THE ECOLOGY OF *ANAPLASMA PHAGOCYTOPHILUM* AND THE BLACKLEGGED TICK, *IXODES SCAPULARIS* IN THE UPPER MIDWEST, U.S.A.

By

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ABSTRACT

Ixodes scapularis, commonly known as the blacklegged tick (or deer tick), is a medically important tick species that is spreading multiple diseases in the eastern USA. It is especially important as the vector for transmitting the Lyme disease pathogen, *Borrelia burgdorferi* and anaplasmosis pathogen, *Anaplasma phagocytophilum (Ap)*. With factors such as habitat restoration, increasing deer densities and climate change, the geographic range of I. *scapularis* has been expanding. Most of the disease cases are reported from two major foci: the Upper Midwest and the Northeast. With the expansion of *I. scapularis* it is important to study the ecology of *I. scapularis* and its associated pathogens. The overall goal in this dissertation was to study the ecology of a tickborne disease and its vector. There are two research chapters and to help understand the context and importance of the research topics, there are two literature review chapters, one to review *Ap* and one to review Species distribution models (SDMs) with particular attention paid to literature on *I. scapularis* SDMs.

The first research chapter examines the host ecology of *A. phagocytophilum* at a highly endemic site for *I. scapularis* in the Upper Midwest. I estimated that eastern chipmunks have relatively greater realized reservoir competence than the white-footed mouse but considering the overall contribution to the enzootic cycle of *A. phagocytophilum*, white-footed mice may play a larger role because they feed a higher proportion of larvae. Most questing nymphs and all the hosts captured that were infected with *A. phagocytophilum* were infected with the human pathogenic strain of *A. phagocytophilum*, *Ap*-ha. This means that if humans and/or companion canines are bitten by an *A. phagocytophilum*-infected tick at this field site, there is a high risk that of disease. I found the phenology patterns of infection prevalence of hosts, on-host larvae, and the density of infected nymphs follow that of the phenology patterns of questing nymphs and larvae. Blood and

biopsy samples can be used for assaying *A. phagocytophilum*, but I suggest conducting xenodiagnoses experiments to determine empirically the length of transmission of *A. phagocytophilum* by each tissue type into larvae. Conclude with what is novel and/or important about the findings.

The second research chapter centers on developing species distribution models for *I. scapularis* in Michigan. In this chapter I looked at the environmental predictors that were important in determining where *I. scapularis* would occur within Michigan and where suitable habitats for the occurrence of *I. scapularis* in Michigan are found. I developed models for the Upper and Lower Peninsula. I used two different modeling methods, a logistic regression and a machine learning technique based maximum entropy modeling. For both peninsulas environmental predictors related to temperature, humidity, presence of maple, beech, birch forest types, presence of white-tailed deer, soil moisture, and soil clay content. In the Lower Peninsula, most of the southern regions were considered to have suitable habitat for the occurrence of *I. scapularis*, while the northern region in the Lower Peninsula had the least suitable habitats for *I. scapularis* occurrence. In the Upper Peninsula, central southern regions as well as regions along the Lake Superior had suitable habitats for *I. scapularis* occurrence, while there were pockets of least suitable habitats across the peninsula. Future studies should develop a species distribution model based on the current distribution of *I. scapularis* in Wisconsin and project it onto Michigan to extrapolate where suitable habitats are found in Michigan. A comparison of that model with the ones we have developed can help us to understand better the invasion process of *I. scapularis* in Michigan. Furthermore, continuing surveillance efforts in the currently predicted least suitable regions will determine if those habitats really are not suitable for *I. scapularis* or if it just has not gotten there. Conclude with what is novel and/or important about the findings.

This dissertation is dedicated to my family and friends.
Thank you for believing in me and supporting me.

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CHAPTER 1: INTRODUCTION

In modern times there is an increasing trend in vector-borne diseases. Globally vector-borne diseases account for 17% of all the world's infectious diseases claiming around 700,000 lives annually (World Health Organization, 2017). In the United States alone, nearly 650,000 cases of vector-borne diseases were reported to the Centers for Disease Control and Prevention (CDC) from 2004 – 2016, out of which 75% of the cases were attributed to tickborne diseases (Rosenberg *et al.*, 2018). In North America, medically and veterinary important ticks have become an immense disease burden on humans and livestock (Berrada and Telford, 2009; Pérez de Leon *et al.*, 2012; Miller, Farnsworth and Malmberg, 2013; Rosenberg *et al.*, 2018; Wisely and Glass, 2019; Rodino, Theel and Pritt, 2020; Eisen and Paddock, 2021). The increase in ticks and tick-borne diseases in North America has occurred largely due to the recent geographic expansion of the range of ticks (Sonenshine, 2018a; Tsao *et al.*, 2021)

Lyme disease, a tick-borne disease, is the most common vector-borne disease in the United States reported annually. Nearly 82% of tickborne diseases are attributed to Lyme disease (Rosenberg *et al.*, 2018). Between the periods of 2010 – 2018, nearly 470,000 cases of Lyme disease were diagnosed in the US with annual number of cases reported ranging between 30,000 – 40,000. (Kugeler *et al.*, 2021) It has been proposed that the annual disease burden of Lyme disease could be estimated to be as much as $1 billion dollars with an average cost of $1200 per patient (Hook *et al.*, 2022). In the US there are two major foci of Lyme disease incidence - the upper Midwest, and the Northeast. These foci lie within the distribution of the vector of Lyme disease in the eastern USA, the blacklegged tick (*Ixodes scapularis*), which has been expanding for more than a half century (Eisen and Eisen, 2018a; Gardner *et al.*, 2020; Burtis *et al.*, 2022).

Ixodes scapularis is also responsible for transmission of several other bacterial pathogens

including *Anaplasma phagocytophilum,* which causes granulocytic anaplasmosis, *Borrelia miyamotoi,* which causes relapsing fever spirochete, and *Ehrlichia muris eauclarensis*, which causes ehrlichiosis. *Ixodes scapularis* is also responsible for transmitting a parasitic organism, *Babesia microti,* which causes babesiosis, as well as a virus, Powassan virus, which causes an encephalitis. These pathogens and associated diseases are also found mainly in the upper Midwest and Northeast (Fleshman *et al.*, 2021). With the spread of the *I. scapularis*, the diseases associated with this tick are also on the rise in the US (Eisen and Eisen, 2018a; Rosenberg *et al.*, 2018)

Distribution of* I. scapularis *in Michigan

Ixodes scapularis have been expanding in range in both the north central and northeastern U.S. for more than half a century (Dennis *et al.*, 1998; Eisen, Eisen and Beard, 2016; Gardner *et al.*, 2020). It is hypothesized that the northern *I. scapularis* populations originated from migrant tick populations from the southern US that dispersed northward during the receding Pleistocene ice sheets (Humphrey, Caporale and Brisson, 2010; Frederick *et al.*, 2023). The expansion of *I. scapularis* beginning in the middle of the last century was due to changes in land use as well as increases in the white-tailed deer (*Odocoileus virginianus*) populations due to conversion of agricultural land to forests and hunting restrictions on white-tailed deer (Barbour and Fish, 1993; Hamer *et al.*, 2010; Ginsberg, Rulison, Miller, Pang, Arsnoe, Hickling, Ogden, LeBrun, *et al.*, 2020). *Ixodes scapularis* is a habitat generalist (Labruna, 2014; Sonenshine, 2018a) and feeds on a wide range of different mammalian hosts which enable them to succeed in environments that humans inhabit. Thus, because of this rapid spread of *I. scapularis* in the Midwest and the Northeast, predicting the spatial risk of contracting *I. scapularis* and its associated diseases is important.

In the Upper Midwest, the first populations of *I. scapularis* were found in western central

Wisconsin in the late 1960s (Gardner et al. 2020). In Michigan the first *I. scapularis* population was first detected in the Upper Peninsula, specifically in Menominee County in the 1980's (Strand et al., 1992; Walker et al., 1994). Then, in 2002 the first *I. scapularis* population was detected in the southwestern corner of the Lower Peninsula of Michigan (Foster, 2004), after which *I. scapularis* has been expanding northward and eastward (Dennis et al., 1998; Eisen et al., 2016; Hamer et al. 2010; Lantos et al., 2017). Today, *I. scapularis* is detected in 63/83 counties of Michigan, some of which border Lake Huron on the east side of the state (Michigan Department of Health and Human Services, 2021, 2022).

With the invasion of *I. scapularis* in Michigan, it is important to characterize the distribution of suitable habitats throughout Michigan and to better understand the abiotic and biotic factors that are important in predicting the future distribution of *I. scapularis*. The first predictive spatial model for *I. scapularis* in Michigan was based on applying a habitat suitability model developed from *I. scapularis* surveillance data in Wisconsin (Guerra *et al.*, 2002), and it was from this model that Foster, and colleagues detected the first populations of *I. scapularis* in southwestern Michigan (Erik Scott Foster, 2004). Since then, there have been other models predicting the spatial distribution of *I. scapularis* within Michigan (Diuk-Wasser *et al.*, 2010; Hahn *et al.*, 2016; Burtis *et al.*, 2022). Developing habitat suitability models specially in an area where *I. scapularis* is invading into, such as Michigan, is important because these models will help to target specific areas for future surveillance efforts. These models also have a public health benefit where we can inform the public as well as healthcare providers on the future risk of not just Lyme disease but other diseases that are caused by *I. scapularis* such as anaplasmosis.

Human anaplasmosis and the ecology of A. phagocytophilum

Although Lyme disease is the leading tickborne disease in the U.S. and therefore is arguably the most important, anaplasmosis is the second leading tick-borne disease (Rosenberg *et al.*, 2018). Human anaplasmosis was discovered in 1994 from western central Wisconsin and is caused by *Anaplasma phagocytophilum,* a gram-negative intracellular bacterium (Chen *et al.*, 1994). Both *A. phagocytophilum* and *B. burgdorferi* share the same vector *I. scapularis* as well as major reservoir hosts such as the white-footed mouse (*Peromyscus leucopus*) (Donahue, Piesman and Spielman, 1987; Levin, Nicholson, Massung and Fish, 2002; Massung, Levin and Priestley, 2004; Stuen, Granquist and Silaghi, 2013). As with Lyme disease, the two major foci for human anaplasmosis are in the Upper Midwest and Northeast. Lyme disease and anaplasmosis share similar symptoms in humans, including flu-like symptoms with fever, headache, and malaise. Similar to Lyme disease, the peak onset of these symptoms is typically common during the late spring/early summer when nymphal *I. scapularis* are active (Chen *et al.*, 1994).

There is no transovarial transmission of *A. phagocytophilum* in its tick vector *I. scapularis*. The two *I. scapularis* life stages that are important in the transmission of *A. phagocytophilum* are the nymphs and the adults. Due to their relative smaller size and the activity period being in summer which overlaps with the time people are outside doing recreational activities, the nymphs possess relatively a greater disease risk than adults. Therefore, we see a lot of disease onset typically during summer periods. There is also a small bump in human disease cases during the fall and early spring.

Because there is no transovarial transmission of *A. phagocytophilum,* the wildlife host community plays a major role in the enzootic cycle of *A. phagocytophilum*. A most comprehensive study that describes the ecology of *A. phagocytophilum* was conducted in the Northeast (Keesing

et al., 2012, 2014). No such study has been conducted in the Midwest which would help to understand the similarities and the differences between how *A. phagocytophilum* is maintained in the two regions. Thus, comparing how *A. phagocytophilum* maintained in the Midwest with that of the Northeast might help us uncover these epidemiological differences.

This dissertation

This dissertation examines two topics. One of the major components of this dissertation investigates an aspect of tick ecology while the other component investigates the ecology of a tickborne pathogen.

The first topic focuses on studying the spread of *I. scapularis* within Michigan using habitat suitability models to describe where suitable habitats currently lie within Michigan. A region with a tick invasion is dynamic and over time habitats with endemic tick populations will expand but developing these models will help us in understanding the patterns of expansion over time. While there have been habitat suitability models developed for Michigan previously at a regional and national levels (Erik Scott Foster, 2004; Hahn *et al.*, 2016; Burtis *et al.*, 2022), I wanted to develop models based only on the comprehensive active surveillance data of *I. scapularis* available for Michigan. Furthermore, another goal in this study was to use two different methods of species distribution modeling to compare how similar or different they would be based on the different assumptions each method makes. Thus, this chapter will help to understand where suitable habitats are currently distributed in Michigan and will be the initial step towards predicting where *I. scapularis* may spread. With these initial habitat suitability models, we can make informed decisions on where our surveillance efforts should be targeted to and think where we should focus future tick prevention and control strategies.

The second aspect of this dissertation focuses on the community ecology of *A.*

phagocytophilum within an *I. scapularis* endemic region in the Midwest. Lyme disease is the leading vector borne disease in the US; therefore, much of the ecology of its enzootic cycle has been studied but less research has been conducted on the ecology of other tickborne diseases. More thorough ecological studies of *A. phagocytophilum* have been conducted in the Northeast and to some extent in the West (where *I. pacificus*, the western blacklegged tick is the vector). Most studies on *A. phagocytophilum* in the Midwest have focused on tick ecology and less so on the host ecology with a few exceptions. Therefore, this chapter attempts to illuminate more the contribution of certain wildlife species on maintenance of *A. phagocytophilum*. With the increase in human anaplasmosis cases from the Midwest, it is important to learn more about the various ecological aspects of the maintenance of *A. phagocytophilum* in nature.

Ixodes scapularis range expansion will result in the increase in disease incidence of multiple diseases. Thus, trying to understand the patterns of the invasion process of *I. scapularis* and how diseases spread by *I. scapularis* are maintained in nature will be helpful to guide prevention and control strategies in regions where the *I. scapularis* is endemic and in regions where *I. scapularis* is invading. Therefore, this dissertation hopes to bridge the knowledge gaps found in both ticks ecology and the ecology of *A. phagocytophilum* to better understand the spread of ticks and the maintenance of tickborne diseases.

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CHAPTER 2: *ANAPLASMA PHAGOCYTOPHILUM*: A REVIEW

ABSTRACT

Anaplasma phagocytophilum is the causative agent of human granulocytic anaplasmosis in the US and Europe. It is an emerging vector borne disease spread by ticks in the *Ixodes ricinus* complex throughout the world within the northern hemisphere. Clinical symptoms of *A. phagocytophilum* are flu-like and symptoms typically are seen in summer and fall, coinciding with the activity periods of the vector stages. It is an intracellular pathogen, generally infecting neutrophils, with intricate mechanisms to infect a host cell and evade the host immune system. *Anaplasma phagocytophilum* is known to infect several vertebrate species, where several serve as important reservoir hosts. Furthermore, *Anaplasma phagocytophilum* seems to show host associations, such that some strains are known to be exclusively circulating within specific species. In this literature review here, I provide a basic understanding of the pathogenesis, ecology, and epidemiology of *A. phagocytophilum*.

Keywords: *Anaplasma phagocytophilum*, pathogenesis, ecology, epidemiology, host, vectors

INTRODUCTION

Anaplasma phagocytophilum is an emerging vector borne pathogen. It is known to cause human granulocytic anaplasmosis, tick-borne fever in ruminants, equine granulocytic anaplasmosis in horses, febrile fever in cats, and canine anaplasmosis in dogs (Rar and Golovljova, 2011). In humans, typical disease symptoms include fever, malaise, myalgia, thrombocytopenia, and leukopenia (Bakken and Dumler, 2015). Symptoms in dogs and cats are very similar, including lethargy, fever, and anorexia (Little, 2010). Most human patients have mild clinical symptoms and do not require hospitalizations; the case fatality rate for *A. phagocytophilum* is relatively low and is about 0.6% (Dumler, 1997).

Distribution and epidemiology of A. phagocytophilum

Anaplasma phagocytophilum has been detected in parts of Europe (e.g., Norway, United Kingdom, Germany, Slovakia, and Switzerland), Asia (e.g., Russia, China, and Korea), and North America, where the major foci are found in Upper Midwest and the Northeast US and where a third much smaller foci occur in western US (Stuen, 2007; Stuen, Granquist and Silaghi, 2013). In the US, it was first discovered in patients from northern Minnesota and Wisconsin in 1992, when one who presented with severe flu-like symptoms two weeks after a tick bite died (Chen *et al.*, 1994). Since then, the number of human granulocytic anaplasmosis cases has increased (Biggs et al., 2016) and has become increasingly important for public health. The incidence rates of human cases have increased from 2.0 cases per million persons from 2000-to 2007 to 6.3 cases per million cases from 2008-to 2012 (Rar and Golovljova, 2011; Dahlgren *et al.*, 2015).

According to the CDC, human granulocytic anaplasmosis is the second most reported vector-borne disease, after Lyme disease (Biggs et al., 2016; Baker et al., 2020; Elias et al., 2020), and areas with high Lyme disease risk also have a high risk of human granulocytic anaplasmosis.

Seroepidemiological data from people suggest that in endemic regions 15% - 36% of the population seem to have been infected with human granulocytic anaplasmosis (Bakken and Dumler, 2015). Human cases are relatively higher among males who are older than 40 years old, while people with a compromised immune system are more susceptible (Jin et al., 2012; Bakken and Dumler, 2015; Biggs et al., 2016; Dumic et al., 2022). Human disease incidence in Europe and Asia seems to be less common compared to that in the US (Dumler *et al.*, 2005; Stuen, 2007; Stuen, Granquist and Silaghi, 2013; Dumic *et al.*, 2022a), which may be due to the low abundance of the *A. phagocytophilum* strains that infects and causes disease in humans. In dogs, canine anaplasmosis was first detected from a dog in California and since then is found throughout the US in regions where *I. scapularis* is endemic and invading; considering serological data, up to about 40% of dogs are found to be seropositive for canine granulocytic anaplasmosis (Beall *et al.*, 2008; Bowman *et al.*, 2009; Carrade *et al.*, 2009; Qurollo *et al.*, 2014; Khatat *et al.*, 2021).

Pathogenesis of infection

Anaplasma phagocytophilum is a gram-negative intracellular pathogen of about 0.4 – 1.3 mm in size (Severo *et al.*, 2012). It can infect mammalian cells of hematopoietic origin; it primarily infects neutrophils, and to a lesser extent, monocytes, macrophages, red blood cells, platelets, and endothelial cells (Rikihisa, 2011). The pathogenesis of *A. phagocytophilum* is poorly understood. *Anaplasma phagocytophilum* is polymorphic bacteria where during infection of a host cell, a dense-cored form binds to the cell, and then once internalized in the host neutrophil; the early forms develop into round reticular structures residing within an early endosome, acquire nutrients through binary fission, and develop into distinct membrane-bound intracytoplasmic bacterial aggregates called morulae, which later on become small dense structures (Chen *et al.*, 1994; Dumler, 1997; Webster *et al.*, 1998; McQuiston *et al.*, 1999; Dumler *et al.*, 2005; Rikihisa, 2010;

Bakken and Dumler, 2015). These morulae are apparent in microscopic slides of stained blood smears and tissue samples prepared from infected patient tissues (Chen *et al.*, 1994; Dumler *et al.*, 2001, 2005).

Anaplasma phagocytophilum has a relatively small genome size of about 1.47 – 148 Mb with the absence of plasmids (Dunning Hotopp *et al.*, 2006; Barbet *et al.*, 2013). *Anaplasma phagocytophilum* lacks the genes needed for the synthesis of lipopolysaccharides and peptidoglycan, and consequently, the outer membrane lacks the peptidoglycan and lipopolysaccharide layers (Lin and Rikihisa, 2003b). Due to this lack of peptidoglycan and lipopolysaccharide, *A. phagocytophilum* cells incorporate host cell cholesterol to survive (Lin and Rikihisa, 2003b). During the initial infection process, *A. phagocytophilum* binds to the P-selectin binding domain of the human neutrophils through the P-selectin glycoprotein ligand (PSGL-1) on the *A. phagocytophilum* cell (Herron *et al.*, 2000). The dense-core form of *A. phagocytophilum* facilitates the adhesion to the host cell by recognizing the human PSGL-1 (Troese and Carlyon, 2009). Most human neutrophil cells are enriched with P-selectin binding domain cell surface receptors, making it easy for *A. phagocytophilum* to attach and enter human neutrophils. When infecting mouse neutrophils, A. *phagocytophilum* P-selectin glycoprotein ligand is not required; instead, fucosyl transferases are required (Rikihisa, 2011). Several surface proteins, such as major surface proteins Msp2, Asp55, and Asp62, are required for *A. phagocytophilum* to bind and infect the host cells (Wang, Kikuchi and Rikihisa, 2006; Ge and Rikihisa, 2007). Entry into the host cell is mediated through lipid rafts known as caveolae (Lin and Rikihisa, 2003a). Through lipid caveolae, *A. phagocytophilum* cells are directed into inclusions within the host cell. These *A. phagocytophilum* inclusions do not have characteristic features of an early cellular endosome such as the early endosome marker Rab5, early endosome antigen 1 (EEA1), the vacuolar (H^+) ATPase

(Webster *et al*., 1998; Mott, Barnewall and Rikihisa, 1999; Yoshiie *et al*., 2000).Once inside these inclusions, replication of *A. phagocytophilum* occurs (Rikihisa, 2011). The replicative form of *A. phagocytophilum* is the reticulate cellular form, and replication occurs by binary fission (Troese and Carlyon, 2009). During the replication process, which takes about 24 hours, the membrane of parasitophorous vacuoles (morulae) increases in size to accommodate the multiplying reticulate form of *A. phagocytophilum* cells (Troese and Carlyon, 2009). After 24 hours of replication, the reticulate forms start to condense into the dense-core form and are ready to burst out of the host cell and infect new host cells (Troese and Carlyon, 2009).

Neutrophils are an essential part of the innate immune system known for their ability to phagocytose invading pathogens and lysing them through the formation of lysosomes. Neutrophils generally have a short lifespan, and a short half-life of about 7 hours (Tak *et al.*, 2013). A characteristic feature of a neutrophil is the ability of spontaneous apoptosis to enable cell turnover and homeostasis in the host system. *Anaplasma phagocytophilum* can inhibit a neutrophil's spontaneous apoptosis ability, thereby increasing the lifespan of the neutrophil. This inhibition process provides the *A. phagocytophilum* bacterium sufficient time to develop and replicate within the infected neutrophil (Scaife *et al*., 2003; Borjesson *et al*., 2005; Ge *et al*., 2005; Tak *et al*., 2013). *Anaplasma phagocytophilum* inhibits spontaneous apoptosis in neutrophils in several ways by upregulating anti-apoptotic genes, by maintaining the membrane potential of mitochondrial membrane within infected neutrophils, and by inhibiting the caspase3 activation pathway required for spontaneous cell apoptosis (Ge *et al*., 2005). Autophagy is a natural process of cellular degradation mediated by lysosomes. In a bacterial infection, autophagy is vital to a cell because it helps clear out intracellular infections and will enable the infected cell to differentiate between self and pathogen antigens to be presented to activate the innate and active immune responses (Amano,

Nakagawa and Yoshimori, 2006). Once *A. phagocytophilum* infects the host cell, it subverts the autophagy response mechanism of the host neutrophil (Niu, Yamaguchi and Rikihisa, 2008). After invading a host neutrophil, *A. phagocytophilum* inclusions show characteristic features of an early autophagosome, including being enveloped by a double-lipid bilayer membrane and localization of essential components of cellular autophagosomes such as microtubule-associated proteins and beclin-1 to the *A. phagocytophilum* inclusions (Niu, Yamaguchi and Rikihisa, 2008). Although *A. phagocytophilum* inclusions shows autophagosome characteristics, it does not mature into a late autophagosome and does not fuse with an autolysosome (Niu, Yamaguchi and Rikihisa, 2008). Thus, *A. phagocytophilum* can develop and replicate safely within these autophagosomes without being lysed. During the infection of *A. phagocytophilum*, patients develop a humoral immunity and launch pro-inflammatory cytokine responses (Dumler *et al.*, 2000). Typically, patients infected with anaplasmosis are treated with doxycycline, and many patients seem to develop high titers of antibodies again anaplasmosis which lasts up 12 – 18 months (Bakken and Dumler, 2015).

Tick vectors

Anaplasma phagocytophilum is transmitted by hard ticks in the *Ixodes ricinus* complex. In the eastern US, it is transmitted by the blacklegged tick, *I. scapularis;* in the western US, it is transmitted by the western blacklegged tick, *I. pacificus;* in Europe, the primary vector is the sheep tick or the castor bean tick, *I. ricinus;* and in Asia and Russia, the primary vector is *I. persulcatus* (Woldehiwet, 2010; Stuen, Granquist and Silaghi, 2013; Dugat *et al.*, 2015). *Anaplasma phagocytophilum* has been detected by PCR in tick species such as *Dermacentor albipictus* (the winter tick), *Haemaphysalis leporispalustris* (the rabbit tick) and *H. longicornis* (the Asian long-horned tick) (Goethert and Telford, 2003; Baldridge *et al.*, 2009; Price *et al.*, 2022), while the vector competence for *D. albipictus* and *H. leporispalustris* has not been shown (Stuen, Granquist

and Silaghi, 2013) whereas *H. longicornis* was shown not to be a competent vector in transmitting *A. phagocytophilum* (Levin *et al.*, 2021).

Ticks in the *Ixodes* genus are three-host feeders, and at each life stage, the tick blood feeds on a new host except for adult male ticks, which do not feed. *Anaplasma phagocytophilum* is not transmitted vertically in *Ixodes* species; thus, only the nymphal and adult (female) stages can transmit the bacterium to mammalian and avian hosts. Therefore, for an *Ixodes* spp. tick to acquire *A. phagocytophilum*, it must feed on an infected host.

In *Dermacentor albipictus* ticks, the transovarial transmission of *A. phagocytophilum* under laboratory conditions has been observed (Baldridge *et al.*, 2009). In addition, several studies have shown that *Ixodes* spp. ticks could acquire *A. phagocytophilum* through co-feeding, where transmission of the pathogen can occur from an infected tick to an uninfected tick feeding in proximity during simultaneous feeding on an uninfected host (Levin and Fish, 2000a; Ogden *et al.*, 2002).

When an *Ixodes* spp. tick feeds on an infected host, *A. phagocytophilum* will reach the ticks midgut through the blood meal. Within a tick, *A. phagocytophilum* survives in the midgut cells and later migrates into the salivary gland cells, and this process will take about 24 hours of feeding on the infected host (Hodzic *et al.*, 2001; Severo *et al.*, 2012). The migration of *A. phagocytophilum* from the midgut to salivary glands is mediated through the hemolymph of the tick. A tick salivary protein called P11 facilitates the migration of *A. phagocytophilum* by enabling infection of tick hemocytes (Liu *et al.*, 2011). The infection of hemocytes by *A. phagocytophilum* is vital to infecting the salivary glands. *Anaplasma phagocytophilum* produces inclusions and has been shown to reside within the secretory salivary acini (Telford *et al.*, 1996). Once an infected tick starts blood-feeding on a mammalian host, the feeding will activate the migration of *A.*

phagocytophilum from the midgut into the salivary glands, and within the salivary glands *A. phagocytophilum* will start replicating (Hodzic *et al.*, 2001).

During the infection within the tick, *A. phagocytophilum* manipulates the secretion of several proteins within the tick to survive and be transmitted into a viable host. For example, *A. phagocytophilum* induces the expression of a salivary protein, salp16, within *I. scapularis* ticks (Sukumaran *et al.*, 2006). Sukumaran *et al*., 2006 have shown that during early infection of the salivary glands, *A. phagocytophilum* requires salp16 protein. Another protein that is upregulated in *I. scapularis* upon the infection of *A. phagocytophilum* is the tick antifreeze protein (Neelakanta *et al.*, 2010), which may enable *A. phagocytophilum*-infected *I. scapularis* to survive better during winter compared to the non-infected *I. scapularis* ticks. Therefore *I. scapularis* ticks may benefit from this interaction with *A. phagocytophilum*, although evidence that *A. phagocytophilum*-infected ticks have higher over-wintering survivorship compared to non-infected ticks in nature has yet to be demonstrated.

Several experiments have shown that transmission of *A. phagocytophilum* from an infected *I. scapularis* tick to a host occurs between 24 and 48 hours after the tick attaches to the host (Hodzic *et al.*, 1998; des Vignes *et al.*, 2001; Levin, Troughton and Loftis, 2021). The transmission from an infected tick to a naïve host is dose-dependent, and in laboratory mice (*Mus musculus*) a median dose of $10^4 – 10^5$ of morulae are required to infect a mouse (Hodzic *et al.*, 1998). Therefore, the initial quantity of *A. phagocytophilum* within the tick may be insufficient to infect a host and *A. phagocytophilum* must replicate within the tick to achieve the infectious dose. The number of *A. phagocytophilum* copies within *I. scapularis* has been shown to increase over a 72-hour tick feeding period allowing a sufficient infectious dose (Levin, Troughton, and Loftis, 2021). Therefore, the longer an infected tick is attached to a naive host, the greater the potential of the

host getting infected with *A. phagocytophilum*; for reference, *I. scapularis* nymphs typically complete their bloodmeal in about 4 days on a laboratory rabbit (Troughton and Levin, 2007).

Nymphal ticks are the most crucial stage in transmitting the pathogen to humans because they are the first life stage capable of infecting a host, numerous, difficult to detect due to their small size, and because of the nymphal activity peaks in late spring/early summer coinciding when humans are highly active outdoors (Biggs *et al*., 2016; Murphy *et al*., 2017). Adult female ticks also can carry *A. phagocytophilum* and infect humans. Generally, since adult female ticks are larger than nymphs and have a reddish abdomen, the probability is greater that they will be discovered before taking a blood meal or before feeding long enough to transmit an infectious dose of *A. phagocytophilum* compared to nymphal ticks (Falco, Richard C., Durland, 1988). As discussed earlier, it takes at least 24 – 48 hours to transmit *A. phagocytophilum* from an infected *I. scapularis* to a host. Due to the relatively shorter transmission time compared with that of the Lyme disease pathogen, which requires at least 36 hours of feeding, adult *I. scapularis* may also play a larger role in human disease transmission for anaplasmosis compared with Lyme disease. The presence of a small peak of human granulocytic anaplasmosis cases seen during the fall when adult blacklegged ticks are active but nymphs are not, and the absence of such a peak for Lyme disease, supports the hypothesis that adult *I. scapularis* may play a relatively larger epidemiological role.

***Reservoir hosts of* A. phagocytophilum**

Anaplasma phagocytophilum utilizes similar reservoir hosts as *Borrelia burgdorferi*, the Lyme disease bacterium (Keesing *et al.*, 2012, 2014; Stuen, Granquist and Silaghi, 2013; Foley *et al.*, 2016; Stephenson and Foley, 2016). Similar to *B. burgdorferi*, the primary reservoir for *A. phagocytophilum* in the northeastern and northern midwestern US is the white-footed mouse

(*Peromyscus leucopus*) (Telford *et al*., 1996; Magnarelli *et al*., 1997; Walls *et al*., 1997; Levin, Nicholson, Massung, Sumner, *et al*., 2002), which is mainly found in woodland habitats (Musser, 1969). Once infected, white-footed mice can launch a strong humoral immune response within 7 – 14 days after exposure (Levin and Fish, 2000b).From laboratory xenodiagnoses experiments, infected laboratory mice (*M. musculus*) can transmit *A. phagocytophilum* to naïve larvae for up to 9 weeks (Levin and Ross, 2004), although the peak transmission period (i.e., infecting the greatest proportion of naïve larvae) occurs within the first 2-3 weeks, after which transmission efficiency decreases. Several studies have shown that *P. leucopus* have very high larval burdens compared to other small mammal species (Keesing *et al.*, 2009; Hersh *et al.*, 2014), which is important in maintenance of the enzootic cycle of *A. phagocytophilum*.

Several other small mammals in the northeastern and upper midwestern US are also known to be competent reservoir hosts for *A. phagocytophilum*. These include the eastern chipmunk (*Tamias striatus*), northern short-tailed shrew (*Blarina brevicauda*), red squirrel (*Tamiasciurus hudsonicus*), flying squirrel (*Glaucomys volans*), gray squirrel (*Sciurus carolinensis*), masked shrew (*Sorex cinereus*), meadow jumping mouse (*Zapus hudsonicus*), red-backed vole (*Clethrionomys gapperi*), and meadow vole (*Microtus pennsylvanicus*) (Walls *et al.*, 1997; Stafford *et al.*, 1999; Levin, Nicholson, Massung and Fish, 2002; Johnson *et al.*, 2011; Keesing *et al.*, 2012, 2014). In addition to these small mammals, raccoons (*Procyon lotor*) and cottontail rabbits (*Sylvilagus floridanus*) can become infected with *A. phagocytophilum* (Levin, Nicholson, Massung and Fish, 2002; Goethert and Telford, 2003). *Procyon lotor* appears to have lower reservoir competence for *A. phagocytophilum* (Keesing *et al.*, 2012) compared to mice; no reservoir competence studies for *S. floridanus* have not been conducted.

In the US, many passerine bird species serve as hosts for *I. scapularis* larvae and nymphal

ticks. For example, the gray catbird (*Dumetella carolinensis*), Swainson's thrush (*Catharus ustulatus*), American robin (*Turdus migratorius*), wood thrush (*Hylocichla mustelina*), eastern towhee (*Pipilo erythrophthalmus*), brown thrasher (*Toxostoma rufum*), Carolina wren (*Thryothorus ludovicianus*), Northern cardinal (*Cardinalis cardinalis*), ovenbird (*Seiurus aurocapilla*), veery (*Catharus fuscescens*), house wren (*Troglodytes aedon*), chipping sparrow (*Spizella passerina*), indigo bunting (*Passerina cyanea*), and common yellowthroat (*Geothlypis trichas*) are some of the common passerine bird species that are commonly infested with nymphal and larval life stages of *I. scapularis* (Anderson *et al.*, 1986; Stafford, Bladen and Magnarelli, 1995; Nicholls and Callister, 1996; Smith *et al.*, 1996; Scharf, 2004; Hamer, Goldberg, *et al.*, 2012; Scott, Anderson and Durden, 2012). Although birds can be infested with *I. scapularis*, the ability of these bird species to act as competent reservoir hosts for *A. phagocytophilum* seems to be relatively low, or *A. phagocytophilum* has not been detected in these species (Daniels *et al.*, 2002; Ogden *et al.*, 2008; Hamer, Goldberg, *et al.*, 2012; Johnston *et al.*, 2013; Dingler *et al.*, 2014; Dumas *et al.*, 2022). For example, in a study in upstate New York in the Northeast, the reservoir competence of *A. phagocytophilum* in four bird species - veery, gray catbird, wood thrush, and American robin - ranged from 2% - 10% (Keesing *et al.*, 2012) which is relatively low compared to some of the small mammal species described previously which was greater than 10%.

In California, the enzootic cycle of *A. phagocytophilum* is slightly different. The wildlife hosts for *I. pacificus* are somewhat different than that in the northern eastern US in that the immature ticks commonly feed on lizards, birds, and small mammals, while adult *I. pacificus* feed on deer, dogs, coyotes, bears, bobcats, and numerous other hosts (Furman and Loomis, 1984). In the western USA, the dusky-footed woodrat (*Neotoma fuscipes*) plays an important role in the enzootic cycle of *A. phagocytophilum* (Nicholson *et al.*, 1999). Other mammalian hosts known to

be infected with *A. phagocytophilum* in the western US include redwood chipmunk (*Tamias ochrogenys*), brush mouse (*Peromyscus boylii),* pinyon mouse (*Peromyscus truei*), western harvest mouse (*Rheithrodontomys megalotis*), western grey squirrel (*Sciurus griseus),* American black bear (*Ursus americanus*), and gray fox (*Urocyon cinereoargenteus*) (Foley *et al.*, 2004; Drazenovich, Foley and Brown, 2006; Foley, Clueit and Brown, 2008; J. E. Foley *et al.*, 2008; Nieto and Foley, 2008; Gabriel *et al.*, 2009; Foley and Nieto, 2011). Lizards and reptiles in California such as northern alligator lizard (*Elgaria coereleus*), sagebrush lizard (*Sceloporus graciosus*), western fence lizard (*Sceloporus occidentalis*), Pacific gopher snake (*Pituophis catenifer*), and common garter snake (*Thamnophis sirtalis*) are also shown to become infected with *A. phagocytophilum*. However, their reservoir competence is very low (Nieto *et al.*, 2009).

In Europe, the reservoir host composition responsible for maintaining *A. phagocytophilum* differs from the United States. In Europe, small mammals, particularly rodents, may not play a significant role in maintaining *A. phagocytophilum* in nature. Several studies have shown rodents species such as a yellow-necked mouse (*Apodemus flavicollis*), wood mouse (*Apodemus sylvaticus*), black-striped field mouse (*Apodemus agrarius*), and several different vole species such as the bank vole (*Myodes glareolus*), common vole (*Microtus arvalis*), field vole (*Microtus agrestis*) and root vole (*Microtus oeconomus*) to be infected with *A. phagocytophilum* at a very low level, but their reservoir competence has not been studied (Jorge S. Liz *et al.*, 2000; Kevin J Bown *et al.*, 2003; Hulínská *et al.*, 2004; Grzeszczuk *et al.*, 2006; Smetanová, Schwarzová and Kocianová, 2006; Barandika *et al.*, 2007; Marumoto *et al.*, 2007; Silaghi, Woll, *et al.*, 2012; Majazki *et al.*, 2013). Few shrew species, such as the common shrew (*Sorex araneus*) and the greater, white-tooted shrew (*Crocidura russula*), are also known to be infected with *A. phagocytophilum* in the UK, Switzerland, and Spain (Ogden *et al.*, 1998; Jorge S. Liz *et al.*, 2000;

Kevin J Bown *et al.*, 2003; Barandika *et al.*, 2007; Bray *et al.*, 2007). A few recent studies in the UK, Germany, and Romania have shown that the European hedgehog (*Erinaceus europaeus*) and the Northern, white-breasted hedgehog (*Erinaceus roumanicus*) have high infection prevalence for *A. phagocytophilum* and are hypothesized to be competent reservoir hosts for *A. phagocytophilum* (Silaghi, Skuballa, *et al.*, 2012; Dumitrache *et al.*, 2013; Földvári *et al.*, 2014).

In Europe, wild ruminants may play a greater role in the enzootic cycle of *A. phagocytophilum*, where roe deer (Capreolus capreolus), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), sika deer (*Cervus Nippon*) are reported to have relatively high *A. phagocytophilum* infection prevalence in the UK, Denmark, Poland, Slovakia, Czech Republic, Germany, Austria, Switzerland, and in Italy (Oporto *et al.*, 2003; Beninati *et al.*, 2006; Carvalho *et al.*, 2008; Veronesi *et al.*, 2011; Overzier *et al.*, 2013; Stuen *et al.*, 2013; Kauffmann *et al.*, 2017; Stigum *et al.*, 2019; Remesar *et al.*, 2020; Silaghi *et al.*, 2020).

In Europe, *I. ricinus* is known to infest several bird species. Many bird species throughout Europe are not competent reservoir hosts for *A. phagocytophilum* due to their inability to become infected (Björsdorff *et al.*, 2001; Skotarczak *et al.*, 2006; Franke *et al.*, 2010; Hildebrandt *et al.*, 2010). Several studies have shown the common blackbird (*Turdus merula*) to be infected with *A. phagocytophilum* and to carry infected larvae indicating that they could be a potential competent reservoir host of *A. phagocytophilum* (Fuente *et al.*, 2005; Skotarczak *et al.*, 2006; Paulauskas, Radzijevskaja and Rosef, 2009; Palomar *et al.*, 2012; Jahfari *et al.*, 2014; Mărcuţan *et al.*, 2014). Apart from the common blackbird, these studies have found the common chaffinch (*Fringilla coelebs*), red wing (*Turdus iliacus*), song thrush (*Turdus philomelos*), house sparrow (*Passer domesticus*), Spanish sparrow (*Passer hispaniolensis*), rock bunting (*Emberiza cia*), woodchat shrike (*Lanius senator*), magpie (*Pica pica*) and long-tailed tit (*Aegithalos caudatus*) either to be

infected with *A. phagocytophilum* or harbor infected larvae, indicating transmission of *A. phagocytophilum* to larvae (Fuente *et al.*, 2005; Paulauskas, Radzijevskaja and Rosef, 2009; Hornok *et al.*, 2014; Mărcuţan *et al.*, 2014).

