and fall throughout the years. Our objectives were: 1) to determine if/when *B. burgdorferi* would be detected in ticks/wildlife at the initial SLBE site and how long it would be delayed from the initial detection of the presence of *I. scapularis*; 2) to determine if the Lyme disease tick and pathogen were present at the second, further north, SLBE site; and 3) to compare *I. scapularis* infestation and infection prevalence over time at both SLBE sites to the endemic reference site from 2007-2011.

Objective 1: "Tick-first" process of invasion at SLBEs: Pathogen how much later?

Estimating pathogen detection time based on late spring sampling period:

Strictly looking at the sampling performed in late May/June is important as it is consistent with the prior work performed by our lab when the invasions of the Lyme disease tick and bacterium were tracked along Lake Michigan. In 2005, our lab had conducted a small trapping effort and did not detect any *I. scapularis* attached to small mammal hosts (n=21, July; Hamer et al. 2010). We did not trap in 2006, but when we resumed trapping in 2007, we detected *I. scapularis* nymphs on a low proportion of mammals. Our small trapping effort in 2005 may have not been sensitive enough to detect a low density of established *I. scapularis*, or larvae may have been dispersed subsequently into the area by hosts (e.g., migratory birds). Beginning the following year, 2008 (Hamer et al. 2010), both larvae and nymphs were found on mammals in each year of the study. Although there were fluctuations early on, in general, there was a trend indicating that infestation rates generally increased annually. Two years after the tick was found on hosts, the first questing adult tick was collected (June 2009), and two years later, the first questing nymphal tick was collected (June 2011). These data support prior findings that small mammals appear to be more sensitive versus drag sampling for detecting invading blacklegged tick populations.

Although the tick was first detected in 2007, it was not until June 2012 when the Lyme disease pathogen first was detected at SLBEs in a white-footed mouse tissue biopsy, ticks removed from eastern chipmunks (larvae and nymphs), and tissue biopsies from chipmunks. Thus, the data support the hypothesis that the blacklegged tick invaded prior to the bacterium at this site, and it appears to be at least a five-year delay between their arrivals (Figure 1.7).

A similar delay between *I. scapularis* arrival and the arrival of *B. burgdorferi* was observed in southern Canada; using surveillance data, it took five years for *B. burgdorferi* to invade an *I. scapularis* established location (Ogden et al. 2013). Together these data support models indicating that a minimum

threshold of ticks must be achieved to allow enzootic maintenance of *B. burgdorferi* (Norman et al. 1999; Ogden et al. 2007; Ogden et al. 2013).

Estimating pathogen detection time based on full sampling:

When *post-spring* sampling was also considered (2007-2015), the first detections of questing adults, nymphs, and larvae were sooner compared with considering only *late spring* sampling, and the time between detection of ticks and pathogen was shorter. The blacklegged tick was still first found attached to small mammals in late May 2007 (Hamer et al. 2010) and the first questing adult and nymph *I. scapularis* were found two and four years later, respectively. Host-seeking larvae appeared one year after questing nymphs were detected, whereas even by 2015, they had not been detected during the *late spring* sampling. Thus, by September 2012, all three life stages of the blacklegged tick had been collected via drag sampling.

When considering *post-spring* sampling, *B. burgdorferi* was detected nearly one year sooner (July 2011) than when only considering *late spring* sampling (June 2012), and was four years after the first blacklegged tick was found attached to mammals (Figure 1.7). Sampling effort and duration obviously impacts inferences about detection. Our estimates of rate of spread and detection of pathogen using *late spring* sampling (Hamer et al. 2010), therefore may underestimate the detection and/or arrival of *B. burgdorferi*, as was seen when we expanded our sampling effort to include other periods during the seasonal activity of the tick.

The first time that *B. burgdorferi* was found (July 2011) it was detected in a white-footed mouse (biopsy) and also in a nymph that was attached to a different, white-footed mouse in which we did not detect infection (Figure 1.7). Based on reservoir competence status, it is reasonable that the first infected host was a white-footed mouse as this species is one of the most competent reservoirs (LoGiudice et al. 2003). Throughout the remainder of our study, however, there were only two other instances in which mice were found to be infected again at SLBEs (June 2012 and July 2014). Thus, this implies that

although we can definitely say that the pathogen arrived at SLBEs, it was still in low prevalence, at least within the white-footed mouse population.

Interestingly, the mouse that was parasitized with the infected nymph was simultaneously infested with eight additional nymphs and twelve larvae. All of the larvae and the other nymphs were negative, thus, highly suggesting that the mouse itself was not infected with *B. burgdorferi*, since larvae are born uninfected (Piesman et al. 1986). This then implies that the nymph was infected previously; either the nymph was infected as a larvae from a local endemic cycle, or it may have been introduced from further away as an infected engorged larvae by an infected host, such as a bird or other reservoir host with a large dispersal distance.

Objective 2: Tick and/or pathogen arrival at SLBEn?

Invasion based on late spring sampling period:

Similar to SLBEs, blacklegged nymphs were first found attached to small mammal hosts at SLBEn when sampling first began (June 2011); however, no *B. burgdorferi* was detected during the *late spring* sampling efforts. Every year thereafter, at least one small mammal was infested with at least one larva and/or nymph, and each year, there was a trend showing white-footed mouse and eastern chipmunk infestation levels comparable or greater than the first year that sampling occurred. Two years after the blacklegged tick was found attached to mammals, the first questing adult and nymph were collected (June 2013). At the same time that questing ticks were found, *B. burgdorferi* was first detected. Thus, there was a two year delay between the tick and the pathogen arrivals (Figure 1.7). The *late spring* sampling supports the "tick-first" invasion scenario at SLBEn; very similar to SLBEs.

Invasion based on full sampling:

Mammal and drag sampling took place *post-spring*, in addition to during the *late spring*, from 2011-2015. When the entire sampling effort at SLBEn was considered, the blacklegged tick was still first

found attached to small mammals in June 2011 but the first questing adult was collected a year earlier (April 2012) than initially determined when only the late May/June efforts were considered; the first detection of a questing nymph did not change (June 2013). However, prior to the collection of questing ticks, *B. burgdorferi* was detected. When the full sampling effort was evaluated, the Lyme disease pathogen was detected in the same year that the site was first sampled, just one month after the tick was found attached to mammals (July 2011; Figure 1.7). Because we did not sample SLBEn prior to 2011, however, the sequence of invasion cannot be determined. It is possible, however, when looking at the entire sampling effort, rather than a "tick-first" scenario of invasion, SLBEn may have undergone a "dual-invasion" scenario, in which both *I. scapularis* and *B. burgdorferi* established concurrently. Although this may have been a consequence as to when sampling began (as described in the Study Limitations section), the dual-invasion process has been observed in Michigan at another recently invaded site south of SLBEn, along the Lake Michigan coast (C3 in Hamer et al. 2010). This abbreviated gap between tick and pathogen establishment, supports what actually would be expected in the Midwest since larval and nymphal ticks are seasonally synchronous and there would be a high number of immigrating infected engorged larvae (Ogden et al. 2013; Hamer et al. 2012).

The first time that *B. burgdorferi* was found, July 2011, it was detected in a nymph that was attached to an eastern chipmunk. This was the only tick on the chipmunk. The chipmunk itself was negative for the presence of *B. burgdorferi*. Therefore, this suggests that as a larva it must have fed upon an infected host in order to obtain the bacterium (Piesman et al. 1986). Similar to SLBEs, since this was the first detection of the pathogen, based on the sensitivity of our methods, it is possible that the infected nymph may have fed as a larva on a migratory bird and consequently dropped off at SLBEn, contributing to the long distance dispersal of the tick and bacterium (Madhav et al. 2004).

Objective 3: SLBEs and SLBEn compared to the reference site and sequence of invasion?

Blacklegged ticks and *B. burgdorferi* were both detected at all three sites across the study period. However, as we expected the Van Buren reference site had a significantly greater abundance of blacklegged ticks and greater *B. burgdorferi* infection prevalence than both SLBE sites, supporting the recent emergence at the National Lakeshore. In general, the average proportion of white-footed mice and eastern chipmunks infested with blacklegged ticks was at least double at the reference site compared to either SLBE site. Of the mammal ticks, larvae on eastern chipmunks were first infected at SLBEs in 2012 and for the next two years continued to increase until the infection prevalence was comparable with the average larval chipmunk infection prevalence at the reference site. Nymphs on chipmunks were also first infected at SLBEs in 2012; while infection prevalence fluctuate across time, the average prevalence at the reference site was about double that of SLBEs. It was not until 2014 that larvae on chipmunks were infected at SLBEn, with an infection prevalence of about 1.5 times lower than the reference site average. It was one year sooner that nymphs were infected on chipmunks at SLBEn; however, the average infection prevalence was still approximately 1.5 times lower than the reference site average. Thus, although the infection prevalence of mammal ticks is generally increasing with time at both SLBE sites, larval and nymphal prevalences are still approximately 1.5 times lower than that of the endemic reference site.

A similar trend was observed with mammal infection with *B. burgdorferi*. The average small mammal tissue infection prevalence at SLBEs was approximately 5 times less than the reference site as it was not until the last three years of sampling until mammals began to be infected. At SLBEn, it was not until the last two years of sampling that infections in mammals were detected; yet, the average infection prevalence was about 1.5 times less than the reference site (the average at SLBEn was greater than SLBEs because mammal trapping only took place for four years at SLBEn compared to eight years at SLBEs). Thus, as would be expected, given that the ticks attached to mammals were approximately 1.5 times less

infected than the reference site, the mammals themselves, which must have been bitten by an infected tick in order to become infected, averaged $\geq$1.5 times lower infection prevalence than the reference site.

The density of questing infected nymphs (DIN) is the metric used for estimating Lyme disease risk. Larval ticks are born uninfected and must feed upon an infected host in order to obtain the pathogen (Piesman et al. 1986). Questing nymphs are responsible for the most cases of human Lyme disease and thus, have epidemiologically the greatest significance (Barbour et al. 1993). The DIN at the Van Buren reference site during this study (2007-2011; late May/June) was 1.75/1000 m^2, which is similar to what was found at this site from 2004-2008 (Hamer et al. 2010) and in the northeastern U.S. (Gatewood et al. 2009). The DIN at SLBEs was 0.03/1000 m^2 and 0.01/1000 m^2 at SLBEn, i.e., 100 times lower. Therefore, the Lyme disease risk at both SLBE sites was still considered minimal at this time.

Based on the northward invasion pattern that was observed within Michigan's Lower Peninsula along the Lake Michigan coast, we hypothesized that SLBEn would have had fewer ticks and lower infection prevalence in comparison to SLBEs (Hamer et al. 2010). The two sites, however, were both undergoing similar invasion dynamics, as there was not a significant difference between small mammal infestations or between small mammal infection prevalence. Yet, *B. burgdorferi* was detected in ticks and mammals at SLBEn after it was found at SLBEs (Figure 1.7). Similarly, at SLBEn, each life stage of the blacklegged tick was collected via drag cloth years after the corresponding tick was found at SLBEs, which does support the northward Lake Michigan invasion (Figure 1.7). In sites where tick populations are not well established due to limited reproduction occurrence, questing larval densities are markedly decreased (Stafford et al. 2003). This was evident at SLBEn in which no questing larval *I. scapularis* have been detected as opposed to SLBEs which host-seeking larvae have been found since 2012. Additionally, while *B. burgdorferi* has been detected from ticks removed from SLBEs white-footed mice since 2011, by the end of the study (2015), no infected ticks removed from white-footed mice at SLBEn have been detected yet. Thus, although similar invasion dynamics are occurring at both SLBE sites as

measured by mammal infestation and infection, in general, the northward Lyme disease invasion trend was supported.

Delayed seasonal peak in questing nymphs?

In southwestern Michigan, the peak nymphal blacklegged tick activity was documented in Hamer et al. 2012 to take place in June, which is also coincident with peak larval activity (Hamer et al. 2012). Questing nymphs are responsible for the most cases of human Lyme disease and thus, have epidemiologically the greatest significance (Barbour et al. 1993). However, further north along Lake Michigan, the average air temperature tends to be lower in the spring (Assel et al. 1995). Therefore, we hypothesized that this may contribute to a delay in the peak of the immature stages of the tick in comparison to the southwest Lower Peninsula.

When the relative nymphal activity at SLBEs within 2012 and 2014 (the two years that consistent monthly sampling occurred) was evaluated, the peak activity occurred in July. Minimal nymphs were collected at SLBEn in 2012 and 2014; however, nymphs were active (i.e. collected) in July as well. This is a month later than what was observed in the southwest Lower Peninsula. Due to a limited number of questing larvae collected during this study, the larval phenology was not determined at either SLBE site. A similar lag was observed in northern sites vs. southern sites across the U.S., with southern sites reaching their nymphal peak prior to the north (Diuk-Wasser et al. 2006). Although our study was only carried out within the state of Michigan, differences in spring ambient temperature between the northern and the southern Lower Peninsula appear to delay the peak activity of nymphs. This is important from a public health standpoint as peak visitation at SLBE and the peak relative nymphal activity both take place in July (NPS stats).

Eastern chipmunks - earlier indicator of *B. burgdorferi*?

In general, in the Midwest and also in the Northeast, white-footed mouse densities tend to be greater than eastern chipmunk densities. In Illinois, the capture rates of white-footed mice was almost twice that of eastern chipmunks (Slajchert et al. 1997), and in Massachusetts, at one site, the ratio of mice to chipmunks was 15:1 (Mather et al. 1989). This held true at our sites during this study as well; on average 70% of the captures were mice and 30% were chipmunks. Some have found that white-footed mice have a significantly higher reservoir potential than other species (characterized by the susceptibility of the host to infection when bitten by an infected vector, the ability of the pathogen to magnify and persist in the host, and the efficiency of the host at transmitting the spirochete to feeding vectors), including eastern chipmunks, as mice were more abundantly infested with larval ticks and possess the greatest infectivity (Mather et al. 1989, LoGiudice et al. 2003). Others argue that the reservoir potential of hosts depends on the habitat and location; chipmunks also play a role as a major reservoir of *B. burgdorferi* (Mannelli et al. 1993). Larval ticks will feed as readily on chipmunks and there is no difference in the ability of larval ticks to molt to nymphs regardless of feeding on mice or chipmunks (Mannelli et al. 1993, Slajchert et al. 1997).

Although mice tend to be in greater densities, chipmunks on average live longer (Tryon and Snyder 1973, Wolff et al. 1988) and can remain infected with the Lyme disease pathogen for at least 4 months and potentially longer compared to mice, which can remain infective for at least 6.5 months, but whose infectivity declines after 2 months (McLean et al. 1993, Donahue et al. 1987). Thus, chipmunk infections will most likely carry over from one year to the next (Slajchert et al. 1997). Studies evaluating the role of eastern chipmunks typically have been carried out in locations where the Lyme disease tick and pathogen are well established; however, in areas that have recently become invaded by *B. burgdorferi* (such as SLBEs and SLBEn), we found that eastern chipmunks tend to be an earlier indicator of the pathogen's presence in comparison to white-footed mice.

White-footed mice typically feed more larvae than chipmunks and eastern chipmunks have higher levels of nymphal infestation than mice (Mannelli et al. 1993, Slajchert et al. 1997, Schmidt et al. 1999). This trend was observed within our data as well. However, eastern chipmunks can still serve as a host for the larval stage of the tick. The first time that larvae were found feeding on hosts at SLBEs, June 2008, they were found on both mice and chipmunks. The same held true at SLBEn. The first time larvae were found feeding on hosts, July 2011, they were found on both mice and chipmunks. Thus, chipmunks do not necessarily serve as an earlier indicator of the presence of the blacklegged tick in a Lyme disease invading location; however, based on the two SLBE sites, they serve as a better indicator of the arrival of the pathogen.

At both SLBE locations, larval ticks were infected when removed from an eastern chipmunk prior to the larval ticks feeding on white-footed mice. At SLBEs, the first infected larval tick removed from an eastern chipmunk was in early June 2012 and it was not until two years later (June 2014) that the first larval tick removed from a white-footed mouse was infected. At SLBEn, the first infected larval tick removed from an eastern chipmunk was in June 2014 and to date, no white-footed mice larval ticks have been infected (Figure 1.7). This trend continued to carry out over time at SLBEs and SLBEn with higher infection prevalence in eastern chipmunk larvae and nymphs compared to white-footed mice. This was further supported when considering *B. burgdorferi* infection within the hosts themselves. Overall, combining sites over time, eastern chipmunk tissue samples from SLBEs and SLBEn had approximately 32 times greater infection prevalence compared to that of white-footed mice ($p < 0.001$). Others have shown this as well with eastern chipmunk ear biopsies having greater *B. burgdorferi* infection prevalence than white-footed mice (Slajchert et al. 1997). Therefore, especially as chipmunks may live longer than mice, we found that in Lyme disease-establishing locations that chipmunks serve as an earlier and more reliable predictor of *B. burgdorferi* than mice.

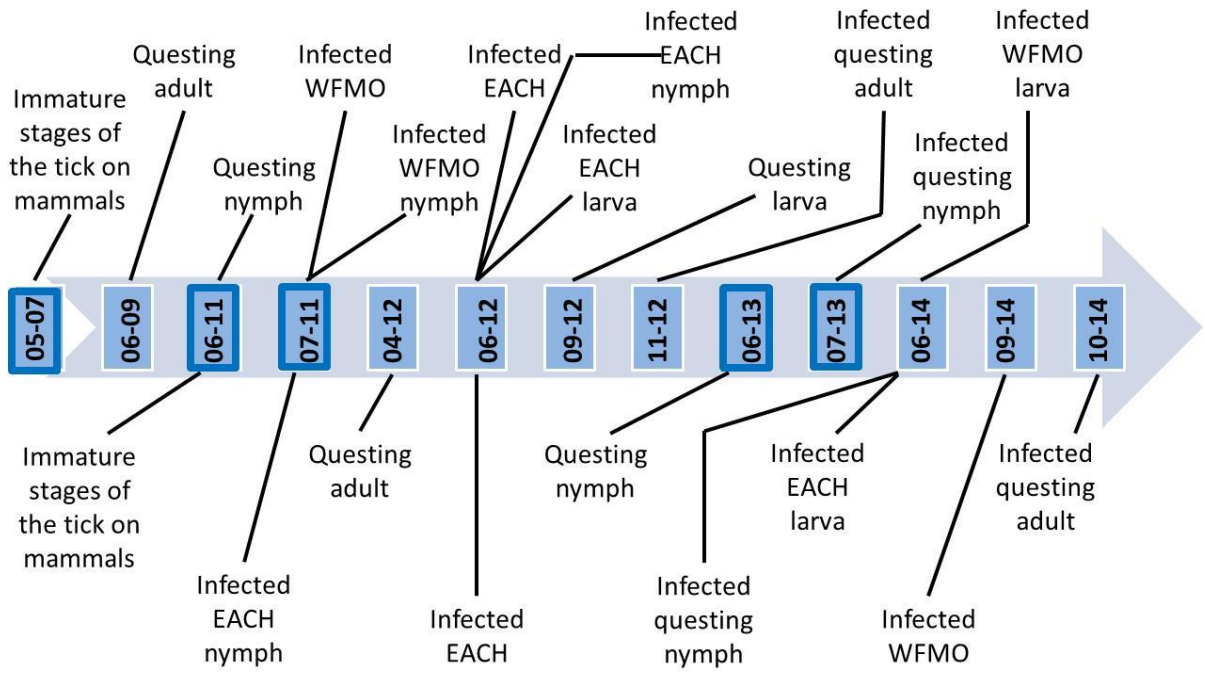

Figure 1.7 Timeline of the first *I. scapularis* and *B. burgdorferi* detections at both SLBEs (above) and SLBEn (below). Note-this timeline is not to scale.

Conclusion:

SLBE is one of Michigan's most visited destinations with millions of national and international visitors annually. From 2004-2008, our lab tracked the invasion of the blacklegged tick and Lyme disease pathogen along two transects of sites, an inland and a coastal, evaluating three different scenarios by which the invasion of the Lyme disease vector and/or pathogen into Lower Michigan may be occurring (Hamer et al. 2010). Within this west coast Lake Michigan model of continuous, contiguous advancement of the tick and pathogen, SLBE was located the furthest north. During this study, no *B. burgdorferi* was detected at SLBE, although blacklegged larval and nymphal ticks had been found attached to a low proportion of mammalian hosts thus, supporting the "tick-first" scenario of invasion at SLBE. Also, no questing blacklegged ticks of any life stage had yet been detected.

We continued mammal trapping and drag sampling for host-seeking ticks at the same SLBE site, SLBEs, from 2009-2015, and by September 2012, all three life stages of the tick were collected via drag cloth. We detected the first presence of *B. burgdorferi* four years (five years if strictly considering late May/June efforts) after the first *I. scapularis* tick was found. In areas undergoing the tick-first invasion, birds and/or deer may be doing the main introduction however; the timing may differ depending on whether birds are dispersing larvae or nymphs. Across this nine-year study at SLBEs, a trend illustrating an increase in *I. scapularis* and *B. burgdorferi* was observed. Determining the delay between tick and pathogen establishment can be advantageous as interventions and early precautions can be enacted.

In 2011, we began sampling at a second (further north) field site within SLBE, SLBEn. At SLBEn, a similar situation was observed in which blacklegged ticks were found during the first sampling effort (June 2011) however, no *B. burgdorferi* was detected. This quickly changed. By the following month, July 2011, *B. burgdorferi* was detected. Thus, supporting the "dual-invasion" scenario of invasion, in which both *I. scapularis* and *B. burgdorferi* established concurrently. However, this may have been a consequence of when sampling began. The dual-invasion process has been observed in

Michigan at another recently invaded site south of SLBEn, along the Lake Michigan coast (C3 in Hamer et al. 2010). By April 2012 and June 2013, the first questing adult and nymph, respectively, were collected at SLBEn.

From 2007-2011, Van Buren State Park served as the reference site for the two SLBE sites as we knew, from previous investigations, that this site was endemic for the blacklegged tick and Lyme disease pathogen (Hamer et al. 2010). Van Buren was sampled until *I. scapularis* was found at both SLBE sites. Overall, as we expected, Van Buren had a significantly greater abundance of blacklegged ticks and greater *B. burgdorferi* infection prevalence than both SLBE sites, supporting the recent emergence at SLBE.

The relative nymphal activity at SLBEs in 2012 and 2014 (the two years that consistent monthly sampling took place) was determined and the peak activity occurred in July. Although a limited number of nymphs were collected at SLBEn in 2012 and 2014, nymphs were active in July as well. This was one month after the peak in the southwest Lower Peninsula, which is important from a public health standpoint as the nymphal stage has epidemiologically the greatest significance and the peak in nymphal activity coincides with the peak in visitation at SLBE (Hamer et al. 2012, Barbour et al. 1993, NPS stats). Furthermore, interestingly, we also found that in areas that have recently become invaded by *B. burgdorferi* (such as SLBEs and SLBEn), eastern chipmunks tend to be an earlier indicator of the pathogen's presence in comparison to white-footed mice. Given the northward invasion of the blacklegged tick and Lyme disease pathogen along the Lake Michigan cost in Michigan's Lower Peninsula (Hamer et al. 2010), we hypothesized that SLBEn would have fewer ticks and lower *B. burgdorferi* infection prevalence versus SLBEs. The two sites, however, are both undergoing similar invasion dynamics, as there was not a significant difference between small mammal infestations or between small mammal infection prevalence. Yet, *B. burgdorferi* was detected in ticks and mammals at SLBEn after it was found at SLBEs. Similarly, at SLBEn, each life stage of the blacklegged tick was collected via drag cloth years after the corresponding tick was found at SLBEs, which does support the

northward Lake Michigan invasion. However, given the level of infestation and infection with *B. burgdorferi*, it is likely that the leading edge of the invasion of *I. scapularis* and *B. burgdorferi* now lies further north than SLBEn.

