OCCURRENCE OF *IXODES SCAPULARIS* AND *BORRELIA BURGDORFERI* IN THE FRAGMENTED LANDSCAPES OF EAST-CENTRAL AND NORTHEAST ILLINOIS

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Natural Resources and Environmental Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

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ABSTRACT

The distribution of *Ixodes scapularis* and *Borrelia burgdorferi* has continued expanding in Illinois over the past twenty years. However, the extent to which this tick vector and etiologic agent of Lyme disease has spread is not well known. In east-central Illinois (*ECIL*), I evaluated habitat diversity and temporal changes of *I. scapularis* occurrence and *B. burgdorferi* infection within a natural area in Piatt County, Illinois. In northeast Illinois (*NEIL*), I assessed the geographic distribution and abundance of *I. scapularis* in forest patches of 32 public-access forest preserves in Cook, DuPage, Lake, and McHenry counties. In *ECIL*, small mammals were trapped and attached ticks were collected in young forest, prairie, old forest, and flood plain sites from 2005 – 2009. Small mammal diversity and abundance were compared. Collected ticks were identified to species level based on morphology, and identification was confirmed molecularly. Prevalence of *I. scapularis* (% mammals infested), mean intensity (*I. scapularis* per infested mammal), and relative density (*I. scapularis* per mammal trapped) were calculated. Multiple *I. scapularis* larvae found on a single mammal were pooled for testing; whereas, *I. scapularis* nymphs were tested individually for *B. burgdorferi* infection using polymerase chain reaction (PCR). *Ixodes scapularis* were most abundant in the young forest and prairie sites. The prairie had the highest diversity of small mammal hosts. Out of 2,446 trapped small mammals, 388 were infested with *I. scapularis*. Prevalence, mean intensity, and relative density of *I. scapularis* and prevalence of *B. burgdorferi* infection were highest for the prairie and young forest sites. The overall *B. burgdorferi* infection of *I. scapularis* in the natural area was 14% (56 / 388). In *NEIL*, timed dragging surveys were conducted from May to October 2008 and April to October 2009. A total of 602 *I. scapularis* of all three life stages (larvae, nymphs, and adults)

were collected from 17 of the 32 sites. The highest abundances of *I. scapularis* were found at coastal forested sites near Lake Michigan, and *I. scapularis* appears to be widely distributed throughout the counties of Cook, DuPage, and Lake where suitable habitat is available. The distribution of *I. scapularis* is encroaching upon developed areas, increasing the risk for human exposure to Lyme disease. This study provides baseline data for further evaluation of emerging Lyme disease foci in Illinois. Habitats and reservoir hosts that were previously overlooked may be suitable for *I. scapularis* and *B. burgdorferi* establishment in a dynamic fragmented landscape. This study reveals mechanisms associated with wildlife – vector – pathogen interactions that could influence disease emergence and exposure, and it emphasizes the need to increase research efforts and public awareness concerning the occurrence of *I. scapularis* and the prevalence of *B. burgdorferi* in Illinois.

ACKNOWLEDGEMENTS

I extend my deepest gratitude to Dick Warner and Nohra Mateus-Pinilla for guiding me and stretching my abilities to new heights throughout my entire graduate school experience. I also thank my committee members, Jeff Nelson and Linn Haramis, for their invaluable input, tick expertise, and support. This project could not have been possible without the efforts of my field and lab assistants: B. Danner, N. Rivera, E. Phillips, K. Carberry, M. Ulrich, S. Alvarez, M. Nickols, J. Sheehan, H. Awobode and volunteers. Acknowledgement goes to M. Buhnerkempe, J. Teisburg, L. Kammin, and S. Rao for their contributions to the initial stages of the RAP study. I thank S. Hamer, J. Tsao, S. Callister, S. Lovrich, and D. Jobe for their laboratory expertise and instruction. Thanks to J. Nelson and T. Velat for sharing their tick dragging experiences with me. Access to field sites and facilities were granted by N. Beccue, D. Becker, and the Allerton Park Staff as well as the Forest Preserve Districts of Cook, DuPage, and Lake Counties. Thanks to G. Kelly (Illinois Department of Natural Resources) and to J. R. Rydzewski for providing field housing.

A special thank you to S. Fredebaugh and my fellow graduate students for being a source of encouragement, support, and diversion, especially at the times I needed it the most. Finally, I graciously thank my loving family and my dear friends for always believing in me and for never allowing me to lose sight of my dreams.

My research was funded by the Department of Natural Resources and Environmental Sciences, the Office of Sustainability, the Illinois Natural History Survey, and The Earth and Society Initiative at the University of Illinois Urbana-Champaign.

"In every walk with Nature, one receives far more than he seeks." –John Muir

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LITERATURE REVIEW

1. Lyme disease

1.1 History of Lyme disease

Lyme disease, or borreliosis, is the most commonly reported vector-borne disease of humans in the Northern hemisphere, and one of the fastest-emerging infectious diseases in the United States (Bacon et al. 2008). In the U.S., 27,444 human cases were reported in 2007, surpassing the previous maximum reported in 2002 by 15% (CDC 2009). Although enhanced surveillance accounts for some of this increase, there is a true rise in emergence in certain areas (CDC 2009). In Illinois, there were 108 reported human cases in 2008 compared to only 35 cases in 2000 (IDPH 2010).

Borreliosis was first identified as a disease based on a group of human cases originally thought to be juvenile rheumatoid arthritis reported near Lyme, Connecticut, in 1975 (Steere et al. 1977). Patients developing erythema chronicum migrans, a bull's-eye-shaped rash, at the site of a tick bite led Steere and other researchers to conclude that this "Lyme disease" was similar to symptoms of tick-borne illness reported in Europe (Steere et al. 1977).

Although Lyme disease became accepted as a manifestation of a tick-borne illness, the actual cause of the disease was still not understood clearly. In 1980, Burgdorfer discovered the etiologic agent to be a spirochete, *Borrelia burgdorferi*, which he isolated both from patients diagnosed with Lyme disease and from tick specimens collected on Shelter Island, New York, a Lyme-endemic area (Burgdorfer et al. 1982). Three genomic groups of *B. burgdorferi* have been identified. All strains found in North America belong to the group, *Borrelia burgdorferi sensu stricto* (Steere et al. 1994). Strains from groups 2 and 3 have been found in Europe. Isolates

from group 2 strains have been named *Borrelia garinii* (Baranton et al. 1992), and isolates from group 3 strains have been named *Borrelia afzelii* (Canica et al. 1993).

1.2 Symptoms of Lyme disease

Early manifestations of the disease often include nondescript flu-like symptoms such as fever, fatigue, malaise, and muscle and joint pain (Shapiro and Gerber 2000, Steere et al. 2004, Wormser et al. 2006). Patients typically develop erythema migrans, a characteristic red, bulls-eye rash at the site of the tick bite within 30 days of infection with *B. burgdorferi* (Bacon et al. 2008). An early infection can usually be treated successfully with broad-spectrum antibiotics. However, late manifestations of the infection spread to other parts of the body making it more difficult to treat and usually leading to life-long morbidity. If left untreated in humans, Lyme disease can permanently affect the skin, musculoskeletal, cardiac, and central nervous systems ultimately leading to severe arthritic joint pain, cardiac abnormalities, and neurological damage (Steere et al. 1980, 2004). In addition to affecting humans, domestic animals such as dogs and horses can also contract the disease and suffer from arthritic manifestations (Anderson et al. 1983, Kornblatt et al. 1985).

Diagnosis of Lyme disease is based on clinically observed symptoms and a history of potential exposure to infected ticks in Lyme-endemic geographic regions (Wormser et al. 2006). Lyme disease can be difficult to diagnose, as testing methods can reveal misleading results such as false-negatives. Therefore, two-tiered serologic testing is recommended to confirm infection in patients (*CDC* 2005). The continual emergence of Lyme disease within the U.S., and the expansion of endemic areas are strong reasons for public education on tick avoidance and early treatment interventions (Bacon et al. 2008).

1.3 Transmission of Lyme disease

Hard-bodied ticks of the *Ixodes ricinus* species complex are the major vectors for transmitting the spirochete, *B. burgdorferi* (Burgdorfer et al. 1982, Barbour et al. 1983, Steere 1994). Ticks from this group are found in almost every geographic region of the world. The vector tick endemic in the eastern half of the United States is *Ixodes scapularis* (Anderson et al. 1983, Callister et al. 1988). *Ixodes dammini* in the northeastern United States and *Ixodes scapularis* in the Midwest were once considered two separate species; however, this classification has been disproven and both are now referred to as *I. scapularis* (Steere and Malawista 1979, Levine et al. 1985, Oliver et al. 1993). *Ixodes pacificus* is the vector for Lyme disease in the western United States (Burgdorfer et al. 1985, Bissett and Hill 1987), *Ixodes ricinus* in Europe (Anderson 1989), and *Ixodes persulcatus* in Asia (Dekonenko et al. 1988).

Upon acquiring *B. burgdorferi* from feeding on an infected host, the engorged tick retains the spirochetes in the mid-gut (Piesman et al. 1990). When the tick feeds on a new host during its next life stage, the spirochetes are activated and pass through the salivary glands into the host. This transmission takes about 24 hours to complete (Piesman et al. 1987, Ribiero et al. 1987). Although these ticks may bite humans at any life stage (larva, nymph, or adult), the infected nymph and adult stages are responsible for transmitting the spirochete (Steere et al. 2004). Vertical transmission of *B. burgdorferi* in *I. scapularis* is negligible because it occurs in less than 1% of all larvae (Piesman et al. 1986, Burgdorfer et al. 1992). However, once a tick does become infected as a larva or nymph, it maintains that infection transtadially (Fish 1993). The nymph stage poses the greatest risk for transmitting *Borrelia burgdorferi* infection because of its small size and its peak activity coinciding with increased human activity outdoors in late spring and early summer (Fish 1993).

1.4 Endemic areas of Lyme disease

Lyme disease occurs in Europe (Stanek et al. 1988), Asia (Dekonenko et al. 1988), and North America (Burgdorfer et al. 1982). In the United States, focal endemic areas of Lyme disease include the northeast, the far-west, and the upper Midwest (Steer and Malawista 1979, Steere et al. 1994). The highest infection rate in ticks and the greatest number of human cases occur in the northeast from Massachusetts to Maryland (Burgdorfer et al. 1982, Steere 2006). In contrast, infection rates in the far-west are much lower because the vector competence of *I. pacificus* may be lower than other ticks in the *I. ricinus* complex based on a variety of physiological and ecological reasons (Lane et al. 1991).

In the Midwest, the initial cases of Lyme disease were diagnosed in southeast Minnesota and northwest Wisconsin (Davis et al. 1984, Callister et al. 1988, 1991). Efforts to assess the distribution of *I. scapularis* and *B. burgdorferi* infection within the Midwest have occurred in: Wisconsin (Callister et al. 1991), Michigan (Hamer et al. 2007), Iowa (Lingren et al. 2005), Indiana (Pinger et al. 1996), and Illinois (Guerra et al. 2002, Jobe et al. 2007). There is a need to continuously monitor changes in the spread of *I. scapularis* distribution and *B. burgdorferi* prevalence in the United States to stay abreast of emerging tick-borne disease foci (Cortinas et al. 2002).

2. Ecology of *Ixodes scapularis*

2.1 Life cycle of Ixodes scapularis

The distribution and occurrence of *Ixodes scapularis*, also known as the blacklegged tick or deer tick, is typically associated with environmental factors such as tick habitat suitability (Kitron et al. 1991a, Guerra et al. 2002) and small mammal host abundance (Anderson et al.

1983, Brownstein et al. 2003). Suitable habitat for *I. scapularis* has been characterized by oak-dominated forested areas with sufficient leaf litter and moist, sandy soils (Kitron et al. 1992, Schulze et al. 1998). The distribution and establishment of *I. scapularis* depends upon favorable vegetation, soil, topography, and climate necessary for questing, molting, diapause, and oviposition (Guerra et al. 2002).

The two-year life cycle of *I. scapularis* consists of three stages: larva, nymph, and adult (Piesman and Spielman 1979, Anderson and Magnarelli 1980). Each stage actively quests for a blood meal before molting into the next stage. As stated previously, *Ixodes scapularis* becomes infected with *B. burgdorferi* while feeding on the blood of infected natural reservoir hosts. *Borrelia burgdorferi* is maintained within a tick transtadially (Fish 1993). Therefore, *I. scapularis* has three opportunities to become infected with the spirochete, and two opportunities to spread the spirochete to uninfected hosts.

The larval stage hatches in late summer and takes its first blood meal before overwintering. The nymph stage becomes active late spring to early summer of the following year. After the second blood meal, it molts into the adult stage during fall of the same year. Occasionally the adults overwinter and emerge in early spring of the next year if they were unable to mate in the fall (Steere 1994). Adult ticks feed and mate on their final host. The male takes a small blood meal while the female feeds for up to a week until engorged to repletion. Before dying, an engorged gravid female lays up to 3,000 eggs that hatch to repeat the cycle (Wilson et al. 1990). The seasonal overlap of infected nymphs emerging and feeding before the newly hatched cohort of larvae emerge is a key factor in continually maintaining the enzootic cycle of Lyme disease.

2.2 Hosts of Ixodes scapularis

Ixodes scapularis is a three-host generalist tick. The immature stages feed on a variety of small and medium-sized mammals and birds (Anderson 1988). In particular, the white-footed mouse (*Peromyscus leucopus*) is the primary reservoir host for maintaining *B. burgdorferi* within the environment (Levine et al. 1985, Donahue et al. 1987, Hofmeister et al. 1999). Other small mammals, specifically rodents, also serve as hosts for immature *I. scapularis* (Anderson et al. 1985, 2006, Markowski et al. 1997, 1998); however, they possess a limited reservoir potential for *B. burgdorferi* (Mather et al. 1989, LoGiudice et al. 2003) (Table 1.1.). Evidence implicating a host as a reservoir of infection has previously been based upon either recovering *B. burgdorferi* from host blood, other tissues, and attached ticks or detecting the presence of *B. burgdorferi*-specific antibodies in the host (Anderson et al. 1983, 1986, Bosler et al. 1983, 1984, Magnarelli et al. 1984, 1988). Reinfection of small mammal hosts is common, as some mammals species remain susceptible regardless of previous built-up antibodies (Bunikis et al. 2004). Therefore, the availability of hosts to maintain the *Borrelia* cycle in the United States is almost limitless.

