The Ecology and Epidemiology of the Blacklegged Tick, *Ixodes scapularis* and the Risk of Lyme Disease in Ontario, Canada

by

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THE ECOLOGY AND EPIDEMIOLOGY OF THE BLACKLEGGED TICK, IXODES SCAPULARIS AND THE RISK OF LYME DISEASE IN ONTARIO, CANADA

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This thesis is an investigation of the ecology and epidemiology of Ixodes scapularis and the risk of Lyme disease in Ontario, Canada. Over the past two decades, there has been rapid range expansion of I. scapularis northward into Canada. This tick is a vector for numerous pathogens of human and animal health significance, including Borrelia burgdorferi sensu stricto, the causative agent of Lyme disease in North America. In 2014 and 2015, tick dragging was conducted at 154 sites across southern, eastern and central Ontario. Ecological data were also collected at each site. Ixodes scapularis were detected at 29 of 154 sites, with a “hot spot” for I. scapularis identified in eastern Ontario. Nine sites had B. burgdorferi-positive ticks. The presence of I. scapularis at a site was positively associated with cumulative degree days above zero and negatively associated with westward longitude, based on mixed multivariable logistic regression. The relative abundance of shrubs, the density of the understory and the interaction of these two variables were also significant. Follow-up field sampling for I. scapularis was conducted at 36 sites in 2016 to assess the spatial spread of the tick. Ixodes scapularis was detected at five new sites in eastern Ontario. These findings were consistent with the estimated speed of range front expansion by Leighton et al. (2012). Colonization of I. scapularis at sites behind this range front is occurring at a slower and heterogeneous rate. Data from field sampling and previous surveillance of I. scapularis were used...
to create an ecological risk indicator to assist public health professionals with risk assessment for reproducing populations of *I. scapularis*. This thesis provides valuable knowledge on the distribution, spread and ecology of an emerging vector and pathogen, and can be used to target public health interventions.
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STATEMENT OF WORK

The preparation of this thesis was the sole responsibility of Katie M. Clow.

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AIC – Akaike information criterion
BLUP – best linear unbiased predictor
CI – confidence interval
CSD – census subdivision
DD>0°C – cumulative degree days greater than zero degrees Celsius
DIN – density of infected nymphs
DNA – deoxyribonucleic acid
GIS – geographic information system
GPS – Global Positioning System
ICC – intraclass correlation coefficient
IGS – intergenic spacer
MAUP – modifiable areal unit problem
MP – Murphy’s Point Provincial Park
NAD – North American Datum
NIP – nymphal infection prevalence
NML – National Microbiology Laboratory
OR – odds ratio
PCR – polymerase chain reaction
PHAC – Public Health Agency of Canada
R0 – basic reproductive number
rRNA – ribosomal ribonucleic acid
RST – ribosomal spacer type
SD – standard deviation
TP – Turkey Point Provincial Park
USA – United States of America
UTM – Universal Transverse Mercator
CHAPTER 1: REVIEW OF THE LITERATURE

INTRODUCTION

Lyme disease is the most common vector-borne disease in North America, affecting tens of thousands of individuals annually (Ogden et al. 2015; Centers for Disease Control and Prevention 2016). Lyme disease in humans commonly presents as a flu-like illness with a characteristic erythema migrans rash (i.e., bull’s eye rash). If left untreated, Lyme disease can cause chronic rheumatologic, cardiac and neurologic symptoms (Wormser et al. 2006). In eastern North America, it is caused by the spirochete bacteria *Borrelia burgdorferi* sensu stricto and vectored by the Ixodid hard tick *Ixodes scapularis* (blacklegged tick) (Burgdorfer et al. 1982). Various small mammal species are the most competent reservoirs for the bacteria (Anderson et al. 1983; Levine et al. 1985; Donahue et al. 1987).

The distribution of the tick and bacteria has been rapidly expanding over the past two decades and encompasses a large geographic area stretching from the northeastern USA south to the Texas-Mexico border and includes areas of the midwest USA and Canada (Ogden et al. 2008c; Feria-Arroyo et al. 2014). Recent spread into Canada is notable, where in 1991 there was one known established population at Long Point in Ontario, and now there are numerous endemic areas in the provinces of Ontario, Quebec, Manitoba, Nova Scotia and New Brunswick (Barker et al. 1992; Ogden et al. 2008c). This range expansion has coincided with a significant increase in the incidence of human Lyme disease (Table 1.1) (Centers for Disease Control and Prevention 2016; Public Health Agency of Canada 2017). It is believed that the distribution of *I. scapularis* and the risk of Lyme disease in Canada extend far beyond these endemic sites (Ogden et al. 2006c; Koffi et al. 2012; Nelder et al. 2014).

Interest in the ecology of Lyme disease has grown recently with the potential impacts that climate change may have on the spread of disease (Estrada-Pena et al. 2013). An understanding of disease ecology is pertinent to determine current disease risk, predict the future distribution and spread of disease, and inform public health of effective prevention and control measures (Fish and Childs 2009; Franke et al. 2013).
The quest to understand the abiotic and biotic factors associated with Lyme disease ecology has been admirable. However, despite the wealth of information available on tick phenology, host relationships, and the influence of habitat and climate, no simple conclusions are available that apply widely across geographic areas. The impact of climate change, habitat change and biodiversity on Lyme disease distribution and spread remain areas of active research and scientific debate.

To synthesize the wealth of information available on Lyme disease ecology, we conducted a literature review on the abiotic and biotic factors associated with the distribution and spread of Lyme disease in eastern North America. This review provides a comprehensive, up-to-date picture of the known factors impacting Lyme disease ecology. We also present the current perspectives on the ecological drivers for the changing distribution of the vector and pathogen to highlight areas where there may be gaps in knowledge, and to help shape future research initiatives.

METHODS

The literature review was conducted using a pre-determined systematic process. Four databases were chosen based on their extensive coverage: PubMed®, Agricola®, CAB Direct® and Web of Science®. Search results were restricted to publication dates from 1990 to present (December 2014) and English language only. The following keywords (related terms are listed in brackets) were used as search terms in each database: Lyme disease (Lyme borreliosis, tick-borne disease, vector-borne disease); *Borrelia burgdorferi* sensu stricto (*Borrelia burgdorferi*); *Ixodes scapularis* (deer tick, blacklegged tick, hard-bodied tick); climate (climate change, temperature); habitat (forest, fragmentation); reservoir host (white-tailed deer, white-footed mouse, small mammal); disease emergence; ecology (biodiversity); North America (eastern United States, Canada, Ontario). Search terms were combined when appropriate using Boolean expressions. All articles were stored using RefWorks®, and duplicates were removed.

The initial search yielded 900 articles. Following the search, article titles were scanned for relevance to the topic. Any article deemed irrelevant to the focus of the literature search was
discarded, leaving 405 articles. For the purposes of this review, irrelevant articles included those examining: species other than *I. scapularis* and *B. burgdorferi* sensu stricto; geographic scope outside of eastern North America; microbiology and genetics of *B. burgdorferi*; and clinical human disease including diagnostic methodology and treatment regimens. Abstracts of retained articles were assessed for relevance, and irrelevant articles were discarded. All remaining articles (197) were reviewed in full. Seven more were discarded. Citation searching was completed to address any gaps in the literature review process, and yielded 13 additional articles. The total number of articles used for this review was 203.

**REVIEW OF THE LITERATURE**

**Climate**

Climate impacts the development, lifecycle, activity and ultimate survival of *I. scapularis*, as well as the transmission patterns of *B. burgdorferi*. It is most valuable to examine the influence of climatic variables at the level of the tick, as microclimatic measurements directly reflect the tick’s environment and can be significantly different from more course climatic measurements (Estrada-Pena et al. 2013; Estrada-Pena and de la Fuente 2014).

For the tick to successfully complete its lifecycle and reproduce, it must develop from egg to larvae to nymph to adult. At the larval, nymphaal and adult stages, the tick quests to find a host for a blood meal, and then undergoes a period of inactivity, called diapause, when it develops to the next life stage (Spielman et al. 1985; Anderson and Magnarelli 1993; Keirans et al. 1996). This is a simplistic view of the tick’s lifecycle, but sufficient for the purposes of this review.

Development is influenced by both climatic factors as well as factors intrinsic to the tick (Awerbuch and Sandberg 1995; Rodgers et al. 2007a). The rate of development exhibits a power relationship with temperature. As temperature increases, there is an exponential increase in the rate at which the tick moves from one life stage to the next. At higher temperatures, the rate of development no longer increases dramatically. Temperatures exceeding 30°F can be detrimental
to development at any life stage. The effect of lower temperatures varies by life stage. Females will not lay their eggs at temperatures below 4°C. For larvae to successfully develop from these eggs, the temperature needs to be above 10°C (Ogden et al. 2004). Therefore, in areas where the winter is long and the summer is short, the tick may deplete its energy stores before it is able to develop to the next life stage. The most vulnerable period is the time from when the engorged female lays her eggs to when the larvae acquire a blood meal. If this period is longer than two years, the larvae cannot survive (Ogden et al. 2005).

Lindsay et al. (1995) conducted field trials to examine the influence of climate on tick development. The study was conducted at Long Point, Ontario, where there is an established population of *I. scapularis*, as well as at three other northern locations in Ontario where there were no tick populations. In areas with cooler temperatures, development was prolonged, especially the time to egg laying and time to hatching.

Diapause is influenced by photoperiod and temperature (Belozerov and Naumov 2002; Ogden et al. 2005), as well as morphogenetic and behavioural factors (Ogden et al. 2004). The relative impact of each factor appears to vary based on the climate, with temperature having a greater impact than photoperiod in cooler, more northern areas (Ogden et al. 2005).

Relative humidity and temperature determine the tick’s questing activity and behaviour. Questing is highest when the temperatures are lower and the relative humidity is higher. This is generally in the early morning, or evening (Schulze et al. 2001a; Schulze and Jordan 2003). In vitro experiments show that the distance moved to quest and the posture of the tick are influenced by temperature, while relative humidity determines the height at which they quest. Questing height is greatest at 100% relative humidity (Vail and Smith 2002).

*Ixodes scapularis* is highly sensitive to desiccation, and if ticks are exposed to relative humidity levels less than 82% for greater than four hours, they experience decreased survival (Rodgers et al. 2007b; Berger et al. 2014b). Mortality is also high at temperatures above 30°C (Ogden et al. 2005). Overwintering survival rates are generally high, provided ticks are in a
protected site with sufficient snow cover (Lindsay et al. 1995). However, if the tick is not in a protected area, it will freeze, especially if the moisture level is high (Brunner et al. 2012).

Seasonal climatic variations in terms of minimum, mean and maximum temperature, as well as vapour pressure deficit impact the annual pattern of the tick’s lifecycle; commonly referred to as phenology (Gatewood et al. 2009). There are two distinct patterns for *I. scapularis*. In the northeast, peak activity occurs at different times for all life stages. This asynchrony is most relevant to larval and nymphal life stages. Peak nymphal activity occurs in early summer. If the nymphs are infected with *B. burgdorferi*, they can transmit the bacteria to the host during the blood meal. Peak larval activity occurs in late summer. Since there is negligible transovarial transmission of *B. burgdorferi*, larvae can only acquire the bacteria to continue the transmission cycle when they feed on an infected reservoir host (Piesman et al. 1986; Magnarelli et al. 1987). When the life stages are asynchronous, the host needs to remain infective for an extended period to ensure pathogen transmission (Ogden et al. 2007). In the midwestern USA, there is synchrony of larval and nymphal stages in the spring and early summer. This means that transmission of *B. burgdorferi* from infective nymphs to larvae via a reservoir host occurs within a short period of time. There is also an opportunity for transmission during co-feeding. These seasonal differences in phenology have impacted the relative abundance of the strains of *B. burgdorferi*. In the northeast, strains that have longer infectivity in the host (e.g., strain RST 1) predominate (Kurtenbach et al. 2006; Gatewood et al. 2009; Hamer et al. 2012b).

When studying Lyme disease, climate is also commonly assessed on a coarser scale. As previously mentioned, this must be viewed with caution as course scale data, including data from weather stations, remote-sensed data or aggregated data at the regional or national level, may not be reflective of the microclimate conditions to which the tick is exposed (Berger et al. 2014a; Lee et al. 2014). Different patterns can appear depending on which scale is used and may not be relevant to the ecology of the tick (Estrada-Pena 2001; Pardanani and Mather 2004).

Effort has been made to use climatic data to make predictions for the upcoming season. Although Moore et al. (2014) did find an association between an earlier onset of the Lyme disease season and an increase in the cumulative growing degree-days above 10° C, this finding
was not uniform across the study area. Higher precipitation levels correlated with a delayed onset of the Lyme disease season, but these data were not valuable for advanced prediction. When temperature and precipitation data from local weather stations were compared to tick abundance over a 12-year period, no relationships were evident (Schulze et al. 2009).

Numerous studies have indicated that there may be a relationship between rainfall and tick abundance (Ashley and Meentemeyer 2004). Since *I. scapularis* is vulnerable to desiccation, drought can directly impact its survival. Equally important are the indirect effects drought can have on the survival of the tick. Climate can impact vegetation and host populations, which then impact *I. scapularis* populations. For example, in drought conditions, the understory decreases. This leads to less food and protection for mice, one of the primary hosts for immature ticks (Jones and Kitron 2000). White-footed mice populations decline and this has been tied to lower nymphal numbers two years post-drought (Subak 2003). Higher rainfall in the spring has been correlated with increased nymphal abundance and Lyme disease risk two years later (Subak 2002). Indirect effects will be discussed in more detail in subsequent sections.

Climate at a broad level can effectively be used to determine the areas that are theoretically suitable to support a reproducing population of ticks (Killilea et al. 2008). This knowledge can then be incorporated with other ecological variables to more precisely determine an area’s suitability (Brownstein et al. 2003). Risk maps have been created to examine large climatic patterns (Brownstein et al. 2003; Ashley and Meentemeyer 2004; Ogden et al. 2005; Waller et al. 2007). For example, Ogden et al. (2005) created a process-based dynamic model to determine the theoretical maximum distribution of *I. scapularis*. The model incorporated temperature data and known values for temperature-dependent development rates and was used to create a map to determine the geographic range where *I. scapularis* can successfully establish a reproducing population. This was a valuable exercise as the results indicated a much wider potential distribution than previously thought.
Habitat

Many habitat features are intricately related to climate, as temperature and precipitation influence the vegetation species and structure (Reisen 2010). Habitat can also overcome climatic extremes, providing a protective microclimate in areas otherwise considered to be hostile (Schulze and Jordan 2005).

Research examining the influence of habitat features on the survival, reproduction and abundance of *I. scapularis* abound in the literature. Although habitat features vary across the landscape, characteristic patterns have emerged. *Ixodes scapularis* populations are established both along the coast and near bodies of water, as well as at inland sites (Sonenshine et al. 1995; Clark et al. 1998; Bunnell et al. 2003; Raizman et al. 2010). Forest cover is a basic habitat feature for the presence of *I. scapularis* (Schulze and Jordan 2005). However, this relationship is not simple. Increased forest cover is not necessarily associated with higher risk (Nicholson and Mather 1996). Higher tick abundance has been linked to fragmented forests with less canopy cover (Schulze and Jordan 2005; Killilea et al. 2008). Tree species composition also appears to be important. Numerous studies have illustrated a positive association between deciduous forests and the presence of *I. scapularis* (Guerra et al. 2002; Schulze and Jordan 2005; Killilea et al. 2008). However, this association does not mean that *I. scapularis* cannot survive in forests predominated by coniferous species. On Fire Island, New York, a study was conducted to compare tick abundance on two separate forests plots, and found no difference in tick abundance between coniferous and deciduous forests (Ginsberg et al. 2004).

The structure and composition of the forest influence the understory and leaf litter layer. When the canopy cover is less dense, more sunlight is available for growth of the understory (Prusinski et al. 2006). Understory, including shrubs and plants, as well as a thick leaf litter layer provide a protective microclimate for *I. scapularis* that reduces desiccation and prevents large temperature fluctuations (Mather et al. 1993; Lindsay et al. 1999a; Schulze and Jordan 2005). Moreover, understory provides shelter and food for host species, and can be used by the tick for questing (Carroll 2002; Prusinski et al. 2006). Tick abundance has repeatedly been positively
associated with a shrubby understory and a prominent leaf litter layer (Lindsay et al. 1998; Lubelczyk et al. 2004; Schulze and Jordan 2005).

A study conducted by Lindsay et al. (1998) illustrates the influence of habitat characteristics on tick survival and development. They monitored a tick population over a four-year period in four distinct habitats: maple forest, oak savannah, white pine and cottonwood dune. Egg laying, hatching success, molting and overwinter survival of fed females were highest in the maple forest. This habitat had a well-developed understory and leaf litter layer. Survival and development were always lowest in the cottonwood dune, which offered relatively little protection for the ticks.

Soil impacts the tick’s survival directly and indirectly. The level of moisture in the soil and microhabitat is partly determined by the soil composition. Although *I. scapularis* is highly susceptible to desiccation, evidence shows that sandy soil and other soil types with large particle size that promote water drainage provide a better microhabitat for the ticks (Guerra et al. 2002; Bunnell et al. 2003). A high level of moisture, such as in clay soil, favours the growth of fungi (Guerra et al. 2002). Many fungal genera are ubiquitous in the environment and can be pathogenic to *I. scapularis* under the appropriate conditions (Greengarten et al. 2011). Soil composition also influences the vegetation and host community (Guerra et al. 2002; Bunnell et al. 2003; Ogden et al. 2006a).

Most studies have examined habitats in which the ticks are currently established. There is also value in studying other habitat types for suitability for *I. scapularis*. Ogden et al. (2006a) looked at larval development within egg masses in habitats typical in areas in the Lower Great Lakes and St. Lawrence Plain region. This area was outside of the known distribution of *I. scapularis* at the time of the study. Survival was low in the sand dunes and dry-fresh sugar maple deciduous forest, most likely due to inadequate moisture. In contrast, the sugar maple deciduous and deciduous swamp supported larval development, indicating that other habitats outside the current range are suitable and may be at risk for *I. scapularis* invasion.
Within suitable habitats, *I. scapularis* are non-uniformly distributed (Estrada-Peña 1998; Wilson 1998). There does not appear to be any significant association with tick distribution and host movement (Schulze et al. 2001b; Prusinski et al. 2006) or at the edges of the habitat (e.g., forest boundaries, cleared trails) (Siegel et al. 1991).

Although these habitat characteristics help elucidate the factors that influence the suitability of an area for *I. scapularis*, it is important to note that they may not directly relate to the risk of disease. Peri-domestic exposure is believed to account for the largest proportion of Lyme disease cases (Barbour and Fish 1993). Efforts have been made to examine the habitat associated with suburban residential areas with a high incidence of Lyme disease. There is a positive relationship between residences with areas of forest cover (Killilea et al. 2008). It is not the total area of forest cover that is of significance, but rather the interspersion of forested area and amount of forest-herbaceous edge habitat (Jackson et al. 2006). Edge habitat is believed to be where contact with *I. scapularis* occurs (Tran and Waller 2013). This is not because the actual abundance of ticks is higher in these areas, but that the level of human activity is greater (Horobik et al. 2006). Habitat risk factors may be valuable for use in landscape planning (Schulze et al. 1991; Ward and Brown 2004) in conjunction with human behaviour patterns (Eisen et al. 2012).

Despite the extensive work that has been conducted to determine what factors contribute to suitable habitat for *I. scapularis*, it is still not clear why a tick population thrives in one area, yet in a similar area no population exists (Schulze et al. 1991; Killilea et al. 2008).

**Hosts**

*Ixodes scapularis* are host generalists, and will acquire a blood meal from a variety of hosts including large, medium and small-sized mammals as well as many species of birds and lizards (Hanincova et al. 2006). However, each life stage appears to have host preferences. These preferences differ based on habitat type and geography, which influence the community of hosts available to the tick (McCoy et al. 2013).
Major differences exist between species regarding host quality for *I. scapularis* (Wilson et al. 1990). Tick feeding success, the quality of blood meal, degree of engorgement, and molting success vary by host (LoGiudice et al. 2003; Brunner and Ostfeld 2008). Intraspecific variation also exists (Brunner and Ostfeld 2008; Brunner et al. 2011). Several factors influence the quality of the host including permissiveness, grooming behaviour, immune response to tick attachment, and prior exposure to the tick (Brunner and Ostfeld 2008), as well as age and sex (Lindsay et al. 1997; Hofmeister et al. 1999; Bouchard et al. 2011).

Reservoir competency for *B. burgdorferi* also varies across species, and this has a profound impact on disease transmission. A host species’ role in disease transmission can be quantified in two ways. Realized reservoir competence is the probability that the blood meal acquired from a host will lead to infection of the tick (Giery and Ostfeld 2007). Reservoir potential incorporates realized reservoir competence and the number of vectors feeding on the host to determine the proportion of infected ticks that are contributed by each host species (Brunner et al. 2008). Many strains of *B. burgdorferi* are not species-specific and can efficiently be transmitted to multiple species (Hanincova et al. 2006).

Hosts can transport ticks into new areas, and their role in dispersion is highly dependent on their home range and seasonal movement patterns, as well as the level of infestation. Small mammals appear to have a limited role in dispersal, while deer may contribute to greater localized spread. Migratory birds have the greatest capacity for dispersal (Deelman et al. 1996; Madhav et al. 2004).

**Small mammals**

In the northeastern USA and southern Ontario and Quebec, it is believed that 80 to 90% of ticks become infected with *B. burgdorferi* from four small mammal species: *Peromyscus leucopus* (white-footed mouse), *Tamias striatus* (eastern chipmunk), *Blarina brevicauda* (short-tailed shrew) and *Sorex cinereus* (the masked shrew) (Brisson et al. 2008).
The white-footed mouse is a habitat generalist and has long been considered the preferred host for sub-adult *I. scapularis* and the most important reservoir host for *B. burgdorferi* (Mannelli et al. 1993a). Longitudinal studies have examined the transmission dynamics of *B. burgdorferi* in natural populations of *P. leucopus* (Lindsay et al. 1997; Hofmeister et al. 1999; Bunikis et al. 2004; Oliver et al. 2006). *Borrelia burgdorferi* is only transmitted to white-footed mice via an infected tick. No vertical or horizontal transmission occurs. Infection levels in juvenile mice are generally very low, which may be due to immunity from maternal antibodies. Older mice are significantly more likely to be infected and in highly endemic areas, infection prevalence can reach levels >90% (Hofmeister et al. 1999). Mice remain infectious only for a short duration of time (~1-2 weeks), although the bacteria can be detected in tissues for many months. Re-infection can also occur throughout the season (Lindsay et al. 1997; Bunikis et al. 2004). There is no evidence that infection with *B. burgdorferi* impacts survival or longevity of *P. leucopus* (Hofmeister et al. 1999). However, results from one study suggested that individuals infected with *B. burgdorferi* used a greater amount of space for foraging, which may lead to increased opportunities for tick contact (Schwanz et al. 2011).

Because of the significant role *P. leucopus* plays in the transmission of *B. burgdorferi*, many control mechanisms have been designed to interfere with transmission at the mouse-tick interface. These attempts have been met with mixed success. Stafford (1991, 1992) examined the impact of providing permethrin-treated cotton balls to be used in the nests of white-footed mice. Despite a significant reduction in the number of sub-adult ticks on white-footed mice, no significant change was noted in host-seeking nymphs or infection prevalence. Bait boxes have been used to apply fipronil to white-footed mice. This strategy successfully reduced the abundance of host-seeking nymphs and mice infestation levels. However, this study was only conducted in an island environment and it is unknown if this strategy would be suitable for a more open habitat, such as a mainland site (Dolan et al. 2004). Vaccination of white-footed mice and other wildlife is currently being explored. One vaccination has shown promise in trials (Voordouw et al. 2013), but many challenges associated with wildlife vaccination administration must still be overcome, including differing host immune responses, wide home ranges of some species and variation in diet (Tsao et al. 2012).
Although the eastern chipmunk is not a habitat generalist, it plays an important role in the transmission of *B. burgdorferi*. *Tamias striatus* is easily infected with *B. burgdorferi*, exhibits spirochetemia and maintains prolonged tissue infection (McLean et al. 1993a). In areas where *T. striatus* and *P. leucopus* co-exist, sub-adult ticks exhibit feeding preferences. Studies suggest that the proportion of nymphs found on *T. striatus* compared to *P. leucopus* is much higher, and the opposite pattern is seen for larval *I. scapularis* (Bouchard et al. 2011). It was initially hypothesized that these feeding preferences contribute to elevated risk of Lyme disease in the peri-domestic environment, as chipmunks frequent residential and suburban areas. However, no clear patterns in host abundance or tick infestation level were evident between locations (Schulze et al. 2005).

In other geographic areas, studies have been conducted to elucidate the role of native small mammal species. Although *P. leucopus* has a wide geographic distribution, its abundance is lower in the southeastern United States. *Peromyscus gossypinus* (cotton mouse), *Mus musculus* (house mouse), and *Sigmodon hispidus* (cotton rat) are all *B. burgdorferi*-competent reservoir species in this area (Magnarelli et al. 1992; Sonenshine et al. 1995; Oliver et al. 2003; Durden et al. 2004). Despite high infection prevalence of *B. burgdorferi* in many of these species, the incidence of Lyme disease remains low. Further research is needed to understand the transmission dynamics in the southeastern United States, including the role of other tick species as well as lizard species (Clark et al. 1998, 2002; Clark 2004; Anderson et al. 2006). In Minnesota, *Clethrionomys gapperi* (the red-backed vole) is common in coniferous and mixed forests. Laboratory studies suggest reservoir potential of this species, but its actual role in nature has yet to be determined (Bey et al. 1995).