Study Limitations and Future Research:

When the sampling took place within the calendar year: Sampling was only performed consistently across the two SLBE sites and also at the reference site during late May/June. This was done because we were replicating the prior sampling efforts performed by our lab to track comparably the invasion of the Lyme disease tick and bacterium in the same area (Hamer et al. 2010). However, we also wanted to conduct more detailed sampling, which was carried out at the SLBE sites during additional months over the years (i.e. *post-spring*). It was through these *post-spring* efforts that we were able to first detect the presence of *B. burgdorferi* at SLBEs and SLBEn. This then raises the question - could *B. burgdorferi* have been found earlier at these two sites (as well as those sampled in Hamer et al. 2010) if additional sampling had been routinely performed across the years?

If this specific study were to be repeated with the goal of detecting the presence of *I. scapularis* and *B. burgdorferi* with limited sampling effort, we would recommend that consistent sampling among sites be performed in July rather than late May/June given differences in peak larval and nymphal activity periods. The peak larval and nymphal blacklegged tick activity periods in southwest Michigan may occur in June, (Hamer et al. 2012); however, we found that the questing nymphal peak in the northern Lower Peninsula was a month later. The first detection of larval ticks at SLBEn was in July, and the first time that *B. burgdorferi* was detected at both SLBE sites was in the month of July. Another recommendation for increasing the probability of detecting *B. burgdorferi* would be to ensure trapping of eastern chipmunks. We found throughout this study that eastern chipmunks tended to be an earlier indicator of the pathogen's presence in comparison to white-footed mice. Eastern chipmunks are most active in the early summer and the fall, with activity level in early and mid-July approaching the June level and a surge

in male activity occurring in the beginning of July, thus, further supporting the July sampling efforts (Dunford 1972). However, the month that consistent sampling takes place should be dependent on geographical location of the study since the tick phenology varies with southern vs. northern sites (Diuk-Wasser et al. 2006), therefore, the July recommendation would be of greatest benefit for northern Michigan areas.

When the sampling started: Additionally, immature stages of the tick were found attached to mammals during the first trapping efforts at SLBEn. This suggests that the presence of *I. scapularis* may have been detected sooner if sampling began prior to 2011. Ideally, we would have liked to begin sampling when neither blacklegged ticks nor pathogen were detected, to increase our sensitivity for determining the tick's arrival and to estimate the time lag (if any) between tick and pathogen establishment.

Furthermore, we proposed that SLBEn underwent a "dual-invasion" scenario of invasion, since the pathogen was detected at approximately the same time as the tick. However, since we detected both during the first couple of months of sampling, the tick or the pathogen may have arrived prior to the other thus, allowing SLBEn to have undergone either the "tick-first" or the "spirochete-first" scenarios of invasion. Regardless which invasion scenario occurred, since questing larvae and infected ticks from white-footed mice have yet to be detected at SLBEn, this is highly suggestive that this location is a recently invaded site for both the Lyme disease tick and the pathogen. Also, this abbreviated gap between tick and pathogen establishment, supports what actually would be expected in the Midwest since larval and nymphal ticks are seasonally synchronous and there would be a high number of immigrating infected engorged larvae (Ogden et al. 2013; Hamer et al. 2012). The first infected mammal was found the second year of sampling at SLBEn, therefore, further supporting that the pathogen just recently arrived to the area. Questing ticks were also found the second year, in low densities, supporting that the tick also just recently arrived. Thus, this adds support for the "dual-invasion" scenario at this location.

Future research: This work was a continuation and expansion of the study previously performed in our lab (Hamer et al. 2010). A multi-year (nine years) continuous monitoring effort at designated sites within Michigan allowed for not only the first presence of the pathogen to be detected but also the gradual increase in tick abundance and hence disease risk to be followed. This study provided updated data for Michigan's Lyme disease risk map (MDHHS 2016-b). As previous work has shown, vector-borne disease risk maps are an extremely important tool for public health guidance and intervention (Benedict et al. 2007; Rakotomanana et al. 2007; Ogden et al. 2008).

Although there are different possible scenarios of Lyme disease invasion into an area (Hamer et al. 2010), it appears that there are locations, such as southern Canada and SLBEs, that undergo the "tick-first" scenario, where there is a delay before the pathogen arrives. In order to have human and/or domestic animal Lyme disease cases, the pathogen, the host (reservoir and susceptible), and the environment must overlap, thus forming a triangle (i.e. epidemiologic triangle); without all entities present and in line with one another human/domestic animal disease will not be possible. Thus, when one piece is missing, as in the case with the delayed appearance of *B. burgdorferi*, interventional strategies can be attempted in order to minimize the potential disease outbreak, when and if the epidemiologic triangle becomes complete and overlap occurs (CDC Principles of Epidemiology in Public Health Practice, Third Ed.). Potential interventional strategies include, but are not limited to, human tick checks, tick control via area-wide acaricides and vegetation management, deer-targeted acaricides, elimination or exclusion of deer, and human prophylactic treatment after tick bites (Hayes et al. 2003; Sood et al. 1997; Stafford et al. 1991; Curran et al. 1993; Pound et al. 2000; Wilson et al. 1988; Nadelman et al. 2001). However, due to the difficulties and/or public acceptance of the above control measures, others argue that the primary interventional technique is public education (Hayes et al. 2003; Steere et al. 2004; Malouin et al. 2003).

A crucial element of combat against emerging pathogens is surveillance from multidisciplinary research efforts (Woolhouse 2002), and this real-time disease invasion tracking study is such an example.

However, this invasion work should not stop here. Given the level of infestation and infection with *B. burgdorferi* at both SLBE sites, it is likely that the leading edge of the invasion of *I. scapularis* and *B. burgdorferi* lies further north than SLBEn. Future work should involve mammal trapping and drag sampling at locations north of SLBEn in order to determine the current leading edge of the Lyme disease invasion in Michigan's Lower Peninsula in addition to neighboring counties to the east, thus, creating an even more up-to-date disease risk map which could serve as a source for the public's education.

APPENDIX

APPENDIX

Table 1.3 Total number of non-white-footed mouse and eastern chipmunk captures at the three sites (Van Buren reference site, SLBEs, and SLBEn) during the *late spring* and *post-spring* trapping efforts.

Mammalian species	Reference	SLBEs	SLBEn	
Southern flying squirrel	4	9		Late Spring
Glaucomys volans		1	1	Post-Spring
Meadow vole			1	Late Spring
Microtus pennsylvanicus			5	Post-Spring
Northern short-tailed shrew		4	5	Late Spring
Blarina brevicauda		10	13	Post-Spring
American red squirrel		2		Late Spring
Tamiasciurus hudsonicus		2		Post-Spring
Meadow jumping mouse		1		Late Spring
Zapus hudsonius			1	Post-Spring
Fox squirrel		1		Late Spring
Sciurus niger				Post-Spring
Long-tailed weasel				Late Spring
Mustela frenata		2	3	Post-Spring
Virginia opossum				Late Spring
Didelphis virginiana		2		Post-Spring
Eastern gray squirrel				Late Spring
Sciurus carolinensis		3		Post-Spring

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CHAPTER 2

Success of mammalian hosts for maintaining the Lyme disease vector Ixodes scapularis in the absence of white-tailed deer

Abstract:

How the Lyme disease tick spreads and becomes established is of great public health importance. White-tailed deer are believed to be the most important hosts for adult *Ixodes scapularis* and critical for its spread and maintenance, but few opportunities exist to investigate tick and pathogen dynamics in their absence. Two Lake Michigan islands, North/South Manitou Islands, presented this opportunity as one island was home to a deer population and the other was completely devoid. On the deer-less island, it was assumed that the tick population was less able to reproduce, unless other medium-sized mammals were serving as alternative hosts for the adult stage. As expected, the island with the deer had a more established tick population and presented as a greater public health concern. However, unlike what was hypothesized, all three life stages of the tick were found on the deer-free island. To determine how the blacklegged tick population was being maintained in the absence of deer, potential alternative hosts for the adult tick (snowshoe hares (*Lepus americanus*), passerine birds, and coyotes (*Canis latrans*) were trapped. Coyotes were determined to be maintaining the tick population in the absence of deer. Furthermore, eastern chipmunks (*Tamias striatus*) were found to play a crucial role with maintaining the juvenile stages of the tick on the islands. This has implications for locations undergoing deer reductions for disease management purposes given that even if deer are not present, an established tick population can still occur if alternative medium/large mammalian hosts, such as coyotes, are present.

Introduction:

Lyme disease is the most commonly reported vector-borne disease of humans in Michigan and the U.S. (MDHHS 2016; Mead 2015). The disease is caused by the bacterium *Borrelia burgdorferi*,

which is transmitted in eastern North America by the blacklegged tick, *Ixodes scapularis* (CDC 2015). Lyme disease is a significant public health problem as the number of human cases has been and is continuing to increase nationally.

In Michigan's Lower Peninsula, the blacklegged tick and the bacterium are spreading northward and inland along the Lake Michigan shoreline (Hamer et al. 2010). One of Michigan's most visited landmarks, Sleeping Bear Dunes National Lakeshore (SLBE), was at the leading edge of this invasion in 2009 (Figure 2.1). SLBE includes two large offshore islands-North Manitou Island (NMI) and South Manitou Island (SMI). Following a report by a deer hunter of abundant blacklegged ticks on NMI in Fall 2010 (Foster 2010), Michigan State University (MSU), Michigan Department of Health and Human Services (MDHHS), and the National Park Service (NPS) began an investigation of ticks on the islands in Summer 2011 and subsequently followed this initial investigation with four additional years.

Prior to this hunter report, Lyme disease ticks had not been reported from the Manitou Islands. The known wildlife on both islands included small rodents such as white-footed mice (*Peromyscus leucopus*) and eastern chipmunks (*Tamias striatus*), as well as snowshoe hares (*Lepus americanus*), coyotes (*Canis latrans*), and white-tailed deer (*Odocoileus virginianus*) (NMI only). The immature stages of the blacklegged tick (the larval and nymphal stages) typically feed on white-footed mice and eastern chipmunks, both of which are competent reservoirs for *B. burgdorferi* (Donahue et al. 1987, Mather et al. 1989, LoGiudice et al. 2003). The adult stage, on the other hand, typically feeds on large mammals such as white-tailed deer, thus making this host crucial for the completion of the tick's life cycle; deer, however, are incompetent hosts for *B. burgdorferi* (Telford et al. 1988). In 1926, humans introduced deer to NMI and as of 2006, the estimated deer herd size was 80-100, or 1.4-1.7 deer/km^2 (National Park Service 2016-a, Jennings 2012). With the absence of white-tailed deer on SMI, it was assumed that the tick population was less able to reproduce, unless other medium-sized mammal species were serving as alternative hosts for the adult stage. Therefore, given the different host communities and

how they were a primary tourist destination, the Manitou Islands presented a valuable opportunity for studying key aspects about Lyme disease ecology that also had great public health relevance.

Our objectives for this study were: 1) Determine if the Lyme disease tick and pathogen were present and to what degree in small mammal hosts and questing ticks on both the island with the deer and also the island devoid of deer; 2) Compare mammal and host-seeking infestation and infection prevalence from one island to the other; 3) Evaluate the role of alternative hosts for maintaining the Lyme disease tick on the island devoid of deer.

We hypothesized in the absence of deer and given a depauperate community of medium/adult mammals, there would be a lower blacklegged tick population. Since birds still played a role introducing ticks, we predicted that nymphal and adult ticks would be present, albeit in low numbers, on the deer-less island. Yet, no larval ticks would be detected (Elias et al. 2011), unless other hosts served as alternatives for the adult tick.

Materials and Methods:

Site selection and sampling regime:

Sampling Sites: North and South Manitou Islands (NMI, SMI), Leelanau County, are part of Seeping Bear Dunes National Lakeshore (SLBE) (Figure 2.1). NMI is approximately 12 miles from Leland, MI which is a small town on the mainland. NMI has a land area of 58 km^2 with 20 miles of shoreline. SMI is approximately 5 km from NMI and has a land area of 21.4 km^2 with 10 miles of shoreline (National Park Service 2016-b). Both islands consist of deciduous/mixed forests in addition to sandy, open dunes with a small lake located within the islands. Currently, no year-around human residents reside on either island and no domestic pets are permitted. Prior to the report received from a deer hunter on NMI after the annual week-long deer hunt, in Fall 2010, MDHHS and MSU had not

received any reports of blacklegged ticks on the islands nor had any structured tick surveys been performed on NMI or SMI.

Sampling Regime-Both islands were sampled from 2011-2015, with small mammal trapping and drag sampling taking place at minimum in June of every year (except in 2015), when all three life stages of *I. scapularis* would be active. Coyote trapping took place in the fall when the adult stage of *I. scapularis* would be in peak. Additional drag sampling, small mammal trapping, bird mist-netting, and rabbit trapping took place throughout the calendar year as listed in Appendix 2.1.

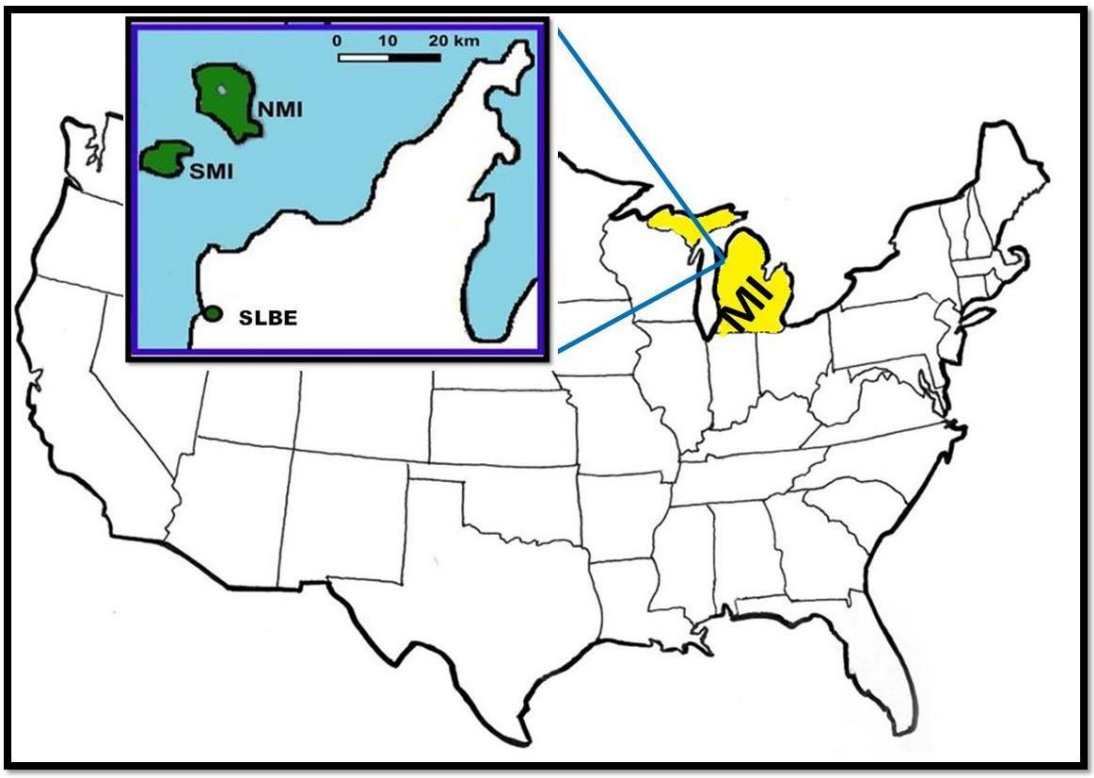


Figure 2.1 Map of the United States with Michigan highlighted in yellow. The two islands, North Manitou Island (NMI) and South Manitou Island (SMI) that are part of Sleeping Bear Dunes National Lakeshore (SLBE) are shown.

Small mammal trapping:

Small mammals, targeting white-footed mice and eastern chipmunks, were trapped following the protocol used in Hamer et al. 2010. Briefly, at each site, small mammals were trapped along six transects of 25 live traps (H. B. Sherman Traps, Tallahassee, FL) spaced 10 m apart and baited with crimped oats for two consecutive nights. Trapping took place at least once per month when immature stages of the tick were active. Traps were set in the evening and checked the following morning. All captured mammals were identified to species and sex, examined for ticks, and biopsied in both ears using a 2-mm biopsy punch (Miltex Instruments, York, PA). Ticks and ear biopsies were stored separately in 70% ethanol. Recaptured animals that were caught the previous day were strictly examined for ticks with no additional ear biopsies taken; animals recaptured from a previous trapping period, however, were biopsied again. All captured mammals were released back at the point of capture. The number of trap nights per trapping period was adjusted for tripped traps as follows: number of traps set - (0.5*no. of tripped traps). Animal handling procedures were approved by Michigan State University's Institutional Animal Care and Use Committee Animal Use Form #: 06/12-103-00.

Questing tick sampling:

Each site was sampled for questing ticks by dragging a 1 m^2 corduroy cloth along the forest floor (Hamer et al. 2010). This was performed along the same transects (5-10 m on either side of the transect) that were used for small mammal trapping. A minimum of 1000 m^2 (i.e., 4 transects) was dragged during each site visit. The cloth was examined every 20 m and any ticks that were found attached to the cloth or to the individual performing the sampling were collected and stored in 70% ethanol.

Snowshoe hare trapping:

Snowshoe hares (*Lepus americanus*) were trapped on SMI from June-September, 2012 and 2014. Snowshoe hares were the only specie of rabbit present on SMI (Sleeping Bear Dunes National Lakeshore 2000). Four to six wooden box traps were baited with cut apples and set in the evenings and then checked the following morning. Trapping took place at minimum for two consecutive nights per month. The traps were placed throughout the SMI "village," the southeast side of the island, where the hares appeared to be in greatest abundance and most active. Any captured hares were brought back to a central processing location. One technician properly restrained the hare while a second technician checked for ticks and biopsied in both ears using a 2-mm biopsy punch (Miltex Instruments, York, PA). Ticks and ear biopsies were stored separately in 70% ethanol. Recaptured animals that were caught the previous day were strictly examined for ticks with no additional ear biopsies taken; animals recaptured from a previous trapping period, however, were biopsied again. To minimize stress, a towel was used to cover the eyes and the tick checking was limited to ten minutes. After which time, the hare was released back into the "village." Animal handling procedures were approved by Michigan State University's Institutional Animal Care and Use Committee Animal Use Form #: 06/12-103-00.

Bird mist-netting:

On each island, six 12-m mist-nets (Avinet, Dryden, NY) were used to capture birds and were placed throughout the "village" on both islands, on the southeast side of SMI and eastern side of NMI. The protocol used in Hamer et al. 2010 was followed. Briefly, nets were run from 0600-1200 h, weather permitting, and were checked every 30 minutes. Captured birds were weighed, identified to species and sex, measured, searched for ticks, and leg-banded with federally issued bands prior to release. In 2014, birds were captured on both islands June-August and then on SMI in September-October. Mist-netting was performed under the U.S. Geological Survey Bird Banding Laboratory Federal Bird Banding Permit #23629.

Coyote trapping:

Coyotes (*Canis latrans*) were lived trapped using 3.5 EZ grip padded leg hold traps with off-set jaws and swivel (Olsen et al., 1986; Phillips et al., 1996; Frame and Meier, 2007). A total of twenty-six traps were set throughout SMI in locations were prior visual inspection indicated the presence of coyote feces and/or foot prints. Trapping took place in October, 2014 after the public ferry stopped running, thus, no other humans were on the island during the trapping session aside from the research personnel. Four traps were set for nine consecutive nights and then the remaining twenty-two traps were set for eight consecutive nights. Traps were visually inspected and checked every 6-8 hours. Species-specific scent lures were used near the traps.

Once a coyote was captured, one technician restrained the animal using a catch poll while the second used a 42" jab stick pole in order to anesthetize using ketamine hydrochloride (Ketaset; Fort Dodge, Overland Park, KS) and xylazine hydrochloride (Rompun; Bayer Health Care, Kansas City, KS) followed by reversal with administration of yohimbine hydrochloride (Antagonil; Wildlife Laboratories, Fort Collins, CO). Once anesthetized, the trap was removed and a blindfold was used to cover the face in order to minimize sensory stimuli. The animal was identified to species and sex by inspection, examined for ticks, blood sampled via jugular vein, biopsied in both ears using a 4-mm biopsy punch (Miltex Instruments, York, PA), and ear-tagged (National Band and Tag, Newport, KY). Tick checking was limited to ten minutes.

In order to further verify the viability of coyotes as hosts for maintaining blacklegged ticks, adult engorged female blacklegged ticks were kept alive by placing them in individual tubes with approximately two blades of grass and a finely mesh top. The females were brought back to Michigan State University's Containment Facility and were kept in secured chambers in which temperature and humidity were monitored. The females were kept until oviposition was observed, after which time, the females were either discarded or stored in 70% ethanol. All other non-engorged adult ticks and ear biopsies were stored separately in 70% ethanol in the field. Animals were released at the point of capture.

Wildlife procedures were approved through Michigan State University's Institutional Animal Care and Use Committee Animal Use Form #: 06/12-103-00 and National Park Service's Institutional Animal Care and Use Committee Animal Use Protocol Number: MWR_SLBE_Sidge_Coyote.Foxes_2014.A2.

Pathogen detection:

All ticks were identified morphologically to species and life stage using dichotomous keys (Keirans et al. 1978; Sonenshine 1979; Durden et al. 1996). Total DNA from ticks and ear biopsies, with the exception of tick samples from 2014, were extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's animal tissue protocol with the modifications described previously (Hamer et al. 2010). Only *I. scapularis* ticks were assayed; any other species found was strictly recorded and stored for potential future analysis. In situations where more than three adult or nymphal ticks of the same species, life stage, and sex were removed from an individual animal, three were randomly selected for testing (Hamer et al. 2010). *Borrelia burgdorferi* was detected using a quantitative polymerase chain reaction (qPCR) targeting a fragment of the 16S rDNA gene (Tsao et al. 2004). With the exception of the 2014 tick samples, the qPCR protocol was performed as previously described (Hamer et al. 2010). Reactions for the qPCR were performed with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The 2014 tick samples were extracted and tested by collaborators at the Centers for Disease Control and Prevention, Division of Vector Borne-Infectious Diseases. Total DNA from the 2014 ticks were extracted and *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Babesia microti* were detected following procedures as described in Hojgaard et al. 2014 (Appendix 2.2). The consistency between the two assays was compared prior to screening the 2014 samples. Of 304 ticks and tissue biopsies that were tested by both labs, there was 97.4% agreement; there was no significant difference between the two assays (Fisher's exact test: $p = 0.798$).

Statistics:

Linear regression was used to assess trends in adult and nymphal tick densities within the sites over time. Fisher's exact test was used to assess differences in overall capture success between sites and also the pathogen testing procedures used at Michigan State University versus what was used at the CDC. This was performed using GraphPad Software, Inc. (La Jolla, CA), with an alpha level of 0.05. In order to assess differences in infestation and infection prevalence in addition to annual capture success between sites and also within the same site over time, the z-score and associated two-tailed probabilities were calculated. This was performed using a web-based calculator, Social Science Statistics (Stangroom 2016), with the assumption of a normal distribution and equal variance and using an alpha level of 0.05. Error bars correspond to 95% binomial confidence intervals for proportions (Agresti and Coull 1998) and were created using GraphPad Software, Inc. (La Jolla, CA).

Results:

Small mammal captures:

On NMI, from 2011-2014, with 5167 adjusted trap nights, we captured a total of 907 white-footed mice, of which 16% were captured the previous day, and a total of 838 eastern chipmunks, of which 15% were caught the previous day. On SMI, from 2011-2014, with 5121 adjusted trap nights, we captured a total of 486 white-footed mice, of which 16% were captured the previous day, and a total of 769 eastern chipmunks, of which 20% were caught the previous day.

Overall, with all trap years combined, there was not a statistically significant difference between white-footed mouse capture success on NMI in comparison to SMI ($p = 0.097$) nor with eastern chipmunk captures success between the islands ($p = 1.000$). Although, on SMI, with previous day recaptures excluded, there was a statistically significant greater number of eastern chipmunks captured than white-footed mice ($p < 0.001$). With previous day eastern chipmunk and white-footed mouse captures excluded

on NMI, there was not a significant difference between the proportion of chipmunks and mice caught (p = 0.070). However, when considering each year individually, there was a significant difference between species captured in three of the four capture years on NMI and all four years on SMI (Figure 2.2).

