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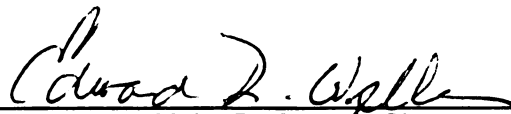
***IXODES SCAPULARIS (ACARI: IXODIDAE) AND
BORRELIA BURGDORFERI IN SOUTHWEST
MICHIGAN: POPULATION ECOLOGY AND
VERIFICATION OF A GEOGRAPHIC RISK MODEL***

presented by

Erik Scott Foster

has been accepted towards fulfillment
of the requirements for the

M.S. degree in **Entomology**



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***Ixodes scapularis* (Acari: Ixodidae) and *Borrelia burgdorferi* in
Southwest Michigan: Population Ecology and Verification of a
Geographic Risk Model**

By

Erik Scott Foster

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ABSTRACT

***IXODES SCAPULARIS* (ACARI: IXODIDAE) AND *BORRELIA BURGDORFERI* IN SOUTHWEST MICHIGAN: POPULATION ECOLOGY AND VERIFICATION OF A GEOGRAPHIC RISK MODEL**

By

Erik Scott Foster

Ixodes scapularis and *Borrelia burgdorferi* sensu stricto have been identified and isolated from field sites in Southwest Michigan. The region, historically non-endemic, shows evidence of a recent biological invasion by the vector and pathogen. A geographic risk association model, published for Wisconsin and Illinois, has been applied to the aforementioned region under invasion. Field methods including flagging, small mammal trapping, and hunter-killed deer checks were applied in an attempt to elaborate and verify the utility of the model in a six county area of Southwest Michigan. Of 80 sites investigated, 22 sites were found to have *I. scapularis*, and *B. burgdorferi* has been isolated from 16 of the 22 sites. Of the 22 tick positive sites, a significant association was found between habitats predicted by the risk model and locations of establishing populations. One population of *I. scapularis* in Covert Township was studied longitudinally over two field seasons, and its seasonal life cycle and infection rate is described.

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**For
Robert Benjamin Foster**

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Chapter 1: Introduction

Tick-Borne Disease

Ticks are blood-sucking arthropods found in virtually all terrestrial regions of the earth. They are important vectors of human and animal diseases. The biomedical and economic importance of ticks led to a great interest in the study of these arthropods in the twentieth century, although the interest has existed since ancient times. Homer mentioned the occurrence of ticks on Ulysses' dog in the year 850 B.C. An even earlier reference to "tick fever" is from an Egyptian papyrus scroll dated 1550 B.C. Pliny, Cato and Aristotle referred to ticks as disgusting parasitic animals, which were very troublesome (Obenchain and Galun, 1982). Despite this early recognition of the damage to animals caused by ticks, an understanding of tick-borne disease dynamics was gained only at the end of the nineteenth century.

In 1857, Livingstone published the results of 16 years of exploration in southern Africa. In regions that are now Angola and Mozambique, he documented an illness of humans following the bite of a tick, known regionally as the "tampan" or "carapato." This brief description was the first written account of tick-borne relapsing fever caused by the spirochete *Borrelia duttoni*. Murray subsequently described *Ornithodoros moubata*, the tick vector, in 1877. Dutton and Todd (1905) reported that *O. moubata* transmitted spirochetes to monkeys while feeding; the first demonstration that ticks were capable vectors of relapsing fever spirochetes. In the United States Smith and Kilbourne (1893) made the historic discovery that *Boophilus annulatus* (Say) transmits the protozoan agent of Texas cattle fever, *Babesia bigemina*. Close behind was the

description of Rocky Mountain Spotted Fever by the ear, nose, and throat specialist Edward Ernest Maxey (1899), and the description of the disease agent *Rickettsia rickettsii*, by Howard T. Ricketts.

Ticks rank second only to mosquitoes as vectors of life threatening, or debilitating human and animal diseases. The unique number of disease agents, which ticks transmit both to their hosts and, transovarially, to their offspring, increases the potential health hazard manifold since they are both the vectors and the reservoirs of these diseases. The global cost of controlling ticks and tick-borne diseases, together with the loss of livestock and diminished productivity is in the range of billions of dollars annually (Sonenshine, 1991).

Since the beginning of the 1980's, more than 15 new tick-borne bacterial diseases have been described in the world (Parola and Raoult, 2001), and emerging tick-borne pathogens in the United States have become recognized as significant causes of human disease. Babesiosis, human monocytic ehrlichiosis, human granulocytic ehrlichiosis, Powassan Encephalitis, and Lyme disease have caused resurgence of tick-borne disease research (Beasley *et al.*, 2001, Telford *et al.*, 1997). Lyme disease, a spirochetosis caused by *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner (Johnson *et al.* 1984) has reached epidemic proportions in some regions of the United States, with over 23,000 cases reported to the CDC in 2002 (CDC, 2004). The highest risk of transmission occurs in the Northeastern, Mid-Atlantic, and Midwest United States (Wilson 1998). I will review the history, biology, distribution, and epidemiology of Lyme disease, and present the results of recent investigations of Lyme disease ecology in southwest Lower Michigan.

Lyme Disease

History: Lyme disease was discovered with the help of two concerned mothers in Old Lyme, Connecticut. These individuals started to see an uncommonly high number of Juvenile Rheumatoid Arthritis (JRA) cases in children in their community. They brought this to the attention of the Connecticut Department of Public Health, and through a collaboration of local physicians, local public health, and the Yale University Rheumatology Section, in particular Dr. Alan Steere, organized a surveillance system. Thirty-nine children and 12 adults were found to have a similar type of arthritic condition, characterized by brief but recurrent attacks of asymmetric swelling and pain in a few large joints, especially the knee. An epidemiological analysis showed that the overall prevalence rate of the condition was 4.3 per 1000 residents; among children, it was 12.2 per 1000, a frequency 100 times greater than that of JRA (Steere *et al.*, 1977, Steere *et al.*, 1989).

There was also striking geographic clustering, with half of the affected Lyme residents living in heavily wooded areas on two adjoining country roads, as did half of those in East Haddam (an affected community nearby). Clustering within families was also observed with 6 families having more than 1 affected member. The majority of patients noted onset of symptoms in summer or early fall, with familial cases occurring over several years. These epidemiologic features seemed most compatible with an arthropod-vectored illness (Steere *et al.*, 1977).

Many patients reported an expanding lesion, identified as Erythema Migrans (EM) around the area thought to be an insect bite. In Europe, Erythema Chronicum Migrans (ECM) was described by Afzelius (1921) in Sweden, and is associated with the

bite of *Ixodes ricinus* (Linne) ticks. Patients in Europe presenting with ECM often later showed signs of neurologic abnormalities, but not arthritis. Later, studies following patients with EM in Connecticut found many developed arthritis, but also neurologic or cardiac abnormalities. Thus the illness in Lyme residents was thought to be a previously unrecognized clinical entity, a complex, multi-system disorder, and was named Lyme disease (Steere *et al.*, 1989, Sonenshine, 1993).

The identification of patients with EM also helped identify the domestic vector of the disease. Many patients remembered being bitten by a tick at the site of EM a median of 12 days before onset. One patient saved the implicated tick and it was identified as *Ixodes scapularis*, the black-legged or deer tick. Acarological surveys showed high abundance of the ticks on white-footed mice and white-tailed deer in forested areas surrounding patient's homes, and the etiologic agent *Borrelia burgdorferi* was subsequently identified from culturing rodent and tick tissues in the early 1980's (Burgdorfer, 1984, 1989).

The earliest known North American cases of Lyme disease occurred in Cape Cod in the 1960's. However, *B. burgdorferi* has been identified by PCR in museum specimens of ticks and mice from Long Island dating from the late 19th and early 20th centuries, and the infection has probably been present in North America for millennia (Dennis and Hayes, 2002). Lyme disease has since become epidemic in the U.S., where it has spread rapidly throughout many regions. It is also widespread in Europe, Asia, Japan and parts of Australia (Sonenshine, 1993). Although regional variations in *Borrelia* species and strains occur, the symptomology of the disorder is similar world-wide (Steere, 1989).

Symptoms: Lyme disease in humans is not a life-threatening illness, but if left untreated, may cause severe chronic manifestations. It is most often a mild illness mimicking a summer flu, but serious problems involving the heart, joints and nervous system may develop in some individuals. Lyme disease is usually classified into three distinct stages.

Acute Localized: In its early stages (7-14 days post infection) Lyme disease is recognized by a characteristic “bullseye” rash, erythema migrans (EM) in 70-80% of patients, and spirochetes are restricted to the peripheral edge of the rash (Steere et al., 2004).

Accompanying the rash may be headache, nausea, fever, and muscle or joint aches. At this stage, Lyme disease can be successfully treated with antibiotics, and long-term sequelae rarely occur (Wormser *et al.*, 2000).

Early Disseminated: Weeks to months after initial exposure to the bacterium or after the first symptoms appear; some people may develop complications involving the heart and/or nervous system. Specific disorders may include various degrees of heart block, nervous system abnormalities such as meningitis, encephalitis and facial paralysis (Bell's palsy), and other conditions involving peripheral nerves. Painful joints, tendons, or muscles may also be noted during this stage of the disease (Steere *et al.*, 2004).

Late Disseminated: If left untreated, the most common objective manifestation of late disseminated Lyme disease is intermittent swelling and pain of one or a few joints, usually large, weight-bearing joints such as the knee. Some patients develop neurologic disorders, or encephalopathy, the latter usually manifested by cognitive disorders, sleep disturbance, fatigue, and personality changes. Infrequently, Lyme disease illness may be severe, chronic, and disabling (Centers for Disease Control, 2003).

Etiology: Lyme disease is caused by the Gram-negative, microaerophilic spirochete, *Borrelia burgdorferi*. It is a member of the Family Spirochaetaceae, which includes similar spirochetes that cause syphilis and relapsing fever. There are currently 37 species of spirochetes in the genus *Borrelia*, many of which cause disease in humans and domestic animals (Schwan and Piesman, 2002). Ticks transmit all species, except for *B. recurrentis*, the cause of louse-borne relapsing fever. *Borrelia burgdorferi* can only be cultured in Barbour, Stoenner, Kelly (BSK) media, developed by Barbour (1984).

The principle tick vectors of Lyme disease spirochetes in North America are *Ixodes scapularis* and *Ixodes pacificus* (Cooley & Kohls). *Borrelia burgdorferi* is maintained in the environment through a complex tick, small mammal zoonosis (Ostfeld and Keesing, 2000). When a susceptible tick ingests spirochetes from infected reservoir hosts, the bacteria quickly multiply to high levels in the tick midgut until the next molt. At this point, the spirochete numbers drop to their lowest level, and are restricted to the lumen of the midgut (Piesman *et al.*, 1990). In the case of nymphal stage ticks, upon commencement of feeding activity, which lasts 3-4 days, spirochete numbers may multiply >300-fold (de Silva and Fikrig, 1995). In addition to spirochete multiplication, changes in the expression of outer surface proteins (Osp) lay the foundation for eventual transmission to the host animal through the tick salivary glands (Schwan and Piesman, 2002).

Spirochetes in the midgut of an unfed, infected nymph, predominantly express OspA. This protein is also the surface antigen expressed by the spirochetes *in vitro*. As the nymphal tick begins to feed, spirochetes begin to multiply, cease expression of OspA, and begin to express OspC. OspC expression reaches its peak at 48 hours after the

commencement of feeding, and is associated with the migration of spirochetes from the midgut, dissemination through the hemolymph, and passage through the salivary glands (Schwan and Piesman, 2000). Thus, the presence of OspA is thought to bind the spirochetes to the midgut; and the repression of OspA, and upregulation of OspC is thought to promote spirochete dissemination to the host. The factors that regulate the shift in Osp expression are likely varied and complex, but may include: Temperature (Schwan and Piesman, 2000), cell density (de Silva *et al.*, 1999), or pH (Carroll *et al.*, 1999).

Most people bitten by infected ticks do not become ill with Lyme disease. This is mainly due to prompt removal of attached ticks, and the transmission dynamics of *B. burgdorferi*, which are associated with the differential expression of OspA and OspC. Virtually no transmission occurs during the first day of tick attachment, inefficient transmission takes place during the second day of feeding, and extremely efficient transmission occurs during the third day of feeding (Piesman and Dolan, 2002). These observations are consistent with the timing of spirochete multiplication, differential expression of outer surface proteins, and dispersal within the tick. Understanding these concepts are important for health care providers in endemic areas, as antibiotic prophylaxis is not needed in instances of prompt tick removal (Wormser *et al.*, 2000, Schwan and Piesman, 2002).

Ecology

Vectors and Hosts: In the northeastern and north central United States, a highly efficient, horizontal cycle of *B. burgdorferi* transmission occurs among larval and nymphal *I. scapularis* ticks and certain rodent hosts, particularly white-footed mice and chipmunks

(Mannelli *et al.*, 1993, Slajchert *et al.*, 1997, Ostfeld and Keesing, 2000). This cycle may result in high infection among rodents and nymphal ticks, and many human cases of Lyme disease during the late spring and summer months. White-tailed deer, which are the preferred host of adult *I. scapularis*, are not involved in the life cycle of the spirochete, but seem to be critical for the survival of the ticks (Wilson *et al.*, 1985, Telford *et al.*, 1988, Rand *et al.*, 2003).

Ticks are classified as Arachnids, and fall within the dominant subclass Acari, which includes the mites and ticks. Terrestrial arthropods are believed to have evolved during the Paleozoic Era (543-248 million years ago), aided by their strong, supportive exoskeletons. Chelicerates and Uniramians are thought to have diversified during the late Silurian and early Devonian periods (441-362 million years ago), and all of today's orders of arachnids were present by the Carboniferous period (354-290 million years ago) (Hoogstraal, 1985). Ticks belong to the order Parasitiformes (mites and ticks), and the subsequent suborder Ixodida. Members of this order are obligate blood-sucking parasites with a hypostome bearing enlarged teeth to secure the tick to its host. The first tarsus bears a distinctive sensory organ, the Haller's organ, which takes the place of antennae in the Insecta (Sonenshine, 1991).

The suborder Ixodida is comprised of three families: (1) the Ixodidae (hard ticks), (2) the Argasidae (soft ticks), and (3) the Nuttalliellidae, represented by only a single species. The family Ixodidae is the largest and most economically important family. The Ixodidae are characterized by the presence of a sclerotized plate on the dorsum, the scutum (Figure 1-1); elsewhere on the body, the cuticle is characterized by many tiny

invaginations that penetrate only partially into the cuticle. Soft ticks, in contrast, have a leathery cuticle (Sonenshine, 1991).

Behaviorally, most ixodid ticks quest for hosts in vegetation rather than exhibiting den-associations. They are dispersed widely, but tend to accumulate along pathways, trails or bedding areas frequented by their hosts. Most species of the genus *Ixodes*, however, are den-associated species (Sonenshine, 1991). Ticks of this genus are easily separated from other ixodids by the presence of a ventral groove, anterior to and enclosing the anus, and the absence of ornamentation, or eyes on the lateral margin of the scutum.

***Ixodes scapularis* (Say), the Black-legged Tick**



Figure 1-1. Questing adult, female *Ixodes scapularis*. The scutum separates members of this family from other ticks. Images in this thesis are presented in color.

Ixodes scapularis is the primary vector of Lyme disease in the United States. Specimens from the Northeastern and Midwestern range of the species were originally classified as *Ixodes dammini*, and *Ixodes scapularis* was thought to occupy the southeast and south central United States. Morphological and genetic evidence revealed that the two species were actually one

species with a wide geographic range, slight regional morphological divergence, and a wide host range (Oliver, 1993).

Ixodes scapularis ticks complete their life cycle over 2 years and during that time pass through four developmental stages. Adult female ticks lay their eggs in the spring. The eggs hatch into larvae, which have 6 legs, and are approximately 1 mm in size. The

larvae seek out hosts, often a small mammal or a bird, in low-lying vegetation and leaf litter in forested habitats. Larvae take a single blood meal from the host during the summer, and subsequently drop off, and molt into the 8-legged nymphal stage. The nymphal stage overwinters, often in a bedding of leaf litter, and then emerges during the late spring or early summer, to seek a host. Nymphal ticks are generalist feeders and may feed on a variety of small to medium sized mammals, as well as birds and lizards. After obtaining a single blood meal, the nymph drops off its host and molts into the final stage, an adult. There is no sexual dimorphism until the adult stage. In the fall of that year, the adult female tick seeks a host, often a white-tailed deer (*Odocoileus virginianus* Zimmermann), for its final blood meal. Adult male ticks will seek out the deer to find a mate (Ostfeld and Keesing, 2000). The adults may find each other on large hosts by assembly or “arrestment” pheromones. Recent research has shown significant assembly of ticks in response to cast larval skins, and the chemicals guanine and xanthine, (Sonenshine, 1993, Sonenshine *et al.*, 2003). After mating and obtaining a final blood meal, the female tick drops off its host and overwinters before laying her eggs the following spring, male ticks subsequently perish. Some adult ticks may overwinter unfed or unmated until the next spring, and resume host seeking and egg laying activities in the early spring.