Although birds in Europe and North America may not play a prominent role in the enzootic cycle of *A. phagocytophilum*, they may contribute to the dispersal of infected ticks. Several of these bird species were shown to have infected *I. ricinus* nymphs indicating the potential threat of birds dispersing infected ticks into unexplored areas where previous *A. phagocytophilum* infections were unknown. Further investigation into the common blackbird's role in the enzootic cycle of *A. phagocytophilum* should be studied as it is a relatively common bird species in Europe.

***Genetic diversity of* A. phagocytophilum**

Europe has been at the forefront of strain diversity studies for *A. phagocytophilum*, in part perhaps of the importance to livestock. Anaplasmosis in Europe predominantly is a disease of livestock. The strains circulating in Europe mainly cause tick-borne fever in ruminants, especially in sheep and cattle (Ladbury *et al.*, 2008; Beugnet and Marié, 2009; Atif, 2015).

Based on the conserved *16S rRNA* gene in the US, two *A. phagocytophilum* strains are commonly found, including a human pathogenic strain (*Ap*-ha), which causes disease in humans, and a deer variant strain (*Ap*-v1) that is not known to cause disease in humans (Massung *et al.*, 1998, 2002). Although both strains have been detected in multiple wildlife species (Keesing *et al.*, 2014) the primary reservoir host for *Ap*-ha is the white-footed mouse (*P. leucopus*), which is only weakly competent for *Ap*-v1, while white-tailed deer serve as the primary reservoir host for *Ap*-v1 and are incompetent for *Ap*-ha (Massung *et al.*, 1998, 2003, 2005; Stafford *et al.*, 1999). Although *Ap*-v1 (and closely related strains) was first discovered in the north central US (Massung *et al.*, 1998; Michalski *et al.*, 2006) very little work has been conducted to investigate the ecology,

including host associations, of different strains. White-footed mice, chipmunks, and raccoons showed lower reservoir competence to *Ap*-v1 than *Ap*-ha (Yabsley *et al.*, 2008; Keesing *et al.*, 2014). Considering birds, catbirds, veeries, and wood thrush showed a low reservoir competence for *Ap*-ha, while American robins had low reservoir competence for both Ap-v1 and Ap-ha (Keesing *et al.*, 2012).

Of note, *Ap*-ha is rarely detected in Europe although cases of human granulocytic anaplasmosis have (Rar, Tkachev and Tikunova, 2021; Dumic *et al.*, 2022a) been reported (Atif, 2015; Lagler *et al.*, 2017; Tsiodras *et al.*, 2017; Dumic *et al.*, 2022a), and there have not been enough studies conducted in Asia to understand its prevalence there. In California (US), researchers have explored the genetic diversity of *A. phagocytophilum* more thoroughly than in the northeastern and north central US, using multiple genetic markers.

Several early studies differentiating genetic strains of *A. phagocytophilum* used 16S rRNA. In Norway, several variants of 16S rRNA were found in sheep with distinct clinical manifestations (Stuen *et al.*, 2002, 2003). In addition, early European studies used the *16S rRNA* gene to distinguish variants of *A. phagocytophilum* circulating in red deer and roe deer (Zeman and Pecha, 2008). However, different studies have concluded that the level of variation within the *16S rRNA* gene may not be enough to delineate among strain types in Europe, which led to researchers investigating other genes(Bown *et al.*, 2009; Scharf *et al.*, 2011; Silaghi, Liebisch and Pfister, 2011), including those encoding the ankyrin protein (*ankA* gene), the groESL operon (*groES* gene, and *groEL* gene), and the major surface protein 2 (*msp2* gene). Researchers in Europe and Russia further have developed a multilocus sequence typing (MLST) system to investigate *A. phagocytophilum* genetic diversity (Huhn *et al.*, 2014; Mukhacheva, Shaikhova and Kovalev, 2019; Mukhacheva *et al.*, 2020).

The gene that encodes ankyrin protein (*AnkA* gene) has been one commonly used gene to differentiate *A. phagocytophilum* strains mainly in Europe. Ankyrin proteins are essential in different protein-protein interactions and modulate gene transcription within the host cells (Caturegli *et al.*, 2000; Loewenich *et al.*, 2003; Park *et al.*, 2004; Ijdo, Carlson and Kennedy, 2007; Scharf *et al.*, 2011; Majazki *et al.*, 2013; Mukhacheva *et al.*, 2020). The *ankA* gene target can differentiate between five *A. phagocytophilum* variant clusters from different host species (Scharf *et al.*, 2011; Dugat *et al.*, 2015). The first gene cluster was including sequences taken from humans, dogs, cats, horses, and a few ruminants (Rar and Golovljova, 2011). This *ankA* gene cluster branched into two variants, grouped geographically (i.e., European and versus American regions origins) (Rar and Golovljova, 2011). The rest of the gene clusters were all from different host species in Europe, where the second gene cluster comprised variants from roe deer and red deer; the third gene cluster comprised variants from cattle, sheep, roe deer, and red deer; the fourth gene cluster comprised variants from roe deer; and the fifth gene cluster comprised variants from rodents (Rar and Golovljova, 2011; Scharf *et al.*, 2011). Therefore, *ankA* gene cluster may show varying degrees of host association.

The groESL operon in *A. phagocytophilum* covers the region of two encoding genes, *groES*, and *groEL*, which are essential in heat shock protein production. Much of the *groESL* genotyping has been conducted in Europe and Russia. Four major *groESL* clusters of *A. phagocytophilum* have been delineated (Jahfari *et al.*, 2014) and are referred to as ecotypes that appear to be host specific. The most common of these gene clusters is ecotype I, comprising isolates from humans, dogs, cattle, horses, hedgehogs, red deer, sheep, and mouflons. Ecotype II isolates are primarily from roe deer; ecotype III isolates are from rodents and questing *I. persulcatus*; and ecotype IV isolates are often associated with birds- mainly the common blackbird

(Jahfari *et al.*, 2014). Since both *AnkA* and *groESL* genes show similar clustering patterns, there is more evidence to support the hypothesis that *A. phagocytophilum* strains show host tropisms. In both gene targets, gene sequences originating from America cluster separately from the European sequences, indicating distinguishing variants of *A. phagocytophilum* circulating within different regions.

A major surface protein gene of *A. phagocytophilum* (*msp2*) also shows a regional clustering pattern, where the American variants cluster separately from the European variants (Morissette *et al.*, 2009; Silaghi, Liebisch and Pfister, 2011). This clear regional clustering pattern may be due to differences in host species and host species composition between the two regions as well as divergence due to geographic separation. In California, a genetic variant based on the *msp2* gene of *A. phagocytophilum* cycles exclusively within dusky-footed woodrats (Foley et al., 2008). This variant does not infect horses or dogs, is distinct from *Ap*-ha and *Ap*-v1 variants and from the *ankA* gene variants (Trost *et al.*, 2018). Dusky-footed woodrats frequently show high infection prevalence and seropositivity in California (Nicholson *et al.*, 1999; J. Foley *et al.*, 2008).

Recent advancement in strain typing *A. phagocytophilum* uses multi-locus sequence typing (MLST) techniques. This approach uses seven housekeeping genes to determine the sequence types. However, there have been only four studies done using MLST for *A. phagocytophilum*, and all are based on samples collected in Europe and Russia. Overall, using MLST, it was found that the sequence typing showed similar patterns with the *16S rRNA* and *ankaA* loci (Chastagner *et al.*, 2014; Huhn *et al.*, 2014; Mukhacheva, Shaikhova and Kovalev, 2019; Mukhacheva *et al.*, 2020).

The future in strain typing of *A. phagocytophilum* should be based on whole-genome sequencing. However, only a few studies have sequenced the genome of *A. phagocytophilum* (Dunning Hotopp *et al.*, 2006; Barbet *et al.*, 2013; Dugat *et al.*, 2015). Thus, there have been no

publications, to our knowledge, using whole genome sequencing to investigate genetic diversity and host association of *A. phagocytophilum*, indicating the need to sequence more variants to identify different strains circulating within different regions and within different host species.

Conclusion

There are many gaps in knowledge in the ecology of *A. phagocytophilum* especially in the Upper Midwest, where it is endemic. There are also many gaps in knowledge in the strain diversity of *A. phagocytophilum* in the Upper Midwest and the Northeast of the US. With the improvements seen in whole genome sequencing techniques in the last few years, looking at strain diversity within endemic regions of the eastern USA would be important in understand how *A. phagocytophilum* strains are maintained in nature and their importance to human disease risk. Furthermore, not a lot is known about the pathogenesis of *A. phagocytophilum* as its transmitted from the tick to the host and vice versa. With the continued range expansion of *I. scapularis* in the eastern U.S., further investigating the ecology, strain diversity and pathogenesis of *A. phagocytophilum* will be crucial in reducing disease risk.

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CHAPTER 3: THE ECOLOGY OF *ANAPLASMA PHAGOCYTOPHILUM* AT A SITE IN THE UPPER MIDWEST USA (FORT MCCOY, WISCONSIN)

ABSTRACT

Anaplasma phagocytophilum is an intracellular bacterium that causes canine and human granulocytic anaplasmosis, and most cases in the United States occur in two main foci: the Upper Midwest and the Northeast. In these regions, *A. phagocytophilum* is vectored by the blacklegged tick (= deer tick), *Ixodes scapularis* Say 1821. Although human granulocytic anaplasmosis is the second most common vector-borne disease in the US, few ecological studies have been conducted in the Upper Midwest. The main objective of this study was to characterize the ecology of *A. phagocytophilum* at a site in central Wisconsin where blacklegged ticks have been established for more than half a century and human anaplasmosis is endemic. Sampling was conducted from May – September in 2010 – 2012 every 2-3 weeks during which questing ticks and small and medium-sized animals were live-captured on 3 1-ha grids. A subset of questing ticks, attached ticks, host biopsies, and host blood were assayed for infection with *A. phagocytophilum* by a real time PCR followed by a confirmatory nested PCR. Over the three years, 1284 number ticks were collected; the average infection prevalence of questing nymphs and adults for *Ap*-ha was 15.3% and 20.4% respectively. All infected ticks were infected with the *Ap*-ha strain. The infection prevalence of *A. phagocytophilum* of small to medium sized mammals comprising 10 species (N = 1244 individuals) ranged from 5.7% to 89%. *Peromyscus leucopus* (white-footed mouse) was the most frequently captured species (N = 571, infection prevalence = 28.0%) and *Tamias striatus* (eastern chipmunk) had the highest infection prevalence (88.9%). Based on the fully engorged larvae collected from white-footed mice and eastern chipmunks, the realized reservoir competence of eastern chipmunks (88.9%) was significantly greater than white-footed mice (12.9%), but white-

footed mice fed a significantly greater number of larvae (71.7% of the total larvae collected from animals) compared to eastern chipmunks (1.2% of the total larvae collected from animals). Therefore, the relative contribution of white-footed mice to the enzootic cycle of *A. phagocytophilum* may be more significant at this field site. The phenology of infection prevalence of hosts and on-host larvae showed a similar pattern where infection prevalence increased from May to June and then decreased. This pattern is comparable with the phenology of questing infected *I. scapularis* nymphs. We also compared the infection prevalence between blood and ear biopsies of white-footed mice (N=367) that were obtained during the same capture event. There was no significant difference in infection prevalence in mice from which *A. phagocytophilum* was collected from blood only, biopsy only, and both types simultaneously for each pairwise analysis. Interestingly, the infection prevalence of on-host larvae was highest when collected from mice from which *A. phagocytophilum* was detected in both ear biopsies and blood samples at the same capture event. Future ecological studies should be conducted across the Midwest to assess similarities and differences in the enzootic maintenance cycle; furthermore, they should also explore the roles of birds and other hosts not captured (or not thoroughly studied here), making certain to differentiate among *A. phagocytophilum* strains. Future research may also look deeper into the infection, transmission, and immunity dynamics of highly infected hosts such as the eastern chipmunk to better understand the host-pathogen interactions. Finally, laboratory-based studies should also compare the temporal dynamics of detection of *A. phagocytophilum* from ear, blood, and transmission to feeding larvae in xenodiagnoses experiments to better understand the relevance of detection of *A. phagocytophilum* in blood or biopsy samples given the logistical advantages for collecting biopsies in field work.

Keywords: *Anaplasma phagocytophilum*, Upper Midwest, blacklegged tick, white-footed mouse, eastern chipmunk

INTRODUCTION

Anaplasma phagocytophilum is an intracellular bacterium and is known to cause granulocytic anaplasmosis in humans, equine granulocytic anaplasmosis in horses, febrile fever in cats, and canine granulocytic anaplasmosis in dogs (Chen *et al.*, 1994; Stuen, Granquist and Silaghi, 2013; Dugat *et al.*, 2015). Human granulocytic anaplasmosis is an infectious zoonotic disease in North America, Europe, and Asia (Stuen, 2007; Stuen, Granquist and Silaghi, 2013; Silaghi *et al.*, 2017). In the United States, after Lyme disease, anaplasmosis is the second leading vector-borne and tick-borne disease. Furthermore, like Lyme disease, the reported human case incidence for anaplasmosis is greatest in the Northeast and the Upper Midwest (Dahlgren *et al.*, 2015).

Anaplasma phagocytophilum is vectored by ticks that belong to the *Ixodes ricinus* complex, the same species complex that vector the agents of Lyme disease in North America and Eurasia. In the US, as with the Lyme disease agent *Borrelia burgdorferi*, *A. phagocytophilum* is vectored by *Ixodes pacificus* in the western and *Ixodes scapularis* in the eastern USA (Barlough et al., 1997; Pancholi et al., 1995; Richter Jr. et al., 1996; Telford et al., 1996). In the US, there are two major strains of *A. phagocytophilum* circulating within the same enzootic cycle, one strain is the human pathogenic strain *Ap*-ha, which causes disease in humans, and the other strain is the deer variant *Ap*-v1, which is not known to cause disease in humans.

Although many studies have been conducted to elucidate the eco-epidemiology of Lyme disease, less effort has been focused on that of human anaplasmosis. Less research also has been conducted on the ecology of the agent of anaplasmosis in the Upper Midwest compared to in either the northeastern or western US. Limited data suggest that the ecology of the enzootic cycle maintaining *A. phagocytophilum* in the Upper Midwest US should be similar to that in the

Northeast, but that there may be differences based on regional differences in the ecology of *I. scapularis* (e.g., differences in phenology, Ogden et al. 2007; Gatewood et al. 2009; Ogden et al. 2018). Thus, using samples from a study originally designed to study the ecology of the Lyme disease pathogen, we had the opportunity to better characterize the ecology of *Ap*-ha in central Wisconsin, an area in the Upper Midwest highly endemic for *I. scapularis*-borne diseases. Our objectives were: 1) to estimate the prevalence of infection of questing *I. scapularis* (nymphs and adults) for *Ap*-ha; 2) to estimate the prevalence of infection of small and medium-sized mammals for *Ap*-ha; 3) to characterize the realized reservoir competence for *Ap*-ha of small and medium-sized mammals often parasitized by *I. scapularis*; and 4) to characterize the seasonal enzootic dynamics of infection between ticks and hosts. As part of this work, we also compare the use of blood versus ear biopsies for inferring the infection and transmission status of wildlife.

MATERIALS AND METHODS

Field site and study design

We investigated the ecology of *I. scapularis* and *A. phagocytophilum* at Fort McCoy Military Installation, in Monroe County, western central Wisconsin (WI; 44.0391 N, −90.6766 W). *Ixodes scapularis*, *B. burgdorferi* and Lyme disease have been endemic at Fort McCoy for decades (Anderson, Duray and Magnarelli, 1987). *Anaplasma phagocytophilum* has been detected there previously (Steiner et al. 2008; Hamer et al. 2014). The field site, experimental design, and sampling protocols have been described previously in Ginsberg et al., 2021, Ogden et al., 2018, Rulison et al., 2013. Below we summarize the most pertinent aspects and refer the reader to prior studies for additional details. Sampling was carried out on three 1-ha grids that are dominated by oak (*Quercus* spp.), pines (*Pinus* spp.), red maples (*Acer rubrum*), and have a shrub layer consisting of mainly tree saplings (Rulison *et al.*, 2013; Arsnoe *et al.*, 2015). Each grid was

separated by ≥ 3 km. Grids were sampled either every two (2010) or three (2011-2012) weeks from May to September, with some additional sampling outside that period.

Questing ticks

Sampling for questing ticks was conducted on rain-free days. Questing ticks were collected by a combination of dragging and flagging a 1 m^2 flannel cloth (Rulison *et al.,* 2013; Ogden *et al.,* 2018). Each grid comprised of a 7 x 7 array with 15 m between grid points. Each sample period comprised 8 parallel 90-m transects (alternating dragging and flagging), for a total of 720 m^2. The cloth was inspected every 15 m. All ticks were removed and placed into vials of 95% ethanol. All three grids were sampled for a total of 2,160 m^2 per sample period (also referred to as a 'trapping session' below).

Wildlife hosts

Sampling was carried out as described in Ginsberg et al., 2020 and Ogden et al., 2018. Each trapping session comprised of 2 trap nights. Small mammals were live captured from metal traps, pitfall traps, and occasionally wooden and metal coverboards. One long-folding aluminum Sherman trap (Sherman Traps, Tallahassee, FL) was placed at each of the 49 grid points (Appendix, Figure 3.10). Traps were baited with crimped oats. One pitfall array was placed external to and along each side of the grid. Each array comprised of two 40-m long aluminum flashing, buried into the soil, and bisecting each other perpendicularly. One five-gallon bucket was sunk into the ground at the end of each of the four arms, as well as the center of the array, for a total of five buckets per array and 20 buckets per grid. Twenty metal and twenty wooden (61 cm x 61 cm) coverboards were placed in pairs at 20 evenly spaced locations on the trapping array. To trap medium-sized mammals, one medium-sized Tomahawk trap (Tomahawk Live Trap Co. Tomahawk, WI) was placed at the midpoint of each edge of the grid, just outside of the grid for a

total of 4 traps. Traps were baited with one can of sardines. All traps were set in the evening and checked 12 hours later the next morning.

Captured small mammals were identified to species, sexed, weighed, and marked with a uniquely numbered metal ear tag (Monel #1, National Band & Tag Company, Newport, KY). Medium-sized mammals were anesthetized and processed as described in Ogden et al., 2018. To minimize stress to the animal and to standardize sampling, we systematically inspected each animal for ticks for up to 5 minutes. All detected ticks were collected. We obtained ear biopsies (2 cm diameter) and blood samples (1% of body weight, up to 3 ml for medium-sized mammals) from each individual the first time we captured it within a trap session. If individuals were recaptured during the same trapping session, they were processed as described, but no additional ear biopsy nor blood sample was collected. Individuals recaptured in another trapping session were processed as if they were first time captures (i.e., with biopsy and blood samples collected). After animals were processed and were alert, we released them at the point of capture. Because of the lack of external ear pinnae, and because of their high metabolism, to minimize stress, we did not tag shrews individually and we did not sample blood. We prioritized sampling shrews first and then released them immediately before processing all other small mammals. All ticks and ear biopsies were stored in microcentrifuge tubes with 95% ethanol. We collected blood from hunter-harvested deer in Fort McCoy, Wisconsin. All animal handling procedures were approved by the Michigan State University's Institutional Animal Care and Use Committee (AUF # 06/09-094-00).

Pathogen detection

Questing ticks and ticks removed from hosts were identified at Michigan State University using dichotomous morphological keys (Clifford, Anastos and Elbl, 1961; Sonenshine, 1979; Keirans and Litwak, 1989; Durden and Keirans, 1997). For on-host ticks, to reduce the probability

that we would assay on-host larvae that had not fed long enough to acquire *A. phagocytophilum* from an infected host, we selected engorged ticks based on Han, 2019. Briefly, we used an 8-point scale Engorgement Index (EI) ranging from 1 (0-hour, attachment time point) to 8 (> 72 hours, natural drop-off time point). Up to 5 engorged larvae with EI7 (72 hours, right before natural drop-off time point) were selected by microscopic examination from each captured animal.

Genomic DNA was extracted from questing ticks, ear biopsies, blood samples, and a subset of on-host *I. scapularis* ticks (both on-host nymphs and larvae based on the engorgement status), using Qiagen DNeasy Blood and Tissue kits (Valencia, CA) as per Hamer et al., 2010. The extracted DNA samples were assayed for *A. phagocytophilum* using a real-time PCR (rt-PCR) targeting a 122 bp region of the *msp2* gene, which encodes a major surface protein (Drazenovich, Foley and Brown, 2006). A TaqMan probe-based rt-PCR on ABI QuantStudio 7 Flex PCR System was used at the Michigan State University Research Technology Support Facility Genomics Core. A total volume of 15 ml reaction consisted of 150 nM probe and each primer at 150 nM and 300 nM to assay tick DNA and mammalian tissues respectively. Bovine serum albumin at 0.25 ml was used as an additive in each reaction mixture to improve rt-PCR efficiency (Han, 2019). For the positive control, *A. phagocytophilum* human pathogenic Strain USG3 isolate derived from a beagle that was purposely exposed to infected adult *I. scapularis* ticks was used (Yeh *et al.,* 1997) and was obtained from the Centers of Disease Control and Prevention (Yeh *et al.,* 1997). For the negative control, ultrapure PCR water was used.

Samples positive by the rt-PCR (i.e., "suspect positives") were confirmed using a nested PCR that targets a 546 bp region of the *16S rRNA* gene (Massung *et al.,* 1998). The samples that were positive by the confirmatory nested PCR were sequenced by Sanger sequencing to distinguish between *Ap*-ha (human pathogenic strain) and *Ap*-v1 (deer variant strain) strains based on a two-

base pair difference (Massung *et al.*, 2003) in the *16S rRNA* gene. DNA sequencing was conducted by using the ABI Prism 7900HT Sequence Detection System and ABI 3730 xl DNA Analyzer (Applied Biosystems, Foster City, CA) at the Michigan State University Research Technology Support Facility Genomics Core.

Definitions and data analysis

Several objectives of this study include characterizing trends in *A. phagocytophilum* infection among questing *I. scapularis*, wildlife hosts, and transmission to on-host larval ticks. The prevalence of infection (%) of *A. phagocytophilum* in questing nymphs and adults was estimated by dividing the number of ticks confirmed *A. phagocytophilum* positive by rt-PCR divided by the total number of ticks tested for each respective life stage. Due to the relatively low infection prevalence of *A. phagocytophilum* in Wisconsin (on average 4-6% in nymphs (Lehane et al 2021; Foster et al. 2022) and 9-10% in adults (Foster et al. 2022; Lehane et al. 2021), to increase the reliability and representativeness in trends, we combined data from all grids and from all three years.

Although all individuals were inspected for ticks each time they were captured, for ecological analysis, only the numbers of ticks collected during the first captures of individuals within a trapping period were used for estimating *I. scapularis* burden (i.e., number of ticks of a given life stage per individual). This is because for an individual captured for the first time within the trapping period, the number of ticks attached would comprise all ticks contacted for multiple days prior to capture. Because our protocol was to remove ticks from captured animals, however, the subsequent number of ticks on an individual recaptured the next day would not be comparable, as they would comprise newly parasitizing ticks and those that we missed on the first day of capture. Thus, for consistency of analyses, larval and nymphal burdens were estimated using only values

from an individual's first capture within a trapping period. Because our trapping periods were 2-3 weeks apart, a duration exceeding that for larval and nymphal *I. scapularis* to feed to repletion, data from animals recaptured in different trapping periods were included in all analyses. A negative binomial regression analysis was conducted to estimate if there were an effect of host species, sex, mass, trapping period (trapping week), trapping year and trapping array on the larval and nymphal counts on individual animals.

Although *A. phagocytophilum* is an intracellular, blood-borne organism, researchers have used ear biopsy and spleen tissue biopsies to detect *A. phagocytophilum* in addition to using blood (Hodzic *et al.*, 2001; Lane *et al.*, 2005; Granquist, Aleksandersen, *et al.*, 2010; Granquist, Stuen, *et al.*, 2010; Szekeres *et al.*, 2015; Rosso *et al.*, 2017). Here we assayed both sample types (blood and ear biopsies), as each sample type was not always available from each animal or species (e.g., we did not collect blood samples from shrews). We scored any individual as positive (at a given time of capture) if at least one tissue type - blood or skin biopsy - was positive.

The realized reservoir competence of a host species represents the probability that a larva feeding on an individual of a particular host species will become infected with *A. phagocytophilum* (Keesing *et al.*, 2012). It was estimated as the mean percentage of on-host *I. scapularis* larvae infected by an individual host of a particular species, considering host infection prevalence (Keesing *et al.*, 2012) (i.e., considering how not all individuals of a given host species are infected).

Given equal sampling effort across sampling periods, the phenology, or seasonality, of questing ticks per month was estimated as the density of questing ticks (or questing infected ticks) detected in that month. Similarly, the phenology of on-host ticks (or infected on-host ticks) was calculated as the average number of larvae (or nymphs) per individual per host per month.

We used a Chi-square test and Fisher's exact test (for $n < 5$ for any category) to compare the

infection prevalence between questing life stages and among hosts, applying a Bonferroni correction to the p-value when making multiple comparisons. We used logistic regression to compare the infection prevalence of *A. phagocytophilum* from biopsy samples and blood samples among species. A generalized linear model was used to model the *A. phagocytophilum* infection status of individual hosts as a function of host nymphal burden, host species, sampling grid, sampling year, trapping week, sex, and mass. Analyses were conducted using RStudio (Version 1.2.1335). All error bars shown for infection prevalence are 95% binomial confidence intervals.

RESULTS

The basic tick sampling and mammal trapping results were reported in Ogden et al. 2018, Ginsberg et al. 2020 and Han et al. 2019, but to understand *A. phagocytophilum* infection prevalence and dynamics, they are presented here as well. Furthermore, differences in presentations of results exist based on the different questions being asked.

Questing ticks and pathogen detection

Over three years and among all three grids, we collected 662 and 622 questing nymphal and adult *I. scapularis*, respectively. We found no significant differences in infection prevalence of questing nymphs and adults among the grids and sampling years, in part potentially due to low infection prevalence and sample sizes. Therefore, for subsequent analyses, the infection prevalence was combined overall years and grids. The overall infection prevalence for nymphs (15.3%, 12.6 - 18.2 95% CI) was lower than that of adults (20.4%, 17.3 - 23.8 95% CI, Fisher's exact test p = 0.02301). Except for one nymph that was infected with the *Ap*-v1 variant, all other positive ticks (n=41 nymphs and n= 42 adults) were infected with the *Ap*-ha variant. All negative extractions and PCR controls were negative; all positive controls for PCR were positive.

Wildlife hosts

Over three years, there were 2151 capture events comprising 14 small to medium-sized mammal species: white-footed mouse (*Peromyscus leucopus*), southern red-backed vole *(Myodes gapperi*), long-tailed shrew species (*Sorex* spp.), northern short-tailed shrew (*Blarina brevicauda*), raccoon (*Procyon lotor*), eastern chipmunk (*Tamias striatus*), meadow vole (*Microtus pennsylvanicus*), meadow jumping mouse (*Zapus hudsonicus*), Virginia opossum (*Didelphis virginiana*), star-nosed mole (*Condylura cristata*), southern flying squirrel (*Glaucomys volans*), fisher (*Pekania pennanti*), eastern mole (*Scalopus aquaticus*), and gray fox (*Urocyon cinereoargenteus*). *Peromyscus leucopus* and *P. lotor* were the most frequently captured small and medium-sized mammal species, respectively (Table 3.1).

On-host ticks

We collected a total of 8918 blacklegged ticks (8279 larvae, 623 nymphs, and 16 adults) from 2151 hosts (including recaptures), which comprised of 10 species (Table 3.1). When only the first-time captures within a trapping period were considered (including recaptures across trapping periods), we collected a total of 7204 larvae and 568 nymphs from 1822 individuals (Table 3.1).

Table 3.1. Small and medium-sized mammals captured at Fort McCoy, Wisconsin (2010 - 2012) and their average larval and nymphal *I. scapularis* burdens (number of ticks per individual host), median and range per host species. *First time captures could include recaptured individuals only if recaptures were caught during a different trapping period.

Species	**First time captures within a trapping period***	**Tick infestation of first time captures within a trapping period**		**Tick Burdens**	
		Total number of ticks collected (% prevalence)		**Average number of ticks per individual (median, range)**	
		Larvae	**Nymphs**	**Larvae**	**Nymphs**
White footed mouse (*Peromyscus leucopus*)	1055	5126 (73.3%)	274 (16.6%)	4.86 (3, 0-43)	0.26 (0, 0-6)
Southern red-backed vole (*Myodes gapperi*)	374	390 (40.6%)	54 (10.2%)	1.04 (0, 0-19)	0.14 (0, 0-5)
Long-tailed shrew (*Sorex* spp.)	164	626 (55.5%)	8 (4.5%)	4.07 (1, 0-42)	0.05 (0, 0-2)
Northern short-tail shrew (*Blarina brevicauda*)	119	650 (69.7%)	14 (4.2%)	5.46 (3, 0-43)	0.12 (0, 0-4)
Raccoon *(Procyon lotor*)	52	221 (38.5%)	116 (55.8%)	4.25 (0, 0-50)	2.23 (1, 0-21)
Eastern chipmunks (*Tamias striatus*)	27	84 (51.9%)	71 (51.9%)	3.1 (1, 0-21)	2.63 (1, 0-10)
Meadow vole (*Microtus pennsylvanicus*)	10	21 (60.0%)	5 (20%)	2.1 (1, 0-13)	0.5 (0, 0-4)
Meadow Jumping mouse (*Zapus hudsonius*)	8	6 (12.5%)	5 (0%)	0.75 (0, 0-6)	0
Virginia opossum (*Didelphis virginiana*)	7	22 (42.9%)	22 (51.1%)	3.14 (0, 0-11)	3.14 (0, 0-13)
Star-nosed mole (*Condylura cristata*)	3	150 (66.70%)	0 (0%)	5 (7, 0-8)	0
Southern flying squirrel (*Glaucomys volans*)	2	1 (50 %)	2 (100%)	0.5 (0.5, 0-1)	1 (1, 1-1)
Gray fox (*Urocyon cinereoargenteus*)	1	0 (0 %)	2 (100%)	0	2 (2, 0-2)

A negative binomial regression model indicated that host species, sex, mass, trapping period (trapping week), trapping year and trapping grid were significant factors in explaining the distribution of larval counts on individuals (Appendix, Table 3.5). The white-footed mouse, 2010, grid A, and males served as the reference levels. Southern red-backed vole had 0.19 times (95% confidence intervals (CIs) = 0.16 – 0.24, p-value = < 2e-16) fewer ticks, and the meadow vole had 0.35 times (95% CIs = 0.15 – 0.97, p-value = 0.03) fewer ticks compared to white-footed mice. Female mice had 0.65 times (95% CIs = 0.55 – 0.77, p-value = 3.70e-07) fewer larval counts compared to male mice. For each 1 g increase in mass, the larval count decreased by 1 (95% CIs 0.996 – 0.998, p-value=0.01). Larval counts per individual in 2011 were 0.52 times (95% CIs = 0.43 – 0.62, p-value = 9.96e-13) lower compared to 2010. With the progression of capture week over the season, the numbers of larvae parasitizing hosts decreased by 0.98 times per week (95% CIs 0.96 – 0.99, p-value = 0.0021). Individuals captured on grids B and C had 0.83 times (95% CIs = 0.69 – 0.99, p-value = 0.04) and 0.71 times (95% CIs = 0.59 – 0.87, p-value= 0.0006) fewer larvae compared to individuals captured on grid A (Appendix, Table 3.6).

A negative binomial regression model indicated that host species, sex, mass, trapping period (trapping week), trapping year and trapping array were significant factors in explaining the distribution of nymphal counts on individuals (Appendix, Table 3.5). Again, the white-footed mouse, grid A, and male mice were used as the references levels. Compared to the white-footed mouse, the northern short-tail shrew, southern red-backed vole, and long-tailed shrew species had 0.10 times (95% CIs = 0.01 – 0.78, p-value = 0.03), 0.36 times (95% CIs = 0.25 – 0.53, p-value = 2.33e-07), and 0.24 times (95% CIs = 0.06 – 0.91, p-value = 0.04) fewer attached nymphs per individual. Conversely, the Virginia opossum, raccoon, and eastern chipmunk had 35 times (95% CIs = 9.1 – 132, p-value = 2.00e-07), 28 times (95% CIs = 8 – 101, p-value = 4.60e-07), and 8

times greater (95% CIs = 3.9 – 15.2, p-value = 5.09e-09) nymphs per individual respectively compared to the white-footed mouse. Compared to individuals captured on grid A, individuals captured on grid C had 0.6 times fewer nymphs (95% CIs = 0.42 – 0.87, p-value = 0.007). As the capture week progressed the number of nymphs on individual hosts significantly decreased by 0.89 times (95% CIs = 0.86 – 0.91, p-value = < 2e-16). With a 1 g increase in mass, the nymphal counts on hosts decreased by 1 time (95% CIs = 1.01 – 0.99, p-value = 0.03) (Appendix, Table 3.7).

Pathogen detection

Table 3.2 shows the pathogen detection results of 10 mammal species for which we had blood and/or biopsy samples. Individuals may have been captured in more than one trapping period, but blood (n = 516) and biopsy (n = 1212) samples were only collected once per trapping period. We were not able to collect blood or ear tissue species from every individual when captured (e.g., no blood was obtained from any shrew); hence the numbers of blood and ear biopsy samples are different.

Table 3.2. *Anaplasma phagocytophilum* infection of small to medium-sized mammals captured at Ft. McCoy, Wisconsin from 2010 – 2012 by sample type. *Includes multiple ear biopsies and blood samples from individuals captured from multiple trapping periods. ** The final number (%) of infected individuals comprised those from whom *A. phagocytophilum* had been detected from at least one tissue type.

Host species	**Number of samples assayed by tissue type**			**Number of host tissues positive (infection prevalence %)**		
	All tissues	**Blood**	**Ear Biopsy**	**All tissues****	**Blood***	**Ear Biopsy***
White-footed mouse	755	442	678	213 (28.2)	125 (28.3)	136 (20.1)
Southern red-backed vole	269	5	269	34 (12.6)	2 (40.0)	32 (11.9)
Long-tailed shrews spp.	75	0	75	5 (6.7)	-	5 (6.7)
Northern short-tail shrew	53	1	52	3 (5.7)	0 (0)	3 (5.8)
Raccoon	51	40	47	17 (33.3)	11 (27.5)	10 (21.3)
Eastern chipmunk	18	16	15	16 (88.9)	12 (75.0)	13 (86.7)
Meadow vole	9	1	9	2 (22.2)	0 (0)	2 (22.2)
Virginia opossum	7	6	7	4 (57.1)	2 (33.3)	2 (33.3)
Meadow jumping mouse	7	1	7	1 (14.3)	1 (100.0)	0 (0)
Southern flying squirrel	2	0	2	0 (0)	-	0 (0)
Star-nosed mole	1	1	1	0 (0)	0 (0)	0 (0)
Gray fox	1	1	1	0 (0)	0 (0)	0 (0)

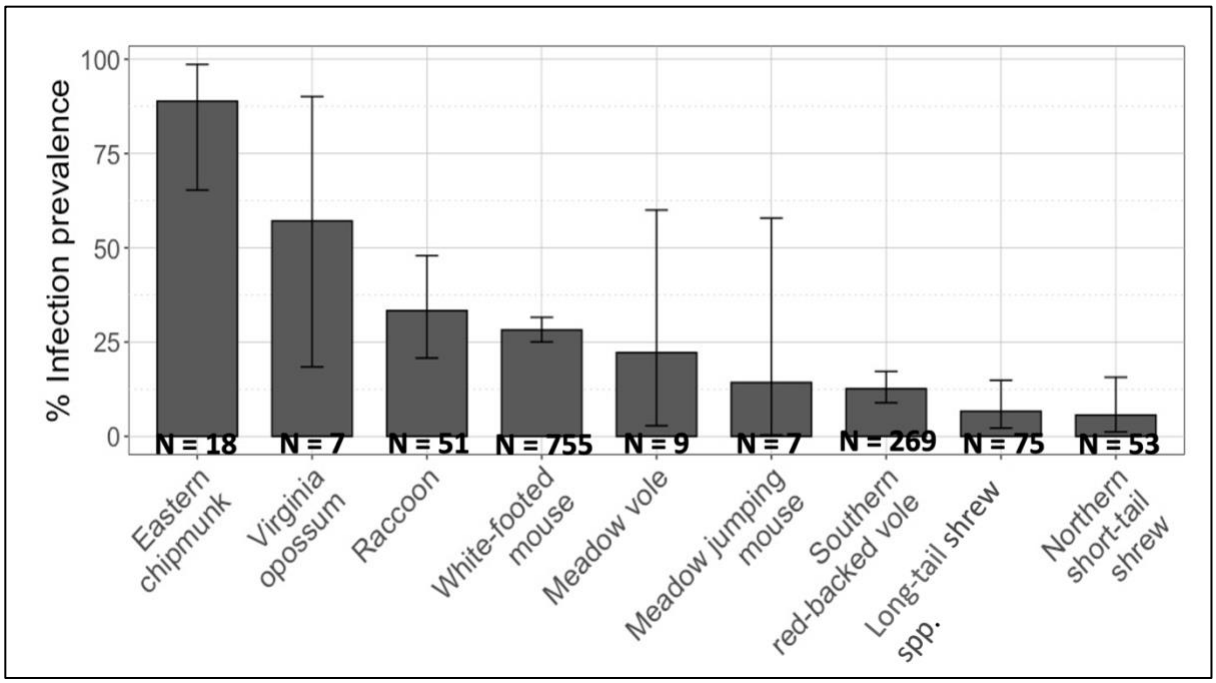

Figure 3.1. Infection prevalence (95% binomial confidence intervals) of *A. phagocytophilum* for nine mammal species captured at Fort McCoy, Wisconsin from 2010 - 2012. Numbers of individuals assayed are shown beneath each bar.

Anaplasma phagocytophilum was detected in 9 of the 12 mammal species captured (Table 3.2), and the overall infection prevalence of hosts captured was 23.6% (n=1248). Only *Ap*-ha strain was detected among infected individuals. Host species that had a greater sample size of 5 were used in subsequent analyses. The infection prevalence of eastern chipmunks (88.9%, n = 18) was highest and was significantly greater than that of the white-footed mouse, raccoon, southern red-backed vole, meadow vole, meadow jumping mouse, northern short-tailed shrew, and *Sorex spp.* (long-tailed shrews) (adjusted for multiple comparisons, pairwise Fisher's exact p-value < 0.001, Figure 3.1). There was no significant difference (adjusted for multiple comparisons, pairwise Fisher's exact p-value = 0.425) in the infection prevalence between the white-footed mice (28.0%, n = 762) and the raccoon (33.3%, n = 51). In addition, we screened 100 deer blood samples from

hunter-harvested deer. Only 3% of the deer were infected and all were infected with *Ap*-V1 strain.

Host infectivity & realized reservoir competence

We collected 7,146 larvae from nine host mammal species that were first-time captures within a trapping period, and which were of known sex (Table 3.1). White-footed mice (72%), northern short-tailed shrews (9%), long-tailed shrews spp. (9%), and southern red-backed voles (5%) comprised 95% of the attached larvae detected on captured hosts (Figure 3.2).