**Medium-sized mammals**

Relatively fewer studies have examined the role of medium-sized mammals as hosts for *I. scapularis*. *Ixodes scapularis* have also been found on coyotes (*Canis latrans*) and black bears (*Ursus americanus floridanus*) (Kollars et al. 1999; Yabsley et al. 2009; Levi et al. 2012; Way and White 2013). Results are inconclusive for raccoons (*Procyon lotor*). In New York State, raccoons are highly abundant, but have minimal tick burdens (Wilson et al. 1990), while in
Illinois the raccoons collected had large larval burdens (Mannelli et al. 1993b). Sub-adult ticks have been collected in large numbers on opossums (*Didelphis virginiana*) (Mannelli et al. 1993b). Interestingly, more in depth examination of opossums shows that ticks appear to preferentially feed on this species. Opossums are not considered good quality hosts though and feeding survival is low. This phenomenon has been termed an ‘ecological trap’, and species that fit this description may have a role in decreasing host-seeking tick population numbers as well as associated pathogen transmission (Keesing et al. 2009). Raccoons and opossums that were sero-positive for *B. burgdorferi* have been noted, but the prevalence is low. Opossums also have a limited geographic range within Canada, which influences their potential role as a host. Overall, there are large gaps in our knowledge of the role these species play in transmission of *B. burgdorferi* (Oliver et al. 1999).

**White-tailed deer**

White-tailed deer (*Odocoileus virginianus* Zimmerman) are the primary blood meal hosts for female *I. scapularis* (Rand et al. 2003). There is evidence that adult ticks preferentially attach to white-tailed deer (Wilson et al. 1990), which may be a result of the tick’s ability to respond to deer urine and interdigital gland secretions (Carroll 2000, 2001). Although white-tailed deer play an important role in the reproductive stage of the tick, they are not competent reservoirs for *B. burgdorferi* and therefore do not have a biologic role in the pathogen transmission cycle (Telford et al. 1988).

Many studies have found a positive relationship between tick abundance and deer density (Rand et al. 2003). Early studies focused on deer exclusion or removal and were met with mixed success (Daniels et al. 1993). Daniels et al. (1993) used fencing to exclude deer at 5 sites for 25 years. While the prevalence of *B. burgdorferi* did not change, there was a significant decline in the number of larvae and nymphs, which contributed to a decreased risk of infection. Other studies have found similar findings (Stafford 1993; Rand et al. 2000, 2004). It appears that in the absence of deer, ticks find other hosts. This is commonly a small mammal host and if this species is a competent reservoir, infection prevalence may increase. This paradoxical rise in infection prevalence may not be permanent and if deer are not reintroduced into the system, its disappears.
within 2 to 3 years as adult tick abundance declines (Rand et al. 2004). However, *I. scapularis* are not eliminated in these areas because reintroduction may occur via bird and small mammal movement (Daniels et al. 1993; Elias et al. 2011).

In open, mainland areas, complete deer removal is not feasible or necessarily desirable (Rand et al. 2003). It appears that once deer reach a certain density, the size of the population is no longer a key determinant of tick abundance (Amerasinghe et al. 1992; Ostfeld et al. 2006). However, it is unknown how much the deer population must be reduced to have an impact on tick abundance (Daniels et al. 1993; Rand et al. 2003; Jordan et al. 2007). Incremental decreases in deer density by almost 50% in one American county did not contribute to a significant change in tick abundance (Jordan et al. 2007). Deer population reduction of 87% in another community did lead to a significant decline in tick abundance as well as the incidence of Lyme disease. Such community-based programs require an ongoing active effort to see continued results (Kilpatrick et al. 2014).

In recent years, efforts have shifted away from deer population control towards using deer as a tool to target the tick population. Deer treatment stations were first used to provide ivermectin-treated corn. Unfortunately, it was difficult to ensure that deer consumed enough feed to achieve an acaricidal concentration of ivermectin in their blood and success was limited (Rand et al. 2000). Four-poster deer treatment stations were later piloted. At these stations, acaricide is applied to deer as they feed. Initial trials saw a large reduction in questing ticks within two to three years (Solberg et al. 2003; Hoen et al. 2009). However, other studies have experienced challenges, such as inadequate usage by deer, heavy labour demands and inconsistent results (Schulze et al. 2008; Miller et al. 2009). The viability and widespread applicability of these stations remains an active area of research.

Deer abundance has also been associated with the presence of chalid wasps (*Ixodiphagus* sp.). Chalid wasps are natural parasitoids of *I. scapularis* that kill the nymphal stage, and it is believed that they use white-tailed deer to locate the tick. Despite high levels of these parasitoids in isolated areas of the eastern United States, they do not appear to impact the tick population (Stafford et al. 2003).
Since deer are the primary hosts for adult ticks, they have been used for surveillance for *I. scapularis* and *B. burgdorferi*. Examining hunter-killed deer may have some use in introductory scanning for *I. scapularis*, but this method has low specificity and sensitivity (Amerasinghe et al. 1992; Cortinas and Kitron 2006; Keefe et al. 2009; Bouchard et al. 2013b; Lee et al. 2013). There is no value in using deer to detect *B. burgdorferi*, as deer produce a short-term antibody response, and can have a borreliacidal effect on feeding ticks (Gallivan et al. 1998; Raizman et al. 2013).

**Birds**

Many species of birds are parasitized by *I. scapularis*. In total, over 71 species have been identified as hosts for *I. scapularis*, with the most common species being ground-foraging and ground-nesting songbirds like wood warblers (*Phylloscopus sibilatrix*), brown thrashers (*Toxostoma rufum*), wrens (Troglodytidae family), and thrushes (Turdidae family) (Giardina et al. 2000; Brinkerhoff et al. 2011a; Raizman et al. 2013). Tick acquisition rate and burden varies by species and season (Mitra et al. 2010).

Numerous bird species are also competent reservoirs for *B. burgdorferi*. Interest in their potential role in transmission first arose when fed-larval ticks removed from birds tested positive for *B. burgdorferi*. Natural infection with *B. burgdorferi* in a song sparrow (*Melospiza melodia*) was subsequently described (McLean et al. 1993b). Widespread field studies have examined the potential role birds may play in pathogen transmission (Nicholls and Callister 1996; Durden et al. 1997; Scott et al. 2010). Species including the American robin (*Turdus migratorius*), Swainson’s thrush (*Catharus ustulatus*), common yellowthroat (*Geothlypis trichas*), and song sparrow (*Melospiza melodia*) are competent reservoirs (Richter et al. 2000; Ginsberg et al. 2005; Scott et al. 2012; Raizman et al. 2013). Infection prevalence is generally low, and the duration of infectivity is not known for all species (Ginsberg et al. 2005). Genotypic analysis of *B. burgdorferi* strains isolated from birds shows great variability. Most of the strains identified are the same as those detected in host-seeking nymphs, but the frequency differs (Brinkerhoff et al. 2010), and it is unclear if the most frequently detected strains in birds are infective to mammals (Brinkerhoff et al. 2010, 2011a). This is of concern as birds frequent the peri-domestic
environment (Ginsberg et al. 2005). Overall, bird species have a much lower reservoir potential when compared to small mammal species (Giardina et al. 2000), and there is limited evidence of elevated risk for humans in areas where birds are present (Townsend et al. 2003; Hamer et al. 2012a).

Birds have been implicated in both the local and widespread dissemination of *I. scapularis* (McLean et al. 1993b). Birds travelling along the Atlantic flyway can introduce *I. scapularis* from the northeastern United States into southeastern Canada, and birds following the Mississippi flyway can introduce *I. scapularis* into central Canada from the midwestern United States (Morshed et al. 2005). Genetic evidence exists to support these dissemination patterns (Morshed et al. 2006; Ogden et al. 2010, 2011; Mathers et al. 2011). At the time of spring migration, nymphs are active in the northeast and both nymphs and larvae are active in the midwest (Brinkerhoff et al. 2011b). This may have implications for both the establishment of a reproducing population of *I. scapularis* as well as *B. burgdorferi* transmission. Since larvae are important for population establishment, and field studies have shown that birds coming from the Midwest have higher infection prevalence and are more likely to be co-infested with nymphs, it is believed that the introduction of *I. scapularis* and *B. burgdorferi* will be more efficient from the mid-west United States than the Northeast (Klich et al. 1996; Brinkerhoff et al. 2011b).

Ogden et al. (2008b) conducted a widespread surveillance study of birds in Canada. Over 39,000 birds were collected. Approximately 1% of birds were infested with *I. scapularis*, and 15.4% of ticks were positive for *B. burgdorferi*. Although these numbers appear small, when they are considered in light of the 3 billion birds that migrate north annually in North America, it is estimated that 50 to 175 million *I. scapularis* are introduced each year. Most of these ticks will not establish a reproducing population, but if the area is suitable for tick survival, risk exists. Additionally, if infected sub-adult ticks survive, they may transmit the pathogen to the next host during the blood meal.
Lizards

The role of lizards in Lyme disease transmission remains unclear. Lizards are abundant in the southern United States, where the incidence of Lyme disease is much lower. It has been hypothesized that these species may be of importance for reduced pathogen transmission (Clark et al. 2001). One preliminary study examined museum specimens of 13 species of lizards for *I. scapularis*. Ticks were found on 135 individuals, predominately of the *Eumeces* genus (Oliver et al. 1993). Clark et al. (2005) analyzed 10 species for *I. scapularis* and *B. burgdorferi*. Sub-adult ticks were collected from 3 species: *Plestiodon laticeps* (broad-headed skink), *Plestiodon inexpectatus* (southeastern five-lined skink) and *Ophisaurus ventralis* (eastern glass lizard). Fifty-four percent of serological samples from nine species were positive for *B. burgdorferi* sensu lato, including several samples that were identified as *B. burgdorferi* sensu stricto. *Plestiodon laticeps* and *P. inexpectatus* were identified as important hosts for nymphs and larvae in Missouri. The tick burden on these two species was higher than on white-footed mice (Bey et al. 1995).

In the northeastern USA, there are a small number of native lizard species. *Ixodes scapularis* were present on 3 of the 5 species examined. *Eumeces fasciatus* (five-lined skink) had the highest tick burden. Only one tick sample was positive for *B. burgdorferi*, and it was removed from *Sceloporous undulatus* (eastern fence lizard) (Giery and Ostfeld 2007). Further analysis of *S. undulatus* illustrated minimal reservoir potential (Rulison et al. 2014).

Clearly, there are differences between lizard species in host quality for *I. scapularis* and reservoir competence for *B. burgdorferi*. Do some lizard species play a role in pathogen transmission (Clark et al. 2005)? Do species such as *E. fasciatus* act as dilution hosts by feeding large numbers immature ticks without contributing to the transmission cycle (Giery and Ostfeld 2007)? These questions remain to be answered and may provide insight into the ecology of Lyme disease in areas where lizard species predominate.
UNDERSTANDING FUTURE DISTRIBUTION AND SPREAD

Climate change

The impact of climate change on infectious disease has been an area of great debate in the scientific community, and Lyme disease is no exception. Risk maps have been produced to forecast the impact of climate change on the distribution of *I. scapularis*. Brownstein et al. (2005) used predictive logistic regression to identify areas that may be suitable based on seasonal temperature and relative humidity projections for 2080. Currently, there are large areas where the climate is suitable that have not been colonized by *I. scapularis*. In North America, this area will increase by 68.9%, with Canada experiencing an increase of 212.9%. Although the overall net change is positive, there will be some loss of suitable habitat at lower latitudes due to extreme increases in temperatures. It is important to note that these findings were overlaid with projections for human population distribution and the actual risk of human exposure is not anticipated to significantly change. Ogden et al. (2008d) incorporated climate projections, habitat factors and bird migration to create risk maps for current and future Lyme disease distribution. Again, findings were consistent with an increase in suitable area in 2020, 2050 and 2080. In this model, climate change not only contributed to warmer temperatures at higher latitudes, but also led to earlier and faster bird migration and therefore a wider, more efficient distribution of the tick (Ogden et al. 2008d).

Expanding upon these data, climate and habitat change were used to determine the rate of invasion of *I. scapularis*. It is estimated that the tick’s range will expand northward by approximately 46 km each year (Leighton et al. 2012). The expansion front of *B. burgdorferi* is predicted to expand much more slowly. Currently, there appears to be a 5-year lag between *I. scapularis* population establishment and a sustained transmission cycle of *B. burgdorferi* in eastern Canada (Ogden et al. 2013a). With climate change it is estimated that the pathogen will move north 3.5 – 11 km each year (Simon et al. 2014). This rate is accelerated in the Midwest (Ogden et al. 2013a), and there is evidence that it may occur concurrently with or before *I. scapularis* introduction (Hamer et al. 2010, 2011).
Climate change is also predicted to impact tick phenology, and transmission patterns of *B. burgdorferi* (Ogden et al. 2006b; Ogden et al. 2014). Transmission is studied using the basic reproductive number, \( R_0 \) (Zhao 2012; Dunn et al. 2013; Ogden et al. 2013b). In simple disease models, this parameter can be defined in two ways. For microorganisms, it is the number of secondary cases that result from one infectious individual given the entire population is susceptible. For macroparasites, \( R_0 \) is the number of new female parasites produced by one female, in the absence of density-dependent limitations (Wu et al. 2013). In the case of Lyme disease, \( R_0 \) is more complex given it is a multi-host system, involving both a microorganism (i.e., *B. burgdorferi*) and macroparasite (i.e., *I. scapularis*). In general, it is hypothesized that areas at lower latitudes will become more synchronous, while areas at higher latitudes will remain asynchronous (Ogden et al. 2006b). Ogden et al. (2008a) studied \( R_0 \) based on the projections for climate change in 2020, 2050 and 2080. Two transmission patterns were possible. Larvae became infected either by: (a) feeding in the summer on an infectious host that acquired the pathogen from an infectious nymph in the spring, or (b) feeding in the spring on an infectious host that acquired the pathogen from an infectious nymph the previous fall. Both transmission patterns are asynchronous, but differ in the efficiency of pathogen transmission. When a host must remain infective over the winter to facilitate transmission, the transmission efficiency declines. In 2020 and 2050, it is predicted that pattern (a) will predominate and this could lead to an elevated risk of Lyme disease. *Borrelia burgdorferi* strains that are short-lived and may have greater pathogenicity in the host will be favoured. By 2080, pattern (b) is most common and transmission efficiency declines and potentially the risk of Lyme disease.

Although these studies provide evidence that climate change will increase the distribution of *I. scapularis* and potentially the risk of Lyme disease, it is not accurate to simply state that climate change inevitably means increased risk of disease (Gage et al. 2008). Climate change may impact many other factors, including extreme weather events, human demographics, habitat change, and host distribution.

Extreme weather events are predicted to increase in association with climate change. Floods and drought will negatively impact *I. scapularis* populations, but to what degree is unknown (Berger et al. 2014a).
The Carolinian forests are predicted to expand. In Canada, these forests are currently restricted to an area along the northern shores of Lake Erie and Lake Ontario. They provide ideal habitat to the tick, and therefore expansion of this habitat may lead to an increased distribution of *I. scapularis* in Canada (Ogden et al. 2006a).

Finally, many questions remain as to the impact of climate change on host abundance and distribution. As previously mentioned, bird migration is anticipated to occur earlier and at an accelerated rate, facilitating distribution of the tick (Ogden et al. 2008b). The range of the white-footed mouse has been expanding, and may be impacted by habitat change as well (Simon et al. 2014). The rate of expansion has been quantified at approximately 15 kilometres per year in the Midwest and 10 kilometres per year in Quebec (Roy-Dufresne et al. 2013). Climate change is not anticipated to directly affect white-tailed deer populations (Brownstein et al. 2005), but it is unknown if other factors related to climate change will influence distribution and abundance (Ogden et al. 2008d). Although there remain several gaps in our knowledge on the ecological role of lizard species in the cycle of Lyme disease, if they do serve as dilution hosts, and their range shifts northward as predicted with climate change, there may be a subsequent decrease in the risk of Lyme disease (Brownstein et al. 2005; Ginsberg et al. 2014).

**Biodiversity**

The ‘host community model’, created by LoGiudice and colleagues (2003), launched the debate on the impact of biodiversity on Lyme disease. Preliminary work conducted by Ostfeld and Keesing (2000a) showed a significant negative relationship with species richness at the state or multi-state level and the per capita number of Lyme disease cases. The ‘host community model’ examined this relationship at a much finer scale to determine how increasing a site’s species richness would affect nymphal infection prevalence (NIP). The rationale for building the model was that disease transmission potential is highly dependent on the host on which larvae acquire a blood meal. If there were other hosts in the community that would draw larval blood meals away from the competent reservoir *P. leucopus*, then the nymphal infection prevalence would decline. This is termed the ‘dilution effect’.
Hosts vary in their dilution potential; a good dilution host would occur at high density, feed many ticks and have low to no reservoir potential (LoGiudice et al. 2003). White-footed mice will always be a reservoir host, eastern squirrels will always be a dilution host, and other species such as the short-tailed shrew can act as a dilution host or rescue host, depending on the abundance of white-footed mice. Rescue hosts are species that have high reservoir potential and can maintain pathogen transmission in the absence of white-footed mice (LoGiudice et al. 2008). Songbirds exhibit low to no dilution potential (Giardina et al. 2000). Although these concepts exhibit great potential benefit for both species conservation and public health, further research has illustrated that the conclusions are not so simple (Wood et al. 2014).

There appears to be a lack of consistency of how to define biodiversity and measure Lyme disease risk. Biodiversity is commonly expressed as either species richness, which is simply the number of species present, or species evenness, which is the proportion of each species in the community. These terms do not provide any information on the species present or their potential role in the host community (Ostfeld and Keesing 2000a; LoGiudice et al. 2008). The ‘host community model’ assesses Lyme disease risk with nymphal infection prevalence. Because NIP does not consider the density of ticks, using the density of infected nymphs (DIN) may be a more appropriate measure (Ogden and Tsao 2009; Wood and Lafferty 2013).

The dilution theory is based upon four key assumptions: (1) host species vary in reservoir competence; (2) tick infection will decrease as incompetent hosts increase; (3) biodiversity will always favour non-competent hosts; (4) tick density will not increase (Wood and Lafferty 2013). What happens if the species that are added to the community have no effect on the competent reservoir species, and tick density increases (Ogden and Tsao 2009)?

Ogden and Tsao (2009) found that the ‘dilution effect’ only occurs in a limited number of situations; otherwise there may be amplification of disease risk, as there are more feeding opportunities for ticks and therefore a higher density of ticks. This becomes evident when the outcome of interest is DIN (Ostfeld and Keesing 2000b). For dilution to occur, additional hosts must either compete with or predate white-footed mice. White-footed mouse density would subsequently decline, although in nature this is deemed unlikely. Dilution can also occur if the
alternate hosts kill the ticks, or nymphs preferentially feed on the alternate hosts to break the transmission cycle (Ogden and Tsao 2009).

Findings from field studies are inconclusive. No relationship between biodiversity and Lyme disease risk was evident on Block Island, Rhode Island (States et al. 2014). Habitat fragments occupied by white-footed mice and relatively few other species characterize this area. Despite low biodiversity, no elevations in NIP or DIN were noted between the island and mainland, where biodiversity was much higher. Werden et al. (2014) again examined island habitats, this time in Thousand Islands National Park, Ontario. Their results indicated that the effect of species richness is moderated by the relative abundance of P. leucopus. An increase in species richness was correlated with a decline in NIP, but this was only significant when the relative abundance of P. leucopus was low. Fieldwork conducted by Bouchard and colleagues (2013a) in southern Quebec also found inconclusive results. Although the infestation level of sub-adult ticks decreased as species richness increased, the density of P. leucopus also increased and this ultimately led to a minor increase in nymphal abundance. Interestingly, in this study, biodiversity was not only viewed in terms of the host community, but vegetation as well. A significant negative relationship between mature tree diversity and nymphal infestation on rodents was noted. Additional research is needed to understand this finding.

More large-scale field studies are required to elucidate the complex relationship between biodiversity and the risk of Lyme disease. Future work needs to examine not only nymphal infection prevalence, but also the density of infected nymphs (Ogden and Tsao 2009; Wood and Lafferty 2013). Biodiversity should also be viewed in a more holistic manner, not simply as species richness or species evenness. This includes examining the diversity of all plant and animal species beyond the community of small mammal hosts (Ostfeld and Keesing 2000b; Bouchard et al. 2013a; Wood and Lafferty 2013).

**Habitat change**

Reforestation is hypothesized to be one factor that contributed to the resurgence of Lyme disease (Barbour and Fish 1993; Barbour 1998). Human activity continues to change the
landscape and several factors associated with habitat change may be contributing to an increased risk of Lyme disease (Reisen 2010).

Development of forest and greening of suburban and urban areas has led to a patchwork landscape of small forest fragments. Allan et al. (2003) examined the influence of forest plot size and the density of infected nymphs. They hypothesized that as habitat size decreases, biodiversity would also decrease. However, in this scenario, species that thrive in marginal habitats, especially *P. leucopus*, would likely thrive and have limited predation (Ostfeld and Keesing 2000a; Rogic et al. 2013). This would ultimately lead to an increase in the density of infected nymphs. The density of larvae would also increase because deer would be likely to frequent these areas and introduce gravid females. Field studies demonstrated that the density of nymphs was higher in smaller patches of forest. This negative relationship only existed in patches less than 2 hectares in area. There was no significant increase in the density of larvae, indicating that deer may not be as prominent in these fragmented habitats as initially thought (Allan et al. 2003). Forest fragmentation continues to be an area of research, and the results to date are mixed. Several studies have illustrated similar findings (Jackson et al. 2006; Killilea et al. 2008), while others have not (LoGiudice et al. 2008; Tanner et al. 2010). Wilder and Meikle (2004) did not detect a relationship with tick abundance and fragment size. Their research focused on forest patches within an agricultural area. It is unknown if habitat type or classification of patch size influenced these results.

Introduction of invasive or alien plant species results in a change in the structure and species of forest habitats. Invasive understory species are more commonly found in areas that have been recently disturbed by human or animal activity (Jordan and Schulze 2005; Mize et al. 2011). These species form a dense understory, which is typically resistant to deer browsing due to the unpalatable nature of the plants (Elias et al. 2006). This not only creates a suitable microclimate for *I. scapularis*, but also provides excellent shelter for many of the tick’s hosts including small mammals, birds and deer. Significantly higher abundance of nymphal and adult ticks has been found in areas dominated by invasive species (Lubelczyk et al. 2004; Elias et al. 2006). It is the invasion of exotic plant species that has been attributed to the growth of the *I. scapularis* population in Wisconsin. Large areas of red pine stands have suffered loss due to the

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red pine pocket decline (Coyle et al. 2013; Lee et al. 2014). The death of these trees has allowed for the invasion of exotic shrubs and forbs (Coyle et al. 2013). Surveillance in these sites has found an increase in tick abundance, which is much higher when compared to other parks and forests (Lee et al. 2014).

CONCLUSIONS

Numerous abiotic and biotic factors influence the ecology of Lyme disease and these have and will continue to impact the spread and distribution of the vector and pathogen in the future.

Although a wealth of knowledge exists on Lyme disease ecology, there are no simple relationships that fully explain the distribution of *I. scapularis* across all areas. It is important to consider all the potential contributing factors to create a realistic, dynamic ecological picture. In this scenario, one must consider the host-parasite relationship, the host-habitat relationship and the parasite-habitat relationship, all within the context of climate (Mize et al. 2011).

All components of the ecological picture interact and research conducted by Lindsay et al. (1998, 1999b) clearly illustrates the complex relationships. When examining survival and development of *I. scapularis* at Long Point, the tick abundance was much higher than expected in the oak savannah. This habitat offers relatively little protection for ticks when compared to other sites, but deer routinely visit this area and may explain the increased abundance of ticks (Lindsay et al. 1998). The same area was also used to examine the correlation between the density of white-footed mice and the abundance of ticks. Although it has been well-established that white-footed mice are important hosts for sub-adult ticks, there was minimal correlation between the density of white-footed mice and the abundance of ticks. Rather, habitat features had a much stronger influence on the tick population, and moderated the impact of white-footed mice on tick abundance (Lindsay et al. 1999b).

The relative influence of ecological variables varies with time and space. Ostfeld et al. (2006) examined the role of deer density, small mammal density, temperature, precipitation and
the presence of acorns on tick abundance. The strongest association was between nymphal abundance and acorn levels two years prior. Acorns are a source of food for small mammals and deer. Following years of oak mast, it appears that there is an increase in white-footed mouse and chipmunk density. These hosts provide a blood meal to many larvae, which subsequently increases the nymphal abundance in the following season. Although the presence of acorns is not a universal predictor of Lyme disease risk, as acorns are not present at all sites or in every year, it warrants recognition as an important ecological relationship within the Lyme disease system (Schauber et al. 2005).

Clearly, if a single factor or a small group of factors are examined, without consideration of the broader picture, important relationships will be missed and our understanding of Lyme disease ecology will be plagued with misconceptions.

As we move forward, a more comprehensive approach will be necessary to enhance our understanding of the impact of climate change, biodiversity and habitat change on Lyme disease risk. It is accurate to say that these drivers will shift the distribution of *I. scapularis* and the risk of Lyme disease over time. Continued research on the ecology of Lyme disease, with focus on climate change, biodiversity and habitat change, is necessary if we hope to understand and predict the changing landscape of Lyme disease in the future.