Transovarial transmission of *B. burgdorferi* spirochetes rarely occurs; therefore virtually all larval ticks hatch from eggs free of infection (Piesman *et al.*, 1986). Larval ticks that take their blood meal from an infected vertebrate host, however, may acquire the spirochete and retain the infection transtadially throughout the remainder of their lives. The later stages of a tick infected as a larva can therefore transmit spirochetes to

their subsequent hosts. The cycle of Lyme disease is therefore dependent on the seasonal periodicity of each stage of tick. Nymphal ticks, which emerge in the early spring, feed on small mammal hosts within endemic foci. If these nymphs were infected as larvae the previous year, they will pass on infection to their hosts. *Borrelia burgdorferi*-free larvae, which emerge after the nymphs, may then feed on these infected hosts and continue the infection cycle. The presence of overlapping cohorts is therefore essential for the maintenance of the pathogen in the environment (Spielman *et al.*, 1985, Randolph *et al.*, 2002). An example of the tick-host-infection cycle is described in Figure 1-2.

***Peromyscus leucopus* (Rafinesque), the white-footed mouse**



Figure 1-3. *Peromyscus leucopus*, the white-footed mouse. Images in this thesis are presented in color.

White-footed mice serve as the primary hosts for the immature stages of *I. scapularis* due to their wide range and generally high abundance. In addition, these small rodents are competent and efficient reservoirs of *B. burgdorferi* (Levine *et al.*, 1985, Mather *et al.*, 1989). *Peromyscus leucopus*,

once infected by a nymphal stage tick, reaches its maximum infectivity at 2-3 weeks, and may remain infective for several weeks more. Moreover, white-footed mice tolerate high levels of tick infestation. This increases its capacity and influence as a reservoir of Lyme disease spirochetes in endemic regions (Sonenshine, 1993). The mice show no apparent signs of disease, and do not pass infection to offspring.

The white-footed mouse is broadly distributed across eastern North America, and generally dwells in wooded areas, from early successional and edge-habitat to mature forests (Hall, 1981). The nest is dug in the ground or built in the hollow of a tree. The

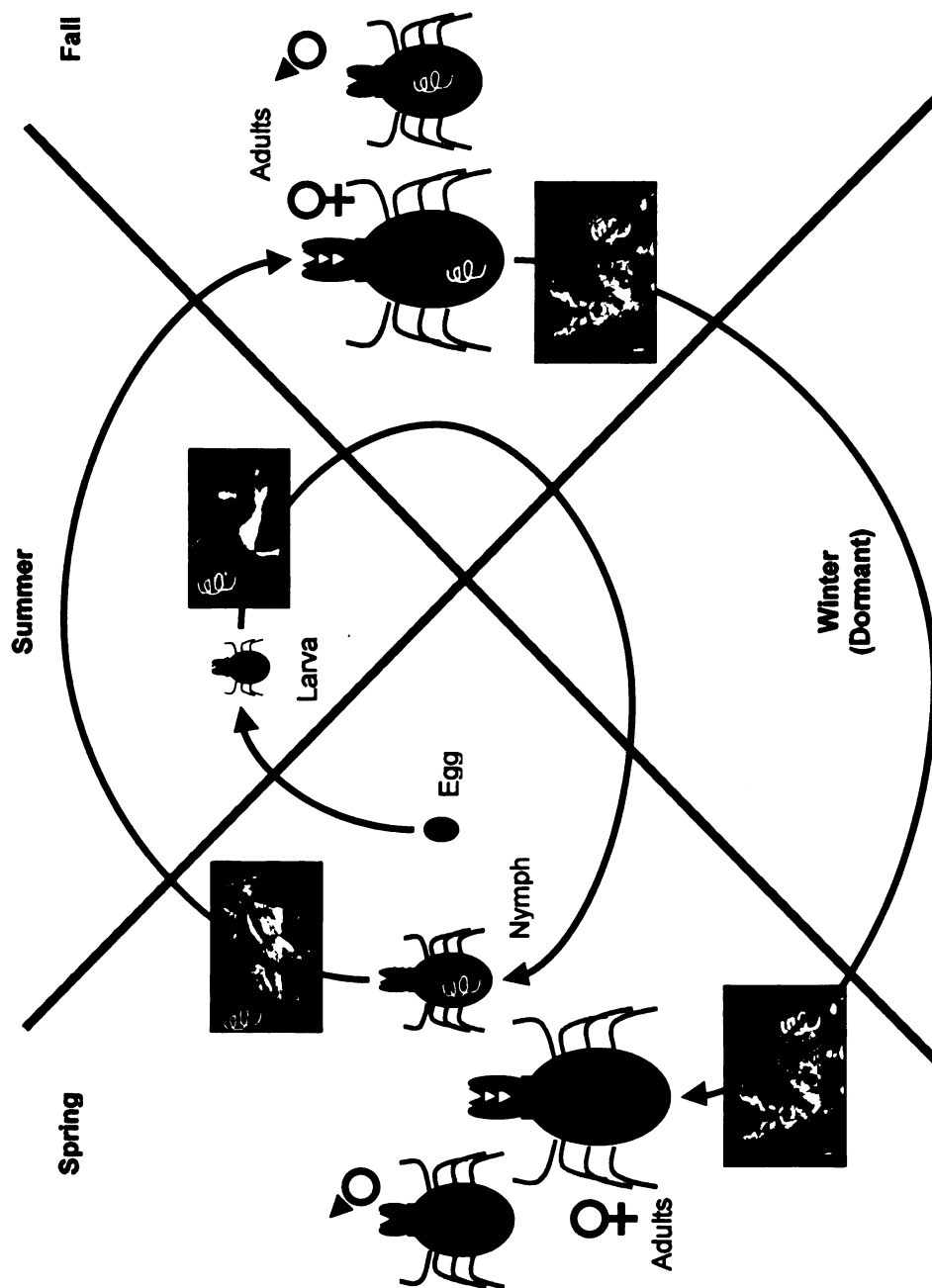


Figure 1-2. Seasonal life cycle of *Ixodes scapularis*. Eggs hatch in the early summer and larvae feed on small mammal hosts. After digesting the blood meal, larvae molt to nymphs and overwinter. During the following spring, nymphs emerge and feed on a small mammal host. Upon digestion of the blood meal, nymphs molt into the adult stage and actively seek hosts, primarily white-tailed deer. During the fall adult females take a blood meal, and males often mate the females on the host. Fed and unfed adults overwinter until the next spring. Upon mating and digestion of the blood meal, females lay a large clutch of eggs and die. Male *I. scapularis* are not capable of ingesting large blood meals and die soon after mating. Images in this thesis are presented in color.

species is nocturnal, and omnivorous, feeding primarily on insects in spring and summer, and on cached seeds and nuts through the winter (Baker, 1983). Mating usually takes place February-November, but may vary with latitude. There can be up to 4 litters a year, each with 3-5 young. The general abundance, and ability of the species to inhabit small forest patches, makes this species one of public health interest, as it is a reservoir and has implications in the spread of Lyme disease.

***Tamias striatus* (Linnaeus), the eastern chipmunk**



Figure 1-4. *Tamias striatus*, the eastern chipmunk. Images in this thesis are presented in color.

Chipmunks are competent reservoirs of the Lyme disease spirochete, and may serve as a primary source of disease amplification in certain regions and habitats, being more highly parasitized by nymphal stage ticks. They may also be an important secondary host for *I.*

scapularis larvae (Mather *et al.*, 1989, Slajchert *et al.*, 1997). The eastern chipmunk is distributed widely across the eastern half of North America, and prefers deciduous forest habitats, although it is able to survive in peridomestic habitats (Hall, 1981).

T. striatus leads a more or less solitary existence and is active during daylight hours. They feed on seeds, fruit, nuts, and insects and carry food in the pouches in their cheeks. Food is hoarded back to the nest, which is generally a burrow dug in the ground. Mating usually takes place in March-April and sometimes again in July-August. Each litter has 2-8 young, which are independent after 8 weeks (Anderson, 1982).

***Odocoileus virginianus* (Zimmermann), the white-tailed deer**



Figure 1-5. *Odocoileus virginianus*, the white-tailed deer (female). Images in this thesis are presented in color.

Population levels of white-tailed deer have been associated with *I. scapularis* tick abundance in Lyme disease endemic regions of the United States (Wilson *et al.*, 1985, Rand *et al.*, 2003). Attempts to control tick populations by completely removing the predominant host of the adult tick stage have shown success in scientific studies (Wilson *et al.*, 1988, Rand *et al.*, 2004). The studies, however, were

conducted on isolated islands, in the absence of emigration or immigration of deer.

White-tailed deer do not play a role in the maintenance of *B. burgdorferi* in the environment, as they are reservoir incompetent (Telford *et al.*, 1988). They are important, however, in the maintenance of *I. scapularis* populations, and serve as the predominant host of adult stage ticks. They also play a role in the migration of ticks into new areas.

Often viewed as an agricultural pest, white-tailed deer are ubiquitous across North America, and the northern half of South America. They gather in herds, usually of not more than a dozen animals of the same sex. They prefer to feed on tender grass and herbs in the summer months, and in winter survive on branches and leaves of bushes. After mating, the female gives birth to 1 or 2 fawns after 7 months gestation (Anderson, 1982).

Distribution of Lyme disease in the Upper Midwest: In the upper Midwest, Lyme disease occurs only in areas populated by *Ixodes scapularis*. The first discovery of *I.*

scapularis in the Midwest was made by Jackson and DeFoliart (1970) in northern Wisconsin. This discovery was coupled with a case report of erythema chronicum migrans associated with tick bite in the state (Scrimenti, 1970), laying the foundation for future investigations and description of additional foci of disease (Kitron and Kazmierczak, 1997). Populations of *I. scapularis* have since been described in Minnesota (Drew *et al.*, 1988), Iowa (Novak *et al.*, 1991), Illinois (Nelson *et al.*, 1991), and Indiana (Pinger *et al.*, 1996).

In Michigan, *I. scapularis* and *B. burgdorferi* are not wide spread, and have historically been restricted to sites in Menominee County, Upper Peninsula (Strand *et al.*, 1992, Walker *et al.*, 1994). *Borrelia burgdorferi* transmission to the human population was also documented (Stobierski *et al.*, 1994), establishing the endemicity of Lyme disease in that region of Michigan. Because of the close geographic proximity of Lyme endemic regions to Michigan, and increasing case reports of human Lyme disease in southern Michigan, surveillance and field assessment was conducted by the Michigan Department of Community Health (MDCH), Michigan State University (MSU), and the Michigan Department of Agriculture (MDA) from 1988-1998 (MDCH, 1998).

Results of passive tick surveillance for *I. scapularis* through identification of ticks submitted by citizens corroborated previously determined foci of established tick populations in Menominee County and adjacent areas, but seemed to discount the widespread presence of *I. scapularis* across southern Michigan (Figure 1-6). Of all the southern counties of Michigan, however, Berrien County shows the highest submission over the surveillance period of 17 years. This southwest region of the state is of major concern, as populations of infected *I. scapularis* ticks have been found in northwestern

Indiana (Pinger *et al.*, 1996), and in Illinois (Nelson *et al.*, 1991). It is expected that if a range expansion of *I. scapularis* is to occur, that this region is where it will progress.

From 1990 to 1998, sentinel physician and active surveillance were conducted in Lower Michigan in an attempt to discover *I. scapularis* populations or to isolate *B. burgdorferi* from wildlife, or patients presenting with Lyme disease-like illness. MDCH epidemiologists conducted over 1,000,000 patient-seasons of surveillance with participants from 48 Lower Michigan counties. Physicians were prompted to submit skin biopsies of suspected EM lesions in patients presenting with Lyme disease-like symptoms. Biopsies were cultured for the presence of *B. burgdorferi*, and no culture confirmed infections were discovered during the surveillance time span in southern Michigan. Culture confirmation was made in 10 human cases from Menominee County during the same period (MDCH, 1998).

Active, field surveillance was conducted as “case follow-up” of Lyme disease-like illness of suspected local exposure. Sixty investigations were conducted in 25 counties from 1990 to 1998 (Figure 1-7). Researchers from the MDA and MSU conducted small mammal trapping in an attempt to isolate *I. scapularis* and *B. burgdorferi* from sites in Lower Michigan. Sites were also sampled in Menominee County, after human Lyme disease exposures. A total of 967 rodent specimens were examined and tested for infection. In total, 132 infected rodents were discovered, all from Menominee County. Larval and nymphal ticks were also discovered on animals from the same area. Of the sites investigated in Lower Michigan, no infected rodents or *I. scapularis* were found (MDCH, 1998).

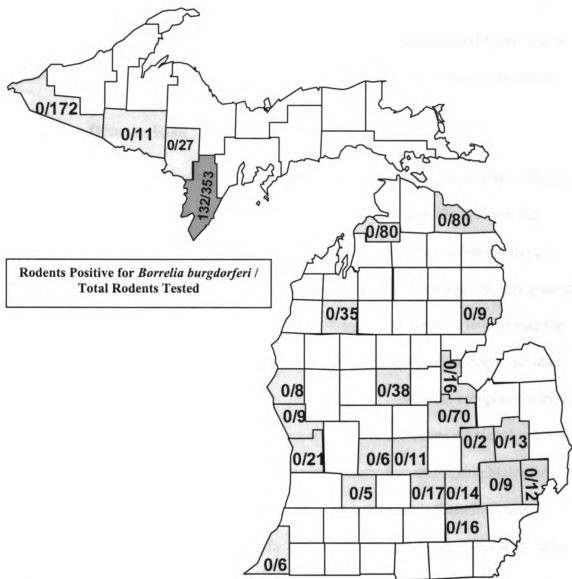


Figure 1-7. Results of case follow-up investigations of Lyme disease-like illness in Michigan, 1990-1998. Sixty investigations were conducted including small mammal trapping and flagging. *Borrelia burgdorferi* positive rodents were present only in Menominee County. No *Ixodes scapularis* ticks were found outside of Menominee County. Images in this thesis are presented in color.

These investigations supported the hypothesis that Lower Michigan is not, as a whole, endemic for Lyme disease transmission, and that the expansion of Lyme disease infected ticks into the region, would constitute a relatively recent biological invasion.

Modeling Disease Risk

Landscape Epidemiology focuses on the biotic and abiotic factors that influence the manner in which disease agents or vectors are distributed spatially between sub-populations of hosts and their habitats. Populations of hosts and parasites are largely determined by the spatially variable, often fragmented, biotic and abiotic factors present in their landscape. With the advent of GIS technology, researchers are able to map the distributions of hosts and disease incidence, and create risk association maps that may predict outbreaks across large areas. By incorporating environmental components from remotely sensed and ground surveyed sources, it is possible to detect relational patterns in the distribution of species; such patterns may yield insight into underlying biological mechanisms. In the case of vector-borne disease, the landscape is therefore mapped in terms of spatially variable infection risk factors and disease incidence (Hess *et al.*, 2002).

Risk maps can be developed on varying scales, from local or village level to regional and continental levels. The choice of spatial resolution determines the degree of precision of a risk map, and is important in modeling disease systems, or vector populations, which in the case of Lyme disease, are highly focal (Kitron, 2000). For instance, the Centers for Disease Control and Prevention (CDC) is the organization looked at to provide current information on the distribution of disease incidence in the United States. The scale at which the CDC reports regions of endemicity for Lyme disease or Lyme disease risk, is at the county level (CDC, 2004). Specifically, if a

number of ticks, or human cases are reported in a county, the entire county is therefore classified as endemic, or at a high risk. However, because the risk of acquiring Lyme disease is highly dependent upon contact with populations of infected vectors, and the vector of Lyme disease is found under specific environmental and ecological conditions, maps of such broad scale have limited use in the actual prediction of infection risk, or potential transmission interventions. They are important, however, in educating the general public and the medical community to include Lyme disease in their “index of suspicion,” especially in regions where *I. scapularis* populations are expanding.