Adult *I. scapularis* feed and mate on larger animals such as their primary host, the white-tailed deer (*Odocoileus virginianus*) (Piesman et al. 1979, Bosler et al. 1984). Without deer in a natural area, adult *I. scapularis* lack a host on which to find each other and mate, likely resulting in lower tick densities (Wilson et al. 1988). Although deer are not a competent reservoir for *B. burgdorferi*, they play an important role in *I. scapularis* dispersal along riparian corridors (Telford et al. 1988). Increased populations of deer in the Midwest, especially in Illinois, have been associated with the increasing dispersal of *I. scapularis* to new areas via deer movement along riparian corridors (Cortinas and Kitron 2006). The spread of *I. scapularis* by deer

contributes to the increase of immature tick occurrence among potentially infected small mammals (Steere 1994, Jobe et al. 2006).

Both the white-footed mouse and the white-tailed deer are considered habitat generalists (Adler and Wilson 1987, Rosenblatt et al. 1999). Their population density, productivity, and survival can shift as environmental conditions change in quality. This adaptability to a variety of habitat conditions results in population flexibility that enables these generalists to occupy poorer habitats (Adler and Wilson 1987). Fragmentation of habitat into small forest patches has been associated with decreased vertebrate species diversity and increased densities in *P. leucopus* (Adler and Wilson 1987, Rosenblatt et al. 1999). The net effect of decreasing habitat patch size is an increasing fraction of *I. scapularis* feeding on a *B. burgdorferi* competent reservoir, thus increasing the risk for nymphal infection prevalence (Ostfeld and Keesing 2000, Allan et al. 2003). Although the ecological and spatial determinants of variation in *I. scapularis* distribution and habitat use are still not well understood at the local-scale level (Goodwin et al. 2001), declining habitat quality, increased habitat fragmentation, and decreased biodiversity appear to have significant roles in the overpopulation of the white-footed mouse and white-tailed deer (Rosenblatt et al. 1999), the establishment of *I. scapularis* (Ginsberg 1994), and an increased risk of Lyme disease (Van Buskirk and Ostfeld 1995, LoGiudice et al. 2003).

Some studies have shown that increased biodiversity leads to a "dilution effect" of immature *I. scapularis* acquiring *B. burgdorferi* infection because the ticks feed on more hosts with decreased reservoir-competency (Van Buskirk and Ostfeld 1995, Ostfeld and Keesing 2000, LoGiudice et al. 2003). The "dilution effect" implicates the need to maintain biodiversity as a tool to reduce disease risk (Schmidt and Ostfeld 2001). In contrast, Estrada-Peña (2009) concluded that neither species richness nor host density alone had a significant effect on the level

of *B. burgdorferi* infection within a site. Infection index varied at the habitat patch level compared to either the county-level or state-level of infection (Estrada-Peña 2009). A comprehensive evaluation of all vertebrate diversity in a habitat could reveal a better understanding of existing ecological interactions influencing the survival of *B. burgdorferi*-competent reservoirs and creating a potential dilution, or amplification, effect (Ogden and Tsao 2009).

3. Collecting and Testing Methods

Densities of questing *I. scapularis* within a natural area can be estimated if surveyed during the peak activity season for each life stage. Common methods developed to survey for *I. scapularis* in a site include: walk sampling, drag sampling, small mammal trapping, and CO_2-baited traps (Ginsberg and Ewing 1989, Falco and Fish 1992). The most successful methods for collecting immature *I. scapularis* include small mammal trapping and drag sampling (Falco and Fish 1992). However, larvae reside in the leaf litter on the ground and are difficult to see on a drag cloth. Therefore, small mammal trapping provides a more accurate estimate of larval *I. scapularis* abundance, especially detection at low population levels (Ginsberg and Ewing 1989).

Drag sampling, which involves pulling a white flannel or corduroy cloth along low vegetation, leaf litter, and trail edges in deciduous forest habitats, is a common method to collect adult ticks (Callister et al. 1991, Diuk-Wasser et al. 2006). Dragging effort is determined by either set transects of a specified distance or by a time allotment. Sampling should be repeated at least three to six times during the season to maximize tick collection during peak times of activity (Diuk-Wasser et al. 2006).

Collected ticks must be accurately identified to species and life stage based on morphology using microscopy and tick identification keys (Sonenshine 1979). Identifying endemic Lyme disease foci where *I. scapularis* have become established has been accomplished by isolating *B. burgdorferi* from collected tick specimens or host tissues (Anderson et al. 1985, Callister et al. 1988, 1991). *Borrelia burgdorferi* is commonly identified from tick or tissue samples by culture (Barbour 1984, Johnson et al. 1984, Callister et al. 1990) and by polymerase chain reaction (PCR) techniques (Picken et al. 1997, Piesman et al. 2001).

To culture *B. burgdorferi*, the mid-gut and salivary glands removed from live *I. scapularis* ticks, or host tissue such as the spleen, kidney, bladder, or ear biopsy, are placed in Barbour-Stonner-Kelly Medium (BSK II) (Barbour 1984). The cultures are then examined for viable spirochetes by dark field microscopy for 10 to 21 days. Culturing tick and host tissue has aided in determining the risk of Lyme disease in a given area (Nelson et al. 1991, Callister et al. 1991, Jobe et al. 2007).

Borrelia burgdorferi can also be isolated from tick and mammal tissue and amplified to an identifiable level using the polymerase chain reaction (PCR) method (Piesman et al. 2001). DNA from tick or host tissue can be tested for the presence or absence of spirochetes (Sinsky and Piesman 1989). Gene segments of *Borrelia* that are commonly amplified through PCR include: *Osp*A (Malloy et al. 1990), *Osp*C (Jauris-Heipke et al. 1995), segments of the flagellin gene (Lebech et al. 1991, Picken 1992), and 16S rRNA (Marconi and Garon 1992, Liebisch et al. 1998). Sensitivity of PCR amplification between studies and type of gene segment amplified varies, but many are sensitive enough to detect positive samples containing a minimum 10^0 to 10^{-1} organisms (Bunikis et al. 2004).

4. *Ixodes scapularis* and *Borrelia burgdorferi* in Illinois

Populations of both the vector tick and the spirochete were once limited to a small endemic area in Minnesota and Wisconsin (Davis et al. 1984, Callister et al. 1988). Although *I. scapularis* was initially detected along the Rock River in Ogle County in the late 1980s (Bouseman et al. 1990), Illinois has not historically been considered a Midwestern endemic focus for Lyme disease (Picken et al. 1995). Studies assessing the occurrence of *I. scapularis* near major metropolitan areas such as Milwaukee and Chicago in the early 1990s showed no evidence of tick populations within these areas (Callister et al. 1991). However, Picken et al. (1995) suggested that Illinois may eventually be considered an area of focal endemicity for both *I. scapularis* and *B. burgdorferi* based on the positive findings of several studies (Kitron et al. 1991b, Nelson et al. 1991). Additional foci of established *I. scapularis* distribution have been detected along the Rock and Mississippi Rivers, and more recently along the Illinois River (Cortinas et al. 2002). By 2007, Jobe et al. confirmed the presence of *B. burgdorferi*-infected *I. scapularis* at sites in northeast Illinois located less than one mile from city limits, and recognized that Lyme disease could become a significant health concern around the Chicago metropolitan area. The risk of Lyme disease exposure is increasing throughout Illinois, most recently emerging in the northeast area of the state (Jobe et al. 2006, 2007).

The landscape of Illinois is highly fragmented and characterized by extensive farming, expanding development, and urbanization. Therefore, forest habitats suitable for *I. scapularis* establishment are discontinuous, occurring primarily along riparian corridors abutting residential and recreational areas (Guerra et al. 2002, Cortinas et al. 2002). Most of the remaining natural areas in Illinois have become state parks, nature preserves, and wildlife areas with high volumes of visitor traffic (Cortinas et al. 2002). Because these natural areas are small and fragmented,

they hold high potential for dense establishment of *I. scapularis*. This situation creates concern for increased disease transmission in Illinois. Assessing the habitat diversity and the spatial distribution of *I. scapularis* and the prevalence of *B. burgdorferi*, especially in regions of the state where this information is unknown, is essential to develop preventive measures where a potentially serious disease may be encroaching upon urban and suburban areas (Guerra et al. 2002, Jobe et al. 2007). People who never considered being at risk for coming into contact with *I. scapularis* or *B. burgdorferi* in developed landscapes of Illinois may become vulnerable to an increased exposure to Lyme disease.

Bouseman et al. (1990) hypothesized that the likely routes for the expansion of *I. scapularis* distribution and *B. burgdorferi* prevalence in Illinois would follow along major rivers. Riparian corridors provide suitable tick habitat and act as dispersal and migratory routes for white-tailed deer and birds (Cortinas et al. 2002). Kitron et al. (1991a) added validity to this hypothesis by finding that infested deer tended to cluster around the Rock and Mississippi Rivers. In 2006, Cortinas and Kitron confirmed that *I. scapularis* in northwest Illinois has continued expanding its range southward along the Illinois River. Based on the evidence for the riparian corridor hypothesis thus far, it is possible that expansion of *I. scapularis* distribution could increase along the Fox and Des Plaines Rivers of northeast Illinois and the Sangamon River of east-central Illinois. A Midwest model of *I. scapularis* habitat suitability predicted suitable forested habitats along the Des Plaines River in northeast Illinois (Guerra et al. 2002).

To date, mapping the spatial distribution of *I. scapularis* establishment within Illinois has been limited to nonstandardized reporting (IDPH 2009). There are numerous parks and forest preserves throughout the fragmented landscapes of northeast and east-central area of the state with suitable *I. scapularis* that have not been evaluated. Many of these natural areas were

predicted to be ideal habitat for small mammal hosts and *I. scapularis* populations (Guerra et al. 2002). Therefore, *I. scapularis* may be established in areas thought to be free of the tick and the Lyme disease-causing spirochete. In reality, these areas could merely be overlooked due to lack of reporting (Madhav et al. 2004). Several studies have clarified the need to maintain surveillance of *I. scapularis* range expansion and *B. burgdorferi* prevalence and to identify focal endemic areas of Lyme disease risk (Callister et al. 1991, Falco et al. 1995).

Overall, my research builds upon previous work assessing *I. scapularis* distribution in northeast and east-central Illinois. More specifically, it highlights the significance of *I. scapularis* expanding its range in the state, potentially increasing the endemic range of *B. burgdorferi*. Emerging Lyme disease foci create health concerns of increased disease risk among humans, especially in heavily populated metropolitan areas. This study provides baseline distribution data that will be useful to further evaluate the occurrence of *I. scapularis* and *B. burgdorferi* in Illinois to improve disease prevention efforts.

Chapter 1 meets the formatting requirements for *Vector-Borne and Zoonotic Diseases.*

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6. Tables

Table 1.1. Known natural reservoir hosts of *Borrelia burgdorferi* and their reservoir competence of infecting *Ixodes scapularis*.

Reservoir Host	Scientific name	Reservoir Competence (%)	Source
White-footed mouse	*Peromyscus leucopus*	90	Mather et al. 1989
Chipmunk	*Tamias striatus*	75	Mather et al. 1989
Sorex shrews	*Sorex spp.*	51.2	LoGiudice et al. 2003
Northern short-tail shrew	*Blarina brevicauda.*	41.8	LoGiudice et al. 2003
Grey squirrel	*Sciurus carolinensis*	14.7	LoGiudice et al. 2003
Meadow vole	*Microtus pennsylvanicus*	5.5	Mather et al. 1989

CHAPTER 2:

IXODES SCAPULARIS AND *BORRELIA BURGDORFERI* AMONG DIVERSE HABITATS WITHIN A NATURAL AREA IN EAST-CENTRAL ILLINOIS

1. Introduction

Steadily increasing in the number of reported cases over recent years, Lyme disease is the most common vector-borne disease in the U.S. In 2007, 27,444 human cases were reported, surpassing the previous maximum reported in 2002 by 15% (CDC 2009). Although enhanced surveillance accounts for some of this increase, there is a true rise in emergence in certain areas (CDC 2009). In Illinois, there were 108 reported human cases in 2008 compared to only 35 cases in 2000 (IDPH 2010).

Lyme disease is caused by the spirochete *Borrelia burgdorferi sensu stricto* and is transmitted by the blacklegged tick, *Ixodes scapularis*, in the Northeast and Midwest United States (Burgdorfer et al. 1982, Davis et al. 1984, Lane et al. 1991). In humans, *B. burgdorferi* infection can result in dermatologic, musculoskeletal, cardiac, or neurologic abnormalities (Shapiro and Gerber 2000, Steere et al. 2004, Wormser et al. 2006). If left untreated, Lyme disease leads to persistent arthritis and neurological damage (Spielman et al. 1985).

Borrelia burgdorferi is transmitted to immature *I. scapularis* during a blood meal from an infected host (CDC 2009). *Ixodes scapularis* is a three-host generalist tick that feeds on a wide variety of small mammal hosts during its immature stages (Mather et al. 1989). Once infected, *I. scapularis* ticks maintain the spirochete transtadially (Fish 1993). The white-footed mouse (*Peromyscus leucopus*) is the primary reservoir host for both *I. scapularis* and *B. burgdorferi* (Levine et al. 1985, Donahue et al. 1987, Hofmeister et al. 1999). Other small mammals,

specifically rodents, also serve as hosts for immature *I. scapularis* (Anderson et al. 1985, 2006, Markowski et al. 1997, 1998); however, they possess a limited reservoir potential for *B. burgdorferi* (Mather et al. 1989, LoGiudice et al. 2003). Reinfection of small mammal hosts is common, as some species remain susceptible regardless of previous built-up antibodies (Bunikis et al. 2004a). Adult *I. scapularis* feed and mate on larger mammals such as the white-tailed deer (*Odocoileus virginianus*). Although deer are not competent reservoirs for *B. burgdorferi*, they play an important role in tick dispersal along riparian corridors. A gravid female *I. scapularis* can drop from a deer in a new area and lay up to 3,000 eggs that hatch to feed on potentially infected small mammal populations (Jobe et al. 2006, Steere 1994).