**STUDY RATIONALE AND OBJECTIVES**

With the rapid and ongoing range expansion of *I. scapularis* northward in Ontario and the concurrent rise in the incidence of human cases of Lyme disease, there is a pressing need to monitor the distribution and spread of the tick and its pathogens (Ogden et al. 2008c, 2015). Moreover, given the complexity of *I. scapularis* ecology, we need to understand the ecological factors that facilitate the establishment of *I. scapularis* populations in woodland habitats in Ontario. This foundational knowledge is required for the effective implementation of public health preventative measures and ongoing surveillance.
Specific research objectives were to:
1) Establish a baseline distribution of *I. scapularis* and *B. burgdorferi* across southern, eastern and central Ontario (Chapters 2 and 3);
2) Determine the influence of site-level ecological factors on the presence of *I. scapularis* in woodland habitats of Ontario (Chapter 3);
3) Assess the spatial spread of *I. scapularis* and emergence of *B. burgdorferi* in a zone of emergence (Chapter 4); and
4) Develop an ecological risk indicator for field sampling of *I. scapularis* by public health professionals in Ontario, Canada (Chapter 5).
Table 1.1: The number of reported cases of human Lyme disease and incidence per 100 000 in Canada and the United States of America from 2009 to 2015.

<table>
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<th>USA²</th>
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<td>Incidence</td>
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<td>2015</td>
<td>917</td>
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</tr>
</tbody>
</table>

¹ Public Health Agency of Canada 2017
² Centers for Disease Control and Prevention 2016
REFERENCES


Lee X, Hardy K, Johnson DH, Paskewitz SM. 2013. Hunter-killed deer surveillance to assess changes in the prevalence and distribution of *Ixodes scapularis* (Acari: Ixodidae) in


CHAPTER 2:
DISTRIBUTION OF TICKS AND THE RISK OF LYME DISEASE AND OTHER TICK-BORNE PATHOGENS OF PUBLIC HEALTH SIGNIFICANCE IN ONTARIO

This chapter was previously published as: Clow KM, Ogden NH, Lindsay LR, Michel P, Pearl DL, Jardine CM. 2016. Distribution of ticks and the risk of Lyme disease and other tick-borne pathogens of public health significance in Ontario, Canada. Vector-Borne Zoonotic Dis 16:215–222. Copyright permission was granted by the journal for use in this thesis.

ABSTRACT

Over the past two decades the northward spread of *Ixodes scapularis* across Ontario, Canada has accelerated and the risk of Lyme disease has increased. Active surveillance is a recognized and effective method for detecting reproducing populations of *I. scapularis*. In this study, we conducted field sampling consistent with an active surveillance approach from May to October 2014 at 104 sites in central, eastern and southern Ontario to determine the current distribution of *I. scapularis* and other tick species, and enhance our understanding of the geographic risk associated with *Borrelia burgdorferi* and other tick-borne pathogens of public health significance in this region. *Ixodes scapularis* was present at 21 of the 104 sites visited. Individuals of the tick species *Dermacentor variabilis, Haemaphysalis leporispalustris* and *Ixodes dentatus* were also collected. *Ixodes scapularis* were positive by polymerase chain reaction (PCR) for *B. burgdorferi* at 5 sites. These sites formed a significant spatial cluster in eastern Ontario. No ticks were PCR-positive for *Borrelia miyamotoi, Anaplasma phagocytophilum* and *Babesia microti*. This study provides an up-to-date picture of the distribution of *Ixodes scapularis* and other ticks species, and the risk of *B. burgdorferi* and other pathogens of public health significance in central, eastern and southern Ontario. This information may allow for more effective surveillance efforts and public health interventions for Lyme disease and other tick-borne diseases in this region.
INTRODUCTION

Globally, ticks represent one of the most significant public health risks, second only to mosquitoes as vectors for pathogen transmission (Parola and Raoult 2001). Although tick-borne diseases have been recognized for centuries, interest has intensified in the recent past due to the potential impact climate change may have on the expanded distribution of tick species, and subsequent introduction of tick-borne diseases (Estrada-Peña and De La Fuente 2014).

Lyme disease is the most important tick-borne disease in North America, with over 22 000 cases reported in the United States of America in 2012 (Centers for Disease Control and Prevention 2015), although the true annual incidence may be in the order of 300 000 cases (Hinckley et al. 2014). In the eastern USA and Canada, the causative agent of Lyme disease, Borrelia burgdorferi, is transmitted by the blacklegged tick, Ixodes scapularis (Burgdorfer et al. 1982). Ixodes scapularis can also transmit other pathogens of public health significance, most notably Anaplasma phagocytophilum (Dumler and Bakken 1995), Babesia microti (Spielman et al. 1984), as well as the recently-recognized Borrelia miyamotoi (Scoles et al. 2001).

Prior to 1990, only one isolated population of I. scapularis was known to exist in Canada (Watson and Anderson 1976). Over the past two decades the northward spread of I. scapularis has accelerated. Large numbers of ticks are carried northward annually via migratory birds (Ogden et al. 2008a), and the areas into which these ticks are being introduced are becoming increasingly suitable for tick development due to climate change (Ogden et al. 2008c, 2014c). In Ontario, there are now eight recognized endemic sites, although the distribution of this tick is believed to be much wider (Sider et al. 2012; Ogden et al. 2014b). The spread of I. scapularis has coincided with a notable increase in the incidence of human Lyme disease cases (Ogden et al. 2015a).

Surveillance for ticks to assess the risk of tick-borne disease has been a long-standing practice in Ontario (Scholten 1977). Passive surveillance, whereby ticks that are found by the public are brought to public health units and health care providers, is an important component of the surveillance program. These ticks are identified and I. scapularis are sent to the National
Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC) for pathogen screening by polymerase chain reaction (PCR) (Ogden et al. 2006b). In the mid 1900’s, the predominate tick species collected via passive surveillance were *Dermacentor variabilis* and *Ixodes cookei* (Scholten 1977). As the northward expansion of *I. scapularis* accelerated, the number of *I. scapularis* submitted grew substantially and by 2009, exceeded any other tick species (Nelder et al. 2014). Passive surveillance has been instrumental in monitoring the risk of Lyme disease and tick-borne disease in the province, but enhanced analyses of these passive surveillance data are needed to further understand the risk of disease (Ogden et al. 2010; Koffi et al. 2012).

Active surveillance is recognized as an effective approach for the detection of *I. scapularis* populations. As outlined at the Consensus conference on Lyme disease (Health Canada 1991), active surveillance involves collecting ticks from the environment via drag sampling, and testing *I. scapularis* for the presence of *B. burgdorferi*. Small mammal trapping is also indicated. Although small mammal trapping is the most sensitive method for detecting ticks, it is not as frequently used as drag sampling due to the associated costs and intensive time and labor demands (Ogden et al. 2014a).

In this study, we conducted field sampling consistent with an active surveillance approach across a widespread area of central, eastern and southern Ontario from May to October 2014 to determine the current distribution of *I. scapularis* and other tick species, and to enhance our current understanding of the geographical risk associated with *B. burgdorferi* and other tick-borne pathogens of public health significance in the province.

**METHODS**

**Site Selection**

Sites were selected to compare the prevalence of *I. scapularis* positive sites between the three ecoregions of central, eastern and southern Ontario (Lake Erie – Lake Ontario ecoregion 7E, Lake Simcoe – Rideau ecoregion 6E, and Georgian Bay ecoregion 5E) (Figure 2.1).
Ecoregions are defined by the Ministry of Natural Resources, Government of Ontario and are based on coarse-scale climate, demographic and watershed analysis (Ministry of Natural Resources 2007). We chose these divisions as they represent three distinct ecological regions where the climate (annual accumulated number of degree-days >0°C) is likely sufficient to support *I. scapularis* development (Ogden et al. 2005). Areas with known established populations of *I. scapularis* were excluded (Sider et al. 2012). The sample size was calculated to detect a difference in the prevalence of *I. scapularis* positive sites between ecoregions of 0.6 and 0.2. These values reflect the expected prevalence in an area with highly suitable habitat and climatic conditions for *I. scapularis* and an area with relatively limited suitability, respectively (Brownstein et al. 2003; Diuk-Wasser et al. 2006). Based on these prevalence values, a power of 80% and an alpha of 0.05, 28 sites per ecoregion were required. We opportunistically selected forested sites with a minimum area of 0.25 km² for inclusion in this study. Sites were limited to forested land cover since this environment is the preferred habitat of *I. scapularis*, which was the target tick species in this study (Lindsay et al. 1998). Permission to access each site was secured by field personnel.

**Field sampling**

Field sampling was conducted from May to October 2014. 104 sites within ecoregions 5E, 6E and 7E were visited once during this timeframe. Handheld global positioning systems (GPS) were used to record the longitude and latitude values at each site. Ticks were collected by dragging a 1 m squared white flannel drag cloth attached to a 1.25 m pole across the forest floor and over surface vegetation in parallel transects for the equivalent of three person-hours per site. Every three minutes, the timer was stopped and the drag cloth and researcher’s clothing were examined for ticks. All adults, nymphs and larvae were removed and counted. If the larval number was high (>100), an estimate was made, rounding to the nearest tens. Adults and nymphs were collected and stored in 70% ethanol for further processing. When only larvae were found, they were collected and stored in 70% ethanol for species identification. Tick dragging was not conducted on days when it rained.
Laboratory analyses

Nymphal, adult and when required, larval ticks samples were submitted to the National Microbiology Laboratory (NLM, Public Health Agency of Canada, Winnipeg, Manitoba) for species identification. Adult and nymphal ticks identified as *I. scapularis* were tested for the presence of *B. burgdorferi, B. miyamotoi, A. phagocytophilum* and *B. microti* by real-time PCR as previously described (Ogden et al. 2006b, Dibernardo et al. 2014). Briefly, QIAGEN DNeasy 96 tissue kits (QIAGEN Inc., Mississauga, Ontario) were used for DNA extraction. A duplex screening assay was chosen to screen the samples for *Borrelia* spp. using the 23S rRNA real-time polymerase chain reaction (PCR) assay, and *A. phagocytophilum* using the msp2 real-time PCR assay (Courtney et al. 2004). Analysis for *B. microti* was conducted using the methods described by Nakajima et al. (2009) for the detection of the CCTeta gene. All *Borrelia* spp.-positive samples were subsequently tested for *B. burgdorferi* using a confirmatory ospA real-time PCR assay, and *B. miyamotoi* using an IGS real-time PCR assay. *B. miyamotoi*-positive samples were further verified using the glpQ real-time PCR assay (Dibernardo et al. 2014). To account for possible contamination during DNA extraction and PCR reactions, water or "blank" controls were included in all extraction and PCR runs. All of these controls were negative during the course of this study.

Statistical analysis

Exact logistic regression was conducted to determine if there was a difference in the prevalence of positive sites for each tick species (*I. scapularis, D. variabilis, Haemaphysalis leporispalustris* and *I. dentatus*) between ecoregions and to evaluate the difference in the prevalence of *B. burgdorferi* positive sites between ecoregions. Exact logistic regression was chosen because for each regression analysis at least one ecoregion had a small (<5) number of sites with a positive outcome. All statistical analyses were completed in STATA version 13.1 (STATA Corp, Texas, USA; 2014).

The spatial scan statistic was implemented using SaTScan 9.4 (www.satscan.org, 2015). A purely spatial retrospective analysis using the Bernoulli probability model was chosen to
assess areas of high prevalence. The spatial window was set at a maximum spatial cluster size no greater than 50% of the population at risk. Monte Carlo replications were limited to 999 replications. Only statistically significant secondary clusters with no geographical overlap were reported; hierarchical and Gini clusters (i.e., clusters based on the Gini index) were reported if they met these criteria (Kulldorff 2015). The spatial scan statistic was conducted twice. The first analysis examined the site data categorized according to the presence or absence of *I. scapularis* and the second analysis examined the data based on whether a site was positive or negative for *B. burgdorferi*. A significance level of $\alpha=0.05$ was used for all statistical and spatial analyses.

Spatial data were prepared using QGIS version 2.6.0 Brighton (www.qgis.org; 2014). Briefly, the vector layer for ecoregion (Ontario Ministry of Natural Resources 2007) was accessed through the Scholars GeoPortal at the University of Guelph (http://geo2.scholarsportal.info). Ecoregions 5E, 6E and 7E were selected to form the base layer. Site data were plotted using the recorded longitude and latitude values. In the first spatial display, two GIS layers were created to categorize the findings of field sampling. The presence or absence of *I. scapularis* at each site was used as the first layer, and an additional layer was added to classify each site based on the presence of another tick species. A second spatial display was created for the presence of *I. scapularis* and detection of *B. burgdorferi*. Using one data layer, the sites were categorized as ‘absent’ if no *I. scapularis* were collected and ‘present’ if at least one *I. scapularis* of any life stage was collected. Each *I. scapularis* ‘present’ site was further categorized as ‘negative for *B. burgdorferi*’ if all samples analyzed were negative for the bacteria, ‘positive for *B. burgdorferi*’ if at least one sample was positive for the bacteria, and ‘only larvae’ if only larvae were collected and therefore no laboratory testing was completed.

**RESULTS**

Four species of ticks were found at the 104 field sampling sites. *Ixodes scapularis* was the most prevalent tick species collected and was present at 21 sites (20.2% (95% confidence interval (CI) 13.0-29.2%)) across all three ecoregions (Table 2.1). A total of 626 *I. scapularis* were collected, comprised of 31 adult, 35 nymphal and 560 larval ticks. A total of 152 *H. leporispalustris* were found at 9 sites (8.7% (95% CI 4.0-15.8%)) within ecoregions 5E and 6E,
and a total of 18 *D. variabilis* were found at 7 sites (6.7% (95% CI 2.7-13.4%)) in ecoregions 6E and 7E. The least prevalent tick species was *I. dentatus*, with only one tick collected at one site in ecoregion 7E (1.0% (95% CI 0.02-5.2%)). The odds of detecting a site that was positive for *H. leporispalustris* were significantly less likely in ecoregion 7E, when compared to ecoregion 5E. There were no other significant differences in the prevalence of sites with *I. scapularis* or the two other tick species amongst the ecoregions (Table 2.2).

More than one tick species was present at 6 sites. When more than one tick species was present, it was either *I. scapularis* and *D. variabilis*, or *I. scapularis* and *H. leporispalustris* together.

*Borrelia burgdorferi* was detected in *I. scapularis* from 5 sites (4.9% (95% CI 1.6-11.2%); Table 2.1). In total, 10 *I. scapularis* were positive for *B. burgdorferi*. The number of positive ticks at each site ranged from 1 – 3, with a median of 2. There was no significant difference in the prevalence of *B. burgdorferi* between the ecoregions (Table 2.1). *Borrelia miyamotoi*, *A. phagocytophilum* and *B. microti* were not detected in any of the 31 adult and 35 nymphal *I. scapularis* tested (0% (one-sided 97.5% CI (0-5.4%)).

A spatial cluster of *I. scapularis* positive sites was identified in eastern Ontario, covering parts of ecoregions 5E and 6E (Figure 2.1). A spatial cluster of *B. burgdorferi* positive sites was found in the same area of eastern Ontario (Figure 2.2).

**DISCUSSION**

Passive surveillance has been invaluable for studying the emergence of Lyme disease in Canada. Analysis of samples submitted from 1990 to 2003 by Ogden et al. (2006b) indicated that the distribution of *I. scapularis* may be much greater than initially thought and expand far beyond the endemic areas. Monitoring the number of ticks submitted and the infection prevalence has been used as an indication for *I. scapularis* establishment within an area (Ogden et al. 2010) and epidemiological risk factors for tick acquisition and disease exposure have been elucidated from demographic information collected from submitters (Nelder et al. 2014).
However, there are a number of drawbacks associated with passive surveillance. Passive surveillance lacks specificity due to the incidence of adventitious ticks, which are ticks that are sporadically introduced into an area, most commonly by migratory birds (Ogden et al. 2014a). Furthermore, sensitivity can be low because an absence of submissions does not necessarily indicate an area without ticks. This is especially true in areas of low human population density, where there is a very low probability of gathering sufficient data through passive surveillance (Nelder et al. 2014). Low specificity and sensitivity can lead to an elevation of false positive and false negative areas, respectively. For these reasons, active surveillance has been considered the gold standard method for identifying areas where *I. scapularis* ticks have become established.

Passive surveillance also has reduced capacity to detect all the life stages of the tick. Adult ticks are the most common instar of ticks submitted by the public. Nymphs and larvae are difficult for the public to recognize due to their small size and are rarely submitted via passive surveillance (Ogden et al. 2006b). However, these stages provide valuable information on the tick population and disease risk. The presence of the immature stages of ticks, and/or the presence of more than one stage of tick at a site provides stronger evidence of a reproducing population of *I. scapularis* (Koffi et al. 2012). Nymphs also provide the best indication of disease risk. Peak nymphal activity occurs in early summer, when humans are more likely to engage in outdoor activities; often in clothing that provides limited protection from tick bites. This factor, along with the decreased detection as a result of their small size, leads to an elevated risk of transmission of *B. burgdorferi* from nymphal *I. scapularis* (Ogden et al. 2008b).

Using an approach consistent with active surveillance, we detected *I. scapularis* in all three ecoregions of southern, eastern and central Ontario. These sites represent new areas outside of the known endemic sites of Point Pelee National Park, Rondeau Provincial Park, Long Point Provincial Park, Turkey Point Provincial Park, Wainfleet Bog Conservation Area, Prince Edward Point National Wildlife Area and Saint Lawrence Islands National Park (Figure 2.1) (Sider et al. 2012). No significant differences were noted in the prevalence of sites positive for *I. scapularis* or *B. burgdorferi* between ecoregions. Spatial clusters indicated a high prevalence of sites with *I. scapularis* and positive for *B. burgdorferi*-infected ticks in the area of eastern Ontario.
Based on these findings, eastern Ontario may represent a ‘hot spot’ for *I. scapularis* and the transmission of *B. burgdorferi*. We hypothesize that this ‘hot spot’ is a result of a large area of highly suitable habitat, as well as an intensified rate of localized spread via small mammals and white-tailed deer. More research is required to investigate this hypothesis. Eastern Ontario has previously been labeled as an area of elevated risk due to a significantly higher rate of tick submissions from the public. Our findings validate and strengthen these data gathered by passive surveillance (Ogden et al. 2010; Nelder et al. 2014).

It may be expected that if a significant cluster was detected, there would also be a significant difference between the ecoregions; however, this was not the case. This discrepancy is likely due to the inclusion of portions of both 5E and 6E within each cluster, and therefore minimal difference in the prevalence of positive sites between each ecoregion. It is also important to consider the modifiable areal unit problem (MAUP) (Waller and Gotway 2004). In our study, we used the boundaries of each ecoregion to aggregate the site data. The unit of ecoregion was chosen because the boundaries define areas with coarse-scale ecological differences, and these differences may have an impact on the ecology of Lyme disease. However, it appears that these boundaries may not be the most appropriate level at which to aggregate and analyze the data. The criteria used to create these ecoregions may not reflect the significant ecological factors present in the microhabitat of the tick (Estrada-Peña et al. 2013) and may explain our inability to detect differences based on our regression analyses. In contrast, the spatial scan statistic has a flexible scanning window and was not restricted to the ecoregion boundaries. It also should be noted that we did not reach our minimum sample size for ecoregion 5E due to weather and time constraints, and this may have affected our ability to detect a significant difference between the ecoregions (Type II error).

Importantly, we did not detect *I. scapularis* at a large number of sites. Many of these negative sites were located in areas with lower population density where passive surveillance is inadequate (Nelder et al. 2014). Both positive and negative findings are of significant benefit to public health. Currently, there is no human vaccine for *B. burgdorferi* or widespread, effective tick control measures. Therefore, disease prevention focuses on public health education and
preventive measures that include personal protective measures and landscape modification. These efforts need to be targeted to the areas of elevated risk because they require considerable effort and investment by the public (Ogden et al. 2015b). Knowledge of the presence and absence of *I. scapularis* populations assists with risk assessment and appropriate planning of public health interventions (Ogden et al. 2008b).

Seasonal life stage patterns as well as changes in daily tick activity due to temperature and humidity fluctuations can affect the results of tick dragging (Estrada-Peña et al. 2013). We conducted tick dragging throughout the active season of *I. scapularis*, and collected adult, nymphal and larval stages in varying abundance. Although this should be acknowledged as a limitation of this study, our approach also provided a number of benefits. The presence of host-seeking larvae (and to a lesser extent, nymphs) indicates that reproducing populations of ticks are establishing at some of our study sites, and highlights key areas that should be targeted for ongoing surveillance. Additionally, nymphal ticks represent the greatest risk of disease. Our tick dragging efforts were effective in collecting nymphal ticks, and illustrate the necessity of an active surveillance approach to more accurately determine disease risk. Conducting field sampling throughout the season is supported by work conducted by Ogden et al. (2014a), who illustrated that tick dragging can be completed at any time during the season to provide adequate evidence of a risk area.

*Anaplasma phagocytophilum, B. microti* and *B. miyamotoi* were not detected in any *I. scapularis* ticks collected in this study. Since we did not collect a large number of ticks, the power of our sample size was low, limiting our ability to detect these pathogens in our study. Previous studies report a low infection prevalence of 0.3% for both *A. phagocytophilum* (Nelder et al. 2014) and *B. miyamotoi* (Dibernardo et al. 2014). Based on the one-sided 97.5% confidence interval, we expect the true infection prevalence to be between 0% and 5.4%, so larger sample sizes of ticks would need to be tested in order to rule out the presence of these pathogens at *I. scapularis*-positive sites.
Dermacentor variabilis, H. leporispalustris and I. dentatus were also collected via dragging. Haemaphysalis leporispalustris was significantly less likely to be detected at a site in ecoregion 7E, when compared to ecoregion 5E. Research conducted by Gabriel-Rivet and colleagues (2015) in New Brunswick, Canada found significant associations with the presence of H. leporispalustris and a number of ecological variables including degree-days >0°C, proportion of clay in the soil, site elevation, season, and mean annual precipitation. Ecological differences exist between ecoregions 5E and 7E and these differences may help to explain the presence of this tick at specific sites. More field research examining the preferred habitat of H. leporispalustris should be conducted in Ontario to investigate this hypothesis.

Haemaphysalis leporispalustris and D. variabilis pose a potential public health risk from zoonotic pathogens other than B. burgdorferi (e.g., Rickettsia rickettsii, the causative agent of Rocky Mountain Spotted Fever, and Francisella tularensis, the causative agent of tularemia), although in Canada the prevalence of infection with these pathogens associated with these tick species is usually very low (Wood and Artsob 2012). Ixodes dentatus rarely feed on humans and therefore poses little, if any risk to public health (Kollars and Oliver 2003).

It is important to note that the field sampling approach employed in this study was biased towards detecting I. scapularis. Each species of tick has a preferred habitat (Estrada-Peña et al. 2013; Gabriele-Rivet et al. 2015), and the field sampling sites were selected based on the preferred habitat of I. scapularis. Nonetheless, our findings illustrate that an active surveillance approach can provide the added benefit of monitoring other species of ticks, including emerging or invasive species that may pose a risk to public health.

The findings of our widespread field sampling can be used to establish a current baseline for I. scapularis distribution in Ontario. As this tick species continues to spread northward via migratory birds (Ogden et al. 2008a) and is able to establish populations in areas at higher latitudes that have become suitable for tick development due to climate change (Ogden et al. 2014c), the risk of Lyme disease will increase, and it is therefore pertinent for risk assessment to have a baseline for comparison. This baseline measurement allows for more effective
monitoring of risk and can be used to appropriately direct future surveillance efforts and public health interventions.

In addition to surveillance efforts to monitor ticks and tick-borne disease, the ecology of each tick and the cycle of pathogen transmission must be understood. This will greatly enhance surveillance efforts and the implementation of effective control strategies (Estrada-Peña et al. 2013). The ecology of Lyme disease is complex, involving a number of abiotic and biotic factors. These factors can differ depending on the geographic area, which has been illustrated by extensive research conducted in many states, as well as in the province of Quebec (Ostfeld and Keesing 2000; Bouchard et al. 2011). Smaller scale studies in Ontario have begun to elucidate some of the ecological factors influencing the establishment and spread of *I. scapularis* and Lyme disease in the province, including the role of habitat type in egg hatching and tick survival, and the influence of deer and microclimatic factors on tick abundance (Lindsay et al. 1998; Ogden et al. 2006a; Werden et al. 2014). Widespread ecological research is warranted in Ontario to complement the current body of knowledge, including data gathered by passive and active surveillance.

**CONCLUSIONS**

In this study, we used an active surveillance approach to better understand the distribution of *I. scapularis* and the risk of *B. burgdorferi* in Ontario, as well as other tick species and tick-borne pathogens of public health significance. *Ixodes scapularis* and *B. burgdorferi* were detected outside of the known endemic areas, including a spatial cluster of positive sites in eastern Ontario, which indicates that this region may represent a ‘hot spot’ for Lyme disease risk. This information can help to direct future surveillance efforts and public health interventions. Furthermore, we can now expand upon our knowledge of tick distribution and disease risk by conducting studies on the ecology of *I. scapularis* and *B. burgdorferi* to better understand the abiotic and biotic factors influencing the establishment and spread of Lyme disease in Ontario.
Table 2.1: Number of *Ixodes scapularis* and the prevalence of *Borrelia burgdorferi* in nympha and adult *I. scapularis* from ecoregions 5E, 6E and 7E.