Tick-borne diseases are geographically localized and occur only in foci with optimal conditions for the ticks and animals involved in the circulation of the pathogens (Parola and Raoult, 2001, Randolph *et al.*, 2002). Lyme disease risk in a given area depends upon the distribution of established populations of infected tick vectors. Therefore, determining the actual range, and predicting the potential range, of *I. scapularis* is vital to Lyme disease risk assessment. Recently, a habitat suitability model for *Ixodes scapularis* was developed in Wisconsin and northern Illinois (Guerra *et al.*, 2002) on a much finer geographic scale (300 x 300 m) than previous risk models (CDC, 2004). The model attempts to predict the risk for acquiring Lyme disease, based on the ecologic factors present that influence tick population maintenance. Areas where tick presence (suitability for population maintenance) is predicted are therefore associated with an increased risk of acquiring Lyme disease. In Wisconsin and northern Illinois, the model correctly classified tick absence at 83% and tick presence at 88%, an acceptably accurate result for a model based on relatively simple multivariate techniques, such as discriminant analysis and logistic regression (Hess *et al.*, 2002).

As discussed earlier, the lower peninsula of Michigan has been studied for the presence of *I. scapularis* and *B. burgdorferi* broadly, and human cases of Lyme disease-like illness have been investigated without substantial evidence of locally acquired infection. Many of the geographic and ecologic features that were found to be associated with the presence of *I. scapularis* in Wisconsin and Illinois are also widespread in lower Michigan. These features were predicted to be dry to dry/mesic deciduous forests with fertile, sandy or sandy/loam soils overlying sedimentary bedrock (Guerra *et al.*, 2002). As populations of infected *I. scapularis* ticks have been found in northwestern Indiana (Pinger *et al.*, 1996), and in Illinois (Nelson *et al.*, 1991), it is increasingly important to determine whether *I. scapularis* and *B. burgdorferi* are present in lower Michigan.

Various entities share responsibility for surveillance efforts of tick-borne diseases in Michigan, including MDCH, MDA, and MSU. The CDC are also interested in determining and communicating the risk of tick-borne disease in the United States. The risk model developed by Guerra *et al.* (2002), is a preliminary, working model to be refined and expanded, and to eventually predict Lyme disease risk across the entire Eastern United States. To assess the regional applicability of the model, however, a field test is needed using independent data. In a region historically non-endemic for the presence of the vector tick, but under suspicion of recent biological invasion, the models predictive accuracy can also be tested. Verification or invalidation would come in the form of statistical association between independently sampled sites and the predictions of the model. Through the cooperation of Michigan's various entities and the University of Illinois at Urbana Champaign, the risk model was expanded to include Michigan, with

the intent of predicting where *I. scapularis* will establish, if and when it is found in lower Michigan.

Since it is unknown whether populations of *I. scapularis* are present in southern Michigan, I propose to study the southwestern region based on its proximity to known populations in northwestern Indiana, and northeastern Illinois. It is my intent to attempt to discover *I. scapularis* individuals or populations, and to correlate tick presence or absence with the predictions of the preliminary risk model as applied to Michigan. If *I. scapularis* is discovered, it is also my intent to study whether *B. burgdorferi* is present in tick and small mammal specimens. This information is important to communicate the risks of Lyme disease to the public and to the health care community, and to raise the awareness of a regionally expanding, potentially debilitating, yet preventable disease.

This study can be accomplished by utilizing a multi-faceted sampling approach, including:

- Flagging – The direct collection of ticks from vegetation. Optimal for adult and nymphal stages.
- Small mammal captures – Removal of larval and nymphal stage ticks from small mammals. Ear punch biopsies are also taken from animals for culture or PCR identification of *Borrelia burgdorferi* infection.
- Hunter-killed deer screening – The collection of adult ticks from hunter-killed deer during the firearm hunting season.
- Infection rates will be determined by testing all nymphal and adult *I. scapularis* and ear punch biopsies.

Study Objectives

Based on historical record in Michigan, and recent ecological studies in the North central United States, it is my hypothesis that southwest Lower Michigan is in the early stages of invasion by *Borrelia*-infected ticks. My study objectives are four-fold (Figure 1-8):

- 1) Using a multi-faceted sampling approach, determine if *Ixodes scapularis* is in the process of establishment in southwest Lower Michigan by investigating sites within an eight county area.
- 2) Determine if *Borrelia burgdorferi* is present within tick and small mammal populations.
- 3) Describe the seasonal periodicity of *Ixodes scapularis* populations in Lower Michigan by longitudinally studying an established population.
- 4) Attempt to verify the predictive ability of the previously published habitat-based, geographic risk model (Guerra *et al.*, 2002), as applied to Michigan.

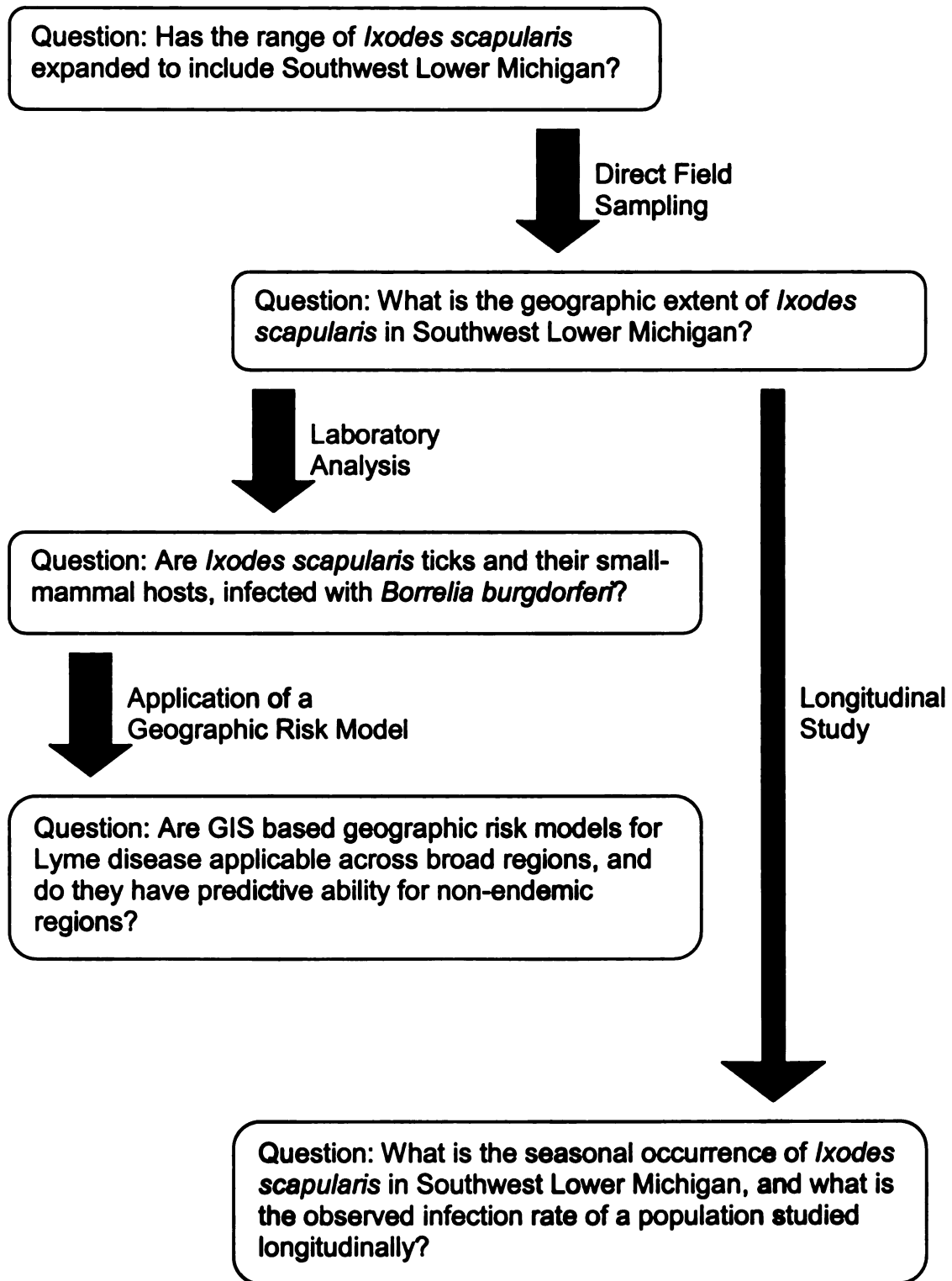


Figure 1-8. Flowchart demonstrating the questions of interest in the study, and the direction of the investigation. Images in this thesis are presented in color.

Chapter 2: Materials and Methods

Direct Field Sampling for the Presence of *I. scapularis* in Michigan

Field Sites: Sampling sites were restricted to the southwestern region of Michigan's Lower Peninsula due to the proximity of tick populations described in Illinois (Nelson *et al.*, 1991, Guerra *et al.*, 2002) and Indiana (Pinger *et al.*, 1996) (Figure 2-1). Regional topography varies from flat and rolling terrain (inland field sites) to upland dune habitat (shoreline field sites). Forage and row crops, upland and lowland mixed deciduous forest tracts, orchards, and sparse urban development dominate the landscape. The shoreline from Berrien County in the south to Muskegon County in the study area is dominated by northern hardwood associated, and mixed deciduous/coniferous sand dune habitat (Figure 2-2).

From May 2002 to October 2003, 80 sites were surveyed for the presence of *I. scapularis* by "flagging", small-mammal trapping, or both. The sites were geocoded into a geographic information system (GIS) and mapped using ArcMap 8.1 (ESRI, Redlands, CA) (Table 2-1, Figure 2-3). Field sites were selected on the basis of three criteria:

- Accessibility
- Land Use/Land Cover
- Deer screening results

A number of state, county, and municipal parks/game areas are accessible in the eight county area of southwestern, lower Michigan under investigation. These sites provided a starting point for tick investigations due to their accessibility, geographic extent, and availability of undisturbed forest tracts. Scientific collection permits were obtained from

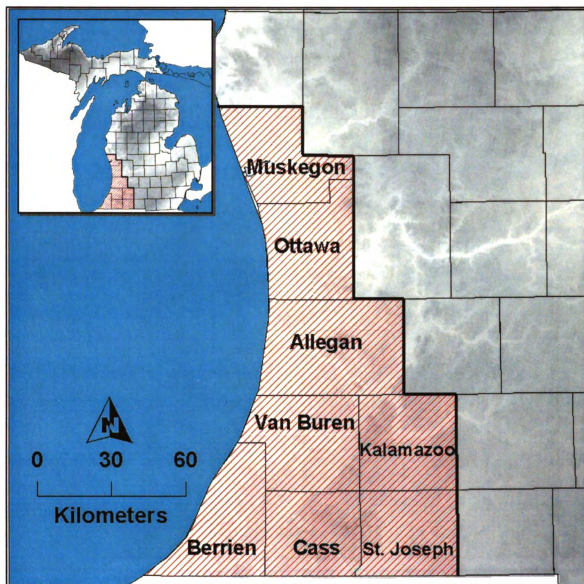


Figure 2-1. Map of the geographic location of southwestern, lower Michigan, and the region of interest in the current study. Images in this thesis are presented in color.

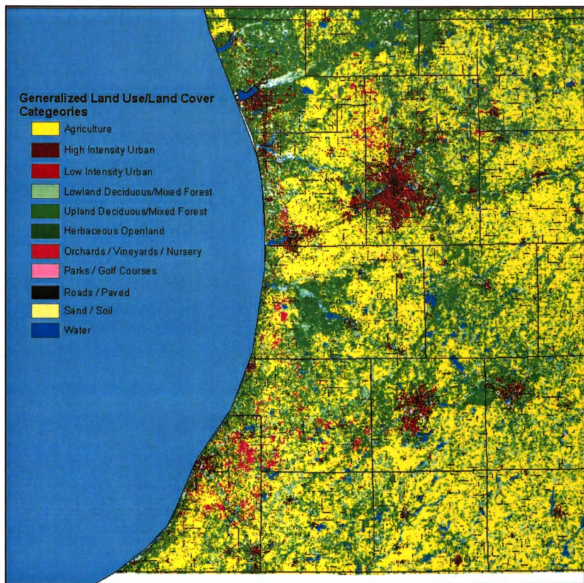


Figure 2-2. Land cover and Land use classification of southwestern, lower Michigan, based on data derived from classification of Landsat Thematic Mapper (TM) imagery. Originators: MDNR, Forest, Mineral and Fire Management Division. Images in this thesis are presented in color.

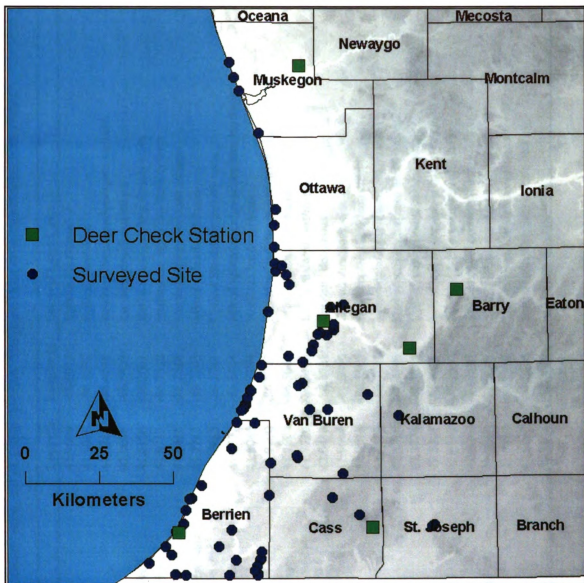


Figure 2-3. Location of sites directly surveyed in southwestern, Lower Michigan from November 2001 to November 2003. Sites investigated directly by flagging or small mammal trapping (blue circle); Deer check stations, operated November 15-16, 2001-2003 (green square). Images in this thesis are presented in color.

Table 2-1. List of directly sampled sites by site ID, county, name, and geographic location. The general land cover classification is also listed.

Site ID	County	Name/Location	Latitude	Longitude	General Land Cover
1	Berrien	Warren Dunes SP	41.9030	-86.6012	Upland Shrub/oak
2	Berrien	Warren Dunes SP	41.9016	-86.6082	Upland Shrub/oak
3	Berrien	Warren Woods SP	41.8319	-86.6248	Lowland deciduous/emergent wetland
4	Berrien	Grand Mere SP	42.0038	-86.5425	Lowland deciduous/maple
5	Berrien	Bond Rd. St. Joseph East Bank	41.8156	-86.2601	Herbaceous open/low density trees
6	Berrien	Bond Rd. St. Joseph East Bank	41.8215	-86.2558	Herbaceous open/low density trees
7	Berrien	Plym Park Rd. NW, Niles	41.8416	-86.2538	Northern hardwood association
8	Berrien	Bertrand Rd. St. Joseph West Bank	41.7743	-86.2695	Mixed upland deciduous
9	Berrien	NE Witmer and River Rd.	41.7983	-86.2741	Upland Shrub/low density trees
10	Berrien	Red ArrowHwy / tracks	41.8571	-86.6484	Mixed upland deciduous/northern hardwood
11	Berrien	RedArrow/M12trail	41.8048	-86.7184	Northern hardwood/oak
12	Berrien	Madeline Bertrand Park	41.7700	-86.2700	Mixed upland deciduous/maple-elm
13	Berrien	Olive Branch & Avery Bridge	41.7690	-86.5640	Mixed upland deciduous
14	Berrien	Martin Rd.	41.7720	-86.6100	Lowland deciduous/maple
15	Berrien	Rudnick Park	42.0433	-86.5031	Lowland deciduous
16	Berrien	Warren Dunes SP	41.9004	-86.6040	Upland shrub/oak-elm
17	Berrien	Grand Mere SP	42.0018	-86.5505	Mixed upland deciduous/maple
18	Van Buren	Van Buren SP West	42.3377	-86.3041	Mixed upland deciduous/oak-maple
19	Van Buren	Van Buren SP East	42.3360	-86.3006	Herbaceous open/sparse deciduous/oak
20	Van Buren	Firelane 1	42.2974	-86.3215	Upland mixed deciduous/oak-maple
21	Allegan	Monroe & 42nd	42.5301	-85.9558	Mixed upland deciduous/oak-beech/herb open
22	Allegan	2 track Deer Dump	42.5262	-85.9557	Mixed upland deciduous/oak
23	Allegan	S.E. of 115 & 42nd	42.5225	-85.9557	Mixed upland deciduous/oak
24	Allegan	2nd 112 Turnoff W of 44th	42.5066	-85.9861	Mixed upland deciduous /conif/oak
25	Allegan	N.E. of 112 & 48	42.5137	-86.0132	Upland shrub/low density oak-beech
26	Allegan	N.E. of 112 & 49	42.5083	-86.0226	Mixed upland deciduous/oak-beech
27	Van Buren	Cal Haven Trail	42.4153	-86.2556	Mixed upland deciduous

Table 2-1. (contd.)