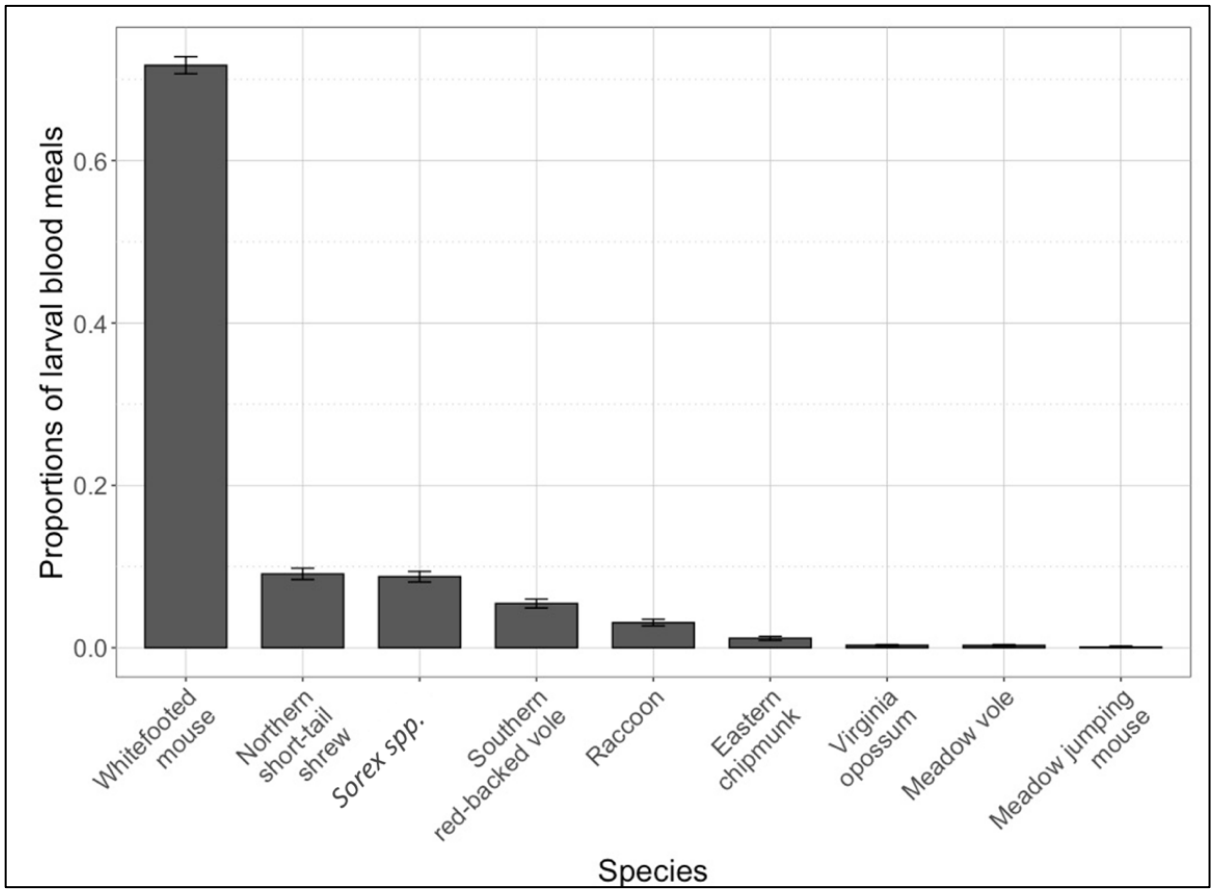

Figure 3.2. Distribution of attached larvae detected among small to medium-sized mammalian species live captured at Fort McCoy, Wisconsin from 2010 – 2012. Error bars represent the 95% confidence intervals.

Of the 7146 larvae, we selected 199 nearly fully engorged ticks to assay for *A.*

phagocytophilum (Table 3.4). These engorged larvae were collected predominantly from white-footed mice (78.4%) and eastern chipmunks (16.6%), from which *A. phagocytophilum* was detected in 18.6% (29/156) and 100% (33/33) of engorged larvae respectively. Given *A. phagocytophilum* was detected in 36% of white-footed mice and 81% of eastern chipmunks (Figure 3.1), the realized reservoir competence of these two host species is estimated as 12.9% (95% CIs 0.126 – 0.133) and 88.9% (95% CIs 0.861 – 0.813) respectively (Table 3.3). Although *A. phagocytophilum* was detected from many raccoons, southern red-backed voles, and shrews, no inferences about infectivity nor realized reservoir competence can be made as few or no engorged larvae were collected from these hosts that were infected with *A. phagocytophilum*.

Table 3.3. The realized reservoir competence of white-footed mice and eastern chipmunks capture from 2010-2012 at Ft. McCoy, Wisconsin for *Anaplasma phagocytophilum*.

Species	**Infection prevalence (%) (Total number of captures assayed)**	**Total number of fully engorged larvae from infected hosts**	**Total number of larvae infected with Ap**	**Reservoir Competence* (SE)**	**Realized Reservoir Competence** (Confidence Intervals)**
White-footed mouse	28.2% (755)	48	22	45.8% (9.3%)	12.9% (0.126 – 0.133)
Eastern chipmunk	88.9% (18)	33	33	91.7% (8.3%)	88.9% (0.861 – 0.913)

*Reservoir competence of a given species is calculated as the average infection prevalence of *A. phagocytophilum* in attached larvae sampled from *A. phagocytophilum* -infected hosts.

**Realized reservoir competence is calculated as the mean percentage of larvae infected with *A. phagocytophilum* by an individual host of a given species (Keesing et al., 2012), taking into consideration that not all individuals are infected.

Factors affecting the infection status of a host

A logistic regression model indicated that host species (Wald's test p-value = 3e-09), *B. burgdorferi* infection status (Wald's test p-value = 6.4e-05), trapping week (p-value = 0.000769), sex (Wald's test p-value = 0.01), trapping grid (Wald's test p-vale = 4.9e-07) and mass (Wald's test p-value = 0.02) were significant factors for predicting *A. phagocytophilum* infection status (Appendix, Table 3.8). The nymphal burdens on hosts were not able to predict the variation in *A. phagocytophilum status* or the infection prevalence of host species (Figure 3.3, Appendix, Table 3.9)

The odds of individuals being infected with *A. phagocytophilum* increased significantly by 2 times (95% CIs = 1.42 – 2.83, p-value = 8.62E-05) when individuals were infected with *B. burgdorferi*. Compared to white-footed mice, the odds of long-tailed shrew spp. being infected with *A. phagocytophilum* was 0.47 times less (95% CIs = 0.29 – 0.74, p-value = 0.001); the odds of meadow vole being infected was 15.3 times less (95% CIs = 1.75 – 173.5, p-value = 0.02); and the odds of the eastern chipmunk being infected was 23.44 times greater (95% CIs = 2.49 – 252.23, p-value = 0.005). The odds of females being infected with *A. phagocytophilum* was 1.5 times (95% CIs = 1.09 – 2.08, p-value = 0.013) lower compared to that of males. The odds of being infected with *A. phagocytophilum* decreased by 1 time (95% CIs = 1.00 – 0.99, p-value = 0.02) with a 1 g increase in mass (Appendix, Table 3.9). With the progression of capture week, the odds of being infected with *A. phagocytophilum* decreased by 0.95 times (95% CIs 0.92 – 0.98, p-value = 0.001). Compared to individuals captured on grid A, individuals trapped in grid B, the odds of being

infected were 2.89 times greater (95% CIs = 1.92 – 4.43, p-value = 5.93E-07) and for the individuals trapped on grid C, the odds of being infected increased by 1.5 times (95% CIs = 0.95 – 2.39, p-value = 5.93E-07).

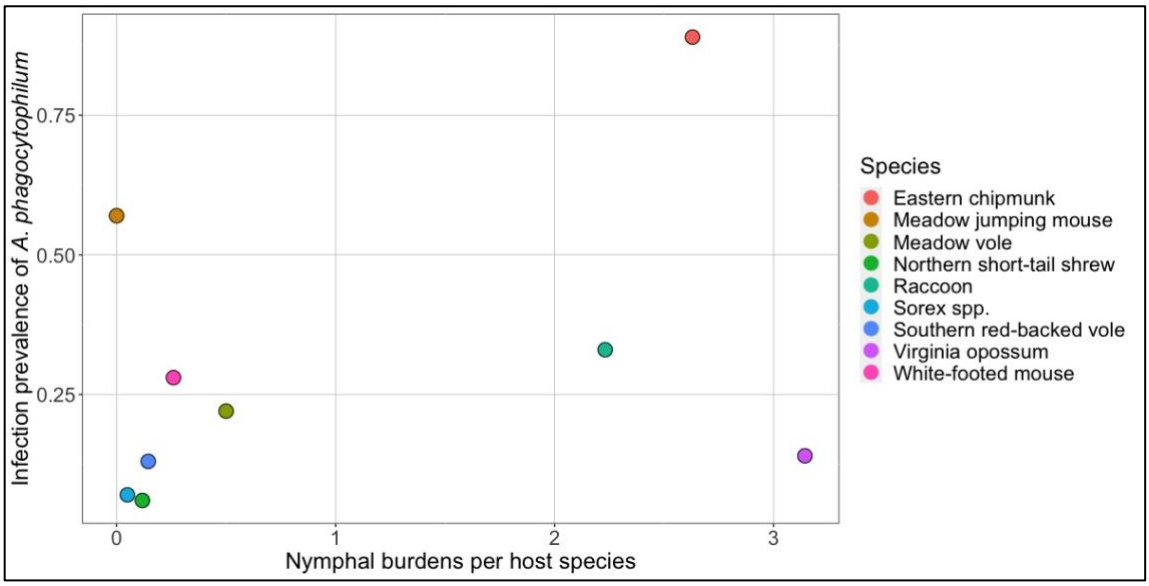

Figure 3.3. The relationship between the *Anaplasma phagocytophilum* infection prevalence and nymphal burdens among host species captured in Fort McCoy, Wisconsin from 2010 – 2012. The nymphal burden is the average number of nymphs per individual of a given host species.

Enzootic cycle: phenology of interactions between ticks, white-footed mice, and* A. phagocytophilum *infections

To examine the dynamics of infection between one generation of ticks to the next via a reservoir host, we focused on white-footed mice because they are considered an important reservoir host in the Northeast; there are an important host for juvenile blacklegged ticks at our field site, and because they formed the largest number of hosts we captured. Figure 3.4 depicts the phenology of questing *I. scapularis* ticks collected from Fort McCoy, Wisconsin averaged over the three study grids and years. The nymphal activity peaked in June and then gradually dropped

off as summer progressed, while the larval activity had a bimodal distribution with peaks in June and August (Figure 3.4).

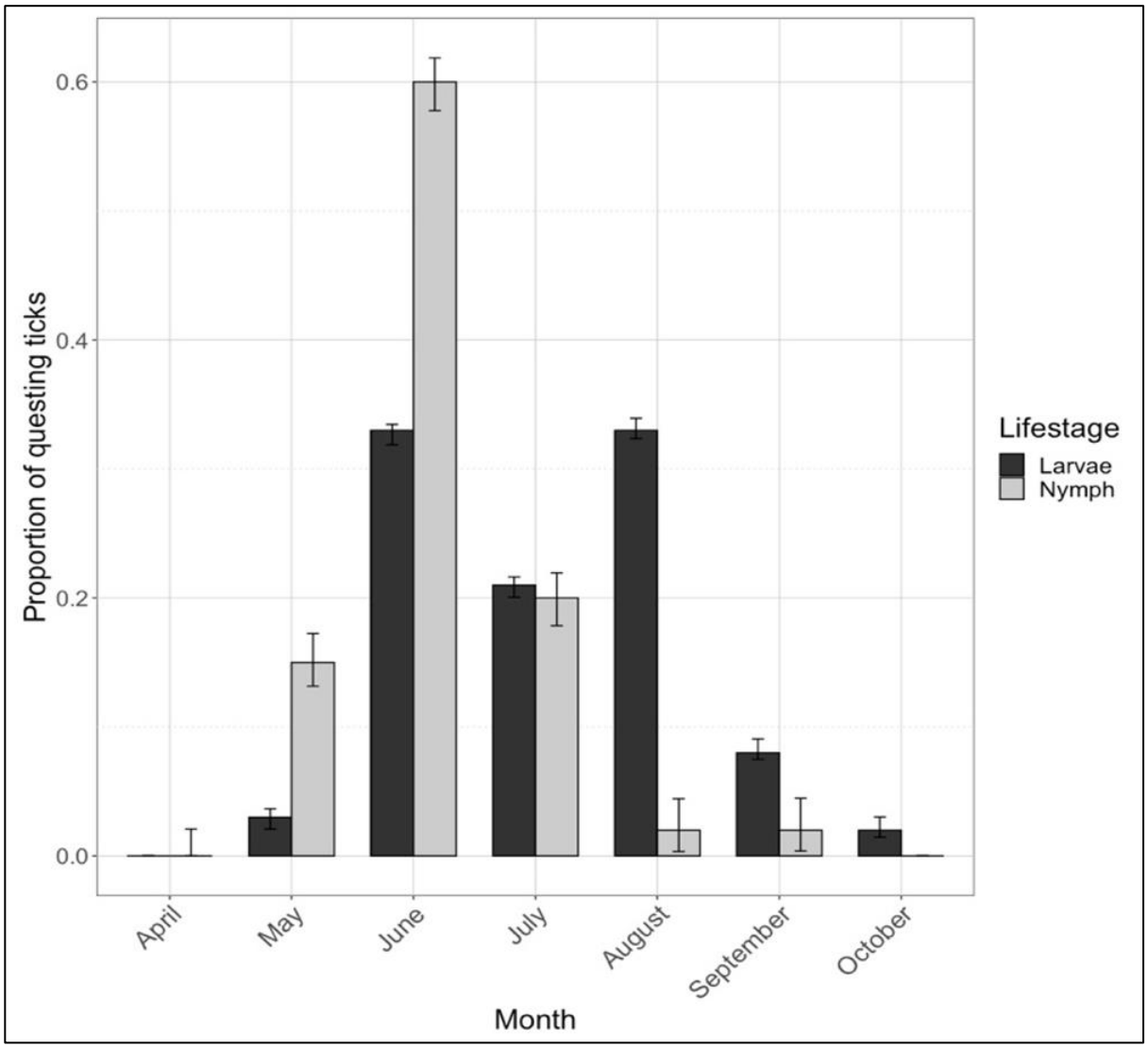

Figure 3.4. The phenology of questing *I. scapularis* ticks collected from Fort McCoy, Wisconsin from 2010 - 2012. The error bars show the 95% binomial confidence intervals.

Similarly, we looked at the phenology of on-host *I. scapularis* ticks parasitizing all mammalian hosts (during their first-time captures within a trapping period) from May to October (Figure 3.5). Nymphs and larvae were observed on hosts in every month hosts were captured. The

phenology pattern was very similar to the questing tick phenology. On-host nymphal proportions showed an increase initially from May to July, but as the summer progressed, the proportions on-

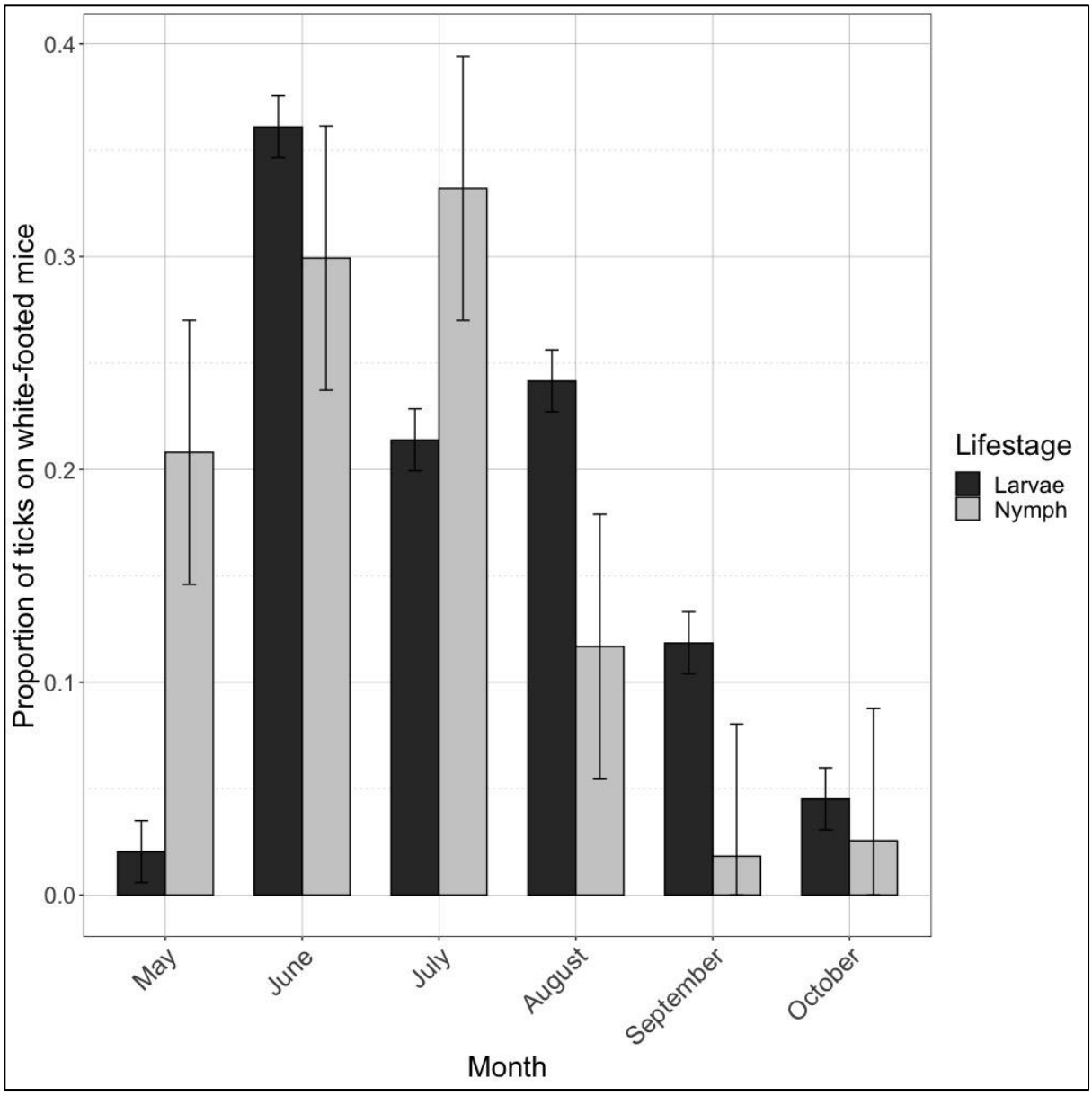

Figure 3.5. Phenology of *I. scapularis* parasitizing all mammal hosts captured at Ft. McCoy, Wisconsin, 2010-2012. The error bars represent the 95% binomial confidence intervals.

host decreased by about 50%. Similar to the questing larvae, on-host larvae show a bimodal phenology, where the proportions of on-host larvae peaked in June and August.

We next looked at the phenology of the infection prevalence of *A. phagocytophilum* for all mammals together with the phenology of infection prevalence of *A. phagocytophilum* in on-host larvae parasitizing all hosts (Figure 3.6). Although not statistically significant, the trend in infection prevalence of *A. phagocytophilum* in all mammalian hosts increased from May – July and then decreased as the summer progressed. The infection prevalence of on-host larvae for A. phagocytophilum shows a similar pattern to that in mammalian hosts (Figure 3.6); although not statistically different, there is a trend that the larval peak occurs one month earlier.

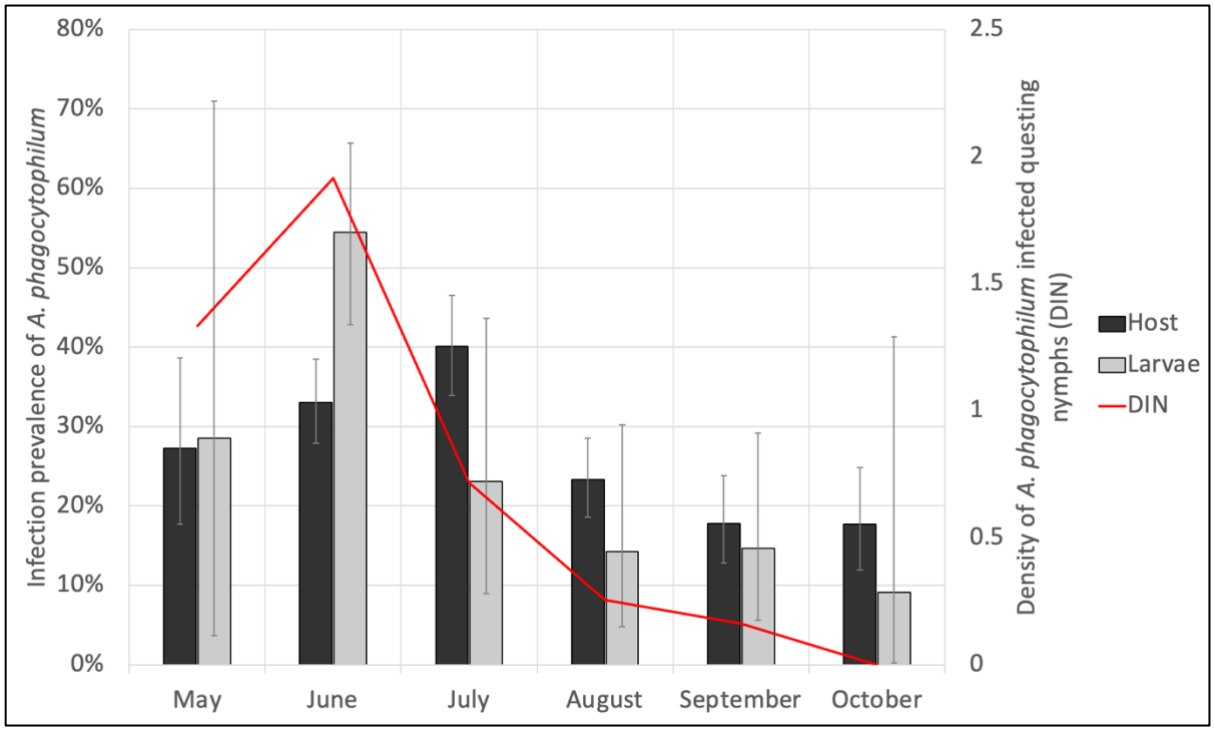

Figure 3.6. The phenology of infection prevalence of *Anaplasma phagocytophilum* in all captured mammals and on-host larvae and the density of questing infected nymphs (DIN) at Ft. McCoy, Wisconsin (2010-2012). The error bars represent the 95% binomial confidence intervals.

Mouse blood v. biopsy v. transmission of* A. phagocytophilum *to on-host larvae

In total, we assayed 1183 biopsy samples and 516 blood samples collected from 10

different host species (Table 3.4, Figure 3.7). The total host infection prevalence of *A. phagocytophilum* based upon all the biopsy samples was 18.7%, while based upon all blood samples, was 29.5% was significantly different (chi-square p-value < 0.00001).

There were 427 animals from which both the blood and biopsy samples were taken during the same capture event. When considering only those host species that had at least 5 capture events, of which the white-footed mouse comprised the majority (Table 3.4), there was no significant difference in infection prevalence of *A. phagocytophilum* when detected by blood only, biopsy only and both types of tissues (Fisher's exact test p-value ranged from 0.1 – 0.93) for each pairwise analyses.

Table 3.4. *Anaplasma phagocytophilum* infection assay results from hosts with both blood and biopsy samples collected during the same capture event at Fort McCoy, Wisconsin from 2010 - 2012. Species listed had at least 5 capture events from different trapping periods.

Host species	**Total number of capture events**	**Host tissues positive for *A. phagocytophilum* (infection prevalence %)**		
		Blood only	**Biopsy only**	**Both types of tissues (Blood and Biopsy)**
White-footed mouse	367	53 (14.4)	37 (10.1)	48 (13.1)
Raccoon	36	6 (16.7)	5 (13.9)	4 (11.1)
Eastern chipmunk	13	0 (0)	2 (15.5)	9 (69.2)
Virginia opossum	6	2 (33.3)	2 (33.3)	0 (0)
Southern red-backed vole	5	2 (40.0)	0 (0)	0 (0)

Because we had many *A. phagocytophilum* positives from blood and biopsy samples for white-footed mice (N=367), we conducted further analyses to compare blood and biopsy samples.

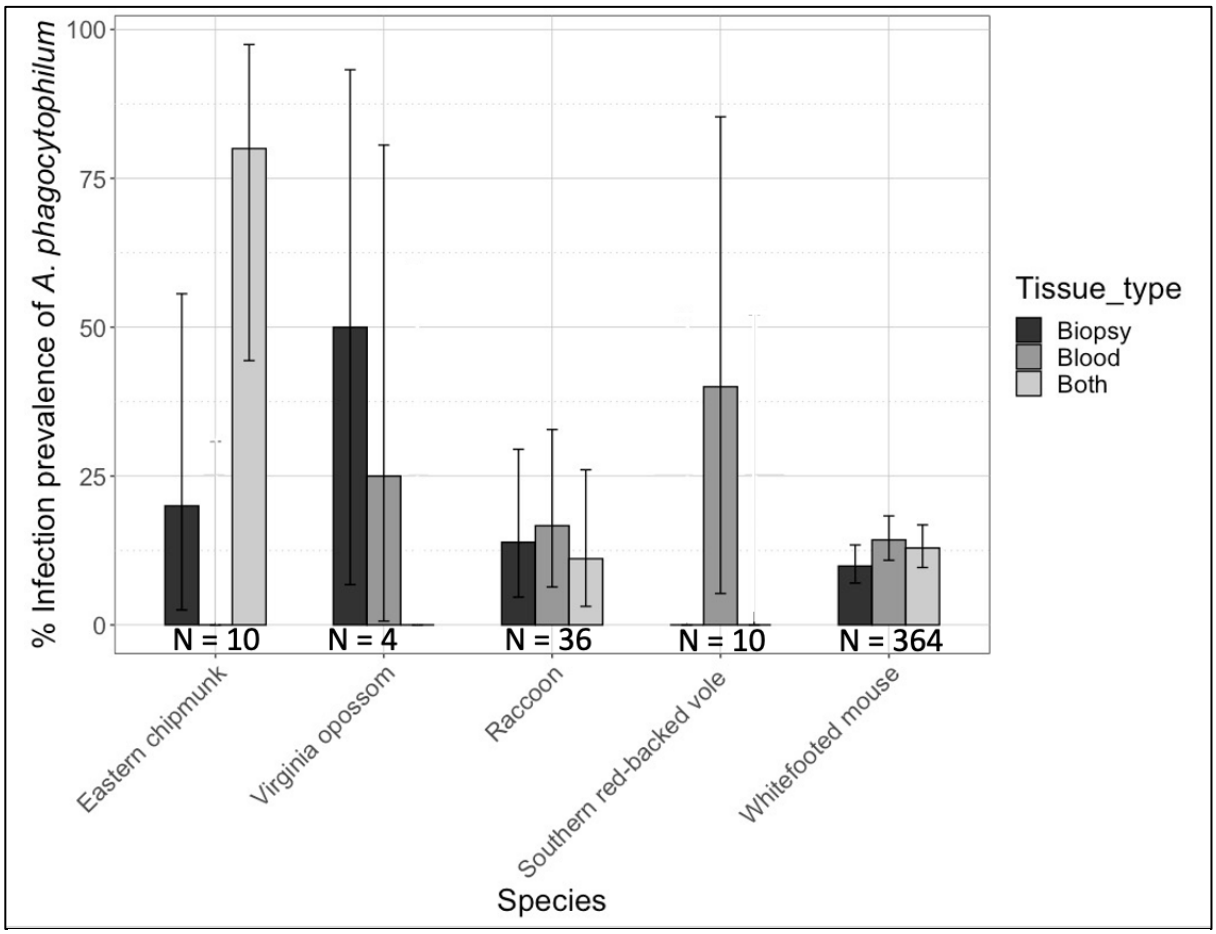

Figure 3.7. Infection prevalence (95% binomial CI) of *A. phagocytophilum* in host animals where both a blood and biopsy tissue were sampled from the same capture event from Fort McCoy, Wisconsin from 2010 - 2012. N represents the total number of paired samples tested where some proportion of pairs tested positive for A. phagocytophilum in only the biopsy, in only the blood, or only in both the blood and biopsy.

The infection prevalence of mice based on blood only, ear biopsies only, and both simultaneously were 14.4%, 10.1%, and 13.1% respectively. Overall, according to the pairwise Fisher's exact test

with a Bonferroni correction there was no significant difference between detection of *A. phagocytophilum* among the sample types (p-values ranged between 0.08 – 0.2). We then compared the infection prevalence of mice that were positive by blood or biopsy regardless of if they were also positive by the other sample. In this case, again, there was no significant difference

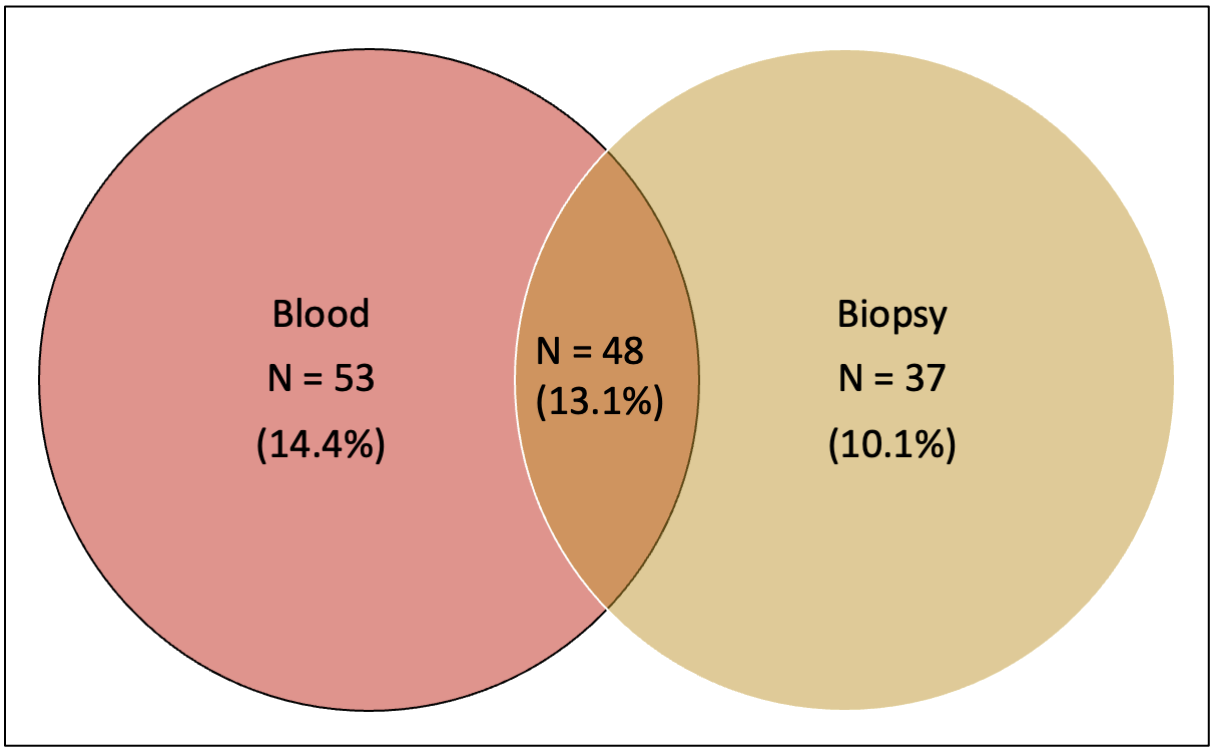

Figure 3.8. A Venn diagram of detections (%) of *Anaplasma phagocytophilum* from pairs of blood and biopsy samples collected from white-footed mice during the same capture event from Fort McCoy, Wisconsin (total N= 367 paired samples). In total, *A. phagocytophilum* was detected from 27.5% of blood (N = 53) and 23.2% of biopsy samples (N = 37).

between the infection prevalence of mice when considering blood (27.5%; 95% CL 23.1 – 32.4%) or biopsies (23.2%; 95% CL 18.9 – 27.8%, Fisher's exact value = 0.203 and $p < 0.05$).

We next looked at how the infection prevalence of mice changed from May to October (compiled over all three years and three grids) (Figure 3.9). According to the pairwise Fisher's

exact test with a Bonferroni correction, there was no significant difference in infection prevalence of *A. phagocytophilum* using blood only, biopsy only and both type of tissues simultaneously for each month (p-value ranged between 0.34 – 1).

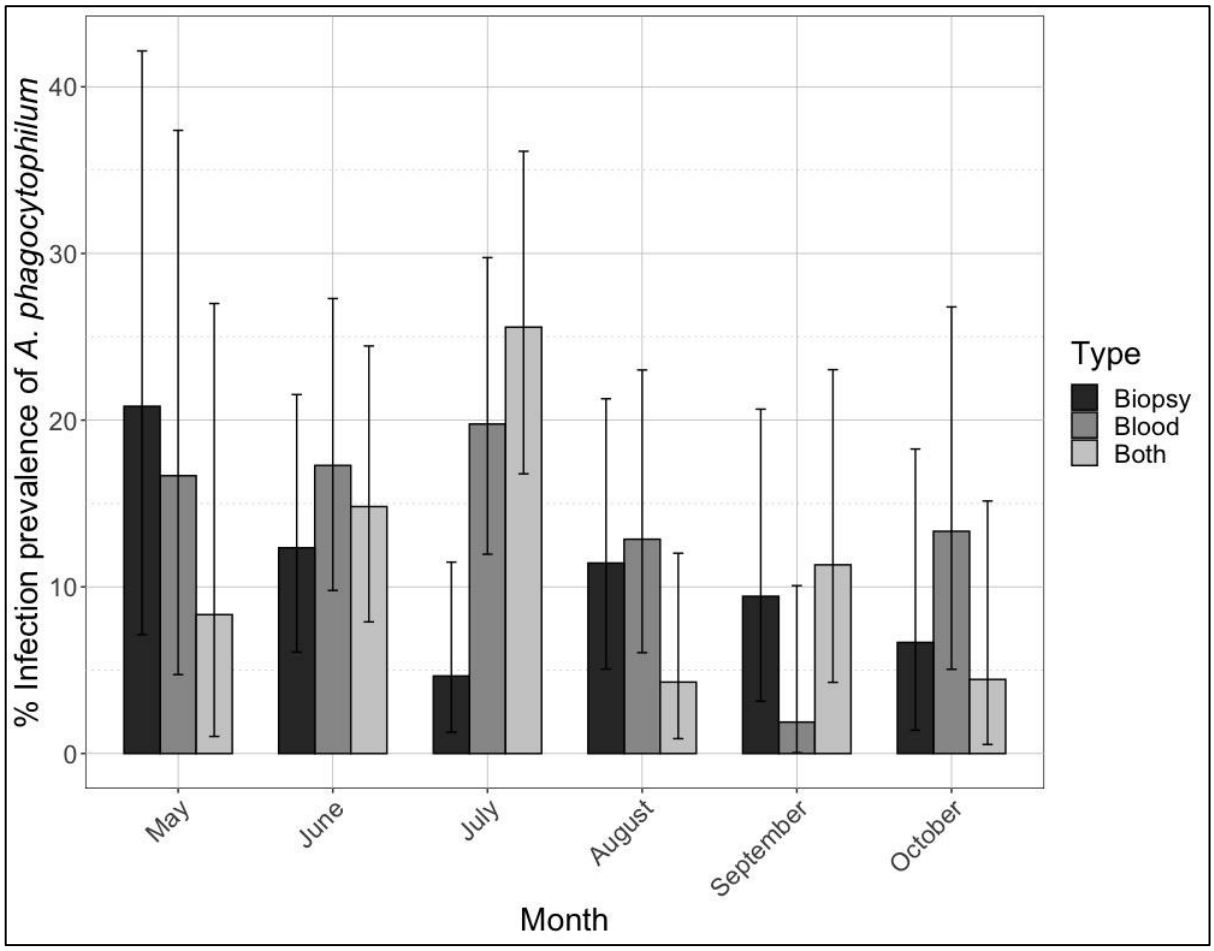

Figure 3.9. The infection prevalence of *Anaplasma phagocytophilum* in blood only, biopsy only and in both types of tissues simultaneously obtained from white-footed mice live captured at Fort McCoy, Wisconsin from 2010 - 2012. The error bars represent the 95% binomial confidence intervals.

We conducted a logistic regression analysis to examine whether detection of *A. phagocytophilum* in the blood only, biopsy only, or both tissue types simultaneously, was related

to the *B. burgdorferi* infection status, the numbers of nymphs, sex, mass, trapping week and trapping array. In the first model, where *A. phagocytophilum* infection status by blood only was the response variable, capture year was a significant variable (Wald's test p-value = 0.02), where the odds of being infected in 2012 was 2.69 times greater (95% CIs = 1.36 – 5.47, p-value = 0.005, Appendix, Table 3.10) compared to that in 2010. In the second model where *A. phagocytophilum* infection status by biopsy only was the response variable, the odds of being infected increased 1.08 times (95% CIs = 1.01 – 1.17, p-value = 0.034, Appendix, Table 3.11) with a 1 g increase in mass. In the third model where *A. phagocytophilum* infection status by both types of tissues simultaneously was the response variable, there was a significant difference in infection status among the capture years (Wald's test p-value = 0.006), where compared to being captured in 2010, the odds of being infected in 2012 was lower by 0.23 times (95% CIs = 0.09 – 0.54, p-value = 0.001, Appendix, Table 3.12). With the progression of capture week, the odds of detection of *A. phagocytophilum* in both tissues simultaneously decreased by 0.92 times (95% CIs = 0.86 – 0.98, p-value = 0.02, Appendix, Table 3.12). No other factors significantly predicted infection status in each of the three models.

We looked at the transmission of *A. phagocytophilum* from white-footed mice to larvae and how detection of *Ap* in each tissue type relates to the probability that a parasitizing larva would be infected. We developed a logistic regression model where the response variable in the model was the *A. phagocytophilum* infection status of a larva parasitizing an individual white-footed mouse, while the predictor variables were infection status for *A. phagocytophilum* by blood only, by biopsy only, or by both types of tissues simultaneously, the *B. burgdorferi* infection status of individuals, the number of parasitizing nymphs, sex, mass, trapping week and trapping array. In this model, the odds of detecting *A. phagocytophilum* in on-host larvae increased by 675 times

(95% CIs = 27 – 185660, p-value = 0.002, Appendix, Table 3.13) when *A. phagocytophilum* was detected in both types of tissues simultaneously compared to when *A. phagocytophilum* not detected only individuals. Also in this model, the odds of detecting *A. phagocytophilum* from on-host larvae increased by 1.47 times (95% CIs = 1.08 – 2.32, p-value = 0.04, Appendix, Table 3.13) with every 1 g increase of mass when *A. phagocytophilum* was detected in both types of tissues simultaneously.

To try to infer the kinetics of infection from field data, we analyzed the temporal pattern of detecting *A. phagocytophilum* in individuals that were captured at least twice during the summer from different trapping periods, which were at least 2, if not 3, weeks apart. Appendix, Figure 3.11. shows the chronology of positive and negative assay results for *A. phagocytophilum* of individual mice when captured by Julian date. In total there were 40 mice that were captured multiple times that were positive for *A. phagocytophilum* by at least one type of tissue (blood, biopsy, or both) during at least one capture event (Appendix, Figure 3.11).

As shown in Figure 3.11 there was no clear pattern in infection status of white-footed mice over time, but looking at general trends we noticed that animals become biopsy positive or remained biopsy positive at the end of summer (Panel A, Figure 3.11) between Julian days 250 – 300. Overall animals that become positive by blood only or remained blood positive and the animals that become positive by both tissues simultaneously and remained positive by both tissues simultaneously were found early to mid-summer (Panel B and Panel C, Figure 3.11) from Julian days 100 – 250. Most of the recaptured mice were not infected by both tissue types by the end of summer from Julian days 250 – 300. Early on in summer during the first 150 Julian days there were no animals that were infected by both types of tissues (blood and biopsy) at the same time.