<table>
<thead>
<tr>
<th>Ecoregion</th>
<th>5E (27 sites)</th>
<th>6E (43 sites)</th>
<th>7E (34 sites)</th>
<th>Total (104 sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of sites with <em>I. scapularis</em> (95% confidence interval (CI))</td>
<td>22.2 (8.6 – 42.3)</td>
<td>25.6 (13.5 – 41.2)</td>
<td>11.8 (3.3 – 27.5)</td>
<td>20.2 (13.0 – 29.2)</td>
</tr>
<tr>
<td>Total number of ticks collected</td>
<td>49</td>
<td>569</td>
<td>8</td>
<td>626</td>
</tr>
<tr>
<td>Total number of adult ticks collected</td>
<td>10</td>
<td>16</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td>Total number of nympha ticks collected</td>
<td>15</td>
<td>20</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Median number of adult and nympha ticks/site (range)</td>
<td>2 (2 – 15)</td>
<td>2 (1 – 10)</td>
<td>1 (1 – 4)</td>
<td>2 (1 – 15)</td>
</tr>
<tr>
<td>Prevalence of sites with <em>B. burgdorferi</em> infected ticks (95% CI)</td>
<td>7.4 (0.9-24.3)</td>
<td>7.1 (1.5 – 19.5)</td>
<td>0</td>
<td>4.9 (1.6 –11.2)</td>
</tr>
<tr>
<td>Number of adult and nympha ticks infected with <em>B. burgdorferi</em></td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

1 As outlined by the Ontario Ministry of Natural Resources (2007)
2 Based on the results of 42 sites. One site did not have laboratory results, as only *I. scapularis* larvae were collected.
3 Based on the results of 101 sites. Three sites did not have laboratory results, as only *I. scapularis* larvae were collected.
The odds of a site being positive for *B. burgdorferi* in either ecoregion 6E or 7E compared to the referent 5E.

| Median number of adult and nymphal ticks infected with *B. burgdorferi*/site (range) | 1 (1 - 2) | 2 (2 - 3) | 0 | 2 (1 - 3) |
| Odds ratio\(^1\) (95% CI; p-value) | Referent | 0.96 (0.1 – 12.28; 1.0) | 0.34 (0 – 4.45; 0.41) |

\(^1\) The odds of a site being positive for *B. burgdorferi* in either ecoregion 6E or 7E compared to the referent 5E.
Table 2.2: Prevalence of sites with *Ixodes scapularis*, *Haemaphysalis leporispalustris*, *Dermacentor variabilis* and *I. dentatus* and the association between ecoregions 5E, 6E and 7E in Ontario and the prevalence of sites with each tick species based on exact logistic regression.

<table>
<thead>
<tr>
<th>Tick Species</th>
<th>Ecoregion¹ 5E (27 sites)</th>
<th>Ecoregion 6E (43 sites)</th>
<th>Ecoregion 7E (34 sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I. scapularis</em></td>
<td>Prevalence (95% confidence interval (CI))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.2 (8.6 – 42.3)</td>
<td>25.6 (13.5 – 41.2)</td>
<td>11.8 (3.3 – 27.5)</td>
</tr>
<tr>
<td>Odds ratio² (95% CI; p-value)</td>
<td>Referent</td>
<td>1.20 (0.34 – 4.59; 0.98)</td>
<td>0.47 (0.09 – 2.28; 0.45)</td>
</tr>
<tr>
<td><em>H. leporispalustris</em></td>
<td>Prevalence (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.5 (6.3 – 38.1)</td>
<td>9.3 (2.6 – 22.1)</td>
<td>0</td>
</tr>
<tr>
<td>Odds ratio (95% CI; p-value)</td>
<td>Referent</td>
<td>0.46 (0.08 – 2.37; 0.45)</td>
<td>0.10³ (0 – 0.79; 0.03)</td>
</tr>
<tr>
<td><em>D. variabilis</em></td>
<td>Prevalence (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>9.3 (2.6 – 22.1)</td>
<td>8.8 (1.8 – 23.7)</td>
</tr>
<tr>
<td>Odds ratio (95% CI; p-value)</td>
<td>Referent</td>
<td>3.52² (0.42 – ∞; 0.27)</td>
<td>3.20² (0.33 - ∞; 0.33)</td>
</tr>
<tr>
<td><em>I. dentatus</em></td>
<td>Prevalence (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>2.9 (0.07 – 15.3)</td>
</tr>
<tr>
<td>Odds ratio (95% CI; p-value)</td>
<td>Referent</td>
<td>1 (0 - ∞)</td>
<td>0.79² (0.2 - ∞; 1.0)</td>
</tr>
</tbody>
</table>

¹ As outlined by the Ontario Ministry of Natural Resources (2007)
² The odds of a site being positive for *I. scapularis* in either ecoregion 6E or 7E compared to the referent 5E.
³ Median unbiased estimate
Figure 2.1: 104 research sites in ecoregions 5E, 6E and 7E were surveyed by drag sampling from May to October 2014. Four species of ticks were collected: *Ixodes scapularis* (black star = present, white circle = absent), *Dermacentor variabilis* (grey diamond), *Haemaphysalis leporispalustris* (grey square) and *I. dentatus* (grey triangle). An area with a high prevalence of sites that were positive for *I. scapularis* was detected in eastern Ontario (semi-translucent circle). Endemic sites are provided for reference (black asterisk).
Figure 2.2: The presence (triangles) and absence (white circles) of *Ixodes scapularis* and *Borrelia burgdorferi* at 104 research sites in ecoregions 5E, 6E and 7E was determined by drag sampling from May to October 2014. Thirteen sites had the presence of *I. scapularis*, but tested negative for *B. burgdorferi* (dark grey triangles). Five sites had both the presence of *I. scapularis* and tested positive for *B. burgdorferi* (black triangles). An area with a high prevalence of sites that were positive for *B. burgdorferi* was detected in eastern Ontario (semi-translucent circle). The status of three sites was unknown as only the larval stage was collected (light grey triangles).
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*scapularis*, in Canada now and with climate change. *Int J Health Geogr* 7:24.


CHAPTER 3:
THE INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON
THE INVASION OF *IXODES SCAPULARIS* IN ONTARIO

This chapter was previously published as: Clow KM, Ogden NH, Lindsay LR, Michel P, Pearl DL, Jardine CM. 2017. The influence of abiotic and biotic factors on the invasion of *Ixodes scapularis* in Ontario, Canada. *Ticks Tick Borne Dis* 8(4):554-563. Copyright permission was granted by the journal for use in this thesis.

ABSTRACT

In northeastern North America, the blacklegged tick, *Ixodes scapularis*, is the vector of numerous tick-borne pathogens, including the agent of Lyme disease, *Borrelia burgdorferi* sensu stricto. Since 1990, there has been a rapid spread of *I. scapularis* northward into the province of Ontario, Canada. Climate change has been implicated as one of the driving factors for the spread of this vector. Other ecological factors also influence survival of *I. scapularis* populations and may facilitate invasion. The objective of this study was to identify local abiotic and biotic factors of significance for the invasion of *I. scapularis* in Ontario. The presence of ticks was determined by drag sampling at 154 sites in southern, eastern and central Ontario from May to October in 2014 and 2015. At each site, data on site aspect, forest cover, understory density and composition, soil moisture and composition, and the depth of litter layer were collected. Cumulative degree days above zero °C, total precipitation and elevation were attributed to each site using a geographic information system. A mixed multivariable logistic regression model was created to assess the impact of the ecological factors on the presence of *I. scapularis*. In total, *I. scapularis* was found at 29 sites (18.8%) across the study area. The density of the understory, the presence of shrubs and the interaction of these two ecological factors were statistically significant, as well as longitude and cumulative degree days above zero. Our findings illustrate that local ecological factors are of importance for the invasion of *I. scapularis* into Ontario, and may be used to enhance local public health interventions and current predictive models and risk maps for *I. scapularis*. 

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INTRODUCTION

Within the last two decades, there has been a rapid expansion of the population of *Ixodes scapularis* within the province of Ontario, Canada (Ogden et al. 2014b). Long Point peninsula, located on the north shores of Lake Erie, was home to the first known population of *I. scapularis* in this province. Ticks existed at that site for decades with relatively little known spread (Watson and Anderson 1976). Now, *I. scapularis* is found at numerous locations across the southern portion of the province and in eastern Ontario (Ogden et al. 2014b; Clow et al. 2016). Ticks that are positive for *Borrelia burgdorferi* sensu stricto, the primary causative agent for Lyme disease in northeastern North America, have also been detected in these areas and the expansion of *I. scapularis* has coincided with a significant increase in the incidence of Lyme disease in humans (Ogden et al. 2015).

Climate change has been implicated as one factor facilitating the northward spread of the tick (Ogden et al. 2008b, 2014c). Large numbers of ticks are brought into Ontario every year on migratory birds, and as temperatures increase, the conditions are becoming more suitable for the development and survival of these newly introduced *I. scapularis* (Ogden et al. 2008a, 2008b, 2014c; McPherson et al. 2017). However, there are clearly ecological factors other than temperature that determine if introduced *I. scapularis* can become self-sustaining, reproducing populations (Ogden et al. 2013).

The ecology of Lyme disease is complex, involving several abiotic and biotic factors. Extensive ecological research has been conducted across the northeastern United States, examining the influence of hosts, habitat and climate on *I. scapularis* and *B. burgdorferi* (e.g., Lubelczyk et al. 2004; Ostfeld et al. 2006; Schulze et al. 2009). Although many patterns have been elucidated, some relationships appear to be specific to factors at the local level, suggesting the need to examine the ecology of the tick and pathogen in each specific environment.

In Ontario, a limited number of small-scale studies have been conducted. Lindsay and colleagues (1998, 1999a, 1999b) examined the role of habitat type, and host density on the development, survival and abundance of *I. scapularis* at Long Point peninsula. Maple forest was
consistently the most suitable habitat, yet deer density could modify this relationship. Suitability of more northern forest habitats in the Great Lakes Plains – St. Lawrence River basin were examined to determine if ticks could inhabit these areas as climatic conditions became appropriate. They found that fresh-moist sugar maple deciduous forests, and possibly ash mineral deciduous swamp, can support *I. scapularis* populations (Ogden et al. 2006), although the findings of this study are not consistent across the tick’s range (Dister et al. 1997; Guerra et al. 2002). Abiotic and biotic factors were studied across different sites in the Thousand Islands archipelago. Ground temperature and deer density were positively associated with tick abundance, while small mammal species richness was negatively associated with tick abundance, with the relationship modulated by the relative abundance of white-footed mice (Werden et al. 2014).

It is unknown if these patterns can be generalized across Ontario, and whether they are important for the spread of the tick. Our objective was to examine the influence of site-level abiotic and biotic factors on the local patterns of invasion of *I. scapularis* populations across southern, eastern and central Ontario.

METHODS

A subset of the data for this study (104 sites sampled in 2014) were collected contemporaneously with data collected for Clow et al. (2016), in which the current occurrence and distribution of *I. scapularis* and *B. burgdorferi* in Ontario were explored.

Site selection

The criteria for site selection have been previously described (Clow et al. 2016). Briefly, a minimum of 50 sites were selected from each of the three main ecoregions of southern Ontario (Lake Erie-Lake Ontario ecoregion 7E, Lake Simcoe-Rideau ecoregion 6E, and Georgian Bay ecoregion 5E; Figure 3.1). These ecoregions were defined by the Ontario Ministry of Natural Resources according to course-scale climatic and ecological factors (Ontario Ministry of Natural Resources 2007; Crins et al. 2009) and represent distinct areas of ecological variability within the area of Ontario determined to have a sufficient number of degree days above zero required
for *I. scapularis* development (Ogden et al. 2005). Sites were chosen opportunistically and consisted primarily of provincial parks and conservations areas. Each site had to be forested and encompass a minimum area of 0.25 km$^2$ to provide sufficient space for sampling. Approval to access all areas was secured by the field team. Localities where blacklegged tick populations were known to occur were not eligible for site selection (Sider et al. 2012).

**Field sampling**

One hundred and fifty-four sites were visited once in 2014 or 2015 during the months of May through October. Fifty sites were visited in ecoregion 7E, 54 in ecoregion 6E and 50 in ecoregion 5E (Figure 3.1). Sites were visited on dry days only (i.e., not raining, surface vegetation dry to the touch).

At each site, drag sampling for ticks was conducted for a total of three person-hours (Ogden et al. 2014a). A 1 m$^2$ white flannel drag cloth attached to a 1.25 m wooden pole was pulled across the forest floor and ground vegetation in parallel transects. Field personnel checked each drag cloth and their clothing at 3-minute intervals, during which the timer was stopped. The life stage of all ticks collected was recorded and they were placed into tubes containing 70% ethanol for species identification.

Site-level ecological data were collected after every 15 minutes of dragging, resulting in 12 sets of observations per site. Geographical coordinates were recorded in longitude and latitude using a geographical positioning system (GPS), as well as site aspect (i.e., slope of the land), forest type, understory density, understory type and relative abundance, soil composition and moisture, and depth of litter layer (Appendix 3.1) (Lee et al. 1998). These ecological data were linked to the tick dragging results from the previous 15 minutes of dragging.

**Climate Data**

Climate data for each site were provided by Natural Resources Canada through the regional, national and international climate modeling project (McKenney et al. 2011; Natural Resources Canada 2016). This project applies thin plate spline smoothing algorithms
(ANUSPLIN) to field measured climatic data to create spatially continuous climate models for each year, as well as for 30-year periods (i.e., climate normal data). These data are especially valuable to create robust measurements for areas that are remote and do not have a nearby weather station (McKenney et al. 2011). For our study, we chose climate normal data (1980-2010) because the tick lifecycle occurs over 2 to 3 years and the timing of tick invasion at each specific site is unknown. Mean annual precipitation, elevation and mean minimum and maximum monthly temperatures were obtained. Annual cumulative degree days above zero (DD>0 °C) were calculated using the average monthly temperature (based on mean minimum and maximum values). If the average monthly temperature was above zero, the temperature was multiplied by the number of days in the month. The monthly values for each site were summed to determine the annual DD>0 °C for each location as per previous studies (Gabriele-Rivet et al. 2015).

**Laboratory analyses**

All adult, nymphal, and a subset of larval *I. scapularis* were sent to the National Microbiology Laboratory (NML) at Winnipeg (Public Health Agency of Canada, Winnipeg, Manitoba, Canada) for species verification and diagnostic testing (Clow et al. 2016).

**Statistical analyses**

The prevalence of sites in each ecoregion with *I. scapularis* was calculated along with the 95% confidence intervals (CI).

Descriptive statistics were examined for all explanatory variables. Categories were collapsed when the data were sparse, but only if the combined categories had biological relevance (Appendix 3.2 and 3.3).

Continuous variables for the percentage of each soil component, the depth of litter layer, DD> 0°C, elevation, longitude, latitude and mean annual precipitation were assessed graphically for linearity. We considered the assumption of linearity to be met if the lowess curve formed a straight line against the log odds of the outcome. All continuous variables except elevation and
longitude violated the linearity assumption. When linearity was not present, each variable was modelled using a quadratic function, if appropriate. When the quadratic function did not represent the relationship, continuous variables were converted into categorical variables, based either on biologically meaningful cut-points (site-level variables) or quartiles (climate and geographical data) (Appendix 3.4).

Collinearity was assessed using either the Pearson’s or phi correlation coefficient depending on the functional form of the variables (i.e., continuous versus categorical/dummy variables). Any variables with a coefficient greater than $|0.8|$ were considered highly correlated, and not included together in the same model. Collinearity was checked throughout the modeling process as variables were created or categories combined.

A causal diagram was created in preparation for model building. Potential confounding variables were identified and subsequently assessed throughout model building. A variable was considered a confounder if its removal resulted in $\geq 30\%$ change in the coefficient of the explanatory variable, assuming the variable was not an intervening variable. Potential intervening variables were also identified from the causal diagram, and were not included in the same model as the antecedents (Dohoo et al. 2009).

Univariable analyses were undertaken for all variables (measured and constructed) on the presence of *I. scapularis* at a site (outcome variable). A liberal significance level (i.e., $\alpha = 0.2$) was used to determine variables for inclusion in the multivariable model. Two-way interactions were explored separately for all significant main effects from the univariable analyses. When the model did not converge, the descriptive statistics were examined and if appropriate, the variable categories were collapsed to account for sparse data. A significance level of $\alpha = 0.05$ was used for evaluation of interaction terms with the main effects and all subsequent modeling.

Mixed multivariable logistic regression models were fitted to estimate the effect of all significant main effects and interaction terms; random effects for site and county were included to account for clustering. If the variables were not significant, they were removed in a step-wise fashion, while assessing for confounding. For categorical variables or groups of variables,
significance was assessed globally using the likelihood ratio test. Any variables that showed
evidence of acting as confounders or were part of a significant interaction term were kept in the
model regardless of significance level.

If more than one model was possible, these final models were compared using the Akaike
information criterion (AIC); a lower AIC value indicated superior model fit (Dohoo et al. 2009).

Outliers were identified by graphically examining the Pearson residuals. Model fit was
assessed by examining the homoscedasticity and normality of the Best Linear Unbiased
Predictors (BLUPs).

STATA version 13.1 (STATACorp, College Station, TX; 2014) was used for all
statistical modeling.

RESULTS

Field sampling

The overall prevalence of sites positive for *I. scapularis* was 18.8% (29 of 154 sites; 95% 
CI 13.0-25.9%). The prevalence of sites positive for *I. scapularis* in each ecoregion was 16.0% 
(8 of 50 sites; 95% CI 7.2-29.1%) in 5E, 24.1% (13 of 54 sites; 95% CI 13.5-37.6%) in 6E and 
16.0% (8 of 50 sites; 95% CI 7.2-29.1%) in 7E (Figure 3.1).

Statistical analysis

The following categorical and continuous variables were statistically significant based on
univariable analyses and a liberal p-value (p<0.2): understory density, relative abundance of non-
woody vegetation, relative abundance of trees, relative abundance of shrubs, predominant soil
type, depth of litter layer, DD>0 °C, mean annual precipitation, elevation, longitude, and latitude
(Table 3.1). Significant interactions were present between the relative abundance of shrubs and
the density of the understory, the relative of abundance of non-woody vegetation and the relative
The final mixed multivariable logistic regression model included statistically significant associations between understory density, relative abundance of shrubs, and the interaction of these two latter main effects, longitude and cumulative degree days above zero and the presence of *I. scapularis* at a site (AIC = 314.96) (Table 3.2). A site with high understory density with a low relative abundance of shrubs had significantly lower odds of having *I. scapularis* when compared to a site with a low understory density with a medium to high relative abundance of shrubs (Table 3.3). The odds of detecting *I. scapularis* was lower if the site was further west (OR = 0.12; 95% CI 0.04-0.36; p<0.001). DD>0 °C exhibited a quadratic relationship with the presence of *I. scapularis*. When examined over a range of observed DD>0 °C values, there was a gradual increase in the log odds of the outcome, until a maximum value of approximately 3500 DD>0 °C (Figure 3.2). The relative abundance of non-woody vegetation was included as a confounding variable for understory density.

The correlation between observations at the site-level was high (intra-class correlation (ICC) = 81.4%; 95% CI 65.0% – 91.2%), while the correlation between observations from different sites within the same county was lower (ICC = 30.3%; 95% CI 8.6% – 66.8%). No outliers were identified by examination of the Pearson residuals. The BLUPs fulfilled the assumptions of normality and homoscedasticity.

**DISCUSSION**

Modelled projections of future climate suggest that *I. scapularis* populations will continue to spread across Ontario in the coming decades. Current projections predict that by 2050 our entire research area will be considered moderate to high risk for *I. scapularis* (Ogden et al. 2008b, 2014c; McPherson et al. 2017). Invasion of *I. scapularis* into the province is therefore dynamic, and our research should be considered in light of the evolving nature of this process. Our findings illustrate that the ecological factors of cumulative degree days above zero, density of the understory, relative abundance of shrubs, and the interaction of understory density and
shrub abundance as well as the geographic location of the site are statistically significant factors for the presence of *I. scapularis*.

Cumulative degree days above zero considers the magnitude, and the duration of time of the temperature above zero, both of which have been well-established as factors important for tick survival, development and activity (Lindsay et al. 1995; Ogden et al. 2004, 2005). As temperature increases, so does the speed of development, and the temperature needs to remain elevated for a sufficient period for development to occur from one life stage to the next without depletion of the tick’s energy stores (Lindsay et al. 1995). Temperature conditions during the periods of spring to autumn each year must be above threshold for survival of a reproducing population of *I. scapularis* (Ogden et al. 2014c). The importance of temperature for *I. scapularis* is illustrated in our study. The log odds of detecting *I. scapularis* gradually increased over a range of observed DD>0 °C values, until a maximum value of approximately 3500 DD>0 °C. The log odds of the presence of *I. scapularis* was greater than zero at all observed DD>0 °C values. The tick’s current distribution includes areas of much warmer climate (Ginsberg et al., 2014), so we do not anticipate higher DD>0 °C values in our study area to be detrimental now, or in the near future with climate change (Ogden et al. 2008b).

When examining the ecological factors associated with *I. scapularis*, it is critical to study the micro-habitat features (Estrada-Pena and de la Fuente 2014). The microhabitat can dramatically differ from the macro-habitat as certain ecological features, such as the litter layer and understory, can protect against dramatic weather fluctuations that otherwise would be detrimental for the tick (Schulze and Jordan 2005). The microhabitat also supports the resident host population (Prusinski et al. 2006).

Based on our results, the forest understory is an important component of this microhabitat. The forest understory serves many purposes; for the tick, understory is a substrate on which it can quest, and be protected from weather extremes; for the tick hosts, it provides a suitable habitat and potential food source (Lubelczyk et al. 2004; Schulze and Jordan 2005; Prusinski et al. 2006). In our study, we not only examined if understory was present or not, but further classified it based on its density and the relative abundance of three types of vegetation:
shrubs, immature trees and non-woody vegetation. The most influential characteristics were the density and the relative abundance of shrubs. Most notably, when understory density was high with a low relative abundance of shrubs, the risk of *I. scapularis* decreased, relative to a site with a low understory density that has a medium to high relative abundance of shrubs. We predict that this association exists because shrubs contribute to a suitable habitat for ticks and small mammal hosts when compared to other types of vegetation. In the future, it would be beneficial to identify the species of shrubs in each area. Several studies have found an association with the establishment of *I. scapularis* following the growth of non-native, invasive shrub species (Elias et al. 2006; Coyle et al. 2013). These plants provide an ideal habitat for small mammals and ticks. Additionally, most types of invasive shrubs are not palatable to deer, so these habitats are more resistant to destruction by deer browsing (Elias et al. 2006; Coyle et al. 2013).

The significance of longitude in the model illustrates that as the location of a site moves westward, the odds of detecting *I. scapularis* decreases. The most probable explanation for this relationship is the proximity to endemic areas in the northeastern United States. Migratory birds bring millions of *I. scapularis* into Ontario each year along the Atlantic and Mississippi flyways (Ogden et al. 2008a). The study sites that are further west are greater distances from these endemic areas and may have a lower number of ticks introduced via migratory birds. Longitude may also act as a proxy measure for potentially unmeasured ecological factors that vary based on geographic location. For example, our study did not incorporate characteristics of the host population, such as deer density, and small mammal abundance and diversity, which previous studies have shown to be of importance (Lindsay et al. 1999a, 1999b; Werden et al. 2014). Future research endeavors should consider exploring these factors. Latitude was not included, as it was highly correlated with DD>0 °C and the multivariable model with DD>0 °C had a lower AIC value (AIC=314.96) than the multivariable model with latitude (AIC=320.69).

Interestingly, several ecological factors which had been previously highlighted as important for *I. scapularis* were not significantly associated with the presence of the tick in our models. Deciduous forests, an increasing depth of litter layer and higher relative proportion of sandy soil have been identified as risk factors (Lindsay et al. 1999a; Guerra et al. 2002; Bunnell et al. 2003; Killilea et al. 2008), while coniferous forests, and low precipitation have been
identified as protective factors against *I. scapularis* (Guerra et al. 2002; Subak 2002, 2003; Ashley and Meente 2004; Greengarten et al. 2011). There are several potential reasons why these factors did not remain in our model. Simply, these factors may not be of significance at the local level for the presence of *I. scapularis* in Ontario. We suspect that several of these variables were intervening variables, but we acknowledged this in our causal diagram and conducted our analyses with consideration of the relationships between variables. Alternatively, our sample size may not have been large enough to detect a difference, especially if there was minimal variability in these factors between sites. Sample size was affected by several missing observations. This occurred for two reasons; we did not take multiple measurements per site or include understory characteristics until site 14 (i.e., at sites 1-13, only 1 observation was recorded per variable and no understory data were collected), and due to a lack of resources we visited a small number of sites with additional volunteers who were only trained in tick dragging, not ecological data collection.

When interpreting these results, it is again important to consider the dynamic nature of this process. A site can be negative for one of three reasons: 1) the site is not ecologically suitable (true negative); or 2) the invasion process has not yet progressed to that area (false negative); or 3) the tick was not detected by dragging (false negative). This creates a non-differential misclassification of the outcome, which will bias the odds ratio towards the null (Dohoo et al. 2009). As the spread of the tick progresses to sites that are currently ecologically suitable but negative for *I. scapularis*, we would expect this bias to decrease and the odds ratios to reflect the true measures of association.

Tick dragging has been used reliably to estimate environmental risk of Lyme disease (Duik-Wasser et al. 2012). When compared to the ‘gold standard’ of small mammal trapping, the sensitivity to detect *I. scapularis* is lower, especially in areas of low tick density (i.e., newly emerging tick populations) (Ogden et al. 2014a). Additionally, the sensitivity and specificity are not constant because tick abundance varies by season and life stage (Ogden et al. 2007). Since many of our field sites were in areas of *I. scapularis* emergence, we must acknowledge these limitations and consider that some of our field sites were classified falsely as negative (Ogden et
al. 2014a). We also know that it is not possible to standardize our findings into a rate or density of ticks, and thus, we restricted our analysis to logistic regression (i.e., binary outcome).

We also need to reflect upon the methods applied to measure each ecological variable. For example, soil was classified on a crude percentage scale. Laboratory analysis of soil samples may have provided a more reliable estimate of soil composition. Precipitation was analyzed using a 30-year average, which allowed us to assess the conditions that ticks of previous cohorts may have been exposed to at that site. However, it limited our ability to detect extreme annual changes, such as drought, which may be more influential on the tick population (Jones and Kitron 2000).