Site ID	County	Name/Location	Latitude	Longitude	General Land Cover
28	Berrien	Sarret Nature Preserve	42.1566	-86.3798	Lowland mixed deciduous/oak-beech
29	Allegan	Saugatuck Dunes	42.7025	-86.1970	Lowland mixed deciduous/coniferous
30	Berrien	Warren Dunes Group Site	41.9261	-86.5771	Lowland mixed deciduous/oak
31	Allegan	125th & 38th ASGA	42.5987	-85.9165	Mixed upland deciduous/coniferous
32	Allegan	42nd & Monroe ASGA	42.5386	-85.9554	Mixed upland deciduous/oak-aspen
33	Van Buren	Firelane 2	42.2940	-86.3231	Upland mixed deciduous/oak-maple
34	Van Buren	Firelane 3	42.2908	-86.3206	Upland mixed deciduous/oak-maple
35	Van Buren	27 1/2 & 28th	42.3252	-85.8161	Upland mixed deciduous/oak
36	St. Joseph	Three Rivers North Site	41.9245	-85.5448	Lowland scrub/mixed deciduous
37	St. Joseph	Three Rivers South Site	41.9202	-85.5553	Lowland scrub/mixed deciduous
38	Cass	Crane Pond SGA 1	41.9188	-85.8027	Mixed upland deciduous/oak-maple
39	Cass	Crane Pond SGA 2	41.9548	-85.8524	Mixed upland deciduous/oak-maple
40	Allegan	Fennville #1	42.5792	-86.2273	Upland shrub/low density oak
41	Berrien	Bennie Lane	41.9091	-86.3765	Mixed upland deciduous
42	Berrien	Red Bud Trail & Wildon Ct.	41.8169	-86.3586	Lowland mixed deciduous/maple
43	Berrien	Weaver Rd. Forest Fragment	41.7848	-86.2803	Mixed upland deciduous
44	Ottawa	Holland SP	42.7774	-86.2050	Upland dune shrub/beech-maple
45	Allegan	52nd St. ASGA	42.4770	-86.0405	Mixed upland deciduous/oak
46	Allegan	7th Day Compound	42.4583	-86.0470	Mixed upland deciduous/beech-maple
47	Van Buren	15th Ave.-Van Buren Co.	42.3600	-86.0871	Mixed upland deciduous/maple
48	Van Buren	M-140, South Haven	42.3765	-86.2665	Herbaceous open/sparse maple-aspen
49	Van Buren	Palisades Park Community	42.3136	-86.3154	Mixed upland deciduous/oak-maple
50	Van Buren	Firelane 4	42.2862	-86.3282	Mixed upland deciduous/oak-maple
51	Van Buren	Firelane 6	42.2803	-86.3302	Mixed upland deciduous/oak-maple
52	Van Buren	70th & 84th	42.1148	-86.2183	Mixed hardwoods
53	Cass	Beechwood	42.0150	-86.2228	Oak Dom/mixed Hardwood
54	Van Buren	681 & 18th	42.3513	-86.1042	Lowland Decid/Maple Dom

Table 2-1. (contd.)

Site ID	County	Name/Location	Latitude	Longitude	General Land Cover
55	Van Buren	38th & 52nd	42.2771	-86.0566	Mixed Upland Deciduous/Oak Dom
56	Van Buren	38th & 46th	42.2774	-85.9825	Mixed Hardwood
57	Berrien	48th & Johnson	42.2368	-86.2847	Beech/Maple and Oak
58	Allegan	68th ave & 104th st	42.4428	-86.1455	Lowland mixed decid/maple dominant
59	Allegan	Warner Camp, Grand Junction	42.4231	-86.0858	Mixed lowland decid/conifer/shrub
60	Van Buren	92nd ave & 36th st	42.0820	-85.9187	Mixed HW & Conifer/Maple
61	Cass	Russ County	42.0078	-85.9633	Mixed upland deciduous /conif
62	Van Buren	CR 681& CR 352	42.1294	-86.1063	Mixed lowland HW Oak Dom
63	Van Buren	Redfield & Fir Rd.	42.1364	-86.1089	Mixed lowland deciduous
64	Berrien	Oak Forest Rd.	41.7705	-86.3860	Mixed lowland deciduous
65	Allegan	Old Allegan Rd.	42.6611	-86.1425	Mixed hardwood/conifers
66	Allegan	Wakerobin Estate	42.7267	-86.2013	Mixed upland deciduous /oak-beech-maple-pines
67	Berrien	Coveney	41.8583	-86.4309	Mixed hardwood/conifers
68	Allegan	62nd & Blue Star	42.6917	-86.1526	Mixed upland deciduous /Beech-Maple/herb open
69	Allegan	64 & 140	42.7183	-86.1741	Mixed lowland coniferous/Pines
70	Muskegon	PJ Hoffmaster State Park	43.1285	-86.2727	Mixed upland deciduous/Oak-pines
71	Allegan	Allegan State Game Area	42.5926	-85.9695	Mixed upland deciduous /Oak dom
72	Ottawa	Butternut and Lakeshore	42.8917	-86.1978	Mixed upland deciduous /Oak dom
73	Ottawa	168 & Ransom	42.8446	-86.2033	Mixed upland deciduous /Oak-pines
74	Berrien	Martin and Taube	42.2373	-86.3490	Mixed upland deciduous /Oak
75	Berrien	Livingston and Red Arrow	41.9667	-86.5662	Mixed upland deciduous/Oak
76	Kalamazoo	West of Kalamazoo	42.2585	-85.6884	Mixed upland deciduous/Herbaceous open
77	Van Buren	Firelane 8	42.2761	-86.3391	Upland mixed decid/oak-maple
78	Muskegon	River and Scenic	43.2979	-86.3778	emergent wetland/pines
79	Muskegon	Duck Lake State Park	43.3455	-86.3960	lowland/upland/decid mixed/herb open
80	Muskegon	Muskegon State Park	43.2560	-86.3551	Upland mixed decid/Oak ass/herb open

the Michigan Department of Natural Resources, and park access was negotiated. Sites of interest on private land were investigated under permission of the landowner.

Based on previous studies of the habitat preferences of *I. scapularis* (Kitron *et al.*, 1992, Glass *et al.*, 1994, 1995, Guerra *et al.*, 2002) sites were also selected by browsing GIS coverages of Land Use/Land Cover and selecting likely habitats. Sampling attempts were conducted by random stops at small forest patches along predetermined transects. Locations of deer infested by *I. scapularis* during the previous field season and noted on county level maps, were also used to direct collection attempts. Site selection was not standardized due to the nature of the investigation. A major goal of the study was to identify establishing populations of ticks in a region of the state where *I. scapularis* has never been collected directly from the environment. To this end, many sites were investigated using the above means, and not randomly selected. This afforded a better opportunity to discover ticks in small habitat patches across a large region.

Hunter-Killed Deer Screening: Deer screening was conducted during the opening two days of firearm hunting season in southwest Michigan during fall 2001-2003.

Volunteer collectors teamed with Michigan Department of Natural Resources (DNR) biologists examined white-tailed deer (*Odocoileus virginianus*) carcasses at check stations located in state game areas and at a regional DNR operations station (Figure 2-4, Table 2-2). MDNR personnel verified age and sex of the deer, and locational data was provided by the hunter voluntarily on county level maps. Deer were examined along the head and shoulders for the presence of ticks by parting the fur with a comb and forceps. Ticks were placed into vials containing a moist substrate, and later identified to species

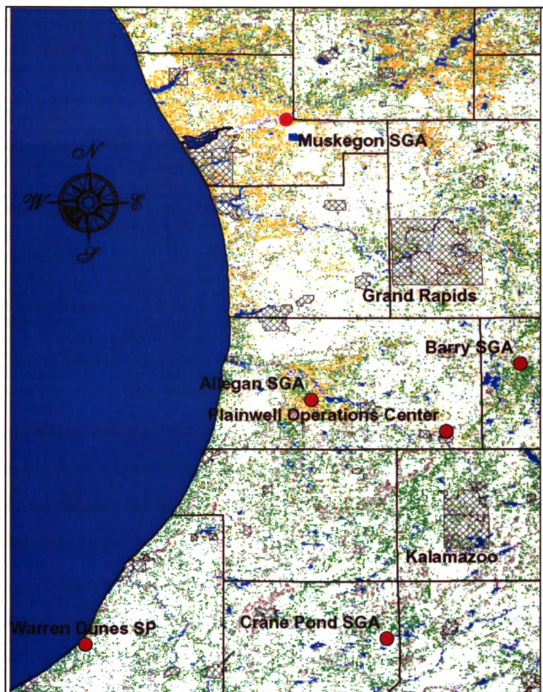


Figure 2-4. Location of deer screening stations during the firearm-hunting seasons, 2001-2003. Images in this thesis are presented in color.

Check Station	Dates Sampled
Allegan State Game Area (SGA)	Nov. 15-16, 2001 Nov. 15-16, 2002 Nov. 15-16, 2003
Barry SGA	Nov. 15, 2003
Crane Pond State Game Area	Nov. 15-16, 2001 Nov. 15-16, 2002 Nov. 15, 2003
Muskegon SGA	Nov. 16, 2003
Plainwell Operations Center	Nov. 15-16, 2001 Nov. 15-16, 2002 Nov. 15-16, 2003
Warren Dunes State Park	Nov. 15, 2002 Nov. 15-16, 2003

Table 2-2. Deer check stations manned by volunteers for tick screening. Hunter-killed deer surveys were conducted during the firearm hunting season during 2001-2003.

using Keirans and Clifford (1978).

Ixodes scapularis ticks were tested by DNA extraction and PCR for detection of *B. burgdorferi* infection (see below).

“Flagging”: “Flagging” is the collection of questing ticks using a drag cloth (approx. 1-m²) made of flannel or corduroy (Figure 2-5).

The drag cloth is pulled behind the collector through the undergrowth

and leaf-litter at the forest floor. Ticks waiting for a suitable host on the vegetation will attach to the cloth as they would a passing animal. This method is useful for collecting nymphal and adult ticks, but larvae, which are highly focal within the environment, are more likely collected by means of small mammal surveys.

Flagging was conducted during peak adult and nymphal questing periods to identify new populations of *I. scapularis* in southwestern, Lower Michigan. Based on studies of tick seasonality in Menominee County, flagging was conducted from May to July, then again in October (Walker, personal communication). Collectors visited sites and sampled in an approximately 150 meter radius around a geocoded point using a Garmin® GPS 12XL, during timed visits. This confined sites to an area of roughly 300 meters in diameter, which is the smallest scale predictive ability of the GIS based risk model developed by Guerra *et al.* (2002). Tick drags were checked at approximately 20-

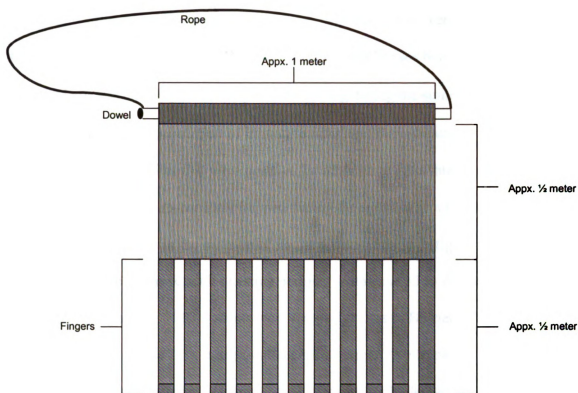


Figure 2-5. Diagram of a drag cloth or "flag" for directly sampling ticks from their questing locations in the environment. The flag is constructed using sewn corduroy cloth, rope, and a wooden dowel. "Fingers" are sewn to the trailing edge of the flag so that a portion of the cloth is always in contact with lowest vegetation and leaf-litter. Images in this thesis are presented in color.

meter increments for attached ticks. Ticks were removed from the cloths using fine-tipped forceps, stored in 70% ETOH, and identified to species using Keirans and Clifford (1978), and Durden and Keirans (1996). *Ixodes scapularis* ticks were later analyzed for the presence of *B. burgdorferi* by DNA extraction and PCR (see below).

Small Mammal Surveys: The purpose of trapping during the summers of 2002 and 2003 was to assess the population of immature ticks, and *Borrelia* infection among ticks and small mammals. Sites that were positive for adult stage ticks during the adult questing periods were subsequently trapped during the summer months, and several sites of interest were trapped in the absence of flagging data when investigations were conducted outside the peak adult questing period. From June 13 to August 22, 2002, and May 7 to September 17, 2003, rodents were trapped at sites in Berrien, Van Buren, and Allegan Counties (Table 2-3). Large, collapsible Sherman live-traps (3 x 3.5 x 9") were used to capture small mammals (Figure 2-6A). Traps were baited with a mixture of whole oats, black sunflower seeds, and placed along linear transects approximately 5 meters apart. Placement of traps was conducted subjectively according to the habitat trapped, and mammal signs in the environment (obvious rodent burrows, feeding trails). All traps were set in the late afternoon and checked early the following morning. Transects varied in the number of traps and number of nights trapped at each location. Standardized transects and trapping methods are often used to estimate population size and geographic focality. In this study trapping methods were not standardized because the main purpose of trapping was to collect as many rodents as possible, and examine them for presence of *B. burgdorferi* and *I. scapularis* without reference to host population size.

Table 2-3. Sites investigated for the presence of immature *Ixodes scapularis* by small mammal trapping. All sites were investigated for immature *I. scapularis* after adult ticks were identified by flagging, except (*) which were only investigated by small mammal trapping.

Trapping Site	County	Trap Nights	Latitude	Longitude	General Land Cover
112 & 48	Allegan	395	42.5136	-86.0132	Upland shrub/low density oak and beech
125 & 38	Allegan	80	42.5987	-85.9165	Mixed upland deciduous/coniferous
168 & Ransom	Allegan	100	42.8446	-86.2033	Mixed upland deciduous /coniferous
48 & Johnson	Berrien	360	42.2368	-86.2847	Beech, maple, and oak
64 & 140	Allegan	100	42.7182	-86.1741	Mixed lowland coniferous/pines
*Allegan State Game Area	Allegan	200	42.5925	-86.9695	Mixed upland deciduous /oak
*Butternut & Lakeshore	Ottawa	150	42.8916	-86.1978	Mixed upland deciduous /oak
Firelane 1	Van Buren	900	42.2974	-86.3215	Mixed upland deciduous /oak and maple
Firelane 2	Van Buren	320	42.2939	-86.3231	Mixed upland deciduous /oak and maple
Firelane 3	Van Buren	128	42.2907	-86.3206	Mixed upland deciduous /oak and maple
Firelane 4	Van Buren	346	42.2861	-86.3282	Mixed upland deciduous /oak and maple
Fennville	Allegan	160	42.5791	-86.2273	Upland shrub/low density oak
Grand Mere State Park	Berrien	500	42.0001	-86.5519	Mixed upland deciduous /maple
Madeline Bertrand Park	Berrien	100	41.7700	-86.2700	Mixed upland deciduous /maple and elm
Palisades Park	Van Buren	290	42.3136	-86.3154	Mixed upland deciduous /oak and maple
Saugatuck State Park	Allegan	300	42.7025	-86.1970	Lowland mixed deciduous /coniferous
Van Buren State Park	Van Buren	406	42.3376	-86.3041	Mixed upland deciduous /oak and maple
Wakerobin Estate	Allegan	200	42.6617	-86.1422	Mixed upland deciduous /coniferous
WarrenDunes State Park	Berrien	623	41.9260	-86.5771	Mixed lowland deciduous /oak
Weaver Rd., Niles	Berrien	360	41.7847	-86.2803	Mixed upland deciduous /oak and maple
Total		6018			



Figure 2-6. A) Large collapsible mammal trap set along the base of a tree, and baited with a whole oat/sunflower seed mix. Sticks are used to brace the trap against mischievous visitors (raccoons, black-squirrels, etc.); B) Trapped rodents are emptied into plastic holding bags for easier handling, and maneuvering into the inhalation chamber; C) The animal is lightly anesthetized with methoxyfluorane; D) Ticks are gently pulled from their feeding locations, usually on the head and ears of the animal, and stored for identification and testing by DNA extraction and PCR; E) An ear punch biopsy is taken while the animal is anesthetized, the sample is immediately stored in a phosphate buffered saline/30% glycerol solution, on dry ice or liquid nitrogen. Ear punches can then be cultured, or undergo DNA extraction and PCR for the presence of *B. burgdorferi* in the lab. Images in this thesis are presented in color.