In the Midwest, populations of both the vector tick and spirochete were once limited to southeast Minnesota and northwest Wisconsin (Davis et al. 1984, Callister et al. 1988, 1991); however, the focal endemic area has expanded farther into Illinois (Bouseman et al. 1990, Cortinas and Kitron 2006) and the surrounding states (Pinger et al. 1996, Walker et al. 1998, Lingren et al. 2005, Hamer et al. 2007) in the past twenty years. In Illinois, Lyme disease has mainly been identified in the northern third of the state (Kitron et al. 1991, Nelson et al. 1991, Jobe et al. 2006, 2007); however, *I. scapularis* has been both identified and predicted in several parts of the state (Guerra et al. 2002) and its distribution has continued to increase in recent years (IDPH 2009).

The distribution and occurrence of *I. scapularis* is typically associated with environmental factors such as tick habitat suitability (Kitron et al. 1991, Guerra et al. 2002) and small mammal host abundance (Anderson et al. 1983, Brownstein et al. 2003). Although the ecological and spatial determinants of variation in *I. scapularis* distribution and habitat use are not well understood at the local-scale level (Goodwin et al. 2001), fragmentation of habitat into

small forest patches has been associated with decreased vertebrate species diversity and increased densities in *P. leucopus* (Adler and Wilson 1987, Rosenblatt et al. 1999). The net effect of decreasing patch size is an increasing fraction of *I. scapularis* feeding on a *B. burgdorferi* competent reservoir, thus increasing the risk for nymphal infection prevalence (Ostfeld and Keesing 2000, Allan et al. 2003).

Suitable habitat for *I. scapularis* has been characterized by oak-dominated forested areas with sufficient leaf litter and moist, sandy soils (Kitron et al. 1992, Schulze et al. 1998). The importance of habitat suitability is emphasized by a predictive model for the distribution and establishment of *I. scapularis* in Illinois based on vegetation, soil, topography, and climate necessary for questing, molting, diapause, and oviposition (Guerra et al. 2002). To my knowledge, recent studies of *I. scapularis* distribution and abundance have only been conducted in forested sites.

The spatial variation and dynamism of the Lyme disease system highlights the need for a more comprehensive approach to study *I. scapularis* and *B. burgdorferi* habitat suitability in small natural areas (Killilea et al. 2008). Previous studies show consistent findings that some natural areas have a higher association with risk and incidence of Lyme disease than others (Allan et al. 2003, Killilea et al 2008). Both decreased biodiversity and increased habitat fragmentation appear to have significant roles in the overpopulation of *P. leucopus*, the establishment of *I. scapularis*, and the risk of Lyme disease in a natural area.

Illinois, once dominated by prairie, is now characterized by a highly fragmented landscape where forest patches are surrounded by urban areas, remnant prairie patches, and agricultural lands (Rosenblatt et al. 1999). In this study, I evaluated habitat diversity and temporal changes of *I. scapularis* occurrence and the maintenance of *B. burgdorferi* among small

mammal hosts within a natural area in east-central Illinois. Prior to this study, the presence of *B. burgdorferi* has not been evaluated in this region; although, *I. scapularis* has been identified.

The objectives of this study were 1) to determine *I. scapularis* occurrence and *B. burgdorferi* infection across four habitats within the natural area, and 2) to evaluate differences in *I. scapularis* occurrence and *B. burgdorferi* infection within the same natural area over a five-year period. To accomplish these objectives, I live-trapped small mammals and collected attached ticks; tested *I. scapularis* for *B. burgdorferi* infection using PCR and qPCR assays; measured small mammal diversity and abundance; and calculated *I. scapularis* prevalence, mean intensity, and relative density (Margolis et al. 1982).

2. Materials and Methods

2.1 Study area

The study was conducted between 2005 and 2009 in Robert Allerton Park (RAP), a 614 ha natural area owned by the University of Illinois located 6.4 km southwest of Monticello, in Piatt County, Illinois. The park is bisected by the Sangamon River; the south half is a natural area consisting of a river corridor, a flood plain, upland and bottomland forests, meadows, and prairie surrounded by an intensively farmed agricultural landscape (Fig. 2.1.). I chose four sites representative of the habitats found within the park. Site 1 is an upland young successional forest comprised of oak-hickory (*Quercus-Carya*) stands and heavy undergrowth (Wang et al. 2008). Site 2 is a restored tall-grass prairie mainly comprised of big bluestem and Indiangrass (*Andropogon gerardii*, *Sorghastrum nutans*). Site 3 is a mature successional forest comprised of old-growth oak-hickory (*Quercus-Carya*) stands. Site 4 is a floodplain of the Sangamon River

dominated by silver maple and green ash (*Acer saccharinum, Fraxinus pennsylvanica*) (Wang et al. 2008).

2.2 Sampling frame

A 100 m x 100 m grid consisting of 100 trapping stations set 10m apart was established within each habitat site similar to the methods described by Kitron et al. 1991. Two 5.08 x 6.35 x 16.51cm Sherman live traps (H.B. Sherman Traps, Tallahassee, FL) baited with sunflower seeds were placed 1m apart at each trapping station for a total of 200 traps per night at each site. Traps were set in the late afternoon and retrieved the following morning. Trapping took place from June through October 2005 – 2009 with the exception of two trap nights in May 2007 and one in November 2008. Each site was trapped between 3 – 11 nights per year (mean = 6.3, SD = 0.26), and between 30 – 33 nights over the study period (mean = 31.5, SD = 1.29).

2.3 Small mammal processing

Captured mammals were restrained, identified, sexed, weighed, ear-tagged (National Band and Tag, Newport, KY), and examined for ticks. Ticks were removed using tweezers and placed in 70% ethanol vials for later identification and lab analysis. One 2-mm ear punch biopsy was taken from each mammal using a sterilized circular ear punch (National Band and Tag, Newport, KY) and placed in 70% ethanol (Sinsky and Piesman 1989). Recaptured mammals were similarly processed and their ear tag numbers were recorded. Following examination, the small mammals were released at the trapping site. All procedures were approved under animal care and biosafety protocols at the University of Illinois Urbana-Champaign.

2.4 Lab analysis

Identification of the ticks to life stage and species was performed under a dissecting microscope using an identification key (Sonenshine 1979). Multiple *I. scapularis* larvae from a single host were then pooled and tested as one unit; whereas, nymphs were tested individually. Total DNA from the tick-pools and ear biopsies was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following a modified version of the Qiagen supplementary protocol: Purification of Total DNA from Ticks for Detection of *Borrelia* DNA. The DNA samples were tested for *B. burgdorferi* at Michigan State University using either 1) a nested polymerase chain reaction (PCR) for the 16S – 23S rRNA intergenic spacer region (IGS) of *Borrelia* spp. (Bunikis et al. 2004b) followed by visualization with gel electrophoresis or 2) a quantitative PCR (qPCR) of a region of the 16S rRNA of *B. burgdorferi* (Tsao et al. 2004). Preliminary experiments showed that both tests were comparable in sensitivity and were able to detect positive samples containing a minimum $10^0 – 10^{-1}$ organisms.

2.5 Statistical analysis

The number of small mammals and *I. scapularis* were calculated by habitat and by year. I used three measures to evaluate habitat diversity and temporal changes of *I. scapularis* occurrence: prevalence of *I. scapularis* infestation (proportion of mammals that were infested), mean intensity of infestation (number of *I. scapularis* per infested mammal), and *I. scapularis* relative density (number of *I. scapularis* per mammal). Prevalence of *B. burgdorferi* infection was quantified by the proportion of positive tick-pools from infested mammals. Differences in number of small mammals, number of *I. scapularis*, mean intensity of infestation and *I. scapularis* relative density across habitats and years were compared using negative binomial

regression. Ratios (R) of means were reported and used to quantify the differences. Because number of trapnights did not distribute equally among all sites and years, it was adjusted in the regression model. Prevalence of *I. scapularis* infestation and *B. burgdorferi* infection were evaluated using logistic regression. Odds ratios (OR) were used to quantify the differences in prevalence. If sparse data were observed (expected count < 5), an exact test was used. Statistical analyses were performed using SAS software (Version 9.1.3; Statistical Analysis System Institute Inc., Cary, NC). A $P \leq 0.05$ was considered significant in this study.

3. Results

3.1 Small mammal abundance and diversity

A total of 2,446 small mammals captured (including recaptures) were examined for ticks from 2005 through 2009. The white-footed mouse (*Peromyscus spp.*) comprised the vast majority (96%) of individuals captured in all habitats. Other species processed include: the meadow jumping mouse (*Zapus hudsonius*) in the young forest, flood plain, and prairie; and the prairie vole (*Microtus ochrogaster*) and the Western harvest mouse (*Reithrodontomys megalotis*) only found in the prairie. The abundance of small mammals captured was significantly different ($P < 0.001$) across the four habitats. The abundance was highest for the flood plain (n = 844) followed by the old forest (n = 739) and young forest (n = 724), then the prairie (n = 139). Despite the prairie having the lowest abundance of mammals, it had the highest diversity of species captured (Table 2.1.).

3.2 Ixodes scapularis abundance

A total of 1,009 immature *I. scapularis* ticks (977 larvae and 32 nymphs) were collected from the small mammals (Table 2.1.). In addition, I identified 290 immature *Dermacentor*

variabilis ticks, found primarily in the old forest and flood plain, but did not include them in the analysis. The abundance of *I.* scapularis was also different ($P < 0.001$) across the four habitats. The young forest had the highest abundance of *I. scapularis* (n = 676), followed by the prairie (n = 176), the old forest (n = 127), and the flood plain (n = 30).

3.3 Ixodes scapularis occurrence

Looking at differences in *I. scapularis* occurrence between habitats, prevalence of *I. scapularis* infestation did not vary between the prairie and the young forest (the reference group for site) ($P = 0.983$) (Table 2.2.). However, the odds of infestation per trapnight for the young forest were 5 and 37 times higher compared to the old forest ($P < 0.001$) and the flood plain ($P < 0.001$) respectively (Table 2.2.). Intensity of infestation showed the same pattern with the greatest number of *I. scapularis* per infested mammal found in the prairie and the young forest ($P = 0.498$). These two habitats had, on average, twice as high an intensity of infestation as both the old forest ($P < 0.001$) and the flood plain ($P = 0.001$) (Table 2.2.). Based on the outcome of the previous measures, the density of *I. scapularis* was also highest for the prairie and the young forest ($P = 0.913$), followed by the old forest ($P < 0.001$), and finally the flood plain ($P < 0.001$) (Table 2.2.).

Over the study period, the odds of infestation prevalence per trapnight compared to 2005 (the reference group for year) only increased significantly in 2009 (OR = 2.4 (1.5, 3.9), $P < 0.001$) (Fig. 2.2a.). In addition, 2009 had the highest relative density (0.6 *I. scapularis* per mammal), and the second highest mean intensity (3.0 *I. scapularis* per infested mammal) (Figs. 2.2b, 2.2c.). Interestingly, 2006 had the second highest abundance of mammals (n = 738) with the lowest prevalence of infestation (11.2%), yet the highest mean intensity of infestation (3.7 *I.*

scapularis per infested mammal) (Figs. 2.2a, 2.2b). Despite a considerably small number of mammals captured in 2007 (n = 55), prevalence and mean intensity of *I. scapularis* infestation were third highest, and relative density was second highest (Figs. 2.2a – 2.2c).

3.4 Borrelia burgdorferi prevalence

In total, 388 *I. scapularis* pools and 861 ear tissue samples were tested for *B. burgdorferi* by either PCR or qPCR. Out of 388 *I. scapularis* pools, 56 were positive, for an overall infection prevalence of 14% (Table 2.3.). The highest proportion of positive tick pools was found in the prairie (27%). The odds of *I. scapularis* pools testing positive in the prairie were twice as high as the young forest; however, the *P* value is at the maximum limit of significance ($P = 0.05$) (Table 2.3.). The sample sizes of *I. scapularis* pools testing positive for *B. burgdorferi* were sparse in the old forest (n = 5) and the flood plain (n = 1) making it difficult to accurately compare differences with the number of positives in the young forest (Table 2.3.). Because of the small sample size of total positive *I. scapularis* pools (n = 56), more data are demanded to confirm the differences across sites. Although the abundance of *I. scapularis* varied across years ($P < 0.001$), the prevalence of *B. burgdorferi* infection within RAP did not significantly vary over the study period ($P = 0.992$). The exception being 2006, when none of the samples tested positive (Fig. 2.3).

Out of 861 ear biopsies that were tested, only two samples were positive for *B. burgdorferi*: a *Z. hudsonius* from the flood plain in 2005 and an *M. ochrogaster* from the prairie in 2009. The DNA sequence of the 16S – 23S rRNA IGS of *B. burgdorferi*, extracted from the *Z. hudsonius* ear tissue, was determined to validate the PCR assay and determine the strain identity. The IGS product was purified (Qiagen PCR Purification Kit; Qiagen, Valencia, CA)

and the sequence was determined using the inner primers on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequence was aligned with the prototypical strains published in Bunikis et al. (2004b) using the program MEGA (Tamura et al. 2007). The isolated *B. burgdorferi* strain was IGS type 4D, which is a ribosomal spacer type (RST) 3 spirochete. All 3 RST groups of *B. burgdorferi* have been associated with human skin lesions and cultures (Liveris et al. 1995).

Given the large sample size of ear biopsies, I expected a higher proportion of them to test positive. Because *B. burgdorferi* infection in *I. scapularis* pools averaged 14% overall, the infection prevalence of the ear biopsies should be similar. Reasoning for this low positive outcome in the ear biopsies is discussed in the next section.

4. Discussion

The effect of habitat diversity on the occurrence of *I. scapularis* and the prevalence of *B. burgdorferi* infection among small mammal hosts in a natural area is not well known. In the highly fragmented region of east-central Illinois, the Sangamon River is a vital riparian corridor among small forest and remnant prairie patches surrounded by agricultural fields and urban areas (Rosenblatt et al. 1999). This landscape produces a complex but relatively small network of habitat diversity with unknown effects on the dynamics of *I. scapularis* occurrence and *B. burgdorferi* infection among small mammal hosts. The results of this study support the hypothesis that *I. scapularis* distribution continues to expand in Illinois (Cortinas and Kitron 2006, IDPH 2009) and that environments suitable for transmission of Lyme disease continue to emerge, specifically in the east-central region of the state.

The data of this study indicate differences in *I. scapularis* prevalence, mean intensity, and relative density between habitats, with the prairie and young forest having greater measures of *I. scapularis* occurrence than both the old forest and the flood plain ($P < 0.001$). The prevalence of *I. scapularis* infestation among trapped small mammals ranged from 40% in the prairie to 33% in the young forest, 11% in the old forest, and 2% in the flood plain. Although these results are comparable to small mammal studies in forest patches in northwestern Illinois (Kitron et al. 1991, Mannelli et al. 1994, Slajchert et al. 1997), this study is the first to report a prairie habitat supporting the highest prevalence of *I. scapularis* infestation compared to the forested sites within the same natural area.