We also looked at how long *A. phagocytophilum* would be detected in an individual by

biopsy, blood and both types of tissue samples. On average, *A. phagocytophilum* was not detected after ~37- 38 days, with the range being ~16 – 93 days since first detection of infection. From the 40 individuals that were recaptured, individuals who were positive by both types of tissues simultaneously, we were able to detect *A. phagocytophilum* for about 18 days (16 – 93 days); for animals that were positive by biopsy only we were able to detect *A. phagocytophilum* for 74 days (range 33 – 93 days); and for animals who were positive only by blood we were able to detect *A. phagocytophilum* for 36 days (range 34 – 37 days).

To try to infer transmission dynamics, using this same dataset, we used a logistic regression model to analyze if detection of an infected on-host engorged larva were related to trapping week, host infection status (0 = uninfected; 1 = biopsy only; 2 = blood only; 3 = blood and biopsy), sex, mass, nymphal burden, and trapping grid. In this model, the odds of larvae being infected with *A. phagocytophilum* significantly increased by 164.7 times (95% CIs value 17.6 – 4131.6, p-value = 1.4E-04) when mice were infected with *A. phagocytophilum* by both types of tissues simultaneously compared to individuals who were not infected, while the odds of larvae being infected with *A. phagocytophilum* significantly increased by 1.31 times (95% CIs value 1.07 – 1.70, p-value = 0.02, Appendix, Table 3.14) with every 1 g increase in mass.

DISCUSSION

The first detected *A. phagocytophilum*-infected patient in the US was reported from Wisconsin and Minnesota in the early 1990s (Chen *et al.*, 1994), and retrospective analysis of adult *I. scapularis* ticks collected from northern Wisconsin in 1982 and 1991 revealed that 10.3% were infected with *A. phagocytophilum* (Pancholi et al. 1995). *Anaplasma phagocytophilum* had not been detected previously in North America and was first named *Ehrlichia phagocytophila*, and the disease was called human granulocytic ehrlichiosis (HGE) (Chen *et al.* 1994). The Upper Midwest,

namely Wisconsin and Minnesota, continues to be one of two major foci for human and canine anaplasmosis in the US. To better understand the ecology of the enzootic cycle of *A. phagocytophilum* in the Upper Midwest, we took advantage of an on-going field study by our lab to investigate the ecology of the Lyme disease bacterium at Fort McCoy, Monroe County, in the central western region of Wisconsin (Ogden et al., 2018; Ginsberg et al., 2020). An established population of *I. scapularis* has existed at Fort McCoy since at least since 1983 (Godsey *et al.*, 1987), and several studies have demonstrated that *B. burgdorferi, A. phagocytophilum,* and other *I. scapularis*-borne pathogens are endemic at Ft. McCoy (Anderson, Duray and Magnarelli, 1987; Belongia *et al.*, 1997; Steiner *et al.*, 2008; Stromdahl *et al.*, 2014; Han, Hickling and Tsao, 2016; Han *et al.*, 2021). Prior investigations of *A. phagocytophilum*, however, either were focused only on ticks, or were conducted in the late 1990s, when *A. phagocytophilum* had not been detected and/or was present at below the level of detection in those studies. Here we had the opportunity to study the enzootic cycle where the prevalence of infection of *A. phagocytophilum*, and specifically that of the human pathogenic variant (*Ap*-ha), is quite robust. From a field study carried out over three years, we estimated the infection prevalence of *A. phagocytophilum*-ha in questing *I. scapularis* ticks; tick burdens on several host species; and compared the realized reservoir competence (Keesing *et al.*, 2012, 2014) for *A. phagocytophilum* in several common small to medium-sized mammalian hosts that often-feed *I. scapularis* juvenile ticks. To infer the temporal dynamics of *A. phagocytophilum* transmission between *I. scapularis* and reservoir hosts, we characterized the phenology of *A. phagocytophilum* infection among questing nymphal *I. scapularis*, hosts, and attached larval *I. scapularis*. Finally, we also compared the detection of *A. phagocytophilum* between blood and ear tissue biopsies and related that to the detection of infected attached larval ticks.

Fort McCoy, an epidemiologically risky site for human granulocytic anaplasmosis

The estimates for nymphal (15.3%, 12.6 - 18.2 95% CI) and adult (20.4%, 17.3 - 23.8 95% CI) infection prevalence for *A. phagocytophilum* observed at Ft. McCoy, Wisconsin from 2010 – 2012 are at the upper range of that previously reported in the literature for sites throughout Wisconsin (Pancholi et al. 1995; Lee et al., 2014; Murphy et al., 2017; Stauffer et al., 2020; Westwood et al., 2020), the Upper Midwest (Lehane *et al.*, 2021; Burtis *et al.*, 2022; Foster *et al.*, 2022), as well as in the Northeast and Mid-Atlantic states (Lehane *et al.*, 2021).

Limited data from questing adult blacklegged ticks collected at Ft. McCoy suggests that *A. phagocytophilum* may have been emerging at Fort McCoy over the last two to three decades. Jackson et al., 2002 reported no detection from 713 adults *I. scapularis* ticks (nor 104 blood samples collected from white-footed mice) in 1997. The infection prevalence was 14% (n=100, 14% 95% CI 8.53-22.14) in adult ticks sampled in 2006 (Steiner et al., 2008) and 11.44% (n = 341, 8.48 – 15.42 95% CI) in 2006-2007 (Hamer et al., 2014). These data support the trend seen in Foster et al., 2022, which reports a marginally significant increase in adult infection prevalence for *A. phagocytophilum* at established sites that were sampled repeatedly from 2005-2019 in Minnesota and Wisconsin.

Historically, most studies (Lehane *et al.*, 2021) have not genetically differentiated *A. phagocytophilum* when detected in ticks or wildlife. As Massung et al., 1998 writes, however, lack of differentiation may be misleading since *Ap*-v1 has not been associated with human (nor canine) disease. In our study, apart from one nymph that harbored *Ap*-v1 (a deer variant strain), all infected questing nymphs and adults we collected harbored the *Ap*-ha variant. This finding agrees with that of the few prior investigations in Wisconsin where *A. phagocytophilum* has been typed (Steiner *et al.*, 2008; Lee *et al.*, 2014; Murphy *et al.*, 2017), perhaps contributing to high anaplasmosis risk to

both humans and companion animals in this state. The prevalence of infection with *Ap*-ha in questing ticks appears to be more variable in the Northeast (Yeh *et al.*, 1997; Courtney *et al.*, 2003; Keesing *et al.*, 2014; Edwards *et al.*, 2019; Jordan, Gable and Egizi, 2022), where there are areas where *Ap*-v1 predominates (Massung *et al.*, 1998; Edwards *et al.*, 2019; Jordan, Gable and Egizi, 2022; Prusinski *et al.*, 2023). More research is needed to understand under what conditions *Ap*-ha prevalence is high. One hypothesis is that *Ap*-v1 (and other variants) may compete with and reduce the prevalence of infection of *Ap*-ha, like that seen between different species of *Rickettsia* bacteria and *Rickettsia rickettsii* (Massung *et al.*, 1998).

Ixodes scapularis *parasitizing wildlife at Fort McCoy, Wisconsin*

Ixodes scapularis is known to feed on many different host species, including mammals, birds, and lizards (Keirans *et al.*, 1996). The juvenile stages of *I. scapularis* are responsible for the enzootic maintenance of non-vertically transmitted pathogens like *B. burgdorferi* and *A. phagocytophilum*. In our original study, Fort McCoy was one of eight field sites, where the objective was to compare the ecology of *I. scapularis* and *B. burgdorferi* over a latitudinal gradient from Wisconsin and Massachusetts to Florida. Specifically, the study was conducted to elucidate the tick-microbe-wildlife host interactions, focusing on the juvenile stages, as they are responsible for the enzootic maintenance of *B. burgdorferi,* which like *A. phagocytophilum*, is not vertically transmitted from adult females to larvae. Based on the known ecology at the time, limited resources, and capacity, we focused only on small and medium-sized mammals. Note, not reported in this dissertation, using the same PCR assays as reported here for Ft. McCoy, I detected 0% infection prevalence in both adults (N = 44) and nymphs (N = 9) at our field site in Tennessee and 4.9% in adults (N=142) and 2.3% (N = 256) in nymphs at our field site in Massachusetts respectively, supporting the clinal distribution of *A. phagocytophilum* observed by others (Lehane

et al. 2021).

Using a variety of capture methods, over three years we captured 2151 hosts, comprising 14 species of small and medium-sized hosts. Most hosts comprised of small mammals, and of those, the white-footed mouse was frequently captured. The larval and nymphal burdens on captured hosts at our site resembled that previously reported where larvae most commonly are observed infesting small mammals such as white-footed mice, whereas nymphal life stages commonly infest larger hosts, including eastern chipmunks, raccoons and opossums (Anderson and Magnarelli, 1980; Davidar, Wilson and Ribeiro, 1989; Mather et al., 1989; Wilson et al., 1990; Shaw et al., 2003; Brunner and Ostfeld, 2008; Barbour et al., 2015; Jones et al., 2015). In particular, white-footed mice had the greatest average number of larvae per individual animal (4.86), while eastern chipmunks had the greatest average number of nymphs per individual (2.63), trends supported at other sites and studies (Mannelli *et al.*, 1993; Slajchert *et al.*, 1997; Schmidt, Ostfeld and Schauber, 1999; Shaw, Ostfeld and Keesing, 2001; Hamer *et al.*, 2010; Han *et al.*, 2021; Sidge *et al.*, 2021) although differences between mouse and chipmunk larval loads may vary and may not be that different in some scenarios (e.g., Sidge et al. 2021, Keesing et al. 2009). Southern red-backed voles were the second most captured host species, but they had fewer on-host larvae and nymphs compared with white-footed, which has been seen in other studies (Main *et al.*, 1982).

Female hosts had significantly lower larval and nymphal infestations compared to males, which has been reported in other studies (Schmidt, Ostfeld and Schauber, 1999; Shaw, Ostfeld and Keesing, 2001; Brunner and Ostfeld, 2008; Jones *et al.*, 2015). It has been hypothesized that males have more parasites on them on average, perhaps due to having relatively larger home ranges and having differences in reproduction and growth rates compared to females (Moore and Wilson, 2002; Krasnov *et al.*, 2005; Butler *et al.*, 2020). Temporally, although the three sampling grids

were similar in habitat and the number of questing larvae and questing nymphs collected were similar, but on-host larval and nymphal counts were lower in 2011. Seasonally, the phenological patterns of questing and on-host nymphs and larvae reflected the synchronous pattern, generally peaking in July, described for the Upper Midwest (Gatewood *et al*. 2009).

Hosts infected with* A. phagocytophilum *in Fort McCoy, Wisconsin

Because there is no transovarial transmission for *A. phagocytophilum*, its enzootic cycle is maintained through horizontal transmission. Naïve larvae may acquire *A. phagocytophilum* by feeding on an infected host. Alternatively, although there are contradictory results in the laboratory studies (Levin and Fish, 2000b, 2000a), field data suggest that blacklegged ticks may acquire *A. phagocytophilum* through non-systemic transmission by co-feeding in proximity with an infected nymph on the same host (Levin, Des Vignes and Fish, 1999; Levin and Fish, 2000b, 2000a). Conversely, the hosts can only become infected through the bite of an infected nymphal tick which obtained the pathogen as a larva. Thus, wildlife species that tend to be infested by both nymphs and larvae facilitate the maintenance of the enzootic cycle of *A. phagocytophilum.*

At our study site the overall infection prevalence of mammals for *A. phagocytophilum* was about 24% (N = 1248). To our knowledge, this study is the first to investigate the infection prevalence of *A. phagocytophilum* among a community of small to medium sized mammals in the Midwest, and thus we cannot compare trends at the community level. If we focus on white-footed mice, our study found ~28% of the white-footed mice (n = 755) captured from 2010 – 2012 infected with the human pathogenic strain of *A. phagocytophilum*. Two studies conducted in the same region in Wisconsin but 10-14 years prior to ours found no white-footed mice infected with *A. phagocytophilum*. These studies were conducted at several study sites located near La Crosse, Wisconsin (Jackson *et al.*, 2002) and within Fort McCoy, Wisconsin (Hofmeister *et al.*, 1998).

These studies may represent a time just prior to the emergence of *A. phagocytophilum*.

A more contemporaneous study conducted in northern Wisconsin from 2012 - 2014 found 1.7 % (n = 237) of the white-footed mice infected with *A. phagocytophilum* (Larson, Lee and Paskewitz, 2018); this difference may also reflect the emerging nature of *A. phagocytophilum* or ecological differences in host communities or climate influencing enzootic dynamics. A more recent study in north-central Wisconsin (2018-2019) showed that only 1% (n = 94) white-footed mice and 0.6% (n=318) deer mice (*Peromyscus maniculatus*), a closely related species, to be infected with *A. phagocytophilum* (Larson *et al.*, 2021), As the authors point out, the first case of anaplasmosis from a human patient was detected in the county of their study, and thus in this case, it does not appear that *A. phagocytophilum* infection prevalence, at least in the small mammal community twenty years later, is very high. In Minnesota infections of *A. phagocytophilum* in white-footed mice varied from 11.4% (n = 158) in 1995 (Walls *et al.*, 1997) to 46.8% (n=98) in 2000 and 20% (n=150) in 2001 at Camp Ripley, Minnesota (Johnson *et al.*, 2011) Although these are only two studies, they may suggest that *A. phagocytophilum* became established earlier in eastern Minnesota.

In the Northeast several studies have estimated *A. phagocytophilum* infection prevalence and reservoir competence within mammal communities and the infection prevalence ranged between 14.1% - 57.9% (Stafford *et al.*, 1999; Levin, Nicholson, Massung and Fish, 2002; Massung *et al.*, 2002; Keesing *et al.*, 2012, 2014). Realized reservoir competence of a host species is estimated as the mean percentage of ticks infected by an individual of that host species (Keesing *et al.*, 2012), and reflects on an animal's ability to become infected by a pathogen and the ability to transmit the pathogen to a competent vector. According to Keesing et al., 2012, in a study conducted in southern New York, it was found that white-footed mice, eastern chipmunks, and short-tailed

shrews had greater realized reservoir competence for *A. phagocytophilum* (> 10%), compared to the other small to medium size mammals (as well as a few bird species captured at their study site in upstate New York). At our field site, the infection prevalence of the eastern chipmunk was significantly greater than any other small to medium-sized mammals captured at our field site and the eastern chipmunk had a higher realized reservoir competence compared to the white-footed mouse. In our study all engorged larvae that were tested from infected eastern chipmunks were infected with *A. phagocytophilum*, whereas not all engorged larvae collected from infected white-footed mice were infected. The realized reservoir competence values for both white-footed mouse and eastern chipmunk are comparable to those given in Keesing *et al*, 2012. All our host animals were infected with only *Ap*-ha which was slightly different from what Keesing *et al,* 2014, where most mammals species supported both strains, although most species had a higher reservoir competence for *Ap*-ha. A possible reason for this difference may be due in part to most questing ticks at our field site harboring mainly the *Ap*-ha strain as mentioned previously.

The greater infection prevalence observed in eastern chipmunks compared with other species we captured and tested may be due to having on average higher nymphal burdens (Table 1, Figure 3). But interestingly nymphal burdens were not important in determining the infection status of *A, phagocytophilum.* Nymphal *I. scapularis* is the main life stage that is responsible for the transmission of *A. phagocytophilum* to small and medium-sized mammals. Having greater nymphal burdens ostensibly would result in a greater chance of *A. phagocytophilum* transmission to hosts. If the duration of detection of *A. phagocytophilum* in the blood and/or tissue were longer in eastern chipmunks, that might also result in having a greater infection prevalence compared to the white-footed mice. Although no laboratory transmission studies have been conducted with eastern chipmunks, in laboratory infected white-footed mice were able to launch an immune

response against *A. phagocytophilum* within 2 weeks of infection and antibodies remained in the blood for several months (Levin and Fish, 2000b). Furthermore, xenodiagnosis experiments using laboratory mice (*Mus musculus*) showed that majority of transmission to larvae occurred around 1-2 weeks post-infection of the mice although there was some variation among *A. phagocytophilum* isolates (Levin and Ross, 2004). Interestingly, there has been one study conducted on the congener redwood chipmunk (*Tamias ochrogenys*) in California, which was found to be PCR positive for *A. phagocytophilum* between four to seven weeks; and more importantly, even after 30 days post-infection was able to transmit *A. phagocytophilum* to xenodiagnostic larvae (Nieto and Foley, 2009). Thus, perhaps the duration of infection lasts longer in eastern chipmunks. Another hypothesis to explain the higher infection prevalence observed in eastern chipmunks is how the shorter life span and relatively higher reproductive rate of white-footed mice may dilute the infection prevalence over the season, especially if many offspring are born after the peak nymphal questing period.

Even if eastern chipmunks were to have higher realized reservoir competence, however, white-footed mice may still play a greater role in terms of their relative contribution to the enzootic cycle of maintaining *A. phagocytophilum*. Given their high relative densities in habitats where *I. scapularis* is abundant, white-footed mice feed a relatively greater number of larvae compared to any other host species (Figure 2) and thus may transmit *A. phagocytophilum* to a greater number of larvae compared to other host species. In our study, we used multiple capture methods (Sherman live traps, pitfall traps, cover boards) with different capture biases, which may have allowed us to catch a greater diversity and number of small mammals compared to just using Sherman live traps. Amongst the species we captured, our relative numbers of animals caught per species suggest that the abundance of white-footed mice was likely greater compared to other host species captured.

With greater abundance and greater larval burdens, white-footed mice thus may contribute more to the maintenance of *A. phagocytophilum* compared to other hosts we trapped at our study site at Ft. McCoy, Wisconsin.

Regarding the factors that might affect the infection status of a an individual, we found that that the *B. burgdorferi* infection status, host species, sex of the host species, mass of hosts species, trapping array and capture week were significant variables that explained the variation seen in the *A. phagocytophilum* infection status. In our model individuals who were infected with *B. burgdorferi* had two times the odds of being infected with *A. phagocytophilum*, which may not be surprising since both pathogens share the same vector and several reservoir host species.

As seen by the estimates of infection prevalence, eastern chipmunks had greater odds of being infected with *A. phagocytophilum* compared to white-footed mice. Furthermore, in our comparison meadow vole and *Sorex* spp. had lower odds of becoming infected. Both these species, however, we did not have a larger sample size to determine accurately the infection prevalence. For meadow voles, the habitats we trapped may not have been optimal for them. In comparing the infection status between males and females, females had lower odds of being infected compared to males, for reasons discussed previously. A laboratory study reported that male laboratory mice had significantly higher loads of *B. burgdorferi* as well as higher prevalence in ear tissue (Zinck *et al.*, 2022). The variation in *A. phagocytophilum* infection dynamics within hosts among different species and between sexes similarly should be further explored to understand factors influencing heterogeneities in transmission to larval *I. scapularis*.

***Phenology of ticks and infection of* A. phagocytophilum**

To better understand the dynamics of *A. phagocytophilum* transmission between *I. scapularis* and wildlife hosts, we investigated the phenology of questing and on-host *I. scapularis*,

infection of *A. phagocytophilum* among the host community, and infection of *A. phagocytophilum* in on-host larvae. Phenological patterns of immatures are important to consider given the duration of infectivity of wildlife reservoir hosts for *A. phagocytophilum*, which is not vertically transmitted and for which some co-feeding, non-systemic transmission may occur. For *A. phagocytophilum* to be maintained enzootically, naïve larvae must acquire *A. phagocytophilum* from hosts that had been previously infected by an infected nymph (i.e., systemic transmission) or must co-feed near an infected nymph on the same host (i.e., non-systemic transmission). The shorter the duration of infectivity of the reservoir host, the more important the amount of overlap (or synchrony) between the nymphal and larval host-seeking activity periods. For instance, for the Lyme bacterium, *B. burgdorferi*, many epidemiologically important strains can infect white-footed mice for life. In this case, the duration of infectivity of a mouse is less important; larvae can host-seek later compared to nymphs; and factors such as survivorship and reproduction rates of the mouse population may be more important. At the other extreme, for tick-borne encephalitis (in Europe) and perhaps Powassan encephalitis virus (in North America), where co-feeding non-systemic transmission is believed to be the main route of horizontal transmission, enzootic maintenance necessitates great overlap of nymphal and larval host-seeking periods.

A transmission experiment with laboratory mice (*Mus musculus*) showed that transmission to naive larvae (i.e., xenodiagnostic larvae) can occur for at least twelve weeks for some *A. phagocytophilum* isolates (Levin and Ross, 2004) but that higher transmission efficiency occurs 1-3 weeks post-infection, where 70-81% of xenodiagnostic larvae are infected. A second peak of transmission occurs about 3.5-7 weeks post-infection, when 36-64% of xenodiagnostic larvae become infected. In another experiment with laboratory bred white-footed mouse, Levin, and Fish (2000) presented data that suggest that mice can clear the infection by 11- and 15-weeks post-

infection. Furthermore, partial immunity reduces susceptibility to infection, transmission efficiency (in mice that do become infected) to xenodiagnostic larvae (~6-7% v. 83%) (Levin and Fish 2000), as well as non-systemic transmission. The percentage of larvae that acquired *A. phagocytophilum* from co-feeding infected nymphs was 10% on naïve mice and 1% on mice that had been infected previously (Levin and Fish, 2000a, 2000b).

The phenologies of nymphal and larval host-seeking at Ft. McCoy resemble that previously observed for the Upper Midwestern region, where generally, there is broad overlap in the nymphal and larval host seeking phenologies (Gatewood *et al.*, 2009; Hamer, Hickling, *et al.*, 2012; Ogden *et al.*, 2018). Given what is known about *A. phagocytophilum* infection and infectivity dynamics, the synchrony in nymphal and larval host-seeking phenologies at Ft. McCoy, and the Midwest in general, may facilitate both systemic and co-feeding transmission of *A. phagocytophilum*. This phenology of larvae differs from that typically found in the Northeast, where there is generally one peak of host-seeking larvae which occurs in late summer/early fall, about 2-3 months after the nymphal peak, and thus, where co-feeding transmission between nymphs and larvae may not occur as frequently (Gatewood *et al.*, 2009; Hamer, Hickling, *et al.*, 2012; Ogden *et al.*, 2018).

When we examine the phenology of the infection prevalence of *A. phagocytophilum* within the host community, it appears to lag by one month from the phenology of the density of questing infected nymphs and appears to match better the phenology of on-host nymphs. If we extrapolate from lab experiments with mice to the whole captured host community (which predominantly comprised white-footed mice), given peak infectivity of a host to larvae may occur 1-3 weeks post-exposure to an infected nymph, then the temporal trends in host infection prevalence generally suggest that at our study site, more than 60% of hosts are infected by July. After July, hosts still are acquiring infection, but at a lower rate, reflecting the decline in the density of questing infected

nymphs and on-host nymphs (Figure 6). A study conducted in Minnesota reported similar phenology in infected white-footed mice where the infection prevalence increased from May to June and then decreased as summer progressed (Johnson *et al.*, 2011).

Especially as we are uncertain about the relative abilities of hosts to transmit *A. phagocytophilum* based on detection of the DNA from blood, ear biopsy, and/or both tissue types, it is probably most informative to examine the phenology of acquisition of *A. phagocytophilum* by on-host larvae. Even though *A. phagocytophilum*-positive on-host larvae are detected May through October, a much larger proportion occur in the first half of the season - specifically, about 58 and 74% of all on-host larvae that test positive for *A. phagocytophilum* are collected by June and July respectively. This may reflect in part the phenology of on-host larvae, but interestingly the proportion of larvae in which *A. phagocytophilum* is detected appears to peak earlier than that in the host population. Though certainly not definitive, these data support the hypothesis that co-feeding transmission might contribute to the enzootic maintenance of *A. phagocytophilum*. The decline in infection in hosts and on-host larvae later in summer may reflect the clearance of *A. phagocytophilum*, reduced rate of exposure to infected nymphs (exacerbated by the birth of susceptible hosts), reduced susceptibility of hosts to re-infection and co-feeding transmission due to partial immunity (Levin and Fish 2000).

In the Northeast, most larval activity peaks about 2.5 months after the nymphal activity peak. Thus, all else equal, phenological differences in *I. scapularis* activity between the upper Midwest and the Northeast should result in lower nymphal infection prevalence for *A. phagocytophilum*, but this has not been observed in limited data (Lehane et al 2021), and thus other factors (e.g., different strains, host differences in duration of infection, different host communities) may also be important. In study conducted between 2008 – 2012 the human granulocytic anaplasmosis case

incidence was higher in the Upper Midwest states compared to the Northeast (Dahlgren *et al.*, 2015), but this no longer appears to be the case (Centers for Disease Control and Prevention, 2022). Thus, again, other factors, including those above as well as overall densities of ticks and differences in human behavior may be important.

Biopsy vs Blood assays: What could we use to assay for* A. phagocytophilum *in field studies?

Because *A. phagocytophilum* is a blood borne pathogen, for field studies involving wildlife, blood samples are generally collected. Our study determined that the animals frequently can be rt-PCR positive not just by blood but also by ear biopsy tissues. We compared assay results from matched ear biopsy and blood samples taken during the same capture event and found no significant difference in infection prevalence although pathogen detection results were not always congruent for samples taken at the same time. We then compared the infection prevalence by blood only, biopsy only and both tissues simultaneously for a few hosts species and within white-footed mice. Again, there was no significant difference in infection prevalence of *A. phagocytophilum*. We also did not see any difference in the phenology of infection prevalence by blood, biopsy or both types of tissues.

We next considered just white-footed mice since we had relatively large sample size of matched individuals, and conducted a logistic regression analysis to see if the host factors or environmental factors would affect the likelihood that an individual would be infected by blood only or biopsy only or both types of tissues simultaneously. In each model, we had different factors that came out as significant, but it is unclear what these factors reveal about the infection dynamics of *A. phagocytophilum* by blood, biopsy or both types of tissues simultaneously.

When considering transmission of *A. phagocytophilum* to larvae from white-footed mice we wanted to see if there were any association with the detection of *A. phagocytophilum* by tissue

type. Our model showed that when an individual mouse was infected with both types of tissues simultaneously, the odds of detecting *A. phagocytophilum* in on-host larvae were 675 times greater (95% CIs = 27 – 185660, p-value = 0.002) than if *A. phagocytophilum* were detected in only the blood or the biopsy. It could mean that when *A. phagocytophilum* is detected in both types of tissues at the same time, there may be a higher load of bacteria within the hosts and therefore the probability of transmission is higher. The best method to further examine these factors would be to conduct a xenodiagnostic lab experiment like that of Levin and Fish 2000 and Levin and Ross *et al.* 2004, where one would infect white-footed mice with *A. phagocytophilum* and simultaneously assay biopsy, blood, and transmission efficacy to larval *I. scapularis* for at least 16 weeks, a time frame that would simulate natural enzootic dynamics in the Upper Midwest.

Several studies in Europe have considered using ear biopsy samples over blood samples because *A. phagocytophilum* might be short-lived in the blood (J S Liz *et al.*, 2000; Kevin J. Bown *et al.*, 2003; Bown *et al.*, 2008; Baráková *et al.*, 2014; Rosso *et al.*, 2017), which might explain why there might be relatively fewer animals infected by blood only later in the season. To test this hypothesis, conducting a laboratory transmission experiment using white-footed mice and measuring the length of detection *of A. phagocytophilum* by blood and biopsy would be useful. A study conducted on dogs found that even after dogs seroconverted, *A. phagocytophilum* could be detected in ear biopsies (Berzina *et al.*, 2014), and it was hypothesized in separate study that the skin may be a site for persistent infection of *A. phagocytophilum* (Ladbury *et al.*, 2008; Granquist, Aleksandersen, *et al.*, 2010) which could be the reason for our observations in this study. But currently no study exists to explain how *A. phagocytophilum* can persist in the skin.

Anaplasma phagocytophilum is the second most common vector-borne disease in the United States (Adams *et al.*, 2017). The human case incidence of granulocytic anaplasmosis in the

US drastically increased from ~ 340 cases in 2000 to over 5600 cases in 2019 (Biggs, Behravesh, K. Bradley, *et al.*, 2016; Adams *et al.*, 2017; Dumic *et al.*, 2022b). With the increase in and expansion of I. *scapularis* populations, the human disease incidence of granulocytic anaplasmosis will increase, therefore investigating the ecology of *A. phagocytophilum* should help in prevention of human granulocytic anaplasmosis (e.g., how well would bait tubes designed to kill *I. scapularis* on mice reduce anaplasmosis risk to humans?). Our investigation focused on examining dynamics over three years at one site where *I. scapularis* and associated diseases have been endemic. Given the variation in climate and vegetation throughout the Midwest, more ecological studies should be conducted to better understand factors affecting the heterogeneity in the enzootic cycles leading to variation in spatial risk of anaplasmosis. Future studies should include bird species known to harbor *I. scapularis* ticks, especially to better appreciate the potential of birds for dispersing infected *I. scapularis* ticks into emerging regions. Additional studies examining strain diversity and wildlife host species in the Midwest may help to further understand heterogeneity of *A. phagocytophilum* risk and predict risk as it continues to emerge throughout the eastern U.S.

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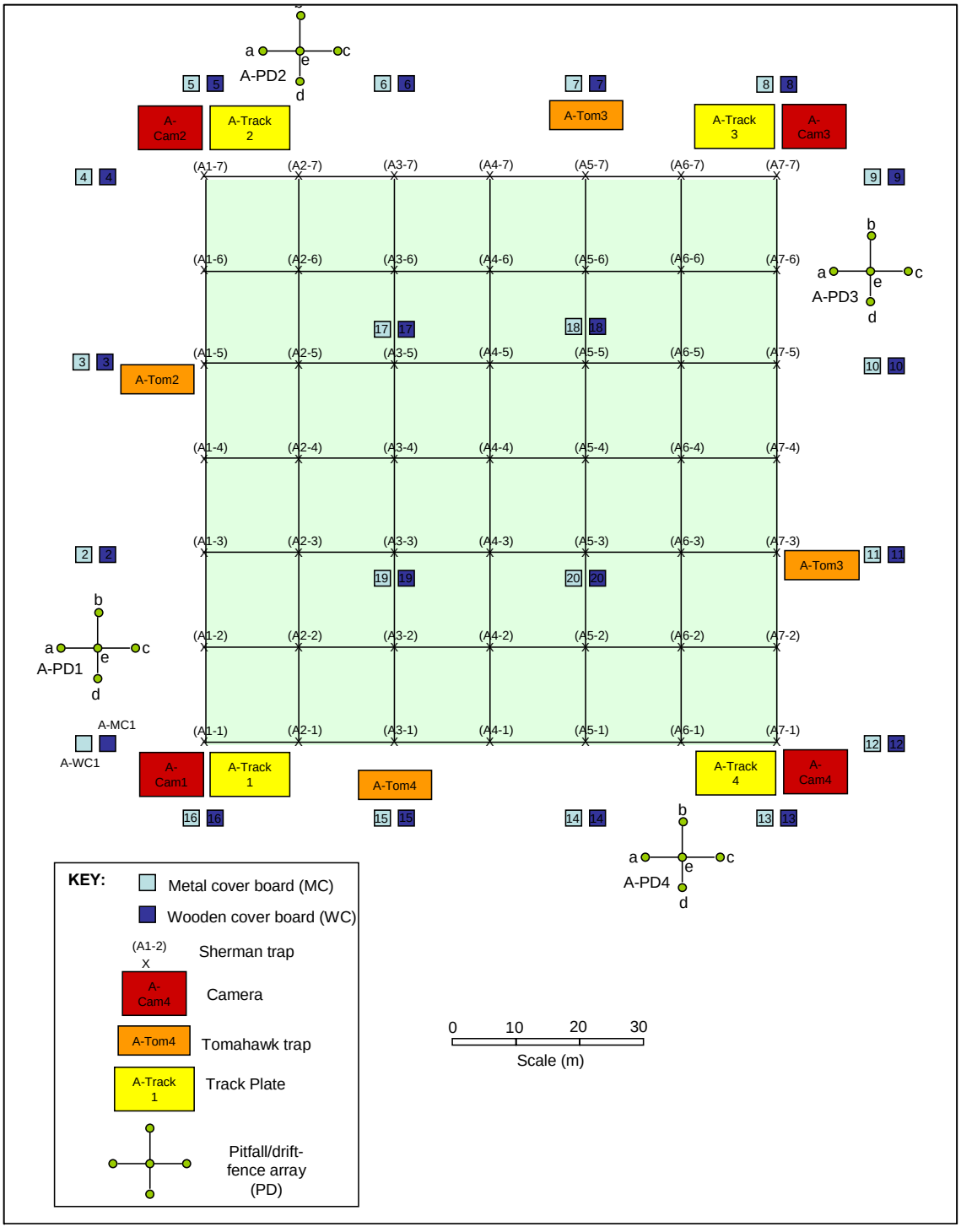

Figure 3.10. The set-up of traps within a sampling grid at Fort McCoy, Wisconsin.

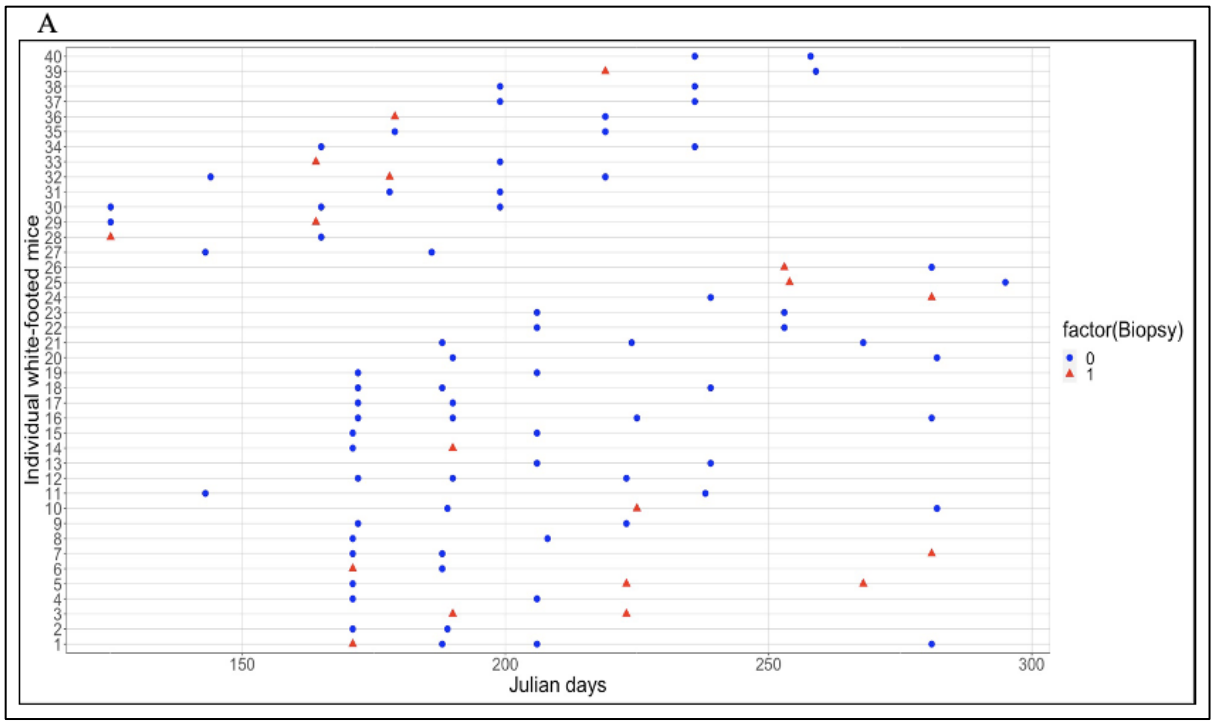

Figure 3.11. *Anaplasma phagocytophilum* infection status through time in individual recaptured white-footed mouse (rows) at Fort McCoy, Wisconsin 2010-2012. Each panel represents the infection status of an individual mouse by a particular tissue type: biopsy only (A), blood only (B) and by both tissues simultaneously (C). "●" represents animals from which *A. phagocytophilum* was not detected in that tissue type, while a "▲" represents animals which *A. phagocytophilum* was detected in that tissue type. For reference Julian date 121, 152, 182, 213, 244, 274 and 305 represents May 1st, June 1st, July 1st, August 1st, September 1st, October 1st, and November 1st.

Figure 3.11 (cont'd)

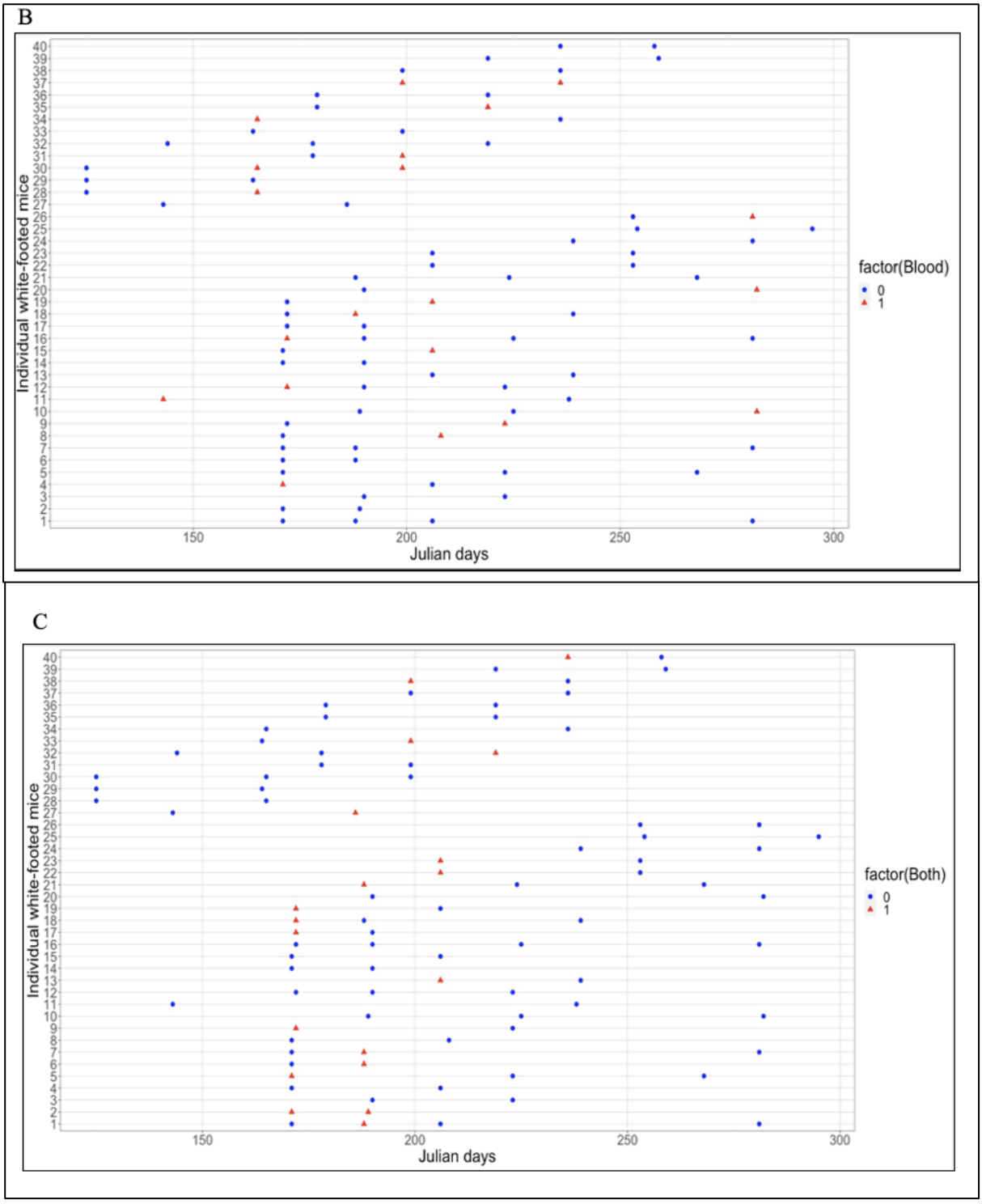

Table 3.5. The negative binomial regression model coefficients and the incident rate ratios for the *Ixodes scapularis* larvae parasitizing small to medium sized mammals.