Additional small mammal species that were captured from 2011-2014 on NMI included, meadow voles (*Microtus pennsylvanicus*) (n = 122), northern short-tailed shrews (*Blarina brevicauda*) (n = 3), and fox squirrels (n = 3). On SMI from 2011-2014, additional small mammal captures included northern short-tailed shrews (n = 9).

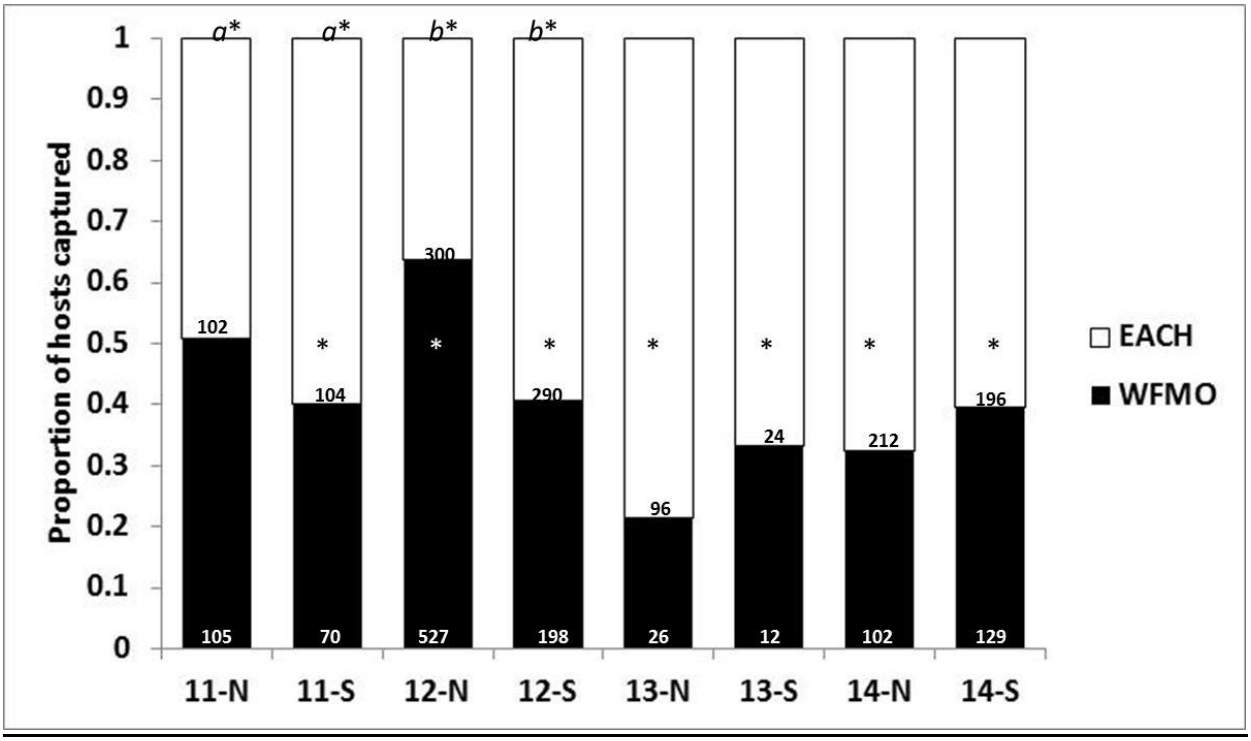


Figure 2.2 Proportion of white-footed mice (WFMO; black) and eastern chipmunks (EACH; white) captured each year (with the last two digits of the year depicted) on NMI (N) and SMI (S). The number within each bar indicates the total number of mice (black) and chipmunks (white) captured. The asterisk on the bar itself indicates that there is a statistically significant difference (z-score) between WFMO and EACH success for that year/site. The letter and asterisk above the bar indicates a statistically significant difference (z-score) between sites (NMI vs. SMI) with the proportion of WFMO and EACH captured (*a**: 2011; *b**: 2012)

Ixodes scapularis infestation on small mammals:

To make sure counts were independent and not affected by tick removal the prior day, we estimated tick infestation prevalence using data from the first time an animal was captured, regardless if it were captured on the first day or second day of the trapping period. Counts from individuals captured in multiple trapping periods were considered independent.

Overall, both immature stages of the blacklegged tick were found attached to captured small mammals on SMI and NMI. Immature stages were detected on small mammals every June during the study on both islands. In general, NMI had approximately 3.5 times greater mammal infestation than SMI. When the proportion of mice and chipmunks infested with larvae, nymphs, and/or both were combined, NMI small mammals were significantly more infested than SMI in June of 2011, 2013, and 2014 ($p < 0.001$ each year; Figure 2.3, 2.4).

On SMI, there was not a significant difference between larval vs. nymphal infestation on white-footed mice or eastern chipmunks in June each year aside from June 2014, when the chipmunks had approximately 8.5 times greater nymphal infestation than larval ($p < 0.001$; Figure 2.3, 2.4). On NMI, however, during three of the four study years, the mice had a greater larval infestation than nymphal (p= 2012: <0.001; 2013: 0.011; 2014: 0.005). On the NMI chipmunks, there was not a difference between the infestation of the two immature stages of the tick in June each year aside from 2014, when none of the chipmunks were infested with larvae but approximately 22% were infested with nymphs ($p = 0.001$; Figure 2.3, 2.4).

The seasonal dynamics of white-footed mice and eastern chipmunks within a calendar year on the two islands were compared to a Lyme disease endemic site located along the Lake Michigan coast of Michigan's Lower Peninsula (Figure 2.5).

White-footed mice: There was a gradual increase in white-footed mice abundance within the year on both of the islands and the endemic site, with mice feeding more larvae than nymphs. At both the endemic site and the islands, larval abundance peaked in June and August and there was a slight peak

with nymphs in June in both locations. When only these peak tick abundance months (June and August) were considered, the island white-footed mice, which were less abundant, fed significantly less larvae and nymphs than the island chipmunks (Figure 2.6). In general, there was a trend that mice were less abundant on the islands in comparison to the endemic site (Hamer et al. 2012).

Eastern chipmunks: On the islands, there was relatively consistent chipmunk abundance throughout the summer and early fall, unlike at the endemic site which had varying abundance. At both locations, however, chipmunks fed more nymphs than larvae except in the late summer/early fall on the islands. In addition, there was a nymphal peak prior to a larval peak on the islands which was not observed at the endemic site. In general, there was a trend showing greater chipmunk abundance on the islands than at the endemic site downstate (Hamer et al. 2012).

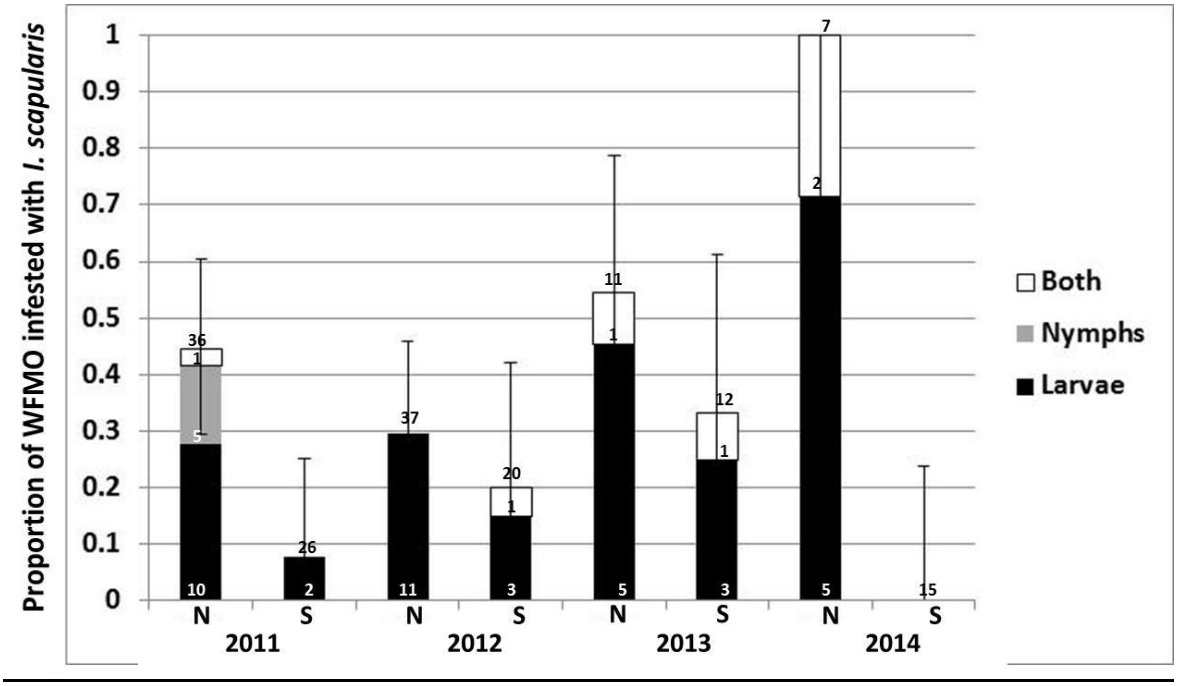


Figure 2.3 The proportion of white-footed mice (WFMO) infested with *I. scapularis* larvae (black), nymphs (gray), and both larvae and nymphs (white) on North Manitou Island (N) and South Manitou Island (S) in June 2011-2014. This is a non-cumulative stacked graph. The (95% binomial confidence interval) error bars correspond to the overall proportion of mice infested with at least one *I. scapularis* tick. The number of individual WFMO infested with each life stage is indicated within the corresponding shaded bar. The number above each bar represents the total number of unique white-footed mice captured.

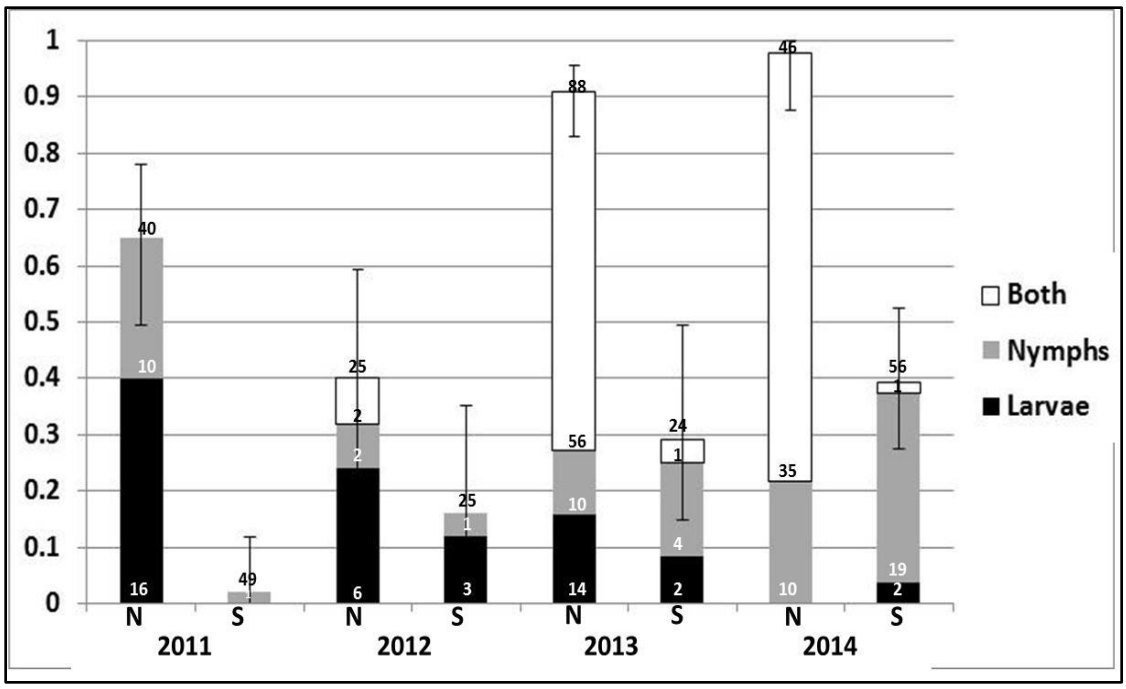


Figure 2.4 The proportion of eastern chipmunks (EACH) infested with *I. scapularis* larvae (black), nymphs (gray), and both larvae and nymphs (white) on North Manitou Island (N) and South Manitou Island (S) in June 2011-2014. This is a non-cumulative stacked graph. The (95% binomial confidence interval) error bars correspond to the overall proportion of mice infested with at least one *I. scapularis* tick. The number of individual EACH infested with each life stage is indicated within the corresponding shaded bar. The number above each bar represents the total number of unique chipmunks captured.

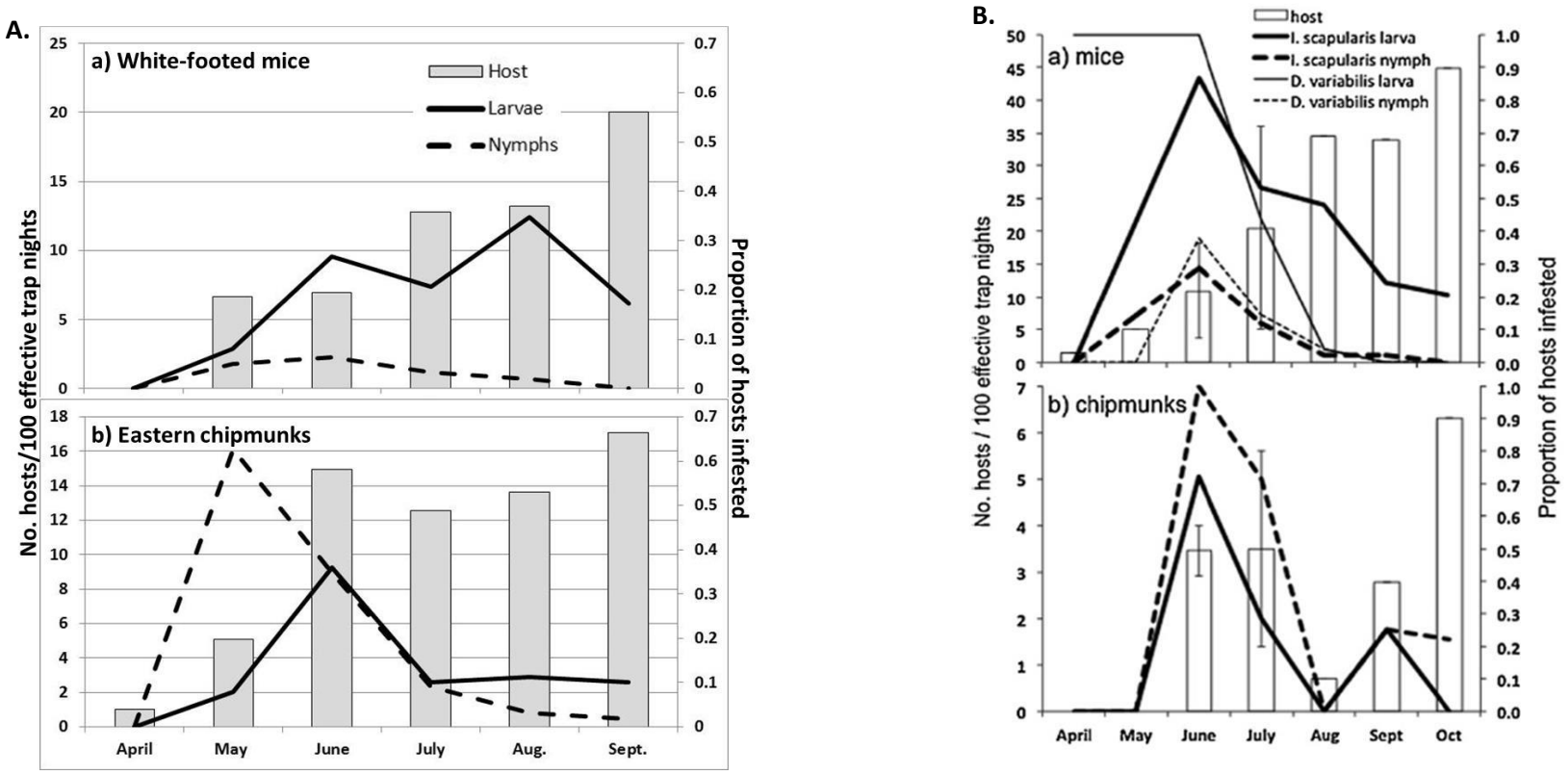


Figure 2.5 Seasonal dynamics of a) white-footed mice and b) eastern chipmunks within a calendar year on NMI and SMI in the top graph (A). The number of hosts/100 effective trap night (bars), and the proportion that each host specie was infested with *I. scapularis* larvae (solid line) and nymphs (dashed line) were combined from NMI and SMI each month from 2011-2014. The graph to the right, B, depicts the seasonal dynamics of tick infestation of white-footed mice (a) and eastern chipmunks (b) from a Lyme disease endemic field site approximately 223 miles south of the Manitou Islands along the west coast of Michigan (Hamer et al. 2012).

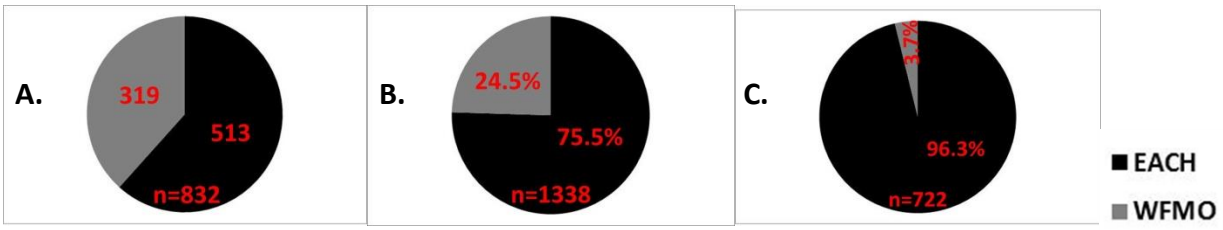


Figure 2.6 Number of white-footed mice (WFMO; gray) and eastern chipmunks (EACH; black) captured on NMI and SMI in June 2011-2014 and August 2012, 2014; with the total number of mice and chipmunk captures indicated at the bottom of the pie chart (A). The percent of larvae (B) and nymphs (C) that were removed off of NMI and SMI eastern chipmunks (EACH; black) and white-footed mice (WFMO; gray) from June 2011-2014 and August 2012, 2014, with the total number of larvae (B) and nymphs (C) collected indicated at the bottom of the corresponding pie chart.

Borrelia burgdorferi infection in *I. scapularis* removed from small mammals:

Borrelia burgdorferi was detected in both immature stages of the tick removed from small mammals on the two islands (Table 2.1).

On SMI, the Lyme disease pathogen was not found in the June mammal ticks until the last two years of the study. *Borrelia burgdorferi* was detected in larvae removed from both mice and chipmunks, and nymphs on chipmunks but not on white-footed mice.

On NMI, in June of every year within the study, larvae and nymphs were found to be infected from mice and chipmunks with the exception of white-footed mice nymphs in June 2012 when zero ticks were tested. Aside from the chipmunk larvae which had infection prevalences fluctuating with time, white-footed mice larvae and nymphs and eastern chipmunk nymphs, had a steady increase with June infection prevalence over time. Furthermore, there was a significant increase in infection prevalence from June 2011 (first year) vs. June 2014 (last year) in larvae and nymphs removed from both mice and chipmunks (p = mice larvae: 0.002; chipmunk larvae: 0.001; mice nymphs: 0.001; chipmunk nymphs: <0.001).

Table 2.1 Infection prevalence of *I. scapularis* larvae (a) and nymphs (b) removed from captured white-footed mice (WFMO) and eastern chipmunks (EACH) on NMI and SMI in June from 2011-2014, with the total number of ticks of each life stage tested indicated in parentheses.

a)	WFMO		EACH	
	NMI	SMI	NMI	SMI
2011	7.14% (14)	0.00% (1)	18.92% (37)	0.00% (1)
2012	18.18% (11)	0.00% (3)	62.50% (8)	0.00% (3)
2013	71.43% (7)	20.00% (5)	72.86% (70)	0.00% (3)
2014	71.43% (7)	N/A (0)	52.17% (69)	66.67% (3)

b)	WFMO		EACH	
	NMI	SMI	NMI	SMI
2011	23.08% (13)	0.00% (1)	20.00% (30)	N/A (0)
2012	N/A (0)	0.00% (1)	25.00% (4)	0.00% (1)
2013	100% (3)	0.00% (1)	70.37% (162)	25.00% (4)
2014	100% (8)	N/A (0)	73.09% (275)	42.42% (33)

Borrelia burgdorferi infection in small mammals:

A total of 299 and 394 white-footed mouse tissue biopsies were tested from SMI and NMI, respectively. A total of 357 and 420 eastern chipmunk tissue biopsies were tested from SMI and NMI, respectively. Every June throughout the study, small mammals were infected with *B. burgdorferi* on SMI and NMI, with the exception of SMI in 2011. Although the pathogen was detected from 2012-2014 on SMI, this was only within eastern chipmunks. None of the June white-footed mice on SMI were infected. On the other hand, both mice and chipmunks were infected every year on NMI. Overall, small mammal (mice plus chipmunk) infection prevalence was significantly greater on NMI than SMI (p = 2011: 0.003; 2012: <0.001; 2013: <0.001; 2014: 0.002).

Across the calendar year, white-footed mice infection prevalence peaked in June on NMI. The peak of the eastern chipmunk infection prevalence lasted longer than the white-footed mice, with the peak occurring in June and July on NMI (Figure 2.7). The only month that mice were found to be infected on

SMI was in September (of 2014). On SMI, the chipmunks were infected June-September, with the proportion infected relatively consistent aside from a drop in September (Figure 2.7).

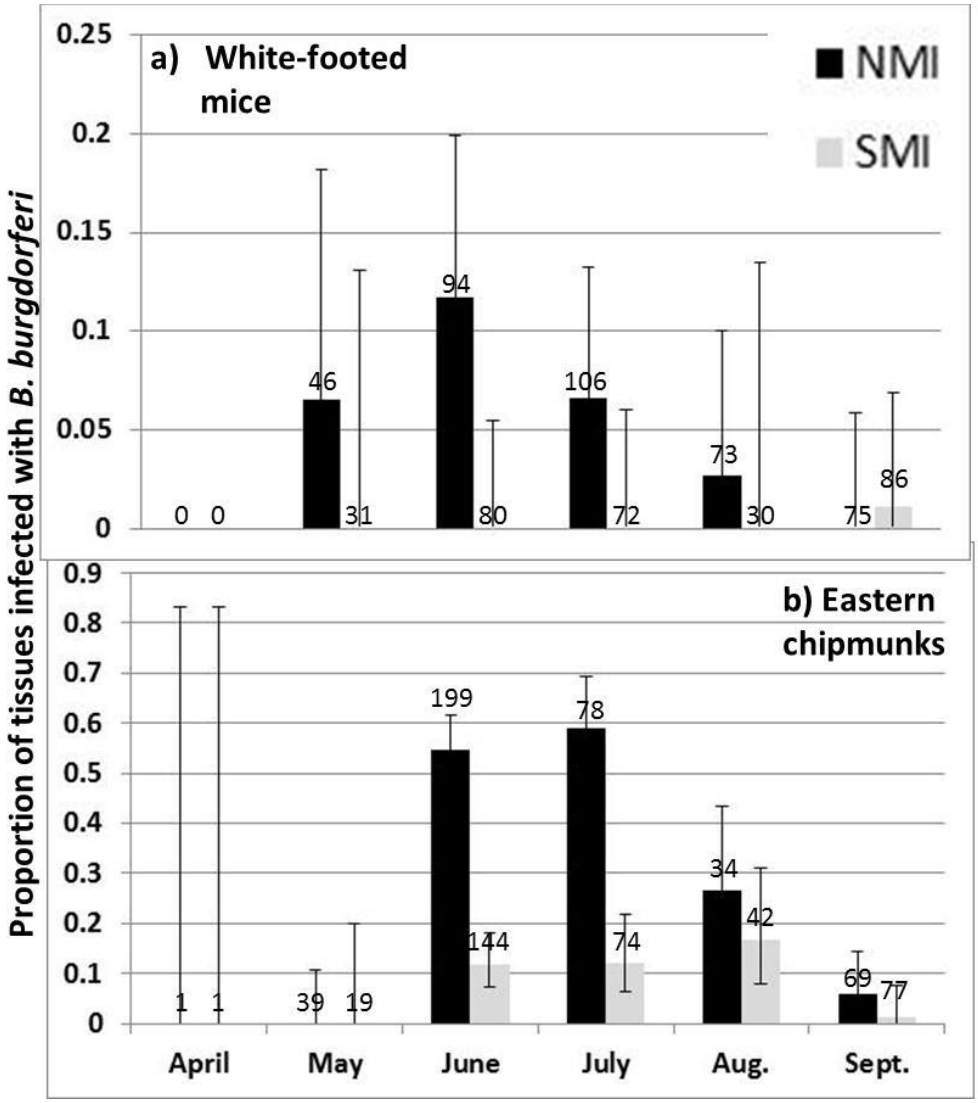


Figure 2.7 Proportion of infected tissue biopsies from a) white-footed mice and b) eastern chipmunks across the calendar year. The samples from NMI (black) and SMI (gray) each month were combined from 2011-2014 with the number of samples tested indicated above each bar. Error bars correspond to 95% binomial confidence intervals.