Another important outcome of this study indicates that *B. burgdorferi* is present in the park. The highest prevalence of *B. burgdorferi* infection was found in *I. scapularis* pools from the prairie (27%) followed by the young forest (15%), the old forest (6%), and the flood plain (6%). This result is of pivotal importance to evaluate the risk of Lyme disease transmission in an area where the mechanisms of host-vector coexistence and the maintenance of *B. burgdorferi* remain equivocal. Although *B. burgdorferi* is present within the park, there was no significant temporal change of *B. burgdorferi* infection across the study period, with the exception of zero positive samples in 2006. Upon investigation, no issues regarding the sample collection, DNA extraction, or PCR assays were discovered that would cause the negative data for 2006.

Surprisingly, only two ear biopsies were positive for *B. burgdorferi*. Because 14% of the *I. scapularis* pools tested positive, a similar number of ear biopsies should be positive as well. The ear punch method has shown successful sensitivity for both culture and PCR (Sinsky and Piesman 1989, Barthold et al. 1991, Hofmeister et al. 1992, Marshall et al. 1994). However, there are several factors that may have contributed to the low number of positive ear biopsies in

my study. The density of spirochete growth has been shown to increase as the position of the ear punch moved inward toward the center and base of the ear (Sinsky and Piesman 1989, Hofmeister and Childs 1995). These researchers found that most negative samples were taken from peripheral ear tissue. In addition, by repeatedly sampling individuals, the proportion of positive punches noticeably decreased at 16 weeks post-infection (Sinsky and Piesman 1989). The location of the ear punches (peripheral versus central tissue) most likely varied within my study. Non-infested mammals may have been infected with *B. burgdorferi* more than 16 weeks prior to capture, suggesting low to absent spirochetemia in ear tissues at the time of capture.

It is also understood that the number of visible spirochetes in infected tissue greatly decreases as infection progresses, as seen in the histology study of Barthold et al. (1991) and in human Lyme disease tissues. Marshall et al. (1994) tested preserved museum specimens of *P. leucopus* for *B. burgdorferi*. Only 2 of 280 samples were positive using nested PCR for *Osp*A. In this case, low positive results were most likely caused by inhibitory effects of specimen preservation methods. However, Marshall et al. (1994) state that a small sample of skin (2 mm) may contain low copy numbers of *Borrelia* DNA. Distribution of spirochetes may vary across the tissue, especially between the ear periphery and the center. Tick larvae feed for 2-3 days (Fish 1993), so they may accumulate a greater spirochetal load compared to the ear tissue, leading to a higher proportion of *I. scapularis* pools testing positive compared to the ear biopsies.

Small mammals from the flood plain accounted for 35% (844) of total captures, yet very few *I. scapularis* were found -- 3% (30) of total *I. scapularis* collected. Out of 16 *I. scapularis* pools from the flood plain, only one was positive for *B. burgdorferi*. Therefore, the flood plain accounts for many of the negative ear biopsies based on high mammal abundance but extremely low *I. scapularis* occurrence and *B. burgdorferi* prevalence. In addition, 64% (555) of ear

biopsies were collected in 2006, when no *I. scapularis* pools or ear biopsies tested positive, thus accounting for another large portion of negative samples. However, further investigation is needed to resolve this discrepancy between *B. burgdorferi* prevalence in *I. scapularis* pools and in mammal ear biopsies.

In contrast to small mammal studies in hyper endemic areas of Lyme disease, the overall prevalence of *B. burgdorferi* infection in RAP (14%) was lower compared to 21% in northwest Illinois (Kitron et al. 1991), 29% in Massachusetts (Levine et al. 1985), 29% in Maryland (Anderson et al. 2006), 20-55% in Wisconsin (Godsey et al. 1987), and 94% in New York (Fish and Daniels 1990). Although this study utilized PCR assays to test for *B. burgdorferi*, the overall prevalence within the park is also lower than the prevalence of *I. scapularis* infection (32-37%) reported from tick dragging studies in northeast Illinois utilizing culture methods (Jobe et al. 2007). The prevalence of *B. burgdorferi* at RAP is still relatively low; nonetheless, it has been recommended that a person bitten by a tick in areas where the percentage of *B. burgdorferi*–infected *I. scapularis* exceeds 20% receive prophylactic antibiotic treatment (Nadelman et al. 2001, Wormser et al. 2006, Jobe et al. 2007).

Surprisingly, the prairie had the highest occurrence of *I. scapularis* (although not statistically different from occurrence in the young forest), despite having the lowest abundance of small mammals. The prairie also had the highest observed diversity of small mammals, including *Peromyscus spp.* as well as *M. ochrogaster*, *R. megalotis*, and *Z. hudsonius*. These additional species may serve as substitute or additional hosts for *I. scapularis* where *P. leucopus* is less abundant (Mannelli et al. 1993, Markowski et al. 1997).

Of additional interest in this study, all positive *I. scapularis* pools and one positive ear biopsy from the prairie came from the prairie vole, *M. ochrogaster*. Although the meadow vole

(*Microtus pennsylvanicus*) has been reported as a competent reservoir for *B. burgdorferi*, (Anderson 1988, Markowski et al. 1998), no reports to date describe *M. ochrogaster* as a potential reservoir host for *B. burgdorferi*. Mather et al. (1989) reported that the prairie does not qualify as suitable habitat for *I. scapularis* populations, so the potential for small mammal reservoir hosts of *B. burgdorferi* in this habitat may have been overlooked.

Based on previous studies, decreased biodiversity and increased habitat fragmentation appear to have significant roles in the overpopulation of *P. leucopus* (Rosenblatt et al. 1999), the establishment of *I. scapularis* (Ginsberg 1994), and the risk of Lyme disease (Van Buskirk and Ostfeld 1995, LoGiudice et al. 2003). Fragmentation of habitat in RAP has perhaps led to increased densities of *P. leucopus* in the forested areas, as the majority of small mammals captured in this study were *Peromyscus spp.* (96%). Yet, this study shows an increase in *I. scapularis* occurrence and *B. burgdorferi* prevalence in the prairie, where small mammal diversity is greatest.

Conclusions from prior studies indicate that an increase in biodiversity leads to a "dilution effect" of immature *I. scapularis* acquiring *B. burgdorferi* infection because of decreased reservoir-competency in hosts (Van Buskirk and Ostfeld 1995, Ostfeld and Keesing 2000). Despite evidence of this hypothesis (LoGiudice et al. 2003), the results of my study suggest otherwise. The presence of *M. ochrogaster* in the prairie could serve as a competent reservoir, thus having an opposite effect, as Ogden and Tsao suggest (2009), by amplifying the abundance of infected *I. scapularis*. However, this study is limited in that it only includes small mammal diversity. A comprehensive evaluation of all vertebrate diversity in these habitats may lead to a better understanding of existing ecological interactions influencing the survival of *B.*

burgdorferi-competent reservoirs and creating a potential dilution, or amplification, effect (Ogden and Tsao 2009).

The observation made by Brown and Burgess (2001) that habitat heterogeneity leads to host diversity and increased rodent density may explain why the occurrence of *I. scapularis* is highest in the prairie as well as the young forest. Given the relatively small size of RAP (614 ha), *I. scapularis* abundance and *B. burgdorferi* prevalence may be dynamic, or continue to shift and change within and among the habitats of the park. The diverse habitats of this natural area are all clustered within a close range and bordered by agriculture fields and urban areas (Rosenblatt et al. 1999). Such habitat composition favors a high degree of connectivity enabling vertebrate host movements between suitable habitat patches, which, based on literature, is an important factor correlated with *I. scapularis* distribution and abundance (Estrada-Peña 2003). For example, this landscape could facilitate deer movement between habitats enabling them to utilize the riparian corridor of the Sangamon River near the young forest as a dispersal route, the prairie as a bedding area, and the agriculture fields as a food source. Such deer habitat use could explain the high occurrence of *I. scapularis* in the prairie and young forest. As habitat patches become smaller in size due to changes in land use toward agriculture and urbanization, *I. scapularis* may increase in abundance and density in remaining habitat (Ginsberg 1994).

Given the difficulties associated with surveying only one study site, *I. scapularis* distribution can easily vary from one local-scale site to the next. However, Piesman and Gray (1994) point out that there are a number of exceptions to generalized tick-habitat associations. Landscape context can influence the abundance of *I. scapularis* (Madhav et al. 2004, Estrada-Peña 2009). This study proves the possibility of *I. scapularis* establishment within habitats other than forested areas.

Continued sampling over the five-year period has increased understanding of the relationship between *I. scapularis* occurrence, *B. burgdorferi* infection, habitat diversity, and small mammal abundance within a small natural area surrounded by a fragmented landscape. Based on these findings, I conclude that estimating *I. scapularis* occurrence and *B. burgdorferi* prevalence in an area may depend on a comprehensive evaluation of the surrounding habitat diversity and species composition. Monitoring the distribution of *I. scapularis* and the prevalence of *B. burgdorferi* in this natural area of east-central Illinois will allow Lyme disease preventive measures to be focused in high-risk areas of concern.

Chapter 2 meets the formatting requirements for *Vector-Borne and Zoonotic Diseases.*

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6. Tables and Figures

Table 2.1. Summary of small mammals captured and Ixodes scapularis collected from sites in Robert Allerton Park, Piatt County, Illinois, 2005-2009.

Habitat	Species	n	Ratio[a] (95% CI)	*I. scapularis*	Ratio[b] (95% CI)
Young Forest[a,b]	*Peromyscus spp.*	723			
	Z. hudsonius	1			
	Total	724	---	676	---
Prairie	*M. ochrogaster*	64			
	Peromyscus spp.	51			
	Z. hudsonius	15			
	R. megalotis	9			
	Total	139	0.2 (0.17, 0.32)	176	0.2 (0.1, 0.3)
Old Forest	*Peromyscus spp.*	739	1.1 (0.9, 1.5)	127	0.1 (0.05, 0.21)
Flood Plain	*Peromyscus spp.*	832			
	Z. hudsonius	12			
	Total	844	1.5 (1.1, 1.9)	30	0.04 (0.02, 0.09)
Total		2,446		1,009	

[a] Ratio of mean number of mammals captured per trapnight, adjusting for year and month. Young Forest served as the reference group for comparison.

[b] Ratio of mean number of *Ixodes scapularis* collected per trapnight, adjusting for year and month. Young Forest served as the reference group for comparison.

Table 2.2. *Ixodes scapularis* occurrence in Robert Allerton Park, Piatt County, Illinois, 2005-2009.

Habitat	Prevalence[a] % (n)	Odds Ratio[d] (95% CI)	Mean Intensity[b] (I)	Ratio[d] (95% CI)	Relative Density[c]	Ratio[d] (95% CI)
Young Forest[d]	32.7 (724)	---	2.9 (237)	---	0.9	---
Prairie	39.6 (139)	1.0 (0.7, 1.5)	3.2 (55)	1.1 (0.9, 1.4)	1.3	1.0 (0.7, 1.5)
Old Forest	10.8* (739)	0.2 (0.16, 0.28)	1.6* (80)	0.6 (0.4, 0.7)	0.2*	0.2 (0.12, 0.22)
Flood Plain	1.9* (844)	0.03(0.02, 0.05)	1.9* (16)	0.5 (0.3, 0.9)	0.04*	0.03 (0.02, 0.05)

[a]Prevalence = infested mammals / trapped mammals. n is number of trapped mammals.

[b]Mean Intensity = *I. scapularis* / infested mammals. I is number of infested mammals.

[c]Relative Density = *I. scapularis* / trapped mammals.

[d]Odds Ratio and Ratio are values per trapnight, adjusting for year and month. Young Forest served as the reference group for comparison.

*Value significantly lower than young forest and prairie ($P \leq 0.001$).

Table 2.3. *Ixodes scapularis* pools from infested small mammals tested for *Borrelia burgdorferi* in Robert Allerton Park, Piatt County, Illinois, 2005-2009.

Habitat	No. of infested mammals	Pools positive for *B. burgdorferi* % (n)	Odds Ratio[a] (95% CI)
Young Forest[a]	237	14.8 (35)	---
Prairie	55	27.3 (15)	2.2 (1.0, 4.5)
Old Forest	80	6.3 (5)	x
Flood Plain	16	6.3 (1)	x
Total	388	14.4 (56)	

[a]Odds Ratio of *B. burgdorferi* infection prevalence per trapnight, adjusting for year and month. Young Forest served as the reference group for comparison.

x Small sample size of Old Forest and Flood Plain did not allow accurate comparisons.

Fig. 2.1. Study site in east-central Illinois. Robert Allerton Park (RAP) is located 6.4 km southwest of Monticello, in Piatt County, Illinois. Site 1 = Young Forest, Site 2 = Prairie, Site 3 = Old Forest, Site 4 = Flood Plain.