Independent Variables	Coefficients	Std Error	Z-value	p-value	Incidence rate ratio (95% CIs)
Intercept	2.36	0.13	18.07	< 2e-16	10.53(8.07 - 13.81)
Compared to white-footed mice					
Northern short-tail shrew	-0.02	0.23	-0.09	0.93	0.98 (0.64 - 1.56)
Southern red-backed vole	-1.65	0.10	-15.71	< 2e-16	0.19 (0.16 - 0.24)
Virginia opossum	-0.05	0.63	-0.08	0.94	0.95 (0.32 - 3.86)
Meadow vole	-1.05	0.47	-2.21	0.03	0.35 (0.15 - 0.97)
Raccoon	0.94	0.57	1.65	0.10	2.55 (1.07 - 6.94)
Sorex spp.	-0.30	0.26	-1.17	0.24	0.74 0.45 - 1.27)
Eastern chipmunks	-0.42	0.31	-1.36	0.18	0.66 (0.37 - 1.25)
Meadow Jumping mouse	-1.89	0.67	-2.80	0.00	0.15 (0.04 - 0.60)
Compared to sampling year 2010					
2011	-0.66	0.09	-7.13	9.96e-13	0.51 (0.43 - 0.62)
2012	-0.03	0.09	-0.35	0.73	0.97 (0.82 - 1.15)
Capture week	-0.02	0.01	-3.21	0.001	0.98 (0.96 - 0.99)
Compared to sampling grid A					
Grid B	-0.19	0.09	-2.09	0.04	0.83 (0.69 - 0.99)
Grid C	-0.34	0.10	-3.44	0.0006	0.71 (0.59 - 0.87)
Compared to males					
Female	-0.43	0.09	-5.08	3.70e-07	0.65 (0.55 - 0.77)
Mass	-0.00021	0.00	-2.55	0.010821	1.00 (0.9997 - 0.9999)

Table 3.6. The negative binomial regression model coefficients and the incident rate ratios for the *Ixodes scapularis* nymphs parasitizing small to medium sized mammals.

Independent Variables	**Coefficients**	**Std Error**	**Z-value**	**p-value**	**Incidence rate ratio (95% CIs)**
Intercept	0.11	0.23	0.50	0.62	1.12 (0.72 - 1.76)
Compared to white-footed mice					
Northern short-tail shrew	-2.29	1.04	-2.20	0.03	0.1 (0.01 - 0.78)
Southern red-backed vole	-1.01	0.20	-5.17	2.33e-07	0.36 (0.25 - 0.53)
Virginia opossum	3.55	0.68	5.20	2.00e-07	34.66 (9.11 - 131.93)
Meadow vole	0.48	0.71	0.67	0.50	1.61 (0.40 - 6.50)
Raccoon	3.32	0.66	5.04	4.60e-07	27.73 (7.62 - 100.91)
Sorex spp.	-1.44	0.69	-2.09	0.04	0.24 (0.06 - 0.91)
Eastern chipmunks	2.04	0.35	5.84	5.09e-09	7.66 (3.87 - 15.17)
Meadow Jumping mouse	-29.03	893500	0.00	1	2.98E-13 (0.00 – Inf)
Compared to sampling year 2010					
2011	0.69	0.17	4.18	2.94e-05	1.99 (1.44 - 2.75)
2012	2.08	0.17	1.25	0.21	1.23 (0.89 - 1.70)
Capture week	-0.12	0.01	-9.084	< 2e-16	0.89 (0.86 - 0.91)
Compared to sampling grid A					
Grid B	-0.20	0.16	-1.24	0.22	0.81 (0.59 - 1.11)
Grid C	-0.50	0.18	-2.70	0.007	0.61 (0.42 - 0.87)
Compared to males					
Female	-0.59	0.17	-3.53	0.0004	0.56 (0.4 - 0.77)
Mass	-0.00021	0.00010	-2.12	0.03	1.00 (1.01 - 0.999)

Table 3.7. The Wald's test p-values for the effect of each categorical variable on the *Anaplasma phagocytophilum* infection status of host mammals.

Variable	**Wald's test p-value**
Effect of species	3.00E-09
Effect of capture year	0.12
Effect of sex	0.01
Effect of grids	4.90E-07

Table 3.8. The logistical regression model coefficients and the odd ratios for the *Anaplasma phagocytophilum* infection status of hosts mammals.

Independent Variable	**Coefficients**	**Std Error**	**Z-value**	**p-value**	**Odds Ratio**
Intercept	-1.27	0.32	-5.24	1.57E-07	0.19 (0.10 - .035)
Borrelia burgdorferi infection status	0.69	0.18	3.93	8.62E-05	2.00 (1.42 - 2.83)
Compared to white-footed mice					
Northern short-tail shrew	-0.77	1.13	-0.69	0.492	0.46 (0.02 - 3.08)
Southern red-backed vole	15.47	392.10	0.04	0.969	5213287 (4.6e-6 - Inf)
Virginia opossum	-1.27	1.10	-1.16	0.246	0.28 (0.01 - 1.67)
Meadow vole	2.73	1.15	2.38	0.017	15.34 (1.75 - 173.51)
Raccoon	-0.34	0.66	-0.511	0.609	0.71 (0.16 - 2.32)
Sorex spp	-0.76	0.24	-3.19	0.001	0.5 (0.29 - 0.74)
Eastern chipmunks	3.15	1.13	2.79	0.005	23.43 (2.50 - 252.23)
Meadow Jumping mouse	-1.01	1.10	-0.92	0.359	0.37 (0.02 - 2.21)
Number of nymphs parasitizing individual hosts	0.15	0.08	1.74	0.082	1.16 (0.98 - 1.37)
Compared to sampling year 2010					
2011	0.39	0.22	1.74	0.082	1.47 (0.95 - 2.28)
2012	-0.08	0.19	-0.42	0.678	0.92 (0.64 - 1.33)
Capture week	-0.05	0.02	-3.36	0.001	0.95 (0.92 - 0.98)
Compared to sampling grid A					
Grid B	1.06	0.21	4.99	5.93E-07	2.89 (1.92 - 4.43)
Grid C	0.41	0.24	1.72	0.085	1.50 (0.95 - 2.39)
Compared to males					
Male	0.40830	0.16380	2.493	0.013	1.50 (1.09 - 2.08)
Mass	-3.99E-04	1.71E-04	-2.33	0.020	1.00 (1 - 0.999)

CHAPTER 4: SPECIES DISTRIBUTION MODELING AND APPLICATION TO *IXODES SCAPULARIS*

ABSTRACT

Species distribution modeling (SDM) is a tool to identify environmental predictors important for a species and to predict suitable habitats and therefore spatial distribution of a species. Here I present a limited review of SDM approaches and then review SDMs of *Ixodes scapularis,* the tick vector that is responsible for many vector-borne diseases in the U.S. There are several methods of developing SDM's, which overall are based on either regression-based approaches or machine learning approaches. Each method incorporates presence-absence or abundance data and is a based on different sets of assumptions. With the development of many species distribution modeling techniques, many have been applied to vector borne disease systems to identify potential environmental variables and habitats that are important to sustaining vectors and therefore predict where vectors and their associated diseases may spread in the future. Developing SDMs enable researchers and public health agencies to strategize better surveillance efforts, especially given limited resources. Species distribution models can be used to inform the public and healthcare workers about the risk in contacting a vector and associated pathogen(s) in regions where the vector previously was not known to occur or be established. These models can also be important when developing control and prevention plans. Developing SDMs for suitable habitats for vectors were first attempted in the early 1970s for mosquitoes, and since then there have been a marked improvement in using SDMs to model potential habitats for mosquitoes especially in the developing world. With the increase in ticks and tickborne disease in the US, due in part to expansion of geographic ranges of several vector species, SDM's have been applied to predict the spread of several tick species. The blacklegged tick, *Ixodes scapularis,* is responsible for transmitting the

most common vector borne disease in the US, Lyme disease. With land use changes, forest management, wildlife management, and climate change, *I. scapularis* has been expanding its range for several decades. In the late 1990s, when *I. scapularis* populations in Wisconsin were still limited geographically, the first *I. scapularis* SDM was developed. Since then, several SDMs for *I. scapularis* have been developed using different types of data, different spatial scales, and different modeling approaches.

Keywords: Species distribution models, vector borne diseases, *Ixodes scapularis*, range expansion

INTRODUCTION

Species Distribution Modeling

Species distribution modeling (SDM) has become one of the most important modeling approaches in ecology. These models incorporate ecological concepts with those of the natural history of a species using statistical methods (Elith and Leathwick, 2009). The origin of SDMs stems from early studies based on mapping out species distribution across landscape along with their associations with geographic and environmental features (Elith and Leathwick, 2009). Many early studies used a combination of climatic features with environmental elements to explain different vegetation patterns seen across the world.

These models could be applied to species from any taxa, both fauna and flora from marine to terrestrial ecosystems. Species distribution models can provide insight into where suitable habitats lie for a species of interest, such as one that is endangered, or alternatively one that is invasive. SDMs typically consider the known localities for a species, which could be occurrence or abundance data, and then try to identify environmental predictors which are associated, and therefore may influence, the distribution of the species of interest (Duarte, Whitlock and Peterson, 2018).

The niche concept of a species forms the central theorem for SDMs. In terms of SDMs, a species' niche is defined as the place an organism exists within its environment or community with all the abiotic and biotic interactions it has (Grinnell, 1917; Whittaker, Levin and Root, 1973). For SDMs, the niche is refined further into a fundamental niche, which encompasses the broad range of environmental features an organism can survive in, and the realized niche, which is the actual environment the organism occupies within the fundamental niche due to different environmental constraints (Whittaker, Levin and Root, 1973; Duarte, Whitlock and Peterson, 2018). A SDM will

essentially predict a species' ability to survive within an environmental space using a statistical method that will quantitatively describe the environmental space (fundamental niche) that is suitability for that species to survive in (Duarte, Whitlock and Peterson, 2018). The final species distribution model will display suitable habitats within a geographic extent that will have all the estimated suitable environmental conditions conducive for the species to survive and reproduce.

Response and Predictor Variables in SDMs

A critical component for building a SDM is to measure all the known occurrence points of a species of interest within a geographic extent. Essentially species occurrence points could be measured as presence/absence of that species or abundance. The level of measurement will depend upon what data are available and which model type is used. The next critical component that is needed for model development are the predictor variables, i.e., the environmental variables that will be used to estimate environmental suitability. Typically, climatic, habitat and soil variables are the most used predictors. The next step is to determine the modeling algorithm to use.

Species distribution modeling algorithms

Species distribution modeling algorithms fall into two main categories: 1) regression-based methods and 2) machine learning based methods. I will briefly review them here.

1. Regression-based methods

Regression-based models like generalized linear models (GLMs), and its non-parametric form a generalized additive model (GAM), are straightforward and the most frequently used approaches. Depending on what type of variable is the response variable, the form of the GLM would change. For example, if the response variable is presence and absence points of a species, a logistical regression model is used, while if the response variable is counts of individuals of a species, a Poisson regression may be used. Other types of linear regression may be used depending

on the distribution of counts (e.g., negative binomial or zero-inflated negative binomial). Generalized additive models closely resemble GLMs but tend to be more flexible because the model is fit using smoothing and piecewise linear splines (Duarte, Whitlock and Peterson, 2018). In general, a GAM has lower bias than a GLM but tends to have a higher variance because the coefficients (bs) of GLM are replaced by a smoothing spline in GAMs (Elith and Franklin, 2013). A more advanced form of a GAM currently being used is a multivariate adaptive regression spline (MARS), which is computationally faster than a GAM and the predictions can be easily converted into a geographical information system (GIS) mapping format (Duarte, Whitlock and Peterson, 2018). But MARs can only be used when the response variable data are converted into a normally distributed dataset, not when the responses are at a presence/absence data level.

2. Machine learning-based methods

A relatively new development in SDMs is the use of machine learning and data mining techniques to predict the distribution of species. These methods include A) boosted regression trees; B) random forests; C) artificial neural networks; D) genetic algorithms; and E) maximum entropy methods.

A. Boosted Regression Trees

Boosted regression trees will combine several models using two algorithms, a decision or regression tree and a boosting regression (Elith, Leathwick and Hastie, 2008). In a decision tree, a set of predictor variables are used, and the input data are split repeatedly according to the predictor variables (Elith, Leathwick and Hastie, 2008). The decision to split the data at the node is to increase the information gained from the tree. The splitting of data will continue until a stopping condition is put into the model. The decision trees on its own is not very accurate because the tree will depend on the input sample data and the features. A slight change in the sample data can result

in a completely different sequences of node splits which might not reflect on the actual relationship. Therefore, each decision tree is boosted to improve the accuracy in this model. Boosted regression trees will build several simple iterative decision trees to the training dataset and combine these trees to give a more accurate representation between the distribution of the species and the environmental variables (Li and Wang, 2013).

B. Random Forests

Random forests are like boosted regression trees in that this technique uses many iterative decision trees which are then bootstrapped to find the optimal model (Drew, Wiersma and Huettmann, 2011). Random forests are based on the Breiman's random forest algorithm, where random decision trees are built depending on the training dataset. The final model will be chosen by the number of votes or classifications each of the trees gets in the random forest; the tree which has the highest vote will be the final model (Breiman, 2001; Drew, Wiersma and Huettmann, 2011). Both the boosted regression trees and random forests are based on ensemble methods where several models or trees are combined to obtain the best model to explain the distribution of the species. Both these techniques are powerful computational machine learning methods.

C. Artificial Neural Networks

Artificial neural networks are another complex modeling method which involve a network of simple processing elements or artificial neurons (Li and Wang, 2013). This method looks at the links between the environment layers and the species distribution dataset which acts as neurons. The artificial neural networks contain hidden layers with neurons receiving information from the input data which are then summed up and added with a constant, which usually is a bias. These then are transformed using a fixed function (Li and Wang, 2013; Zhang and Li, 2018).

D. Genetic Algorithms

Genetic algorithms are based on presence only data of a species and use preset mathematical rules which are called genes. These rules are combined randomly to develop models to explain patterns of distribution for a species (Stockwell and Peters, 1999; Li and Wang, 2013).

E. Maximum Entropy

The maximum entropy (Maxent) method is also a presence only data model. The concept behind the Maxent method is to estimate a target probability distribution by finding the probability distribution that is at a maximum entropy (either spread out or closest to a uniform distribution) that is subjected to a set of environmental constraints (Phillips, Anderson and Schapire, 2006).

Each method has its own set of advantages and disadvantages. The most important aspect is choosing the method that seems most appropriate to predict the distribution of a species of interest. The decision to use a specific method of SDMs will depend mainly on the input sampling data set. For example, if a study has conducted a passive surveillance, there is only information about presence points; therefore, using a machine learning technique which only uses presence points is more appropriate. Once the model is developed, model evaluation is conducted, if possible, to estimate the accuracy of the developed distribution map. The most common way to evaluate a model is assessing the area under the curve (AUC) of the receiver operating curve (ROC) area. The AUC is based on the sensitivity (when the model predicts presence points accurately, i.e. true positives) and specificity (when model predicts absence points accurately i.e., true negatives) of the model data, where the data are split into a training set to develop the model, and the testing data set to test how well the model performs (Fielding and Bell, 1997a). The AUC values range from 0 – 1, where when the AUC values are greater than 0.5, the model will be able to classify suitable habitats better than by chance or at random (Jiménez-Valverde, 2012). Using the AUC to evaluate

a model is beneficial when we have known absence points for a species in the model. But methods that use presence only data also incorporate AUC as a common method to evaluate the model by incorporating likeness of pseudoabsene points spread around the study region. The AUC method of model evaluation is not dependent upon a preset threshold of the prevalence of positive sites vs the prevalence of absence sites in the dataset, but there are other methods of evaluation which are threshold dependent. One such method is called true skill statistic (TSS). The TSS compares the number of true positives against those that are ascribed to random guessing (i.e., false positives) (Allouche, Tsoar and Kadmon, 2006). One benefit of using TSS is that it is not affected by the numbers of prevalence of positives in the data set. The range of values for TSS vary from -1 to 1, where TSS values closer to 1 indicate that the model is predicting the actual number of positives correctly and not just due to random chance. Another method of evaluation that has been used traditionally is the Kappa statistic, which is quite like TSS, but used less frequently as it is very dependent upon the prevalence of positives in the data set (Fielding and Bell, 1997a; Allouche, Tsoar and Kadmon, 2006).

Species Distribution modeling applied to vectors and pathogens

Species distribution modeling has now become an important part of spatial epidemiology and is currently widely used to model pathogens and disease spread. In modern times the spread of different zoonotic pathogens has sparked the importance in making accurate predictions of future spread in pathogens. Species distribution modeling is important in epidemiology because it helps to visualize the current spatial patterns and trends in disease incidences and disease vectors or reservoirs; it helps understand which environmental factors support disease transmission and maintenance; and helps in predicting how risk of exposure may change in the future under different environmental and socioeconomic scenarios (Eisen and Eisen, 2011; Hay *et al.*, 2013; Purse and

Golding, 2015). The eventual purpose of developing SDMs for pathogens and associated vectors is prevention of disease spread. In epidemiology SDMs have been used for multiple purposes including to plan national level intervention strategies, aid decision makers during assessments, help to inform individuals about decision to obtain vaccination/prophylaxis before travel (Eisen and Eisen, 2011; Hay *et al.*, 2013). Understanding the ecology and epidemiology of the pathogen in a particular area or region is critical such that the process of developing and interpreting the SDM is better informed (Rogers and Randolph, 2003).

Species distribution models applied to vector borne disease systems identify associations between the vector or the vector-borne disease data (could be presence/absence or abundance data) and environmental or socioeconomic predictor variables (Eisen and Eisen, 2011). The SDMs for vectors and their vector-borne diseases result in the development of maps that display potential suitable locations for the vector or the vector-borne disease to thrive, which can be extremely helpful for indicating areas of risk where surveillance data were lacking (Eisen and Eisen, 2011). These maps, which are still hypotheses, can enable public health officials and researchers to direct limited resources to monitor most efficiently areas of disease risk. These models can also be combined with human demographic data to assess the proportion of people that are potentially at a risk of exposure to vectors and vector-borne diseases (Eisen and Eisen, 2011).

The earliest form of developing modern species distribution model to predict suitable habitats for disease-causing vectors was applied for mosquito control in New Orleans (National Aeronautics and Space Administration, 1973). The study was conducted by the National Aeronautics and Space Administration (NASA) and used color infrared photography to map vegetation to identify suitable habitats for mosquito *Aedes sollicitans* larval sites within marshes of New Orleans. Similar to the study by NASA, a study conducted in Michigan mapped forested

wetlands and open marshes to find suitable mosquito breeding habitats around the Saginaw Bay area of Michigan, which was then used directly for targeting mosquito control measures (Wagner *et al.*, 1979). Several other studies have used early photointerpretation techniques to identify potential breeding habitats for various species of mosquitoes within Texas (Welch *et al.*, 1989), Lousiana (Cibula, 1976; Barnes and Cibula, 1979), Nebraska, and South Dakota (Hayes *et al.*, 1985). Building upon these early methods, land satellite data were used to model habitat features that are important in Rift Valley Fever activity in Kenya (Linthicum *et al.*, 1987; Pope *et al.*, 1992).

After the introduction of West Nile Virus to the United States in the late 90's, several studies were conducted to generate risk maps of West Nile virus (Brownstein *et al.*, 2002) and predict suitable habitats in the US for several of the West Nile Virus mosquito vectors (Diuk-Wasser, Brown, *et al.*, 2006; Larson *et al.*, 2010; Rochlin *et al.*, 2011). Many studies have been conducted using species distribution modeling to predict suitable habitats for the malaria mosquito vector *Anopheles* spp. as malaria remains to be one of the leading causes of death in the developing world (Guerra, Snow and Hay, 2006; Hay and Snow, 2006; Kulkarni, Desrochers and Kerr, 2010; Obsomer, Defourny and Coosemans, 2012; Gwitira *et al.*, 2015, 2018; Akpan *et al.*, 2018; Frak *et al.*, 2020). Most of these studies have been conducted within the African continent as majority of malaria deaths are reported in within this region. The majority of these models use maximum entropy techniques to identify areas that have suitable habitat conditions for several species of *Anopheles* spp. (Kulkarni, Desrochers and Kerr, 2010; Obsomer, Defourny and Coosemans, 2012; Gwitira *et al.*, 2015; Akpan *et al.*, 2018). With the increase in invasive mosquito species such as *Aedes aegypti* and *Aedes albopictus*, which serve as vectors for viruses such as dengue, Japanese encephalitis, chikungunya and Zika, modeling potential suitable habitats have become vastly important. Many studies have been conducted globally to predict the distribution of these invasive

mosquitoes (e.g., Kobayashi, Nihei and Kurihaha, 2002; Brady et al., 2014; Kraemer et al., 2015; Messina et al., 2016; Kamal et al., 2018; Leta et al., 2018).

***Species distribution modeling of* Ixodes scapularis**

Tick-borne diseases have become a global threat affecting humans, domestic animals, and livestock. In the United States alone in the past decade there has been a gradual increase in tickborne disease cases, where currently at least 75% of the vector borne disease cases reported to the Centers for Disease Control and Prevention (CDC) is attributed to tick borne pathogens (Rosenberg *et al.*, 2018). Of those reported tick-borne disease cases, Lyme disease accounts for at least 82%, making Lyme disease the most common vector borne disease in the United States (Eisen and Eisen, 2018b; Rosenberg *et al.*, 2018). Recently ~34,000 cases of Lyme disease are reported annually to the CDC (Rosenberg *et al.*, 2018; N. C. for E. and Z. I. D. D. of V.-B. D. Centers for Disease Control and Prevention, 2022). Along with Lyme disease, other tickborne diseases such as anaplasmosis and ehrlichiosis have also increased in case numbers since 2011 (Rosenberg *et al.*, 2018). The expansion of the geographic range of disease-vectoring ticks species, and the subsequent increase in tickborne pathogen detection awareness among clinicians and improved diagnostic methods have contributed to the increase in tickborne diseases cases in the US (Eisen and Eisen, 2018b; Sonenshine, 2018b)

Of the tick species of interest in the United States, the eastern blacklegged tick (*Ixodes scapularis*) plays an important role in the spread of tickborne disease since it is the primary vector for several zoonotic pathogens including, six bacterial agents: *Borrelia burgdorferi* and *B. mayonii*, the causative agents of Lyme disease*; Anaplasma phagocytophilum,* the causative agent of human granulocytic anaplasmosis; *Borrelia miyamotoi,* the causative agent of hard tick relapsing fever borreliosis*;* and *Ehrlichia muris eauclarensis,* the causative agent of ehrlichiosis. *Ixodes scapularis*

is also the vector for a protozoan parasite, *Babesia microti* the causative agent of babesiosis, and a potentially fatal flavivirus, Powassan virus, the causative agent of Powassan virus encephalitis. The majority of *I. scapularis* borne disease cases are concentrated into two major foci in the United States, one being the upper Midwest and the other being the Northeast (Hahn *et al.*, 2016).

The earliest understanding of the range of *I. scapularis* show it to be predominantly in the southeastern US, from the Gulf Coast reaching north along the Atlantic Coast to southern Massachusetts, in the central Midwest to Iowa and Indiana, and few specimens from southern Ontario (Bishopp and Trembley, 1945). The earliest record for *I. scapularis* in the northern U.S. was in the 1920s near Cape Cod, Massachusetts (Spielman *et al.*, 1985). Populations of *I. scapularis* were reported sporadically by the 1940s along the Northern Atlantic coast (Bishopp and Trembley, 1945; Eisen and Eisen, 2018b). After the 1970s more records of *I. scapularis* were reported along the New England coastline, in Rhode Island, southern New York, northwestern Wisconsin, and Ontario (Jackson and DeFoliart, 1970; Good, 1973; Watson and Anderson, 1976; Ruebush *et al.*, 1977; Hyland *et al.*, 2000). It is hypothesized that *I. scapularis* probably was distributed throughout the upper Midwest and Northeast pre-European settlement (Tsao et al. 2021). By the late 1800s and early 1900s, however, due to rapid deforestation and severe depopulation of the white-tailed deer (*Odocoileus virginianus*) (the main host for the reproductive stage), the abundance and distribution of *I. scapularis* was probably nearly extirpated in these areas except for a few refugia (Eisen et al., 2017; Spielman et al. 1985). But by the turn of the 20th century reforestation efforts began, along with managed hunting and reintroduction of the white-tailed deer, eventually resulting in the increase and spread of *I. scapularis* in the Northeast and upper Midwest U.S. (Spielman *et al.*, 1985; Lane, Piesman and Burgdorfer, 1991; Dennis *et al.*, 1998; Eisen *et al.*, 2017; Eisen and Eisen, 2018b; Sonenshine, 2018b). While similar dynamics regarding forests and white-tailed deer

populations occurred in the southern U.S. (Paddock and Yabsley, 2007), effects on southern populations of *I. scapularis* have not been discussed in the literature (or our knowledge), perhaps in part because southern populations of blacklegged ticks historically have not been recognized as vectors of disease (Lane, Piesman and Burgdorfer, 1991)

The increase in surveillance efforts have helped to map the distribution of *I. scapularis* within the eastern USA (Centers for Disease Control and Prevention (CDC), 2022). Dennis et al. 1998 published the first county-wide distribution map of *I. scapularis* within the US. This early distribution map was based on records collected through surveys and publicly available data, and it was at the county scale since most available data was by county, but also the scale at which public health metrics are reported state-wide and nationally (Dennis *et al.*, 1998).This study was the first to introduce a scale to define establishment of ticks populations, where counties were considered to be "reported" if at least one tick of any life stage had been identified within one calendar year. Counties were considered to have "established" *I. scapularis* if at least 6 ticks from one life stage or 2 life stages (larvae, nymph, adult) had been detected within one calendar year (Dennis *et al.*, 1998). This classification of defining tick populations is the current standard used by the CDC not just for *I. scapularis*, but for other vector tick species. Based on this classification method the distribution of *I. scapularis* has rapidly expanded with more counties reporting established populations (Eisen, Eisen and Beard, 2016; Beard, Eisen and Eisen, 2021). With this rapid expansion of *I. scapularis* populations, cases of Lyme disease and anaplasmosis cases have increased as well (Centers for Disease Control and Prevention, 2023b, 2023a). This increase in disease incidence is mainly due to the expansion of the *I. scapularis* population within the eastern USA. Interestingly, the cases of these diseases have also increased in western US., where *Ixodes pacificus* is the vector, whose known range has expanded beyond what was reported in Bishopp

and Trembley (1945), but not as dramatically as that of northern populations of *I. scapularis* (Dennis et al. 1998; Eisen et al. 2015). Thus, increased cases of these diseases may be due mainly to increased surveillance and reporting and local ecological changes.

With this rapid expansion of *I. scapularis*, efforts to incorporate species distribution models to predict the spread of *I. scapularis* has become vital. Therefore, it is important to understand the importance of several different environmental factors that impact the survival and expansion of *I. scapularis* (Estrada-Peña, 2002). Global climatic changes likely will also influence the distribution of *I. scapularis*, and thus species distribution models may be able to help predict future disease risk. For example, climate change may create suitable environmental conditions allowing *I. scapularis* to invade into areas previously deemed unsuitable for *I. scapularis* survival; likewise, climate change may decrease the suitability of other areas, leading to range contraction. The suitable habitats in the United State for the development of *I. scapularis* seem to be undergoing a rapid geographic change allowing *I. scapularis* to survive in habitats where previously *I. scapularis* was not detected (Estrada-Peña, 2002).

The earliest *I. scapularis* species distribution model was that of Guerra et al. (2002), which identified several environmental factors associated with suitable habitats for *I. scapularis* establishment primarily in Wisconsin and northern Illinois but also Menominee County in the Upper Peninsula of Michigan. Using a logistic regression model, Guerra et al. (2002) showed soil features such as bedrock geology, quaternary geology, soil order and texture; habitat features such as land cover, forest types and elevation; and climatic features such as annual precipitation and snow fall were important variables that determined habitat suitability for the introduction and establishment of *I. scapularis*. Although Wisconsin is recognized as the center of the major foci of *I. scapularis*, at the time the model was developed, the distribution of *I. scapularis* was still limited

to certain areas, and thus it was a very timely to use species distribution modeling to predict habitats in the rest of Wisconsin and northern Illinois (Guerra *et al.*, 2002). Later, Foster and colleagues (Foster 2004) tested the ability of the model in Guerra et al. (2002) to predict suitable habitats for *I. scapularis* in Michigan, which led to the discovery of established populations of blacklegged tick in southwestern Michigan (Erik Scott Foster, 2004).

Soon after the publication of Guerra et al. (2002); Brownstein et al (2003) published the first eastern U.S. species distribution map, which also was a logistic regression model but was based only on climate variables. Brownstein et al. (2003) found that climatic extremes and variation in humidity were major indicators of a suitable habitats. When looking at the expansion of *I. scapularis*, maximum temperatures, minimum temperatures and the vapor pressure played an important role determining the range expansion of *I. scapularis* within the United States (Brownstein, Holford and Fish, 2005). In addition to expansion into southern Canada, they predicted an expansion of *I. scapularis* by 2080 within areas of Virginia, North Carolina, Georgia, Minnesota, Iowa and Michigan (Brownstein, Holford and Fish, 2003).

The largest species distribution map developed for *I. scapularis* was developed by Diuk-Wasser et al. (2006, 2010). The geographic extent of this species distribution model was from the 100th meridian to the Atlantic Ocean and from the border with Canada to the Gulf Coast. This area was divided into 2-degree grids, from which blacklegged ticks were systematically sampled by drag cloth over four years, where the zero-inflated negative binomial regression model was used. Because this map was focused on modeling the spatial risk of Lyme disease, it focused efforts on modeling the density of questing *I. scapularis* nymphs (Diuk-Wasser et al. 2010) and then the density of questing infected nymphs (Diuk-Wasser et al. 2012). Nymphs are the epidemiologically most important life stage. Interestingly, although *I. scapularis* is widespread through the southern

U.S., nymphs have a different questing behavior, such that they rarely contact humans, and thus Lyme disease risk is concentrated when northern populations of *I. scapularis* are. Thus, after the first year, after questing nymphs were rarely dragged in the southern areas, researchers focused and increased sampling in the northern areas above approximately the 39th parallel. They found that the most important factors predicting nymphal density were altitude, monthly mean vapor pressure deficit and spatial autocorrelation, but other factors such as forest fragmentation and soil texture were not. Since this study, there has not been another large-scale species distribution model developed estimating the risk of Lyme disease based on systematic active surveillance, perhaps because efforts to systematically sample for *I. scapularis* are too resource intensive.

Species modeling of *I. scapularis*, however, has not stopped. As *I. scapularis* has been spreading, there have been more and more surveys for the tick conducted. Even though sampling efforts have not been conducted systematically, presence data are available and can be modeled. Two models were developed for the northern regions of *I. scapularis'* range based on presence and absence of *I. scapularis* modeled at the county-scale (Hahn *et al.*, 2016; Burtis *et al.*, 2022). The courser scale does not allow for more accurate predictions about *I. scapularis* distribution at finer scales when tick densities and environmental factors may be heterogeneous throughout a county. But, for public health departments at the state and federal level, they provide some guidance for prevention measures for which the county level is probably adequate. These maps also provide state and local health departments guidance on how to allocate limited resources for surveillance. As more surveillance for ticks are conducted, more SDMs can be conducted at a finer scale either using presence absence-based models (Lippi, Gaff, White, St. John, *et al.*, 2021; Kopsco *et al.*, 2023) or using tick abundance or density of nymphs (Diuk-Wasser, Brown, *et al.*, 2006; Diuk-Wasser *et al.*, 2010).

Currently we see an increase in ticks and tickborne diseases (Bacon, Kugeler and Mead, 2008; Adams *et al.*, 2017; Rosenberg *et al.*, 2018). With the impact of climate change, habitat modification, habitat fragmentation, along with changes in hosts communities (Rocklöv and Dubrow, 2020; Couper, MacDonald and Mordecai, 2021; Nuttall, 2022), the spread of *I. scapularis* and other vector tick species such as *Amblyomma americanum* (lone star ticks), *Dermacentor variabilis* (the American dog tick) and *A. maculatum* (Gulf Coast tick) are predicted to change (Sonenshine 2018). As such, the risk for the pathogens they vector are also predicted to change. By developing SDMs for these tick species and even their pathogens if data allow, we can identify the distribution of potential suitable habitats for certain tick species, which will help guide future efforts to prevent ticks and tickborne diseases.

Conclusion

Michigan is a state that is at the leading edge of a *I. scapularis* invasion in the Upper Midwest (Dennis *et al.*, 1998; Eisen, Eisen and Beard, 2016; Lantos *et al.*, 2017; Burtis *et al.*, 2022). *Ixodes scapularis* was first discovered in the 1990s in the Upper Peninsula in Menominee a county bordering Wisconsin (Strand, Walker and Merritt, 1992) and then in the early 2000s in the southwestern Lower Peninsula in Berrien a county at the edge of Michigan bordering Indiana (Erik Scott Foster, 2004). Since this initial discovery *I. scapularis* has been gradually spreading along the western coastline of Lake Michigan Northward, along the eastern coastline of Lake Huron and interior southern regions in the Lower Peninsula (Dennis *et al.*, 1998; Hamer *et al.*, 2010, 2014; Eisen, Eisen and Beard, 2016). Slower but continued spread has also occurred in the Upper Peninsula, especially in the western region. With increasing tick populations in Michigan over time we see that human case incidences have increased as well (Michigan Department of Health and Human Services, 2021, 2022). Because there is a gradual, persistent spread of *I. scapularis*, using

SDMs to predict the spread of *I. scapularis* will be important for surveillance and reducing public health risk. Our study in developing SDMs for *I. scapularis* based on the presence-absence data will guide future investigations into potential regions that have suitable sites for the establishment of *I. scapularis* which can then serve also to improve future models as well as guide prevention of tick-borne disease.

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CHAPTER 5: SPECIES DISTRIBUTION MODELING OF THE BLACKLEGGED TICKS (*IXODES SCAPULARIS*) IN MICHIGAN

ABSTRACT

The increase in *Ixodes scapularis*-borne diseases have been attributed to the rapid expansion of the tick throughout the Northeast and the Upper Midwest. In the Upper Midwest, historically endemic populations of *I. scapularis* were first detected in Wisconsin in 1965, after which populations spread throughout the state. In comparison, in the neighboring state of Michigan, *I. scapularis* was first discovered in the early 1990s in the Upper Peninsula, in a county bordering Wisconsin, and in the early 2000s in the southwestern corner of the Lower Peninsula. As in Wisconsin, populations of *I. scapularis* have been steadily expanding in Michigan.

With this invasion occurring in real time, we examined 1) the spread of *I. scapularis*; 2) predicted the environmental predictors important in those suitable habitats by developing the models, and 3) predicted the distribution of suitable habitats for *I. scapularis* in Michigan. We sampled for ticks using drag sampling from 2017 to 2021, where we sampled in 315 sites, although sampling sites differed among years. Due to the Lower and Upper Peninsula having very different ecological, climatic, and geological features we modelled each Peninsula separately using two modeling methods, a regression-based approach using a generalized linear model (GLM) and a machine learning method called maximum entropy (MaxEnt) modeling. We began with 29 initial environmental predictors, which was reduced to 8 for the Lower Peninsula and 9 for the Upper Peninsula in the final GLM models. The final MaxEnt models included 20 predictors for the Lower Peninsula and 18 for the Upper Peninsula. For the climatic predictors, variables related to temperature, humidity and for the ecological predictors features related to soil, elevation and forest types were important.

In evaluating each model, the area under the curve values (AUC) for the GLM and MaxEnt models in the Lower Peninsula were 0.78 and 0.94 respectively, while for the Upper Peninsula they were 0.85 and 0.93 respectively, indicating that all models performed better at classifying sites as positive (or negative) for *I. scapularis* than by random chance. The true skill statistics (TSS) values for the Lower Peninsula GLM and MaxEnt were 0.41 and 0.45, while for the Upper Peninsula they were 0.64 and 0.35 respectively, again, for all models indicating the presence sites were actual presence sites. In both modeling methods similar regions were identified to have high to low suitability habitats for *I. scapularis.*

Through this study we hoped to find suitable regions in the Upper and Lower Peninsula where we can guide our surveillance effort to detect established populations of *I. scapularis*. With the increase in Lyme disease cases in Michigan it is important to predict where suitable sites for establishment of *I. scapularis* are found in Michigan to guide prevention and control strategies as soon as possible. Because Michigan is at the leading edge of an invasion of *I. scapularis*, model predictions may change, where some of the habitats currently predicted as unsuitable may become suitable for *I. scapularis.* When training the models, the data we used may have sites that are "false negatives" where *I. scapularis* was not detected because the tick still has not gotten a chance to get to those sites, to invade and become established yet. Surveillance and/or experimental tick survivorship studies conducted in the future in these putatively unsuitable regions can test these hypotheses.

Keywords*: Ixodes scapularis*, species distribution modeling, Michigan, environmental predictors

INTRODUCTION

Spread of I. scapularis *in the Great Lakes region*

The blacklegged tick, *Ixodes scapularis,* is the vector for several important tick-borne pathogens including the *Borrelia burgdorferi*, *B. miyamotoi* and *Anaplasma phagocytophilum*, *Babesia microti,* and Powassan virus (Nelder *et al.*, 2016; Eisen and Eisen, 2018a; Wolf, Watkins and Schwan, 2020). The historic range of *I. scapularis* encompasses much of the eastern United States, although populations have been expanding into new areas, particularly along the northern edge of their range (Eisen and Eisen, 2018a). The abundance of *I. scapularis* has also been increasing through much of the northern range (Dennis *et al.*, 1998; Eisen, Eisen and Beard, 2016; Eisen and Eisen, 2018a; Fleshman *et al.*, 2021).

Ixodes scapularis has been considered endemic to parts of the Midwest and the Northeast for more than half a century (Dennis *et al.*, 1998). It has been hypothesized that land use changes (Barbour and Fish, 1993; Pfäffle *et al.*, 2013; VanAcker *et al.*, 2019; Diuk-Wasser, Vanacker and Fernandez, 2021), habitat modifications (Pfäffle *et al.*, 2013; Couper *et al.*, 2020; Swei *et al.*, 2020), increased deer population densities (Witmer and Decalesta, 1991; Khatchikian *et al.*, 2015; Kugeler *et al.*, 2016; Gourley *et al.*, 2018; Fish, 2021), and possibly climate change (Süss *et al.*, 2008; Ostfeld and Brunner, 2015; Ogden and Lindsay, 2016; Bouchard *et al.*, 2019; Rocklöv and Dubrow, 2020; Nuttall, 2022) allowed ticks from limited populations to expand throughout these regions. Both the geographic expansion of tick populations and increased abundance have likely contributed to the rising incidence of human tick-borne diseases throughout the eastern United States (Adams *et al.*, 2017; Eisen and Eisen, 2018a).

The first populations of *I. scapularis* recorded from the Great Lakes region were from northcentral Wisconsin in the early 1960s (Jackson and DeFoliart, 1970; Gardner *et al.*, 2020).