Questing ticks:

A total of 74,246 m^2 and 64,850 m^2 were dragged on NMI and SMI, respectively throughout the study. All three life stages were collected via drag cloth on both islands. On NMI, a total of 1084, 130, and 605 *I. scapularis* adults, nymphs, and larvae were collected, respectively. On SMI, a total of 104, 7, and 7 *I. scapularis* adults, nymphs, and larvae were collected, respectively.

There was a trend indicating that each year, the adult and nymphal density in June on NMI was greater than on SMI (Figure 2.8). Using linear regression, over time, there was not a significant change to the adult and nymphal densities in June on either island nor was there a significant change to adult density in the fall on NMI (Figure 2.8).

Borrelia burgdorferi infection in questing ticks:

Overall on NMI from 2011-2015, 36.9% (n = 868) of the adult blacklegged ticks were infected with *B. burgdorferi* and 26.0% (n = 127) of the nymphs were infected. On SMI, a total of 19.2% (n = 104) of the adult ticks were infected with *B. burgdorferi* and 0% (n = 7) of the nymphs were.

On NMI, the proportion of infected adults in June increased over time (2011 with 0.04 infection vs. 2014 with 0.7 infection; $p < 0.001$) as did the proportion of infected adults in the fall (2011 with 0.3 infection vs. 2014 with 0.6 infection; $p < 0.001$). Although there was not a significant increase in infected nymphs in June over time on NMI, there was a spike in 2013 with nymph collection and infection (Figure 2.8). On SMI, when the June drag data was considered, infected adults were only found in 2013 (n = 1) and 2014 (n = 1) (Figure 2.8).

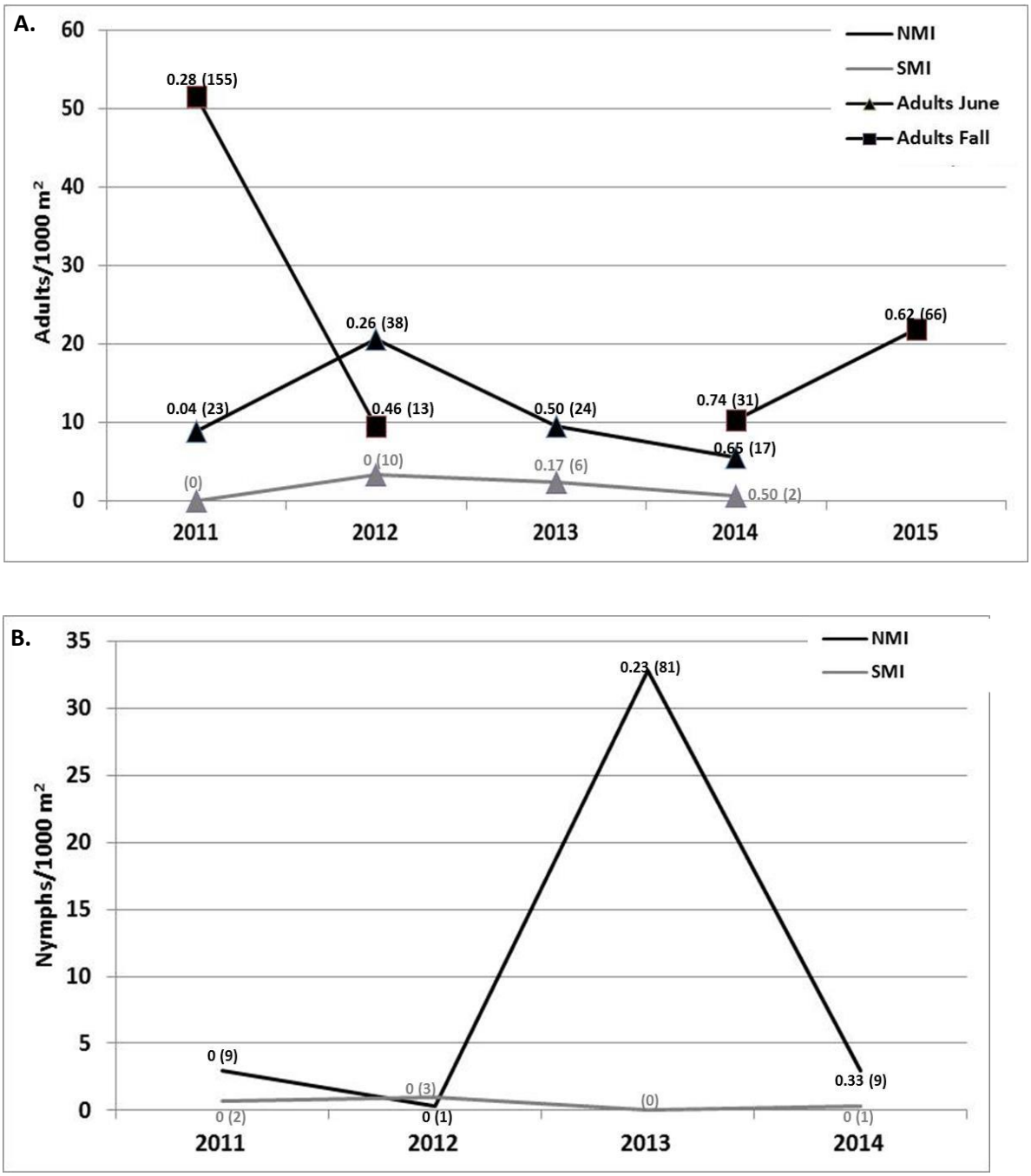


Figure 2.8 Questing tick density/1000 m^2 on NMI (black) and SMI (gray) of: A) *I. scapularis* adults in June (triangle) every year (2011-2014), adults in the fall (square) every year (2011-2015) except 2013, and B) nymphs each June (2011-2014). Fall questing tick sampling only took place on NMI. The proportion of ticks infected is indicated near each point with the total number of ticks tested indicated in parentheses. The proportion infected is color coded by site (NMI black; SMI gray).

Snowshoe hare results:

A total of 23 snowshoe hares were captured (15 in 2012 and 8 in 2014), none of which were re-captured from the previous day, season, or year. None of the hares were infested with any stage of *I. scapularis* ticks or *Ixodes* species in general. However, 74% were infested with one or more *Haemaphysalis leporispalustris* ticks. A total of 3,491 H. *leporispalustris* were removed, with 48% being larvae, 37% nymphs, and 15% adults. A total of 311 larvae (27 batches), 33 nymphs, and 55 adults were tested for *B. burgdorferi*. One nymph was found to be infected.

In 2014, seven rabbit tissues biopsies were tested for the presence of *B. burgdorferi* and/or *Borrelia miyamotoi*. Of these samples, only one was infected with *B. miyamotoi*. No rabbits were infected with *B. burgdorferi*.

Bird results:

Throughout the 2014 season, a total of 167.6 and 350.8 net hours were accumulated on NMI and SMI, respectively.

On SMI, 30 different bird species were captured and 127 unique (i.e. not previously captured) birds were caught. Of these birds, two (1.6%) were infested with at least one *I. scapularis* tick(s). The infested birds were both American redstarts (*Setophaga ruticilla*). A total of 20 *I. scapularis* larvae were removed, of which one larva was tested and it was determined to be positive for *B. burgdorferi*.

On NMI, six different bird species were captured, all of which were also caught on SMI. These six overlapping species included: chipping sparrow (*Spizella passerine*), American redstart (*Setophaga ruticilla*), red-eyed vireo (*Vireo olivaceus*), northern waterthrush (*Parkesia noveboracensis*), gray catbird (*Dumetella carolinensis*), and American robin (*Turdus migratorius*). A total of 49 unique (i.e. not previously captured) birds were caught. Of these birds, six (12.2%) were infested with at least one *I. scapularis* tick(s). The infested birds were five American robins and one American redstart. A total of

two larvae and six nymphs were removed and then tested for *B. burgdorferi*. Approximately 4.1% of the captured birds were found to be infested with infected ticks (two American robins). There was a 50% larval infection prevalence and 33.3% nymphal infection prevalence within the tested ticks.

Coyote results:

A total of two adult female coyotes were captured, neither of which were previously caught. Both coyotes were infested with male and female adult *I. scapularis* ticks; no other tick species were found. A total of 67 adult ticks were removed from the two coyotes, of which 46.3% were female and 53.7% were male. Oviposition was observed in seven of the females; all larvae hatched. One of the two coyotes was tested for the presence of *B. burgdorferi*; it was negative.

Discussion:

One of Michigan's most visited landmarks, Sleeping Bear Dunes National Lakeshore (SLBE), includes two large offshore islands-North Manitou Island (NMI) and South Manitou Island (SMI). In the fall of 2010, there was a report of blacklegged ticks on NMI. Given that neither island had previously been evaluated for the Lyme disease tick and bacterium, SLBE hosts >1.5 million visitors annually, and the Lakeshore was presumed to be at the leading edge of the blacklegged tick invasion within the Lower Peninsula (NPS stats, Hamer et al. 2010), a collaborative study amongst Michigan State University (MSU), Michigan Department of Health and Human Services (MDHHS), and the National Park Service (NPS) was performed and carried out from 2011-2015.

The Manitou Islands presented as a unique opportunity to study Lyme disease ecology as this had direct implications for varying host contributions to the disease cycle. Although both islands were known to be home to small rodents such as white-footed mice (*Peromyscus leucopus*) and eastern chipmunks (*Tamias striatus*), as well as medium sized mammals such as snowshoe hares (*Lepus americanus*) and

coyotes (*Canis latrans*), only NMI maintained a population of white-tailed deer (*Odocoileus virginianus*). SMI was completely devoid of deer. Given that white-tailed deer play a critical role within the Lyme disease system as they are the main hosts for the adult life stage, with the absence of deer on SMI, it was hypothesized that tick reproduction would be significantly reduced, unless other medium-sized mammal species were serving as additional hosts for the adult stage. Therefore, given the different host communities and how they were a primary tourist destination, the Manitou Islands allowed us to study key aspects about Lyme disease ecology that also had great public health relevance.

Our objectives for this study were: 1) Determine if the Lyme disease tick and pathogen were present and to what degree in small mammal hosts and questing ticks on both the island with the deer and also the island devoid of deer; 2) Compare mammal and host-seeking infestation and infection prevalence from one island to the other; 3) Evaluate the role of alternative hosts for maintaining the Lyme disease tick on the island devoid of deer.

Objective 1: Is the tick and/or the pathogen found on SMI and NMI small mammal and/or questing?

The Lyme disease tick, *I. scapularis*, and the Lyme disease pathogen, *B. burgdorferi*, were both found on NMI and SMI within the small animal populations and also in host-seeking ticks.

On SMI and NMI, every June, both immature stages of the blacklegged tick were found attached to either white-footed mice and/or eastern chipmunks. Larvae and nymphs were found attached to both mice and chipmunks throughout the study. June was evaluated on a yearly basis because this month was consistently sampled every year on both islands and it was in June that *I. scapularis* nymphal and larval ticks are active within the Midwest, in particular, within Michigan (Hamer et al. 2012). Additionally, a portion of the ticks that were removed from the small mammals on both islands were found to be infected with *B. burgdorferi*. On SMI, a trend with infectivity from June 2011-June 2014 could not be shown as the Lyme disease pathogen was not found in the June mammal ticks until the last two years of the study.

However, on NMI, there was a significant increase in infection prevalence from June 2011 to June 2014 in larvae and nymphs removed from both mice and chipmunks, which could support increasing Lyme disease potential. *Borrelia burgdorferi* was also detected within the mammals themselves on both islands. Although none of the June mice were infected on SMI, infected larvae removed from white-footed mice were detected, thus, supporting that a portion of the mice were indeed infected as well.

Furthermore, on both islands, all three life stages of the blacklegged tick were collected via drag cloth, with the majority of the ticks being adults. This supports that SMI does have an established tick population but, less in comparison to NMI. The Lyme disease pathogen was only detected within the adult stage on SMI, which shows that the Lyme disease risk to visitors albeit present was still minimal as questing nymphs are responsible for the most cases of human Lyme disease (Barbour et al. 1993).

Objective 2: SMI vs. NMI infestation and infection prevalence in small mammals and questing ticks

Although the land area on NMI is almost three times as large as SMI, there was not a significant difference between the capture success of white-footed mice on NMI vs. SMI or the capture success of eastern chipmunks on NMI vs. SMI when all of the trap years were combined, thus, making small mammal comparisons possible between islands. We hypothesized that since SMI was devoid of deer, in comparison to NMI, the tick population would not be as well established (if it was established at all). Although by the end of the study, we determined that there was an established tick population on SMI, it was, as we expected, less than what was found on NMI.

When the proportion of mice and chipmunks infested with larvae, nymphs, and/or both were combined, NMI small mammals were significantly more infested than SMI in June of 2011, 2013, and 2014, thus, supporting the hypothesis that the island with deer had a more established tick population. Also, the infection prevalence of the larvae and nymphs removed from these small mammals was approximately 1.3 and 1.7 times, respectively, greater on NMI than on SMI. On NMI, approximately

10.4, 18.6, and 86.4 times as many host-seeking adults, nymphs, and larvae were collected than on SMI and the overall adult infection prevalence was almost double on NMI. Additionally, infected host-seeking nymphs were collected on NMI supporting a more robust Lyme disease cycle on the island with deer.

Objective 3: Role of alternative hosts on SMI

Islands are areas in which species can live and flourish surrounded by an area (e.g., water) that prohibits or drastically limits colonization (Diamond 1975). Depending on the rates of immigration and emigration, the ecology of island species can be more strongly affected by biotic interactions compared to mainland communities. The equilibrial number of species that can exist on an island is directly related to the size of the island and the distance it is from other areas. Islands that are larger and less isolated can sustain a greater number of species (Diamond 1975). Understanding island ecology, particularly the role that different hosts play within the Lyme disease system, was one of the primary goals of this investigation.

On SMI, during every year of the study, a significantly greater number of eastern chipmunks were captured than white-footed mice. On NMI, during three of the four years, there was either not a significant difference between the proportion of chipmunk and mouse captures or there were a greater number of eastern chipmunks captured than white-footed mice. This is unlike what would be expected at mainland sites in the Midwest and also the northeast. In Illinois, the capture rates of white-footed mice was almost twice that of eastern chipmunks and in Massachusetts, at one site, the ratio of mice to chipmunks was 15:1 (Mather et al. 1989, Slajchert et al. 1997). This was also the case at multiple sites within Michigan as well, including two sites on SLBE's mainland (Hamer et al. 2010; Sidge 2016).

Previous research has shown that white-footed mice typically feed more larvae than eastern chipmunks and chipmunks have higher levels of nymphal infestation than mice (Mannelli et al. 1993, Slajchert et al. 1997, Schmidt et al. 1999). In general, when small mammal infestation for both SMI and

NMI were combined, this pattern with white-footed mice feeding more larvae and eastern chipmunks more nymphs, held true. However, this was not the case toward the end of the summer and early fall when the chipmunks were infested with more larvae than nymphs. When strictly the month of June was considered, which previous research showed was when both the larval and the nymphal stage were in peak (Hamer et al. 2012) there was not a significant difference during three of the four years between larval and nymphal infestation on mice or chipmunks on either island (aside from the NMI mice which had more larvae than nymphs for three of the four years). Furthermore, in addition to the June larval peak, on the islands, larvae peaked again in August. When June and August island mice and chipmunks were combined and separated by species, the chipmunks fed a greater number of larvae and also a greater number of nymphs than the mice. Thus, this illustrates that on the two Manitou Islands, eastern chipmunks play a critical role in feeding both immature stages of the blacklegged tick.

In the northeast and also in the Midwest, it was found that eastern chipmunk activity or trap success decreases in the summer also known as the "summer lull." The chipmunks were found to be in peak activity in the early summer (June and early July) and then again in the fall (Dunford 1972, Hamer et al. 2012). This chipmunk "summer lull" was not observed on SMI or NMI. On the two islands, chipmunk activity peaked in June and then was maintained throughout the entire summer and into the fall. Furthermore, the island chipmunks were in greater abundance as indicated by number of chipmunks caught/100 effective trap nights.

Eastern chipmunks also influenced questing tick populations on the islands. On NMI, the overall nymphal questing density for the month of June was 8.8/1000 m^2 with the greatest density in June of 2013 with 32.8/1000 m^2. On SMI, the overall June nymphal density was 0.5/1000 m^2 with the greatest density in 2012 with 1/1000 m^2. At a Lyme disease endemic site in the southwest portion of Michigan Lower Peninsula along the Lake Michigan coast, the peak May/June nymphal density was 29.7/1000 m^2. Although, the NMI peak of nymphal density was comparable with the endemic site in Michigan's Lower Peninsula, in general, the nymphal density was less on both islands. Although the lower density on SMI

could be explained by the lack of deer, on NMI the lower nymphal questing density could be explained by the greater chipmunk capture success on the islands, thus, allowing more questing nymphs to find a host rather than be collected via drag cloth. Ginsberg et al. 1999 found that if host densities were high enough so that a large proportion of questing ticks found hosts, the loss of free living ticks could influence density estimates from flagging. This was illustrated on Fire Island, NY when adult densities were much lower on the island as a result from the ticks being unavailable to flag since they were on the deer hosts. On NMI, there was an earlier chipmunk nymphal infestation peak, within the calendar year, in comparison to the endemic site further south within Michigan's Lower Peninsula. The nymphs on the islands were able to find a chipmunk host sooner than the southern Lyme disease endemic site chipmunks, thus, in general, equating for fewer nymphs to be host-seeking and collected during our drag cloth efforts.

The overall NMI infection prevalence for dragged nymphs throughout this study was 26.0%, which was greater than the overall infection prevalence in dragged nymphs along the Michigan Lower Peninsula invasion zone (8.6%; Hamer et al. 2010) and in some locations within the endemic Northeast (15%; Gatewood et al. 2009). This implies that a greater number of larvae were becoming infected out on the islands. Since we showed that each June there was not a significant difference between the proportion of chipmunks infested with larvae or nymphs, aside from 2014 when the chipmunks on both islands fed a significantly greater number of nymphs than larvae, more larvae could be feeding on chipmunks and acquiring this increased nymphal infectivity than the endemic site in Michigan's Lower Peninsula or in some areas within the Northeast. Previous research has shown that since chipmunks typically live longer and can maintain the Lyme disease pathogen for a potentially longer period of time, their infections tend to carry over from one year to the next (Tryon and Snyder 1973, Wolff et al. 1988, McLean et al. 1993, Donahue et al. 1987, Slajchert et al. 1997). Furthermore, on the islands, a greater proportion of chipmunks were infected throughout the summer months compared to white-footed mice. When the seasonal dynamics were considered, from the mid-summer to the early fall, unlike the endemic site in southwest Michigan, on the islands, the chipmunks fed more larvae than nymphs. Thus, there was an

increased probability that the NMI larvae would feed on an infected chipmunk and hence become an infected nymph equating for the increased nymphal drag infection prevalence on NMI.

This increased nymphal infection prevalence within the questing tick population on NMI would seem to present as a public health significance since the nymphal stage is responsible for the most cases of human Lyme disease (Barbour et al. 1993). However, on NMI there were fewer nymphs questing but a greater infection rate in comparison to the Lyme disease endemic site on the west coast in Michigan's Lower Peninsula. The density of infected nymphs, which is the metric used to measure Lyme disease risk, on NMI (1.9/1000 m^2) was approximately 1.3 times greater than the endemic site in Michigan's Lower Peninsula (1.5/1000 m^2, Hamer et al. 2010). Thus, if a host-seeking nymph happens to come across a summer visitor on the island prior to finding one of the numerous chipmunks or even a mouse, then this individual would have a slightly greater probability of contracting the disease given the increased infection prevalence.

Therefore, eastern chipmunks play a critical role within the Lyme disease system on both islands; however, their contribution primarily falls with the immature stages of the tick and maintaining the bacterium. Larger mammals, in particular, white-tailed deer are typically responsible for feeding the adult stage of the tick and completing the tick's life cycle. On SMI, given that the island is absent of deer, we had hypothesized that there was not going to be an established tick population. However, unlike what we had anticipated, immature stages of the tick were detected both on small mammal hosts and also questing within the vegetation implying that alternative medium/large mammal hosts were present on SMI and able to maintain the tick population in the absence of white-tailed deer.

Although rabbits have previously been shown to feed blacklegged ticks and become infected with the Lyme disease pathogen, this was not observed on SMI (Burgdorfer and Gage 1986, Telford and Spielman 1989). Throughout this study, twenty-three unique hares were captured (June-September 2012, 2014) and none were infested with any stage of *I. scapularis* ticks. Furthermore, of the tissue samples

that were tested, none were infected with *B. burgdorferi*. Thus, this supported that snowshoe hares did not play a role within the SMI Lyme disease cycle and were not responsible for maintaining the tick population.

Passerine birds given their migratory patterns play a crucial role for long distance dispersal of blacklegged ticks (Madhav et al. 2004). Migratory birds have been estimated to disperse 50-175 million *I. scapularis* across Canada every spring (Ogden et al. 2008). Although the mist-netting efforts were limited on SMI, we still were able to show that passerine birds were playing a role on SMI by dropping off immature stages of *I. scapularis* ticks. Elias et al. 2011, showed using uncertainty analysis that following complete deer removal on Monhegan Island, ME, the tick density was equivalent to imported tick densities, supporting that all *I. scapularis* ticks on the island were now bird-derived. However, unlike Monhegan Island after the deer removal which found no more *I. scapularis* ticks on small mammal hosts (i.e. Norway rats, *Rattus norvegicus*), no *B. burgdorferi* in the rat tissue, and no additional questing nymphs (Rand et al. 2004; Elias et al. 2011), even though deer were absent on SMI, we still found *I. scapularis* on the small mammal hosts, *B. burgdorferi* in the mammal tissue, and questing nymphal ticks, thus, supporting that on SMI the ticks were being maintained not just due to adventitious ticks from introductive avian species.

Thus, in the fall of 2014, coyotes were trapped as this was the only other known potential host on SMI that could be maintaining the blacklegged tick population in the absence of white-tailed deer. Two coyotes were captured and we found that both were indeed infested with adult *I. scapularis* ticks. Previous research confirmed that *B. burgdorferi* antibodies have been detected in coyotes (Kazmierczak and Burgess 1989). After oviposition was observed in multiple engorged females that were feeding on the SMI coyotes, this confirmed that coyotes were viable hosts for adult *I. scapularis* to feed and complete their life cycle upon and thus, were the hosts responsible for maintaining an established tick population on the deer-free island.

Implications for Lyme disease management:

Investigating questions about the maintenance of the Lyme disease tick and pathogen in the absence of deer (i.e. SMI), has implications for Lyme disease risk management. Previous island research has found that immediately following complete deer removal, adult tick density and infection prevalence increased significantly. Yet, four years later, no adult ticks were found on small mammals and minimal numbers were found questing, which could be due to avian contributions (Rand et al. 2004). Other island research has shown that on islands without deer, the Lyme disease pathogen nor vector were found on captured mice (Anderson et al. 1987).