Captured animals were removed from the traps into plastic zipper-bags for handling (Figure 2-6B), and anesthetized using a Metofane (Methoxyfluorane) inhalation chamber fashioned from a 50 ml conical tube and cotton balls (Figure 2-6C). Animals were then sexed and identified to species. Ticks were removed using fine-tipped forceps, and placed into collection tubes for later identification and testing (Figure 2-6D). Ear punch biopsies were taken (2-4/rodent) to facilitate the isolation/identification of *Borrelia* infection (Figure 2-6E). Ear biopsies were immediately placed into a PBS/30% Glycerol solution, and flash frozen on dry ice or liquid nitrogen. This was done to allow for laboratory culture of *B. burgdorferi* spirochetes from the tissue. Captured animals were allowed to recover in sheltered containers, and then released back into their natural environment. Ticks identified as *Ixodes scapularis* were stored in 70% ETOH at -20° C for later PCR analysis. Small mammals were collected under a scientific collectors permit, issued by the Michigan Department of Natural Resources Wildlife Division.

Culture and PCR Detection of *Borrelia burgdorferi* from Tick and Rodent Biopsy Samples

Tick samples were analyzed using a modified Phenol-Chloroform extraction protocol (Reineke *et al.*, 1998). After extraction of the DNA, a nested PCR was performed using a primer set specific to the flagellin gene of *B. burgdorferi* (Johnson *et al.*, 1992). The PCR protocol was adapted from the Invitrogen® PCR SuperMix™ Kit. PCR products were mixed 5:1 with loading dye to monitor the DNA as it migrated across the gel. Samples were run on a 0.5% (w/v) agarose gel, stained in a 50 µg/ml ethidium bromide solution, and photographed using an AlphaImager™ system (Alpha Innotech, San Leandro, CA) (Figure 2-7).

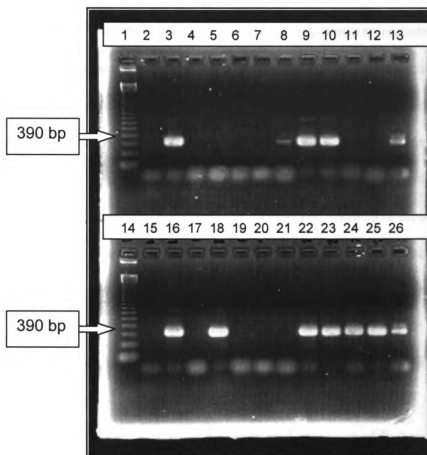


Figure 2-7. Photograph of a 26 well agarose gel loaded with DNA extracted from ticks. The gel is dyed in ethidium bromide and photographed over fluorescent light, exposure is 1 second. A band at 390 base pairs (bp) indicates amplification of the *flagellin* inner gene target of *Borrelia burgdorferi*. Description of wells: 1 & 14 – Marker lanes, 123 bp ladder; 2 & 15 – Negative control lanes, specimens run through the extraction process with no specimen, extraction reagents only; 3-12 & 16-25 – Samples from field sites in southwestern, lower Michigan; 13 – Extraction positive control, a *B. burgdorferi* B-31 strain infected tick is run through the extraction and PCR process; 26 – PCR positive control, a sample of tick or mammal DNA known positive for *B. burgdorferi*. Images in this thesis are presented in color.

Rodent punch biopsies were analyzed using both culture and PCR techniques. Both methods allow for further molecular analysis of tick-borne spirochetes isolated from field sites in southwestern, Lower Michigan. Culture, while more time-consuming, allows for the isolation of live organisms which can be frozen for long periods and resuspended in media for further experimentation. Biopsies from 2002 were cultured in modified Barbour-Stoenner-Kelley (BSK) II medium (Barbour, 1984) with antibiotic supplementations as described by Walker et al. (1994) and allowed to incubate at 34°C for 3 weeks. The samples were checked weekly for spirochete growth using phase-contrast light microscopy at 400X. When spirochetes were noted, cultures were filtered, grown to high density, aliquots mixed with glycerol to 20%, and frozen at -70°C. Isolates were later confirmed as *B. burgdorferi* by DNA isolation and PCR using the flagellin nested primer set described above.

DNA from 2003 punch biopsies was extracted using a modified version of the Qiagen Qiamap Mini Kit tissue protocol. After DNA extraction, PCR was performed according to methods listed previously.

Longitudinal Study at Covert Township Sites, 2002-2003

Seasonal activity has been described for immature ticks (Wilson and Spielman, 1985), and for adult ticks (Sonenshine, 1993, figure 23.8) in the northeast United States, and these life cycles are well established. Seasonal periodicity, however, may vary depending upon climactic and landscape variation. Previous studies in the upper Midwest have diverged slightly from accepted life cycles based on tick populations in the northeast United States (Platt *et al.*, 1992, Walker ED, unpublished). An assessment of the seasonal periodicity of *I. scapularis* is lacking in Michigan, as the only previously

described endemic region of the state is in an isolated location of Menominee County in the Upper Peninsula, and results of longitudinal studies at these sites are unpublished.

A longitudinal study was conducted in an Upland Oak predominated forest patch of approximately 3-km² on the shore of Lake Michigan in Van Buren County (Figure 2-8). The aim of the longitudinal study was to describe the seasonal periodicity of the life-stages of *I. scapularis* in Lower Michigan, and to assess the infection rate of the two infectious tick stages, and their small mammal hosts at established foci. Adult and nymphal ticks were collected using 1-m² corduroy flags during timed visits. Small rodents were sampled on live trap transects for immature ticks and ear tissue biopsy. Flagging was conducted on 26 dates and small mammal trapping on 51 dates, represented by 1694 trap-nights from June 2002-October 2003. Tick infection rates were determined by nested PCR, and rodent infection rate by ear biopsy culture in BSKII media, or DNA extraction and PCR.

Application of a Geographic Risk Model to Michigan

To verify the predictive ability of the landscape risk model published by Guerra *et al.* (2002) for Northern Illinois and Wisconsin, the model was applied to a GIS database containing the corresponding geographic and ecological variables for Michigan. The landscape risk model is based upon associations of land cover and other landscape features with presence or absence of *Ixodes scapularis*, by sampling sites in northern Michigan, throughout Wisconsin, and in northern Illinois. The map's function is to highlight areas of high risk for the establishment or maintenance of *I. scapularis* populations, and subsequently, the risk of Lyme disease (Figure 2-9).



Figure 2-8. Location of longitudinal study sites in Covert Township encompassing approximately 3-km². Points are buffered by an area of radius 150-m to illustrate total area surveyed. Images in this thesis are presented in color.

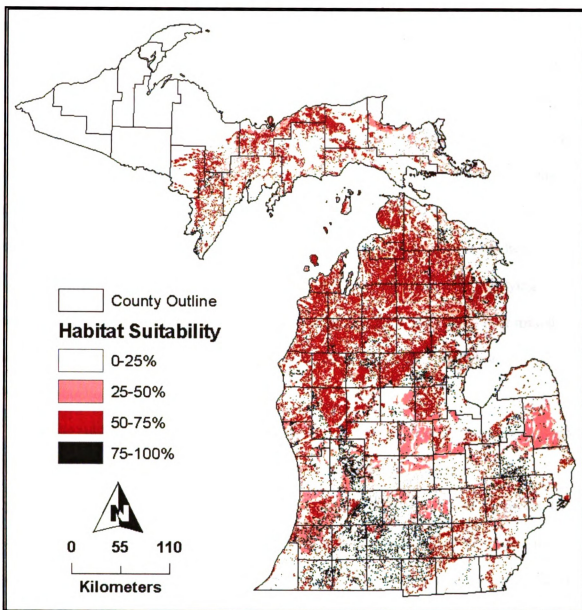


Figure 2-9. Map of Michigan displaying the predictions of the habitat suitability model published by Guerra *et al.* (2002), as applied to Michigan's corresponding habitat variables. Outputs of the model range from 0.00 – 0.999 and are converted to percentiles, and classified into four categories of habitat suitability using equal intervals. Images in this thesis are presented in color.

A model emerged from the land cover layers as follows. Contingency table analyses of the 2 x X format (ticks present/absent x landcover categories within landscape types) were carried out, revealing statistical associations with land cover, soil order, soil texture, forest type/moisture gradient, and bedrock geology. Discriminant functions for tick presence and tick absence were developed and were highly informative, because the equations correctly predicted ticks to be absent in 83% of sites where indeed they were absent; and predicted ticks to be present in 88% of sites where they were indeed present. From the above studies, habitat for *Ixodes scapularis* was predicted to be dry to dry/mesic deciduous forests with fertile, sandy or sandy/loam soils overlying sedimentary bedrock. Unsuitable habitat included grasslands and coniferous or mixed forests, wet forests, relatively infertile soils of clay and silt texture, overlying Precambrian bedrock.

The model was projected onto Michigan using existing datasets available through the Michigan Department of Natural Resources Geographic Data Library (www.mcgi.state.mi.us/mgdl). When displayed using ArcMap 8.1 (ESRI, Redlands, CA), each 300m x 300m pixel assumes a risk characteristic, based on the logistic regression model. The risk characteristics are then classified by equal interval into two categories of risk (0-50%, 50-100%), or four categories of risk (0-25%, 25-50%, 50-75%, 75-100%). Sampled sites were classified into two categories based on *I. scapularis* presence or absence and *chi*-square analysis was performed using SAS 9.1 (©SAS Institute Inc., Cary, NC) on 2x2 and 4x2 tables to verify the model's predictive accuracy.

Chapter 3: Results

Hunter-Killed Deer Screening

November 15 & 16, 2001: Volunteer collectors accompanied DNR wildlife biologists at three check stations in southwest lower Michigan; Crane Pond State Game Area, Plainwell Operations Center, and Allegan State Game Area. Of 130 hunter-killed, white-tailed deer examined, 4 deer were found to have *I. scapularis* ticks. Three of 4 deer had both male and female *I. scapularis*, indicating the possibility of reproducing populations near the location of deer harvest (Table 3-1). The reported locations of deer harvest included the Allegan State Game Area (Allegan County), the area around Warren Dunes State Park (Berrien County), and along the St. Joseph River in the vicinity of Niles (Berrien County) (Figure 3-1). These areas were subsequently sampled directly by flagging during the 2002 field season.

November 15 & 16, 2002: Collectors were located at the same check stations as in 2001 with the addition of a check station at Warren Dunes State Park. Of 221 hunter-killed, white-tailed deer examined, 5 deer were found to have *I. scapularis* ticks (Table 3-1). Two of the 5 deer had both male and female *I. scapularis*, and 3 of the tick-infested animals were harvested at or near locations where infested deer were identified during 2001 (Figure 3-1). Additional locations implicated by infested deer for follow-up investigation during the 2003 field season included the vicinity of Three Rivers (Cass County) and the Crane Pond State Game Area (St. Joseph County).

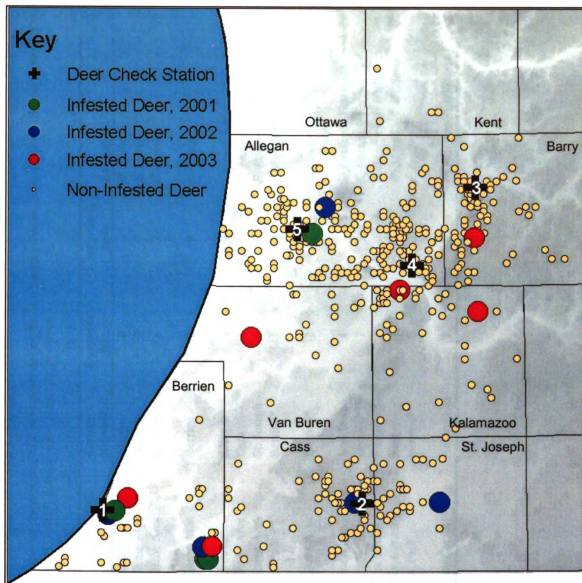


Figure 3-1. Map of the distribution of hunter-killed, white-tailed deer screened during the firearm-hunting season, 2001-2003. Colored circles indicate deer that were found to have *Ixodes scapularis* ticks; Green=2001, Blue=2002, Red=2003. Deer check stations are denoted by numbered crosses; 1=Warren Dunes State Park, 2=Crane Pond State Game Area, 3=Barry State Game Area, 4=Plainwell Operations Center, 5=Allegan State Game Area. Muskegon State Game Area is absent from the figure due to lack of deer specimens examined. Figures in this thesis are presented in color.

Table 3-1. White-tailed deer screening positive for the presence of *I. scapularis* during firearm hunting season, 2001-2003. Each row represents one white-tailed deer (*Odocoileus virginianus*). (*) Denotes previous deer screening positives.

Date	Check		County Harvested	<i>I. scapularis</i>		Examiner
	Station			female	male	
Nov. 15, 2001	Allegan State Game Area		Allegan	0	1	<i>B. Bullard</i>
	Crane Pond State Game Area		Berrien	1	1	<i>E. Foster</i>
	Crane Pond State Game Area		Berrien	1	2	<i>E. Foster</i>
Nov. 16, 2001	Crane Pond State Game Area		Berrien	2	3	<i>E. Foster</i>
Nov. 15, 2002	Allegan State Game Area		Allegan*	1	0	<i>T. Friedrich</i>
	Crane Pond State Game Area		Cass	1	1	<i>E. Foster</i>
	Crane Pond State Game Area		St. Joseph	0	1	<i>E. Foster</i>
	Warren Dunes State Park		Berrien*	1	0	<i>B. Bullard</i>
Nov. 16, 2002	Crane Pond State Game Area		Berrien*	2	4	<i>E. Foster</i>
Nov. 15, 2003	Crane Pond State Game Area		Van Buren	1	1	<i>E. Foster/S. Yaremnych</i>
	Crane Pond State Game Area		Berrien*	7	9	<i>E. Foster/S. Yaremnych</i>
	Plainwell Operations Center		Kalamazoo	1	0	<i>W. Morgan/A. Williams</i>
	Plainwell Operations Center		Barry	1	0	<i>W. Morgan/A. Williams</i>
	Warren Dunes State Park		Berrien*	0	2	<i>J. Tsao</i>
Nov. 16, 2003	Plainwell Operations Center		Kalamazoo	1	0	<i>W. Morgan/A. Williams</i>

November 15 & 16, 2003: Collectors were located at six check stations including two additional stations at the Barry State Game Area, and the Muskegon State Game Area. Of 299 hunter-killed, white-tailed deer examined, 6 deer were found to have *I. scapularis* ticks (Table 3-1). Two of the 6 deer had both male and female ticks. Two deer were harvested from areas previously implicated, including the area around Warren Dunes State Park and the St. Joseph River in the vicinity of Niles. Interestingly, these two locations were consistent throughout the three-year surveillance period, with *I. scapularis* being found on deer harvested every year. New locations for follow up investigation were identified in Van Buren, Barry, and Kalamazoo Counties (Figure 3-1).

Site follow-up investigations of areas where infested deer were harvested during the 2001-hunting season led to the discovery of a focus of *I. scapularis* at Warren Dunes State Park. Ticks were not present at the sites investigated in the Allegan State Game Area, or along the St. Joseph River in 2002. *I. scapularis* activity was further implicated during the 2002-hunting season in the same vicinities, and follow-up investigations revealed two additional population foci in 2003. Small numbers of ticks were found in the Allegan State Game Area, and a reproducing population (indicated by the presence of multiple specimens of each life-stage) was found along the St. Joseph River, south of Niles. The investigation of sites near Three Rivers and the Crane Pond State Game Area revealed no tick activity.

Flagging

Seventy-seven field sites were surveyed for the presence of *I. scapularis* directly by tick-drag during spring and summer of 2002 and 2003. Ticks were collected at 21 sites, in three southwest Michigan counties. Nineteen of 21 positive sites (90.5%) are

within 7 km, and 17 of 21 sites (81%) are within 3 km of the Lake Michigan shoreline. Most sites where *I. scapularis* were found are characterized by sandy soils, and mixed deciduous forest cover (oak/maple dominant). Tick density varied by site and date and is represented by number of ticks dragged per hour. The highest density of adult *I. scapularis* was found in the spring and fall months. Nymphal densities were highest in late spring/early summer. Larval densities are listed, but estimates are better represented by small mammal capture data. The highest densities of ticks were found along the shore of Lake Michigan, in upland dune habitats (Figure 3-2).

Small Mammal Surveys

Twenty sites were investigated by small mammal survey during the summers of 2002 and 2003 (Table 3-2). Trapping efforts were represented by 6,018 total trap nights (number of traps set/date/site). Altogether 690 small rodents were captured (including recaptures) and examined for the presence of feeding *I. scapularis*. *Peromyscus leucopus* was the most commonly captured rodent (83.6% of all trapped mammals) in every study site, with *Tamias striatus* and *Glaucomys volans* comprising most of the remainder (13.5% and 2.3% respectively). *Glaucomys volans* captures were restricted to sites along the shore of Lake Michigan in upland dune habitats (Table 3-2, Figure 3-3).