Since then, populations of *I. scapularis* have been spreading throughout the state and into neighboring contiguous states to the west and south (Bouseman *et al.*, 1990; Hamer *et al.*, 2010; Khatchikian *et al.*, 2015; Fish, 2021). In the neighboring state to the east, Michigan, the first established population of *I. scapularis* was discovered in the Upper Peninsula in a county bordering Wisconsin (Menominee County) in the late 1980's (Strand, Walker and Merritt, 1992). In the early 2000's, a population of *I. scapularis* was found in the southwest corner of the Lower Peninsula (Erik Scott Foster, 2004). Between 2004 to 2008, populations of *I. scapularis* expanded in Michigan at a faster rate northward through counties adjacent to Lake Michigan when compared to the rate of inland spread (Hamer *et al.*, 2010). But by 2016, established populations of *I. scapularis* were detected further inland in the southern regions of the Lower Peninsula and were later found at sites in and near the "Thumb" region adjacent to Lake Huron (Eisen, Eisen and Beard, 2016; Lantos *et al.*, 2017; Fleshman *et al.*, 2021). Since the first population of *I. scapularis* was discovered in the Upper Peninsula of Michigan in 1990, populations of *I. scapularis* were detected in both the westernmost and easternmost counties of Michigan by 1998 (Dennis et al., 1998). By 2016, populations had spread even further into eastern areas of the Upper Peninsula throughout many counties (Eisen, Eisen and Beard, 2016). Currently, *I. scapularis* populations have been reported throughout the Upper Peninsula (Fleshman *et al.*, 2021).

***Species Distribution Models and Ecological Associations for* I. scapularis**

With the constant spread of *I. scapularis* populations into new geographic regions, and the subsequent spread or increase of associated diseases, there is a need to identify potential habitats along the expansion front of *I. scapularis.* Therefore, species distribution models (also referred to as habitat suitability models) have become an increasingly useful tool to identify climatic and ecological factors that may be associated with tick occurrence (Guerra *et al.*, 2002; Bunnell *et al.*,

2003; Lubelczyk *et al.*, 2004; Diuk-Wasser *et al.*, 2010; Johnson *et al.*, 2016) Species distribution models (SDMs) can be used to predict additional areas into which the tick may eventually become established and be of public health concern (Illoldi-Rangel *et al.*, 2012; Feria-Arroyo *et al.*, 2014; Gabriele-Rivet *et al.*, 2015; Lieske and Lloyd, 2018; Soucy *et al.*, 2018; Kessler, Ganser and Glass, 2019; Slatculescu *et al.*, 2020; Glass, Ganser and Kessler, 2021; Kopsco *et al.*, 2023). Species distribution models therefore can be useful in identifying potential suitable habitats in regions surveillance has either been not carried out or have been under sampled especially along the *I. scapularis* expansion front. All the described usages of SDMs will ultimately be used to inform public health considerations.

Identifying climatic and ecological correlates to *I. scapularis* occurrence is a critical first step in identifying potential habitat in emergent areas, such as Michigan. Species distribution models will help in identifying potential climatic and ecological correlates that are important for *I. scapularis* occurrence or establishments (i.e., two life stages of *I. scapularis* or greater than 5 individual *I. scapularis* of any life stage detected within one calendar year) especially along the expansion front of *I. scapularis.* Climatic and ecological factors likely play an important role in the establishment and spread of *I. scapularis* (Estrada-Pea, 2001; Estrada-Peña, 2002; Ostfeld *et al.*, 2006; Diuk-Wasser *et al.*, 2010; Johnson *et al.*, 2016; Ginsberg *et al.*, 2017; Gardner *et al.*, 2020).

Ticks are sensitive to humidity and are prone to desiccation at low humidity (Vail and Smith, 1998; Berger *et al.*, 2014; Elias *et al.*, 2021). Ticks are also highly sensitive to variation in temperature. At high temperatures ticks are susceptible to desiccation, there is a reduction in oviposition success in ticks, and high temperatures will decrease ticks host-seeking activities (Needham and Teel, 1991; Duffy and Campbell, 1994; Eisen *et al.*, 2016) and at extreme low

temperatures there may be a delay in development rates, and increase mortality of *I. scapularis*(Lindsay *et al.*, 1995; Vandyk *et al.*, 1996; Eisen *et al.*, 2016). Precipitation pattern changes are also suggested to affect ticks densities and occurrence. At typical precipitation for example rain, the conditions are kept moist which will promote tick survival (Burtis *et al.*, 2016). Precipitation in the winter seasons which is typically snow, greater snowfall will increase snowpack densities keeping the ground level conditions moist and warm and tick will be sheltered from desiccation and the cold (Linske *et al.*, 2019; Volk *et al.*, 2022). With climate change these conditions seem to demonstrate frequent fluctuations which may affect the ability of ticks to survive.

Local ecological characteristics are also likely to play a role in enabling ticks to survive. Many studies have shown that *I. scapularis* are found within deciduous woods with abundant shrubs and relatively thick layers of leaf litter (Ginsberg and Zhioua, 1996; Ginsberg *et al.*, 2004; Linske *et al.*, 2019). Understory and leaf litter cover likely help maintain moist and cool microhabitats needed by *I. scapularis* to avoid desiccation (Ginsberg and Ewing, 1989; Adler *et al.*, 1992; Ostfeld *et al.*, 1995; Lubelczyk *et al.*, 2004). Despite coniferous cover not being the most optimal habitat type, I. *scapularis* have been found in habitats with coniferous cover, likely because of their wide niche range (Lindsay *et al.*, 1999; Lubelczyk *et al.*, 2004; Elias *et al.*, 2006, 2022; Coyle *et al.*, 2013; Lee *et al.*, 2014). Coniferous forests may be less optimal when compared to deciduous forests, however, since they often provide minimal leaf litter, are associated with drier conditions (Ostfeld *et al.*, 1995; Lindsay *et al.*, 1998; Schulze, Jordan and Hung, 1998; Guerra *et al.*, 2002). Another important ecological feature that may affect a tick's ability to survive, is soil composition. Models have predicted that the presence of sandy soil, which allows drainage of excess water, increases *I. scapularis* suitability and survivorship because it prevents the

introduction of fungi or parasitic nematodes on *I. scapularis* as they overwinter (Kitron *et al.*, 1992; Morgan *et al.*, 1994; Bertrand and Wilson, 1996; Lindsay *et al.*, 1998; Schulze, Jordan and Hung, 1998; Guerra *et al.*, 2002). Acidic soils with high clay content are predicted to be unsuitable since they do not allow drainage of excess water (Guerra *et al.*, 2002).

Using SDMs to predict suitable habitat in areas of recent expansion

Since Michigan is a state with an ongoing invasion of *I. scapularis* (Dennis *et al.*, 1998; Eisen, Eisen and Beard, 2016; Lantos *et al.*, 2017; Fleshman *et al.*, 2021), developing habitat suitability models would be beneficial because it will enable us to predict suitable habitats along the expansion front which will help in planning our surveillance efforts to target regions with suitable habitats. Habitat suitability models will also help in informing the public and healthcare workers of the future risk in ticks and tickborne diseases. The first species distribution model for the Great Lakes region was developed using occurrence records for *I. scapularis* in Wisconsin from 1996 to 1998 (Guerra *et al.*, 2002). According to Guerra et al. (2002), upland forests composed predominantly of oak with sandy soils were high suitability habitats for *I. scapularis*. When the Guerra et al. 2002 model was projected onto Michigan, areas that were predicted to contain highly suitable habitat predominantly distributed in the northern Lower Peninsula and several areas in the southwest along the Lake Michigan shoreline and inland areas along the "Thumb" region of Michigan (Erik Scott Foster, 2004).

Using species distribution models developed from endemic areas and projecting those model predictions onto region of recent emergence is one possible approach that may help to identify suitable habitat patches along the expansion front. Extrapolating data trends from endemic zones, however, may not capture differences in habitat and climatic conditions associated with novel and distinct geographic areas. Michigan, for example, has eco-physiographic contexts that

diverge in some respects from neighboring states. While much of northern Wisconsin and the Upper Peninsula of Michigan fall into the Northern Lakes and Forests ecotype (Omernik and Griffith, 2014), southern areas deviate. Southwestern Wisconsin, for example, falls into the Driftless Area ecoregion, where glacial processes had less pronounced effects on the surrounding landscape, whereas southwestern Michigan is predominantly characterized as Michigan/Indiana Drift Plains (Appendix, Figure 5.9), with predominant landforms and forest types more closely associated with post-glacial drift processes (Leverett and Taylor, 1915; Bertrand and Wilson, 1996; Clayton, Attig and Mickelson, 2001; Fisher, Jol and Boudreau, 2005; Omernik and Griffith, 2014; Dickmann and Leefers, 2016). Another major difference is in central Wisconsin there is a large region of northcentral hardwood forests where in Michigan this ecoregion is restricted to the Traverse City Bay area on northwestern region bordering Lake Michigan. So, while many ecological similarities exist between Michigan and other states in the Great Lakes region, models developed in the endemic areas may not necessarily capture differences in ecological context and which may have important effects on the distributional pattern of *I. scapularis* in the state.

Michigan is also geographically distinct from other states in the Upper Midwest because it is comprised of two geographically distinct peninsulas, which are both surrounded by The Great Lakes. This peninsular geography may create heterogeneity in climatic and ecological conditions relevant to the survivorship and distribution of *I. scapularis* throughout the state. Areas more proximate to the Great lakes have more stable temperatures, precipitation patterns, and snow fall throughout the winter season relative to inland areas (Scott and Huff, 1996). The southern region of the Lower Peninsula is also ecologically distinct from the Upper Peninsula (Omernik and Griffith, 2014). Much of the southern regions of the Lower Peninsula belongs to either the Southern Michigan/Northern Indiana drift plains (Omernik and Griffith, 2014) in the west and

Huron/Erie Lake plains on the east, while the northern regions resemble the Upper Peninsula which predominantly belong to the Northern Lakes and Forests ecoregion (Omernik and Griffith, 2014). The southern region of the Lower Peninsula is dominated by deciduous hardwood tree species like oak, beech, maple, and hickory, while the northern regions of the Lower Peninsula dominated by pure conifers, mixed conifers, and hardwood forests (Dickmann and Leefers, 2016). In the Upper Peninsula the eastern region is much like the northern regions of the Lower Peninsula, but with much more wetlands, which are dominated by swamp conifer forests, while the western region of the Upper Peninsula is dominated by a mix of hardwoods and conifers (Dickmann and Leefers, 2016). Human densities are also higher in the Lower Peninsula (U.S. Census Bureau, 2022), leading to greater degrees of forest fragmentation, urbanization, and agricultural land use (Dickmann and Leefers, 2016). Lastly, and most importantly this unique heterogenous geography and ecology of the Lower and Upper Peninsulas has likely affected the expansion dynamics of *I. scapularis,* such that the populations found in each peninsula represents a distinct focus where populations emerged from different regions.

The objective of this study was to identify climatic and habitat conditions associated with *I. scapularis* occurrence in the Lower and Upper Peninsulas of Michigan and to predict suitable habitat patches. We used two different methods to model the distributions of *I. scapularis* in Michigan, which each use a slightly different dataset and a different set of assumptions. We used Michigan statewide surveillance data collected from 2017 to 2021 to identify ecological correlates to *I. scapularis* occurrence and model suitable habitat. We fit models for the Lower and Upper Peninsulas separately due to differences in ecology, geography, and invasion dynamics between the two peninsulas. We expect these will improve the understanding of where suitable sites for *I.*

scapularis are found and what environmental conditions are important in facilitating the spread and establishment of populations throughout Michigan.

MATERIALS AND METHODS

Sampling sites and tick occurrence data

Sampling efforts were primarily focused to areas with upland deciduous forests, because of previously documented associations between *I scapularis* and deciduous forest cover (Ostfeld *et al.*, 1995; Lubelczyk *et al.*, 2004; Randolph, 2004; Pfäffle *et al.*, 2013; Linske *et al.*, 2019). We also sampled in mixed hardwood forests, which comprised of deciduous hardwood trees mixed with coniferous trees, and a few sites were mainly coniferous. Our sites (total sites = 315; Figure 5.1) consisted of public lands ranging from national and state forests, state, county, and city parks, nature conservancies, nature centers, university properties, and a few individually owned properties (e.g., private residences).

Questing ticks were sampled from April to November, 2017 to 2021, targeting the peak activity periods of nymphal and adult *I. scapularis*. Ticks were sampled by drag cloth (Hamer et al. 2010), where a 1 m^2 white-colored corduroy or flannel cloth or 0.75 m^2 canvas cloth with "fingers" weighed down with curtain weights was dragged on the ground over the leaf litter (Rulison *et al.*, 2013; Centers for Disease Control and Prevention, 2019). At most sites, samples were collected along 1600 m transects that were located along the margins of hiking trails because trail sampling best represents risk of tick-borne exposure to the public. In some cases, sampling 1600 m was not possible, but all sites exceeded the 750 m minimum drag distance suggested by the Centers for Disease Control tick surveillance recommendations (Centers for Disease Control and Prevention, 2019). Field collected ticks were stored in 90% ethanol, and species identification was confirmed in at the Michigan State University laboratory using dichotomous keys (Clifford,

Anastos and Elbl, 1961; Sonenshine, 1979; Keirans and Litwak, 1989; Durden and Keirans, 1997). Surveillance records were used to develop site- and county-level distribution maps using ArcGIS Pro 3.1 (ESRI, Redlands, CA, USA).

Species distribution models: Environmental predictors

We selected 29 environmental predictors (Table 5.1) for our initial model matrix, all of which were commonly evaluated in previous habitat association studies for *I. scapularis* (Springer *et al.*, 2015; Hahn *et al.*, 2016; Lippi, Gaff, White and Ryan, 2021; Lippi, Gaff, White, St. John, *et al.*, 2021; Bacon *et al.*, 2022; Kopsco, Smith and Halsey, 2022). All the environmental predictors were gridded geospatial data stored as rasters. Since the source and original resolution varied among datasets, all rasters were reprojected using a USA Albers's Equal Area Conic projection (USGS version), resampled to a spatial resolution of 1 km^2, and finally masked and cropped to the extent of Michigan using the raster package (version 3.6-20), in RStudio (version 2023.03.1).

Climatic predictors included the 19 bioclimatic variables obtained from WorldClim version 2.1 (Fick and Hijmans, 2017). The bioclimatic variables are a set of variables based on temperature and precipitation measurements taken from global weather stations and averaged across the years from 1970 to 2000, which are meant to represent annual mean global trends, along with seasonality, extreme environmental factors and limiting environmental factors (Hijmans *et al.*, 2005; Fick and Hijmans, 2017).

Michigan is at the northern extent of the distribution of *I. scapularis*, making it more likely that these populations will experience greater seasonal variation in temperature and greater amounts of snowfall relative to southern endemic areas. Because of this, we included two additional climatic predictors, snow water equivalent and cumulative growing degree days, to represent climatic factors that may affect Michigan's tick populations. Snow water equivalent

represents the accumulation of snow during winter months. Snow accumulation insulates the ground, maintaining more humid conditions and higher temperatures than exposed ground (Decker *et al.*, 2003; Templer *et al.*, 2012). This insulating effect likely helps to increase survivorship of *I. scapularis* in winter months (Linske et al., 2019; Volk et al., 2022). Cumulative growing degree days is another important measure that affects the development of *I. scapularis* where temperature changes affect the molting success and molting times of *I. scapularis* (Brunner *et al.*, 2023). We calculated cumulative growing degree days for Michigan by taking the mid-range temperature and then secondarily evaluating the difference between the annual mid-range temperature and a minimum temperature threshold of 0 ºC (McMaster and Wilhelm, 1997). Snow water equivalent and maximum and minimum temperatures were obtained from the Daymet database (version 4; provided courtesy of Oak Ridge National Laboratory, Oak Ridge, TN, USA; Thornton et al., 2022, 1997).

In addition to climate predictor rasters, we also included three categories of ecological predictors in our model: (1) landcover and habitat, (2) soil properties, and (3) host presence. Landcover and habitat predictors included the National Landcover Database (NLCD) land cover types and forest groups. The NLCD land cover type is a raster dataset where each pixel indicates the predominant cover type of an area, with 16 options including open area or water, degree of urban development, barren land, forest cover, shrubland, herbaceous cover and grassland, croplands, and wetlands (Yang *et al.*, 2018; Dewitz and U.S. Geological Survey, 2021; Wickham *et al.*, 2021) provided courtesy of the Multi-Resolution Land Characteristics Consortium (Sioux Falls, SD, USA). The subcategories (16 layers) were combined to form seven raster layers representing the major cover types listed above. In addition to major land-use categories, we also included major forest group classifications in our final model set. The forest groups data used

advanced resolution radiometer imagery to represent 145 forest types spread across the United States (Ruefenacht *et al.*, 2008) provided courtesy of USDA Forest Service Geodata Clearinghouse (United States Department of Agriculture, Washington D.C., USA). The forest groups were separated to form six raster layers: (1) groups comprising of white, red and jack pine forest groups, (2) spruce and fir forest groups, (3) oak and hickory forest groups, (4) elm, ash, and cottonwood forest groups, (5) maple, beech, and birch forest groups, and (6) aspen and birch forest groups.

We incorporated information on the following soil properties in the model set: (1) soil organic carbon density at a depth of 0 to 5 cm, (2) soil water capacity until wilting point at a depth of 0 cm, (3) soil sand content, and (4) soil clay content, which were downloaded as gridded geospatial layers from SoilGrids (Poggio *et al.*, 2021) provided courtesy of the International Soil Reference Information Centre (ISRIC) (Wageningen, The Netherlands). The soil organic carbon density at a depth of 0 to 5cm represents the carbon content of the topsoil layer, mainly consisting of leaf litter, which is likely to be beneficial for the survival of *I. scapularis* (Linske *et al.*, 2019; Volk *et al.*, 2022). The soil water capacity until wilting point represents the soil moisture level. Moisture in the topsoil layer likely plays a role in helping *I. scapularis* to avoid desiccation (Lippi, Gaff, White, St. John, *et al.*, 2021). Both soil sand and soil clay content represent soil properties that are important in water drainage through the soil where high soil sand content has better excessive water drainage ability compared to soil with high clay content, therefore another environmental variable that will keep the topmost soil layer moist such that will prevent desiccation of *I. scapularis* (Guerra *et al.*, 2002).

White-tailed deer (*Odocoileus virginianus*) are important reproductive hosts for *I. scapularis*; therefore, we included a raster which mapped the presence of *O. virginianus* in Michigan (U.S. Geological Survey (USGS) - Gap Analysis Project (GAP), 2018).

Multicollinearity, when the values of two or more predictors in a model matrix are highly correlated with each other (Alin, 2010), is suggested as a possible source of bias. This is because if two variables are correlated with each other, the resulting coefficients are inflated and standard errors are larger (De Marco and Nóbrega, 2018). We tested for multicollinearity among predictors using the variance inflation factor (VIF). The VIF is a numerical index that represents the degree to which variation in one predictor can be attributed to other variables due to underlying correlation structure (Alin, 2010; Vu, Muttaqi and Agalgaonkar, 2015; Cheng *et al.*, 2022). Large VIF values (VIF > 10) may indicate significant multicollinearity between the predictor of interest and others. Therefore, any predictor with a VIF > 10 was removed from the final model matrix prior to subsequent analyses (Cheng *et al.*, 2022).

Table 5.1. A description of the environmental variables used in species distribution modeling.

Variable	Name	Final Resolution	Units	Source
Bio 1	Annual Mean Temperature	1 km^2	ºC	WorldClim data base
Bio 2	Mean Diurnal Range (Mean of monthly (max temp - min temp))	1 km^2	ºC	WorldClim data base
Bio 3	Isothermality (BIO2/BIO7) (×100)	1 km^2	%	WorldClim data base
Bio 4	Temperature Seasonality (standard deviation ×100)	1 km^2	%	WorldClim data base
Bio 5	Max Temperature of Warmest Month	1 km^2	ºC	WorldClim data base
Bio 6	Min Temperature of Coldest Month	1 km^2	ºC	WorldClim data base
Bio 7	Temperature Annual Range (BIO5-BIO6)	1 km^2	ºC	WorldClim data base
Bio 8	Mean Temperature of Wettest Quarter	1 km^2	ºC	WorldClim data base
Bio 9	Mean Temperature of Driest Quarter	1 km^2	ºC	WorldClim data base
Bio 10	Mean Temperature of Warmest Quarter	1 km^2	ºC	WorldClim data base
Bio 11	Mean Temperature of Coldest Quarter	1 km^2	ºC	WorldClim data base
Bio 12	Annual Precipitation	1 km^2	mm	WorldClim data base
Bio 13	Precipitation of Wettest Month	1 km^2	mm	WorldClim data base
Bio 14	Precipitation of Driest Month	1 km^2	mm	WorldClim data base
Bio 15	Precipitation Seasonality (Coefficient of Variation)	1 km^2	%	WorldClim data base
Bio 16	Precipitation of Wettest Quarter	1 km^2		WorldClim data base
Bio 17	Precipitation of Driest Quarter	1 km^2	mm	WorldClim data base
Bio 18	Precipitation of Warmest Quarter	1 km^2	mm	WorldClim data base
Bio 19	Precipitation of Coldest Quarter	1 km^2	mm	WorldClim data base
Snow water equivalent	The average of the daily snow water equivalent (the amount of water contained within the snowpack)	1 km^2	kg m-2	Daymet Database
Tmax	Maximum temperature	1 km^2	ºC	Daymet Database
Tmin	Minimum temperature		ºC	Daymet Database
Elevation	SRTM (Shuttle Radar Topography Mission) elevation data	1 km^2	m	WorldClim data base
Forest groups	6 forest group layers	1 km^2	NA	USDA-FS

Table 5.1 (cont'd)

Land cover classes	Land Cover classes in 2019 put into 7 raster layers	1 km^2	NA	U.S. Geological Survey (USGS) and MRLCR
Soil clay content	Clay content (0-2 micrometer) mass fraction in % at a depth of 0 - 5cm.	1 km^2	mass fraction in ‰	ISRIC World soil information
Soil water retention	Derived available soil water capacity (volumetric fraction) until wilting point at of soil ground observations at a dept of 0cm	1 km^2	volumetric fraction	ISRIC World soil information
White-tailed deer presence	Habitat distribution map for white-tailed deer (*Odocoileus virginianus*) based on 2001 ground conditions	1 km^2	presence-absence	USGS Gap Analysis

Species distribution models: models and evaluation

Two modeling methods were used and compared to develop a species distribution model (SDM) and predict suitable habitats for *I. scapularis* within Michigan. The first approach used a generalized linear model (GLM) with a logit link function. The GLM method incorporates information on both the presence and absence of *I. scapularis*, so both were included in the data set. Presence points were defined as any sample site where *I. scapularis* was detected at least once during the study period, and absence points were defined as any sample site where *I. scapularis* was not detected over the 5-year study period. We originally used different criteria to classify presence points, ranging from the most lenient (single detection at least once during the study period) to more stringent classifications based on CDC establishment criteria (i.e., two or more ticks of any life stage or two life stages collected during a single sampling year, Dennis et al., 1998; Eisen, Eisen and Beard, 2016). We ultimately chose to use simple presence/absence criteria since more stringent criteria did not improve model performance in pilot analyses and we did not have enough data points in the Upper Peninsula (Appendix, Table 5.6). For our second approach we used a machine learning method, maximum entropy modeling (MaxEnt). Unlike the GLM, MaxEnt only requires presence points. We generated 10,000 randomly selected pseudoabsence points to extract background environmental information for MaxEnt model comparisons. The SDM package version 1.1-8 (Naimi and Araújo, 2016) in RStudio were used for GLM analyses, while the MaxEnt analysis was performed using the standalone MaxEnt application (version 3.4.4) in Java (version 8) using the provided graphical user interface (Larson et al., 2022; Phillips and Dudik Miroslav, 2008). Graphical output (e.g., response curves) for the GLM was produced on RStudio, using The SDM package version 1.1-8, while for the MaxEnt produced by the MaxEnt

application. The habitat suitability maps for GLM were produced by The SDM package version 1.1-8 while the habitat suitability maps for MaxEnt was produced by the MaxEnt application.

Because sampling locations were unevenly distributed (Figure 3.1), sampling points were thinned to reduce the possibility of spatial pseudo replication in areas where samples were clustered (within 10 km of each other) prior to model fitting. In cases where thinning was necessary, we used the spThin package version 0.2.0 on R (Aiello-Lammens *et al.*, 2015) to randomly select a single point within clusters that maintained >10 km distance to the next point. For the GLM, environmental data from rasters were extracted using a 10 km radial buffer around each of the sampling points using the raster package version 3.6-23 (Hijmans and van Etten, 2012) on RStudio. We then took the average of each environmental data within that 10 km radial buffer. For the categorical variables such as presence of forest classes and presence of white-tailed deer we took the average value within a 10 km radial buffer which made these variables continuous. For MaxEnt, raster values were extracted from the cell that the sampling point overlaid by the program itself. For both models, we randomly split the data into two subsets, where 70% of the data were included in the training dataset, while 30% of the data were included in the test dataset.

Variable selection was conducted using two separate approaches, depending on the model. For the GLM, a bi-directional stepwise regression analysis was run independently for both Lower and Upper Peninsula analyses prior to fitting the final models for selecting variables that explained the variation seen in the presence of *I. scapularis.* For MaxEnt, variable selection was based on the permutation of importance to evaluate which variables were important in developing the model (Phillips and Dudik Miroslav, 2008; Larson *et al.*, 2022). Permutation of importance can be defined as how the model performs (typically area under the curve values) in the presence of an

environment predictor and in the absence of that particular predictor; the higher the permutation of importance value the greater importance the variable has in explaining the variation in the data.

The predictive performance of both models was evaluated using a repeated K-10-fold cross validation procedure with 10,000 iterations, where for each fold, we randomly split the data into two subsets, where 70% of the data were included in the training dataset, and 30% of the data were included in the test dataset. Model performance was evaluated for both methods based on a threshold dependent criterion which is based on the prevalence of presence points and a threshold independent criterion (Fielding and Bell, 1997b). For a threshold-independent method, the receiver operator curve (ROC) area under the curve (AUC) criteria were used. The ROC considers the true positives (sensitivity) and the false negatives. And the AUC range from 0 – 1 where a value below 0.5 indicates that the presence and absence points are distributed at random by chance and the model could not distinguish between the actual presence points and the false positives, while a value greater than 0.5 indicates the model can distinguish between a true positive and a false positive. Threshold-dependent evaluation methods included the true skill statistic (TSS) and model deviance. The TSS of a model represents the factors of the true positive rates (sensitivity) and the true negative rates (specificity) in a model (Somodi, Lepesi and Botta-Dukát, 2017). The values for TSS range from 0 – 1, where values closer to 0 represent models with low sensitivity and specificity, while a values closer to 1 represent high sensitivity and specificity. The deviance value represents the extent of the deviation of the test data from the training data.

RESULTS

Distribution of I. scapularis *in Michigan*

Our dataset comprised 315 sampling sites throughout Michigan (Figure 5.1) from 2017 to 2021. Sampling effort differed due to the specific surveillance objectives of each year (for a summary see Fowler et al., 2022). In 2017, sampling was focused in the southeast and the "Thumb" region of the Lower Peninsula adjacent to Lake Huron (N = 73 sites; Appendix, Figure 5.9). In 2018 (N = 153 sites), 2019 (N = 95 sites) and 2021 (N = 171 sites), sampling was carried out throughout Michigan and ranged from sites in areas with known established populations of *I. scapularis* to those with no previous record of occurrence (Appendix, Figure 5.9). In 2019 sites were fewer and less dense compared to that in 2018 and 2021 as sites were sampled at least three times, an objective was to obtain a better estimate of nymphal abundance as well as of prevalence of infection with the Lyme disease bacterium. Due to COVID-19 restrictions in 2020, sampling was limited to southern Michigan (N = 81 sites, Appendix, Figure 5.9).

Over the 5 years, *I. scapularis* was detected in 175 sites (Figure 5.1), where 117 sites had established populations in at least one year according to the CDC criteria (Table 5.2). Out of the 83 counties in Michigan, *I. scapularis* was detected in 70 counties, where 63 counties had established populations in at least one year (Appendix, Figure 5.10).

Table 5.2. Sites and counties sampled in Michigan from 2017 to 2021 and the status of *Ixodes scapularis* presence classified as reported or established using the CDC criteria for that year (unlike the CDC, the status of *Ixodes scapularis* populations was determined by the data collected that year and was not contingent upon prior years' categorizations).

Year	**Number of sites sampled**	**Number of counties sampled**	**Number of sites (Number of counties) in which *I. scapularis* is classified as "established"**	**Number of sites (counties) in which *I. scapularis* is classified as "reported"**
2017	74	46	16 (15)	10 (6)
2018	180	79	37 (34)	28 (11)
2019	95	63	57 (44)	18 (11)
2020	80	27	38 (18)	23 (7)
2021	171	83	79 (53)	30 (10)

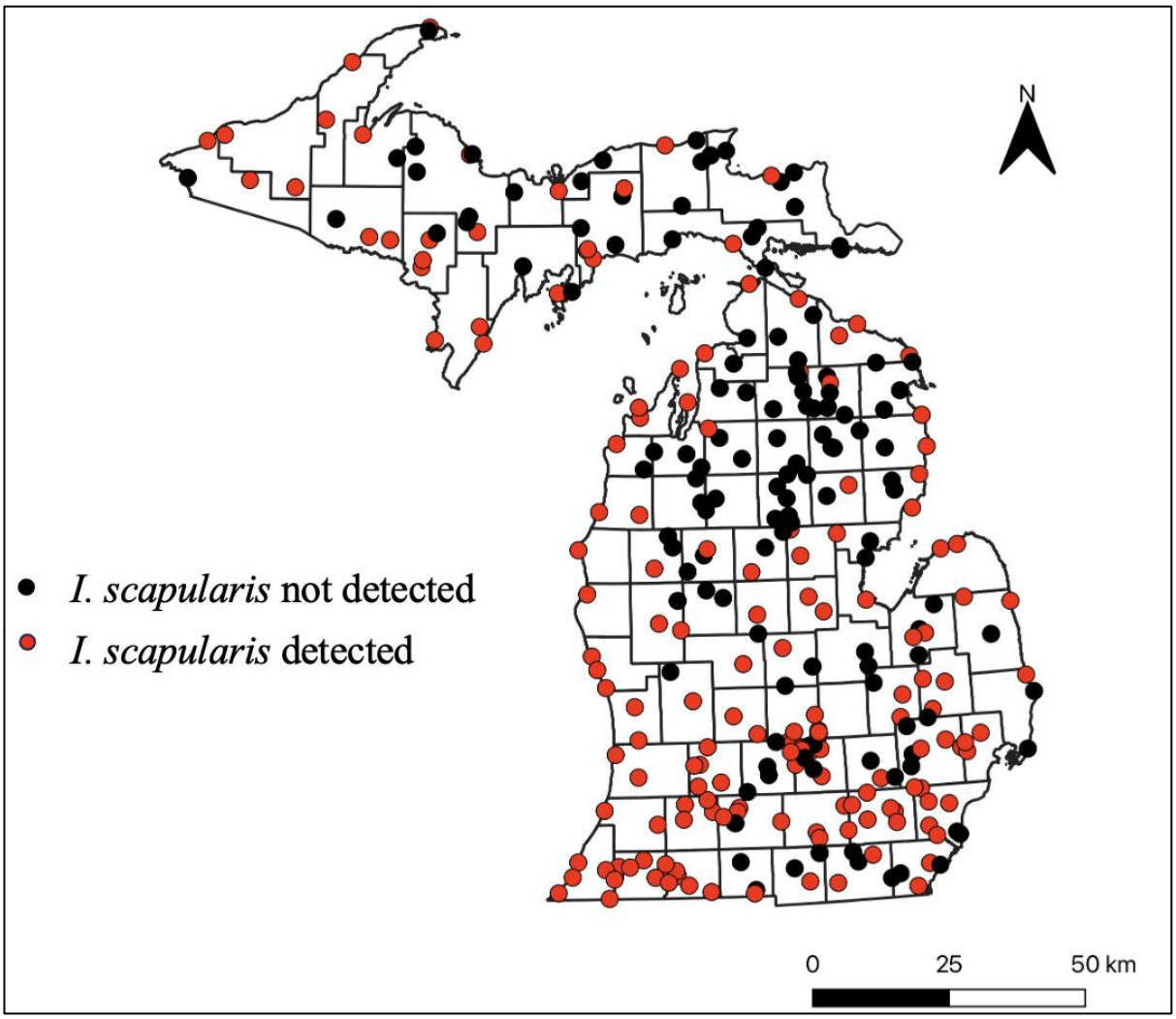

Figure 5.1. Sample sites in Michigan where *Ixodes scapularis* was detected by drag sampling in at least one year from 2017 to 2022.

Environmental variables of importance

Out of the 29 initial environmental predictors, 16 and 19 predictors had a VIF > 10 for the Lower Peninsula and Upper Peninsula respectively, indicating that several predictors were highly correlated. The variables that were highly correlated for both peninsulas were BIO 1, BIO 2, BIO 5, BIO 6, BIO 7, BIO 10, BIO 11, BIO 12, BIO 15, BIO 19, growing degree days, soil sand content, soil organic carbon density, open water, and forests. Furthermore, for the Lower Peninsula, soil water retention was highly correlated with the other variables mentioned above, while for the

Upper Peninsula, BIO 18, soil clay content, barren land and oak and hickory forest groups were highly correlated too. Thus, these variables were removed from subsequent analyses for their respective regions.

Generalized linear model outputs

Bidirectional stepwise regression retained the following predictors in the Lower Peninsula GLM: BIO 3, BIO 4, BIO 8, snow water equivalent, elevation, soil water retention at wilting capacity, the presence of white, red and jack pine forest group, and the presence of white-tailed deer. For the Upper Peninsula, the following predictors were retained in the GLM: BIO 3, BIO 4, BIO 13, and BIO 14, elevation, soil water retention at wilting capacity, the presence of elm, ash and cottonwood forest group, the presence of maple, beech, and birch forest group, the presence of developed land, the presence of crop land, and the presence of wetland. (Table 5.3)

Table 5.3. The stepwise regression coefficients for the environmental predictors for the occurrence of *Ixodes scapularis* that were retained in the GLM for each peninsula. See Table 1 in Methods for a list and description of all variables.

Region	Environmental Predictors	Coefficient	Standard Error	z-value	p-value
Lower Peninsula	Intercept	30.06	15.91	1.89	0.06
	BIO 3	0.47	0.24	2.01	0.04
	BIO 4	-0.05	0.02	-2.86	<0.01
	BIO 8	0.48	0.14	3.41	<0.01
	Snow water equivalent	-0.09	0.05	-1.98	0.05
	Elevation	-0.01	0.01	-3.13	<0.01
	Soil water retention at wilting capacity	0.01	0.002	3.47	<0.01
	White, red and jack pine forest group presence	-1.48	0.96	-1.55	0.12
	White-tailed deer presence	1.98	0.69	2.87	<0.01
Upper Peninsula	Intercept	-81.02	34.58	-2.34	0.02
	BIO 3	-1.80	0.98	-1.85	0.06
	BIO 4	0.14	0.05	2.78	0.01
	BIO 13	-0.26	0.14	-1.80	0.07
	BIO 14	-0.28	0.18	-1.58	0.11
	Elevation	-0.03	0.02	-1.98	0.05
	Soil water retention at wilting capacity	0.05	0.02	2.44	0.01
	Elm, ash, and cottonwood forest groups presence	-52.72	9140.48	-0.01	1.00
	Maple, beech, and birch forest groups presence	3.05	1.82	1.68	0.09
	Developed land presence	-8.15	7.08	-1.15	0.25
	Crop land presence	-21.77	4475.37	-0.01	1.00
	Wet land presence	-4.43	2.85	-1.55	0.12

Looking at the percent contribution of each variable to the Lower Peninsula GLM, BIO 8 and snow water equivalent were two climatic predictors that contributed the most (~44%), and soil water retention at wilting capacity and elevation were the ecological predictors that contributed the most (~46%) (Table 5.4). For the Upper Peninsula GLM, BIO 3, BIO 4, and BIO 13 were the climatic predictors that contributed the most (~44%), and elevation, the presence of maple, birch and beech forest group and the presence of developed land were the ecological predictors that contributed the most (~41%) (Table 5.4)

Table 5.4. Permutation of importance of environmental variables for the occurrence of *Ixodes scapularis* that were used in each the GLM for each peninsula. (See Table 5.1 in Methods for a list and description of all variables).

Region	Environmental Predictor	% Contribution
Lower Peninsula	Soil water retention at wilting capacity	25.3
	Snow water equivalent	24.7
	Elevation	21.1
	BIO 8	19.0
	BIO 4	4.3
	BIO 3	3.9
	White, red and jack pine	1.6
	White-tailed deer	0.1
Upper Peninsula	BIO 3	18.3
	BIO 14	17.2
	Elevation	15.8
	Maple, beech, and birch forest groups	15.3
	Developed land	10.2
	Wet land	9.5
	BIO 4	8.6
	BIO 13	3.7
	Soil water retention at wilting capacity	1.4

In the Lower Peninsula, BIO 3 and BIO 8 have a positive association, while BIO 4 and the snow water equivalent have a negative association with the likelihood of *I. scapularis* occurrence

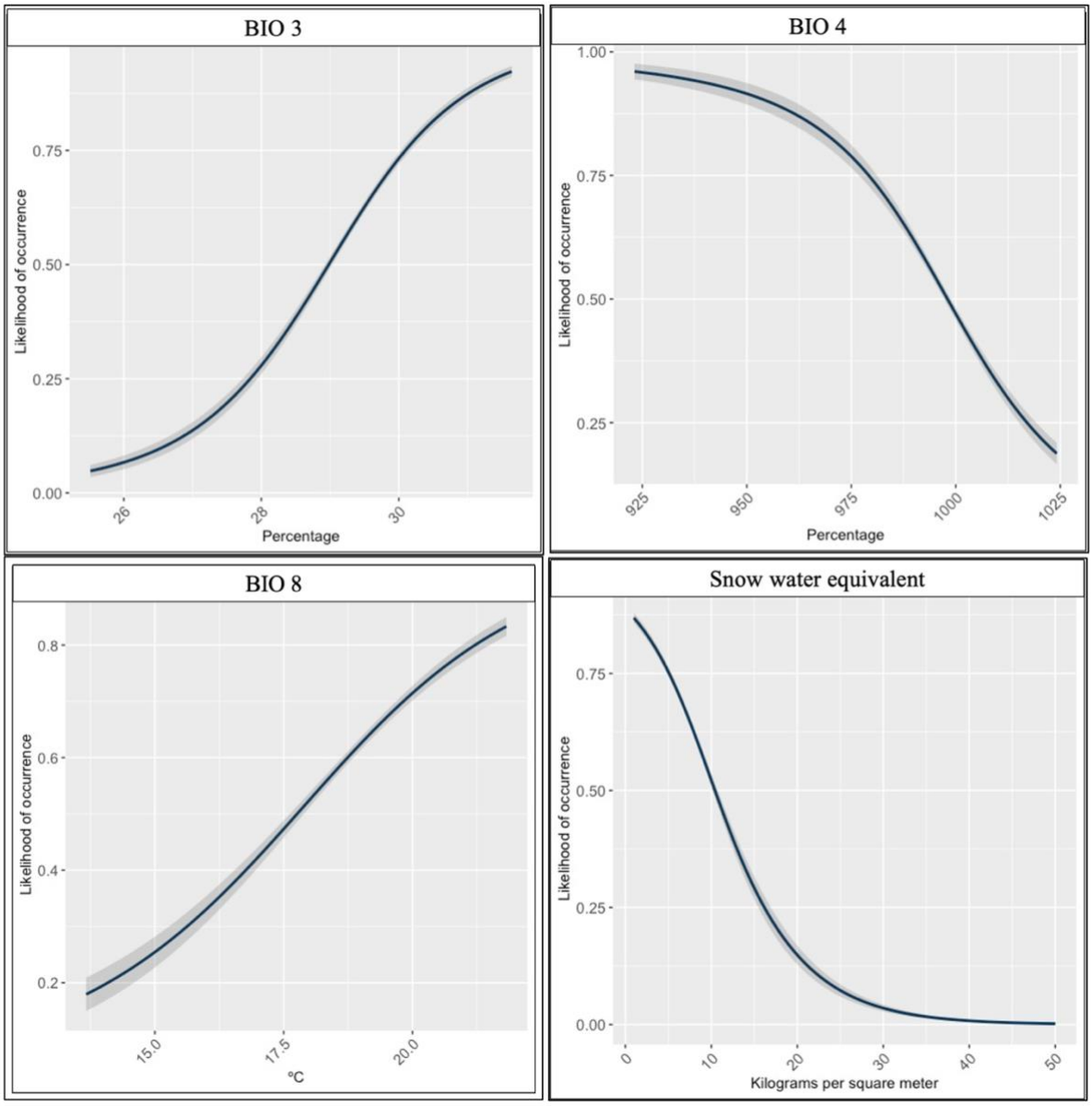

Figure 5.2. The response curves for the climatic predictors of the GLM for the Lower Peninsula for the occurrence of *Ixodes scapularis*. The light grey shade around each curve represents the standard deviations. See Table 1 in Methods for a list and description of all variables.