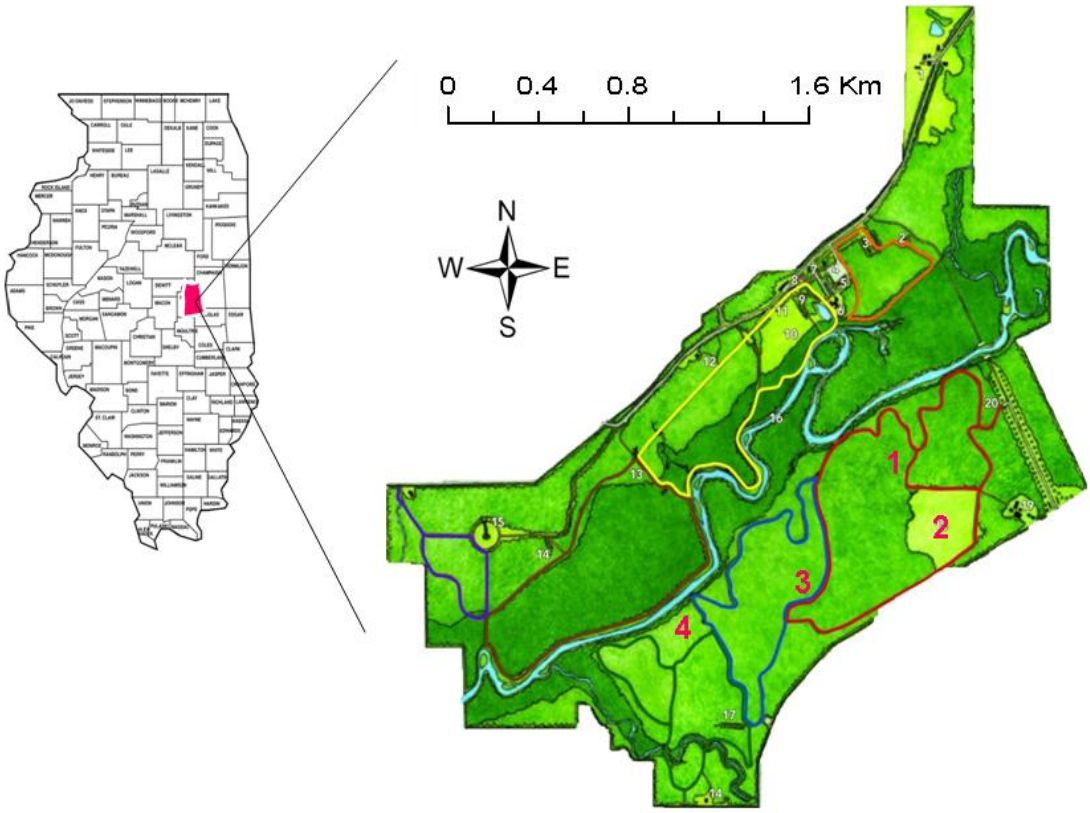


Fig. 2.2a.- c. Temporal changes in *Ixodes scapularis* occurrence (Prevalence, Mean Intensity, and Relative Density) in Robert Allerton Park, Piatt County, Illinois 2005 – 2009. *Value significantly higher compared to 2005 as the reference group ($P \leq 0.001$).

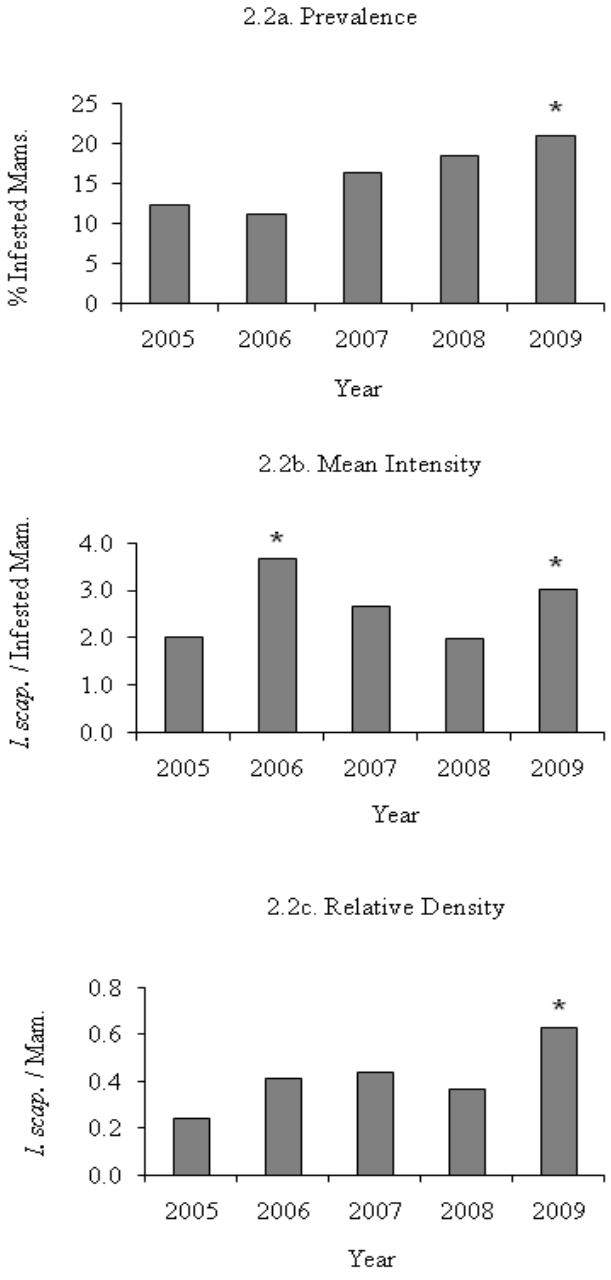
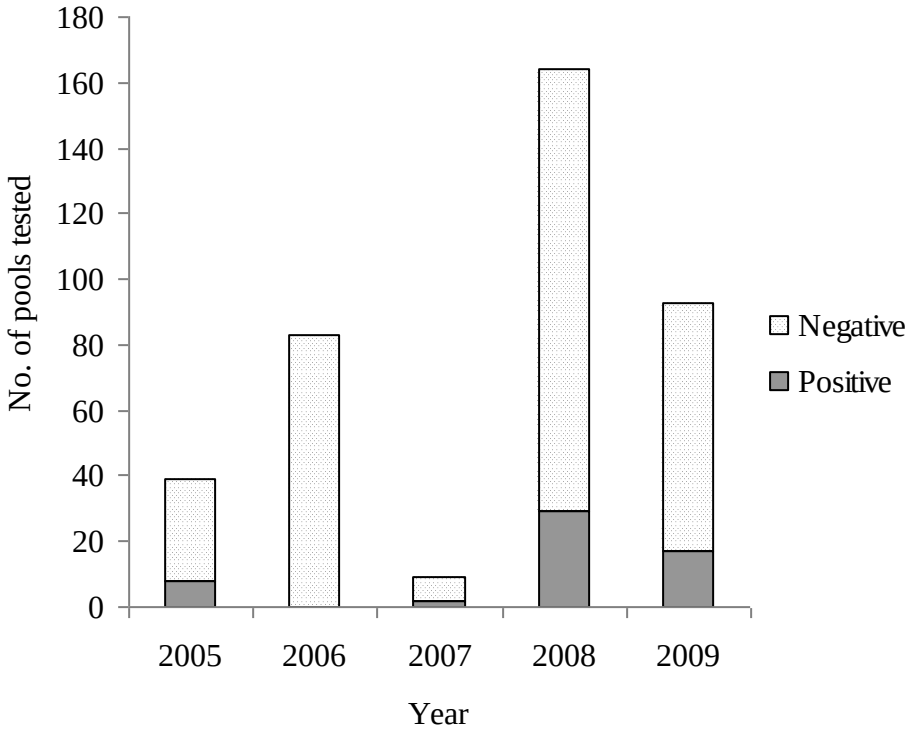


Fig. 2.3. *Ixodes scapularis* pools tested for *Borrelia burgdorferi* in Robert Allerton Park, Piatt County, Illinois 2005 – 2009. % positive pools did not vary significantly between years with the exception of 2006 when no pools tested positive.



CHAPTER 3:

IXODES SCAPULARIS DISTRIBUTION IN NORTHEAST ILLINOIS

1. Introduction

Lyme disease, the most common vector-borne disease of humans in the U.S., is caused by the spirochete *Borrelia burgdorferi sensu stricto* and is transmitted by the blacklegged tick (*Ixodes scapularis*) in the Midwest (Burgdorfer et al. 1982, Steere et al. 1983). The number of human Lyme disease cases in Illinois has quickly increased from 35 reported cases in 2000 to 108 cases in 2008 (IDPH 2010a). If left untreated during the early stages, Lyme disease can affect the skin, musculoskeletal, cardiac, and central nervous systems, ultimately leading to long-term severe joint pain and neurological damage (Steere et al. 2004). Since *B. burgdorferi* occurs naturally in a variety of small mammal and bird reservoir hosts, immature *I. scapularis* become infected when taking a blood meal (Mather et al. 1989, Fish 1993, Bunikis et al. 2004). Humans are incidental hosts of *I. scapularis* and acquire *B. burgdorferi* infection when bitten by an infected nymphal or adult tick.

The two-year life cycle of the vector tick, *I. scapularis*, consists of three stages: larva, nymph, and adult. Each stage actively quests for a blood meal before molting into the next stage. The larvae typically hatch uninfected (Lane 1994). *Ixodes scapularis* maintains the spirochete transtadially, so it can infect a susceptible host while feeding at a later life stage (Piesman et al. 1986, Fish 1993). The nymph stage poses the greatest risk for human infection in the Midwest because of its small size and its peak questing activity coinciding with increased human activity outdoors during late spring and early summer (Fish 1993). Adult ticks feed and mate on larger animals like the white-tailed deer (*Odocoileus virginianus*) (Piesman et al. 1979). Although deer

are not a competent reservoir for *B. burgdorferi*, they play an important role in tick dispersal, especially along riparian corridors (Cortinas and Kitron 2006).

Both the vector tick and spirochete were once limited to a small area in Minnesota and Wisconsin (Davis et al. 1984, Callister et al. 1988). Although *I. scapularis* was initially detected along the Rock River in Ogle County, Illinois, in the late 1980s (Bouseman et al. 1990), Illinois has not historically been considered a Midwestern endemic focus for Lyme disease (Picken et al. 1995). Studies assessing the presence of *I. scapularis* near major metropolitan areas, such as Milwaukee and Chicago, in the early 1990s showed no evidence of tick populations within these areas (Callister et al. 1991). However, Picken et al. (1995) suggested that Illinois may eventually be considered an area of focal endemicity for both *I. scapularis* and *B. burgdorferi* based on the positive findings of several studies (Kitron et al. 1991a, Nelson et al. 1991).

Suitable habitat for *I. scapularis* has been characterized by oak-dominated forests with sufficient leaf litter and moist, sandy soils (Kitron et al. 1991b, Mannelli et al. 1994). The distribution and establishment of *I. scapularis* depends upon favorable vegetation, soil, topography, and climate necessary for questing, molting, diapause, and oviposition (Guerra et al. 2002). Guerra et al. (2002) predicted the distribution of *I. scapularis* in Illinois based on these environmental factors that favor or inhibit *I. scapularis* establishment in new areas. *Ixodes scapularis* abundance is expected to increase with increased habitat suitability. The Centers for Disease Control and Prevention (CDC) define a tick population as "established" in an area if all three life stages or at least six individuals of a single stage are present (Dennis et al. 1998).

Expansion of *I. scapularis* geographic distribution in northern Illinois has typically followed along riparian corridors via dispersal and migration of abundant white-tailed deer populations (Bouseman et al. 1990, Kitron et al. 1991a, Cortinas and Kitron 2006). The Midwest

I. scapularis habitat suitability model predicted suitable forested habitats along the Des Plaines River in northeast Illinois (Guerra et al. 2002). By 2007, Jobe et al. confirmed the presence of *B. burgdorferi*-infected *I. scapularis* at sites in northeast Illinois located less than one mile from city limits, and recognized that Lyme disease could become a significant health concern around the Chicago metropolitan area. Thus, the risk of Lyme disease exposure is increasing throughout Illinois, most recently emerging in the northeast area of the state (Jobe et al. 2006, 2007).

To date, mapping the spatial distribution of *I. scapularis* establishment within Illinois has been limited to nonstandardized reporting (IDPH 2009). There are numerous natural areas, parks, and forest preserves in and around the Chicago metropolitan region. Many homes in this urban-suburban area are also surrounded by dense vegetation and woodlots—ideal habitat for small mammal hosts and *I. scapularis* populations. However, most homeowners are unaware of a potential residential or peridomestic risk of contracting Lyme disease (Guerra et al. 2002, Jobe et al. 2007). Because there is no state-wide system for surveying *I. scapularis*, areas where the tick is present could be overlooked due to lack of reporting (Madhav et al. 2004).

To assess the geographic distribution and abundance of *I. scapularis* throughout the Chicago metropolitan region of northeast Illinois, I surveyed public-access forest preserves frequented by large numbers of visitors. I utilized timed dragging surveys to collect ticks. This study builds upon previous work (Jobe et al. 2006, 2007) assessing *I. scapularis* distribution in northeast Illinois by sampling a larger geographic area over a two-year study period. By collecting ticks over a large geographic range, I clarified whether *I. scapularis* distribution was limited to isolated patches or widespread throughout the northeast Illinois region.

2. Materials and Methods

2.1 Study sites

Thirty-two survey sites were selected from public-access forest preserves throughout Cook (n = 10), DuPage (n = 11), Lake (n = 10), and McHenry (n = 1) counties in northeast Illinois (Fig. 3.1, Table 3.1.). Several sites within each county had previously known presence of *I. scapularis* (Jobe et al. 2007), while the remaining selected sites were not tested previously, or *I. scapularis* presence was unknown (Jeff Nelson, Tom Velat, Mike Adam personal communication). Sites were selected so that they covered a large geographic distribution throughout the tri-county area including 6 sites within Chicago city limits. Each site was surveyed up to five times either between May - June and October 2008, or between April - June and October 2009. Four sites were surveyed in both 2008 and 2009.

2.2 Collection of ticks

Questing ticks were collected by drag sampling for three person-hours per site visit. Sampling was performed during the late morning and late afternoon on days with no rain, little to no wind, and a minimum temperature of 10° C to avoid wet vegetation and excessively hot or cold temperatures. Standardized tick drags, made of 1-m^2 white corduroy attached to a wooden dowel (Mather et al. 1996, Diuk-Wasser et al. 2006), were pulled across leaf litter and low vegetation along trail edges and over short transects (~25 m) perpendicular to trails in forested sites. The drags were checked at 30 second intervals; attached ticks of all three life stages (larvae, nymphs, and adults) were removed and placed in vials filled with 70% ethanol. To maximize tick collection during peak seasonal activity, drag sampling visits per site were repeated up to five times during the field season.

2.3 Laboratory analysis

After transport to the laboratory, ticks were identified to species and life stage based on morphology using a stereo-microscope and tick identification keys (Sonenshine 1979). Tick collection data, including site, date, species, and number of individuals were digitized into a database before specimens were added to the collection. All procedures were approved under biosafety protocols at the University of Illinois Urbana-Champaign, and appropriate research collection permits were obtained from the forest preserve districts. Geographic information systems created in ArcMap® **(**ESRI ArcView 9.2, 2006, Redlands, CA) were used to map tick distribution and relative abundance.