Fifteen sites were found to have small mammals with feeding *I. scapularis*. *Dermacentor variabilis* was also found at all sites with *I. scapularis* present. All dominant rodent species harbored immature *I. scapularis* and *Dermacentor variabilis* at tick positive sites. *Tamias striatus* were significantly more likely to harbor nymphal *I. scapularis* at tick positive sites than other species. *P. leucopus* harbored both larval *I. scapularis* and *D. variabilis* at higher incidence than *T. striatus*. *G. volans* showed lower

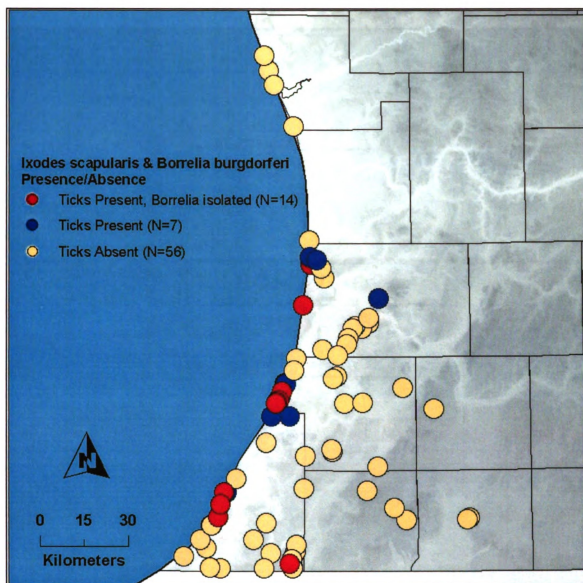


Figure 3-2. Distribution of sites sampled by tick drag. (Red) Fourteen sites revealed *Ixodes scapularis* activity, and *Borrelia burgdorferi* was isolated from tick specimens. (Blue) Seven sites show evidence of *I. scapularis* activity, with no *B. burgdorferi* detected in tick specimens. (Beige) Fifty-six sites show no *I. scapularis* activity. Sampling was conducted during peak adult questing periods from May, 2002-October, 2003. Figures in this thesis are presented in color.

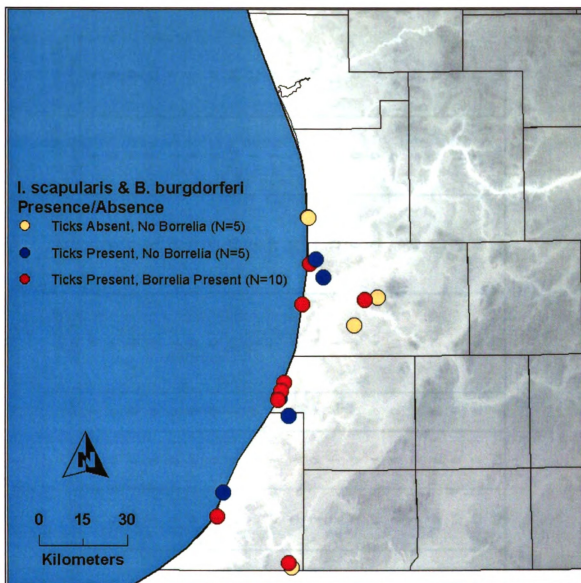


Figure 3-3. Distribution of sites sampled by small mammal trapping (N=20). (Red) Ten sites show *Ixodes scapularis* activity, and *Borrelia burgdorferi* was isolated from small mammal specimens. (Blue) Five sites showed evidence of *I. scapularis* activity, with no *B. burgdorferi* detected in small mammal specimens. (Beige) Five sites showed no *I. scapularis* activity on small mammals. Sampling was conducted during peak immature tick activity periods from May, 2002-September, 2003. Figures in this thesis are presented in color.

Table 3-2. Trapping site, total trap nights, species totals, and tick totals for small mammal surveys conducted during 2002 and 2003 in southwest, lower Michigan.

Trapping Site	Trap Nights	<i>P. leucopus</i>	<i>T. striatus</i>	<i>G. volans</i>	Other	<i>I. scapularis</i> nymph	<i>I. scapularis</i> larvae	<i>D. variabilis</i> nymph	<i>D. variabilis</i> larvae
112 & 48	395	49	1	0	1	0	0	8	6
125 & 38	80	7	0	0	0	0	0	0	8
168 & Ransom	100	8	14	0	0	0	0	0	0
48 & Johnson	360	14	1	3	0	0	1	0	13
64 & 140	100	6	2	0	0	1	2	2	1
Allegan State Game Area	200	45	0	0	0	0	3	8	1
Butternut & Lakeshore	150	12	1	0	0	0	0	4	0
Firelane 1	900	83	15	0	1	19	80	16	36
Firelane 2	320	17	2	0	0	2	7	1	12
Firelane 3	128	7	4	3	0	7	20	1	26
Firelane 4	346	20	2	3	1	8	30	3	28
Fennville	160	28	2	0	0	21	40	6	66
Grand Mere State Park	500	88	2	1	0	1	21	23	179
Madeline Bertrand Park	100	10	1	0	0	0	0	0	0
Palisades Park	290	2	12	4	0	4	3	0	6
Saugatuck State Park	300	17	3	0	0	11	7	7	30
Van Buren State Parks	406	63	3	2	0	9	48	5	50
Wakerobin Estate	200	15	0	0	0	2	12	4	23
WarrenDunes State Park	623	71	15	0	1	8	24	17	40
Weaver Rd., Niles	360	15	13	0	0	4	42	1	11
Totals	6018	577	93	16	4	97	340	106	536

tick incidence for both species and life stages, possibly due to its arboreal nature (Table 3-3). Nymphal *I. scapularis* (N=105) from small mammal hosts were tested for the presence of *B. burgdorferi* by PCR. Infected ticks were frequently associated with *T. striatus*, and of the nymphal ticks associated with *P. leucopus*, none were found to harbor *B. burgdorferi* (Table 3-3).

PCR and culture isolation of *B. burgdorferi* from rodent ear punch biopsies revealed 30 infected animals, with *Tamias striatus* having the highest total infection rate across all captures (Table 3-4). *B. burgdorferi* was not detected in small mammals at any site where *I. scapularis* was absent.

Table 3-4. Comparison of individual infection rate of small mammals with *B. burgdorferi* by species for southwestern, lower Michigan.

<i>Species</i>	No. Individuals Captured	No. Individuals Infected	Infection Rate (%)
<i>Peromyscus leucopus</i>	443	17	3.8
<i>Tamias striatus</i>	85	13	15.3
<i>Glaucomys volans</i>	15	0	0

Longitudinal Study Results

Data from longitudinal studies conducted from 2002-2003 are important to demonstrate the seasonal periodicity of *I. scapularis* at an established site in southwest Lower Michigan. 158 small mammals were screened, and 716 *I. scapularis* were collected (403 questing adults, 176 nymphs, 137 larvae), between early-June and mid-October, 2002, and late-April to mid-October, 2003.

As cohorts of ticks are overlapping and the life cycle of *I. scapularis* is two years, the data are presented as an aggregation of mean density. For tick-drag collection, total ticks/drag-hour is averaged by week across both years. For small mammal data, the total ticks per week are divided by total captures, and averaged by week. Infection rate is

Table 3-3. Larval and nymphal *Ixodes scapularis* presence and infestation rates on the most often trapped species in southwest, lower Michigan. Infection rate of screened nymphs is also compared across species for all specimens collected by small mammal trapping.

<i>Species</i>	No. Individuals Captured	Total Nymphs	Total Larvae	Infestation Rate (No. ticks/capture)			No. Nymphs Screened	No. (%) Positive
				Nymphal <i>I. scapularis</i>	Larval <i>I. scapularis</i>			
<i>Peromyscus leucopus</i>	392	24	296	0.06	0.86		24	0 (0)
<i>Tamias striatus</i>	71	77	41	1.08	0.58		77	26 (33.8)
<i>Glaucomys volans</i>	15	1	2	0.07	0.13		1	0 (0)

described as number of infected ticks over total ticks by life stage and is a “true” description of infection rate, as all adult and nymphal ticks were tested for infection with *B. burgdorferi*.

Trapping success varied from 2.86-42.5%, and was generally associated with trapping effort (Figure 3-4). Both *T. striatus* and *P. leucopus* were broadly active during the trapping period, and *G. volans* activity was noted until mid-July. 13 *P. leucopus* and 7 *T. striatus* were found to be infected with *B. burgdorferi* either by culture or PCR analysis. Infection rates appear seasonally bimodal, but may also be associated with trapping effort (Figure 3-5).

Adult ticks are most prevalent in the early spring, represented by peak populations in the month of April. The earliest collection date was late April (week 18), and represented a peak of 38-adult ticks/drag hour. Whether the peak in the longitudinal data represents the true population density peak is unclear as field collections of adult *I. scapularis* only decline from week 18. Clearly, however, as the peak is witnessed in week 18, it can be inferred that adult activity was present in the previous weeks. Adult tick activity declines into June, and a less substantial peak (13-ticks/drag hour) of activity is seen in the fall months, through November (Figure 3-6).

Pre-adult activity is evident from the spring months to late summer (weeks 19-38). Results of flagging and rodent captures are analyzed using different scales (ticks dragged/hour, mean ticks/capture), and are summarized in Figure 3-6. These results show broad activity throughout the sampling timeframe, and are not clear as to whether populations of immature ticks show a unimodal or bimodal distribution.

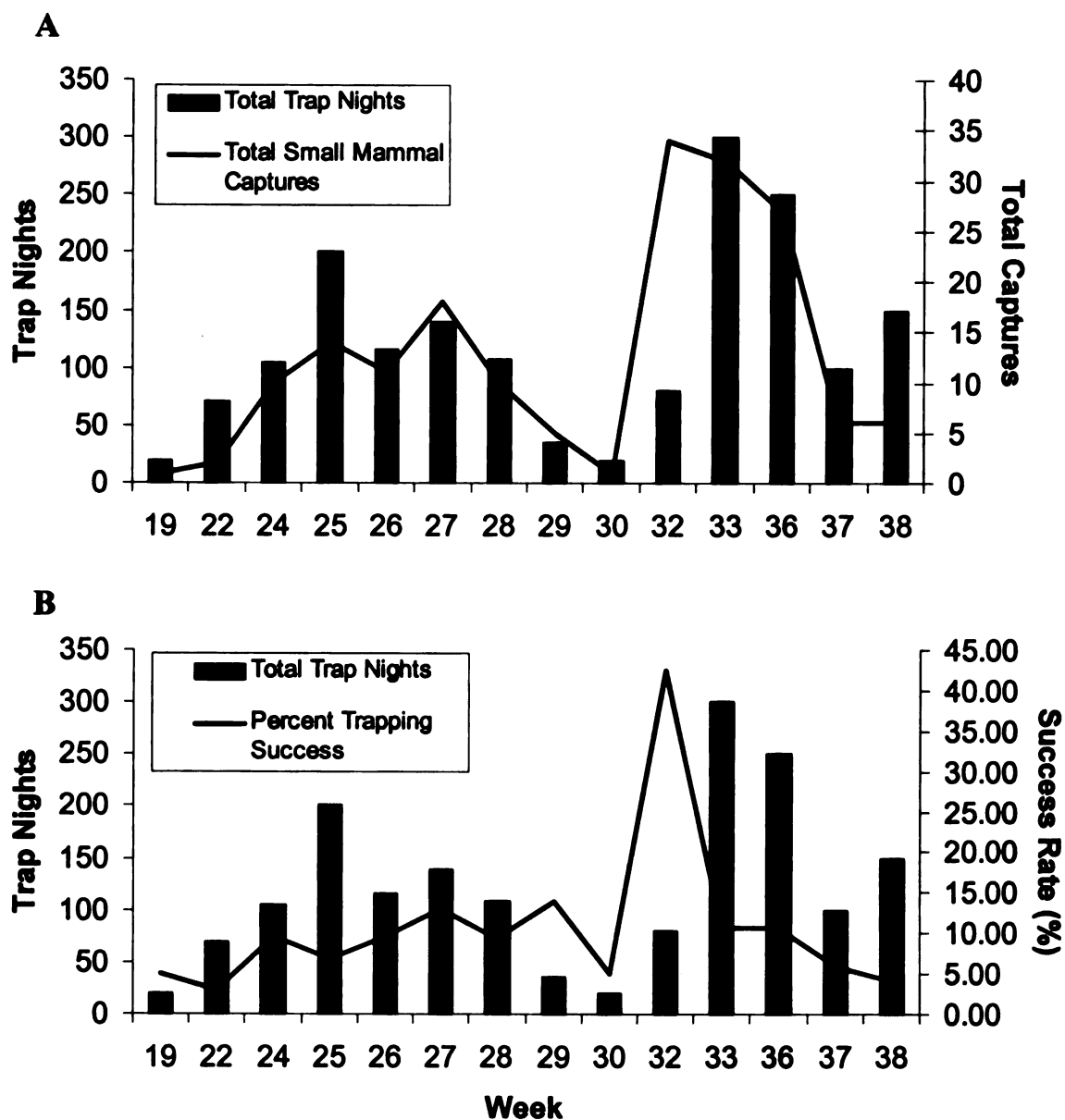


Figure 3-4. Trapping effort, represented by number of trap nights by week, is compared to total captures (A), and capture success rate (B). Data are aggregated by week over the two-year sampling period. Figures in this thesis are presented in color.

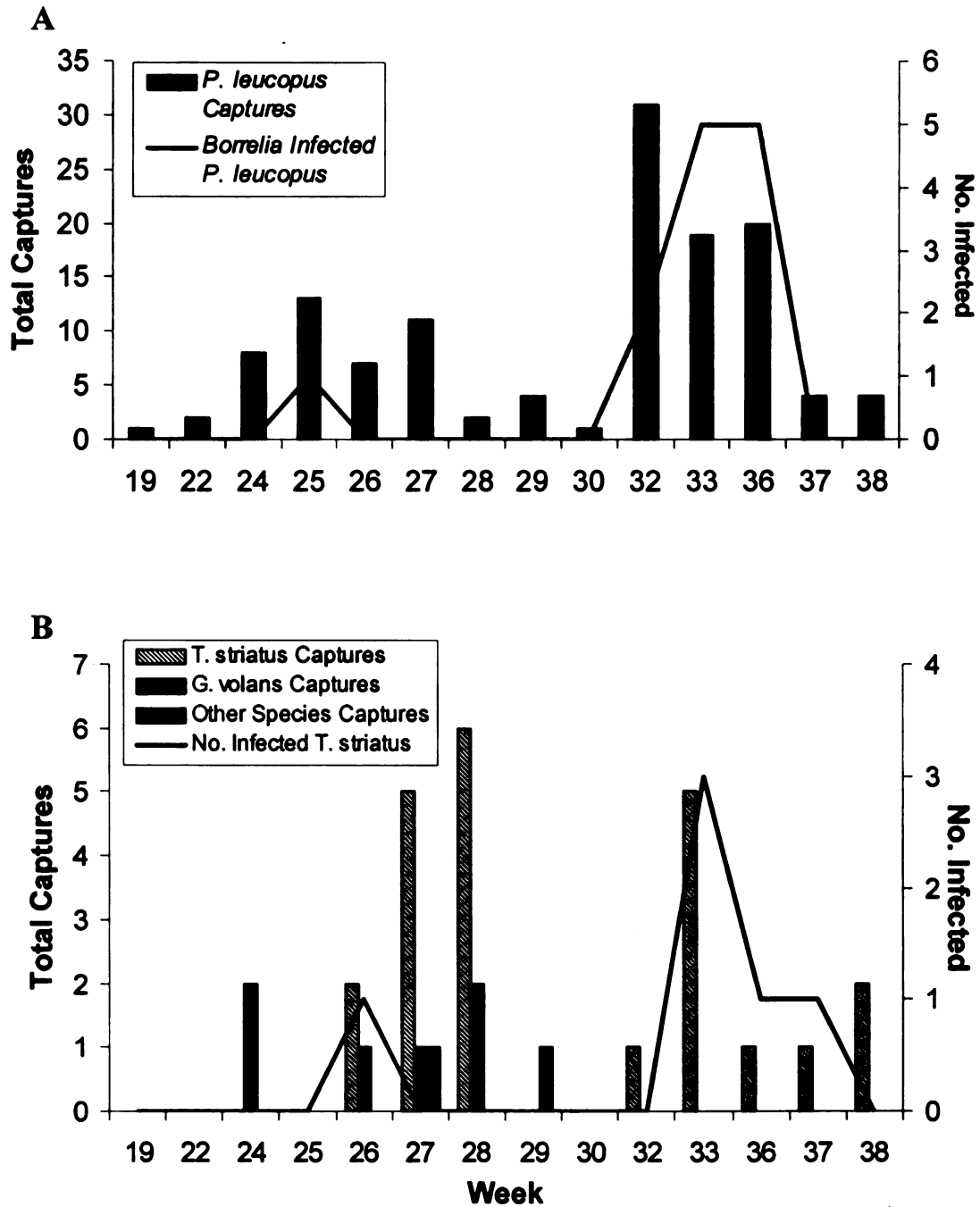


Figure 3-5. Temporal pattern of infection in small mammal captures by week, 2002-2003. A) *Borrelia* infected *Peromyscus leucopus* captures by week vs. total captures; B) *Borrelia* infected *Tamias striatus* captures by week vs. total captures. Images in this thesis are presented in color.