These data support the hypothesis that deer reduction has a basis for Lyme disease management. However, deer culling is extremely controversial. There are clear benefits for reducing a deer population, such as decreased deer-vehicle accidents, vegetation restoration, and possible reduction of tick-borne diseases (Conover 1997, McShea et al. 1997, Cote 2004). When considering tick-borne disease reductions, it is important to consider if the area under investigation is isolated (i.e. NMI or SMI) or if it is ecologically open to immigration (i.e. SLBE mainland). Some studies have found that just using deer fencing or exclosures may significantly reduce the abundance of blacklegged ticks and hence Lyme disease risk, as exclosures had 83% fewer host-seeking nymphs and 90% fewer host-seeking larvae (Daniels et al. 1993). A computer model found that if deer density was reduced from 25 to 0.25 deer/km^2, this would decrease the number of *B. burgdorferi* infected nymphs by 98% (Mount et al. 1997). Meta-analysis indicated that in larger areas if deer were excluded this reduced questing tick densities, yet, as the exclosures become smaller, the questing tick density was increased (Perkins et al. 2006). The larger exclosure areas could refer to SMI.

Thus, not all research clearly supports that decreasing deer density will drastically influence Lyme disease management by reducing tick densities. Kugeler et al. 2016, summarized the current literature regarding deer reduction and Lyme disease incidence and it was suggested that elimination of

deer from isolated areas could have a substantial effect on blacklegged tick reproduction, however, if the deer were strictly being reduced then the results were mixed and the evidence for human Lyme disease risk was limited. Ostfeld et al. 2006 found that the effects of deer on ticks were nonlinear, weak, and variable depending on the life stage. Levi et al. 2012 showed that increases in Lyme disease in the Northeast and Midwest within the previous three decades were often uncorrelated with deer abundance. If only a small or localized area was absent of deer, this would increase tick feeding on rodents and consequently lead to the potential of tick-borne disease hotspots (Perkins et al. 2006).

Research on the Thousand Islands showed that deer abundance was positively associated with tick abundance (Werden et al. 2014). The Manitou Islands have illustrated that even in locations completely devoid of deer, an established tick and Lyme disease pathogen cycle may still be maintained by alternative medium sized mammals. Although, the tick and pathogen levels were less on the deer-free island in comparison to the island with the deer, all three life stages were present and *B. burgdorferi* was detected both in the tick and in mammal tissues, implying that there was still Lyme disease risk present on both islands.

Conclusion:

In Michigan's Lower Peninsula, the blacklegged tick and the bacterium are spreading northward and inland along the Lake Michigan shoreline (Hamer et al. 2010). Sleeping Bear Dunes National Lakeshore (SLBE), Michigan's most visited National Park with >1.5 million visitors in 2015, was at the leading edge of this invasion in 2009 (NPS stats, Hamer et al. 2010). SLBE includes two large offshore islands-North Manitou Island (NMI) and South Manitou Island (SMI). Prior to this study, the tick status and Lyme disease potential on both isolated islands had never been evaluated.

The Manitou Islands are unique in that although the two islands were home to a population of small rodents, including white-footed mice and eastern chipmunks and a limited community of medium

mammals including rabbits and coyotes, only one of the islands contained white-tailed deer. Given that the adult stage of the blacklegged tick typically feeds on white-tailed deer, thus making this host crucial for the completion of the tick's life cycle, these popular islands provided the opportunity to study key aspects about Lyme disease ecology from a varying host perspective, which also had great public health relevance.

From 2011-2014, we performed small mammal trapping on the two islands and from 2011-2015, drag sampling was carried out, after which it was concluded that the Lyme disease tick, *I. scapularis*, and the Lyme disease pathogen, *B. burgdorferi*, were both found on NMI and SMI within the small animal populations and also in host-seeking ticks. On SMI and NMI, every June, both immature stages of the blacklegged tick were found attached to either white-footed mice and/or eastern chipmunks. Furthermore, on both islands, all three life stages of the blacklegged tick were collected via drag cloth, with the majority of the ticks being adults. Given the presence of all three life stages of the blacklegged tick and the detection of *B. burgdorferi* circulating within the ticks and the mammals, this supports that SMI did have an established tick population, which was unlike what we had hypothesized.

Even though SMI did have an established population of ticks and thus, presented as a potential Lyme disease risk area, NMI, as would be expected given the population of deer, had a more established tick population and presented as a greater public health concern. This was supported with a greater: proportion of mammals infested, infection prevalence within the mammal ticks, and number and proportion infected of host-seeking ticks including infected questing nymphs.

Unlike in other locations within Michigan, the Midwest, or the Northeast, eastern chipmunks were determined to play a critical role within the Lyme disease system on both islands. On the Manitou Islands, there was an equivalent or even greater number of chipmunks captured in comparison to white-footed mice, and in June and August throughout the study, the island chipmunks fed more larvae and nymphs than the mice, while remaining active throughout the entire summer without experiencing as

Dunford 1972 explained a "summer lull." On NMI, the density of questing nymphs was lower yet, the overall nymphal infection prevalence was greater in comparison to a Lyme disease endemic site in Michigan's Lower Peninsula along the Lake Michigan coast. The lack of host-seeking nymphs on NMI could also be explained by the abundant number of eastern chipmunks, thus, allowing more questing nymphs to find a host rather than be collected via drag cloth. Additionally, more larvae could be feeding on infected chipmunks on the island, contributing to the increased infection prevalence. Summer visitors and hikers should be made aware of this risk, as the nymphal stage of the tick has the greatest epidemiological significance (Barbour et al. 1993).

In an attempt to determine how the blacklegged tick population on SMI was being maintained, we trapped snowshoe hares in 2012 and 2014, after capturing twenty-three rabbits, it was concluded that they were not responsible for feeding *I. scapularis* ticks as none were found infested on the hares. Mist-netting, albeit a limited amount in 2014, provided the opportunity to illustrate that passerine birds were contributing to the Lyme disease cycle by dropping off infected immature stages of the tick, however, they were not the host for the adult stage of the tick in the absence of deer. In 2014, coyotes were captured on SMI and after catching two, both of which were infested with adult *I. scapularis* ticks and oviposition was observed in multiple engorged females, it was determined that they were responsible for feeding the adult stage of the tick and hence maintaining an established blacklegged tick population in the absence of white-tailed deer.

Investigating questions about the maintenance of the Lyme disease tick and pathogen in the absence of deer (i.e. SMI), has implications for Lyme disease risk management. When considering tick-borne disease reductions, it is important to consider if the area under investigation is isolated (i.e. NMI or SMI) or if it is ecologically open to immigration (i.e. SLBE mainland). Although prior research presents conflicting views as to how much reducing deer populations will aid in disease management, we have shown that on offshore islands, the Lyme disease tick and pathogen prevalence can be reduced if white-tailed deer are completely removed (i.e. SMI). However, even if deer are not present, an established

blacklegged tick population can still occur if alternative medium/large mammalian hosts, such as coyotes, are present. Thus, from a public health perspective, proper precautionary measures should be enacted.

Study Limitations and Future Research:

Historically, SMI was home to the red fox (*Vulpes vulpes*); however, during our five year study on the Manitou Islands, we did not find any evidence of the presence of fox (Sleeping Bear Dunes National Lakeshore 2000). Previous research has shown that the predator-prey relationship between coyotes and foxes can influence the Lyme disease potential in an area. The fox diet is composed of a larger fraction of small animals than the typical coyote diet and small mammals are usually responsible for maintaining the immature stages of the blacklegged tick. Thus, if the fox population increases in a location given the decrease in the coyote population, then the Lyme disease risk will decrease (Levi et al. 2012). Continued research should take place on the Manitou Islands, in particular on SMI, in order to determine if red fox are still present, if not, when and if they arrive again, how the tick population consequently alters.

Prior to this study, medium sized mammals had never been captured on either Manitou Island and aside from periodic trail cameras temporarily installed on SMI to confirm that white-tailed deer were still not present, little is known as to the true mammalian diversity on the islands, specifically, how large the medium sized mammal populations are. Based on the footprints observed while coyote trapping in the fall of 2014, we hypothesized that there were at least four coyotes present on SMI (two adult females were captured and we saw prints resembling a very young pup). However, the relative abundance is not known. Future research could include a medium sized mammal mark recapture study. In addition, in the future, it would be advantageous to install multiple trail cameras throughout the island in order to better determine the general mammalian diversity.

An additional study limitation was that from 2011-2014 when small mammal trapping was performed on the islands, we always set 150 traps, divided amongst six transects. In the future, additional traps should be set in order to better determine small mammal abundances. Furthermore, given the varying landscapes across NMI, small mammal trapping and drag sampling should take place in areas further from the vicinity of the "village." This would allow us to determine if certain locations throughout the island vary in Lyme disease risk.

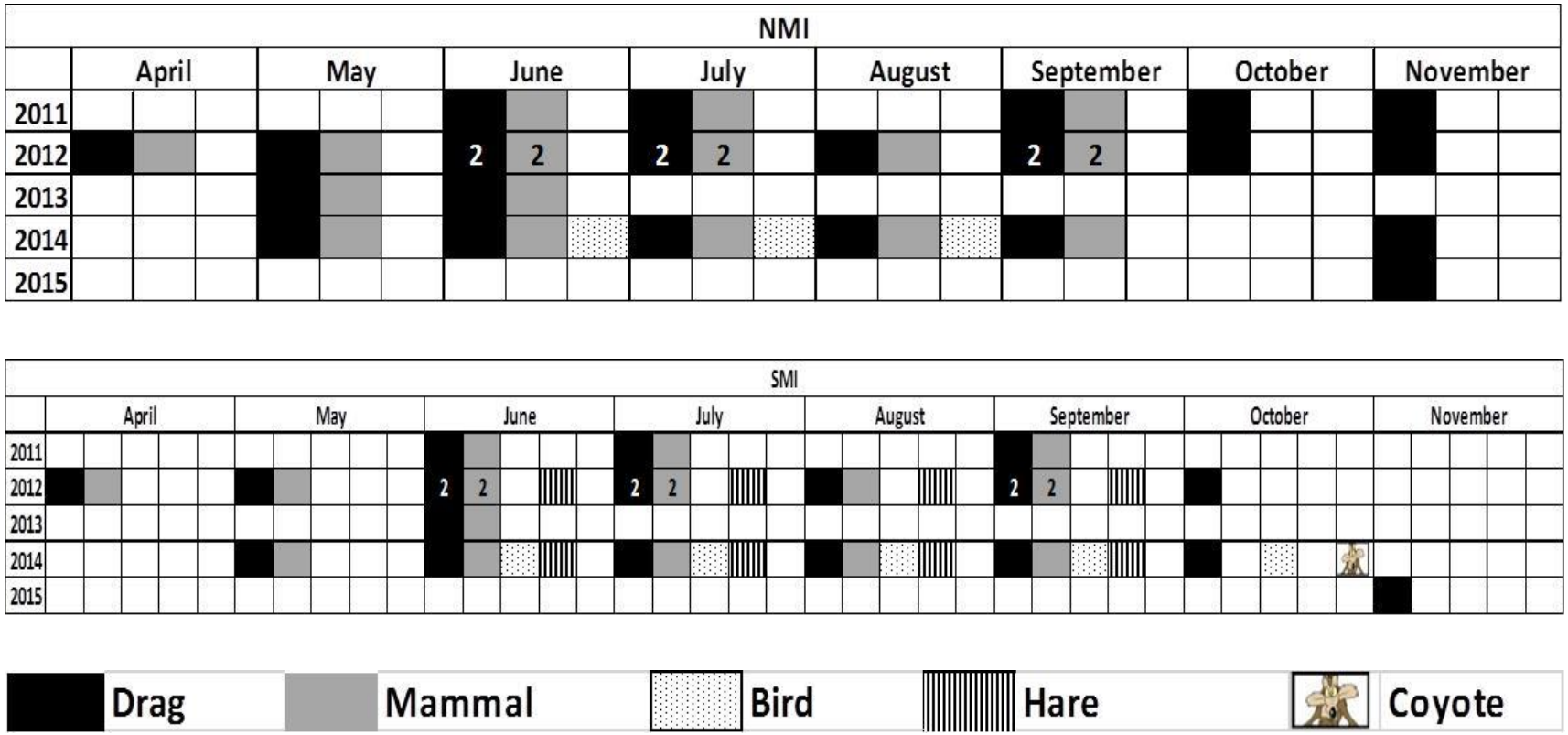
During this study we proposed that the overall lower density of questing nymphs on NMI in comparison to the Lyme disease endemic site in Michigan's Lower Peninsula could be explained by the greater chipmunk capture success on the islands, thus, allowing more questing nymphs to find a host rather than be collected via drag cloth (Ginsberg et al. 1999). In order to confirm this hypothesis, in the future, exclosure work could be performed. The exclosures would need to be constructed in order to prevent small/medium sized animals and deer from entering. Over time, both in and out of the exclosures could be drag sampled and the tick densities compared, with the hypothesis being that inside the exclosures the nymphal questing density would be greater given the lack of hosts for the ticks to attach to.

We only trapped medium sized mammals on SMI as our objective was to determine if alternative hosts were present for the adult stage of the blacklegged tick to feed upon in the absence of deer in order for an established tick population to be present. Future research could also include trapping medium sized mammals on NMI and determining the extent to which they are infested with adult blacklegged ticks given that white-tailed deer are present on that island. Prior research has shown that free-ranging coyotes were infested with adult *I. scapularis* ticks. However, given the isolated nature of our study locations, it would be interesting to compare the role of the medium sized mammals on the two Manitou Islands in regards to the potential to feed blacklegged ticks.

APPENDICES

APPENDIX 2.1: Sampling type and schedule

Table 2.2 Type of sampling (drag, small mammal, bird, rabbit, coyote) that occurred during each month and year on NMI and SMI. The black shading illustrates drag sampling, the gray indicates small mammal trapping, the dots indicate bird mist-netting, the vertical lines indicate rabbit trapping, and the cartoon coyote picture indicates coyote trapping. When sampling was performed twice within a given month, "2" is written on that month/year. (coyote picture: Clip Art)



APPENDIX 2.2: Additional pathogen detection results

In 2014, all of the mammal and questing ticks from both islands were tested by collaborators at CDC, at which time they not only tested the ticks for *B. burgdorferi*, but also for *Anaplasma phagocytophilum* and *Babesia microti*. On NMI, 20 nymphs (4.3%) and 10 larval batches (8.3%) removed from small mammals were infected with both *Anaplasma phagocytophilum* and *B. burgdorferi*. On SMI, one larval batch (5.3%) which was removed from an eastern chipmunk was dually infected with *Anaplasma phagocytophilum* and *B. burgdorferi*. The only questing tick that was infected during this additional pathogen screening was an adult blacklegged tick from NMI which was infected with both *Anaplasma phagocytophilum* and *B. burgdorferi*. No ticks were infected with *Babesia microti* from either island.

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CHAPTER 3

Lyme disease dilution hypothesis test using an isolated, less host-diverse island to a mammalian diverse mainland

Abstract:

The Lyme disease dilution hypothesis predicts that in a community composed of a high diversity of potential hosts for blacklegged ticks to feed upon, ticks would be diverted from feeding on white-footed mice and consequently the infection prevalence of ticks within that community would be less since the white-footed mouse is the primary reservoir for *B. burgdorferi*. Furthermore, host association has been suggested at certain *B. burgdorferi* loci with the prediction, based on the multiple niche polymorphism hypothesis, that diverse host communities would have greater *B. burgdorferi* strain diversity. As a means to test the dilution and multiple niche polymorphism hypotheses, we compared host-limited islands to a host-diverse mainland within the northwest portion of Michigan's Lower Peninsula by comparing *I. scapularis* larval infestation prevalence on white-footed mice, host-seeking *I. scapularis* adult/nymphal infection prevalences and the density of infected nymphs/adults, and also *B. burgdorferi* strain diversity between the two locations. We found mixed support for the dilution hypothesis as the larval infestation on white-footed mice was not supportive yet, the infection prevalence and density of infected host-seeking ticks was greater on the islands as predicted by the dilution effect. The multiple niche polymorphism hypothesis was not supported as the *B. burgdorferi* RST/IGS strain diversity was greater on the host-limited islands. Future research should include testing these two hypotheses by comparing the host-limited island to a host-diverse location with a known long-established tick population.

Introduction:

Lyme disease, which is caused by the bacterium, *Borrelia burgdorferi*, and transmitted in eastern North America by the blacklegged tick, *Ixodes scapularis*, is the most commonly reported vector-borne disease in the United States (Mead 2015). In the northern North America, it is assumed that blacklegged ticks typically complete their life cycle over two years, constituting four developmental stages-egg, larva, nymph, and adult (Yuval and Spielman 1990). The larvae are born uninfected and must feed upon an infected vertebrate host in order to acquire the bacterium (Piesman et al. 1986). Blacklegged ticks are generalist ticks, and this tick has been recorded feeding on more species of vertebrate hosts in comparison to other North American ticks (Anderson et al. 1999). The immature stages of the tick have been detected from a minimum of 52 different species of mammals, 60 species of birds, and 8 species of reptiles (Anderson et al. 2008).

Although the juvenile stages of the blacklegged tick can feed on a wide variety of hosts, the primary reservoir is the white-footed mouse (*Peromyscus leucopus*) (Levine et al. 1985, Donahue et al. 1987). White-footed mice typically have a greater *B. burgdorferi* infection prevalence, density, and infestation prevalence with larval *I. scapularis* in comparison to other potential hosts, thus, making mice one of the most important reservoirs for *B. burgdorferi* (Mather et al. 1989, LoGiudice et al. 2003). The adult stage of the blacklegged tick, on the other hand, restricts their feeding to medium and large mammals, in particular white-tailed deer (*Odocoileus virginianus*) which are considered reservoir incompetent species (Telford et al. 1988).

The 'dilution effect' (Keesing et al. 2006) describes the reduction in pathogen abundance as biodiversity of the host community increases. If a given community is composed of multiple potential vertebrate hosts as opposed to a white-footed mouse dominated area, the proportion of ticks infected with the Lyme disease bacterium would decrease. The ecology of Lyme disease has been described as a system in which the dilution hypothesis may operate, given the wide variety of potential hosts that

blacklegged ticks can feed upon and that vary in *B. burgdorferi* reservoir competence (Ostfeld and Keesing 2000, LoGiudice et al. 2003). However, whether the dilution effect occurs in the Lyme disease system is controversial. Modeling efforts (Dobson 2004) have suggested that host species diversity can result in disease amplification when the transmission is predominately density dependent, meaning if additional hosts are added to the community this could result in increased tick abundance and consequently increased number of infected engorged larval ticks from both the mice and the non-mice hosts. This is similar to the 'rescue effect' in which reservoir-incompetent hosts increase tick numbers and maintain the bacterium in that community when the density of reservoir hosts is too low to otherwise maintain the spirochete (Norman et al. 1999, Ogden and Tsao 2009).

In a modeling study parameterized by field data collected in southern New York (LoGiudice et al. 2003), as greater numbers of host species were added to the community, the nymphal infection prevalence (NIP) decreased. In particular, the key diluting hosts were found to be gray squirrels (*Sciurus carolinensis*) and red squirrels (*Tamiasciurus hudsonicus*) as they had high tick burdens, low reservoir competence, and a high population density (LoGiudice et al. 2003). However, even when these optimal diluting hosts are present within a community, others argue that in order to truly demonstrate evidence of a dilution effect of public health and ecological significance, the NIP cannot be the only means of evaluation; the density of infected nymphs (DIN) must also decrease (Ogden and Tsao 2009), as the DIN affects the risk of encountering an infected nymph (Mather et al. 1996). Thus, the addition of alternative, less reservoir competent hosts may increase tick survivorship, leading to increased tick abundance, and hence increased DIN and amplification of Lyme disease risk (Ogden and Tsao 2009, Salkeld et al. 2010, Randolph and Dobson 2012, and others).

Host diversity within a community may also influence the genetic diversity of the Lyme disease pathogen. It has been suggested that the frequency of *B. burgdorferi* strains in ticks can be explained by the vertebrate species composition, the density of each vertebrate species, the number of ticks that feed on individuals of each species, and the rate at which the ticks acquire various strains (Brisson and Dykhuizen

2006). Host association has been suggested at certain *B. burgdorferi* loci (Brisson and Dykhuizen 2004). Varying host community composition may increase the pathogen diversity by increasing the ecological 'niches' for the pathogen, which would result in the maintenance of polymorphisms within the pathogen population, known as the 'multiple niche polymorphism' hypothesis (Levene 1953, Kurtenbach et al. 2006, States et al. 2014). Thus, in communities composed of a high diversity of potential hosts, *B. burgdorferi* diversity would be greater based on this hypothesis (Brisson and Dykhuizen 2006; States et al. 2014). As a means to test the multiple niche polymorphism hypothesis and the dilution effect, States et al. 2014 compared the *I. scapularis* nymphal infection prevalence, density of infected nymphs, and *B. burgdorferi* genotype diversity (based on ospC diversity) on an island community with low host diversity dominated by white-footed mice to a more host-diverse mainland in the northeast. Although this study did not support the dilution effect, evidence for the multiple niche polymorphism hypothesis was mixed.

In order to further test the dilution effect and as a means to evaluate the multiple niche polymorphism hypothesis using a different *B. burgdorferi* locus, we compared, similar to States et al. 2014, a species-limited island to a more host-diverse mainland in Michigan's Lower Peninsula at one of Michigan's most visited landmarks, Sleeping Bear Dunes National Lakeshore (SLBE). Our objectives were to test the following hypotheses: 1) Given that increased tick burdens would be expected on mice in communities absent of alternative hosts to divert the questing ticks from the mice, *I. scapularis* larval infestation prevalence on white-footed mice will be greater on the island vs. the mainland; 2) the host-seeking *I. scapularis* adult/nymphal infection prevalences and the density of infected nymphs/adults will be greater in the host-limited community vs. the more diverse community; and 3) lower *B. burgdorferi* strain diversity will be present on the island in comparison to the more host-diverse mainland.

Materials and Methods:

Site selection and sampling regime:

Field Sites: Sleeping Bear Dunes National Lakeshore (SLBE) is located in the northwest corner of Michigan's Lower Peninsula, along the Lake Michigan coast within Benzie and Leelanau counties (Figure 3.1). SLBE includes two large offshore islands-North Manitou Island (NMI) and South Manitou Island (SMI). Our lab previously performed studies on both the mainland and the islands (Hamer et al. 2010, Sidge Chapter 1, Sidge Chapter 2). The same two mainland sites, Platte River Campground area (SLBEs) and DH Day Campground area (SLBEn), which were sampled previously (Sidge Chapter 1), were sampled during this study.

NMI is approximately 12 miles from Leland, MI (mainland). NMI has a land area of 58 km^2 with 20 miles of shoreline. SMI is approximately 5 km from NMI and has a land area of 21.4 km^2 with 10 miles of shoreline (National Park Service 2016-a). The known wildlife on both islands includes rodents such as white-footed mice (*Peromyscus leucopus*) and eastern chipmunks (*Tamias striatus*), as well as snowshoe hares (*Lepus americanus*), coyotes (*Canis latrans*), and white-tailed deer (*Odocoileus virginianus*) (NMI only). Both islands consist of deciduous/mixed forests in addition to sandy, open dunes with a small lake located within each island. Currently, no year-around human residents reside on either island and no domestic pets are permitted.