3. Results

I collected a total of 1,067 ticks from 30 out of 32 sites. There were 602 *I. scapularis* ticks (152 larvae, 120 nymphs, and 330 adults) collected from 17 out of 32 sites (Table 3.1.). The CDC defines blacklegged tick establishment in an area if 2 of 3 life stages are found or 6 individuals are collected (Dennis et al. 1998). Therefore, 12 sites meet this requirement. In addition to *I. scapularis*, *Dermacentor variabilis* (n = 321: 1 larva, 1 nymph, 319 adults), *Haemaphysalis leporispalustris* (n = 60: 55 larvae, 5 nymphs), *Ixodes dentatus* (n = 83 larvae), and *Amblyomma americanum* (n = 1 adult) were also collected (Table 3.1.). The proportional number of ticks collected per site separated by species is shown in (Fig. 3.2.). The highest abundance of *I. scapularis* was collected in Lake County (n = 358), followed by DuPage County (n = 140), and Cook County (n = 100). Only one site was surveyed in McHenry County in 2009, and 4 *I. scapularis* were collected. In 2008, 15 sites were surveyed, and 296 *I. scapularis* were

collected (Fig. 3.3.). In 2009, 17 new sites and 4 sites from 2008 were surveyed with 306 *I. scapularis* collected (Fig. 3.4.).

Ixodes scapularis were most abundant at the sites along the shoreline of Lake Michigan (Fig. 3.5.) suggesting suitable tick habitat with ideal microclimate conditions and abundant host availability in this area. The areas where the fewest *I. scapularis* were found, specifically Lake County west of the shoreline and southeast Cook County, are mainly mesic to wet-mesic grassy sites. This type of habitat is not believed to be suitable for the maintenance of abundant *I. scapularis* populations (Brown and Burgess 2001). *Ixodes scapularis* appears to be well distributed throughout northeast Illinois (Fig. 3.6.) where suitable oak-dominated forested habitat is available similar to the predicted areas shown in the Midwest habitat suitability model (Guerra et al. 2002).

4. Discussion

Overall, this study provides a snap-shot of current *I. scapularis* distribution, which can be compared to historical data and aid future studies to monitor changes and predict areas of future establishment of *I. scapularis* populations. During an initial study of the area surrounding Chicago and Milwaukee in the early 1990s, no *I. scapularis* were found and *B. burgdorferi* was only recovered from two small mammals (Callister et al. 1991). These results led to the conclusion that northeastern Illinois and southeastern Wisconsin were not yet included in the Midwestern Lyme disease focus (Callister et al. 1991). By 2006, Jobe et al. began finding *I. scapularis* at sites surrounding the Chicago area in Cook, DuPage, and Lake Counties. In less than twenty years, *I. scapularis* had become established in northeastern Illinois. Because a proportion of those ticks tested positive for *B. burgdorferi*, Lyme disease became an emerging

concern (Jobe et al. 2007). However, only a few sites had been surveyed, so the extent of *I. scapularis* distribution and establishment was still unknown. My current study has clarified the distribution and abundance of *I. scapularis* in areas of northeast Illinois where this information was previously unknown by documenting the location and number of ticks collected through intensive dragging surveys. *Ixodes scapularis* were collected from sites where they were not previously found. This new information is pertinent to build public awareness about the potential exposure to vector ticks and tick-borne disease, such as Lyme disease.

Because *I. scapularis* were found at 17 of the 32 sites between 2008 and 2009, I conclude that *I. scapularis* is widely distributed throughout the study area of northeast Illinois where suitable habitat is available. Similar to the results of a study focused on *I. scapularis* expansion along the Illinois River (Cortinas and Kitron 2006), *I. scapularis* also appears to be expanding its established range along the Des Plaines River and the shoreline of Lake Michigan in northeast Illinois. Likewise, the predictive model for habitat suitability of *I. scapularis* by Guerra et al. (2002) shows the area along the Des Plaines, particularly in Lake County, to be highly suitable habitat for *I. scapularis* establishment. My results support this prediction, as the highest abundance of *I. scapularis* was collected from sites in this geographic area.

The abundance and geographical distribution of *I. scapularis* in an area depend on interactions between microclimate and habitat (Mannelli et al. 1994, Jones and Kitron 2000). Of interest in this study, the eastern Lake - Cook County region appears to have a combination of environmental factors ideal for *I. scapularis* establishment given the large number of ticks collected in this area, especially in coastal sites along Lake Michigan. As Cortinas and Kitron suggested (2006), the distribution of *I. scapularis* in Illinois appears to remain dynamic.

Although *I. scapularis* was the species of interest in this study, a large number of *Dermacentor variabilis* (the American dog tick) were collected, as this tick species is very common throughout the state (IDPH 2010b). Although this species is not a vector for *B. burgdorferi*, it is still a public health concern because it can transmit Rocky Mountain spotted fever, tularemia, and possibly ehrlichiosis to humans (IDPH 2010b). It was surprising to find an adult *Amblyomma americanum* tick in Cook County. Commonly known as the Lone Star tick, its distribution covers the southern half of the state; *A. americanum* is the vector for Southern tick-associated rash illness (STARI) (IDPH 2010b). *Haemaphysalis leporispalustris* and *Ixodes dentatus* are two species of rabbit ticks. Although these host-specific ticks have been found naturally infected with *B. burgdorferi*, they are not competent vectors and rarely bite humans (Anderson 1989, Lane et al. 1991). While drag sampling, the immature stages of these host-specific, nest-dwelling ticks were mostly found in big clusters of 10 or more individuals, mainly in October, suggesting that the tick drag had swept across a nest.

Tick-borne diseases are recognized by the Illinois Department of Public Health as an emerging concern (IDPH 2010b). Therefore, in addition to surveying *I. scapularis*, reporting the relative abundance of these other tick species collected is useful to increase understanding of ecological variables that determine spatial and temporal variation of vector-pathogen interactions. This study provides information to build awareness of prevention efforts against tick bites and to develop environmental management strategies to control further spread of vector ticks and their associated pathogens.

The results of this study yield valuable information about the geographic distribution and abundance of questing *I. scapularis* in northeast Illinois. Based on these results, the public should be aware of *I. scapularis* presence within forested areas of Cook, DuPage, & Lake

Counties. Residents and visitors of the fragmented urban and suburban landscapes of the Chicago metropolitan region may become vulnerable to an increased risk of Lyme disease as the distribution of established *I. scapularis* populations continues to expand. Of interest, this study provides baseline data that will be useful to further evaluate the distribution and abundance of *I. scapularis*. Because *I. scapularis* positive for *B. burgdorferi* were collected (Jobe et al. 2006, 2007) in sites nearby and similar to those of this study, it is important to continue monitoring the prevalence of the spirochete within these areas, especially where *I. scapularis* were found to be abundant.

It is important to continue sampling efforts to track the potential spread of *I. scapularis* and *B. burgdorferi*, as changes in land use can alter wildilfe-vector-pathogen interactions and influence disease emergence. Future research stemming from this study could focus on evaluating environmental factors, such as climate, habitat, and white-tailed deer and small mammal host populations, associated with the identified sites of established *I. scapularis* populations to gain more insight of wildlife-vector-pathogen interactions occurring in the fragmented landscape of northeast Illinois. This further evaluation could identify possible environmental targets for managing or reducing *I. scapularis* distribution and abundance. Because northeast Illinois is so densely populated and the forest preserves are frequently visited by large numbers of people, this study emphasizes the need to increase research efforts and public awareness concerning the occurrence of *I. scapularis* and the prevalence of *B. burgdorferi*. Focusing research of *I. scapularis* and Lyme disease prevention efforts in the Chicago metropolitan region will enable people to make safer decisions when utilizing the outdoor environment for recreation and residence.

Chapter 3 meets the formatting requirements for the *Vector-Borne and Zoonotic Diseases.*

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6. Tables and Figures

Table 3.1. Survey sites visited and ticks collected in northeast Illinois counties 2008 - 2009.

Site	County	Year	Visits	*I. scapularis* [a]	*D. variabilis*	Other	Total Ticks
Brookfield[b]	Cook	2008	4	0/1/0	0	2	3
Hegewisch Marsh	Cook	2008	3	0/0/0	4	40	44
Potawatomi	Cook	2008	1	0/0/0	0	0	0
Powderhorn	Cook	2008	3	0/0/0	8	1	9
Somme Woods	Cook	2008	4	0/2/1	1	1	5
Swallow Cliff South[b]	Cook	2008	4	13/17/12	0	2	44
Brookfield[b]	Cook	2009	4	0/0/0	1	0	1
Caldwell Woods	Cook	2009	1	0/0/0	0	0	0
Forest Glen	Cook	2009	1	0/0/0	0	0	0
Peterson Park	Cook	2009	4	0/1/1	0	0	2
Schiller Woods South	Cook	2009	4	0/0/0	0	0	0
Swallow Cliff South[b]	Cook	2009	2	0/11/41	1	0	53
Blackwell	DuPage	2008	5	0/1/7	5	58	71
Churchill	DuPage	2008	4	1/10/26	7	39	83
Danada	DuPage	2008	5	0/1/22	19	0	42
Fullersburg[a]	DuPage	2008	4	1/2/5	1	0	9
Waterfall Glen[a]	DuPage	2008	3	0/1/26	4	0	31
West DuPage Woods	DuPage	2008	4	0/1/7	5	1	14
York	DuPage	2008	2	0/0/0	0	0	0
Fullersburg[b]	DuPage	2009	2	0/0/1	0	0	1
Meacham Grove	DuPage	2009	4	0/0/0	7	0	7
Pratts Wayne Woods	DuPage	2009	2	0/0/0	10	0	10
Salt Creek	DuPage	2009	4	0/0/0	0	0	0
Waterfall Glen[b]	DuPage	2009	2	0/0/28	0	0	28
West Branch	DuPage	2009	2	0/0/0	10	0	10

Table 3.1. (cont.)

Site	County	Year	Visits	*I. scapularis* [a]	*D. variabilis*	Other	Total Ticks
Fort Sheridan	Lake	2008	4	18/14/92	46	0	170
Ryerson	Lake	2008	4	8/2/5	6	0	21
Grant	Lake	2009	5	0/0/1	34	0	35
Lakewood	Lake	2009	5	0/0/2	57	0	59
Lyons	Lake	2009	4	2/11/23	21	0	57
Nippersink	Lake	2009	5	0/0/0	16	0	16
Raven Glen	Lake	2009	4	0/0/0	25	0	25
Singing Hills	Lake	2009	1	0/0/0	9	0	9
Spring Bluff	Lake	2009	4	109/45/26	4	0	184
Van Patten	Lake	2009	4	0/0/0	2	0	2
Moraine Hills	McHenry	2009	5	0/0/4	18	0	22

[a]larva/nymph/adult

[b]site surveyed in 2008 and 2009

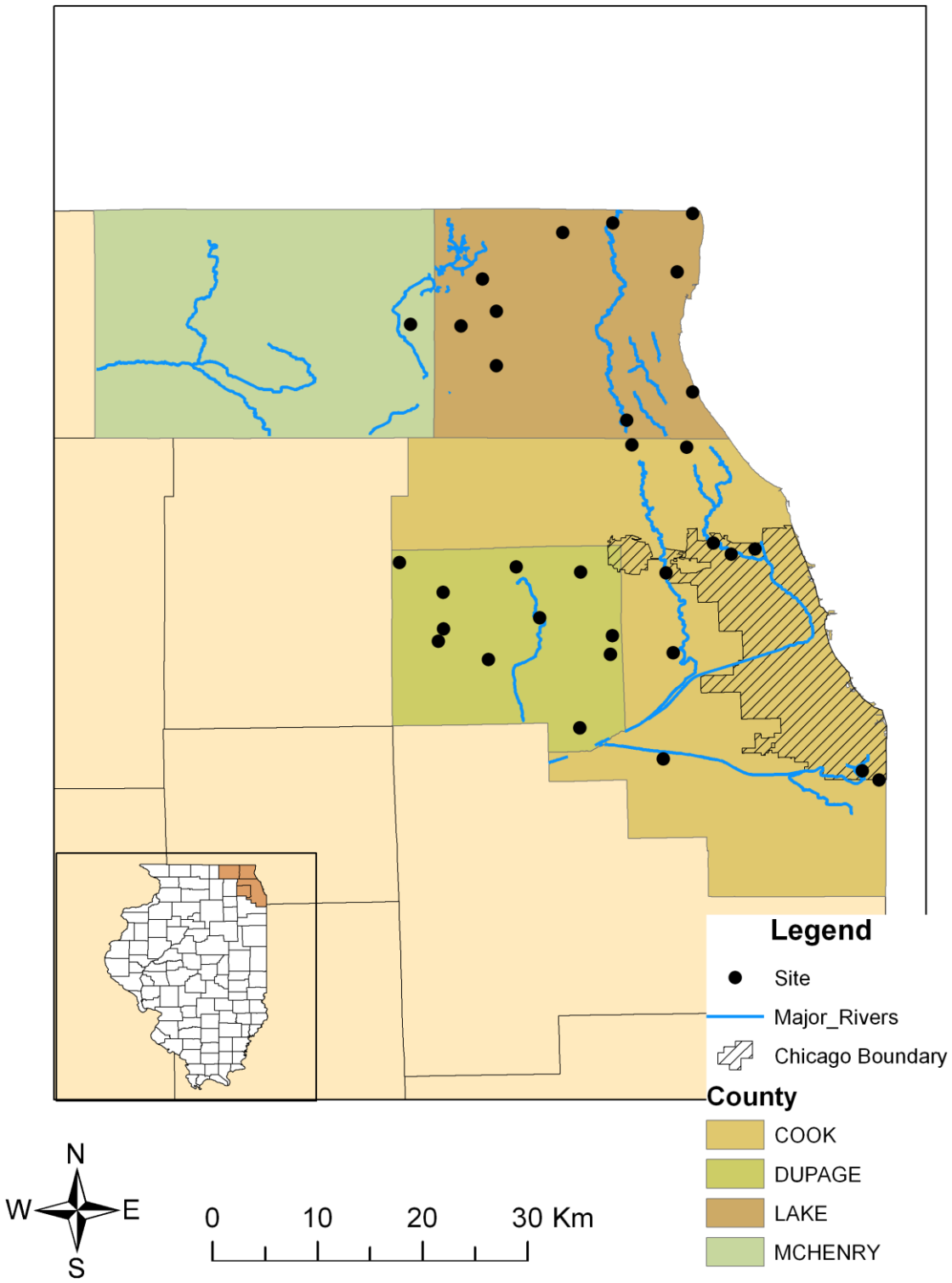

Fig. 3.1. Locations of tick survey sites in Cook, DuPage, Lake, and McHenry Counties of northeast Illinois 2008 - 2009.