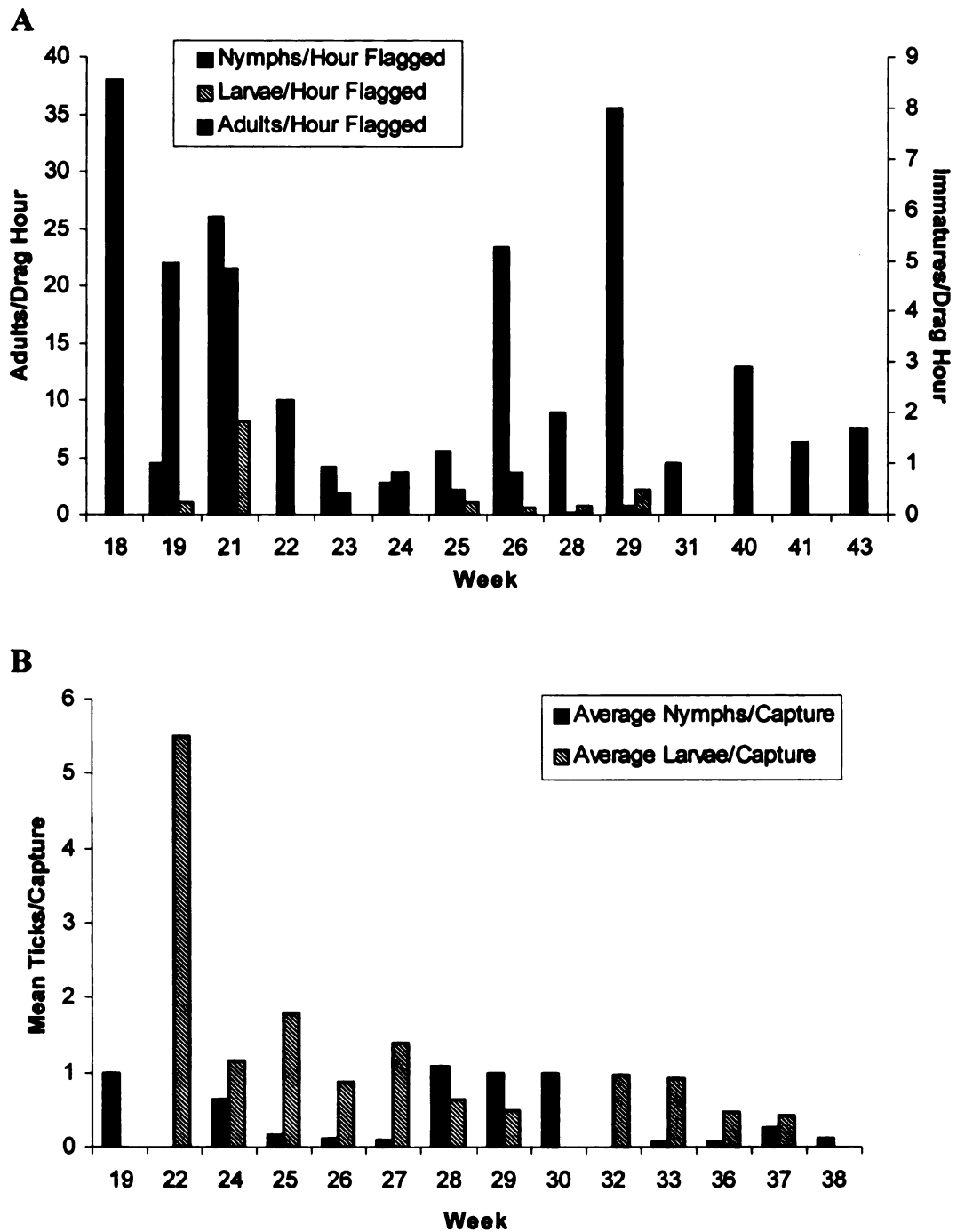


Figure 3-6. *Ixodes scapularis* seasonal periodicity at a longitudinal study site in Covert Twp. A) Seasonal results of flagging efforts, tick abundance is represented by the number of ticks/drag-hour/stage; B) Seasonal results of trapping efforts, tick abundance is represented as the average number of immature ticks/capture/stage. Images in this thesis are presented in color.

A total of 579 nymphal and adult stage ticks were tested for *B. burgdorferi* presence by PCR, with an overall infection rate of 30.22%. Females had the highest infection rate of 40.1% (N=207), followed by males at 33.7% (N=196), and nymphs at 14.8% (N=176) (Figure 3-7B).

A Field Test of a Geographic Risk Model

Field sites were classified into two categories based on *I. scapularis* presence or absence. Data from all field investigations were aggregated to include sites positive by flagging, trapping, or both. 22 of 80 sites investigated in southwest lower Michigan show evidence of *I. scapularis* activity (Figure 3-8) and were assigned to the “present” category, all other sites were assigned to the “absent” category. The geographic risk model published by Guerra *et al.* (2002) was classified into either 2-risk categories of equal interval (0-50%, 50-100%), or 4-risk categories of equal interval (0-25%, 25-50%, 50-75%, 75-100%).

A 2x2 Likelihood Ratio Chi-Square was performed using the 2-risk category classification, and tested against the null hypothesis of no association. Analysis using SAS software showed a significant association between tick presence and the habitat risk profiles predicted by the model (Likelihood Ratio $\chi^2=7.34$, $df=1$, P value=0.0049). Fisher’s exact test also showed a highly significant association (P value=0.0048) indicating the higher risk-category (50-100%) is predictive of *I. scapularis* presence at greater frequency than the lower risk-category (0-50%) based on 80 sites surveyed (Table 3.5). Chi-square analysis of the 2x4 table based on 4-risk categories was also significant (Likelihood Ratio $\chi^2=15.15$, $df=3$, P value=0.0017), verifying the models ability to describe suitable habitat within a historically non-endemic region (Table 3-6).

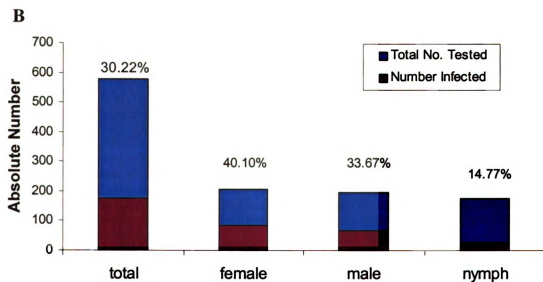
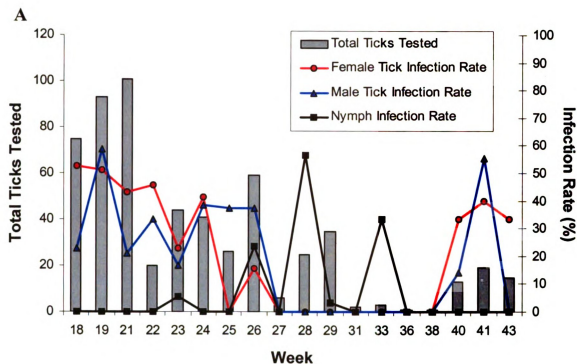


Figure 3-7. A) Infection analysis of ticks collected at a longitudinal site in Covert Twp., by week and developmental stage. Total ticks tested were aggregated by week from 2002 and 2003 collections, and ticks were tested individually. B) Infection rate of all ticks collected in Covert Twp., 2002 and 2003. Images in this thesis are presented in color.

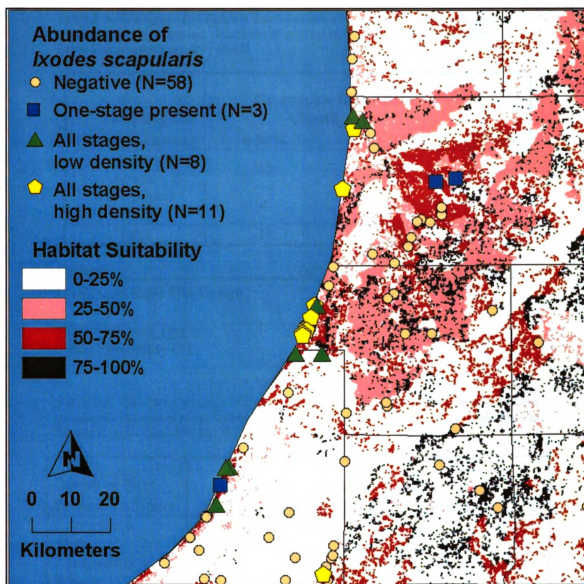


Figure 3-8. Summary map of all investigated sites in southwest, lower Michigan, classified by *Ixodes scapularis* abundance. (Blue square) sites where only one life stage of ticks found; (Green triangle) sites with immature and adult ticks at low density (<5 ticks/drag-hour); (Yellow pentagon) sites with immature and adult ticks at high density (≥ 5 ticks/drag-hour); (Beige circle) ticks absent. The habitat suitability model is also displayed, classified into four categories (0-25%, 25-50%, 50-75%, 75-100%). Images in this thesis are presented in color.

Table 3-5. Chi-square and Fisher's Exact analysis of *I. scapularis* presence /absence vs. the prediction of the geographic risk model as applied to Michigan with 2-classifications. Fisher's Exact Test is significant (P=0.0048).

Sampling Site Presence/Absence	Geographic Risk Model Classification		Total
	0-50%	50-100%	
<i>I. scapularis</i> Absent	30	28	58
<i>I. scapularis</i> Present	4	18	22
Total	34	46	80

<u>Statistic</u>	<u>DF</u>	<u>Value</u>	<u>Probability</u>
Likelihood Ratio Chi-Square	1	7.8985	0.0049

<u>Fisher's Exact Test</u>	
Table Probability (P)	0.0048

Table 3-6. Chi-square analysis of *I. scapularis* presence/absence vs. the prediction of the geographic risk model as applied to Michigan with 4-classifications. Likelihood ratio Chi-Square is significant (P=0.0017).

Sampling Site Presence/Absence	Landscape Model Risk Classification				Total
	0-25%	25-50%	50-75%	75-100%	
<i>I. scapularis</i> Absent	21	9	20	8	58
<i>I. scapularis</i> Present	1	3	17	1	22
Total	22	12	37	9	80

<u>Statistic</u>	<u>DF</u>	<u>Value</u>	<u>Probability</u>
Chi-Square	3	13.3787	0.0039
Likelihood Ratio Chi-Square	3	15.1467	0.0017

Geographic Extent and Population Projection Exercise

The geographic extent of *Ixodes scapularis* ticks found during the study is presented in Figure 3-8, and is classified into four categories based on the presence and abundance of ticks. Sites where ticks were absent were labeled as such and assigned a numeric value of zero. Sites where ticks were present were classified into three categories:

- One stage of *Ixodes scapularis* found – numeric value one
- Immature and adult *Ixodes scapularis* found at low density (<5 ticks/drag-hour) – numeric value two
- Immature and adult *Ixodes scapularis* found at high density (≥ 5 ticks/drag-hour) – numeric value three

GIS applications can prove useful in projecting limited datasets across spatial regions of interest. This is often done by interpolation, which is the process of using points with known values to estimate values at other points. In this instance, inverse-distance interpolation was used. Inverse-distance methods lay a grid on top of the control points (investigated sites), estimate the values at each grid node as a function of distance to the control points, and then interpolate between the grid nodes. The term *inverse-distance* refers to the fact that control points are weighted as an inverse function of their distance from grid points; control points near a grid point are weighted more than control points far away (Slocum, 1999). Inverse distance methods estimate cell values by averaging the values of sample data points in the vicinity of each cell. The closer a point is to the center of the cell being estimated, the more influence, or weight, it has in the averaging process. This method assumes that the variable being mapped decreases in influence with distance from its sampled location. An example can be made of the tick survey data derived from this investigation. The investigated sites with tick density

classifications from Figure 3-8, having numeric values from 0-3, were interpolated using GIS software across southwest, lower Michigan to give a probable tick density estimate across the region, on the same numeric scale (Figure 3-9). The output, therefore, estimates the tick density at sites not sampled, on the same classification scale (0: no ticks – 3: all stages, high density). This simple method however fails to account for regional differences in habitat suitability, assuming instead a homogeneous landscape across which tick populations could expand.

To provide a more biologically meaningful interpolation, however, the analysis was conducted by combining the habitat suitability model with the surface interpolation, and creating a new projection of tick populations across “receptive” habitats (that is to say, habitats predicted to be suitable) only. The habitat suitability model and initial population projection model were then combined. This process was accomplished by multiplying the numeric value of tick density at each pixel, by the predictions of the model. Since the model, when unclassified, gives predictions of habitat suitability from 0.00 (not suitable) to 0.999 (highly suitable) at each pixel, the result should be a more precise estimate of current tick populations on the same scale as the initial projection (Figure 3-10).

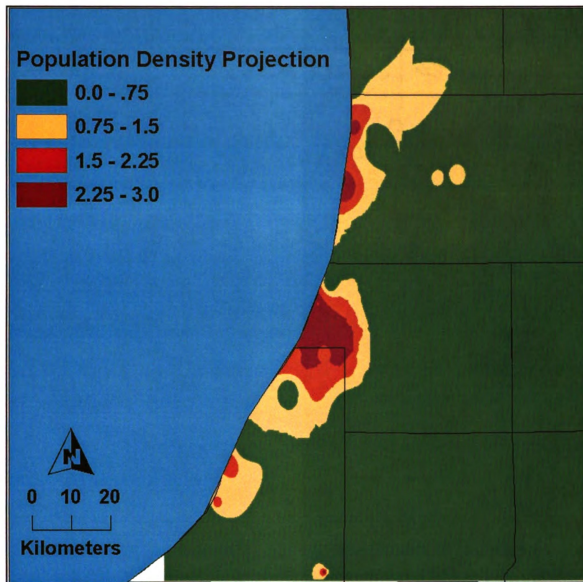


Figure 3-9. Population projection exercise using sites investigated for the presence of *Ixodes scapularis* in southwest, lower Michigan. Using Inverse Distance Weight (IDW) interpolation in ArcMap 8.1 (ESRI, Redlands, CA), tick populations are projected from sampled sites across the landscape with no ecological limitations. Population density is ranked on a scale from no ticks (0.0) to all stages with high density (3.0). This map illustrates population density across a homogeneous landscape, assumed habitable for *I. scapularis*. Images in this thesis are presented in color.

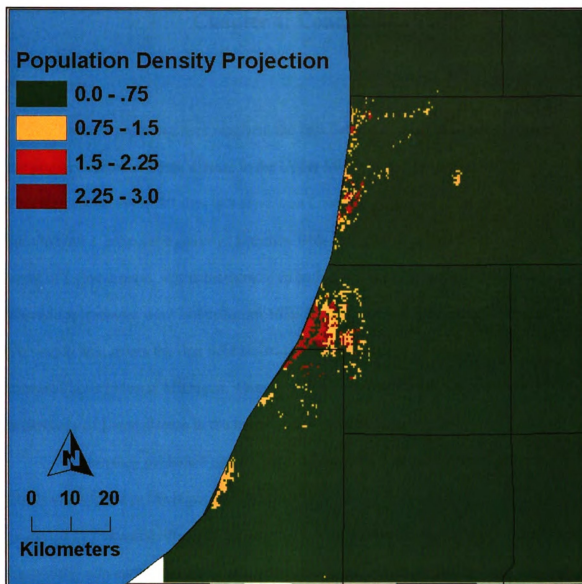


Figure 3-10. Population projection exercise using sites investigated for the presence of *Ixodes scapularis* in southwest, lower Michigan. Using Inverse Distance Weight (IDW) interpolation in ArcMap 8.1 (ESRI, Redlands, CA), tick populations are projected from sampled sites across the landscape using the predictions of the geographic risk model (Guerra *et al.*, 2002) as the ecologically limiting dataset. Population density is projected on a scale from no ticks (0.0) to all stages with high density (3.0), and multiplied by the predicted suitability of the model, which has a scale from 0.0 to 0.999. The resulting density estimates, therefore, conform to the initial scale. This map illustrates population density across a heterogeneous landscape, with varying degrees of habitat suitability for *I. scapularis*. Images in this thesis are presented in color.