We now have an enhanced understanding of the site-level ecological factors facilitating the invasion of *I. scapularis* within the province of Ontario. The results can form the basis of a local approach to public health measures including appropriate site selection for active surveillance, targeted preventative education and landscape management of areas frequented by the public. As we continue to investigate the invasion process, we can conduct more in-depth analysis of site-level ecological variables and incorporate these findings into previously developed climate change models to forecast the future risk of invasion of *I. scapularis*.

**CONCLUSIONS**

The ecology of *I. scapularis* is influenced by several abiotic and biotic factors, and we have elucidated the important role of geographic location, cumulative degree days above zero and a protective microclimate created by certain understory characteristics. As the invasion process continues, this knowledge can be applied to improve public health preventative measures. Current predictive models for *I. scapularis* invasion that account for climate change can also be enhanced by incorporating additional local ecological factors.
Table 3.1: The univariable analyses of site-level ecological variables on the presence of *I. scapularis* at 154 sites in ecoregions 5E, 6E, and 7E in Ontario during the spring, summer and fall of 2014 and 2015 based on mixed logistic regression with county and site-level random effects.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Category</th>
<th>Odds ratio</th>
<th>95% Confidence Interval (CI)</th>
<th>P-value</th>
<th>Wald chi² P-value</th>
<th>County-level variance (95% CI)</th>
<th>Site-level variance (95% CI)</th>
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<td>Ecoregion (n=1705)</td>
<td>5E</td>
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<td>Latitude (°N)</td>
<td>&lt;44.3222</td>
<td>Referent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=1705)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>&lt;44.3222</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01 - 2.11</td>
<td>0.150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(9.84 - 77.23)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(5.97 - 56.90)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(13.57 - 128.96)</td>
<td>(2.11 - 30.89)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.2: Mixed multivariable logistic regression model exploring the association between site-level ecological variables on the presence of *I. scapularis* with random effects for site and county.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Category</th>
<th>e^β</th>
<th>95% CI</th>
<th>P-value</th>
<th>Likelihood ratio test</th>
<th>Wald chi² test</th>
<th>County-level variance (95% CI); ICC (95% CI)</th>
<th>Site-level variance (95% CI); ICC (95% CI)</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Understory density (UD)</td>
<td>Low (bare + sparse)</td>
<td>Referent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>314.96</td>
</tr>
<tr>
<td></td>
<td>High (full + dense)</td>
<td>0.43</td>
<td>0.09-2.00</td>
<td>0.279</td>
<td>0.279</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative abundance of shrubs (S)</td>
<td>Negligible</td>
<td>Referent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.30</td>
<td>0.06-1.47</td>
<td>0.138</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium/high</td>
<td>7.42</td>
<td>1.24-44.34</td>
<td>0.028</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction term UD*S</td>
<td>High UD * Low S</td>
<td>3.96</td>
<td>0.50-31.11</td>
<td>0.191</td>
<td>0.016</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High UD *</td>
<td>0.16</td>
<td>0.02-1.30</td>
<td>0.087</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative abundance of non-woody vegetation</td>
<td>Negligible/low</td>
<td>Referent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.69</td>
<td>0.61-4.74</td>
<td>0.315</td>
<td>0.252</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>2.76</td>
<td>0.83-9.18</td>
<td>0.098</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitude (°W)</td>
<td>0.13</td>
<td>0.04-0.36</td>
<td>&lt;0.00</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative DD&gt;0°C</td>
<td>1.25</td>
<td>0.96-1.64</td>
<td>0.104</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degree days (DD&gt;0°C)²</td>
<td>1.00</td>
<td>0.99-1.00</td>
<td>0.130</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.3: Contrast statements comparing the odds of detecting *I. scapularis* at two sites with different combinations of understory density, relative abundance of shrubs and the interaction of understory density with the relative abundance of shrubs.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Odds Ratio</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site characteristics: Relative to:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High understory density (US) with medium/high relative abundance of shrubs (S)</td>
<td>High US with Low S</td>
<td>1.00</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Low US with Low S</td>
<td>1.69</td>
<td>0.478</td>
</tr>
<tr>
<td></td>
<td>Low US with No S</td>
<td>0.51</td>
<td>0.311</td>
</tr>
<tr>
<td>High understory density with low relative abundance of shrubs</td>
<td>High US with Med/H S</td>
<td>1.00</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Low US with Med/H S</td>
<td>0.07</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Low US with No S</td>
<td>0.51</td>
<td>0.364</td>
</tr>
<tr>
<td>High understory density with negligible relative abundance of shrubs</td>
<td>High US with Med/H S</td>
<td>0.84</td>
<td>0.795</td>
</tr>
<tr>
<td></td>
<td>High US with Low S</td>
<td>0.84</td>
<td>0.811</td>
</tr>
<tr>
<td></td>
<td>Low US with Low S</td>
<td>0.36</td>
<td>0.440</td>
</tr>
</tbody>
</table>
Figure 3.1: 154 sites across ecoregions 5E (light yellow), 6E (light green) and 7E (dark green) were visited once from May to October 2014 and 2015. *Ixodes scapularis* was detected at 29 sites (red stars) and not detected at 125 sites (black dots). Known endemic sites (yellow triangles) and major cities (orange squares) are provided for reference (Clow et al. 2016; Sider et al. 2012). (Spatial data were prepared using QGIS version 2.18.2 [www.qgis.org; 2016]. Base vector layers were accessed through the Scholars GeoPortal at the University of Guelph [http://geo2.scholarsportal.info]).
Figure 3.2: The log odds for the presence of *I. scapularis* based on a range of observed values for cumulative degree days above zero (DD >0°C). The minimum (vertical blue line, M1) and maximum (vertical red line, M2) DD>0 °C for sites in our study, as well as the DD >0 °C for the major Ontario cities of Sudbury (S) (2786 DD >0°C), Ottawa (O) (3285 DD >0°C), Toronto (T) (3530 DD >0°C) and Windsor (W) (4002 DD >0°C) are provided for reference (Government of Canada 2017).
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Schulze TL, Jordan RA, Schulze CJ, Hung RW. 2009. Precipitation and temperature as
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Subak S. 2002. Analysis of weather effects on variability in Lyme disease incidence in the
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Subak S. 2003. Effects of climate on variability in Lyme disease incidence in the northeastern

Watson T, Anderson R. 1976. Ixodes scapularis Say on white-tailed deer (Odocoileus

Geography, deer, and host biodiversity shape the pattern of Lyme disease emergence in the
APPENDICES

Appendix 3.1: Classification of site-level ecological variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site aspect</td>
<td>Crest of hill</td>
<td>Pictorial key provided to field team for reference (adapted from Lee et al. 1998)</td>
</tr>
<tr>
<td></td>
<td>Upper slope of hill</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mid-hill</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower slope of hill</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toe of hill</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>Forest type</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deciduous</td>
<td>&gt;75% deciduous tree species</td>
</tr>
<tr>
<td></td>
<td>Coniferous</td>
<td>&gt;75% coniferous tree species</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>No predominant species evident</td>
</tr>
<tr>
<td>Variable</td>
<td>Understory density</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bare</td>
<td>None present</td>
</tr>
<tr>
<td></td>
<td>Sparse</td>
<td>Partial ground coverage</td>
</tr>
<tr>
<td></td>
<td>Full</td>
<td>Complete ground coverage</td>
</tr>
<tr>
<td></td>
<td>Dense</td>
<td>Complete ground coverage, difficult to walk through</td>
</tr>
<tr>
<td>Variable</td>
<td>Understory type</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trees</td>
<td>Immature trees</td>
</tr>
<tr>
<td></td>
<td>Shrubs</td>
<td>Woody ground vegetation</td>
</tr>
<tr>
<td></td>
<td>Non-woody vegetation</td>
<td>Leafy non-woody plants and grasses</td>
</tr>
<tr>
<td>Relative abundance (of understory type)</td>
<td>Negligible</td>
<td>Minimal to none present</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Low</td>
<td>Small number present</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>Mix of predominant type</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>Predominant type</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soil composition (classified as percentage of each soil type)</th>
<th>Clay</th>
<th>Stiff and sticky, able to roll soil into a ribbon (1 cm of ribbon = 10% clay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loam</td>
<td>Soft organic material</td>
<td></td>
</tr>
<tr>
<td>Sand</td>
<td>Gritty with small rock particulate</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soil moisture</th>
<th>Dry</th>
<th>Dusty, flaky</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>Humid</td>
<td></td>
</tr>
<tr>
<td>Moist</td>
<td>Damp</td>
<td></td>
</tr>
<tr>
<td>Wet</td>
<td>Muddy, boggy</td>
<td></td>
</tr>
</tbody>
</table>

| Depth of litter layer | Centimeters of coverage | Measured using a ruler inserted perpendicularly into the litter layer to the top soil |
Appendix 3.2: Descriptive statistics for all categorical explanatory variables recorded in the field.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Category</th>
<th>Number of observations (^1) (% of total observations)</th>
<th>Number of observations with (I.\ scapularis)</th>
<th>Prevalence of (I.\ scapularis) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecoregion</td>
<td>5E</td>
<td>612 (35.9)</td>
<td>31</td>
<td>5.1 (3.5-7.1)</td>
</tr>
<tr>
<td></td>
<td>6E</td>
<td>570 (33.4)</td>
<td>64</td>
<td>11.2 (8.8-14.1)</td>
</tr>
<tr>
<td></td>
<td>7E</td>
<td>523 (30.7)</td>
<td>27</td>
<td>5.2 (3.4-7.4)</td>
</tr>
<tr>
<td>Season</td>
<td>Spring</td>
<td>421 (24.7)</td>
<td>31</td>
<td>7.36 (5.1-10.3)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>1140 (66.9)</td>
<td>79</td>
<td>6.93 (5.5-8.6)</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>144 (8.5)</td>
<td>12</td>
<td>8.33 (4.4-14.1)</td>
</tr>
<tr>
<td>Site aspect</td>
<td>Crest of hill</td>
<td>31 (2.0)</td>
<td>5</td>
<td>16.1 (5.5-33.7)</td>
</tr>
<tr>
<td></td>
<td>Upper slope of hill</td>
<td>63 (4.0)</td>
<td>2</td>
<td>3.2 (0.4-11.0)</td>
</tr>
<tr>
<td></td>
<td>Mid-hill</td>
<td>121 (7.6)</td>
<td>8</td>
<td>6.6 (2.9-12.6)</td>
</tr>
<tr>
<td></td>
<td>Lower slope of hill</td>
<td>60 (3.8)</td>
<td>2</td>
<td>3.3 (0.4-11.5)</td>
</tr>
<tr>
<td></td>
<td>Toe of hill</td>
<td>30 (1.9)</td>
<td>2</td>
<td>6.7 (0.8-22.1)</td>
</tr>
<tr>
<td></td>
<td>Depression</td>
<td>14 (0.9)</td>
<td>2</td>
<td>14.3 (1.8-42.8)</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>793 (50.1)</td>
<td>57</td>
<td>7.2 (5.5-9.2)</td>
</tr>
<tr>
<td></td>
<td>Variable</td>
<td>470 (29.7)</td>
<td>29</td>
<td>6.2 (4.2-8.7)</td>
</tr>
<tr>
<td>Forest type</td>
<td>Deciduous</td>
<td>831 (53.6)</td>
<td>83</td>
<td>10.0 (8.0-12.2)</td>
</tr>
</tbody>
</table>

\(^1\) An observation refers to one measurement recorded after 15 minutes of dragging. Missing values exist for a subset of observations resulting in variations in the total number of observations.
<table>
<thead>
<tr>
<th></th>
<th>Coniferous</th>
<th>Mixed</th>
<th>Base density of coniferous trees</th>
<th>Mixed Seedlings</th>
<th>Total Basal Area Width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Understory density</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bare</td>
<td>187 (12.1)</td>
<td>532 (34.3)</td>
<td>4</td>
<td>21</td>
<td>3.9 (2.5-6.0)</td>
</tr>
<tr>
<td>Sparse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full</td>
<td>786 (50.0)</td>
<td>223 (14.2)</td>
<td>60</td>
<td>2</td>
<td>0.9 (0.1-3.2)</td>
</tr>
<tr>
<td>Dense</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Relative abundance of non-woody vegetation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negligible</td>
<td>48 (3.1)</td>
<td>515 (32.8)</td>
<td>39</td>
<td>39</td>
<td>7.6 (5.4-10.2)</td>
</tr>
<tr>
<td>Low</td>
<td>315 (20.5)</td>
<td>496 (32.3)</td>
<td>20</td>
<td>23</td>
<td>6.3 (3.9-9.6)</td>
</tr>
<tr>
<td>Medium</td>
<td>578 (37.6)</td>
<td>451 (29.3)</td>
<td>47</td>
<td>47</td>
<td>8.1 (6.0-10.7)</td>
</tr>
<tr>
<td>High</td>
<td>526 (34.2)</td>
<td>118 (7.7)</td>
<td>31</td>
<td>2</td>
<td>5.9 (4.0-8.3)</td>
</tr>
<tr>
<td><strong>Relative abundance of shrubs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negligible</td>
<td>119 (7.7)</td>
<td>473 (30.8)</td>
<td>4</td>
<td>30</td>
<td>6.3 (4.3-8.9)</td>
</tr>
<tr>
<td>Low</td>
<td>315 (20.5)</td>
<td>496 (32.3)</td>
<td>20</td>
<td>23</td>
<td>6.3 (3.9-9.6)</td>
</tr>
<tr>
<td>Medium</td>
<td>578 (37.6)</td>
<td>451 (29.3)</td>
<td>47</td>
<td>47</td>
<td>8.1 (6.0-10.7)</td>
</tr>
<tr>
<td>High</td>
<td>526 (34.2)</td>
<td>118 (7.7)</td>
<td>31</td>
<td>2</td>
<td>5.9 (4.0-8.3)</td>
</tr>
<tr>
<td><strong>Relative abundance of trees</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negligible</td>
<td>167 (10.9)</td>
<td>443 (28.8)</td>
<td>11</td>
<td>27</td>
<td>6.1 (4.1-8.7)</td>
</tr>
<tr>
<td>Low</td>
<td>315 (20.5)</td>
<td>496 (32.3)</td>
<td>20</td>
<td>23</td>
<td>6.3 (3.9-9.6)</td>
</tr>
<tr>
<td>Medium</td>
<td>578 (37.6)</td>
<td>451 (29.3)</td>
<td>47</td>
<td>47</td>
<td>8.1 (6.0-10.7)</td>
</tr>
<tr>
<td>High</td>
<td>526 (34.2)</td>
<td>118 (7.7)</td>
<td>31</td>
<td>2</td>
<td>5.9 (4.0-8.3)</td>
</tr>
<tr>
<td><strong>Soil moisture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>188 (12.0)</td>
<td>188 (12.0)</td>
<td>14</td>
<td>2</td>
<td>7.4 (4.1-12.2)</td>
</tr>
<tr>
<td>Fresh</td>
<td>939 (59.9)</td>
<td>939 (59.9)</td>
<td>69</td>
<td>69</td>
<td>7.3 (5.8-9.2)</td>
</tr>
<tr>
<td>Moist</td>
<td>383 (24.4)</td>
<td>383 (24.4)</td>
<td>21</td>
<td>1</td>
<td>5.5 (3.4-8.3)</td>
</tr>
<tr>
<td>Wet</td>
<td>57 (3.6)</td>
<td>57 (3.6)</td>
<td>1</td>
<td></td>
<td>1.8 (0.04-9.4)</td>
</tr>
</tbody>
</table>
### Appendix 3.3: Descriptive statistics for all continuously measured explanatory variables.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Total number of observations</th>
<th>Mean (standard deviation)</th>
<th>Mean with <em>I. scapularis</em> (standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of litter layer (cm)</td>
<td>1582</td>
<td>2.7 (1.6)</td>
<td>2.7 (1.8)</td>
</tr>
<tr>
<td>Soil composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand (%)</td>
<td>1525</td>
<td>29.9 (32.0)</td>
<td>28.1 (35.7)</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>1525</td>
<td>27.4 (36.1)</td>
<td>21.6 (35.5)</td>
</tr>
<tr>
<td>Loam (%)</td>
<td>1525</td>
<td>42.6 (36.0)</td>
<td>50.2 (40.9)</td>
</tr>
<tr>
<td>Cumulative degree days above zero °C</td>
<td>1705</td>
<td>3107.4 (303.2)</td>
<td>3249.9 (132.5)</td>
</tr>
<tr>
<td>Mean annual precipitation (mm)</td>
<td>1705</td>
<td>941.2 (75.6)</td>
<td>951.0 (27.9)</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>1705</td>
<td>228.2 (87.8)</td>
<td>130.4 (56.6)</td>
</tr>
<tr>
<td>Latitude (°N)</td>
<td>1705</td>
<td>44.3 (1.2)</td>
<td>44.1 (0.8)</td>
</tr>
<tr>
<td>Longitude (°W)</td>
<td>1705</td>
<td>79.4 (2.2)</td>
<td>77.3 (1.9)</td>
</tr>
</tbody>
</table>
### Appendix 3.4: Descriptive statistics for all constructed categorical explanatory variables.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Category</th>
<th>Total number of observations (% of total observations)</th>
<th>Number of observations with <em>I. scapularis</em></th>
<th>Prevalence of observations with <em>I. scapularis</em> (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspect category</td>
<td>Depression</td>
<td>14 (0.9)</td>
<td>2</td>
<td>14.3 (1.8-42.8)</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>794 (50.2)</td>
<td>57</td>
<td>7.2 (5.5-9.2)</td>
</tr>
<tr>
<td></td>
<td>Incline&lt;sup&gt;1&lt;/sup&gt;</td>
<td>305 (19.3)</td>
<td>19</td>
<td>6.2 (3.8-9.6)</td>
</tr>
<tr>
<td></td>
<td>Variable</td>
<td>469 (29.7)</td>
<td>29</td>
<td>6.2 (4.2-8.8)</td>
</tr>
<tr>
<td>Predominant</td>
<td>Absent</td>
<td>20 (1.3)</td>
<td>0</td>
<td>0.0 (0.0-16.8)&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Predominant</td>
<td>Non-woody</td>
<td>526 (34.2)</td>
<td>37</td>
<td>7.0 (5.0-9.6)</td>
</tr>
<tr>
<td></td>
<td>Shrubs</td>
<td>45 (2.9)</td>
<td>3</td>
<td>6.7 (1.4-18.3)</td>
</tr>
<tr>
<td></td>
<td>Trees</td>
<td>259 (16.8)</td>
<td>13</td>
<td>5.0 (2.7-8.4)</td>
</tr>
<tr>
<td></td>
<td>Mix</td>
<td>688 (44.7)</td>
<td>49</td>
<td>7.1 (5.3-9.3)</td>
</tr>
<tr>
<td>Predominant soil type&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Sand</td>
<td>303 (19.9)</td>
<td>22</td>
<td>7.3 (4.6-10.8)</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>357 (23.4)</td>
<td>19</td>
<td>5.3 (3.2-8.2)</td>
</tr>
<tr>
<td></td>
<td>Loam</td>
<td>531 (34.8)</td>
<td>44</td>
<td>8.3 (6.1-11.0)</td>
</tr>
<tr>
<td></td>
<td>Mix</td>
<td>334 (21.9)</td>
<td>12</td>
<td>3.6 (2.0-6.2)</td>
</tr>
<tr>
<td>Sand category</td>
<td>Minimal (&lt;20%)</td>
<td>652 (42.8)</td>
<td>52</td>
<td>8.0 (6.0-10.3)</td>
</tr>
</tbody>
</table>

---

<sup>1</sup> Includes all original categories related to a hill.

<sup>2</sup> Based on the understory type with the highest relative abundance; if two or more species were equal in highest abundance it was categorized as mixed.

<sup>3</sup> One-sided (97.5%) confidence interval.

<sup>4</sup> Based on the soil composition percentages; predominant soil type = soil type ≥50% for each observation.
<table>
<thead>
<tr>
<th>Category</th>
<th>Subcategory</th>
<th>Count (Percentage)</th>
<th>Mean (95% CI)</th>
<th>Std. Dev (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sand mix</strong></td>
<td>20-&lt;50%</td>
<td>422 (27.7)</td>
<td>18</td>
<td>4.3 (2.5-6.7)</td>
</tr>
<tr>
<td></td>
<td>≥50%</td>
<td>451 (29.6)</td>
<td>27</td>
<td>6.0 (4.0-8.6)</td>
</tr>
<tr>
<td><strong>Clay category</strong></td>
<td>Minimal (&lt;20%)</td>
<td>870 (57.1)</td>
<td>67</td>
<td>7.7 (6.0-9.7)</td>
</tr>
<tr>
<td></td>
<td>Clay mix (20-&lt;50%)</td>
<td>202 (13.3)</td>
<td>6</td>
<td>3.0 (1.1-6.4)</td>
</tr>
<tr>
<td></td>
<td>High clay (≥50%)</td>
<td>453 (29.7)</td>
<td>24</td>
<td>5.2 (3.4-7.8)</td>
</tr>
<tr>
<td><strong>Loam category</strong></td>
<td>Minimal (&lt;20%)</td>
<td>497 (32.6)</td>
<td>33</td>
<td>6.6 (4.6-9.2)</td>
</tr>
<tr>
<td></td>
<td>Loam mix (20-&lt;50%)</td>
<td>286 (18.8)</td>
<td>11</td>
<td>3.8 (1.9-6.8)</td>
</tr>
<tr>
<td></td>
<td>High loam (≥50%)</td>
<td>742 (48.7)</td>
<td>53</td>
<td>7.1 (5.4-9.2)</td>
</tr>
<tr>
<td><strong>Depth of litter layer</strong></td>
<td>Low (≤2.5 cm)</td>
<td>805 (50.9)</td>
<td>51</td>
<td>6.3 (4.8-8.2)</td>
</tr>
<tr>
<td></td>
<td>High (&gt; 2.5 cm)</td>
<td>777 (49.1)</td>
<td>56</td>
<td>7.2 (5.5-9.3)</td>
</tr>
<tr>
<td><strong>Latitude (°N)</strong></td>
<td>&lt;43.2767</td>
<td>425 (24.9)</td>
<td>26</td>
<td>6.1 (4.0-8.8)</td>
</tr>
<tr>
<td></td>
<td>43.2767 to &lt;44.3222</td>
<td>427 (25.0)</td>
<td>38</td>
<td>8.9 (6.4-12.0)</td>
</tr>
<tr>
<td></td>
<td>44.3222 to &lt;45.3192</td>
<td>421 (24.7)</td>
<td>58</td>
<td>13.8 (10.6-17.4)</td>
</tr>
<tr>
<td></td>
<td>≥45.3192</td>
<td>432 (25.3)</td>
<td>0</td>
<td>0.0 (0.0-0.9)</td>
</tr>
</tbody>
</table>

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1 One-sided (97.5%) confidence interval.
CHAPTER 4:
SPATIAL ANALYSES OF THE SPREAD OF IXODES SCPULARIS AND BORRELIA BURGDORFERI IN ONTARIO

This chapter has been submitted to PLOS ONE in July 2017 as: Clow KM, Leighton PA, Ogden NH, Lindsay LR, Michel P, Pearl DL, Jardine CM. Northward range expansion of Ixodes scapularis evident over a short timescale in Ontario, Canada.

ABSTRACT

The invasion of the blacklegged tick, Ixodes scapularis into Ontario, Canada poses a significant risk to public health because it is a vector for numerous pathogens, including Borrelia burgdorferi sensu stricto, the causative agent of Lyme disease. Baseline field sampling in 2014 and 2015 detected I. scapularis and B. burgdorferi at sites across southern, eastern and central Ontario, including a hot spot in eastern Ontario. A “speed of spread” model for I. scapularis developed by Leighton and colleagues (2012) estimated that the tick’s range was expanding northward at 46 km/year. In 2016, we revisited a subset of sites sampled in 2014 and 2015 to understand the changing nature of risk, and assess whether the rate of tick invasion is consistent with the speed of spread estimate. Ticks were collected via tick dragging at 17 out of 36 sites, 5 of which were new sites for I. scapularis. Samples were positive for B. burgdorferi at 8 sites. No other I. scapularis-borne pathogens were detected. Centrographic statistics revealed an increase in the dispersion of I. scapularis positive sites in eastern Ontario. Field data for each site were then compared to the model’s predicted year of establishment for each census subdivision. Our findings illustrate that the range expansion of I. scapularis and the emergence of B. burgdorferi is ongoing, and provide short time-scale evidence of the processes associated with I. scapularis spread. The range front appears to be moving at a rate of ~46 km/year, with colonization of the tick behind this range front occurring at a slower and heterogeneous rate. Assessment of site-level ecological factors did not provide any insight into the underlying processes that may be influencing the colonization of I. scapularis in specific areas. Ongoing field sampling is needed to monitor this dynamic process. This study highlights the current geographic risk associated
with Lyme disease, which can be used to target public health interventions to the areas of greatest risk.

INTRODUCTION

The blacklegged tick, *Ixodes scapularis*, has undergone extensive range expansion in the recent past. Reforestation of large areas of the United States created suitable habitat for the tick and the primary host for the adult life stage, the white-tailed deer, *Odocoileus virginianus* Zimmerman (Spielman et al. 1985). *Ixodes scapularis* re-emerged from two foci: one in the northeastern United States, and the other in the Midwest (Hoen et al. 2009; Margos et al. 2012). This tick is a vector for numerous pathogens of public health significance, including *Borrelia burgdorferi* sensu stricto (herein referred to as *B. burgdorferi*), the causative agent of Lyme disease in North America (Burgdorfer et al. 1982). The northern extent of the range of *I. scapularis* is currently expanding into Canada (Diuk-Wasser et al. 2012; Ogden et al. 2015).