The mainland is composed of a 35-mile stretch along the Lake Michigan coast and consists of deciduous/mixed forests with nature beech and sugar maple hardwoods, sandy soils with a leaf litter layer (National Park Service 2016-b). The mainland wildlife community is more extensive than the islands, consisting of, but not limited to, white-footed mice, eastern chipmunks, gray and fox squirrels (*Sciurus carolinensis*, *Sciurus niger*), red fox (*vulpes vulpes*), white-tailed deer, raccoons (*Procyon lotor*), opossums (*Didelphimorphia*), skunks (*Mephitidae*), porcupines (*Hystricidae*), flying squirrels (*Glaucomys volans*), cottontail rabbits (*Sylvilagus floridanus*), and coyotes (National Park Service 2016-

b). Similar to States et al. 2014, an assumption of this study is that the mainland has greater diversity (richness and evenness) compared with the islands. Additional support for the more diverse vertebrate community on the mainland was provided by the variety of captures on the mainland during the limited medium-sized mammal trapping efforts performed previously by our lab (Hamer et al. 2010), namely raccoons and opossums (*Didelphis virginiana*). Other non-white-footed mouse or eastern chipmunk small mammal captures included, fox squirrels, southern flying squirrels, red squirrels (*Tamiasciurus hudsonicus*), meadow jumping mice (*Zapus hudsonius*), and northern short-tailed shrews (*Blarina brevicauda*).

Given similar ecology (i.e. host compositions), invasion history, and the limited number of samples, to test the dilution and multiple niche polymorphism hypotheses, NMI and SMI were combined (the 'islands') and the two mainland sites, SLBEs and SLBEn, were combined (the 'mainland'). Blacklegged tick populations were established on both the mainland and the islands, according to the Centers for Disease Control and Prevention's definition (CDC 1999). Blacklegged ticks were not known to be established in the SLBE region prior to 2007 (Hamer et al. 2010); after which the abundance of blacklegged ticks steadily increased (Hamer et al. 2010, Sidge Chapter 1). The presence of blacklegged ticks on NMI was first reported in fall 2010 by a member of the public, after which sampling revealed established populations on both NMI and SMI.

Sampling Regime: To assess white-footed mouse infestation prevalence and host-seeking tick density/infection prevalence, the islands and mainland were sampled from 2011-2014, with small mammal trapping and drag sampling taking place in June of every year, when all three life stages of *I. scapularis* would be active. Additional mammal trapping and drag sampling took place throughout the calendar year from 2012-2014 (2012: April, November; 2013: May; 2014: May-October) in order to increase sample sizes for strain diversity analysis.

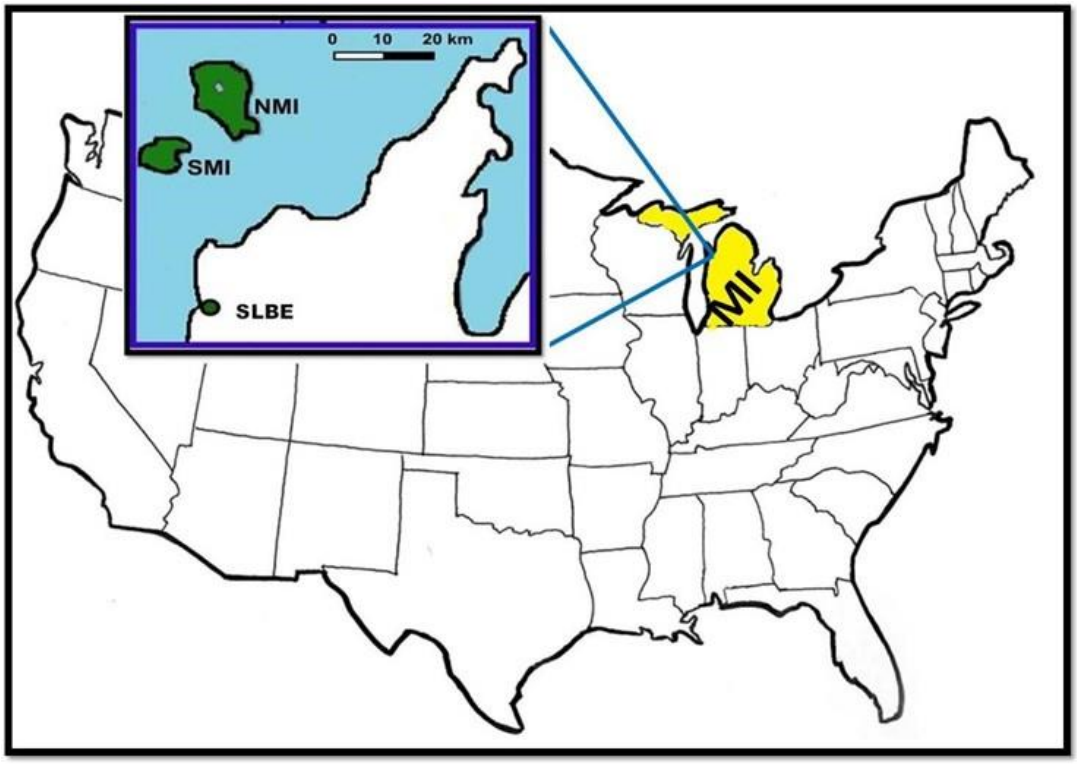


Figure 3.1 Map of the United States with Michigan highlighted in yellow. The two islands, North Manitou Island (NMI) and South Manitou Island (SMI) constitute the "islands" within this study and SLBE (Sleeping Bear Dunes National Lakeshore) constitute the "mainland" sites.

Small mammal trapping:

Small mammals, targeting white-footed mice and eastern chipmunks, were trapped following the protocol used in Hamer et al. 2010. Briefly, at each site, small mammals were trapped along six transects of 25 live traps (H. B. Sherman Traps, Tallahassee, FL) spaced 10 m apart and baited with crimped oats for two consecutive nights. Trapping took place at least once per month when immature stages of the tick were active (i.e. June). Traps were set in the evening and checked the following morning. All captured mammals were identified to species and sex, examined for ticks, and biopsied in both ears using a 2-mm biopsy punch (Miltex Instruments, York, PA). Ticks and ear biopsies were stored separately in 70% ethanol. Recaptured animals that were caught the previous day were strictly examined for ticks with no additional ear biopsies taken; animals recaptured from a previous trapping period, however, were biopsied again. All captured mammals were released back at the point of capture. The number of trap nights per

trapping period was adjusted for tripped traps as follows: number of traps set - (0.5*no. of tripped traps). Animal handling procedures were approved by Michigan State University's Institutional Animal Care and Use Committee Animal Use Form #: 06/12-103-00.

Questing tick sampling:

Each site was sampled for questing ticks by dragging a 1 m^2 corduroy cloth along the forest floor (Daniels et al. 2000, Hamer et al. 2010). This was performed along the same transects (5-10 m on either side of the transect) that were used for small mammal trapping. A minimum of 1000 m^2 (i.e., 4 transects) was dragged during each site visit. The cloth was examined every 20 m and any ticks that were found attached to the cloth or to the individual performing the sampling were collected and stored in 70% ethanol.

Pathogen detection:

All ticks were identified morphologically to species and life stage using dichotomous keys (Keirans et al. 1978; Sonenshine 1979; Durden et al. 1996). Total DNA from ticks and ear biopsies, with the exception of tick samples from 2014, were extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's animal tissue protocol with the modifications described previously (Hamer et al. 2010). Only *I. scapularis* ticks were assayed; any other species found was strictly recorded and stored for potential future analysis. In situations where more than three adult or nymphal ticks of the same species, life stage, and sex were removed from an individual animal, three were randomly selected for testing (Hamer et al. 2010). *Borrelia burgdorferi* was detected using a quantitative polymerase chain reaction (qPCR) targeting a fragment of the 16S rDNA gene (Tsao et al. 2004). With the exception of the 2014 tick samples, the qPCR protocol was performed as previously described (Hamer et al. 2010). Reactions for the qPCR were performed with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) at the Michigan State University

Research Technology Support Facility. The 2014 tick samples were extracted and tested by collaborators at the Centers for Disease Control and Prevention, Division of Vector Borne-Infectious Diseases. Total DNA from the 2014 ticks were extracted and *B. burgdorferi*, *Anaplasma phagocytophilum*, and *Babesia microti* were detected following procedures as described in Hojgaard et al. 2014. The consistency between the two assays was compared prior to screening the 2014 samples. Of 304 DNA samples from randomly selected ticks and tissue biopsies that were tested by both labs, there was 97.4% agreement; there was no significant difference between the two assays (Fisher's exact test: $p = 0.798$).

Nucleotide sequencing:

A subset of ticks and tissue biopsies from all sites that were positive for *B. burgdorferi* via qPCR were then sequenced at the 16S-23S rRNA intergenic spacer region (IGS) of *Borrelia* spp. (Bunikis et al. 2004). The subset of samples focused on positive tissue biopsies from the 2013 and 2014 sampling efforts, positive larval mammal ticks and host-seeking ticks from the mainland and SMI regardless of the year, and positive larval mammal ticks and host-seeking ticks from NMI in 2013 and 2014. Samples were first assayed by a nested PCR followed by visualization with gel electrophoresis as described in Hamer et al. 2010 in order to generate the template for nucleotide sequencing.

The IGS product was purified (EXOSAP-IT; Affymetrix, Santa Clara, CA) and sequences were determined in the forward direction using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) as previously described in Hamer et al. 2010.

Strain level analysis was then performed as described in Hamer et al. 2014. In brief, a 500 nucleotide fragment of the IGS was aligned with the prototypical strains published in Bunikis et al. 2004 and the Midwest strains published in Hamer et al. 2011 and 2014 using the ClustalW algorithms in Mega4 (Tamura et al. 2007). This allowed identification of the 10 main IGS groups (1-10), 20 IGS subtypes (1A, 1B, 2A/C, 2B, 2D, 3A, 3B/C, 3D, 4A, 4B, 5, 6A, 6B, 6C, 7A/B, 8A/C, 8B, 8D, 9, 10), and

the Midwest derived strains (Midwest A-N) (Bunikis et al. 2004, Hamer et al. 2011, Hamer et al. 2014). Furthermore, the sequences were then grouped using the broad ribosomal spacer type (1-3) as per Liveris et al. 1995. All sequence chromatographs were manually evaluated for evidence of poor quality or mixed infections, which were removed from analysis. If a sequence did not match one of the published strains, it was re-sequenced in the forward direction in addition to the reverse direction and if all three of these sequences contained the unique nucleotide polymorphism(s) then it was considered a novel IGS sequence and was assigned the prefix SLBE followed by a chronological number.

Statistics:

Fisher's exact test was used to assess differences in pathogen testing procedures used at Michigan State University and at the CDC. In order to assess differences in mammal infestation and mammal larval burden between locations and also between mammal species within the same location, the z-score and associated two-tailed probabilities were calculated. This was performed using a web-based calculator, Social Science Statistics (Stangroom 2016), with the assumption of a normal distribution and equal variance and using an alpha level of 0.05.

Multiple diversity indices were calculated in order to assess *B. burgdorferi* strain diversity between the host-limited Manitou Islands and the host-diverse SLBE mainland. This included Simpson's diversity without replacement (D) which considered the number of species present and the relative abundance of each species, strain richness (alpha diversity; S) which represented the number of strains in each location, the Shannon diversity index (H') which accounted for both the richness and the evenness of the species, the evenness (J) which measured the relative abundance of the different species, and the Sorensen's similarity index (beta diversity; B) was a means to compare the similarity of the two locations (Simpson 1949, Shannon and Weaver 1949, Krebs 1978, Sorensen 1948).

In order to assess the effect of sample size on strain richness, rarefaction analysis was performed using a web-based calculator, Analytic Rarefaction 1.3, from the UGA Stratigraphy Lab (Holland 2003).

Phylogenetic relationships within the *B. burgdorferi* strains were evaluated by constructing an unrooted neighbor-joining phylogenetic tree using Mega4 (Tamura 2007, Hamer et al. 2014).

Results:

Small mammal captures and *I. scapularis* infestation:

A total of 113 (2004.5 trap nights) and 198 (2354.5 trap nights) white-footed mice (*Peromyscus leucopus*) were captured at SLBE's mainland and islands, respectively, from 2011-2014 in June of each year. Every year, with the exception of 2013 on the mainland, mammal trapping took place for two consecutive nights during the month of June. On the mainland, approximately 90% (n=102) of the total mice were not captured the previous day during the two-day sampling period, and on the islands, 83% (n=164) were not captured the previous day.

In June from 2011-2014, on the mainland, in addition to the white-footed mouse, 111 eastern chipmunks, six northern short-tailed shrews, one southern flying squirrel, and one meadow vole (*Microtus pennsylvanicus*) were captured. The white-footed mouse was the most commonly captured host, consisting of approximately 48.7% of the total captures. On the islands, additional non-white-footed mouse small mammal captures included 413 eastern chipmunks, two northern short-tailed shrews, and three meadow voles. In contrast to the mainland sites, the most commonly captured host on the islands was the eastern chipmunk, which comprised 67% of the total captures followed by the white-footed mouse (32.1% of the captures).

Approximately 24.5% and 30.5% of the white-footed mice were infested with at least one *I. scapularis* tick on the mainland and islands, respectively. Of these infested mice, approximately 19.6% and 27.4% on the mainland and islands, respectively, were infested with at least one *I. scapularis* larva

(mainland vs. islands p=0.147). The mice on the mainland had significantly greater larval burdens than on the islands, with 30.7% of the larvae collected removed from mice on the mainland and 14.3% on the islands (Z-score: -5.4188; p<0.001). However, eastern chipmunks had greater larval burdens in both locations in comparison to the mice. Based on the number of mice and chipmunks captured, on the mainland, the number of larvae removed off of mice was approximately 0.5 larvae/mouse as opposed to the number of larvae removed off of chipmunks was 1.3 larvae/chipmunk. On the islands, the number of larvae removed off of mice was approximately 0.9 larvae/mouse as opposed to the number of larvae removed off of chipmunks was 2.4 larvae/chipmunk.

Questing ticks and *B. burgdorferi* infection:

In June from 2011-2014, a total of nine *I. scapularis* adults and 12 nymphs were dragged on the mainland over a total of 29,000 m^2. On the mainland, 20% (n=5) of the questing adults and 10% (n=1) of the nymphs were infected with *B. burgdorferi*.

On the islands, over the same time period, 22,750 m^2 were dragged and 148 adults and 107 nymphs were collected. Approximately 30% (n=120) of the adults and 20.8% (n=106) of the nymphs were infected with *B. burgdorferi*. There was a trend that the density of adults/1000 m^2, the density of infected adults, the density of nymphs/1000 m^2, and the density of infected nymphs were greater on the islands than on the mainland (Figure 3.2).

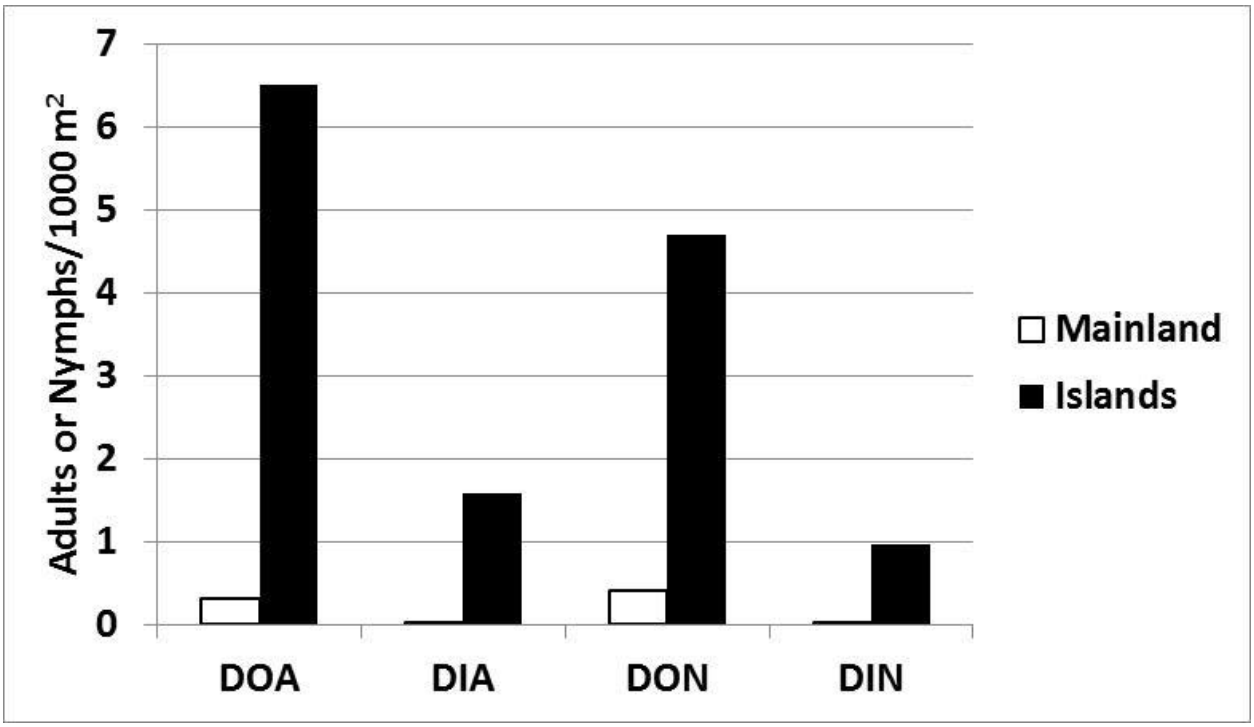


Figure 3.2 Density of questing *I. scapularis* adults (DOA) or nymphs (DON) and the density of infected questing *I. scapularis* adults (DIA) or nymphs (DIN) combined from every June from 2011-2014 on the host-diverse mainland (white bars) and the host-limited islands (black bars) in Michigan's northwest Lower Peninsula.

Borrelia burgdorferi **strain diversity:**

Genotypes- On the islands, a total of 342 *B. burgdorferi* positive samples (41.5% mammal tissue biopsy samples, 23.4% questing ticks, 35.1% ticks removed from mammals) were sequenced at the IGS locus. A total of 53.2% of these samples produced good quality sequences and 15.5% (n=19 mammal tissue biopsies, 10 questing ticks (8 adults, 2 nymphs), and 24 ticks removed from mammals) of the samples showed evidence of mixed-strain infections, the remainder were deemed poor quality. Mixed strain infections were based on the presence of double-nucleotide peaks in the chromatograph at polymorphic sites (Bunikis et al. 2004, Hamer et al. 2014).

On the mainland, a total of 52 *B. burgdorferi* positive samples (55.8% mammal tissue biopsy samples, 13.5% questing ticks, 30.8% ticks removed from mammals) were sequenced, with 59.6% of

these samples producing good quality sequences and 5.8% (n=2 mammal tissue biopsies, and 1 tick removed from mammals) of the samples with evidence of mixed-strain infections, the remainder were deemed poor quality.

On the islands, of the samples that produced a good quality sequence, 34.6%, 32.4% (71.2% adults, 28.8% nymphs), and 33% originated from mammal tissue biopsy samples, questing ticks (divided by life stage), and ticks removed from mammals, respectively. On the mainland, 58.1%, 19.4% (83.3% adults, 16.7% nymphs), and 22.6% of the samples that produced a good quality sequence originated from mammal tissue biopsy samples, questing ticks (divided by life stage), and ticks removed from mammals, respectively.

RST groups-Among the 170 and 26 single strain samples from the islands and mainland, respectively, all three RST groups were represented (Table 3.1, Figure 3.3). RST 3 was in greatest abundance at both locations (islands: 69%, mainland: 61%), followed by RST 2 (islands: 26%, mainland: 27%), and then RST 1 (islands: 5%, mainland: 12%).

Strain richness and diversity-A total of 15 unique strains were found within all 213 samples from the islands and the mainland (Table 3.1, Figure 3.3). The islands had a higher strain richness (n=15) in comparison to the mainland (n=8). Among the strains that were detected at both locations, four were prototypical IGS strains previously published by Bunikis et al. 2004 (IGS 2D, 1A, 6A, 5), two were from previously described bird-associated ticks (Midwest A, F; Hamer et al. 2011), and two were from previously detected Midwest derived questing ticks (Midwest D, H; Hamer et al. 2014). We detected seven strains that were unique to the islands. Among these unique island strains, four were prototypical IGS strains previously published by Bunikis et al. 2004 (IGS 2A/C, 8A/C, 4A, 7A/B); one was from a previously described bird-associated tick (Midwest B; Hamer et al. 2011); one was a previously described Midwest derived questing tick (Midwest C; Hamer et al. 2014); and one was considered a novel strain as it varied from previously published strains (SLBE 1). The type of sample (mammal tissue biopsy,

questing tick, and/or tick removed from a mammal) from which each strain was derived is depicted in Table 3.2. The most common strain among the island samples was Midwest A (RST 2), comprising 20% of the strains. On the mainland, Midwest A (RST 2) and IGS 6A (RST 3) were the most common (23% each) (Figure 3.3).

Of the 213 total samples from the mainland and islands, all 15 strains that were found in this study were found within questing ticks (n=65). The mammal tissue biopsies (n=81) constituted 13 strains and 10 strains were found within the ticks removed from mammals (n=67).

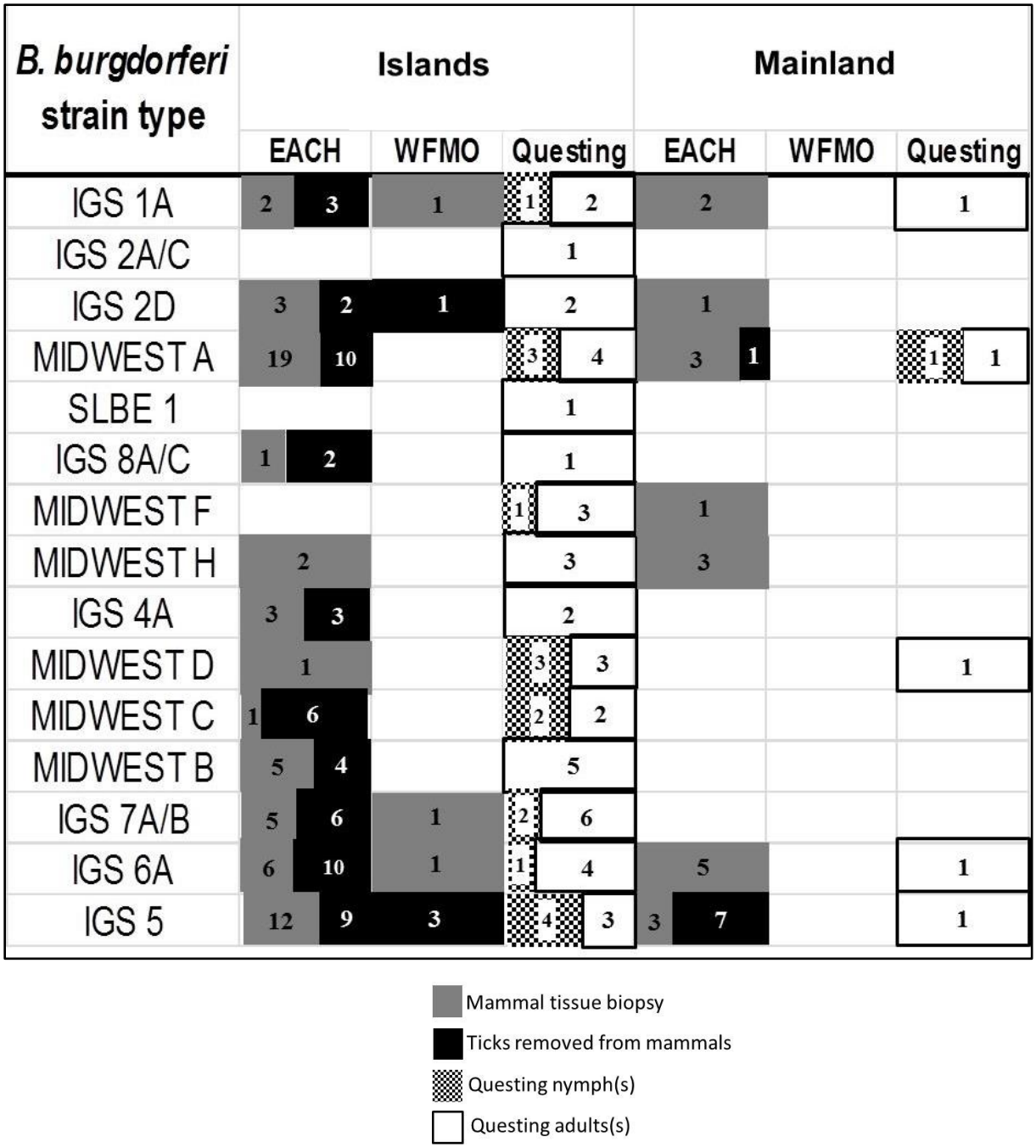
Species richness may be influenced by the difference in sample sizes between the islands and the mainland, thus, the rate in which new species were found per sample size was determined using rarefaction analysis (Figure 3.4; Holland 2003). At a sample size of 16, there was a significant difference in the number of strains between the two locations, with the host-limited island richer than the host-diverse mainland ($p<0.050$).