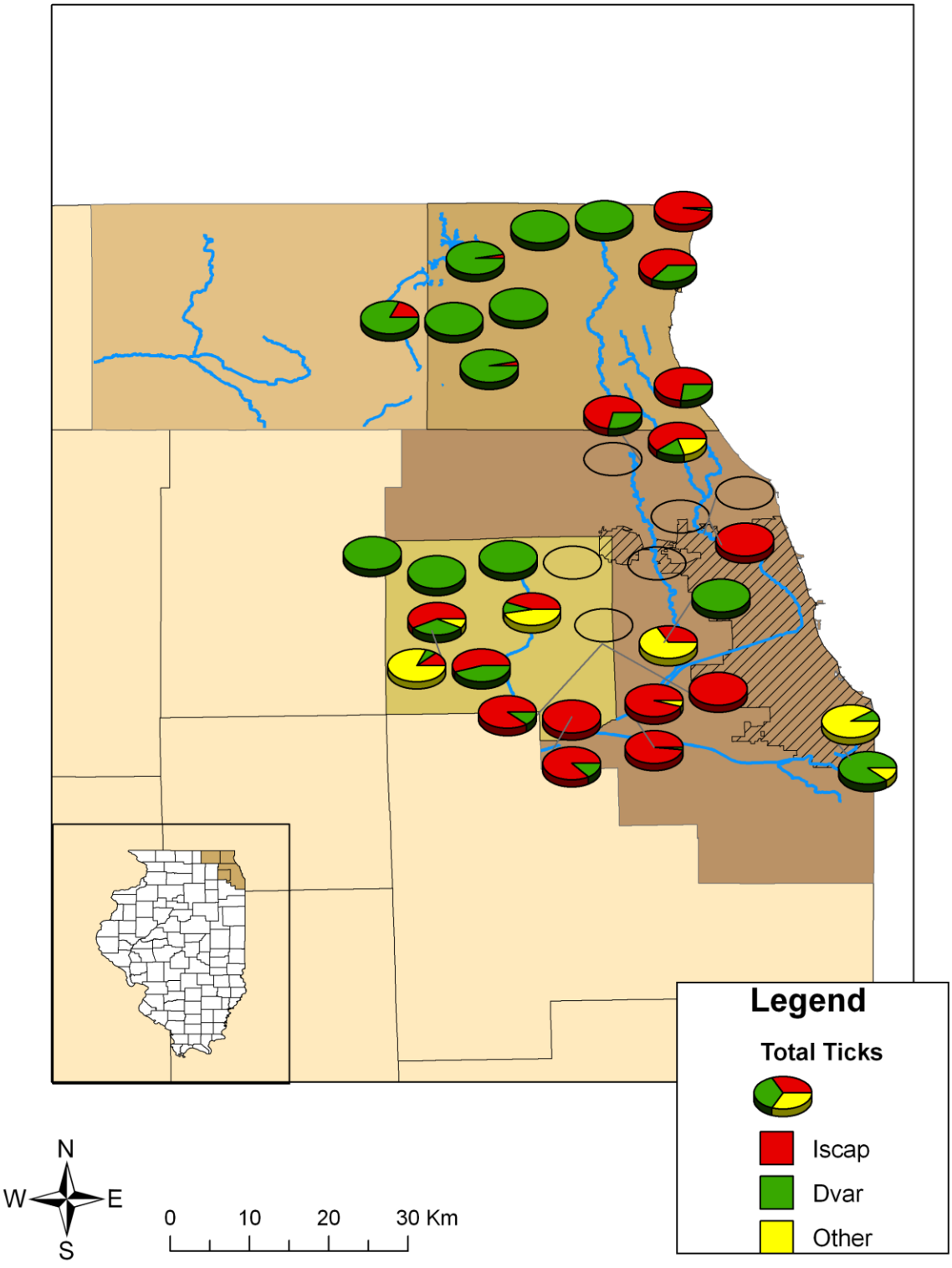

Fig. 3.2. Results of tick surveys in Cook, DuPage, Lake, and McHenry Counties of northeast Illinois 2008 - 2009.

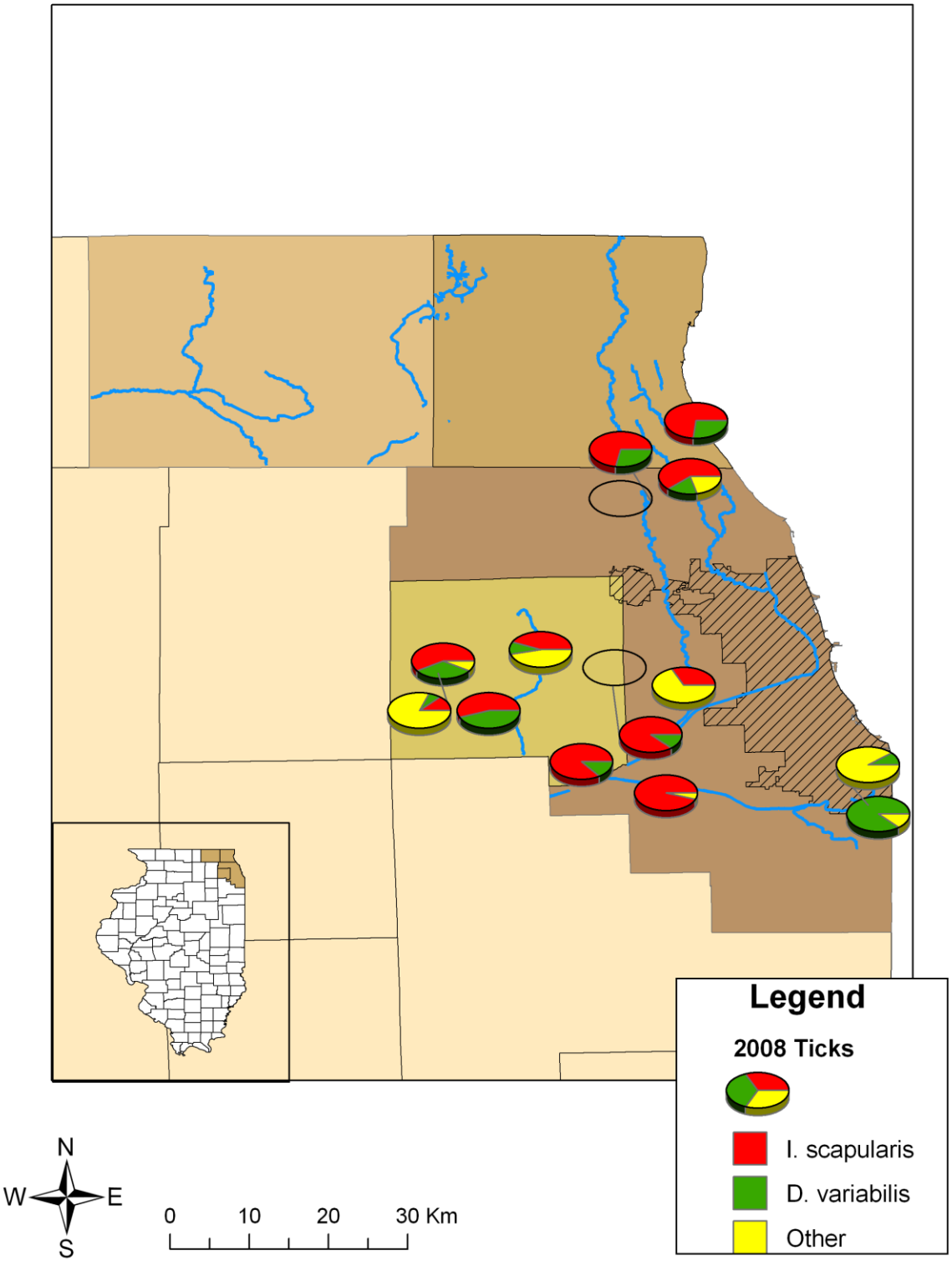

Fig. 3.3. Results of tick surveys in Cook, DuPage, Lake, and McHenry Counties of northeast Illinois 2008.

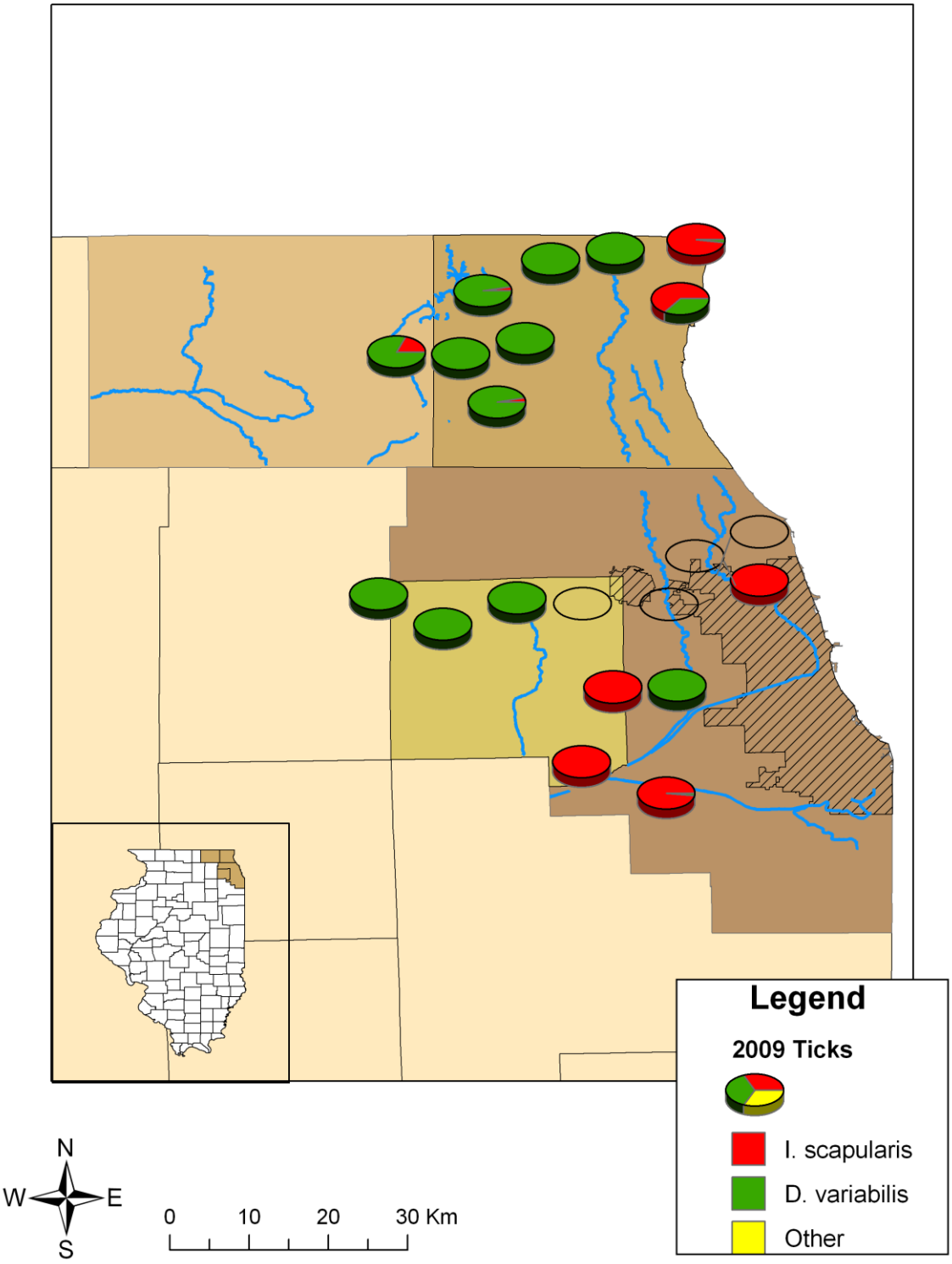

Fig. 3.4. Results of tick surveys in Cook, DuPage, Lake, and McHenry Counties of northeast Illinois 2009.

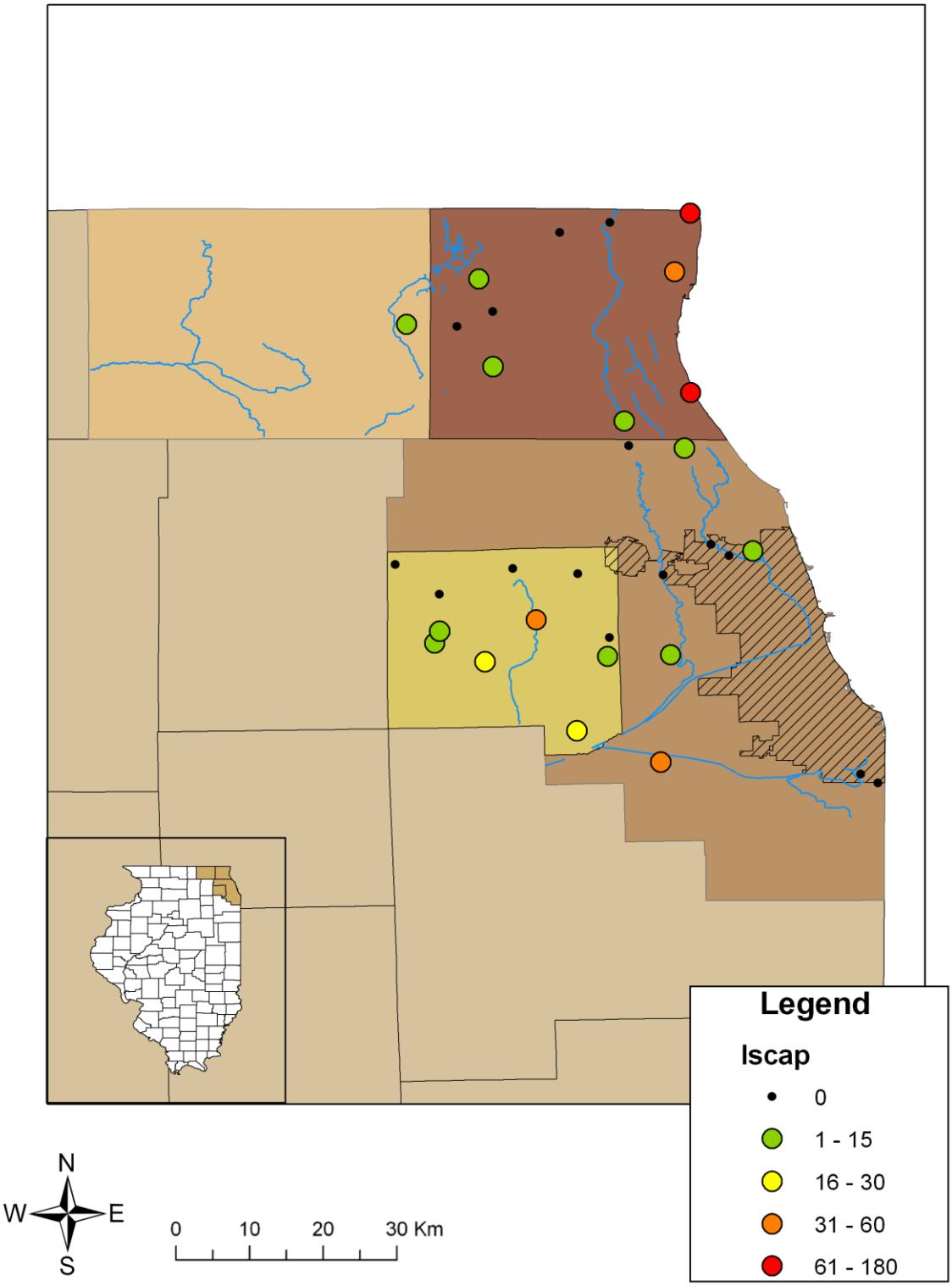

Fig. 3.5. Distribution and relative abundance of *Ixodes scapularis* collected in Cook, DuPage, Lake, and McHenry Counties of northeast Illinois 2008 - 2009.