Invasion of *I. scapularis* into Ontario, Canada involves several dynamic processes. Since 1990, the number of established, reproducing populations of *I. scapularis* has dramatically increased, with a subsequent increase in the incidence of human Lyme disease cases (Ogden et al. 2014, 2015; Clow et al. 2016). Each year, migratory birds likely introduce millions of ticks from the northeastern United States into Canada (Ogden et al. 2008a). This long-distance ($\leq 425$ km) dispersal of ticks seeds new areas and may lead to the establishment of new reproducing populations (Ogden et al. 2008a). With climate change, this process is anticipated to continue, as more northern areas become climatically suitable for the tick (Ogden et al. 2008b, 2014; McPherson et al. 2017).

At a finer scale, there is limited knowledge of the mechanisms of tick dispersal. Ticks only move a few metres on their own during each life stage (Falco and Fish 1991). Therefore, local range expansion is dependent on the movement of hosts, including white-tailed deer, small mammals and ground-dwelling birds (Madhav et al. 2004).
Significant effort has been placed into predicting future expansion of *I. scapularis* and the risk of *B. burgdorferi* in the province. Leighton and colleagues (2012) used 19 years of passive surveillance data to assess the factors determining the northward speed of spread, and then provided an estimate of the speed of spread of the northern range front. The range front was predicted to continue expanding northward at approximately 46 km/year, with expected variation in speed between 35 to 55 km/year depending on temperature conditions. The accompanying colonization or ‘filling in’ of suitable woodland areas behind this range front by *I. scapularis* would occur rapidly afterwards. Stochastic fade-out of early populations can also happen during this time (May et al. 2001). In this region, the spread of populations of the tick has, in general, been followed by the spread of the bacteria that the tick transmits. In eastern Canada, including Ontario, estimates to date have suggested that there is a five-year lag between the establishment of a reproducing population of *I. scapularis* and a detectable, sustained cycle of transmission of *B. burgdorferi* (Ogden et al. 2013).

In 2014, we conducted large-scale field sampling for *I. scapularis* across southern, eastern and central Ontario to determine the distribution of the tick and the risk of Lyme disease (Clow et al. 2016). *Ixodes scapularis* was collected across the study area, with a hot spot of the tick detected in eastern Ontario. Additional field sampling was conducted in 2015 (Clow et al. 2017). Over the two years of sampling, the tick was detected at 29 of 154 sites (18.8%) (Clow et al. 2016, 2017).

With knowledge of the baseline distribution of *I. scapularis* and *B. burgdorferi* in Ontario, we are now able to obtain a snapshot of the processes associated with spread. If the range front of *I. scapularis* is spreading northward at a constant 46 km/year, as estimated by Leighton and colleagues (2012), then the tick should be newly detected at sites within a 46-km radius of areas with high prevalence of *I. scapularis* in 2014 or 2015. If an *I. scapularis* population establishes free of *B. burgdorferi* (Ogden et al. 2013), then *I. scapularis* at newly detected sites should be free of *B. burgdorferi*, and a subset of sites with the presence of *I. scapularis* in 2014 or 2015 may have ticks positive for *B. burgdorferi* in 2016.
In this study, we conducted field sampling for *I. scapularis* at a subset of previously visited sites in Ontario. The objectives were to re-examine the distribution of *I. scapularis* and *B. burgdorferi* within a zone of emergence and apply these field data to assess whether short time-scale changes in tick populations are consistent with the speed of spread estimated by Leighton et al. (2012).

**METHODS**

**Site selection**

Assessment of the speed of spread of the range front requires knowledge on the current geographic limits of *I. scapularis* in the province. Since it was not feasible to conduct field sampling at every possible site, a proxy measure of the current range was required. The primary cluster detected in 2014 contained 15 of the 21 sites with *I. scapularis* and was deemed to be a suitable baseline measurement from which to assess *I. scapularis* spread.

Site selection and initial cluster analysis were previously described (Clow et al. 2016). Cluster analysis was updated to include additional field data from 2015. A spatial scan statistic using a Bernoulli probability model was applied to retrospectively identify areas (i.e., clusters) of high prevalence of *I. scapularis*. No geographical overlap of clusters was permitted, with the maximum size of a cluster set at 50% or less of the total population. Statistical significance was assessed based on 999 Monte Carlo replications. Sites that were positive for *I. scapularis* either in 2014 or 2015 were included as cases, as well as other sites already known to have established populations of *I. scapularis* based on previous surveillance studies in the region (Sider et al. 2012). Any site where *I. scapularis* was not detected was included as a control. Analysis was conducted using SaTScan v9.4.4 (www.satscan.org, 2016) with a significance level of $\alpha=0.05$. The site locations and the results of cluster analysis were mapped using ArcGIS 10.5 (Esri, Redlands, CA; 2016) (Figure 4.1). The Universal Transverse Mercator (UTM) North American Datum (NAD) 1983 projection (zones 18N and 17N) was chosen to accurately calculate distance (Langley 1998). Both 46-km and 92-km buffers were created around the perimeters of the primary and secondary spatial clusters to represent potential spatial spread of *I. scapularis* over
one or two years, respectively, according to the estimate by Leighton et al. (2012). Any previously visited field site that was negative for \textit{I. scapularis} and either within the clusters, or the buffer zones was eligible for selection for analysis of \textit{I. scapularis} spread. Any site within the entire study area that was previously positive for \textit{I. scapularis}, but negative for \textit{B. burgdorferi} was eligible for analysis for the invasion of \textit{B. burgdorferi}.

In total, 36 sites were selected. Specifically, 21 sites were selected to assess spatial spread of \textit{I. scapularis}; 1 site was within the primary cluster, 9 sites were within the 46-km buffer of the primary cluster, 5 sites were within the 92-km buffer of the primary cluster, 4 sites were within the 92-km buffer of the secondary cluster, and 2 sites were outside of both buffer regions. To assess \textit{B. burgdorferi} invasion, 15 sites were selected.

\textbf{Field sampling}

Thirty-six sites were visited once during either the spring (May and June) or fall (September and October) of 2016. Tick dragging was conducted by dragging a 1 m\(^2\) white flannel drag cloth across the forest floor and surrounding vegetation for a total of 3 person-hours. The drag cloth was checked for ticks every 3 minutes, during which time the timer was stopped. All life stages of \textit{I. scapularis} were collected and stored in 70\% ethanol for species verification and laboratory testing.

\textbf{Ecological data}

Site aspect, tree coverage, understory density and type, soil composition and moisture, and depth of litter layer were previously recorded for each site as described in Clow et al. (2017). Multiple measurements were taken for each site for previous analysis, so data were aggregated for each variable to the site-level for this study.
Laboratory analyses

All adult, nymphal and if required, larval *I. scapularis* were sent to the National Microbiology Laboratory (NML) at Winnipeg (Public Health Agency of Canada, Winnipeg, Manitoba, Canada) for species verification. Adult and nymphal *I. scapularis* subsequently underwent testing at NML for *B. burgdorferi*, and three other *I. scapularis*-borne pathogens: *Anaplasma phagocytophilum*, *B. miyamotoi*, and *Babesia microti*.

Laboratory analyses have been previously described (Ogden et al. 2006b). Briefly, DNA was extracted using DNeasy 96 tissue kits (QIAGEN Inc., Mississauga, Canada). Initial screening for *Borrelia* spp. was conducted using the 23s ribosomal RNA real-time polymerase chain reaction (PCR) assay. This was coupled with the msp2 real-time PCR for the detection of *A. phagocytophilum* (Courtney et al. 2004). Samples that tested positive for *Borrelia* spp. were subjected to the ospA real-time PCR to detect *B. burgdorferi* and the IGS real-time PCR for *B. miyamotoi*. *Borrelia miyamotoi*-positive samples were then verified with the glpQ real-time PCR (Dibernardo et al. 2014). Real-time PCR for the CCTeta gene was used to detect *B. microti* (Nakajima et al. 2009). To ensure contamination did not occur during extraction and PCR runs, water blanks were used.

Model exploration

The original output of the parametric survival regression developed by Leighton and colleagues (2012) to determine the speed of spread of *I. scapularis* in Canada was used for model exploration. In building this model, 19 years of passive surveillance data (1990-2008) were incorporated with data on short and long distance tick dispersal and ecological factors of annual cumulative degree days above 0°C (DD>0°C), average annual rainfall, elevation, and percent deciduous or mixed deciduous forest, all at the level of census subdivision (CSD). The final model included DD>0°C, elevation, annual rainfall and both short and long distance dispersal, and estimated range expansion at 46 km/year northward, with a range of 35 km to 55 km, depending on if DD>0°C were cooler or warmer than the period from 1990-2008, respectively (Leighton et al. 2012). The model output included the number of years to establishment from 1991 (number from 0 to n), and the predicted year of *I. scapularis*
establishment with standard error (year from 1991 to n) for each census subdivision (CSD) in our study area. The predicted year of establishment was compared to site status from each year of field sampling to determine if the time of detection of *I. scapularis* aligned with the model output. The standard error was used to calculate the 95% confidence interval for each prediction, and findings of field sampling were also compared to the range of dates. All year predictions were rounded down to the nearest whole number.

Sites were classified as early-to-establish if *I. scapularis* was detected prior to the predicted date for the CSD, on-time if the year *I. scapularis* was detected aligned with the model prediction, or late-to-establish if *I. scapularis* was not detected by the predicted year of establishment. If a site was negative for *I. scapularis* with a future predicted year of establishment, the site was labelled as pre-emergence. Similarly, if a site was positive for *I. scapularis* with a predicted year of establishment in advance of our sampling timeframe, the site was labelled as post-emergence.

To verify if the estimated rate of range expansion could be slower (35 km/year) or faster (55 km/year) based on DD>0°C, the average DD>0°C for each CSD was calculated for the time period directly preceding our study (2009-2013) and compared to the average DD>0°C for 1990-2008 time period. Climatic data were accessed from Environment Canada and calculated as described in Leighton et al. (2012).

**Statistical analysis**

Prevalence and exact 95% confidence intervals (CI) were calculated for sites with *I. scapularis* and sites with *B. burgdorferi*.

Multinomial logistic regression was used to assess the relationship between the number of years to establishment from the speed of spread model (explanatory variable) and the site status for *I. scapularis* (established, risk area, negative). Sites were classified as established if *I. scapularis* was detected both years, and a risk area if *I. scapularis* was detected only in the second year. To test the assumption of linearity, ordinary (binary) logistic regression models were generated for each outcome level. We considered the assumption of linearity to be met if
the lowess curve formed a straight line against the log odds of the outcome. Model fit was assessed using the Fagerland, Hosmer, and Bofin goodness of fit test (Fagerland et al. 2008). The null hypothesis that the model fits was rejected if the goodness-of-fit test was significant.

To determine if ecological factors had an influence on the speed of spread of *I. scapularis*, univariable analysis with logistic regression was conducted. The outcome was the site status late-to-establish (versus all other statuses), and the explanatory variables were the site-level ecological variables, including the difference of DD>0°C between the timeframe preceding the study (2009-2013) and the timeframe of model development (1990-2008). If the number of observations per category was less than 5, exact logistic regression was conducted (Dohoo et al. 2009).

All statistical analyses were conducted using STATA version 14.0 (STATACorp, College Station, TX; 2016) with a significance level of α=0.05.

**Spatial analysis**

Centrographic statistics of mean centre and standard deviational ellipse were calculated for both the baseline field sampling (2014-2015) and follow-up field sampling (2016) timeframes in the area around the primary cluster. The standard deviational ellipse was chosen as it provides a measure of dispersion of sites with *I. scapularis*, and can be used to assess if dispersion changes over time if multiple time points of data are available (Levine 2010a, 2010b). Sites that were positive for *I. scapularis* during that field sampling timeframe were used as point locations. Analysis was conducted using CrimeStat v3.3 (Levine 2010a), and results were projected using ArcGIS (Esri, Redlands, CA; 2016). It was not possible to conduct these calculations for the area around the secondary cluster as there were not sufficient data.
RESULTS

Field sampling

*Ixodes scapularis* was detected at 17 of the 36 sites in 2016 (47.2%; 95% CI 30.4%-64.5%). This was the first detection of *I. scapularis* at five sites (Figure 4.2); four of these sites were within the 46-km buffer of the primary cluster, and the remaining site was within the 92-km buffer of the primary cluster. No changes were detected around the secondary cluster.

Laboratory analyses

Samples of *I. scapularis* from 16 sites were tested (number of ticks = 56; median = 2/site; range = 1-11/site), and 8 sites were positive for *B. burgdorferi* (50.0% (95% CI 24.6%-75.3%)). Seven of these sites were positive for *I. scapularis*, but negative for *B. burgdorferi* in 2014 or 2015. One site was negative for both the tick and bacteria at the first sampling, but positive for *I. scapularis* and *B. burgdorferi* at the second sampling. All 16 sites were negative for *A. phagocytophilum*, *B. microti*, and *B. miyamotoi* (0% (one-sided 97.5% CI 0%-20.6%)).

Model exploration

For 18 sites, the sampling period occurred outside of the timeframe of the prediction (i.e., prediction of establishment pre-2014 or post-2016) (Figure 4.3; Appendix 4.1). Eight sites were sampled after the predicted year of establishment (and confidence interval), and were classified as established. Ten sites were sampled before the predicted year of establishment; six of these sites were sampled outside of the confidence interval of the prediction. All ten sites were negative for *I. scapularis*.

The remaining sites were sampled within the timeframe of the prediction (Figure 4.3; Appendix 4.1). At four sites classified as established, *I. scapularis* was detected prior to the predicted year of establishment. Seven sites were negative for *I. scapularis* after the predicted year of establishment and outside of the confidence interval for the prediction, while four sites were negative for *I. scapularis* after the predicted year of establishment, but within the
confidence interval of the prediction. Three sites were not assessed because *I. scapularis* was detected at the first visit, but not at the follow-up visit.

The average DD>0°C for the time period preceding the study was higher than the average DD>0°C from 1990-2008 for all CSDs (mean 149.5318 DD>0°C; range 53.35 - 216.04). The estimated speed of range expansion therefore could to be greater than 46 km/year, but not exceed 55 km/year.

**Statistical analyses**

The odds of a site being established for *I. scapularis* or a risk area for *I. scapularis* when compared to sites that were absent for *I. scapularis* significantly decreased as the number of years to establishment increased, based on multinomial logistic regression (Table 4.1). The assumption of linearity was met, and the model adequately fit the data.

No ecological variables were significant for sites late-to-establish for *I. scapularis* based univariable logistic or exact logistic regression (p>0.20) (Appendix 4.2).

**Spatial analyses**

The dispersion of sites positive for *I. scapularis* within the area of the primary cluster and buffers, as represented by a standard deviational ellipse, increased between the baseline field sampling season (2014-2015) and the follow-up field sampling season (Table 4.2; Figure 4.4.). The area of the standard deviational ellipse increased by 6391.51 km² northward over the two sampling timeframes (Figure 4.4). Although no calculations could be conducted for the secondary cluster, no changes were noted in *I. scapularis* site status in this area between the field sampling timeframes.

**DISCUSSION**

Range expansion of *I. scapularis* into Canada has been documented since the early 1990s and is predicted to continue northward, in part due to climate change (Ogden et al. 2006b, 2008b;
Leighton et al. 2012). The emergence of *I. scapularis*-borne pathogens, most notably *B. burgdorferi*, has occurred following the introduction of the tick. Previous field sampling has demonstrated that *I. scapularis*, including those carrying *B. burgdorferi*, can be found in southern, eastern and central Ontario, with a hot spot in eastern Ontario (Clow et al. 2016, 2017). In this study, we have illustrated the ongoing invasion of *I. scapularis* and *B. burgdorferi* within Ontario. With these data, we have examined the processes associated with *I. scapularis* spread over a short time-scale, including the speed of range front expansion estimated by Leighton et al. (2012), and *I. scapularis* colonization into suitable woodland behind the range front.

Follow-up field sampling from Clow et al. (2016, 2017) detected *I. scapularis* at 17 out of 36 sites. Five of these sites were newly documented sites for the tick, and located within either the 46-km buffer (4 sites) or the 92 km-buffer (1 site) of the primary cluster. The dispersion of positive sites in eastern Ontario increased in a northward direction between sampling timeframes. This area may represent the range front of *I. scapularis* expansion in eastern Ontario and based on a short time-scale, provide data to support the speed of spread estimate of 46 km/year. However, when these sites are compared with the model prediction for year of establishment of *I. scapularis*, all five sites were late-to-establish. If these sites represent the range front of expansion of *I. scapularis*, then the process may be occurring at a slower rate than estimated, especially since temperature conditions were elevated based on DD>0°C, or be occurring at an inconsistent rate that is not detectable over the short time-scale.

Site status was compared with the predicted year to establishment for each CSD for the remaining sites. For 18 sites, our sampling timeframe was outside of the predicted year of establishment, so it was not possible to establish a temporal relationship. These sites were however in general alignment with the predictions (i.e., post-prediction and established or pre-prediction and absent). For the sites for which we could establish a temporal relationship, 4 were early to establish, and 6 were late-to-establish, when compared to the prediction. These findings illustrate that behind the range front, *I. scapularis* colonization is occurring heterogeneously, and does not consistently align with the same time scale of range front expansion. That being said, there still appears to be a relationship with the predicted year of establishment from the model and field data illustrating the colonization of *I. scapularis*. Based on the multinomial logistic
regression, the predicted number of years to establishment was a significant explanatory variable for site status; the odds of a site being classified as a risk area or as an established area, in comparison to the base level of a negative site, decreased with an increasing number of predicted years to establishment from the speed of spread model.

Both processes of range front expansion and colonization or ‘filling-in’ of woodland habitats behind the range front are dependent on suitable ecological conditions. The speed of spread model incorporates the ecological factors of cumulative degree days above zero (°C), precipitation, elevation, and long-distance and local dispersal at the broad-level of census subdivision (Leighton et al. 2012). These factors most likely influence both processes, and explain the general predictive ability of the model for both processes associated with *I. scapularis* spread.

We know that other ecological factors, such as the density and composition of the understory, the depth of litter layer and the type of forest create favourable habitat for *I. scapularis* (Lindsay et al. 1999a, 1999b; Ogden et al. 2006a; Werden et al. 2014; Clow et al. 2017). Examination of these factors at a finer spatial scale may be needed to understand the colonization process as the microclimate and microhabitat have a significant impact on the tick (Estrada-Pena et al. 2013). To explore what additional ecological factors may influence this colonization process, we conducted univariable analysis using logistic regression. The outcome was the late-to-establish site classification (in comparison to all other site classifications), and the explanatory variables were site aspect, forest type, understory density and type, soil composition and moisture level, the depth of the litter layer (Clow et al. 2017), and the difference in DD>0°C between 1990-2008 and 2009-2013. Based on this analysis, there is no evidence that these ecological factors have contributed to the heterogeneous process of *I. scapularis* colonization. It is important to note that our sample size was small (n=33) and therefore our power to detect a difference was limited.

Consideration also needs to be given to the host population. The role of migratory birds in long distance dispersal (≤425 km) is highly likely, and included in the speed of spread model (Ogden et al. 2008a). Deer and small mammals are important hosts for the adult and immature
life stages, respectively, and have been positively associated with tick abundance in Ontario (Lindsay et al. 1999b; Werden et al. 2014). The role these species play in dispersal on a local level is less well understood. Madhav and colleagues (2004) developed a model that incorporated home range, tick burden and density of white-tailed deer, white-footed mice and American robins to illustrate how *I. scapularis* would disperse over a simple landscape. Deer had the greatest role in dispersal because of their comparatively large home range (~5 km) and large burden of gravid females. White-footed mice showed a negative association with range expansion due to their small home ranges and large burden of immature ticks (Madhav et al. 2004). It is unknown if this model is applicable to more complex multi-host systems in nature, as there has been limited field research in this area. Further investigation into the host dynamics on a local scale may provide additional insight into the process of *I. scapularis* spread, and allow us to refine our predictions for future spread for both range front expansion and colonization.

Of particular interest would be further assessment of habitat and host factors between the areas surrounding the primary and secondary clusters. Based on our field sampling, spread of *I. scapularis* was only detected in eastern Ontario. We suspect that the vast areas of agricultural land, as well as densely populated urban areas in southern Ontario may impact the speed of *I. scapularis* spread. In contrast, eastern Ontario has a greater amount of woodland habitat and less developed areas, which may facilitate *I. scapularis* range expansion (Watkins 2011; Nelder et al. 2014).

It is important to note that surveillance approaches have evolved since the risk of *I. scapularis* was first identified. The passive surveillance program in Ontario has been in place for decades (Scholten 1977). However, passive surveillance can be highly influenced by the level of effort placed into the recruitment of samples. Programs such as ‘Let’s Target Lyme’, led by Public Health Ontario in 2010, have contributed to increased public awareness and sample submission (Nelder et al. 2014). Therefore, the passive surveillance data on which the speed of spread model is founded may have varied in quality and quantity from 1991-2008 and thus impacted the development of the model (Leighton et al. 2012).
Emergence of *B. burgdorferi* in ticks was detected at a subset of sites where *I. scapularis* had been previously documented (either in 2014 or 2015). The tick and the pathogen were detected for the first time concurrently at one site. Three hypotheses exist for the emergence of *B. burgdorferi*: tick-first, pathogen-first or dual invasion (Hamer et al. 2010). Over five years of field sampling in Michigan, Hamer and colleagues (2010) detected evidence of all three processes. In Canada, analysis of passive surveillance data supports the tick-first hypothesis, with an approximate lag in *B. burgdorferi* transmission of five-years (Ogden et al. 2013). As we only had two site visits over a period of three years and collected a small number of *I. scapularis* (median=2/site), we are not able to establish a temporal relationship with the emergence of *I. scapularis* and the transmission of *B. burgdorferi*. We also did not take blood samples from hosts for serology, so rigorous assessment of the pathogen-first hypothesis was not conducted. In general, our findings are consistent with the tick-first hypothesis, as *I. scapularis* at most sites where the ticks recently emerged were free of *B. burgdorferi*, and several sites remained negative for ticks with *B. burgdorferi* over two field sampling seasons. It is necessary to continue to collect field data, including alternative tick species and serological samples from hosts, in and around eastern Ontario over a longer timeframe to better assess the three hypotheses and validate the prediction for the speed of *B. burgdorferi* emergence.

There are several limitations of this study. First, we had a small sample size and sampling timeframe. It would have been ideal to revisit all field sites for more than one follow-up field season. However, this was not feasible for this study, and as a result, we have lower power for statistical analyses (Type II error) and reduced ability to illustrate invasion over time. Also, there are some limitations associated with our method for *I. scapularis* collection. Tick dragging can have low sensitivity, especially in areas of emergence where the tick density is low (Ogden et al. 2014). We expect that some of our sites are falsely negative, and therefore may not be late-to-establish.

Lyme disease has been recognized as an emerging vector-borne disease of public health significance in Canada (Kulkarni et al. 2015). Our study provides valuable information for public health interventions in Ontario. To appropriately target surveillance efforts, public education and physician and veterinarian awareness, it is crucial to know the current distribution of *I.*
I. scapularis and B. burgdorferi. We previously established a baseline distribution, and with our follow-up field sampling, have highlighted new areas of risk. Since these changes were documented over a short period of time, our findings also emphasize the need for the public health, medical and veterinary medical professionals to remain vigilant and aware of the changing risk of I. scapularis-borne disease.

In the future, efforts should be placed into ongoing field sampling, especially around eastern Ontario, which is a hot spot for the tick. Additional research should be considered to understand the role that the local host population plays in the dispersal of I. scapularis.

CONCLUSIONS

We have illustrated the ongoing spread of I. scapularis and the emergence of B. burgdorferi in Ontario, Canada, especially around the known hot spot in eastern Ontario. With field collected data, we explored the speed of spread model for I. scapularis and showed that on a short time-scale, the process of I. scapularis invasion is consistent with the estimated rate of 46 km/year. However, this speed may not be uniform and the following colonization of I. scapularis behind the range front is occurring at a heterogeneous rate. These findings can be used by public health officials to target preventative interventions. In the future, ongoing field sampling is needed to validate the model for the speed of B. burgdorferi invasion, as well as to understand the role host species may play in local dispersal of the tick.
Table 4.1: The association between the number of years to establishment from 1991 and the *I. scapularis* site classification based on multinomial logistic regression.

<table>
<thead>
<tr>
<th>Outcome Levels</th>
<th>Explanatory variable</th>
<th>Relative risk ratio (95% confidence interval)</th>
<th>p-value</th>
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<tr>
<td>Level 1 (base level) = Absent</td>
<td>Years to establishment</td>
<td>0.60 (0.40-0.89)</td>
<td>0.010</td>
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<tr>
<td>Level 2 = Risk area</td>
<td></td>
<td>0.62 (0.44-0.87)</td>
<td>0.006</td>
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<tr>
<td>Level 3 = Established</td>
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**Table 4.2:** Centrographic statistics of the primary cluster and buffer regions for the baseline (2014 – 2015) and follow-up (2016) field sampling seasons.