Additional diversity indices were performed comparing the host-limited islands to the host-diverse community on the mainland (Table 3.3). The mainland had a more uniform number of sequences per strain, as depicted via greater strain evenness. However, the Simpson's and Shannon diversity indices, and strain richness all suggested that the islands had higher *B. burgdorferi* strain diversity. The Sorensen's similarity index indicated that nearly 70% of the strains were in common between the two locations.

Table 3.1 Number (%) of IGS strain types in infected mammal tissue biopsies, questing adults and/or nymphs, and ticks removed from mammals from the host-limited islands and the more host-diverse mainland.

B. burgdorferi strain type	Islands n=170 (%)	Mainland n=26 (%)
IGS 1A	9 (5)	3 (12)
IGS 2A/C	1 (1)	0
IGS 2D	8 (5)	1 (4)
MIDWEST A	34 (20)	6 (23)
SLBE 1	1 (1)	0
IGS 8A/C	3 (2)	0
MIDWEST F	4 (2)	1 (4)
MIDWEST H	5 (3)	3 (12)
IGS 4A	7 (4)	0
MIDWEST D	7 (4)	1 (4)
MIDWEST C	10 (6)	0
MIDWEST B	14 (8)	0
IGS 7A/B	18 (11)	0
IGS 6A	20 (12)	6 (23)
IGS 5	29 (17)	5 (19)
RST 1	5%	12%
RST 2	26%	27%
RST 3	69%	61%

Table 3.2 *Borrelia burgdorferi* strain type found within infected eastern chipmunk (EACH) and white-footed mouse (WFMO) tissue biopsies (gray shading), ticks removed from chipmunks or mice (solid black), or questing nymphs (checkered squares) and/or questing adults (solid white) from the host-limited islands and the more host-diverse mainland. The relative length of the box indicates the proportion of chipmunk (tissues vs. ticks), mouse (tissue vs. ticks), and questing tick (nymphs vs. adults) sequences within that strain type, with the number of sequences indicated inside the symbol boxes.



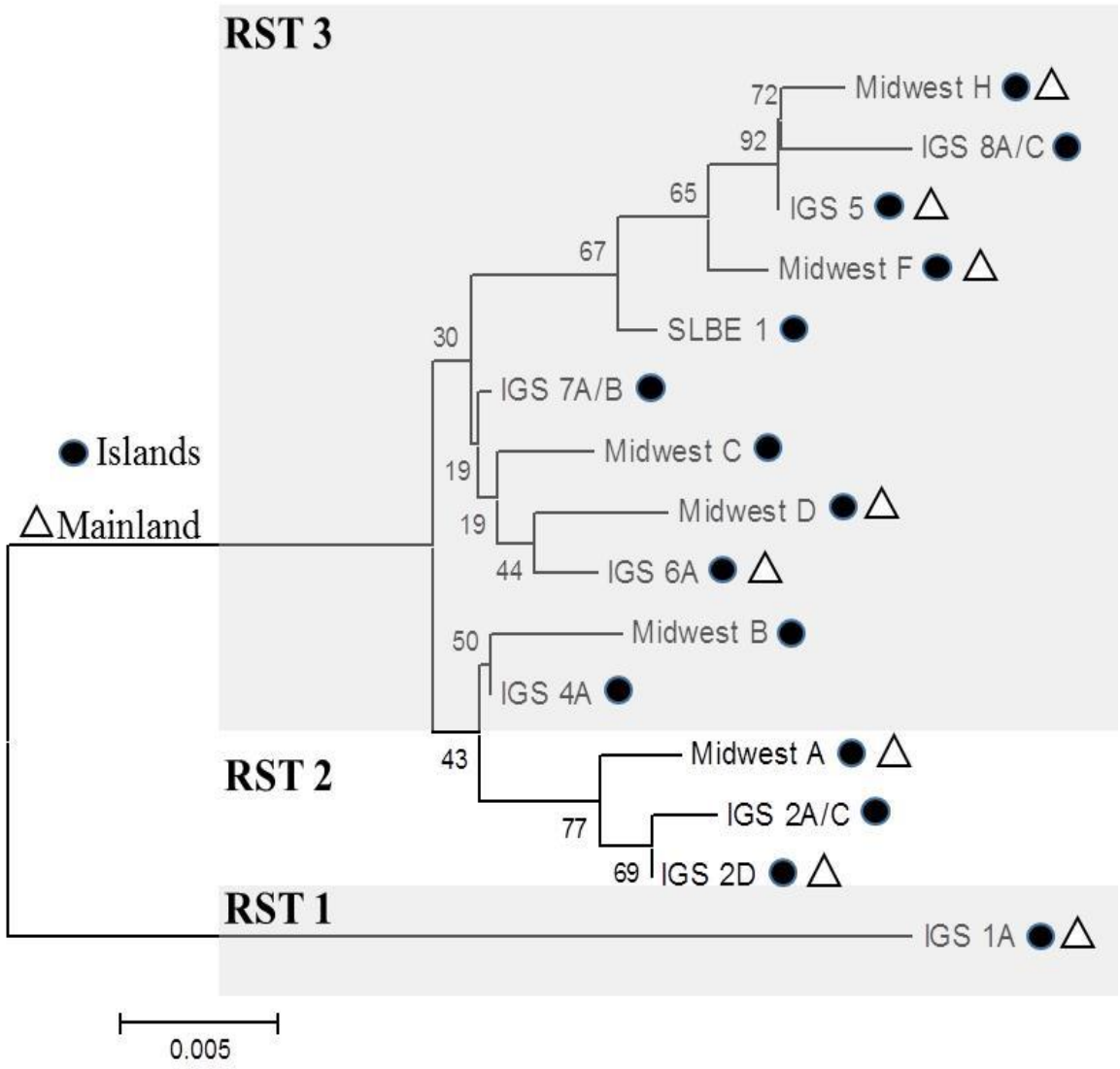


Figure 3.3 Unrooted phylogram of *B. burgdorferi* strain types from host-limited islands (circle) and a more host-diverse mainland (triangle) in Michigan's northwest Lower Peninsula using the neighbor-joining method of a 500 bp fragment of the IGS region. Strains are grouped into three main rRNA spacer types (RST; Liveris et al. 1995). The IGS type is noted in the sequence name as described by Bunikis et al. 2004; 'Midwest' derived names were previously published by Hamer et al. 2011 and 2014; and sequences are labeled as 'SLBE' if no matching sequence has been published. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches

.

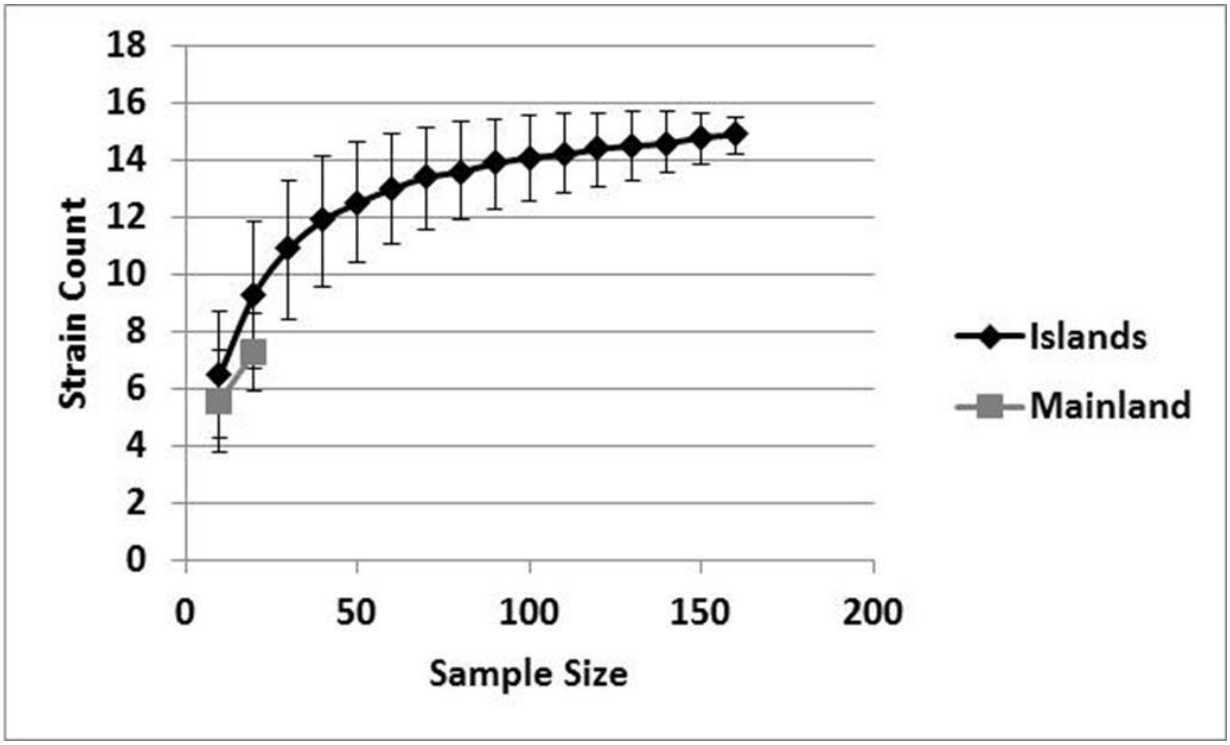


Figure 3.4 Rarefaction curves and 95% confidence intervals as a means to estimate the influence of sample size on the strain richness of *B. burgdorferi* from infected mammal tissue biopsies, questing adult and nymphal ticks, and ticks removed from mammals on the host-limited islands (black) and the more host-diverse mainland (gray) in Michigan's northwest Lower Peninsula. The final data point indicates what was observed, with estimated richness depicted at incremental sample sizes until that point.

Table 3.3 Comparison of the diversity between *B. burgdorferi* strain types found on the host-limited islands to the host-diverse mainland. Bolding indicates the more diverse location for each given ecological index.

Ecological Diversity Index	Islands	Mainland	Interpretation
Simpson's Diversity without Replacement (D)	**0.109**	0.142	Range: 0-1 (high --> low diversity)
Species Richness / Alpha Diversity (S)	**15**	8	Range: 0-N (low --> high number species)
Shannon Diversity Index (H')	**2.371**	1.868	Range: 0-4.6 (low --> high diversity)
Evenness (J)	0.876	**0.898**	Range: 0-1 (uneven --> uniform number individuals per species)
Beta diversity / Sorensen's Similarity Index (B)	0.696		Range: 0-1 (no species in common --> all species in common)

Discussion:

Communities that are composed of a limited composition of mammalian hosts that are predominately white-footed mouse dominated are predicted to have greater *I. scapularis* burdens on mice and maintain the Lyme disease bacterium at greater densities as opposed to communities with a wide-array of potential hosts, as explained by the dilution effect. As alternative, non-mouse, hosts are introduced to an area, they dilute the effect of the white-footed mouse by increasing the proportion of blood meals acquired from less competent hosts (Ostfeld and Keesing 2000, Schmidt and Ostfeld 2001, LoGiudice et al. 2003). Lyme disease presents as an optimal example to test the dilution hypothesis (Norman et al. 1999, Ostfeld and Keesing 2000). Two neighboring communities in which to test the dilution effect are within Sleeping Bear Dunes National Lakeshore (SLBE), in the northwest portion of Michigan's Lower Peninsula.

The SLBE islands are unique as they are home to small rodents (primarily white-footed mice and eastern chipmunks), snowshoe hares, coyotes, and white-tailed deer (one island only). The SLBE mainland, however, is comprised of a greater diversity of potential hosts for juvenile blacklegged ticks, including not only white-footed mice and eastern chipmunks but also a variety of squirrel species, foxes, raccoons, opossums, skunks, porcupines, etc. Although we did not estimate vertebrate species diversity, there are many hypotheses that would predict a greater diversity on the mainland sites compared to the islands. For example, a greater vertebrate biodiversity within mainland communities compared to that of islands is supported by The Equilibrium Theory (McGuinness 1984), in which smaller areas have lower population sizes due to faster species extinction rates. This hypothesis was also supported by the Island Biogeography Theory (Wilson and MacArthur 1967), which hypothesizes increased rates of species immigration and decrease rates of species extinctions on larger versus smaller islands and with the greatest diversity on the mainland. Additionally, the Habitat Diversity Hypothesis (McGuinness 1984) predicts that habitat diversity is positively related with area size, and a greater number of potential

habitats supports the establishment of a greater number of species. Furthermore, the Disturbance Hypothesis (McGuinness 1984) proposes that species become extinct faster on small islands since disturbances are more frequent and more intense in smaller areas. These hypotheses provide support for the proposed greater vertebrate diversity on the SLBE mainland in comparison to the isolated islands.

Furthermore, host association has been suggested at certain *B. burgdorferi* loci with the prediction, based on the multiple niche polymorphism hypothesis, that diverse host communities would have greater *B. burgdorferi* strain diversity (Brisson and Dykhuizen 2004, Levene 1953, State et al. 2014). As a means to test the dilution effect and the multiple niche polymorphism hypothesis, we compared the species-limited islands to the more host-diverse mainland in Michigan's Lower Peninsula, by testing the following hypotheses: 1) *Ixodes scapularis* larval infestation prevalence on white-footed mice will be greater on the island vs. the mainland; 2) the host-seeking *I. scapularis* adult/nymphal infection prevalences and the density of infected nymphs/adults will be greater in the host-limited community vs. the more diverse community; 3) lower *B. burgdorferi* strain diversity will be present on the island in comparison to the more host-diverse mainland.

Objective 1: White-footed mouse larval infestation on the islands vs. the mainland

Previous research has shown that white-footed mice typically feed more larvae than eastern chipmunks and chipmunks have higher levels of nymphal infestation than mice (Mannelli et al. 1993, Slajchert et al. 1997, Schmidt et al. 1999). Furthermore, models have suggested that white-footed mice have the greatest *B. burgdorferi* reservoir competence (LoGiudice et al. 2003), based on the susceptibility of the mice to infection when bitten by an infected tick, the ability of the Lyme disease pathogen to magnify and persist in the mouse, and the efficiency of the mouse at transmitting the spirochete to feeding ticks (Richter et al. 2000, LoGiudice et al. 2003). Within this study, the larval infestation prevalence on the island mice were compared to the mainland mice, with the hypothesis that the larval infestation prevalence would be greater on the host-limited island in comparison to the more host-diverse mainland.

The infestation prevalence was predicted, based on the dilution hypothesis, to be less on the mainland because the mainland community had alternative hosts present that could divert the questing ticks from the mice, thus, making the mice less infested vs. the island mice (Brunner and Ostfeld 2008). This is however, assuming that the total abundance of ticks in the two locations was the same.

We found that there was not a significant difference between the proportion of white-footed mice infested with at least one *I. scapularis* larvae on the island in comparison to the mainland. However, the mice on the mainland had significantly greater larval burdens than on the islands. This is unlike what we hypothesized given the dilution hypothesis and the presence of a wider array of alternative hosts on the mainland. However, this could be supported by the trend of fewer mice captured on the mainland in comparison to the island and a negative correlation between tick burden and mouse density (Ostfeld et al. 1996). This negative trend that Ostfeld et al. 1996 found within their field studies was most likely due to the reduced probability of the mice contacting aggregations of larval ticks. After *I. scapularis* eggs hatch, the larvae remain within close proximity of the egg mass (Daniels and Fish 1990). A similar situation may have occurred on the islands, the island mice may have come across fewer clumps of host-seeking larvae, therefore, contributing to the reduced larval burden that we observed.

Typically when testing the dilution hypothesis within the Lyme disease system, the host-limited community is dominated by white-footed mice (LoGiudice et al. 2003, States et al. 2014). However, in the case of the Manitou Islands, the most commonly captured host was the eastern chipmunk. Previous research analyzed the effect of white-footed mouse and eastern chipmunk population density on juvenile *I. scapularis* (Schmidt et al. 1999). It was determined that high densities of chipmunks reduced the larval tick burden on mice, therefore, supporting the dilution effect of chipmunks on mice. This was evident on the islands, given that the chipmunks had a significantly greater larval burden than the mice. Thus, at the location level (i.e. the islands), some would argue that the dilution hypothesis was supported (Schmidt et al. 1999).

This greater larval burden on chipmunks in comparison to mice was also evident on the mainland. However, there were fewer chipmunks captured on the mainland in comparison to the islands, yet, a similar number of chipmunks and mice were captured on the mainland during this trapping period. This is unlike what would be expected based on previous research in the Midwest and the northeast as white-footed mice typically are captured more often and have higher densities than sympatric eastern chipmunks (Mather et al. 1989, Slajchert et al. 1997). Given the greater chipmunk larval burden on the mainland, this also supported the dilution hypothesis at the location level (i.e. the mainland) since the larvae were being diverted from the white-footed mice. However, previous modeling efforts (Schmidt and Ostfeld unpublished data) determined that in order to indicate that the dilution effect is strong in a given area, non-mouse and non-chipmunk hosts must provide approximately 61% of larval tick meals, which was not evident, based on this study, on the host-diverse mainland.

Objective 2: Questing AIP/NIP and DIA/DIN on the islands vs. the mainland

When the nymphal infection prevalence (NIP) was used as a means to examine the dilution effect within the Lyme disease system (LoGiudice et al. 2003), as new hosts were added to the community, the NIP decreased. This was due to the contribution of key diluting hosts (particularly gray squirrels and red squirrels), which had high tick burdens, low reservoir competence, and a relatively high population density (LoGiudice et al. 2003). However, others have argued that both the NIP and the density of infected nymphs (DIN) must decrease to demonstrate a dilution effect within a community (Ogden and Tsao 2009).

Thus, for this study we examined both the NIP and the DIN, in addition to the adult infection prevalence (AIP) and the density of infected adults (DIA), over a four-year period on the host-diverse mainland and compared it to the host-limited islands. Adult questing ticks were considered, in addition to nymphs, within our study since a limited number of host-seeking nymphs were collected at each of the sites and also because questing adults were included within previous dilution effect models (Schmidt and

Ostfeld 2001). Our hypothesis was that the host-seeking *I. scapularis* adult/nymphal infection prevalences and the density of infected nymphs/adults would be greater on the host-limited islands vs. the more diverse community on the mainland.

We found that the AIP, NIP, DIA, and DIN were greater on the islands than the mainland, as predicted by the dilution hypothesis. Although this may be a consequence of the dilution hypothesis, the duration of establishment of tick populations may have also influenced these findings. In locations where blacklegged ticks have been long-established, there was a trend for these ticks to be more abundant in addition to being significantly more infected than intermediate-established and recently-invaded sites (Hamer et al. 2014).

Based on previous studies that we performed on the mainland of SLBE, we were able to track the arrival of *I. scapularis* ticks and their increase over time leading up to this investigation (Hamer et al. 2010, Sidge Chapter 1). The length of tick establishment on the islands, on the other hand, was not as explicit since blacklegged ticks were collected during the first survey efforts (Sidge Chapter 2). However, we have reason to believe that the tick populations on the islands were established at approximately the same time period as the mainland.

The first report of the presence of blacklegged ticks on the islands was in 2010 by a member of the general public. The islands have been under the ownership of the National Park Service since the late 1970's thus, if the tick population was established much earlier than this initial report, there is reason to believe that the Park Service and/or individuals within the general public would have raised concern either via press releases, visitor awareness/educational messaging, and/or by notifying Michigan Department of Health and Human Services. Furthermore, according to anecdotal accounts the islands have recently (within the last ten years) become invaded by ticks. After speaking with the owners of the public boat transportation for the islands, National Park Service employees who were raised and lived in the area for the vast majority of their lives, and members of the general public who have participated in

the annual NMI deer hunt for 20+ years, all have agreed that they have not noticed and/or encountered a tick on the islands until the last decade.

The difference in the observed tick densities and infection prevalence between the mainland and the islands may be due to the higher density of white-tailed deer on NMI and the greater number of small mammals on the islands in comparison to the mainland. The number of deer harvested per hunter on NMI in 2014 was approximately 0.54 (National Park Service 2014), as opposed to in Leelanau (a county on the mainland) the number of deer harvested per estimated number of hunter was 0.47 (Frawley and Boon 2015). Prior research showed that as the deer population declined, the number of deer seen per hour declined as well (Holsworth 1973). Therefore, this implies that since more deer were taken per hunter on NMI, the population was greater. Additionally, based on our study, there were approximately 3.7 times more eastern chipmunks captured than white-footed mice. With a greater abundance of small rodent hosts for the immature stages of the tick to feed upon and a healthy population of white-tailed deer for the adult stage of the tick, this has allowed the tick population to grow much faster on NMI in comparison to the mainland across the same time period.

Therefore, although the definitive length of tick establishment on the islands is unknown, we believe that the island populations were established at approximately the same time period as the mainland. The greater AIP, NIP, DIA, and DIN on the islands in comparison to the mainland may have been a consequence of the dilution effect given the increased abundance of eastern chipmunks, which have high *B. burgdorferi* reservoir competence (LoGiudice et al. 2003) and also the increased density of white-tailed deer further allowing the tick population to flourish.

Objective 3: *Borrelia burgdorferi* strain diversity on the islands vs. the mainland

Diverse host community composition may increase the pathogen diversity by increasing the ecological ‘niches’ which would result in the maintenance of polymorphisms within that community,

known as the 'multiple niche polymorphism' hypothesis (Levene 1953, States et al. 2014). Thus, in communities composed of a high diversity of potential hosts, and hence more opportunities for transmission, the *B. burgdorferi* diversity would be greater based on this hypothesis (Brisson and Dykhuizen 2006; States et al. 2014). We hypothesized that there would be lower *B. burgdorferi* strain diversity on the host-limited island in comparison to the host-diverse mainland.

Although the mainland had a slightly more even number of sequences per strain, as evident by multiple additional diversity indices, including strain richness, Simpson's diversity, and the Shannon diversity, there was greater *B. burgdorferi* strain diversity found on the host-limited islands in comparison to the host-diverse mainland, which was unlike what we had hypothesized. States et al. 2014 found the opposite, supporting the dilution hypothesis, lower ospC genotype diversity on the less host-diverse island in comparison to the species-diverse mainland. This greater *B. burgdorferi* strain diversity and richness on the islands could be due to importation of non-mouse-adapted genotypes from birds or other immigrating hosts (States et al. 2014). This hypothesis was supported given that the island questing ticks had greater strain richness than what was found in the mammal tissue biopsies. Additionally, the greater sample size from the islands may have contributed to the greater diversity.

When all of the single-strain samples within this study were considered, the mammal tissue biopsies had greater strain richness in comparison to the ticks removed from the mammals. All of the strains that were found within the mammal ticks were also found within the tissue biopsies. *Ixodes scapularis* larvae are born uninfected and must feed upon an infected vertebrate host in order to acquire the bacterium (Piesman et al. 1986). The vast majority of a tick's life span is spent off of a host (Yuval and Spielman 1990). Thus, detecting fewer strains in the mammal ticks would be supported given that the tick falls off of the host after feeding is complete (typically lasting 3-8 days) yet, the infection in the host lasts multiple months (McLean et al. 1993, Donahue et al. 1987). Based on when sampling occurs, the tick may have already completed its blood meal and may no longer be attached yet, if an infected tick

fed on the host prior then the infection and *B. burgdorferi* strain may still be determined equating to more strains being detected from the mammal tissues.