(Figure 5.2). In the Upper Peninsula BIO 14 has a positive association; BIO 3 has stable interaction and then it decreases and increases; while BIO 4 have BIO 13 have a negative association with the likelihood of *I scapularis* occurrence (Figure 5.3).

For the ecological predictors, in the Lower Peninsula elevation and the presence of white, red and jack pine have a negative association, while the soil water content at wilting capacity and the presence of white-tailed deer has a positive association with the likelihood of *I. scapularis* occurrence (Figure 5.4). In the Upper Peninsula, elevation and the presence of developed land have a negative association, while soil water content at wilting capacity, the presence of maple, birch and beech forest group and the presence of wetlands have a positive association with the likelihood of *I. scapularis* occurrence (Figure 5.5).

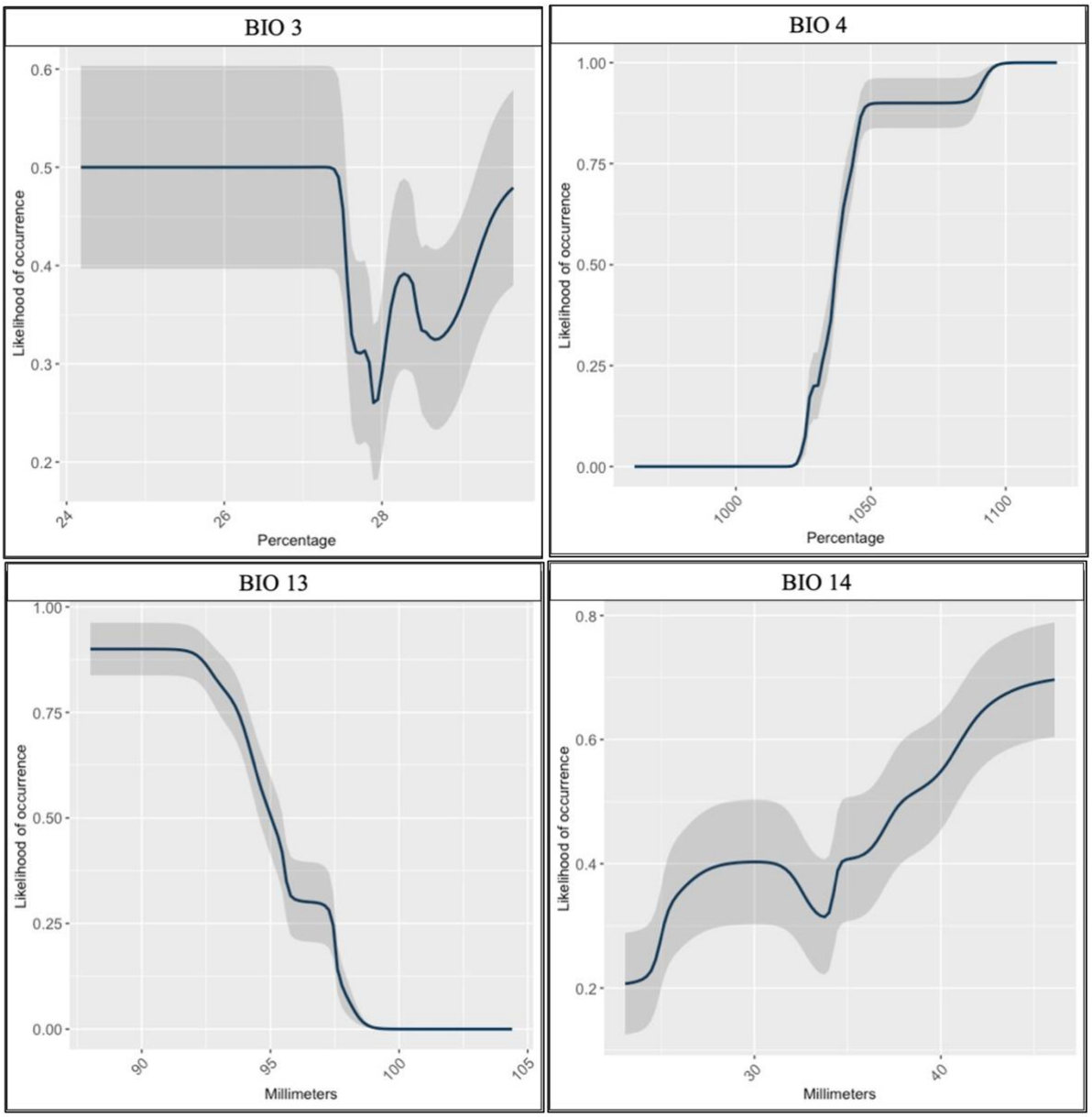

Figure 5.3. The response curves for the climatic predictors of the GLM for the Upper Peninsula for the occurrence of *Ixodes scapularis*. The light grey shade around each curve represents the +/- 1 standard deviations. See Table 1 in Methods for a list and description of all variables.

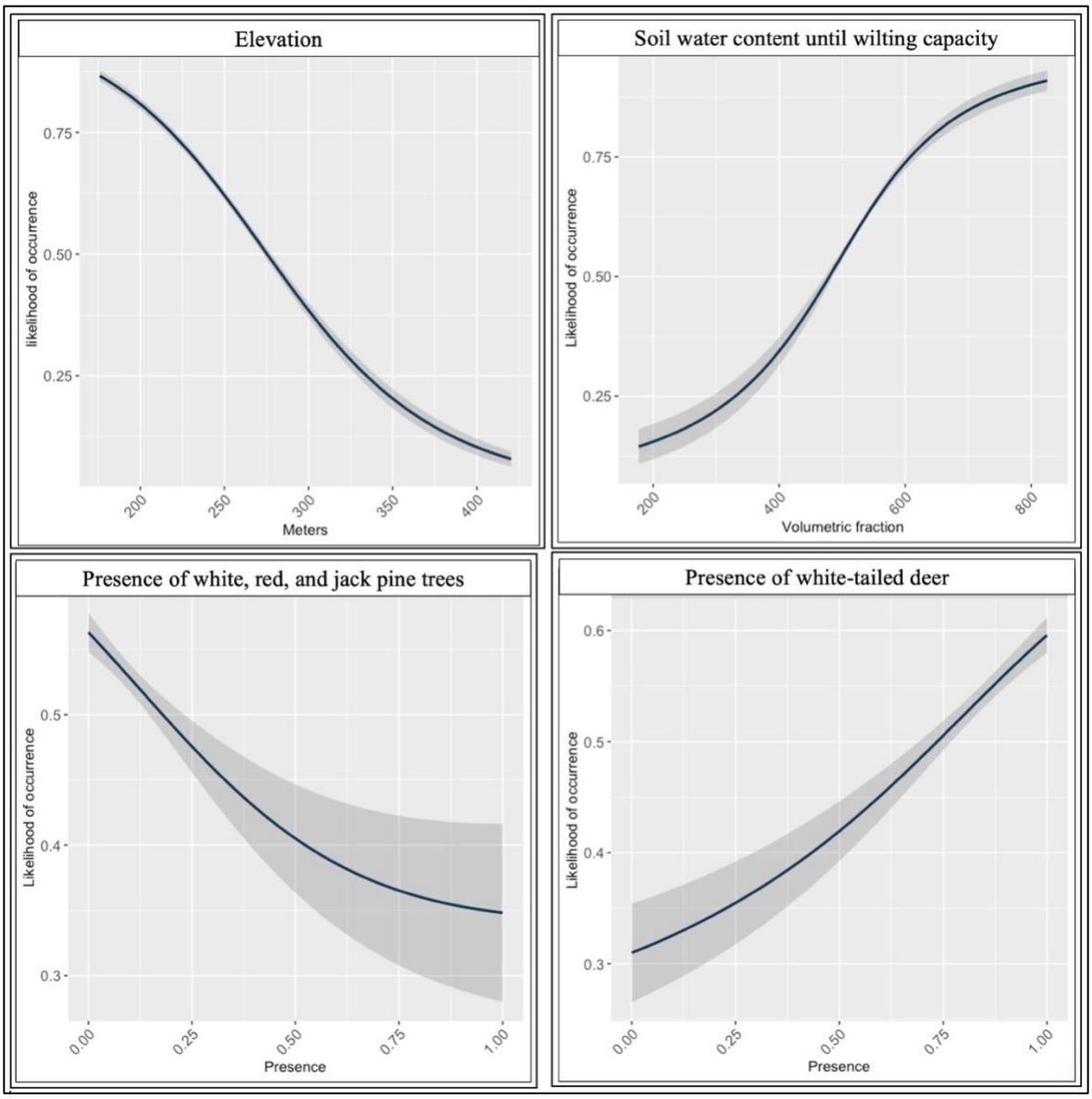

Figure 5.4. The response curves of the ecological predictions of the GLM for the Lower Peninsula for the occurrence of *Ixodes scapularis*. The gray shaded area around each curve represents the +/- 1 standard deviation.

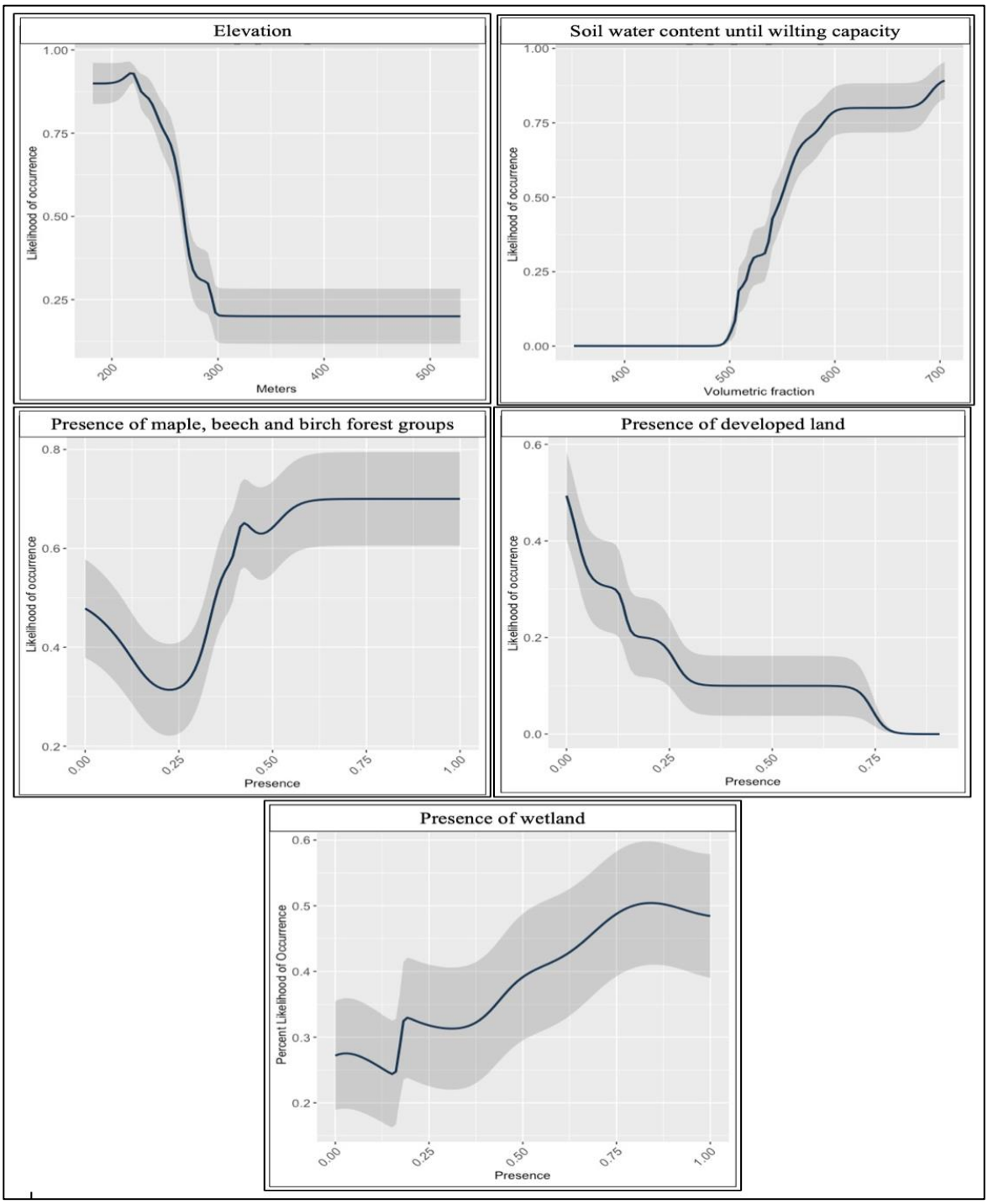

Figure 5.5. The response curves of the ecological predictions of the GLM for the Upper Peninsula. The gray shaded area around each curve represents the +/- 1 standard deviation.

The AUC values for the Lower and Upper Peninsula GLMs are 0.78 and 0.85 respectively (Table 5.5). The TSS values for the Lower and Upper Peninsula models are 0.41 and 0.63 respectively (Table 5.5). The model deviance in the GLM was greater for the Upper Peninsula (deviance = 8.55, Table 5.5) compared to the Lower Peninsula (deviance = 1.18, Table 5.5).

Table 5.5. Evaluation metrics for GLM and MaxEnt models for *Ixodes scapularis* presence data for the Lower and Upper Peninsulas. (AUC = Area Under the Curve; TSS = True Skill Statistic).

Peninsula	**Model Type**	**Evaluation criteria**	**Value**
Lower	GLM	AUC	0.78
		TSS	0.41
		Model deviance	1.18
	MaxEnt	AUC	0.93
		TSS	0.45
		Model deviance	0.67
Upper	GLM	AUC	0.85
		TSS	0.64
		Model deviance	8.55
	MaxEnt	AUC	0.93
		TSS	0.35
		Model deviance	0.67

For the GLM model for the Lower Peninsula, suitable habitats for the occurrence of *I. scapularis* are predicted in a majority of the southwest; patchy areas in the south; and coastal areas in the southwest, southeast in the Thumb, and northeast (Figure 5.6). In contrast, the inland regions in the northwest and north central areas are estimated to be unsuitable by the GLM for the occurrence of *I. scapularis* (Figure 5.6). For the GLM model for the Upper Peninsula, a large portion of the southern mid region (bordering Wisconsin and Lake Michigan, including Menominee County) is estimated to be highly suitable for *I. scapularis.* Pockets of moderately suitable regions occur mainly in the western portion of the Lower Peninsula, while three large

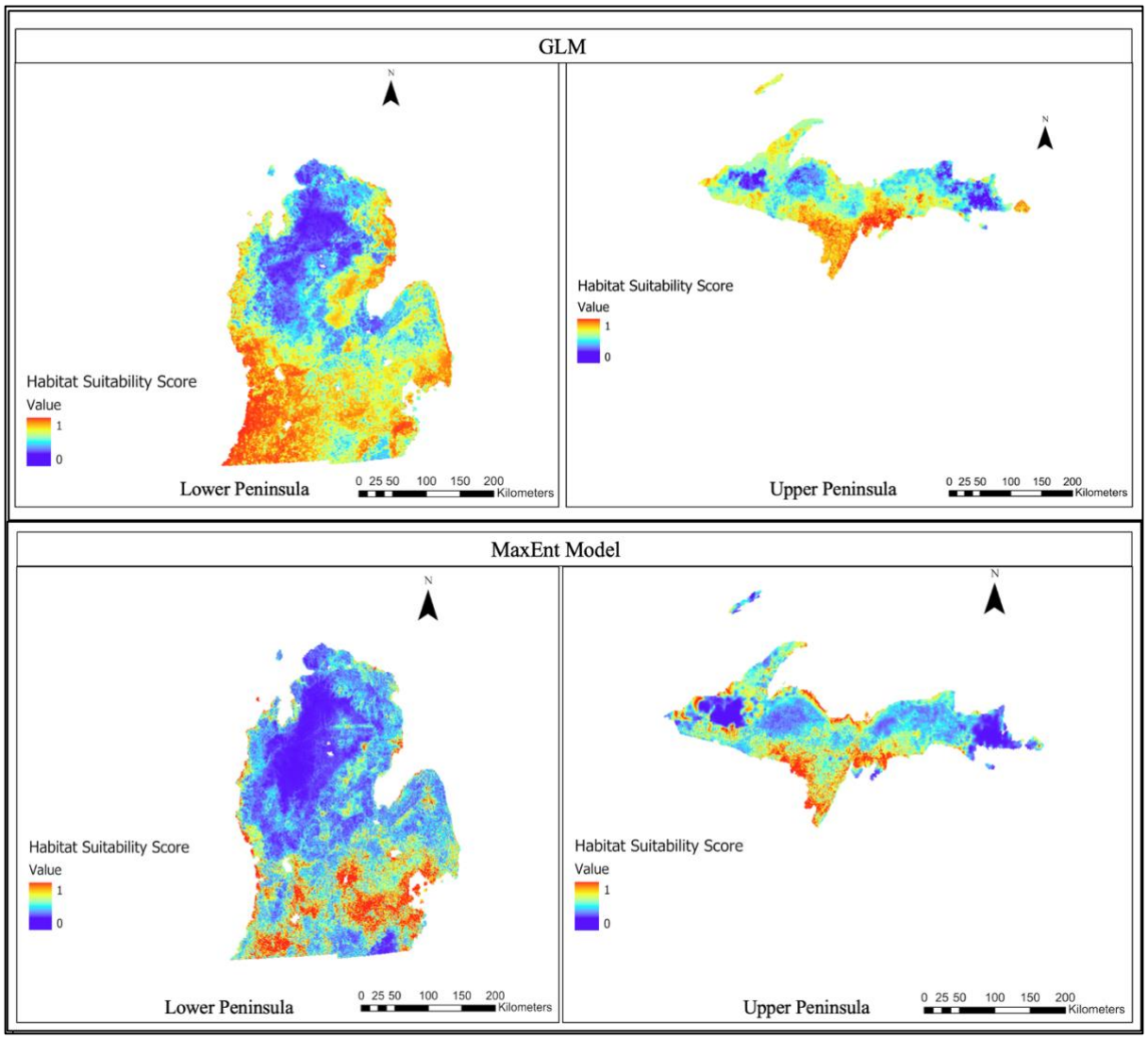

Figure 5.6. The raster outputs of the GLM and the MaxEnt Model for *Ixodes scapularis* presence data for the Lower and Upper Peninsula separately.

sections in the central inland and eastern regions are predicted to be least suitable for *I. scapularis* (Figure 5.6)

MaxEnt model outputs

In the MaxEnt model for the Lower Peninsula, the climatic variables BIO 9, BIO 3, BIO 8, BIO 13, and the snow water equivalent contribute the most (~48%). The ecological variables

elevation, presence of crop land, soil water retention at wilting capacity and the presence of white-tailed deer contributes the most (~33.2%) (Table 5.6).

Table 5.6. Percent permutation of importance of environmental predictors that were retained in the MaxEnt Model for the Lower and Upper Peninsulas. See Table 1 in Methods for a list and description of all variables.

Region	Environmental Predictors	% Contribution
Lower Peninsula	BIO 9	13.3
	Elevation	13.9
	BIO 3	10.6
	BIO 8	10.3
	BIO 13	8.0
	Percentage of crop land	7.4
	Soil water retention at wilting capacity	6.8
	Snow water equivalent	5.5
	Presence of white-tailed deer	5.1
	BIO 4	4.7
	BIO 18	4.3
	BIO 14	2.5
	Presence of wetland	2.2
	Presence of spruce and fir forest groups	2.1
	Presence of aspen and birch forest groups	1.1
	Presence of grass and shrub land	0.8
	Presence of developed land	0.7
	Presence of white, red and jack pine forest groups	0.3
	Presence of barren land	0.2
	Presence of oak and hickory forest groups	0.1
Upper Peninsula	Soil clay content	42.1
	Elevation	10.7
	Snow water equivalent	9.7
	Presence of maple, birch, and beech forest groups	5.8
	BIO 3	4.8
	BIO 13	4.8
	Presence of wetland	4.6
	BIO 4	3.4
	BIO 8	3.1
	Soil water retention at wilting capacity	2.8
	BIO 14	2.6
	BIO 9	1.6

Table 5.1 (cont'd)

	Presence of white, red and jack pine forest groups	1.1
	Presence of spruce and fir forest groups	1.0
	Presence of aspen and birch forest groups	0.8
	Presence of white-tailed deer	0.5
	Presence of grass and shrub land	0.4
	Presence of developed land	0.2

In the MaxEnt model for the Upper Peninsula, the climatic variables did not contribute a large amount to the model, whereas the ecological variables elevation, soil clay content, and the presence of maple, birch and beech forest group contribute the most (~59%) (Table 5.5). In the Lower Peninsula MaxEnt model, BIO 3 and BIO 8 had a positive association, while BIO 9, BIO 13 and the snow water equivalent had a negative association with the log likelihood occurrence of *I. scapularis* (Figure 5.7). For the ecological predictors, elevation and presence of crop land had a negative association, while the soil water content until wilting capacity and presence of white-tailed deer had a positive association with the log likelihood occurrence of *I. scapularis* (Figure 5.8). In the Upper Peninsula MaxEnt model, elevation and soil clay content had a negative association, while the presence of maple, birch and beech forest group had a positive association with the log likelihood occurrence of *I. scapularis* (Figure 5.8). Considering model evaluation, the AUC values for the Upper and Lower Peninsula both are about 0.93; the TSS values are 0.45 and 0.35, respectively (Table 5.5); and the model deviance values for the Upper and Lower Peninsula were 0.67 and 0.67, respectively.

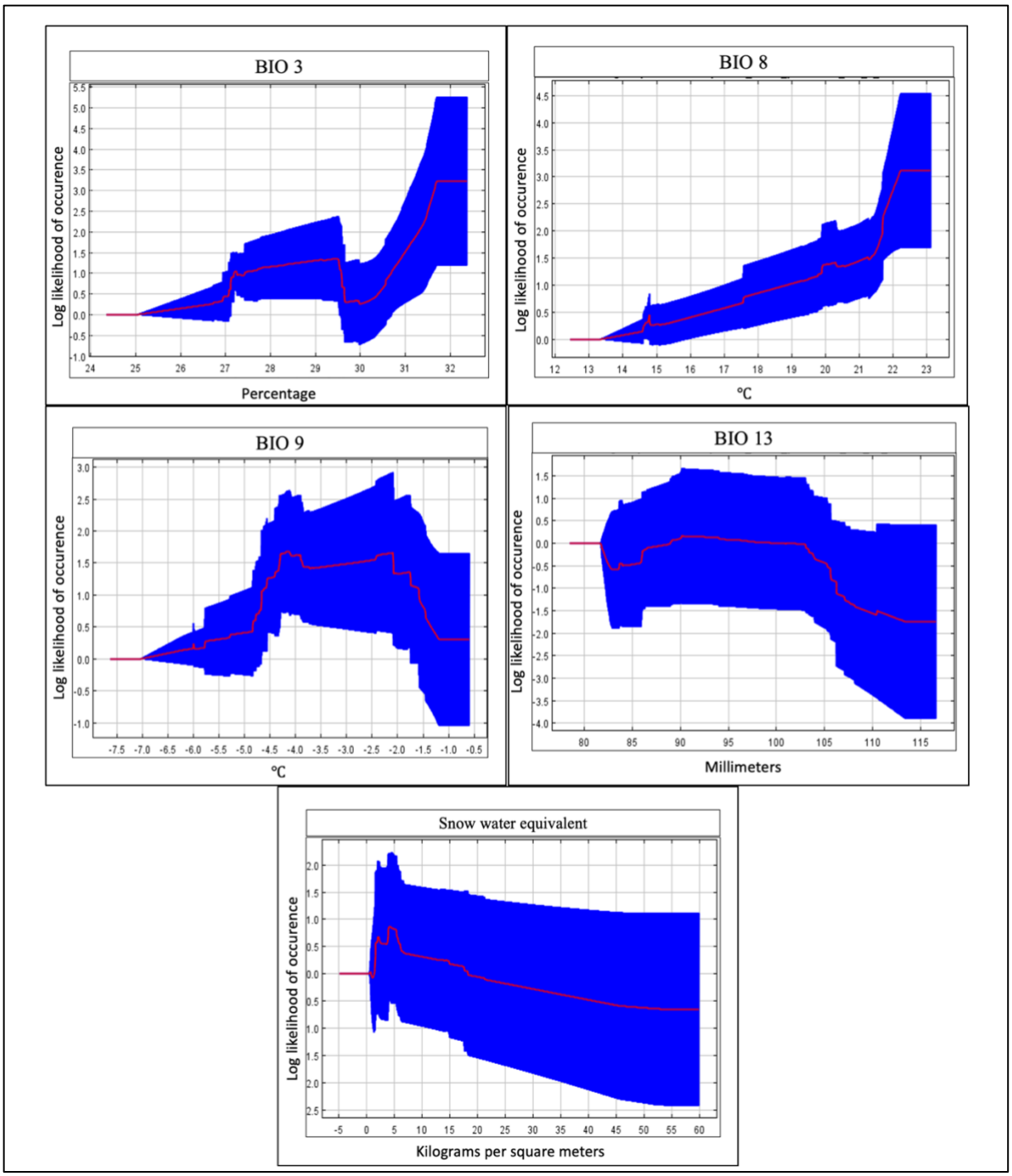

Figure 5.7. The response curves for the climatic predictors of the MaxEnt model for the occurrence of *Ixodes scapularis* for the Lower Peninsula. The blue shaded area around each curve represents the +/- 1 standard deviations. See Table 1 in Methods for a list and description of all variables.

The MaxEnt model raster outputs of the suitable habitats for *I. scapularis* (Figure 5.6) suggest that in the Lower Peninsula, there are pockets of moderate to high levels of habitat suitability mainly in southern region, spanning from Lake Michigan in the west to Lake Huron in the east. A coastal band of moderately suitable habitat of variable width lies along Lake Michigan in the west and along Lake Huron in the east, with some small highly suitable areas dotting the coast in northwestern and northeastern regions. The mid to low suitability habitats for *I. scapularis* are found mainly in the northwestern and north central interior regions. In the Upper Peninsula, the areas of moderate to high suitability are in the south-central region (bordering Wisconsin and Lake Michigan, Menominee County), a few pockets of areas in the west, and a few areas along the coast of Lake Superior along the northern central region (Figure 5.6). Areas estimated to have lowest suitability lie in the far western and eastern regions, and areas with low suitability lie in the interior regions spanning the peninsula (Figure 5.6).

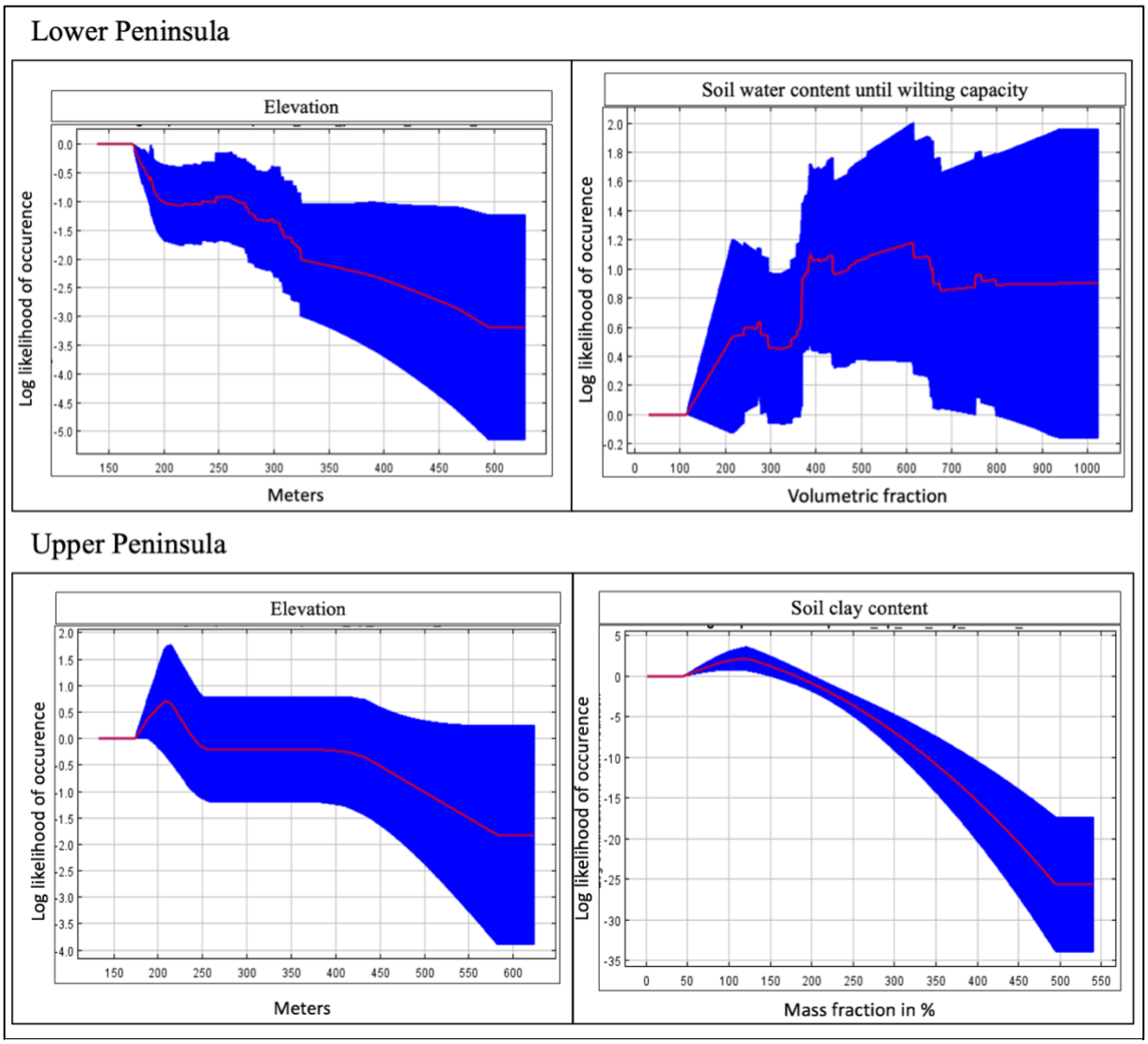

Figure 5.8. The response curves of the continuous ecological predictors of the MaxEnt models for the Lower and Upper Peninsulas. The blue shaded area around each curve represents the standard deviations. No graphs are shown for the binary predictors.

DISCUSSION

For more than half a century *I. scapularis* has been expanding in the north central, northeastern, and mid-Atlantic regions in the eastern U.S. and adjacent areas in southern Canada (Dennis *et al.*, 1998; Eisen, Eisen and Beard, 2016; Fleshman *et al.*, 2021). Due to this range expansion of *I. scapularis*, the incidence of several tickborne diseases such as Lyme disease, anaplasmosis, and babesiosis have been increasing and expanding throughout the eastern U.S. as well (Bacon, Kugeler and Mead, 2008; Dahlgren *et al.*, 2015; Kugeler *et al.*, 2015; Fleshman *et al.*, 2021). Michigan has been at one of the leading edges of the *I. scapularis* range expansion since the first established tick populations were discovered in the Upper Peninsula in the late 1980s (Strand, Walker and Merritt, 1992; EDWARD D Walker *et al.*, 1994) and in the Lower Peninsula (Erik Scott Foster, 2004) in early 2000s. Following the discovery of these populations, *I. scapularis* has expanded in the central and western regions of the Upper Peninsula as well as in the southern and coastal regions in the Lower Peninsula (Hamer *et al.*, 2014; Lantos *et al.*, 2017; Fleshman *et al.*, 2021).

Given *I. scapularis* is still emerging in Michigan, we wanted to identify areas in Michigan where *I. scapularis* may have a high likelihood of invading and becoming established. To do so, we used two methods of species distribution modeling, one was a regression-based method (a generalized linear model), and the other was a machine learning method (MaxEnt) to identify the environmental predictors and the suitable habitats in Michigan for *I. scapularis* occurrence.

Why model the two peninsulas separately?

The 11th largest state in the U.S., Michigan is comprised of a southern and northern peninsula that differ greatly in climatic, habitat and soil conditions. Given differences in geography (Omernik and Griffith, 2014) as well as invasion history, we modeled the two peninsulas

separately. Furthermore, we had many more sampling sites (and more positive sites) in the Lower Peninsula compared to the Upper Peninsula (Figure 5.1), and the model would be weighted towards the conditions in the Lower Peninsula. Altogether from 2017 to 2021, we had 222 and 60 (21.3%) sites in the Lower and Upper Peninsulas respectively, from which *I. scapularis* was detected in 124 and 27 (17.9%) sites respectively. We did in fact explore how different model outputs would be when both peninsulas were modelled together, using both GLM and MaxEnt modeling approaches (Appendix, Table 5.6). Differences in model outputs (between when modeling peninsulas together versus separately) were more pronounced for the Upper Peninsula (Appendix, Figure 5.11). When modeled together, only one small portion of the Upper Peninsula seemed to have suitable habitats for *I. scapularis* (Appendix, Figure 5.11), whereas when modeled separately, more suitable habitats were defined, including several hotspots in the western portion of the state where established populations of *I. scapularis* had been detected previously. We did not observe a drastic difference in the distribution of suitable habitats for *I. scapularis* when the Lower Peninsula was modeled together or separately, perhaps owing to the larger number of sites at which *I. scapularis* had been detected throughout much of the peninsula. The GLM and MaxEnt model results were generally in agreement with each other for both the Lower Peninsula and the Upper Peninsula for estimating both highly suitable regions and unsuitable habitats.

The Lower Peninsula

The first established population in the Lower Peninsula was discovered in the southwest corner of the state in the early 2000s (Foster 2004), potentially invading from neighboring states of Indiana and Illinois where *I. scapularis* was established previously and found questing and on host mammals (Pinger, Timmons and Karris, 1996; Jones and Kitron, 2000). *Ixodes scapularis* continued to spread, initially faster northwards along Lake Michigan than inland and eastwards

(Hamer *et al.*, 2014), but has since become detected and/or established 52 out of 83 counties (Appendix, Figure 5.10) (Dennis *et al.*, 1998; Eisen, Eisen and Beard, 2016; Lantos *et al.*, 2017; Fleshman *et al.*, 2021). Despite becoming more widespread in the Lower Peninsula, there are still many areas and counties where *I. scapularis* has not yet invaded.

The climatic predictors isothermality (BIO 3), mean temperature of the wettest quarter (BIO 8) and snow water equivalent were the three climatic predictors found in common to both the GLM and the MaxEnt in contributing substantially to explaining the variation seen in *I. scapularis* presence sites. Isothermality is a measure of how differences in day to night temperatures vary in relation to differences in summer to winter temperatures (Kessler, Ganser and Glass, 2019). If the isothermality is high, it relates to temperature uniformity while low isothermality relates to greater temperature variation. In both models there was a positive association with isothermality and *I. scapularis* occurrence. In the Lower Peninsula, isothermality is generally high in most of the southern regions while in the Upper Peninsula there is lower isothermality on the eastern region compared to the west. Especially given on average higher temperatures in southern Michigan, having more uniform temperatures is important for successful development of *I. scapularis* where it ensures that there are enough degree days for *I. scapularis* to develop to its next life stage. A similar relationship is seen in *Dermacentor variabilis*, the American dog tick, where it is hypothesized that higher isothermality may aid in developmental rates (James *et al.*, 2015; Kessler, Ganser and Glass, 2019).

The temperature of the wettest quarter has a positive relationship for likelihood of *I. scapularis* occurrence. For Michigan the wettest quarter of the year is April – June which overlaps with the adult and nymphal host-seeking activity periods of *I. scapularis*. An increase in temperature during this quarter (when relative humidity should also be conducive to host-seeking

and survivorship), may lead to increased questing activity of *I. scapularis*, which theoretically should allow ticks greater chances for finding hosts (Vail and Smith, 1998; Valsson and Bharat, 2011; Berger *et al.*, 2014; Elias *et al.*, 2021).

The snow water equivalent relates to the snowpack density. When the snowpack density is higher, it provides an insulation from the lower air temperatures during the harsh winter months and increase *I. scapularis* overwintering success (Volk *et al.*, 2022). Interestingly our model showed a negative association between snow water equivalent and the likelihood of *I. scapularis* occurrence (Figure 5.2). One possibility in the Lower Peninsula is the snow water equivalent shows to be the greatest in the northern region where we did not detect any *I. scapularis*, there by our model predicts this negative association.

For the GLM, the temperature seasonality (BIO 4) had a negative association with *I. scapularis* occurrence. When the temperature variation becomes greater, how different winter and summer temperatures are may impact the developmental patterns and the activity patterns of *I. scapularis* resulting in lower occurrence in regions where there is a greater temperature variation (Vail and Smith, 1998; Schulze, Jordan and Hung, 2001; Burtis *et al.*, 2016). In the Lower Peninsula there is a higher temperature variation inland compared to coastal regions, and within the Upper Peninsula, there is a higher temperature variation in west compared to the east.

Additionally, for the MaxEnt model average temperature of driest quarter (BIO 9) and the amount of precipitation of in the wettest month (BIO 13) were important climatic predictors. The driest quarter of the year in Michigan falls in January – March. In general, there is a positive association with BIO 9 and the likelihood of *I. scapularis* occurrence which corresponds to milder winter temperatures, which may result in higher adult *I. scapularis* host-seeking activity (Valsson

and Bharat, 2011; Burtis *et al.*, 2016; Ogden and Lindsay, 2016; Bouchard *et al.*, 2019; Wallace *et al.*, 2019).

The other climatic variable is the precipitation of wettest month (BIO13), which in Michigan, falls around April. There was a negative association with the amount of precipitation of the wettest month and log likelihood of *I. scapularis* occurrence. High levels of precipitation may create either flooding or occurrence of pathogens like fungal infections on *I. scapularis* which would be unsuitable conditions (Berger *et al.*, 2014; Ogden and Lindsay, 2016; Bouchard *et al.*, 2019).

Considering the ecological predictors, elevation was an important predictor in both the GLM and MaxEnt models, where it was negatively associated with the likelihood of *I. scapularis* occurrence. This finding is consistent with other studies, where it is hypothesized that conditions (e.g., too dry, too cold, too low host abundance) decrease *I. scapularis* survivorship (Diuk-Wasser et al., 2010; Hahn et al., 2016). In these studies, however, elevation differences were referring to elevations above 500 – 800 m, such as the Appalachians. The elevational gain in Michigan, however, is substantially less, especially in the Lower Peninsula, and the change in the elevation may be a proxy for moving from the coast to inland regions. There may be climatic and ecological reasons underlying this association, but it may also be an artifact of the invasion of *I. scapularis* which has spread initially from coastal, i.e., low land areas, within the state. One way to test this hypothesis is modeling the coastal regions and testing it on the inlands regions to see if elevation or the proximity to the coast influences the likelihood of occurrence of *I. scapularis.*

Soil water content at wilting capacity was another variable that was important in both the GLM and the MaxEnt models for the Lower Peninsula. Soil water retention ability correlates to the moisture content in the soil which will keep the soil layers humid such that it will be helpful

for the survival of *I. scapularis* (Schulze, Jordan and Hung, 2001; Rodgers, Zolnik and Mather, 2007; Hayes, Scott and Stafford, 2015; Burtis and Pflueger, 2017; Ripoche *et al.*, 2018; Larson *et al.*, 2022).