<table>
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<tr>
<th>Centrographic statistic</th>
<th>Primary cluster and buffer region</th>
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<tbody>
<tr>
<td></td>
<td>(n=12)</td>
</tr>
<tr>
<td><strong>Mean center x</strong></td>
<td>-76.556008</td>
</tr>
<tr>
<td>(standard deviation)</td>
<td>(1.212581)</td>
</tr>
<tr>
<td><strong>Mean centre y</strong></td>
<td>44.641405</td>
</tr>
<tr>
<td>(standard deviation)</td>
<td>(0.327147)</td>
</tr>
<tr>
<td><strong>Standard Deviational Ellipse</strong></td>
<td></td>
</tr>
<tr>
<td>Clockwise angle of rotation</td>
<td>72.10°</td>
</tr>
<tr>
<td>SD of x axis</td>
<td>29.77 km</td>
</tr>
<tr>
<td>SD of y axis</td>
<td>149.27 km</td>
</tr>
<tr>
<td>Area</td>
<td>13962.89 km$^2$</td>
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</table>
Figure 4.1: Using the spatial scan statistic, two spatial clusters (red lines) were detected in eastern Ontario (primary cluster) and southern Ontario (secondary cluster) using field data from 2014 and 2015 (red stars=field sites where *I. scapularis* was collected, black dots=field sites where *I. scapularis* was not collected, green fill=study area) (Clow et al. 2016, 2017). This statistic captures areas with a significantly higher prevalence of sites with *I. scapularis* relative to the outlying areas. Buffers were drawn 46 km (orange lines) and 92 km (blue lines) from the edges of the clusters to represent one or two years of *I. scapularis* spread, respectively (Leighton et al. 2012). Field sites for 2016 were selected from within the clusters and two buffer regions (hollow black circles). Known endemic sites are provided for reference (yellow triangles) (Sider et al. 2012).
Figure 4.2: Thirty-six field sites were visited during the spring and fall of 2016. *Ixodes scapularis* was collected at 17 sites (large hollow red stars); 19 were negative for the tick (large black diamonds). All sites were previously visited in 2014 or 2015 (*I. scapularis* present = small black stars; *I. scapularis* absent = small black dots) (Clow et al. 2016, 2017).
Figure 4.3: The results from two field samplings at thirty-three field sites were compared to the predicted year of establishment from the Leighton et al. (2012) *I. scapularis* speed of spread model. Eight sites were visited after the predicted year of establishment and were positive (black squares), ten sites were visited before the predicted year of establishment and negative (black dots), four sites were early-to-establish (blue stars) and 11 sites were late-to-establish (orange triangles).
Figure 4.4: Baseline field sampling in 2014 and 2015 detected 12 sites with *I. scapularis* in eastern Ontario (sites = black triangles, mean center = black dot) (Clow et al. 2016, 2017). Repeat follow-up field sampling in 2016 detected *I. scapularis* at 15 sites (sites = red triangles, mean center = red dot). The dispersion of sites with *I. scapularis* increased within this area, based on the standard deviational ellipse (baseline=black ellipse, follow-up=red ellipse).
REFERENCES


Dibernardo A, Cote T, Ogden NH, Lindsay LR. 2014. The prevalence of *Borrelia miyamotoi* infection, and co-infections with other *Borrelia* spp. in *Ixodes scapularis* ticks collected in Canada. *Parasit Vectors* 7(1):183-190.


Estrada-Peña A, Gray JS, Kahl O, Lane RS, Nijhof AM. 2013. Research on the ecology of ticks and tick-borne pathogens--methodological principles and caveats. *Front Cell Infect*


APPENDICES

Appendix 4.1: Comparison of the *I. scapularis* site status over two years of field sampling with the predicted year to establishment based on Leighton et al. (2012) at 33 sites in Ontario.

<table>
<thead>
<tr>
<th>Site ID</th>
<th>I. scapularis status of initial visit in 2014</th>
<th>I. scapularis status of initial visit in 2015</th>
<th>I. scapularis status at follow-up visit in 2016</th>
<th>I. scapularis site classification</th>
<th>Predicted year of establishment</th>
<th>Confidence Interval</th>
<th>Assessment of Prediction</th>
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</tbody>
</table>
Appendix 4.2: The univariable analysis of site-level ecological variables on the late establishment of *I. scapularis* at 33 sites sampled in Ontario during the spring, summer and fall of 2014 or 2015 and again in 2016 based on logistic regression or exact logistic regression (*).  

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Category</th>
<th>Odds ratio (95% confidence interval); p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(number of observations)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspect category*</td>
<td>Flat (19)</td>
<td>REF</td>
</tr>
<tr>
<td></td>
<td>Incline (2)</td>
<td>4.36 (0.32- +Inf.); 0.267</td>
</tr>
<tr>
<td></td>
<td>Variable (12)</td>
<td>0.73 (0.09-4.61); 1.000</td>
</tr>
<tr>
<td>Forest type*</td>
<td>Coniferous (6)</td>
<td>REF</td>
</tr>
<tr>
<td></td>
<td>Deciduous (15)</td>
<td>0.68 (0.07-6.91); 1.000</td>
</tr>
<tr>
<td></td>
<td>Mixed (12)</td>
<td>0.22 (0.012-2.90); 0.352</td>
</tr>
<tr>
<td>Understory density*</td>
<td>Sparse (11)</td>
<td>REF</td>
</tr>
<tr>
<td></td>
<td>Full (16)</td>
<td>0.29 (0.04-1.86); 0.248</td>
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<tr>
<td></td>
<td>Dense (2)</td>
<td>0.85 (0.01-78.36); 1.000</td>
</tr>
<tr>
<td>Predominant understory type*</td>
<td>Mixed (16)</td>
<td>REF</td>
</tr>
<tr>
<td></td>
<td>Non-woody (7)</td>
<td>0.29 (0.01-3.46); 0.551</td>
</tr>
<tr>
<td></td>
<td>Trees (6)</td>
<td>3.15 (0.33-45.11); 0.458</td>
</tr>
<tr>
<td>Predominant soil type*</td>
<td>Mixed (8)</td>
<td>REF</td>
</tr>
<tr>
<td></td>
<td>Clay (6)</td>
<td>0.36 (0.53-6.48); 1.000</td>
</tr>
<tr>
<td></td>
<td>Loam (14)</td>
<td>0.68 (0.08-6.53); 1.000</td>
</tr>
</tbody>
</table>

1 Median unbiased estimate
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Type</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil moisture*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand (5)</td>
<td></td>
<td>2.32 (0.16-44.95); 0.8252</td>
</tr>
<tr>
<td>Dry (3)</td>
<td></td>
<td>REF</td>
</tr>
<tr>
<td>Fresh (17)</td>
<td></td>
<td>1.38 (0.06-94.23); 1.000</td>
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<tr>
<td>Moist (12)</td>
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<td>0.43 (0.01-35.28); 1.000</td>
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<tr>
<td>Wet (1)</td>
<td></td>
<td>1.0*¹ (0.03-+Inf); 1.000</td>
</tr>
<tr>
<td>Depth of litter layer (cm)</td>
<td>Continuous variable (33)</td>
<td>1.10 (0.62-1.96); 0.733</td>
</tr>
<tr>
<td>Loam soil (%)</td>
<td>Continuous variable (33)</td>
<td>1.01 (0.98-1.04); 0.667</td>
</tr>
<tr>
<td>Clay soil (%)</td>
<td>Continuous variable (33)</td>
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</tr>
<tr>
<td>Sand soil (%)</td>
<td>Continuous variable (33)</td>
<td>1.01 (0.98-1.05); 0.453</td>
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<tr>
<td>Difference in average</td>
<td>Continuous variable (33)</td>
<td>1.00 (0.958-1.02); 0.623</td>
</tr>
<tr>
<td>Cumulative DD&gt;0°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Average DD&gt;0°C 2009-2013 - Average 1991 to 2008)</td>
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<td></td>
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</tbody>
</table>

¹ Median unbiased estimate
CHAPTER 5:
AN ECOLOGICAL RISK INDICATOR FOR FIELD SAMPLING
OF IXODES SCAPULARIS AT SITES IN ONTARIO

ABSTRACT

The emergence of the vector *Ixodes scapularis* in Ontario, Canada poses a significant public health risk. Both passive and active surveillance approaches have been employed by public health professionals to monitor for the invasion of this tick. Field surveillance using drag sampling for questing ticks is a recognized and effective method to identify reproducing tick populations. The degree of effort (i.e., number of visits per site) can enhance the sensitivity and specificity of surveillance, but increased effort conflicts with the cost to public health for field surveillance. Here we developed an ecological risk indicator to determine the likelihood of a reproducing population of *I. scapularis* based on field sampling results. Field data from two endemic populations of *I. scapularis* in Ontario were incorporated with previous analyses of surveillance data to create an ecological risk indicator ("the tool"), which is in the form of a scoring system. The tick life stage(s) collected, overall abundance and past surveillance findings from a site are all considered and a risk level is assigned based on current field sampling results. Risk is classified as non-zero (i.e., no *I. scapularis* detected, but risk still present due to adventitious ticks), low, medium or high, and recommendations for future surveillance and public health measures are provided. The tool was validated against field sampling results from five other endemic sites in the province and correctly classified all five endemic areas as high risk. The tool was also applied to field sampling results from 36 sites of unknown status that were visited twice during the period of 2014-2016. There was substantial agreement of site risk classification between measurements, as calculated using a weighted kappa. We also compared the recommended public health actions from the tool with those recommended by the current screening test by Ogden et al. (2014b). The ecological risk indicator may assist public health professionals with the interpretation of field sampling results and direct their efforts for ongoing surveillance and public health interventions for *I. scapularis*-borne diseases, including Lyme disease.
INTRODUCTION

In Canada, Lyme disease has been identified as a vector-borne disease of public health importance (Kulkarni et al. 2015). The primary causative agent, *Borrelia burgdorferi* sensu stricto, is transmitted by the hard tick *Ixodes scapularis*. Since the early 1990s, there has been notable northward expansion of the tick’s range in the province of Ontario and this has coincided with a dramatic increase in the number of human cases (Ogden et al. 2014a, 2015). Range expansion is predicted to continue, in part due to climate change (Leighton et al. 2012; McPherson et al. 2017).

Monitoring this dynamic situation has posed a significant challenge to public health professionals. Passive surveillance, which involves the public submitting ticks collected from themselves or their pets, has been in place in Ontario for decades (Scholten 1977; Nelder et al. 2014). It provides an effective method to detect areas where risk may be emerging, especially with advanced analyses. However, this approach has low specificity and can be heavily influenced by population density (Koffi et al. 2012; Nelder et al. 2014). Active surveillance is recommended in an area once there has been an increase in tick submissions by passive surveillance (Ogden et al. 2014b). This approach has high specificity and is useful for determining the presence of a reproducing population of ticks, as well as the infection prevalence of tick-borne pathogens (Diuk-Wasser et al. 2012).

The first field sampling guidelines were outlined in the Canadian Consensus on Lyme Disease (Health Canada 1991). For an area to be declared endemic for *I. scapularis*, all three life stages of the tick need to be detected, either by dragging or on hosts, for two consecutive years. Although this approach is the ‘gold standard’, it is time and labour intensive and has not been feasible given the need for large-scale field sampling (Koffi et al. 2012; Ogden et al. 2014b).

Acknowledging these challenges, Ogden and colleagues (2014b) developed a screening test for active surveillance. This screening test involves conducting tick dragging once per site for three person-hours any time during May to October. If any *I. scapularis* are detected, the site is declared a 'risk area', as long as the site is in a known area of *I. scapularis* range expansion.
Public Health Ontario has adopted this test, but requires *I. scapularis* to be detected at a site during both spring and fall drags. The site and surrounding area (20-km radius) is then declared a risk area (Ontario Agency for Public Health Protection and Promotion 2015, 2016).

Defining a risk area provides the first step for assessing risk of acquiring a tick bite from *I. scapularis* (and Lyme disease) within an area. There is, however, limited guidance to further categorize risk, based on the results of field sampling, in a way that is translated into public health action to appropriately target limited public health resources to the areas of highest risk.

The main objective of our study was to incorporate the known characteristics of *I. scapularis* populations into a practical ecological risk indicator (herein referred to as the "tool") to assist public health professionals with the interpretation and application of findings from field sampling. To collect information on *I. scapularis* population dynamics in Ontario, we conducted weekly tick dragging at two endemic sites from May to October in 2014. These data provided the foundational parameters of the tool. Additional field sampling was conducted to validate and test the applicability of the tool for risk assessment.

**METHODS**

**Field sampling**

Weekly field sampling was conducted from May to October 2014 (total=24 weeks), alternating between Turkey Point Provincial Park (TP) and Murphy’s Point Provincial Park (MP) (Figure 5.1). Both sites have established, reproducing populations of *I. scapularis* (Sider et al. 2012). Tick dragging was conducted at each site visit by dragging a 1 m² flannel drag cloth over the forest and vegetation for three-person hours. The timer was stopped every three minutes to collect ticks from the drag cloth. All life stages of ticks were counted and recorded. Adult and nymphal *I. scapularis* were collected and stored in 70% ethanol for laboratory analysis.
Laboratory analyses

A subset of adult and nympha I. scapularis were submitted from each site to the National Microbiology Laboratory at Winnipeg (Public Health Agency of Canada, Winnipeg, Ontario). One hundred and sixty-seven adults of the spring cohort, 58 adults of the fall cohort and 60 nymphs were submitted from TP, while 19 adults of the spring cohort, 99 adults of the fall cohort and 11 nymphs were submitted from MP.

All samples were tested for B. burgdorferi, B. miyamotoi, Anaplasma phagocytophilum and Babesia microti. Laboratory analyses have been previously described (Ogden et al. 2006b). In brief, DNeasy 96 tissue kits were used to extract DNA (QIAGEN Inc. Mississauga, Canada). The 23s ribosomal RNA real-time polymerase chain reaction (PCR) was then used to screen for Borrelia spp. If a sample tested positive, it was analyzed with the ospA real-time PCR to detect B. burgdorferi and the IGS real-time PCR to detect B. miyamotoi. The glpQ real-time PCR was then used to verify all B. miyamotoi-positive samples (Dibernardo et al. 2014). For A. phagocytophilum, the msp2 real-time PCR was employed (Courtney et al. 2004), while the real-time PCR for the CCTeta gene was used to detect B. microti (Nakajima et al. 2009). To verify that contamination did not occur during PCR runs, water blanks were used.

Population description

Descriptive statistics of median and range were calculated for the number of each life stage as well as for the total number of I. scapularis collected. Phenology was depicted graphically by site.

Ecological risk indicator development

Both field sampling data and previous research on the dynamics of I. scapularis populations in Ontario were incorporated into the development of the tool. Based on the following information, the tool is divided into criteria on tick life stage, abundance and previous results of tick dragging. Points are assigned accordingly.
Based on the screening test developed by Ogden and colleagues (2014b), the detection of one tick of any life stage is used as the basic criterion of risk. We expand on this criterion by incorporating the life stage of the tick(s) collected. Ticks are deposited annually across Ontario via migratory birds. These ticks, termed “adventitious ticks”, contribute to a non-zero risk of *I. scapularis* across the province and can lead to an increase in false positive sites. Most *I. scapularis* brought into Ontario via migratory birds are engorged nymphal ticks (Ogden et al. 2008, 2010). These molt into adults and can be collected via tick dragging when they quest. If a questing immature life stage is collected, it is much less likely to be an adventitious tick, and more likely a result of local reproduction. As such, immature ticks are an indicator of enhanced risk (Koffi et al. 2012), and receive additional points in the tool.

The abundance of ticks collected reflects the density of *I. scapularis* in that area. When the density is low to medium (i.e., for an emerging population), yield via tick dragging can be low. This is especially true for the immature life stages, which can be more difficult to collect via tick dragging as they quest for small mammals, and remain much lower in the leaf litter and vegetation than adults (Lindquist et al. 2016). The total abundance, as well as abundance for each life stage, was added to the tool to reflect these characteristics, and minimum thresholds were established from field sampling results.

Previous results of tick dragging is the final element. Tick dragging can have low sensitivity in areas of low tick density (i.e., an emerging population), leading to false negative sites (Ogden et al. 2014b). Therefore, if the population is just establishing, it is possible to detect ticks sporadically (i.e., at one visit and then not at the next). To acknowledge this limitation, the scoring system considers the previous findings (if available).

**Ecological risk indicator performance**

The performance of the tool was tested in two ways. First, the tool was applied to tick dragging results from five endemic sites visited in the fall of 2013. If the tool performed adequately, these sites should fall within the ‘high risk’ level.
Next, the tool was applied to 36 sites of unknown *I. scapularis* status for which there was multiple years of field sampling data (these data were collected contemporaneously for Clow et al. 2016, 2017). These sites were first visited in either 2014 or 2015. The tool was applied to the field sampling results and each site was assigned a risk level. These sites were revisited in the fall of 2016, and again the tool was applied to the field sampling results and each site was assigned a risk level. Then, the kappa statistic was used to determine the level of agreement of the tool’s risk assessment between the findings of the two field samplings (Dohoo et al. 2009). If the tool accurately assessed risk, the risk level at a site should either be the same or one level higher between samplings, since we expect there to be continued range expansion of the tick, and/or ongoing establishment of the tick population (Leighton et al. 2012). Both an unweighted and weighted kappa were calculated (Dohoo et al. 2009). The weighted kappa was chosen to indicate that there was a level of agreement between adjacent levels, and this decreased with an increase in the difference between risk levels. The weight matrix assigned was 1.0 for perfect agreement, 0.8889 for partial agreement (one level removed), 0.5556 for limited agreement (two levels removed) and 0.000 for no agreement (three levels removed). The kappa value was interpreted using the guidelines established by Landis and Koch (1977). STATA version 14.0 (STATA Corp, College Station, TX; 2017) was used to calculate the kappa statistics and 95% confidence intervals, with a significance level of $\alpha=0.05$.

**Comparison of risk assessment approaches**

We compared the outcomes of the tool versus the outcomes of the screening test in terms of public health actions (i.e., additional field sampling at a site, preventative measures). Using the criterion established by Ogden et al. (2014b), if one or more *I. scapularis* are detected at a site, the site is declared a risk area. No further field sampling is required, and public health interventions should be initiated.
RESULTS

Field sampling

*Ixodes scapularis* were collected at each site (i.e., MP and TP) every week throughout the sampling season. There were large seasonal variations in the numbers of ticks collected at the two endemic sites (Figure 5.2). However, at least one life stage of tick, with multiple individuals of that life stage was always found, and the total tick abundance was always greater than five ticks (Table 5.1).

Laboratory analyses

The *B. burgdorferi* infection prevalence of the spring and fall adult cohorts at TP were 30.0% (95% confidence interval 20.4%-34.2%) and 31.0% (19.5%-44.5%), respectively, while the nymphal infection prevalence was 11.6% (4.8%-22.6%). At MP, the infection prevalence of the spring and fall cohorts were 36.8% (16.3%-61.6%) and 78.7% (69.4%-86.4%), respectively. The nymphal infection prevalence was 18.2% (2.3%-51.8%). Three *I. scapularis* from MP were positive for *B. miyamotoi* (2.3% (0.5%-6.6%)), one of which was co-infected with *B. burgdorferi*. All samples were negative for *A. phagocytophilum* and *B. microti*.

Population description

The median number of *I. scapularis* collected during each sampling period (i.e., three-person hours) was 134, with a minimum of 6 and a maximum of 756 (Table 5.1). The data are right-skewed due to the high abundance of the larval life stage. Every week, there was always one life stage that had greater than one tick.

Ecological risk indicator development

The tool is created as a scoring system (Figure 5.3). The detection of one tick automatically classifies the site as low risk (at a minimum). If repeated sampling is conducted at a site, previous detection of a tick automatically classifies the site as low risk as well (at a minimum). Additional risk is attributed to the detection of more than one life stage, as well as
more than one tick of the same life stage, and overall abundance. The final score is translated into a risk level (i.e., non-zero, low risk, medium risk and high risk). A description of the risk levels and future recommendations are provided with the tool. The term non-zero risk is applied to a negative site due to the ever-present risk of adventitious ticks (Ogden et al. 2008).

**Ecological risk indicator performance**

The tool assessed all five endemic sites as high risk, based on the field sampling results collected in the fall of 2013 (Table 5.2).

For the field sites of unknown status, the tool assessed 21 as non-zero risk, four as low risk, five as medium risk and six as high risk following field sampling in 2014 and 2015. When the tool was reapplied to assess these sites, based on follow-up field sampling in 2016, 28 sites stayed at the same level, three increased by one level, two increased by two levels and one increased by three levels. Only two sites decreased in risk, one by one level and the other by two levels (Table 5.3). The unweighted kappa was 0.67 (95% CI 0.58-0.75) (p<0.001) and the weighted kappa was 0.74 (95% CI 0.74-0.88) (p<0.001), which is within the range of values supportive of substantial agreement (Landis and Koch 1977).

**Comparison of risk assessment approaches**

The recommended public health actions following each field sampling timeframe using the screening test by Ogden et al. (2014b) and our tool were compared (Table 5.4). The recommendation to repeat field sampling was potentially higher with our tool, while the recommendation to begin preventative measures was less frequent. This is because each site had to meet the criteria of medium or high risk to qualify for this recommendation.

**DISCUSSION**

The ongoing invasion of *I. scapularis* into Ontario, Canada has posed many challenges for public health officials; conducting active field surveillance to determine the geographic risk
of the tick and associated pathogens is one major challenge. In this study, we developed an ecological risk indicator to assist public health officials with the interpretation of field sampling results to determine the likelihood of a reproducing population of *I. scapularis*.

Application of the tool would be straight-forward. The standardized field sampling approach, which was previously outlined in Ogden et al. (2014b), is simple to use. Field sampling can be conducted any time during the active season of *I. scapularis*, which is generally between May to November in eastern Canada (Lindsay et al. 1999a, 1999b). This is supported by our findings at TP and MP. During the known active season, there is a bimodal peak in adult numbers in the spring and fall and a nymphal peak in early summer. There are two larval peaks; a small increase in activity occurs in spring and then the largest period of activity occurs in late summer (Ogden et al. 2007; Lindquist et al. 2016). Daily and diurnal fluctuations in temperature and humidity impact tick activity (Vail and Smith 2002; Berger et al. 2014), while excessively hot temperatures (>30°C) reduce activity (Ogden et al. 2004). Other variations in the numbers of collected ticks may occur for several reasons, which may be associated with methodology. The distribution of *I. scapularis* in the environment is often highly variable, and therefore, tick dragging needs to be conducted over a representative area of the study sites (Schulze et al. 2001; Dobson 2013). If a drag cloth becomes wet (either from rain or dew), the ticks are less likely to attach (Ontario Agency for Health Protection and Promotion 2015). Furthermore, where site vegetation is particularly dense, ticks may become dislodged from the drag, so it may be necessary to check the cloth more frequently.

There are several other caveats to consider for the design of tick surveillance by drag sampling. Selected sites need to be ecologically suitable. *Ixodes scapularis* are found in wooded and brushy areas, and sites for field surveillance should be selected accordingly (Lindsay et al. 1998, 1999b; Schulze and Jordan 2005; Ogden et al. 2006a). If follow-up field sampling is indicated, it is ideal to sample for a subsequent cohort of ticks, as evidence of a new cohort represents successful development and reproduction of the population. In eastern Canada, adults and larvae that are active in the spring are most likely ticks that did not feed successfully in the previous year and subsequently overwintered in an unfed state (Lindsay et al. 1999a). Therefore, if sampling is conducted in the spring for adults, any adults collected in follow-up sampling will
be of a different cohort, while adults collected in the fall and the subsequent spring will most likely be of the same cohort.

The output of the tool is a site-specific risk level; the risk level classifications are non-zero, low, medium or high. Non-zero risk exists when no ticks are detected at a site. The risk of *I. scapularis* cannot be fully negated however, due to the potential introduction of ticks via migratory birds (Ogden et al. 2006b, 2008, 2010). Low risk corresponds to the basic definition of a risk area; either one tick of any life stage is detected, or a tick has previously been detected by field sampling (Ogden et al. 2014b). Medium risk occurs when there is more than one tick, potentially of different life stages. This provides stronger evidence that a reproducing population is present, albeit at low density, and additional monitoring may provide more information.

Consideration should be given to communicating potential risk to the public. The highest level of risk reflects strong evidence of a reproducing population of *I. scapularis*. When this occurs, the focus should shift to implementation of public health measures, such as preventative education. Future field sampling should be considered if additional information on pathogen infection prevalence is required.

The tool performed adequately based on our assessment. First, we assessed the tool for accuracy by comparison against a gold standard. We employed the standardized tick dragging methodology at five endemic sites and used the tool to interpret the results. These sites had previously been declared as endemic by following the gold standard approach outlined in the Canadian Consensus on Lyme Disease (Health Canada 1991). The tool ranked all five endemic sites as high risk and thus they aligned with the gold standard. Next, we used the tool to interpret field sampling results from sites of unknown status that were visited twice during a three-year period. This allowed us to assess the tool for consistency of risk assessment. There was a substantial level of agreement between the two applications of the test at the same site, based both on the unweighted and weighted kappa (Landis and Koch 1977). Since we expect the level of risk to increase over time given the ongoing range expansion of *I. scapularis* (Leighton et al. 2012), and have designed the tool to detect this increase in risk, the weighted kappa provides the most accurate measurement.
When the screening test developed by Ogden et al. (2014b) and our ecological risk indicator were compared, the main difference was the number of sites at which the public health actions (i.e., additional field sampling and preventative interventions) would be warranted. For sites classified as low or medium risk, limited resampling is indicated by Ogden et al. (2014b), versus the encouragement of additional field sampling with our tool. Preventative interventions are suggested by Ogden et al. (2014b) as soon as an area is classified as a risk area, while our approach suggests preventative interventions for medium and high risk sites only. This may result in economies in public health expenditure on interventions that outweigh the costs of revisiting sites at which only one tick was found, although cost-benefit studies are needed. It is important to note that the purpose of the ecological risk indicator is not to replace the screening test, but rather to build upon it and provide another risk assessment tool to assist public health professionals.