One of the mainland chipmunks was infested with 50 larval ticks. For pathogen testing and *B. burgdorferi* strain analysis, these ticks were divided into seven different pools. All seven of these larval pools were found to be infected with the same *B. burgdorferi* strain (IGS 5) as would be expected given that larvae are born uninfected (Piesman 1986). Additionally, fourteen chipmunks from the islands simultaneously had both infected larval ticks and infected tissue biopsies that were *B. burgdorferi* sequence analyzed. In eleven chipmunks, both the ticks and tissue shared the same strain. Interestingly, however, in three chipmunks there was a discrepancy between the tick and the tissue strains. This could be explained given that a mammal may be infected with multiple, sequential infections over time since animals are continually being exposed during the nymphal season, which may consequently result in multiple infections of different strains. Additionally, PCR assays may be biased against the rarer species/strain, which may lead to false negatives (Barbour et al. 2009). Therefore, in the case of these three chipmunks, the chipmunks may have been infected with the same *B. burgdorferi* strain as found within the larval tick(s); however, due to PCR competition for the same primers and probes, different strains were detected.

Lyme disease can cause significant human and canine illness, with different *B. burgdorferi* strains associated with varying disease severity (Wormser et al. 1999). Ribosomal spacer type (RST) and the outer surface protein C (ospC) are the primary genetic typing means to predict the pathogenicity in humans. In this study, the strains were divided by RST. Although severe disease can be caused by RST 2 and 3 strains, previous research has suggested that some RST 1 strains are more strongly associated with disseminated infection in humans, yet, the less invasive RST 2 and 3 strains are typically more common in the Midwest (Derdakova et al. 2004, Wormser et al. 2008, Gatewood et al. 2009, Hamer et al. 2011, 2014). Approximately 95% of the island strains and 90% of the mainland strains were RST 2 and 3, thus, supporting what has been found in other locations across the Midwest. RST 1 strains were present at both

locations, with 5% more RST 1 strains found on the mainland. The prevalence of RST 1 strains at these two SLBE locations was comparable with previously studied Midwest sites (3.4% in Hamer et al. 2012; 6.2% in Hamer et al. 2014) yet, lower than what was detected in the northeast (25.9% in Gatewood et al. 2009). The increased RST 1 prevalence in the northeast has been attributed to the less seasonal synchrony between nymphal and larval activity periods as opposed to the Midwest where the immature stages of the tick were found to be more synchronous (Gatewood et al. 2009). Although, overall, RST 1 strains were in low abundance at both locations, in comparison to the other two RST groups, the potential for visitors to become infected with a strain that may result in increased severity was still present.

The sample type with the greatest strain richness within this study was questing ticks. Host-seeking ticks, particularly the nymphal stage, present the greatest risk to visitors as nymphs are responsible for the most cases of human Lyme disease (Barbour et al. 1993). Although few questing nymphs were collected and thus, included within the strain analysis, *B. burgdorferi* strains that could result in disseminated human Lyme disease originated from questing nymphs found at both locations (Hamer et al. 2014). Thus, from a public health perspective, proper precautionary measures should be enacted on both the mainland and the islands in order to ensure that visitors are properly educated on the potential risk.

Conclusion:

The dilution hypothesis predicts that in a community composed of a high diversity of potential hosts for blacklegged ticks to feed upon, ticks would be diverted from feeding on white-footed mice and consequently the infection prevalence of ticks within that community would be less since the white-footed mouse is the primary reservoir for *B. burgdorferi* (Ostfeld and Keesing 2000, Mather et al. 1989, LoGiudice et al. 2003). Furthermore, host association has been suggested at certain *B. burgdorferi* loci with the prediction, based on the multiple niche polymorphism hypothesis, that diverse host communities would have greater *B. burgdorferi* strain diversity (Brisson and Dykhuizen 2004, Levene 1953, State et al.

2014). As a means to test the dilution and multiple niche polymorphism hypotheses, we compared host-limited islands to a host-diverse mainland within the northwest portion of Michigan's Lower Peninsula.

The first way that the dilution hypothesis was tested was comparing the larval *I. scapularis* prevalence on white-footed mice between the two locations. The proportion of mice infested with at least one *I. scapularis* larvae nor the larval burden on the mice supported the dilution hypothesis. However, when the mainland and the islands were considered independently of one another, the dilution hypothesis was evident at the individual location level. More chipmunks were captured on both the island and the mainland than would be anticipated in the Midwest. This consequentially reduced the larval tick burden on the mice in each community, supporting the dilution effect, similar to what Schmidt et al. 1999 found. However, since eastern chipmunks are considered to be the second most *B. burgdorferi* reservoir competent species (LoGiudice et al. 2003), in order to have a strong dilution effect within an area, more than half of the larval tick meals must be acquired from non-mouse and non-chipmunk hosts, which was not evident within this study (Schmidt and Ostfeld unpublished data).

Host-seeking *I. scapularis* NIP and DIN were also compared between the host-diverse mainland and the host-limited islands. In order to truly demonstrate evidence of a dilution effect of public health and ecological significance, both the NIP and the DIN must decrease, thus, both were considered in this study, in addition to the AIP and DIA (Ogden and Tsao 2009). We found that the AIP, NIP, DIA, and DIN were greater on the islands than the mainland, which supported the dilution hypothesis. We postulated based on recent deer harvest data that the island had a greater abundance of deer compared to the mainland, in addition, to an increased abundance of eastern chipmunks, thus, allowing the tick population to grow at a faster rate than the mainland.

In order to test the multiple niche polymorphism hypothesis, the *B. burgdorferi* RST/IGS strain diversity was compared between the islands and the mainland. As evident by multiple diversity indices, including strain richness, Simpson's diversity, and the Shannon diversity, there was greater *B. burgdorferi*

strain diversity found on the host-limited islands in comparison to the host-diverse mainland, which did not support the multiple niche polymorphism hypothesis and was unlike what we had hypothesized. This may be a result of importation of genotypes from immigrating hosts (States et al. 2014). Additionally, from the strain analysis, we determined that although the vast majority of the strains were RST 2 or 3, as expected within the Midwest, both locations also had strains in RST 1 which may pose greater disease severity in humans (Derdakova et al. 2004, Wormser et al. 2008, Gatewood et al. 2009, Hamer et al. 2011, 2014). The presence of RST 1 strains in nearly 10% of ticks emphasizes the importance of enacting proper tick precautionary measures throughout all of SLBE, including the islands.

Study Limitations and Future Research:

One limitation within this study was that we did not know definitively how long the tick populations have been established on the islands. Although we have reason to believe that the mainland and island tick populations were established at approximately the same time period, if this was not true then length of tick establishment may be a confounding factor within this study. Length of tick establishment may influence *I. scapularis* abundance, infection prevalence, and *B. burgdorferi* strain diversity (Hamer et al. 2014).

An additional study limitation was the low sample size on the mainland when evaluating the *B. burgdorferi* strain diversity. The increased sample size on the islands was most likely due to the ability of the tick population to flourish at a faster rate given the differing ecologies between the two locations. Although ticks and mammals were infected with *B. burgdorferi* on the mainland, it was present in low abundance, consequently resulting in fewer potential samples available for sequencing efforts.

One 'novel' IGS sequence was detected during this study since the sequence did not match one of the previously published strains. This novel sequence was detected in a host-seeking adult tick.

Although, this novel strain was sequenced in the forward direction twice in addition to the reverse direction, a sample size of one was a limitation.

In the future, additional trapping efforts should be performed for medium sized mammals, particularly on the mainland as it was unknown as to the degree in which other potential hosts were contributing to the feeding of blacklegged ticks. We hypothesized since the mainland had a diverse community of potential hosts that these hosts would be diverting the ticks away from white-footed mice and hence supporting the dilution hypothesis. However, in order to verify the role of non-mice and non-chipmunk hosts, additional mammal trapping should be performed.

Additionally, the dilution effect and *B. burgdorferi* strain diversity should be tested by comparing the host-limited island (NMI) to a host-diverse location that has a known long-established tick population. For example, this long-established tick population site could be in WI or Menominee, MI. Although we postulate that the tick population on the mainland of SLBE was established at approximately the same time period as the tick populations on the islands, given the density and infestation prevalence of ticks on NMI (Sidge Chapter 2), a comparison to an additional host-diverse site with a long-established population of ticks may be warranted in the future.

Hamer et al. 2014 began to assess the epidemiological risk associated with selected Midwest strains by determining the direct ospC typing. In the future, ospC typing should be performed on the novel strain that was found within this study in order to further our knowledge on the pathogenicity in humans of novel IGS mutant strains.

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CONCLUSIONS

The Lyme disease tick and bacterium continue to invade and spread across Michigan's Lower Peninsula with Lyme disease being the most commonly reported vector-borne disease in the state (MDHHS 2016). One of Michigan's most visited landmarks, Sleeping Bear Dunes National Lakeshore (SLBE), attracts more than 1.5 million visitors annually with 70% of them coming during the summer months when the immature stages of the blacklegged are host-seeking (NPS stats). Thus, from a public health perspective this research was highly significant in order to determine the current risk and Lyme disease trends across the entire park, including SLBE's two offshore islands (North Manitou Island; NMI and South Manitou Island; SMI), for local, national, and international visitors.

Additionally, SLBE provided unique ecological opportunities. Given that SLBE was presumed to be at the leading edge of the tick invasion within Michigan's Lower Peninsula, this work provided an opportunity to further test the scenario of invasion hypotheses that were proposed in 2010, which would aid in better understanding how Lyme disease may be invading into additional locations throughout the nation (Hamer et al. 2010). Furthermore, SLBE provided a means in order to evaluate the importance of deer and alternative hosts within the Lyme disease system, given that one of the two isolated islands was home to a population of white-tailed deer (*Odocoileus virginianus*) and the other was completely devoid of deer. White-tailed deer are believed to be the most important hosts for adult *I. scapularis* and critical for its spread and maintenance, but few opportunities exist to investigate tick and pathogen dynamics in their absence. Furthermore, since the two islands have a depauperate community of medium/adult mammals as opposed to the wildlife-diverse mainland, SLBE served as a means to test the Lyme disease dilution effect and the multiple niche polymorphism hypotheses.

In Chapter 1, I continued to track the arrival of the blacklegged tick and Lyme disease bacterium on SLBE's mainland, following initial survey efforts by our lab from 2007-2008 (Hamer et al. 2010). During these initial efforts, neither B. burgdorferi nor questing *I. scapularis* were detected, although

blacklegged ticks were found attached to a low proportion of mammalian hosts. This sequence of detection supported the "tick-first" process of invasion at SLBE (Hamer et al. 2010), which hypothesizes that the blacklegged tick is introduced first (e.g., by reservoir incompetent white-tailed deer), and then the Lyme disease agent is introduced second (e.g., by reservoir competent white-footed mice). We were interested in determining the time delay between tick and pathogen invasion; thus we continued surveillance at SLBE until 2015. Furthermore, to see how much further north the blacklegged tick may have invaded along the Lake Michigan coast, an additional coastal site northeast of the initial SLBE site was surveyed beginning in 2011.

I found that there was a four-year delay between the arrival of the tick and the first detection of the bacterium, which has implications for disease management in locations that are undergoing the "tick-first" scenario of invasion. Although all areas that undergo a "tick-first" process of invasion may not experience a four-year delay before the pathogen arrives, modeling surveys have indicated that a minimum threshold of ticks must be achieved prior to *B. burgdorferi* maintenance (Norman et al. 1999; Ogden et al. 2007; Ogden et al. 2013). Therefore, this implies the presence of a delay, although the length of the delay may be variable. For example, in southern Canada using surveillance data, it took five years for *B. burgdorferi* to invade an *I. scapularis* established location (Ogden et al. 2013). Regardless of the length, if an area is determined to be undergoing a "tick-first" process of invasion, interventional strategies may be attempted prior to pathogen arrival.

Additionally, I found that at a second site on SLBE's mainland that the tick and the pathogen, based on our efforts, arrived at approximately the same time supporting the "dual-invasion" scenario, similar to another recently invaded site south of SLBE (Hamer et al. 2010). Yet, we detected both the tick and the pathogen within the first couple of months of sampling, therefore, the tick or the pathogen may have arrived prior to the other implying that this may not have been a true "dual-invasion" scenario. However, this abbreviated gap between tick and pathogen establishment, supports what actually would be

expected in the Midwest since larval and nymphal ticks are seasonally synchronous and there would be a high number of immigrating infected engorged larvae (Ogden et al. 2013; Hamer et al. 2012).

After comparing this mainland work to a reference site, which was previously determined to be endemic for both the tick and the bacterium, as expected, the reference site had a significantly greater abundance of blacklegged ticks and greater *B. burgdorferi* infection prevalence than both SLBE sites, supporting the recent emergence at SLBE. However, at SLBE given the level of infestation and infection with *B. burgdorferi*, it is likely that the leading edge of the invasion of *I. scapularis* and *B. burgdorferi* now lies further northward. Interestingly, we also found that in areas that have recently become invaded by *B. burgdorferi* (such as SLBE mainland), eastern chipmunks tend to be an earlier indicator of the pathogen's presence in comparison to white-footed mice.

In Chapter 2, using SLBE's two islands, I evaluated the success of mammalian hosts for maintaining *I. scapularis* in the absence of white-tailed deer. As expected, I found that the island with deer had a more established tick population and presented as a greater public health concern. However, unlike what was hypothesized, I found all three life stages of the blacklegged tick and detected *B. burgdorferi* circulating within the ticks and the mammals on the deer-free island. Thus, in order to determine how the blacklegged tick population was being maintained in the absence of deer, I trapped potential alternative hosts for the adult tick, including snowshoe hares (*Lepus americanus*), passerine birds, and coyotes (*Canis latrans*). Given that no *I. scapularis* ticks were found on the hares, no adult ticks were found on the passerine birds, and several engorged adult *I. scapularis* ticks were found feeding on multiple coyotes, coyotes were determined to be maintaining the tick population on that island. Previous research has also shown that blacklegged adult ticks have been found on coyotes (Kocan et al. 1999). Furthermore, *B. burgdorferi* antibodies have been detected in coyotes (Kazmierczak and Burgess 1989). Yet, demonstration of coyotes maintaining populations of blacklegged ticks and the enzootic cycle in areas completely devoid of deer has not been documented prior to this study.

Investigating questions about the maintenance of the Lyme disease tick and pathogen in the absence of deer (i.e. SMI), has implications for Lyme disease risk management. When considering tick-borne disease reductions, it is important to consider if the area under investigation is isolated (i.e. NMI or SMI) or if it is ecologically open to immigration (i.e. SLBE mainland). Although prior research presents conflicting views as to how much reducing deer populations will aid in disease management, we have shown that on offshore islands, the Lyme disease tick and pathogen prevalence can be reduced if white-tailed deer are completely removed (i.e. SMI). However, even if deer are not present, an established blacklegged tick population can still occur if alternative medium/large mammalian hosts, such as coyotes, are present.

Additionally, eastern chipmunks were found to play a crucial role with maintaining the juvenile stages of the tick on both islands. On the Manitou Islands, there was an equivalent or even greater number of chipmunks captured in comparison to white-footed mice, and in June and August throughout the study, the island chipmunks fed more larvae and nymphs than the mice, while remaining active throughout the entire summer without experiencing as Dunford 1972 explained a "summer lull." On NMI, the density of questing nymphs was lower yet, the overall nymphal infection prevalence was greater in comparison to a Lyme disease endemic site in Michigan's Lower Peninsula. The lack of host-seeking nymphs on NMI could also be explained by the abundant number of eastern chipmunks, thus, allowing more questing nymphs to find a host rather than be collected via drag cloth (Ginsberg et al. 1999). Additionally, more larvae could be feeding on infected chipmunks on the island, contributing to the increased infection prevalence. Summer visitors and hikers should be made aware of this risk, as the nymphal stage of the tick has the greatest epidemiological significance (Barbour et al. 1993).

In Chapter 3, I tested the dilution effect (Ostfeld and Keesing 2000, Mather et al. 1989, LoGiudice et al. 2003) and the multiple niche polymorphism hypothesis (Brisson and Dykhuizen 2004, Levene 1953, States et al. 2014) by comparing the species-limited islands to the more host-diverse mainland in SLBE. I found that neither the proportion of mice infested with at least one *I. scapularis*

larvae nor the larval burden on the mice supported a prediction of the dilution hypothesis. Based on the dilution effect, the larval *I. scapularis* infestation prevalence on white-footed mice was predicted to be greater on the host-limited island in comparison to the more host-diverse mainland. The infestation prevalence was predicted to be less on the mainland, assuming that the total abundance of ticks in the two locations was the same, because the mainland community had alternative hosts present that could divert the questing ticks from the mice, thus, making the mice less infested vs. the island mice (Brunner and Ostfeld 2008). However, on the islands and the mainland, more eastern chipmunks were captured than anticipated and this may have reduced the larval burden on the mice in each community, thus, supporting a prediction of the dilution effect since the ticks were being diverted away from the mice (Schmidt et al. 1999).

Host-seeking adult/nymphal infection prevalence and adult/nymphal density of infected ticks were greater on the host-limited islands, supporting the dilution hypothesis. Although this may be a consequence of the dilution effect, the duration of establishment of tick populations may have also influenced these findings. In locations where blacklegged ticks have been long-established, there was a trend for these ticks to be more abundant in addition to being significantly more infected than intermediate-established and recently-invaded sites (Hamer et al. 2014). However, although the definitive length of tick establishment on the islands is unknown, I believe based on anecdotal accounts, the postulation that the island had a greater abundance of deer, and the increased abundance of eastern chipmunks which would allow the tick population to grow at much a faster rate than the mainland, that the island populations were established at approximately the same time period as the mainland.

However, *B. burgdorferi* IGS strain diversity was greater on the islands in comparison to the host-diverse mainland, which was unlike what was hypothesized. States et al. 2014 found the opposite, supporting the dilution hypothesis, lower ospC genotype diversity on the less host-diverse island in comparison to the species-diverse mainland. This greater *B. burgdorferi* strain diversity and richness on the islands could be due to importation of non-mouse-adapted genotypes from birds or other immigrating

hosts (States et al. 2014). Additionally, from the strain analysis work, I determined that although the vast majority of the strains were RST 2 or 3, as expected within the Midwest, both the islands and the mainland also had strains in RST 1 which may pose greater disease severity in humans (Derdakova et al. 2004, Wormser et al. 2008, Gatewood et al. 2009, Hamer et al. 2011, 2014). The presence of RST 1 strains in nearly 10% of the ticks emphasizes the importance of enacting proper tick precautionary measures throughout all of SLBE, including the islands.

From the results that were found throughout this dissertation, multiple additional future investigations may now be warranted. For example, given that established populations of the blacklegged tick were found on SLBE's mainland and based on habitat suitability models that deemed Michigan a suitable habitat for *I. scapularis* (Guerra et al. 2002), additional surveillance efforts should be performed within neighboring counties in order to determine if the tick is spreading eastward from the Lake Michigan coast. Thus, this would allow the State of Michigan to create an even more up-to-date disease risk map. Previous work has shown that vector-borne disease risk maps are an extremely important tool for public health guidance and intervention (Benedict et al. 2007; Rakotomanana et al. 2007; Ogden et al. 2008).

From an ecological standpoint, future research in order to better determine the diversity and relative abundance of the various mammalian species present on both of the Manitou Islands would be advantageous. Based on the Manitou Island findings, I would predict that *I. scapularis* ticks and *B. burgdorferi* would be detected on other Lake Michigan islands, such as South and North Fox Islands, Beaver Island, Garden Island, High Island, and Hog Island, with a range of densities and infection prevalences based on the mammalian diversity present. For example, if all of the islands had a population of small mammals, particularly white-footed mice and eastern chipmunks, but varied in medium/large mammal diversity, below are potential *I. scapularis* density and *B. burgdorferi* infection predictions:

1) Lowest tick density/infection prevalence (with only nymphal and adult ticks detected, no larval ticks): no white-tailed deer and no other alternative medium/larger mammals that could serve as a host for the adult stage of the tick are present (Elias et al. 2011). *Borrelia burgdorferi* would still be detected, albeit in a low prevalence, due to passerine species introducing infected ticks to the island (Smith et al. 1996).
2) Intermediate tick density/infection prevalence (with all three life stages of the tick detected): no white-tailed deer but a limited amount of other medium/large mammals are present that could serve as alternative hosts. This situation is similar to SMI, in which coyotes served as an alternative host for the adult tick in the absence of deer.
3) Intermediate tick density/infection prevalence (with all three life stages of the tick detected): white-tailed deer and a large diversity of other medium/large mammals are present. Based on the dilution effect, since the island would be composed of multiple potential vertebrate hosts as opposed to a white-footed mouse dominated area, the proportion of ticks infected with the Lyme disease bacterium would decrease (Keesing et al. 2006). This situation is similar to SLBE mainland.
4) Greatest tick density/infection prevalence (with all three life stages of the tick detected): white-tailed deer and a limited amount of other medium/large mammals are present. This would allow the ticks and the pathogen to flourish, similar to NMI.

Furthermore, in the future, the dilution effect and *B. burgdorferi* strain diversity should be tested comparing the host-limited island (NMI) to a host-diverse location that has a known long-established tick population. For example, this long-established tick population site could be in WI or Menominee, MI. Although we postulate that the tick population on the mainland of SLBE was established at approximately the same time period as the tick population on NMI, given the density and infestation prevalence of ticks on NMI (Sidge Chapter 2), a comparison to an additional host-diverse site with a long-established population of ticks may be warranted in the future.

Lyme disease incidence is continuing to increase across the nation, and as I showed throughout this dissertation, the Lyme disease tick and bacterium are being detected in new areas. In order to have human and/or domestic animal Lyme disease cases, the pathogen, the host (reservoir and susceptible), and the environment must overlap, thus forming a triangle (i.e. Epidemiologic Triangle); without all entities present and in line with one another human/domestic animal disease will not be possible. Thus, when one piece is missing, as in the case with the delayed appearance of *B. burgdorferi*, interventional strategies can be attempted in order to minimize the potential disease outbreak, when and if the Epidemiologic Triangle becomes complete and overlap occurs (CDC Principles of Epidemiology in Public Health Practice, Third Ed.). Therefore, by continuing to track the invasion of the Lyme disease tick and bacterium at SLBE and testing the previously established invasion scenario hypotheses (Hamer et al. 2010), this work provides an example for other locations across the nation that may be undergoing Lyme disease invasion as to how long of a delay may be present between when the tick arrives and when the pathogen is first detected, which has implications for disease management.

Furthermore, this work provided new information as to where the tick has become established within Michigan. However, from a national perspective, this is important as it may be additional evidence for the expansion of vector populations as a consequence of climate change. Some researchers have shown that tick invasion may be due, in part, to on-going climate change in the U.S. (Githeko et al. 2000, Brownstein et al. 2005).

By examining tick and pathogen dynamics in the absence of white-tailed deer, I found that even in locations that are completely devoid of deer, alternative mammals can serve as a host for adult *I. scapularis* ticks, thus, allowing the tick to complete its life cycle. Therefore, in locations that are contemplating deer removal as a means to reduce disease potential, it is important to consider alternative hosts and ensure that proper educational messages are enforced indicating that there still may be Lyme disease risk in that area. Additionally, the island and mainland work have shown that in some areas, eastern chipmunks may play a substantial role within the Lyme disease cycle since I found that

chipmunks may be an earlier indicator of the arrival of *B. burgdorferi* to an area, present in greater abundance, and feed more larvae and nymphs than the mice. Although this may be location dependent, for example, island vs. mainland, invading vs. established tick location, Midwest vs. Northeast, these findings may be relevant for future studies that are geared toward determining if the pathogen is present in an area and/or determining future human risk given that small mammal trapping generally precedes detection of ticks on drag cloths, other mammal trapping, and birds (Hamer et al. 2010).

The *B. burgdorferi* strain analysis work provided an opportunity to test the multiple niche polymorphism hypothesis using a different *B. burgdorferi* locus (Levene 1953, States et al. 2014). Recently, States et al. 2014 evaluated this hypothesis by looking at *B. burgdorferi* genotype diversity based on ospC diversity. Although this work did not support the hypothesis, we found that even in recently invaded locations, such as SLBE mainland and islands, multiple *B. burgdorferi* strains may be present and from a public health perspective, some RST 1 strains that are more strongly associated with disseminated infection in humans (Wormser et al. 2008, Hamer et al. 2014) may also be present in these locations.

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