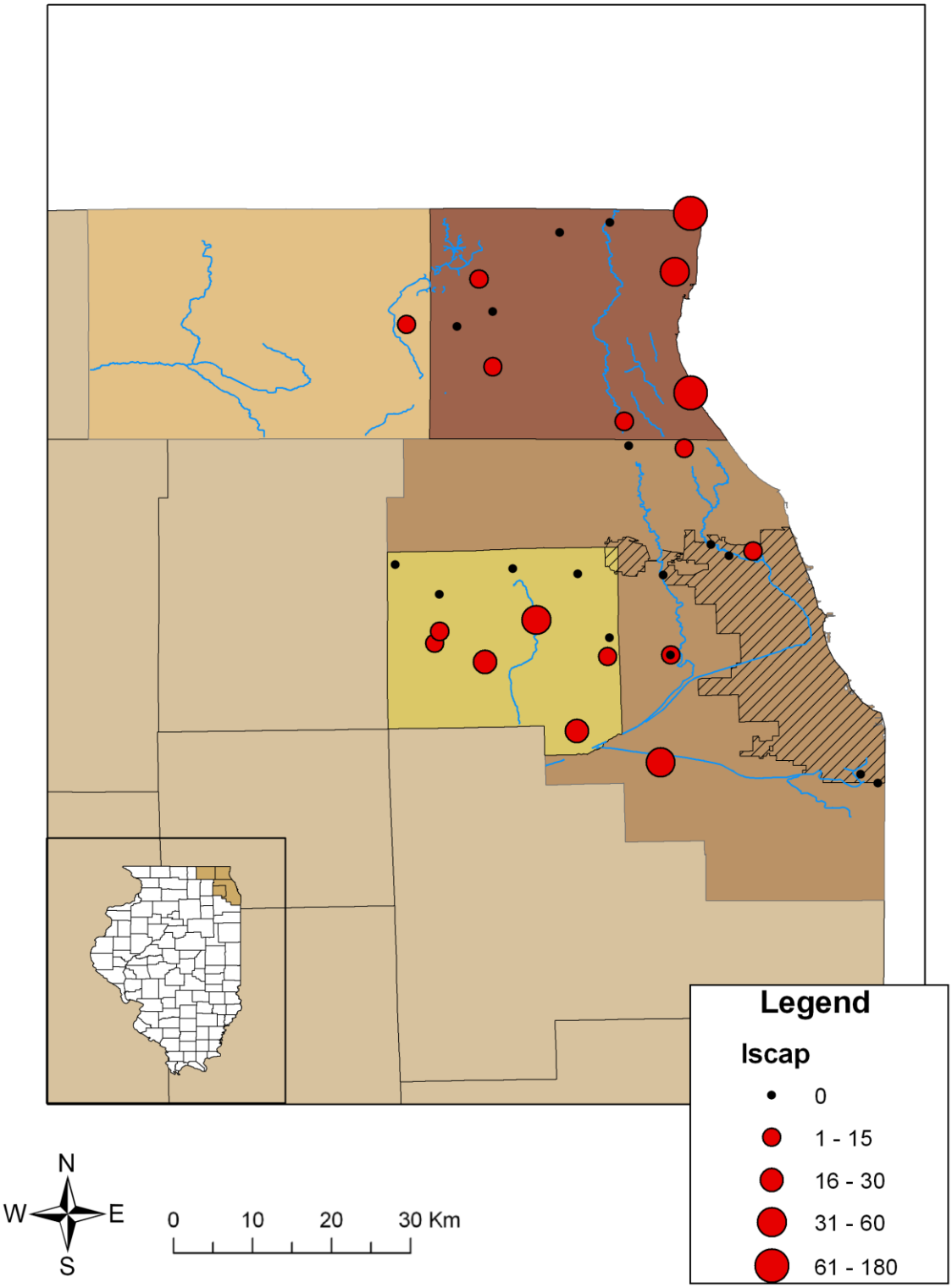

Fig. 3.6. Relative abundance of *Ixodes scapularis* collected at sites in Cook, DuPage, Lake, and McHenry Counties of northeast Illinois 2008 - 2009.

CHAPTER 4:

SUMMARY

The distribution of *Ixodes scapularis* establishment continues to expand in Illinois. At Robert Allerton Park (RAP) in Piatt County, Illinois, *I. scapularis* were most abundant in the young forest and prairie habitats. The prairie had the highest diversity of small mammal hosts. Prevalence, mean intensity, and relative density of *I. scapularis* and prevalence of *B. burgdorferi* infection were highest for the prairie and young forest. The overall *B. burgdorferi* infection prevalence of *I. scapularis* pools from RAP was 14%. Continued sampling from 2005 - 2009 has increased understanding of the relationship between *I. scapularis* occurrence, *B. burgdorferi* infection, habitat diversity, and small mammal abundance within a small natural area surrounded by a fragmented landscape.

In northeast Illinois, *I. scapularis* of all three life stages were collected from 17 of the 32 survey sites. *Ixodes scapularis* appear to be widely established throughout the counties of Cook, DuPage, and Lake where suitable habitat is available. Of interest in this study, the eastern Lake - Cook County region appears to have a combination of environmental factors ideal for *I. scapularis* establishment given the large number of ticks collected in this area, especially in coastal sites along Lake Michigan. My current study has clarified the distribution and abundance of *I. scapularis* in areas of northeast Illinois where this information was previously unknown. *Ixodes scapularis* were collected from sites where they were not previously found. This new information is pertinent to build public awareness about the potential exposure to *I. scapularis* and Lyme disease in the metropolitan region of Chicago.

Surprisingly, the highest abundance of *I. scapularis* were found in habitats not typical or predicted for *I. scapularis* establishment i.e. the prairie habitat in east-central Illinois and the

coastal forested sites along Lake Michigan in northeast Illinois. Taken in context of the overall landscape, it is possible for *I. scapularis* populations to utilize these habitats, especially if they border typical *I. scapularis* habitat (oak-dominated forests). For example, RAP is spatially unique since the prairie is situated between forested areas, a riparian corridor, and agriculture fields. As host mammals, such as white-tailed deer and white-footed mice, adapt to increased development, they utilize habitats in new ways. We may see a reflection of this host habitat shift in *I. scapularis* populations appearing where they previously have not been detected. Therefore, I do not broadly generalize these unconventional *I. scapularis* habitats as ideal locations for their establishment, but it may be possible for *I. scapularis* to take advantage of such habitats and ecotones located near forested habitat in a fragmented landscape.

This study provides baseline data for further evaluation of emerging Lyme disease foci in Illinois. The distribution of *I. scapularis* is encroaching upon developed areas, increasing the risk for human exposure to Lyme disease. Habitats and reservoir hosts that were previously overlooked may be suitable for *I. scapularis* and *B. burgdorferi* establishment in dynamic fragmented landscapes. This study reveals mechanisms associated with wildlife – vector – pathogen interactions that could influence disease emergence and exposure, and it emphasizes the need to increase research efforts and public awareness concerning the occurrence of *I. scapularis* and the prevalence of *B. burgdorferi* in Illinois.

APPENDIX A:

SMALL MAMMAL TRAPPING RESULTS FOR EAST-CENTRAL ILLINOIS

Results of small mammal trapping in four habitats at Robert Allerton Park (RAP) in Piatt County, Illinois 2005 – 2009. I_scap is the sum of *Ixodes scapularis* ticks collected from captured small mammals per trapnight. Mammal is the sum of small mammals trapped, processed, and examined for ticks per trapnight.

Site	Year	Month	Trapnight	I_scap	Mammal
Young Forest	2005	6	1	3	5
Young Forest	2005	6	2	1	4
Young Forest	2005	6	3	0	2
Young Forest	2005	7	4	6	4
Young Forest	2005	7	5	3	2
Young Forest	2005	8	6	14	8
Young Forest	2005	8	7	8	9
Young Forest	2005	8	8	15	7
Young Forest	2005	8	9	9	3
Young Forest	2005	10	10	3	12
Young Forest	2005	10	11	2	16
Young Forest	2006	6	1	2	28
Young Forest	2006	7	2	28	46
Young Forest	2006	7	3	100	41
Young Forest	2006	8	4	94	22
Young Forest	2006	9	5	33	34
Young Forest	2006	10	6	2	43
Young Forest	2007	5	1	1	1
Young Forest	2007	7	2	19	7
Young Forest	2007	10	3	0	4
Young Forest	2008	6	1	1	15
Young Forest	2008	6	2	13	63
Young Forest	2008	7	3	7	29
Young Forest	2008	8	4	100	104
Young Forest	2008	8	5	18	25
Young Forest	2008	9	6	42	64
Young Forest	2008	10	7	6	43
Young Forest	2009	6	1	10	10
Young Forest	2009	7	2	5	12

Appendix A (cont.)

Site	Year	Month	Trapnight	I_scap	Mammal
Young Forest	2009	7	3	41	7
Young Forest	2009	8	4	47	20
Young Forest	2009	9	5	31	17
Young Forest	2009	10	6	12	17
Prairie	2005	6	1	1	2
Prairie	2005	6	2	0	1
Prairie	2005	7	3	0	5
Prairie	2005	7	4	0	1
Prairie	2005	7	5	0	2
Prairie	2005	8	6	0	2
Prairie	2005	8	7	4	9
Prairie	2005	8	8	0	6
Prairie	2005	8	9	0	4
Prairie	2005	10	10	0	1
Prairie	2005	10	11	0	1
Prairie	2006	6	1	0	0
Prairie	2006	7	2	0	1
Prairie	2006	7	3	0	2
Prairie	2006	8	4	18	4
Prairie	2006	9	5	0	1
Prairie	2006	10	6	0	8
Prairie	2007	5	1	0	1
Prairie	2007	7	2	0	2
Prairie	2007	10	3	0	1
Prairie	2008	6	1	0	0
Prairie	2008	7	2	14	5
Prairie	2008	7	3	6	4
Prairie	2008	8	4	4	7
Prairie	2008	9	5	15	9
Prairie	2008	10	6	7	14
Prairie	2009	6	1	0	0
Prairie	2009	7	2	2	3
Prairie	2009	7	3	61	10
Prairie	2009	8	4	29	15
Prairie	2009	9	5	15	10
Prairie	2009	10	6	0	8
Old Forest	2005	6	1	0	6
Old Forest	2005	7	2	0	4

Appendix A (cont.)

Site	Year	Month	Trapnight	I_scap	Mammal
Old Forest	2005	7	3	2	12
Old Forest	2005	8	4	1	8
Old Forest	2005	8	5	0	9
Old Forest	2005	8	6	0	5
Old Forest	2005	9	7	0	12
Old Forest	2005	9	8	1	11
Old Forest	2005	10	9	1	16
Old Forest	2005	10	10	0	13
Old Forest	2006	6	1	0	28
Old Forest	2006	7	2	1	26
Old Forest	2006	7	3	5	33
Old Forest	2006	8	4	4	36
Old Forest	2006	9	5	1	45
Old Forest	2006	10	6	0	54
Old Forest	2007	6	1	0	1
Old Forest	2007	7	2	1	4
Old Forest	2007	10	3	0	11
Old Forest	2008	6	1	1	37
Old Forest	2008	6	2	0	17
Old Forest	2008	7	3	36	76
Old Forest	2008	8	4	38	73
Old Forest	2008	9	5	11	45
Old Forest	2008	10	6	1	29
Old Forest	2009	6	1	0	10
Old Forest	2009	7	2	1	15
Old Forest	2009	7	3	13	23
Old Forest	2009	8	4	8	33
Old Forest	2009	9	5	1	16
Old Forest	2009	10	6	0	31
Flood Plain	2005	6	1	0	15
Flood Plain	2005	7	2	0	1
Flood Plain	2005	7	3	3	3
Flood Plain	2005	8	4	0	11
Flood Plain	2005	8	5	0	12
Flood Plain	2005	8	6	0	8
Flood Plain	2005	8	7	0	13
Flood Plain	2005	9	8	1	11
Flood Plain	2005	10	9	0	28

Appendix A (cont.)

Site	Year	Month	Trapnight	I_scap	Mammal
Flood Plain	2005	10	10	0	16
Flood Plain	2006	6	1	0	62
Flood Plain	2006	7	2	9	70
Flood Plain	2006	7	3	0	11
Flood Plain	2006	8	4	6	67
Flood Plain	2006	9	5	0	49
Flood Plain	2006	10	6	0	27
Flood Plain	2007	6	1	0	0
Flood Plain	2007	7	2	3	23
Flood Plain	2008	6	1	0	14
Flood Plain	2008	7	2	0	35
Flood Plain	2008	8	3	3	81
Flood Plain	2008	8	4	0	45
Flood Plain	2008	10	5	0	27
Flood Plain	2008	11	6	0	26
Flood Plain	2009	7	1	0	41
Flood Plain	2009	7	2	0	30
Flood Plain	2009	7	3	4	50
Flood Plain	2009	9	4	0	30
Flood Plain	2009	9	5	1	22
Flood Plain	2009	10	6	0	16