In the GLM, the presence of white-tailed deer was positively associated with the likelihood of *I. scapularis*. White-tailed deer are important reproductive hosts for the adult stage of *I. scapularis*. Thus, the presence of deer is important for the survival of *I. scapularis*, and other models have shown that the presence of white-tailed deer is important to the distribution of *I. scapularis* (Elias et al., 2021; Kopsco et al., 2023). Conversely, in the GLM, there was a negative association with the presence of white, red and jack pine forests groups and *I. scapularis* occurrence. Pine forests tend to have a thinner layer of leaf litter; be drier; and have more acidic clay soil (Guerra et al., 2002; Lubelczyk et al., 2004), which may be unfavorable for the survival of *I. scapularis*. Similar to elevation, however, it also could be that areas where pines predominate (e.g., central northern Michigan) are those where *I. scapularis* has not yet reached. Alternatively, this finding may also reflect a bias in our sampling design. In our surveillance, we mainly focused on the sites that had deciduous woods or mixed deciduous and coniferous woods, because those are the habitats with sufficient leaf litter that should facilitate survival of *I. scapularis* (Ginsberg, Rulison, Miller, Pang, Arsnoe, Hickling, Ogden, LeBrun, *et al.*, 2020).

Because the GLM and MaxEnt models use different sets of assumptions and different modeling methods, the AUC, TSS and model deviance values are not completely comparable. Although overfitting was not observed in either model, MaxEnt uses machine learning techniques which will maximize the pattern matching of the presence points, which will result in greater AUC values compared to the GLM. In our models the TSS ranged between 0.4 – 0.6 indicating relatively our test data set was able to predict the model based on the training data set well.

The Upper Peninsula

The first established populations of *I. scapularis* discovered in Michigan were discovered in Menominee County, which is in the southern central region of the Upper Peninsula bordering Wisconsin and Lake Michigan (Strand, Walker and Merritt, 1992; Walker *et al.*, 1998). In the final GLM model for the Upper Peninsula, there was one unique climatic predictor that was not present for the Lower Peninsula model- precipitation of the driest month (BIO14) has a positive association with *I. scapularis* occurrence. The driest month in Michigan typically is February, during which time *I. scapularis* generally is expected to be inactive. During winter, larvae, and nymphs most likely are in diapause (Ogden *et al.*, 2018), and only adult ticks can quest, but only when temperatures are above freezing. Thus, at this time of year in northern Michigan, increased precipitation likely would-be increased snow, which might help tick overwintering survivorship. High levels of precipitation keep the topmost soil layer and bottom of the leaf litter layer warm and humid which would be conducive for the survival of *I. scapularis*.

In the Upper Peninsula, the amount of temperature variation over a given period (BIO 4) has a positive association while in the Lower Peninsula it has a negative association. It is possible that great variation indicates that the summers are warm enough, and potentially long enough, to allow for more successful host-seeking success and developmental success from one life stage to another. Colder temperatures are not as much of a concern for *I. scapularis* if ticks are protected under leaflitter and snow, as written previously. Interestingly none of the bioclimmatic predictors for the Upper Peninsula of the MaxEnt model were important in explaining the variation. This may be due to the variation in bioclimmatic predictors not being drastically different among landscape for the MaxEnt model to pick up a drastic difference.

Considering the ecological predictors unique in the GLM, presence of maple beech birch forest group and presence of wetlands have positive associations. The positive association with deciduous forests is not surprising given what is known about *I. scapularis* ecology (Randolph, 2004; Pfäffle *et al.*, 2013), what others have previously found (Brownstein, Holford and Fish, 2003; Diuk-Wasser, Gatewood, *et al.*, 2006; Diuk-Wasser *et al.*, 2010) and may reflect our sample site selection bias. What is surprising, however, is the positive association between the presence of wetlands and *I. scapularis* presence. In general wetlands can be too wet or moist for *I. scapularis* (Larson *et al.*, 2022). There may be regions in the Upper Peninsula, however, which are not covered in water/ice the whole year especially wooded wetlands which might be good habitats for *I. scapularis.* Soil clay content is negatively associated with *I. scapularis* occurrence. Soil with larger clay content is poorly drained, and as mentioned above, too high moisture and water content in the soil is unfavorable for *I. scapularis.*

Evaluating the model performance for the Upper Peninsula again AUC values were > 0.5 for both model approaches, indicating the model can categorize the presence sites and absence sites accurately than by random. It is important to note that the model deviance for the Upper Peninsula in the GLM was high (8.6). Deviance values range from 0 – infinity and smaller values indicate a better fit of the model to the data. We had a relatively smaller sample size of sites and of positive sites for the Upper Peninsula, and when 30% of the data was held back from training in order to be used to test the model, we had so few sites, which may have caused the deviation of model to be greater. To test this hypothesis, future research could add more sites and sample more frequently targeting the Upper Peninsula. One can also artificially randomly reduce the dataset from the Lower Peninsula, the models and see how deviance changes with sample size. It is interesting to note that the deviance values for the MaxEnt for both the two Peninsula were similar,

and this could be partly due to an artifact of the modeling method where the MaxEnt being a machine learning method would maximize pattern recognition among the presence points and decrease the model deviance.

Species distribution models: comparisons with published models

Using two approaches, we modeled potential suitable habitats for *I. scapularis* in Michigan based on the surveillance data collected 2017 to 2021. Because *I. scapularis* is continuing to spread in Michigan, it is unclear how well the maps produced based on a five-year snapshot of data will resemble the future distribution of ticks, such as in another two decades. The GLM and MaxEnt modeling processes are pattern matching approaches – one using regression and one using machine learning – that then assign probabilities of *I. scapularis* occurrence based on relationships identified between the data (i.e., tick presence/absence at various sites) and parameters (i.e., environmental variables) provided. There are many reasons to "believe" in the reliability of these models, such as examining the evaluating criteria mentioned previously like AUC, TSS and model deviance and looking at how well the models predict the known presence sites. There are also reasons to believe that the modeled habitat suitability maps can be improved, such as adding in more presence absence points, using one year's presence-absence points, and then projecting onto the next years presence-absence points to see how well they overlap and projecting a state or an endemic region onto a tick expansion region. To be specific, however, we have modeled the habitat suitability of dynamic *I. scapularis* populations as they invade across Michigan landscapes, and not necessarily the habitat suitability of more equilibria *I. scapularis* populations that have reached an endemic state. The latter would obviously produce a more accurate model, but from the standpoint of making predictions in the nearer term that can still be helpful for public health and our understanding of the invasion process, our models still can be useful.

With time as *I. scapularis* populations expand, potentially most of the state- especially in the Lower Peninsula- might be deemed as having suitable habitats in the future. In distribution models presented by Burtis et al., 2022, they used county level data on *I. scapularis* presence/absence that are available for the eastern U.S. and then modelled the potential suitable habitats at a county scale. According to their models all Michigan counties are predicted to have suitable habitats. The wide range of environmental conditions present in other *I. scapularis*-endemic areas generally must have encompassed that found in Michigan and resulted in all counties estimated to have suitable habitats. In another model developed in Hahn et al., 2016, which used *I. scapularis* distribution data from different literature sources (e.g., Eisen et al., 2016) indicated high suitability counties along the western coast and within the northern central region of the Lower Peninsula as well as almost all regions of the Upper Peninsula, while the Thumb region and the southeastern regions were less suitable. This is slightly different from what was reported in our model as well as the model by Burtis et al., 2022. This may be due to when the models were developed, data used in these models were current up through 2015 (i.e., including areas where *I. scapularis* is established). Data from Michigan that would have been included in analyses would have shown *I. scapularis* populations mainly in the southwestern and western Lower Peninsula (but not in the Thumb nor southeastern Michigan), and limited areas in western Upper Peninsula (Lantos et al. 2017).

The Burtis *et al.* 2022, Hahn *et al*., 2016 models report suitability at the county level, but a similar approach, whereby one uses data from other areas where *I. scapularis* is already endemic, could be conducted to predict habitat suitability at a finer scale. For example, given Michigan shares much of its major environmental conditions with Wisconsin, where *I. scapularis* has been endemic for many years, one could develop a habitat suitability model based on Wisconsin data

and extrapolate onto Michigan. This is exactly what Foster 2004 did; Foster and colleagues discovered the first populations of *I. scapularis* in southwestern Michigan by projecting a habitat suitability model created by Guerra et al, 2002 based on data from Wisconsin onto Michigan. Those data were collected from 1996 – 1998, when similar to the current situation in Michigan, *I. scapularis* was still in the process of spreading in Wisconsin. The model in Guerrra et al. 2002 shows slightly different regions of suitable habitats compared to our models, which is surprising given the different training and environmental data on which the models are built. The most interesting difference is that Guerra et al., 2002 predicted the large area in the interior north central Lower Peninsula to be highly suitable, whereas in our models currently estimate them to be not suitable for *I. scapularis.* Indeed, our model may not be correct because *I. scapularis* has not yet invaded areas with similar abiotic and ecological features to that found in north central Michigan. Thus, MaxEnt may not identify those habitats as suitable, and our absence datapoints for sites in that region would strengthen any negative association in the GLM. Having said that, much of this region is characterized by moderate to low precipitation and the shortest growing seasons in Michigan (Dickmann and Leefers, 2016), and both of which reduce tick survivorship and population establishment of *I. scapularis* (Lindsay et al. 1995). Another model developed using *I. scapularis* nymphal densities is presented in Diuk-Wasser et al., 2006, 2010. These models were based on nymphal densities and during that early time because *I. scapularis* was only detected in the southwestern region of the Lower Peninsula, most of the southern regions were deemed to have low suitability.

Limitations and future research

As mentioned, previously, because Michigan is still undergoing an invasion process, one major limitation to our ability to build an accurate model for habitat suitability of *I. scapularis* in

Michigan is that the presence/absence data may continue to change over time as *I. scapularis* spreads to new areas. We believe our presence data is generally reliable, especially in areas that were sampled in multiple years, and given our experience and the literature that, once *I. scapularis* invades an area, it likely will become established (e.g., Hamer et al. 2010). Regarding our absence data, however, it is unclear whether they will remain negative in the future, again, based on our experience and trends in the literature. Even though some sites from our model may be modeled as unsuitable now, they may become suitable in the future, as predicted by other models using data from other areas in the northern eastern US.

One of the reasons we did not use the same approach as others – to extrapolate models based on data from other states to Michigan - is because of Michigan's unique geography – comprising of two relatively large landmasses in the middle of the continent but being largely surrounded by large bodies of water. Having said that, given the challenge of the on-going invasion, using models based on data from other areas makes sense. Thus, future research should include updating the Guerra et al. 2002 model based on current data from Wisconsin (or more broadly in the Upper Midwest) and projecting it onto Michigan as per Foster, 2004. Wisconsin is the closest and most environmentally similar area with Michigan, even if there are environmental differences. The model from Guerra *et al*., 2002 was based on presence data distributed throughout western Wisconsin with a few exceptions, including sites from northeastern WI and Menominee County (Strand, Walker and Merritt, 1992; Walker *et al.*, 1998). In subsequent years, *I. scapularis* spread throughout the state (as well as into other states) and has had time to become established across the landscape. Thus, a new habitat suitability model may be able to provide an even more reliable and finer scale model. It would be interesting to test and compare predictions made by Guerra *et al*., 2002 on current Wisconsin data as well as projecting it onto Michigan to forecast

suitable habitats. Conducting a similar exercise using tick distribution data from Ontario, Canada may also be useful.

- Another exercise that could shed light on how models are affected by the input data, given the invasion dynamics, is to re-run our analyses (or, better, an updated Wisconsin habitat suitability model run on Wisconsin data) and intentionally train the models on non-random subsets of data to see how similar the resulting models would be. For our model evaluation, models are trained multiple times with random subsets of data to obtain outputs with measures of uncertainty. But our complete data set is influenced heavily by invasion from the southwest area of Michigan in the Lower Peninsula and south-central Upper Peninsula. How different, for example, would the model for the Lower Peninsula currently look if the initial invasion had occurred from southeastern Michigan? Or how would the habitat suitability model for Wisconsin look if presence data originated from the eastern half of the state near Lake Michigan, rather than from the western half and near the Driftless Zone? This is of interest because as *I. scapularis* and other species invade an area, it is important to understand for building species distribution models that current associations with habitats are affected by historical contingencies (e.g., what was the point of introduction?), the ecological context – including what habitats are available and how representative that region of invasion is of the rest of the geographic extent of interest.

Future research to build potentially more precise maps in Michigan include continuing to conduct surveillance, especially in areas where *I. scapularis* currently is absent, as well as to model habitat suitability using abundance data (e.g., with negative binomial regression models or zero-inflated negative binomial models) and not just presence/absence data. Currently the number of sites with abundance data is too low to be able to build a reliable model, but as established

populations continue to increase in size and as the invasion continues, more data should become available.

To test our current habitat suitability model directly and not just rely on using updated models developed in Wisconsin, or to just wait for invasion to occur in Michigan, there are two things we can do. One is to plan active surveillance targeting those potentially suitable and unsuitable regions where we have not detected the presence/establishment of *I. scapularis*. The second is to conduct tick survivorship and development studies of each life stage in these regions by putting them out in semi-natural arenas to measure the effects of local abiotic factors (e.g., Lindsay et al., 1998; Ginsberg et al., 2014; Ogden et al., 2018; Volk et al., 2022).

Future studies can also conduct species distribution modeling on not just *I. scapularis* in general but on ticks infected with various disease agents. Understanding where subsets of *I. scapularis* ticks are infected with the agents of Lyme disease, human granulocytic anaplasmosis, babesiosis, Powassan virus encephalitis, and others may have benefits for directing public health messages regrading prevention, diagnosis, and treatment, but also better understand the variation in ecology of the tick as the pathogens have similar but different ecologies.

With the increase in *I. scapularis* borne diseases it is important to anticipate the patterns of the spread of *I. scapularis*. Currently there are no accepted area-wide tick control methods, unfortunately, so prevention of tick-borne diseases relies mainly on personal protection, and therefore education of the public, public health workers, and medical workers. Species distribution models can serve as a guide for surveillance and a guide to design any control and intervention methods when they become available.

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APPENDIX

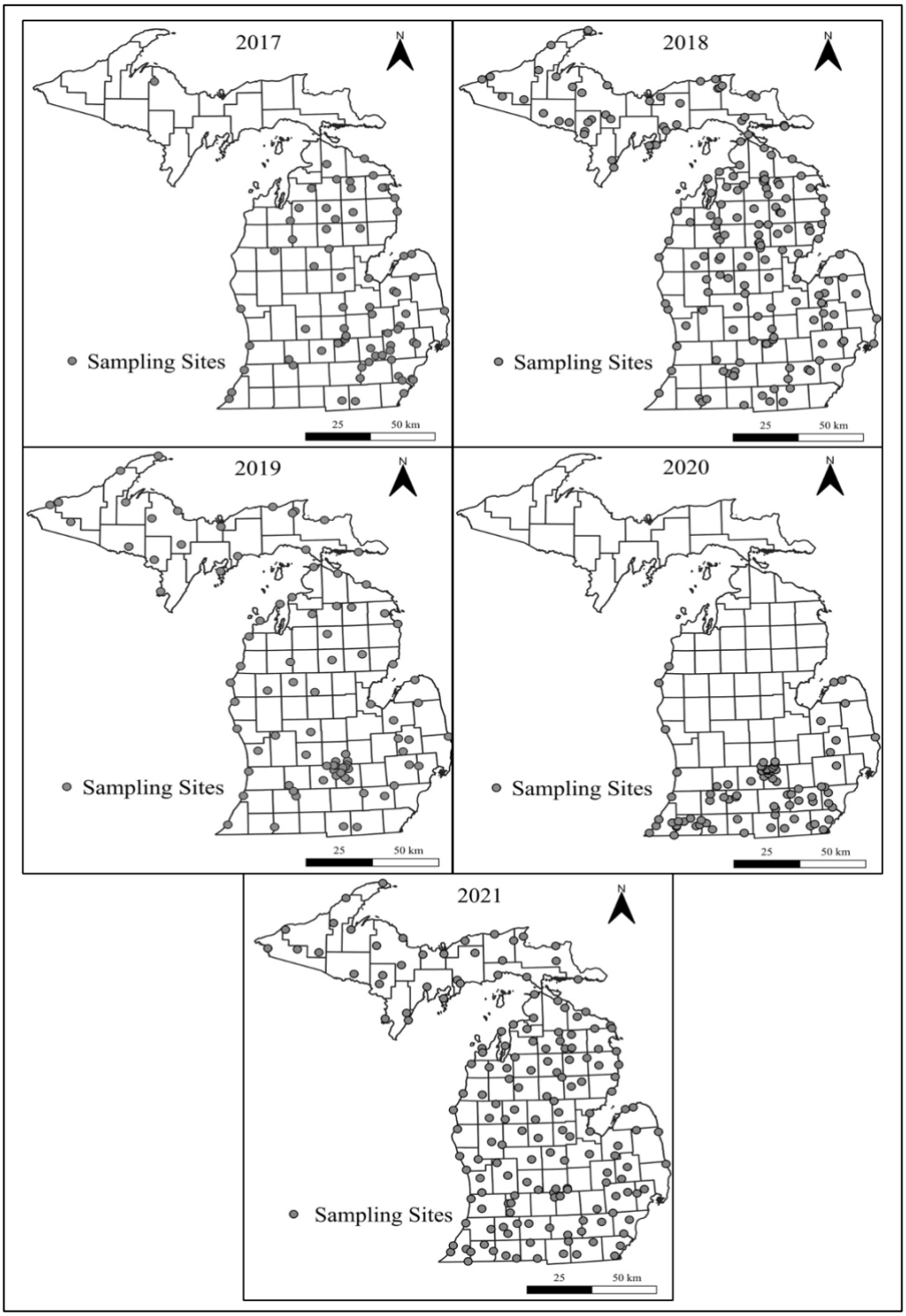

Figure 5.9. The sampling sites of Michigan from 2017 to 2021 for drag sampling *Ixodes scapularis*.

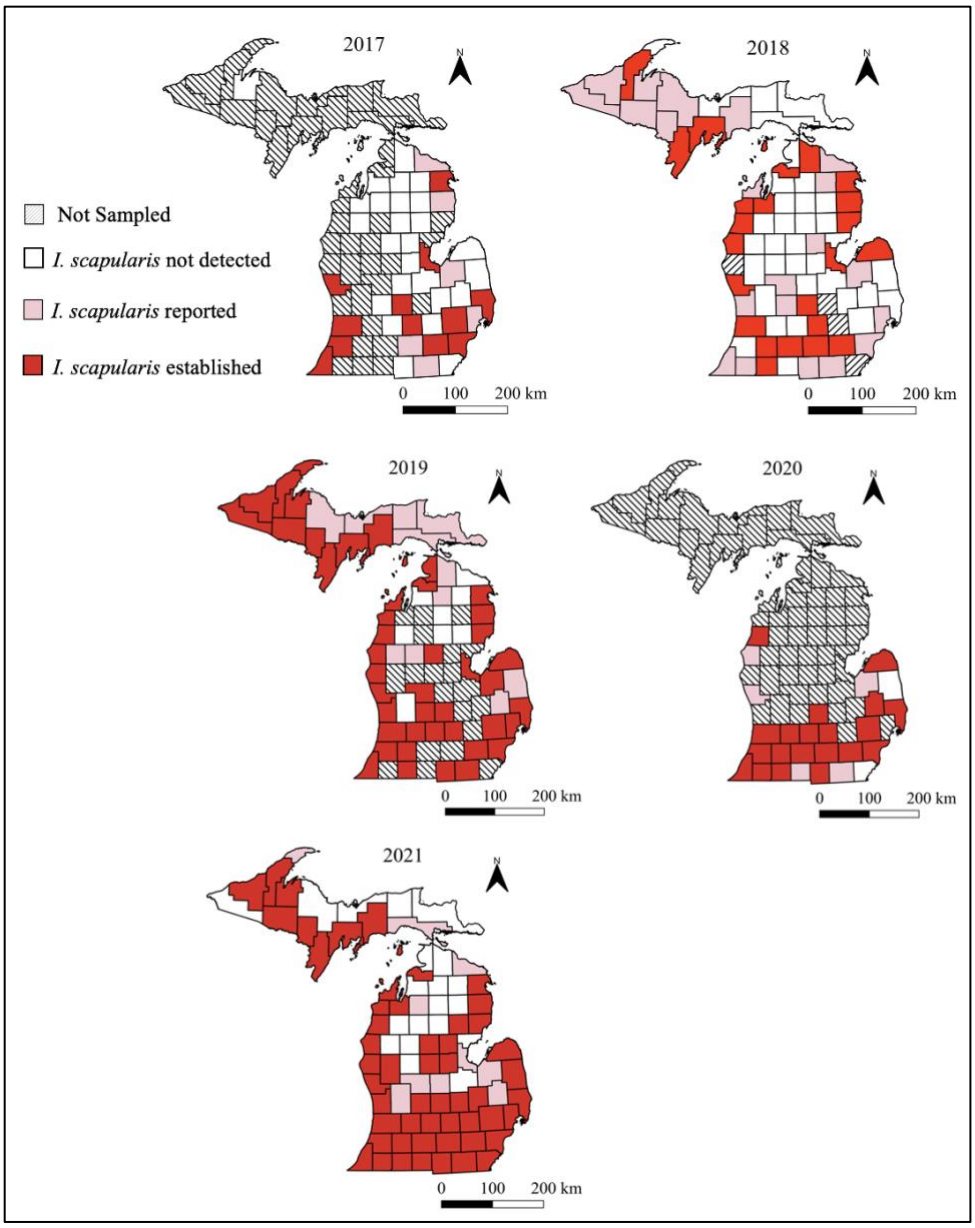

Figure 5.10. County establishment data of *Ixodes scapularis* from 2017 to 2021 according to CDC criteria (Dennis et al. 1998; Eisen et al. 2016). Established criteria correspond to counties that had greater than 5 *I. scapularis* of any life stage detected or 2 life stages of *I. scapularis* detected over one calendar year. Reported criteria correspond to the detection of *I. scapularis* but at levels below that needed to be considered 'established'.

Table 5.7. The *Ixodes scapularis* presence categories that SDMs were based on for the whole state of Michigan.

Category	**Definition**
Lenient	Any site that at least one *I. scapularis* was detected
Moderate	Any sites that had established populations of *I. scapularis* (i.e., sites that had greater than 5 *I. scapularis* of any life stage or 2 life stages of *I. scapularis* detected over one calendar year).
Stringent	Any sites that metboth establishment criteria *I. scapularis* populations present (i.e. sites that had greater than 5 *I. scapularis* of any life stage and 2 life stages of *I. scapularis* detected over one calendar year).

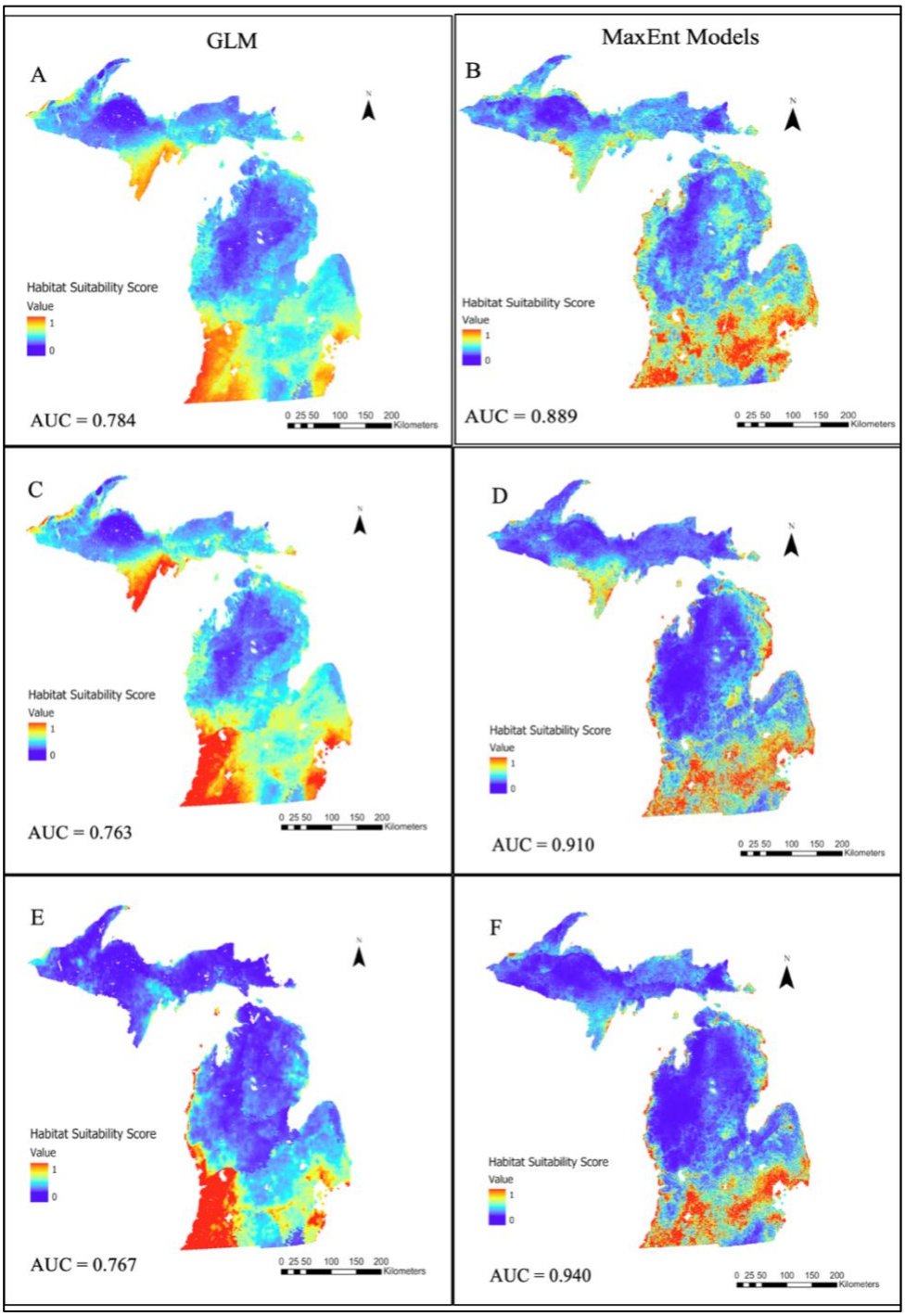

Figure 5.11. The GLM and MaxEnt models for the presence of *Ixodes scapularis* for the entire state of Michigan from the lenient category (A-B) to moderate category (B-C) and the stringent category (E – F) of *I. scapularis* presence/established levels.

CHAPTER 6: CONCLUSION

Ixodes scapularis is rapidly spreading throughout the northeastern and north central regions of the US. With the increase in the geographic distribution of *I. scapularis* there also has been an increase in human diseases that are vectored by *I. scapularis* such as Lyme disease, human granulocytic anaplasmosis, babesiosis and Powassan virus encephalitis (Bacon, Kugeler and Mead, 2008; Kugeler *et al.*, 2015; Adams *et al.*, 2017; Schwartz *et al.*, 2017). Improving our knowledge about the patterns and mechanisms of spread of *I. scapularis* and its associated pathogens will enable us to develop prevention and mitigation strategies.

Lyme disease is the most common vector-borne disease in the US, and much of the knowledge that is derived from ecological studies that have been conducted on the Lyme disease bacterium can serve to inform the ecology of other tick-borne pathogens vectored by *I. scapularis*. Here I wanted to investigate further the disease ecology of *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis, the second most common vector borne disease in the US (Adams *et al.*, 2017). *Anaplasma phagocytophilum*, a gram-negative bacterium, infects white blood cells in mammals. The disease symptoms are very similar to Lyme disease (Dumler et al., 2005; Bakken and Dumler, 2015; Sanchez et al., 2016; Baker et al., 2020), so often in endemic regions of Lyme disease such as the Northeast and the Midwest human granulocytic anaplasmosis may be mistaken for Lyme disease. As the treatment for anaplasmosis is similar to that of Lyme disease, anaplasmosis may be underreported.

Ecology of A. phagocytophilum *at an* I. scapularis *endemic site in the Upper Midwest*

The ecology of *A. phagocytophilum* has been studied at a site in upstate New York where the reservoir competence of small to medium sized mammals were estimated (Keesing *et al.*, 2012, 2014). We wanted to conduct a similar study in the Upper Midwest because there were not any

studies available that looked at several host species over the same time period. We quantified the infection prevalence and the reservoir competence of small to medium sized reservoir hosts; characterized host features that are important in becoming infected with *A. phagocytophilum,* including features that would influence larval and nymphal *I. scapularis* counts on hosts; and characterized the phenology of infection in hosts in relationship to parasitizing nymphs and larvae. We also wanted to compare detection of *A. phagocytophilum* of hosts using two types of tissues (blood vs ear biopsy tissues).

Overall, our results support that found in other studies conducted in the Northeast and limited data in the Midwest when considering larval and nymphal burdens among species. We focused our comparison regarding A. *phagocytophilum*-ha infection among hosts with that of Keesing et al. (2014) because they trapped multiple species of host at one site simultaneously as we had, and because they strain-typed their positives. Keesing et al. (2014) found that the two species with the highest realized reservoir competence were the white-footed mouse (*Peromyscus leucopus*) and the eastern chipmunk (*Tamias striatus*). The realized reservoir competence of eastern chipmunks and white-footed mice for *A. phagocytophilum* at Ft. McCoy from 2010-2012 were much higher than that found at Keesing et al (2014). In fact, the eastern chipmunk had the highest infection prevalence for *A. phagocytophilum*. Because white-footed mice fed the most larvae compared to other species, however, they may contribute the most to infecting larvae with *A. phagocytophilum*. A limitation of this study is that we did not have large enough sample sizes of most mammals to compare the realized reservoir competence, because we were not able to collect enough engorged larvae from other hosts species to have confidence in estimating infectivity; therefore, we only compared the white-footed mouse and eastern chipmunk. If, however, the relative proportion of larvae fed by host species captured in our study is

representative of the host community at Fort McCoy, it still appears that white-footed mice might contribute the most to infecting larvae with *A. phagocytophilum*, and therefore its enzootic cycle.

It has been recognized that the relative timing of questing activity, i.e., phenology, of nymphal and larval *I. scapularis* can be important for the enzootic maintenance of pathogens that are not vertically transmitted (Ogden et al. 2007). For pathogens that have short-lived infections in the host, where the reservoir hosts may be short-lived, and/or where there might be high turnover rate in the reservoir host population, greater overlap (i.e., "synchrony") is needed for maintaining the enzootic cycle. Laboratory studies have shown that although mice may harbor *A. phagocytophilum* infections for twelve weeks (Levin et al. 2004), the highest rates of transmission to larvae occur in the first 1-3 weeks post-infection. This is in comparison with *B. burgdorferi,* which can be transmit by mice at relatively high rates for life (Donahue, Piesman and Spielman, 1987). At Ft. McCoy, although there are questing larvae throughout the summer, the phenology of infected on-host larvae peaks in the first half of summer, which reflects the phenologies of questing infected nymphs and infected mice, which also peak in the first half of summer, as one would expect. The seeming coincident timing of the peak in questing infected nymphs and infected on-host larvae also suggests that co-infection (i.e., non-systemic) transmission might occur. This is of interest because in the Northeast, although the nymphal peak occurs at the same time as in the Midwest, the larval peak occurs in late summer. Our data thus suggest that all else equal, the proportion of larvae that would become infected by feeding on white-footed mice would be much less in the Northeast compared to the Midwest, due to lower rates of systemic and non-systemic transmission. Phenology, among other factors, might contribute to the higher *A. phagocytophilum*-ha infection prevalence observed among questing nymphs, chipmunks, and mice at Ft. McCoy compared to southeastern New York (Keesing et al. 2014).

We looked factors that affect the probability that a host could become infected with *A. phagocytophilum*. Of interest, we found that individuals that were infected with *B. burgdorferi* had relatively a greater chance of being infected with *A. phagocytophilum*. At an *I. scapularis* endemic site like Fort McCoy, Wisconsin the chances of becoming infected with both *B. burgdorferi* and *A. phagocytophilum* would be greater and many hosts species can be coinfected. In other regions where *I. scapularis* is endemic, coinfection of *B. burgdorferi* and *A. phagocytophilum* in hosts is also quite common (Horowitz *et al.*, 2013; Diuk-Wasser, Vannier and Krause, 2016; Westwood, Peters and Rooney, 2020b; Lehane *et al.*, 2021) because of the similar ecologies shared by both pathogens.

Finally, for simply determining the infection prevalence of hosts for *A. phagocytophilum*, our study suggests that once can sample either blood (which is much more commonly performed) or ear tissue biopsies. As *A. phagocytophilum* is an intracellular pathogen that infects white blood cells (Chen *et al.*, 1994), blood is assayed in most cases. There was no significant difference between infection prevalence estimated from blood, biopsy and and/or both types of tissues simultaneously when we tested white-footed mice for whom we had collected both types of samples simultaneously. Ear biopsies have been used in some studies to look at the infection status of *A. phagocytophilum* (Baráková *et al.*, 2014; Rosso *et al.*, 2017) in Europe. A study conducted on canine skin lesions showed that even when dogs seroconverted and no longer showed signs of disease, *A. phagocytophilum* was detected in skin biopsies (Berzina et al., 2014), which was then hypothesized to be due to *A. phagocytophilum* being persistent in skin of hosts (Granquist, Aleksandersen, *et al.*, 2010). Conducting a xenodiagnostic study using mice would help characterize the relationship of the timing of detection of *A. phagocytophilum* [DNA] in blood, biopsy, and transmission to feeding larvae. If skin biopsies could be as used a reliable indicator

of infection of *A. phagocytophilum*, it would be welcome knowledge, as it is less invasive and is easier to store and process.

The case incidence of *A. phagocytophilum* has increased over time with the spread of *I. scapularis* (Biggs, Behravesh, K. K. Bradley, *et al.*, 2016; Adams *et al.*, 2017; Schwartz *et al.*, 2017). Thus, conducting ecological studies across the geographic range of *I. scapularis*, where climate and wildlife host communities may vary, will help us further understand how *A. phagocytophilum* and its various strains are maintained enzootically. We can apply the knowledge gained to predict the level of public health risk of disease as well to control and prevent the further spread of *A. phagocytophilum*.

Species Distribution modeling of* I. scapularis *in Michigan

Michigan is at one of the leading edges of *I. scapularis* invasion. From the time ticks were first discovered in the early 1990s in the Upper Peninsula (Strand, Walker and Merritt, 1992) and in the 2000s in the Lower Peninsula (Erik S. Foster, 2004), blacklegged ticks have spread gradually throughout the state (Dennis *et al.*, 1998; Eisen, Eisen and Beard, 2016; Lantos *et al.*, 2017; Fleshman *et al.*, 2021). Because of this gradual spread of *I. scapularis*, we wanted to develop suitable habitat models to identify the distribution of suitable habitats for the establishment of *I. scapularis to* help inform future disease risk. We also wanted to look at the climatic and habitat predictors that are potentially important in the spread and establishment of *I. scapularis.* In the literature there are several different types of species distribution modeling (SDM) techniques. In our study, we assessed two techniques- one which is based on presence-absence data (a regression-based generalized linear model (GLM) method) and the other, which is based on presence only data (machine learning-based Maximum Entropy (MaxEnt) model).

We used active *I. scapularis* surveillance data collected from 2017 – 2021 from sites

located throughout Michigan. We used 16-19 environmental variables in the final models, where we modeled the Lower and Upper Peninsulas separately given the vast environmental and ecological differences.

Our models had a relatively high area under the curve (AUC) of the receiver operator curve (ROC) values, which ranged from about 0.7 – 0.9, indicating that the model could categorize the presence or presence-absence points better than by random chance. When considering the climatic and habitat predictors that were important for the model, the overall climatic factors that were related to temperature and humidity seem to be most important. Both temperature and humidity are two climatic factors that affect the survival of *I. scapularis* because *I. scapularis* is very susceptible to desiccation (Vail and Smith, 1998; Berger *et al.*, 2014; Elias *et al.*, 2021). The habitat features that were important were factors that were related to the presence of white-tailed deer, presence of deciduous woods, elevation, soil moisture, amount of leaf litter. A caveat is that because our sampling protocol was designed to find *I. scapularis* ticks, we targeted habitats that were believed to be suitable habitats for *I. scapularis;* this may have constrained the habitat variables that came out as important variables in our model.

We found general agreement between the modeling techniques for predicting distributions of suitable habitats for both the Upper and Lower Peninsulas. Suitable habitats in the Lower Peninsula were more concentrated in the southern portion of the state and within pockets along the west and east coast of the state, while in the Upper Peninsula suitable habitats were concentrated in the southern central region bordering Wisconsin and along Lake Superior also in the central region. A recent study conducted using passive and actively collected data collated at the county level from throughout the Northeast and the Midwest showed most of Michigan to have suitable habitat for the establishment of *I. scapularis* (Burtis *et al.*, 2022). It was different from our study

since we found regions in the northern central region of the Lower Peninsula and in the eastern portion of the Upper Peninsula to be unsuitable for *I. scapularis*. Because our model was based on the current *I. scapularis* data from Michigan and because *I. scapularis* is still emerging across Michigan, our model likely is based on data that includes "false negatives". Given our understanding of *I. scapularis* ecology and the range of sites in which it already is established throughout the eastern U.S., we hypothesize that *I. scapularis* eventually will become widespread throughout the state. Therefore, some of the sites and regions that our models predicted to be unsuitable for ticks might be truly unsuitable or may be areas *I. scapularis* has not gotten to yet. A way to test this hypothesis directly would be set up tick gardens (i.e, semi-natural arenas) in those regions that the model predicted to be unsuitable and release *I. scapularis* to measure their survival and development rates. Another would be to continue with periodic active surveillance focused on those regions to detect the presence and establishment of *I. scapularis*.

We could also develop species distribution models based on data from Wisconsin, where *I. scapularis* has time to spread throughout the state. Those models could be projected onto Michigan as Foster (2004) had done with Guerra et al. (2002), leading to the discovery of *I. scapularis* in southwest Michigan. This may provide us with a better understanding if some regions in the Upper and Lower Peninsula are indeed unsuitable. Using Wisconsin as the base model is beneficial because the climatic and habitat variables are similar to Michigan. To further improve our model, we could use other techniques to model the distribution of *I. scapularis* and focus on developing ensemble models, which would give us a better understanding about how each of the SDMs work. Since we have *I. scapularis* abundance data and density data developing models based on these would also give us a better understanding of patterns of invasion of *I. scapularis*.

Developing SDMs is one way to predict species distributions that is relatively inexpensive

and less time consuming if active surveillance, especially in larger regions, cannot be done. Using SDM's also can help guide surveillance activities to regions that have suitable habitats such that one can try to detect invading *I. scapularis* when they are still at low density. These SDMs thus can guide planning prevention and control strategies especially in areas that *I. scapularis* have not been established. As with any type of modeling effort, however, researchers need to be aware of the limitations imposed by the input data and the model assumptions as well as how the models work.

Ticks and tickborne disease have been an important topic in modern times as we observe an increase in several tickborne disease cases like Lyme disease, anaplasmosis, babesiosis, Powassan virus encephalitis, ehrlichiosis, Rocky Mountain spotted fever, as well as the tick-associated red meat allergy. With climate change, habitat fragmentation and modification, tick distributions will shift and/or expand into regions where they have not been observed in recent time. Developing SDMs and trying to gain more insight into how tickborne pathogens are maintained in nature by the wildlife and vector communities will help in the fight for prevention and control of ticks and tickborne diseases.

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