An important point in question is the frequency of resampling. Our tool recommends resampling for the next cohort at sites classified as low and medium risk. Tick abundance usually increases over several years in newly-established populations, and in southeastern Canada *B. burgdorferi* infection prevalence in ticks increases over a 5-year time scale once tick populations become detectable via passive surveillance (Ogden et al. 2013). Early tick populations may not always be detectable by drag sampling when tick densities are very low, or may die out by stochastic fade out (May et al. 2001; Ogden et al. 2010). Under these circumstances, a longer resampling timeframe may be appropriate, especially for areas deemed low risk. However, additional investigation is needed to further explore the phenomenon of stochastic fade out.

Public health surveillance for *I. scapularis* is conducted to determine the risk of disease due to *I. scapularis*-borne pathogens. However, our tool does not consider the presence of pathogens and this was purposively excluded. In eastern Canada, *I. scapularis* populations generally develop free of *B. burgdorferi*; as previously mentioned, current research estimates that there is an approximate five-year lag between tick population establishment and the emergence of *B. burgdorferi* (Ogden et al. 2010, 2013). Adventitious ticks can carry *B. burgdorferi*. The infection prevalence of *I. scapularis* collected from migratory birds was ~15% (Ogden et al. 2008). Therefore, the detection of *B. burgdorferi* is not a useful indicator for early risk
assessment and not incorporated into the tool. Exclusion of *B. burgdorferi* presence also enhances the applicability of the tool. If there is sufficient expertise to identify the species and life stage of the ticks collected, the risk assessment sites can be conducted rapidly, and necessary public health interventions can be initiated without the potential delay associated with laboratory testing.

That being said, laboratory testing for potential pathogens is an important component of public health surveillance and should be considered in conjunction with field sampling. *Ixodes scapularis* is a competent vector for several pathogens of human and animal health significance, including *B. burgdorferi, B. miyamotoi, Anaplasma phagocytophilum, Babesia microti* and Powassan virus (Nelder et al. 2016). In eastern Canada, the infection prevalence of *B. burgdorferi* is highly variable. An area is considered endemic for the pathogen when infection prevalence exceeds 20%, as illustrated at both sites in our study (Wormser et al. 2006). The prevalence of the other pathogens is currently low, but ongoing surveillance is needed to monitor for pathogen emergence and any change in the risk of disease transmission (Nelder et al. 2014; Clow et al. 2016).

There are several limitations that should be acknowledged. First, although tick dragging is a highly valuable surveillance strategy, it can have low sensitivity in areas of low tick density, which leads to an increase in false negative sites (Ogden et al. 2014b). For the tool validation, we had a small sample size of endemic sites. It would be useful to conduct tick dragging at other endemic sites using the standardized methodology and apply the tool to interpret the results. Finally, it is important to note that this tool was developed using data from Ontario and is most suitable for risk assessment in Ontario, and potentially other eastern Canadian provinces. The population dynamics of *I. scapularis* are different in central Canada and therefore this tool should be validated with data from the geographic areas of interest prior to implementation (Ogden et al. 2007, 2013).

Our study has provided a valuable ecological risk indicator for public health professionals to use when conducting field sampling for *I. scapularis*. With this tool, the likelihood of a reproducing population of ticks can be quickly and easily assessed with one site visit, and public
health preventative measures for Lyme disease and other *I. scapularis*-borne pathogens can be initiated in the areas of highest risk.

Now the tool needs to be field-tested with public health professionals. This will ensure it is easy to use by the audience for which it is designed. Qualitative feedback via focus groups and surveys could be of benefit to further refine the tool.
Table 5.1: The median and range of each life stage of *I. scapularis* collected after three person-hours of tick dragging from May to October 2014 at Turkey Point Provincial Park and Murphy’s Point Provincial Park.

<table>
<thead>
<tr>
<th>Life stage</th>
<th>Turkey Point Provincial Park</th>
<th>Murphy’s Point Provincial Park</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (range)</td>
<td>Median (range)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Larva</td>
<td>25 (0-477)</td>
<td>30 (0-756)</td>
<td>28 (0-756)</td>
</tr>
<tr>
<td>Nymph</td>
<td>8 (0-51)</td>
<td>6 (0-23)</td>
<td>8 (0-51)</td>
</tr>
<tr>
<td>Adult</td>
<td>18 (0-198)</td>
<td>18 (0-242)</td>
<td>18 (0-242)</td>
</tr>
<tr>
<td>Total</td>
<td>129 (25-495)</td>
<td>134 (6-756)</td>
<td>134 (6-756)</td>
</tr>
</tbody>
</table>
Table 5.2: The risk assessment scores based on the application of the ecological risk indicator for five endemic sites in Ontario based on tick dragging in the summer and fall of 2013.

<table>
<thead>
<tr>
<th>Site</th>
<th>Field sampling results</th>
<th>1. What life stage(s) did you collect?</th>
<th>2. Did you collect ≥ 2 <em>I. scapularis</em> of the same life stage?</th>
<th>3. Did you collect ≥ 5 <em>I. scapularis</em> (total)?</th>
<th>4. Have <em>I. scapularis</em> been collected at this site before?</th>
<th>Total score and risk level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hill Island&lt;sup&gt;1&lt;/sup&gt;</td>
<td>16 nymphs, 238 larvae</td>
<td>nymph = 2 points</td>
<td>yes = 2 points</td>
<td>yes = 2 points</td>
<td>yes = 1 point</td>
<td>9 = high risk</td>
</tr>
<tr>
<td>Thwartway Island&lt;sup&gt;1&lt;/sup&gt;</td>
<td>9 nymphs, 478 larvae</td>
<td>nymph = 2 points</td>
<td>yes = 2 points</td>
<td>yes = 2 points</td>
<td>yes = 1 point</td>
<td>9 = high risk</td>
</tr>
<tr>
<td>Camelot Island&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2 nymphs, 223 larvae</td>
<td>nymph = 2 points</td>
<td>yes = 2 points</td>
<td>yes = 2 points</td>
<td>yes = 1 point</td>
<td>9 = high risk</td>
</tr>
<tr>
<td>Long Point Provincial Park</td>
<td>1 adult, 25 larvae</td>
<td>adult = 1 point</td>
<td>yes = 2 points</td>
<td>yes = 2 points</td>
<td>yes = 1 point</td>
<td>8 = high risk</td>
</tr>
<tr>
<td>Rondeau Provincial Park</td>
<td>58 adults</td>
<td>adult = 1 point</td>
<td>yes = 2 points</td>
<td>yes = 2 points</td>
<td>yes = 1 point</td>
<td>6 = high risk</td>
</tr>
</tbody>
</table>

<sup>1</sup> Part of Thousand Islands National Park.
Table 5.3: The risk assessment scores based on the application of the ecological risk indicator for sites of unknown status sampled during 2014-2015 and again in 2016 (NZ=non-zero, L=low, M=medium, H=high).

<table>
<thead>
<tr>
<th>Site No.</th>
<th>Field sampling 2014-2015</th>
<th>Field sampling 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
<td>Nymph</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>26</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>49</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>54</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>67</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>69</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>88</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>92</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>95</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>101</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>105</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>115</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>116</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>117</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>119</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>120</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>122</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>128</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>129</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>139</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>149</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>150</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>151</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>152</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>153</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>154</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 5.4: The recommendation for public health actions following each field sampling timeframe based on the screening test outlined by Ogden et al. (2014b) and our ecological risk indicator (i.e., the tool).

<table>
<thead>
<tr>
<th>Risk assessment approach</th>
<th>Field sampling 2014-2015</th>
<th>Field sampling 2016</th>
<th>Outcome comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening test by Ogden et al. (2014b)</td>
<td>Risk area = 15 of 36 sites</td>
<td>Risk area = 5 of 21 sites resampled</td>
<td>• Decreased frequency of resampling</td>
</tr>
<tr>
<td></td>
<td>• Resampling would not be recommended at 15 risk areas</td>
<td>• Resampling would not be recommended at 5 new risk areas</td>
<td>• Potential increased requirements for public health interventions at all risk areas</td>
</tr>
<tr>
<td></td>
<td>• Resampling could be conducted at 21 sites without <em>I. scapularis</em></td>
<td>• Resampling could be conducted at 16 sites without <em>I. scapularis</em></td>
<td>• Of the sites that would not have been resampled, 3 were negative at the second sampling</td>
</tr>
<tr>
<td>Ecological risk indicator</td>
<td>Non-zero = 21 sites</td>
<td>Non-zero = 16 sites</td>
<td>• Potentially increased frequency of resampling</td>
</tr>
<tr>
<td></td>
<td>Low = 4 sites</td>
<td>Low = 7 sites</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium = 5 sites</td>
<td>Medium = 7 sites</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High = 6</td>
<td>High = 6</td>
<td></td>
</tr>
</tbody>
</table>
• Resampling would be recommended for a minimum of 9 sites (low and medium risk)
• Public health interventions would be recommended for 11 sites (medium and high risk)

• Resampling would be recommended for a minimum of 14 sites (low and medium risk)
• Public health interventions would be recommended for 13 sites (medium and high risk)

• More stringent requirements for public health interventions
• Greater information gathered on tick population
Figure 5.1: Field sampling was conducted weekly from May to October 2014, alternating between the endemic sites of Murphy’s Point Provincial Park and Turkey Point Provincial Park (red stars).
**Figure 5.2:** The abundance of each *I. scapularis* life stage collected varied throughout the timeframe of sampling at both (a) Turkey Point Provincial Park and (b) Murphy’s Point Provincial Park in Ontario. Adult *I. scapularis* (green line) demonstrated a bimodal peak in the early spring and fall. Nymphal *I. scapularis* (orange line) were active during late spring to early summer. Larval *I. scapularis* (blue line) were active predominately in late summer, but also had a small peak of activity in late spring. Weekly abundance is presented as the percentage of the total abundance of each life stage collected over the sampling period. The weeks of sampling correspond to each month: 1-3 = May, 4-8 = June, 9-12 = July, 13-16 = August, 17-20 = September, and 21-24 = October.
Figure 5.3: This tool can be used to assess the risk associated with detecting *Ixodes scapularis* during active surveillance at sites in Ontario, Canada. To apply this tool, begin at the top
question marked “START”. Follow the arrows based on the answer to each question, and record the score for each question in the boxes on the right-hand side. Points are allocated based on which criteria are fulfilled, with it being possible to collect points from multiple answers. The minimum and maximum points available from each question are listed under the score box as reference. Once the questions have been completed at “FINISH”, tally the points and match to the corresponding risk level. An example for how to calculate the score based on field sampling results is provided in Table 5.2. (Image source: Centers for Disease Control and Prevention).
REFERENCES


Lindsay LR, Mathison SW, Barker IK, McEwen SA, Surgeoner GA. 1999b. Abundance of *Ixodes scapularis* (Acari: Ixodidae) larvae and nymphs in relation to host density and


CHAPTER 6:
GENERAL DISCUSSION

SUMMARY

Over the past two decades, there has been rapid range expansion of *Ixodes scapularis* in Ontario, Canada. This has posed a significant threat to human and animal health, as this tick is the vector for *Borrelia burgdorferi*, the causative agent of Lyme disease, as well as other pathogens. My work, investigating the ecology and epidemiology of *I. scapularis* and the risk of Lyme disease in Ontario, has filled key knowledge gaps, and highlights numerous areas where additional research is still needed.

In 2014, field sampling via tick dragging was conducted at 104 sites to establish the baseline distribution of *I. scapularis* in southern, eastern and central Ontario. *Ixodes scapularis* was detected at 21 sites (20.2%). These coordinated, widespread surveillance efforts illustrated that the distribution of these ticks was much wider than previously-recognized endemic areas. Cluster analysis revealed a hot spot for the tick in eastern Ontario. Three other species of ticks of potential public health significance were collected via field sampling: *Dermacentor variabilis*, *Haemophysalis leporispalustris* and *I. dentatus*. These initial efforts provided the foundational data for two additional field sampling seasons conducted as components of this thesis.

Fifty additional sites were visited in 2015 to increase the sample size and fill in geographic gaps in the data. The number of sites with *I. scapularis* increased to 29 out of 154 (18.8%). Cluster analysis again identified a hot spot in eastern Ontario, but also revealed a secondary cluster in southern Ontario.

Ecological data were collected in conjunction with field sampling for *I. scapularis* at the 154 sites visited in 2014 and 2015. Research has demonstrated that the rise in temperatures due to climate change has increased the suitability of more northern habitats, and will facilitate the ongoing range expansion of the vector in the province (Ogden et al. 2008; McPherson et al.)
Climate is not the only factor that impacts the tick and consideration needs to be given to other ecological factors, as the tick is also impacted by habitat and hosts. Mixed multivariable logistic regression models were created to assess the influence of site-level ecological factors on the presence of *I. scapularis*. Cumulative degree days above zero (°C) was positively associated with *I. scapularis*, while westward longitude was negatively associated with the presence of *I. scapularis*. The relative abundance of shrubs, the density of the understory and the interaction of these two factors were significant in the model as well, with the net effect related to the overall composition of the two understory characteristics.

With a greater understanding of the baseline distribution of *I. scapularis* and the ecological factors that influence the presence of the tick, follow-up field sampling was conducted in 2016. The primary purpose was to assess the spatial spread of the tick. A ‘speed of spread’ model was previously developed by Leighton and colleagues (2012) and estimated the range front expansion of *I. scapularis* at 46 km per year northward. Thirty-six sites within either a 46-km (one year of spread) or a 92-km (two years of spread) buffer of the spatial clusters were visited. The emergence of *I. scapularis* was documented at five sites. Four of these sites were within the 46-km buffer of the primary spatial cluster, which provides short timescale evidence for the estimated speed of range front expansion. The model's predicted year of establishment of *I. scapularis* was compared to field-collected data from all sites, and the speed at which woodland habitats behind the range front acquire *I. scapularis* populations appears to be heterogeneous.

To determine the geographic risk of Lyme disease and other pathogens transmitted by *I. scapularis*, all *I. scapularis* samples were tested for *B. burgdorferi*, *B. miyamotoi*, *Anaplasma phagocytophilum*, and *Babesia microti*. Of the 29 sites with *I. scapularis* in 2014 and 2015, 9 (31.0%) of the sites were positive for *B. burgdorferi*. A significant spatial cluster of *B. burgdorferi* was detected within the same area as the hot spot for *I. scapularis* in 2014. After follow-up field sampling in 2016, an additional five sites had ticks that were positive for *B. burgdorferi*. Three *I. scapularis* samples were positive for *B. miyamotoi* from Murphy’s Point Provincial Park. All samples were negative for all other pathogens.
Data from extensive field sampling conducted at two endemic sites were incorporated into an ecological risk indicator for public health professionals to assist with the risk assessment of reproducing populations of *I. scapularis* based on tick dragging results. Tick dragging is the recommended approach to assess areas for *I. scapularis*, yet limited guidance exists on how to interpret these results to direct future surveillance efforts and public health interventions. Field sampling data were incorporated with previous surveillance research to build the tool. It expands upon the screening tool developed by Ogden and colleagues (2014) and provides an easy-to-use scoring system to estimate the risk level of a reproducing population of *I. scapularis*. The tool performed adequately based on assessment with field sampling data from known endemic sites and sites of unknown status with multiple years of data.

**LIMITATIONS**

There are several limitations of our field sampling approach that should be acknowledged. Sites were selected according to basic selection criteria, which included: forested area, minimum of 0.25 km$^2$ and accessible. Sites that were easily accessible to the field team were predominately conservation areas, provincial parks and other municipal properties, and did not include private tracts of land. Although this ‘convenience’ sampling allowed us to assess the risk of *I. scapularis* in areas where the public is at greatest risk, it limited our geographic scope, and created gaps in our data.

The tick dragging methodology was chosen for numerous reasons. It can be completed quickly and with relatively limited financial investment, which allowed many sites across a widespread area to be visited within the research budget. Tick dragging has also been shown to have high specificity to detect risk, even with one site visit (Ogden et al. 2014). Public Health Ontario employs tick dragging for surveillance and it was a priority that our field sampling approach aligned with this key public health partner (Ontario Agency of Health Protection and Promotion 2015). Despite these advantages, tick dragging for three-person hours once per year is not the ‘gold standard’ approach outlined in the Canadian Consensus on Lyme disease (Health Canada 1991). It can have low sensitivity, especially in areas where *I. scapularis* is emerging, and it is likely that some of the negative sites were falsely classified. It can be highly variable on
a daily, monthly and yearly basis, even within a site, due to the variability of *I. scapularis* activity, and thus limited our analyses to presence and absence of ticks rather than abundance-based measures, such as the density of infected nymphs (Dobson 2013).

Our sample size was limited for some components of the research. We achieved our target sample size for sites to determine the distribution of *I. scapularis* and the ecological factors that influence the presence of the tick. However, for follow-up field sampling, we were only able to revisit a small portion of the sites. This impacted our ability to adequately assess spatial spread and examine potential ecological factors influencing the spatial patterns. The number of *I. scapularis* samples collected throughout the study was small. Accurate calculations for infection prevalence of *B. burgdorferi* and other *I. scapularis*-borne pathogens could not be completed, so the results are again restricted to presence and absence of pathogens.

**SIGNIFICANCE**

The incidence of tick-borne diseases is on the rise in Canada. Since 2009, when Lyme disease was declared a nationally notifiable disease, the number of human cases has increased from 144 (0.4 cases per 100,000) to over 917 (2.6 cases per 100,000) in 2015 (Table 1.1, Chapter 1) (Public Health Agency of Canada 2017). These numbers are most likely an underestimation, as there has been a large degree of underreporting associated with Lyme disease (Hinckley et al. 2014).

Currently, there is no human vaccine for Lyme disease, nor effective, widespread tick control methods (Ogden et al. 2015b). The prevention and control of Lyme disease and other diseases transmitted by ticks therefore relies on four key elements: (1) avoiding areas with ticks, (2) applying personal protective measures such as wearing protective clothing and insect repellent, and completing ‘tick checks’, (3) decreasing environmental exposure, and if deemed necessary, (4) using prophylactic antibiotics (Ogden et al. 2015b). The findings of this thesis have important public health implications on these four areas of tick-borne disease prevention.
The extensive field sampling conducted from 2014 to 2016 provided a widespread assessment of the current distribution of *I. scapularis* and the pathogens that it can transmit. This information provides the foundation for public health measures. With knowledge on where the tick is located, public awareness can be raised and efforts can be directed to public education on methods of tick bite prevention, as mentioned above. This information also forms the basis of risk assessment for prophylactic antibiotic treatment. Based on the recommendations from the Infectious Diseases Society of America, prophylactic antibiotic treatment should only be administered in areas of high Lyme disease risk (infection prevalence ≥20%) (Wormser et al. 2006).

Preliminary findings on the ecological factors influencing the presence of *I. scapularis* can help guide efforts to decrease environmental exposure. Although additional research is needed to understand the relationship of the understory and *I. scapularis* in the province, in areas frequented by the public (e.g., along walking trails and camping areas), efforts can be made to reduce shrubs so the area is less conducive to *I. scapularis*. This is consistent with other landscape modification recommendations (Stafford 2004).

In addition to informing public health of current risk, the results of this thesis may also enhance future public health efforts. The spread of *I. scapularis* within the province is dynamic, and this will require ongoing surveillance efforts from public health to monitor the distribution of the tick. The ecological risk indicator has direct applications to public health and may be a valuable component of their surveillance efforts to assess the current risk of *I. scapularis* and determine how to most appropriately direct future surveillance and preventative efforts, especially when resources are limited.

Numerous predictive models have been developed to understand and forecast the risk of *I. scapularis* and associated tick-borne diseases in Canada (e.g., Koffi et al. 2012; Leighton et al. 2012; Ogden et al. 2013). It is important that field data be collected to validate these predictions, and this was illustrated in this thesis with preliminary data applied to explore the speed of spread model (Leighton et al. 2012). With field data, we can gain additional insight into the underlying ecological processes, and further refine the models, if required. In places where there are...
discrepancies between the predicted and field data, we can generate hypotheses to guide our ongoing research efforts.

**FUTURE DIRECTIONS**

The current landscape of tick-borne disease in Ontario is one of constant change. It has been predicted that with climate change and other ecological changes, *I. scapularis* will continue to move northward and invade the most heavily humna-populated areas of the province (Ogden et al. 2008; McPherson et al. 2017). This not only poses a risk of Lyme disease, but other *I. scapularis*-borne pathogens. In the northeastern United States, there has been a notable emergence of *B. microti*. Although the invasion of this pathogen has occurred at a slower rate in the USA than *B. burgdorferi* due to lower ecological fitness, it is following a similar geographic trajectory, and the presence of *B. burgdorferi* appears to enhance the transmission of *B. microti* (Diuk-Wasser et al. 2016). There has also been a recent and notable rise in the number of human cases of the highly fatal Powassan virus encephalitis in the northeastern USA (Hermance and Thangamani 2017).

Although the focus of this thesis has been on *I. scapularis*, it is important to apply the lesson learned from *I. scapularis* invasion and to acknowledge the risk other tick species may pose in the future. Although there is no current evidence of reproducing populations of the Lone Star tick, *Amblyomma americanum*, it is the most commonly detected adventitious tick species by passive surveillance in Ontario and transmits diseases of human and animal health significance (Nelder et al. 2014).

With this dynamic landscape, surveillance must be a priority. Surveillance encompasses numerous approaches, which serve different purposes and have accompanying benefits and drawbacks. Throughout the process of this thesis research, significant effort has been placed into field sampling via tick dragging and much reflection has been given on the applications and limitations of this approach, especially given the context of vector emergence. This has prompted me to begin a comprehensive review of surveillance for emerging vector-borne diseases to understand the breadth of approaches and needs for the future.
Surveillance in the context of disease emergence poses significant challenges. For instance, if there is low awareness of the tick, pathogen and potential disease risk, which can be common in areas of emergence, there may be a high degree of underreporting, both with public submissions of ticks and case submissions by physicians (Diuk-Wasser et al. 2012; Hinckley et al. 2014; Nelder et al. 2014). Surveillance efforts are planned and implemented at various scales, from local to regional to national levels, and ticks do not respect these arbitrary borders (Braks et al. 2011). This approach can lead to gaps in data, as well as a variety of forms of data that are not standardized and difficult to compare. The complexity of tick-borne diseases also necessitates the involvement of a multitude of stakeholders in surveillance efforts. In many cases, not all stakeholders are involved in the process, which can again lead to gaps in data as well as repeated efforts (Kulkarni et al. 2015; Nadolny et al. 2015).

Given these challenges, and the reality of finite resources for vector-borne disease surveillance, we need to be strategic in our surveillance efforts (Braks et al. 2011). Building upon the knowledge gained from surveillance conducted in this thesis, and the information available in the literature, the proposed review article will serve as the starting point to design an adaptive framework for surveillance that enhances our capacity to monitor for emerging tick-borne zoonoses in Canada.

Surveillance efforts need to be coupled with ongoing research to understand the ecological processes underlying I. scapularis emergence and spread. Foundational knowledge has been provided by this thesis, while many additional questions remain unanswered. These questions represent opportunities for future research.

More detailed examination of habitat factors should be conducted. Preliminary findings of this thesis indicate that the understory composition of woodland habitats influences the suitability of the site for I. scapularis. In-depth data collection on the understory species would provide more valuable information to elucidate this relationship. Additionally, ongoing site-level ecological data collection should be considered alongside surveillance efforts since the process of invasion is ongoing. The model could thus be refined as the tick establishes in new habitats and regions.
Host dynamics play an integral role in tick ecology and the transmission of disease, and have been extensively studied in the past, including in Ontario (Lindsay et al. 1999; Werden et al. 2014). However, there are many aspects of host dynamics that are not understood, or have been overlooked in past studies. In a recent consensus document on Lyme disease ecology, six of the seven key research gaps were related to host populations and their impact on tick abundance and infection prevalence (Kilpatrick et al. 2017). Data collection on small and medium-sized mammals as well as deer were not included in this thesis. Trapping is time and labour-intensive and was not feasible. Deer pellet counts were not recommended given the wide geographic scope of the study, and unfortunately, provincial data on the white-tailed deer populations are not consistently collected or publicly available.

It would be highly beneficial to conduct a comprehensive longitudinal study of the relationship of the host community with *I. scapularis* in Ontario. The geographic scope could be narrowed from this study to focus on the areas within and surrounding the two spatial clusters for *I. scapularis*. A transect of emergence could be created from each cluster to include sites where the tick has invaded and where the tick is at risk of invading. Comprehensive data on the entire host community, including predators and medium-sized mammals would be collected. Field sampling for all stages of *I. scapularis* would also be routinely conducted. Data collection should span numerous years to monitor the process of *I. scapularis* emergence and localized spread. This research can also contribute to a greater understanding of the emergence of *B. burgdorferi*.

In this thesis, we focused strictly on the presence and absence of *B. burgdorferi*, which provided preliminary information on the risk of disease. However, the relatively short duration of this research, and the lack of host data, did not permit us to assess processes related to the emergence of *B. burgdorferi*.

Research has intensified on *B. burgdorferi* sensu stricto in Canada. In Europe, there is a strong association between the species of *Borrelia*, the reservoir hosts and the clinical manifestations in humans (Rudenko et al. 2011). In North America, it was initially thought that *B. burgdorferi* sensu stricto was a generalist and caused the wide range of clinical signs associated with human Lyme disease (Ogden et al. 2015a). However, genetic analysis of the
bacteria has revealed numerous strain types that may be associated more strongly with specific hosts (Ogden et al. 2015a; Mechai et al. 2016; Vuong et al. 2017). Further, there appears to be significant variations in antibody response that may be attributed to strain type (Ogden et al. 2017).

In the future, efforts should be made to not only identify the bacteria, but also the strain of *B. burgdorferi*. The longitudinal study outlined above could provide valuable data on the processes underlying the emergence of *B. burgdorferi* and potential host – strain associations.
REFERENCES