Chapter 4: Conclusion

Summary

The data presented here represent the first field collection of *Ixodes scapularis*, the primary vector of Lyme disease in the Upper Midwest, in southern Michigan. Acarological survey of 80 sites in 8 southwest Lower Michigan counties has revealed 22 sites to have *I. scapularis* activity. Infection with *Borrelia burgdorferi*, the causative agent of Lyme disease, was demonstrated in both tick and small mammal reservoirs, and *Borrelia* spirochetes were isolated from 16 of 22 sites where *I. scapularis* is present. This study documents the first field isolation of *B. burgdorferi* from tick or small mammal hosts in lower Michigan. Overall, the results conclusively demonstrate endemicity of Lyme disease in the lower peninsula of Michigan.

A previously published geographic risk model for *I. scapularis* (Guerra *et al.*, 2002) was applied to Michigan and tested by direct field sampling across the range of predicted habitat suitability. Chi-square analysis found that the model, when tested in an historically non-endemic region, had predictive value (Likelihood Ratio $\chi^2=15.15$, $df=3$, P value=0.0017). This analysis represents a statistical validation of the habitat model. When sampled sites were plotted against the predictions of the model, 18 of 22 (82%) tick positive sites were correctly classified in the higher habitat suitability categories (50-75%, 75-100%). Three sites (14%) were in the 25-50% category, and only one positive site (4%) was in the 0-25% habitat suitability range.

Discussion

It is now established that *Ixodes scapularis* and *Borrelia burgdorferi* are present, and the Lyme disease zoonotic cycle is establishing at sites in lower Michigan. Based on historical archives of citizen submitted ticks, suspected human cases, and ecologic case follow-up investigations (MDCH, 1998, Walker *et al.*, 1998, MDA, 2003), it is evident that *I. scapularis* is in the initial stages of a biological invasion or range expansion into this region. Concomitant with this invasion is the establishment of *Borrelia burgdorferi* infections in the tick and wildlife populations. Thus, the scenario outlined in this study represents a dual invasion process, first of the tick vector and second of the pathogen itself. This pattern becomes apparent upon inspection of infection rate data in tick and woodland rodent populations presented here. No rodents were infected with *Borrelia* at any sites where ticks were absent. However, certain sites with ticks at low densities lacked *Borrelia* infection in ticks or rodents.

Menominee County in the Upper Peninsula of Michigan is the only other region where *B. burgdorferi* infected ticks and rodents have been described (Walker *et al.*, 1994). It is also the only region in Michigan from which culture confirmed human infection has been demonstrated (Stobierski *et al.*, 1994, MDCH, 1998), save a single culture-confirmed human case from Wexford County with possible travel history (M.G. Stobierski, personal communication). Over a 17-year period, about 400 *I. scapularis* ticks were submitted to the MDA for identification and testing from Menominee County. In contrast, over the same 17-year period, a total of 29 *I. scapularis* were submitted from the 8 county area in this investigation (MDA, 2003). Based on the human population of the two regions in question (Menominee: 25,326 / SW MI: 1,105,024, U.S. Census

Bureau, www.census.gov) if *I. scapularis* were established in southwest lower Michigan, one would expect more contact with the human population, and therefore more submissions of specimens. Results of previous field investigations also provide no evidence of *I. scapularis* or *B. burgdorferi* infection in woodland rodents (MDCH, 1998).

If the populations described by field investigation in southwestern, lower Michigan are indicative of a biological invasion, the question then arises: Why and how is this happening? Many biological, ecological and anthropogenic mechanisms may play a role in the emergence of Lyme disease in lower Michigan. The implication of empirical observations is that suitable habitat and host populations exist in this region for *I. scapularis*, and by extension there is suitable habitat for Lyme disease foci to exist. Populations of infected ticks and wildlife have been described in Illinois and Indiana, within relatively close proximity to the region under investigation (Pinger *et al.*, 1996, Jones and Kitron, 2000, Guerra *et al.*, 2002). It is likely that through movement of wildlife hosts, most notably white-tailed deer, and migratory bird species, the range of *I. scapularis* has expanded into receptive habitats in Michigan (Smith *et al.*, 1996, Klich *et al.*, 1996). The home range of *Peromyscus leucopus*, the dominant host for *I. scapularis* in the study area, is approximately ½ to 1½ acres, which allows for limited dispersal, whereas the home ranges and dispersal tendencies of white-tailed deer and migratory birds are measured in kilometers.

The geographic pattern of tick positive sites in Michigan suggests that migratory birds may indeed play a key role. Tick positive sites were concentrated along the shore of Lake Michigan, and these sites were more often characterized by high-density tick populations than inland positive sites (Figure 3.8). This observation may indicate that

ticks are being introduced along the shoreline, and through vertebrate host dispersal, are slowly expanding eastward to suitable habitats. One may expect that deer dispersal from sites in Illinois or Indiana, would create a less uniform distribution of positive sites across the landscape (Figure 4.1). The presence of small numbers of ticks on deer dispersed across the sampling

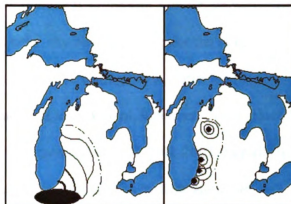


Figure 4-1. Theoretical spread of *I. scapularis* in Michigan. A) Spread by mammals from an endemic point source. B) Multiple focal sites along the coast may implicate birds, with subsequent mammal spread. Adapted from S. Yaremych, unpublished. Images in this thesis are presented in color.

area, however, may be an indication of this phenomenon, or may support the shoreline/bird phenomenon, as you would expect to find many more ticks on deer in an endemic landscape (Wilson *et al.*, 1985, Rand *et al.*, 2003). The latter model of introduction and establishment of *I. scapularis* is supported by the spotty foci as revealed in this study, the largely coastal distribution of the foci, and it suggests regular introductions followed by less regular but some successful population establishments. Introduction and establishment are the two key biological processes of invasion, the third being habitat receptivity (Kolar and Lodge, 2002). All three of these elements exist in the scenario under study here.

An interesting case, by contrast, is the southern most positive site in Berrien County (Site 43, Weaver Rd.). Heavy sampling around the site found no other indication of *I. scapularis* populations, indicating it might be a point source of ticks introduced by avian species. Its location, however, is also within tens of miles of established

populations of infected ticks in northwestern Indiana (Collins and Walker, unpublished). It is reasonable to surmise that white-tailed deer may be responsible for the range expansion. Data from 3 years of hunter-killed deer screening have confirmed the presence of *I. scapularis* on deer from the area. It is important, however, to note that no avian sampling was conducted during the field investigations of 2002-2003. Introduction of ticks by birds over a long range and local movement of ticks by deer and other mammals could both be happening, and could explain the patterns observed in this study. They are not mutually exclusive processes.

Recent hypotheses regarding the ecology of Lyme disease expansion have pointed to anthropogenic changes of the landscape in the increasing risk of Lyme disease. Increasing residential development of rural landscapes causes habitat fragmentation for many domestic mammal species. Small-scale woodlots are negligible for supporting a diverse population of medium and large sized mammals, however, small mammal species may thrive in the absence of predation or competition for resources (Ostfeld and Keesing, 2000). Ironically, this very lack of biodiversity may actually amplify the infection rates of Lyme disease in the small mammal and tick reservoirs. Ticks will have fewer choices of small mammal hosts, and those hosts will predominantly be reservoir competent. With the increase of white-tailed deer populations and their proximity to residential development, this creates a scenario of peridomestic risk for acquiring Lyme disease. The effects of fragmentation on availability and suitability of habitat for tick population establishment are not known.

Most of the sites investigated in this study were undeveloped, but several, which showed *I. scapularis* and *B. burgdorferi* activity, were within close proximity to

residential development. Several discussions with homeowners also revealed that ticks had been removed from family members or pets. Each shoreline State Park including the northernmost sampled park (Saugatuck-Ottawa County) showed tick, and *Borrelia* activity, some at high levels. This indicates that people recreating in, or living in proximity to these areas are at some risk of acquiring Lyme disease. For the medical community, it is imperative that Lyme disease now be added to the “index of suspicion,” because diagnosis during the acute phase of infection followed by prompt antibiotic treatment and resolution of early stage symptoms is the most desired outcome in case management (Wormser *et al.*, 2000).

The predictions of GIS based models are often used to display regional and local risk of acquiring an infectious or vector-borne disease (Cromley and McLafferty, 2002, Brooker *et al.*, 2002). Landscape epidemiology, perhaps more than any other discipline, emphasizes construction of maps representing models of relative risk, but these models are often difficult to test or validate (Graham *et al.*, 2004). Most models are “self-validated” for any given region, that is to say that statistical jackknifing is often used, but it is a form of circular reasoning when the data used to prepare a model are then used to validate it. To determine the applicability of a model across regions not used in developing the model, validation is needed at the ground level with an independent data set. Model validation must be performed in the context of the model’s intended use and performance requirements. Perhaps of greatest importance is the regional transportability of a risk model when the landscape changes appreciably in its structure (Kitron, 2000, Brooker *et al.*, 2002).

The intent of the tick habitat suitability model is to measure positive habitat associations of a disease vector across broad regions for the use of disease prediction, and ultimately to guide public health interventions. Validation is an attempt to determine the biological realism of this model. In this case, falsification would reveal either no associations with particular habitat categories, or negative associations with one or more habitat categories, and tick presence or absence. Support for the model -- equivalent to verification -- is therefore provided by a positive statistical association in a two-tailed test, where appropriate. My aim was to determine if such associations exist and therefore lend support to, or refute the original Guerra *et al.* (2002) model.

GIS spatial analysis applications can prove useful in projecting limited datasets across spatial regions of interest. Biological surveys are often tedious and expensive over the long term. When faced with the responsibility for surveillance and control of vectors in large regions, these applications may be useful in designing vector control strategies with limited ground surveillance. In the case of vector-borne disease, visiting every location in a study area to determine the presence or magnitude of a phenomenon is usually difficult or expensive. Instead, strategically dispersed sample input point locations can be selected, and a predicted value can be assigned to all other locations. Input points can be either randomly or regularly spaced points containing presence, magnitude, or infection rate measurements.

The assumption that makes interpolation a viable option is that spatially distributed objects are spatially correlated; in other words, things that are close together tend to have similar characteristics (Slocum, 1999). For instance, if there are chipmunks

on one side of County Road 65, you can predict with a high level of confidence that there are chipmunks on the other side of the road. When dealing with complex biological phenomenon, however, there are limitations. You would be less sure if there were chipmunks across town at the rock quarry, and less confident still about the state of small mammal populations in the next county. In the case of *Ixodes scapularis*, it is well established that specific ecological conditions influence distribution and population maintenance (Parola and Raoult, 2001, Guerra *et al.*, 2002, Randolph *et al.*, 2002). Applying ground tested habitat suitability models, or other such risk association models to estimates of species or risk distribution, gives the projection a greater biological significance. In the future, this may be accomplished using methods similar to the population projection exercise undertaken in this investigation, however I am unaware of other studies which have taken this approach (Kitron 2000, Brooker *et al.* 2002, and see review in Graham *et al.* 2004).

There are, however, some limitations to my investigations of *I. scapularis* and *B. burgdorferi* in southwest lower Michigan. Due to the timeframe in which sampling was conducted, the life cycles presented are actually incomplete. To estimate adequately the seasonal life cycle of *I. scapularis*, sampling must begin earlier in the spring, preferably first thaw, and continue through November on a weekly or bi-weekly basis. Sampling intensity should also be uniform across the sampling period, so as not to influence the results.

In an attempt to discover establishing populations of ticks, site selection was not conducted uniformly along transects or in a grid. There was a bias in the sampling program towards selecting sites with habitat signatures historically associated with tick

presence; this bias also exists in other similar studies (Kitron *et al.*, 1992, Glass *et al.*, 1994, 1995, Guerra *et al.*, 2002). While not an impediment for an observational study, it does affect the conclusions one can draw on tick absence from under-sampled or non-sampled habitats, and the use of population projections. The interpolation exercise discussed previously is an example. Spatial interpolations are most accurately made by sampling a grid, and interpolating the known point data (investigated site) to the space between the evenly spaced points (uninvestigated sites). The tick occurrence and density data from my investigation leaves broad areas unsampled, and also clumps sampling points together within the region.

Future research on the ecology of Lyme disease in Michigan should focus on the mechanisms of dispersal from hyper-endemic sites. As the seasons pass, and without control interventions, the populations of *I. scapularis* along Lake Michigan are likely to increase in density and spread to neighboring areas. The role of small mammals, birds, and white-tailed deer in population dispersal may be assessed in a biological invasion, or emergence scenario. The role of other small and medium sized mammals in the maintenance of *B. burgdorferi* can also be assessed to estimate the rate at which ticks will be infected based on the mammal community structure at any given foci. This leads to the further refinement of the habitat suitability model. By including biotic and temporal components into the model, not only will projections of habitat suitability based on abiotic and biotic factors be more precise, but a seasonal component can also be important in projecting Lyme disease risk.

I. scapularis is also a vector of other human disease causing agents, such as human granulocytic ehrlichiosis and babesiosis (Telford *et al.*, 1997). As the range of

this tick species expands in the region, it is imperative that the status of these diseases be quantified in tick and wildlife reservoirs, so as to provide the public and healthcare communities with adequate and timely information.

References

- Afzelius, A. 1921. Erythema Chronicum Migrans. Acta. Derm. Venereol. (Stockholm) 2:120-125.
- Anderson, S., editor. 1982. Simon & Schuster's Guide to Mammals. Simon and Schuster Inc., New York.
- Baker, R. H. 1983. Michigan Mammals. Michigan State University Press, East Lansing, Michigan.
- Barbour, A. G. 1984. Isolation and cultivation of Lyme disease spirochetes. Yale Journal of Biology and Medicine 57:521-525.
- Beasley, D. W., M. T. Suderman, M. R. Holbrook, and A. D. Barrett. 2001. Nucleotide sequencing and serological evidence that the recently recognized deer tick virus is a genotype of Powassan virus. Virus Research 79:81-89.
- Brooker, S., S. I. Hay, and D. A. P. Bundy. 2002. Tools from ecology: useful for evaluating infection risk models? Trends in Parasitology 18:70-74.
- Burgdorfer, W. 1984. Discovery of the Lyme Disease Spirochete and its Relation to Tick Vectors. Yale Journal of Biology and Medicine 57:515-520.
- Burgdorfer, W. 1989. Vector/host relationships of the Lyme disease spirochete, *Borrelia burgdorferi*. Rheumatic Disease Clinics of North America 15:775-787.
- Carroll, J. A., C. F. Garon, and T. G. Schwan. 1999. Effects of environmental pH on membrane proteins in *Borrelia burgdorferi*. Infection and Immunity 67:3181-3187.
- Centers for Disease Control. Division of vector-borne infectious disease. 2003. Lyme disease diagnosis. Retrieved December 15, 2003 from the World Wide Web: <http://www.cdc.gov/ncidod/dvbid/lyme/diagnosis.htm>
- Centers for Disease Control. 2004. Lyme Disease, United States 2001-2002. Morbidity and Mortality Weekly Report 53:365-369.
- Cromley, E. K., and S. L. McLafferty. 2002. GIS and Public Health. The Guilford Press, New York.
- de Silva, A. M., and E. Fikrig. 1995. Growth and migration of *Borrelia burgdorferi* in *Ixodes* ticks during blood feeding. Am. J. Trop. Med. Hyg. 53:397-404.

- de Silva, A. M., N. S. Zeidner, Y. Zhang, M. C. Dolan, J. Piesman, and E. Fikrig. 1999. Influence of Outer surface protein A antibody on *Borrelia burgdorferi* within feeding ticks. *Infection and Immunity* 67:30-35.
- Dennis, D. T., and E. B. Hayes. 2002. Epidemiology of Lyme Borreliosis. Pages 251-280 in O. Kahl, J. S. Gray, R. S. Lane, and G. Stanek, editors. *Lyme borreliosis: biology, epidemiology, and control*. CABI Publishing, Oxford, United Kingdom.
- Drew, M. L., K. I. Loken, R. F. Bey, and R. D. Swiggum. 1988. *Ixodes dammini*: occurrence and prevalence of infection with *Borrelia* spp. in Minnesota. *Journal of Wildlife Diseases* 24:708-710.
- Durden, L.A., J.E. Keirans. 1996. Nymphs of the Genus *Ixodes* (Acari: Ixodidae) of the United States: Taxonomy, Identification Key, Distribution, Hosts, and Medical/Veterinary Importance. Entomological Society of America.
- Dutton, J. E., and J. L. Todd. 1905. The nature of human tick-fever in the eastern part of the Congo freestate with notes on the distribution and bionomics of the tick. Liverpool School of Tropical Medicine *Memoir* XVII:1-18.
- Glass, G. E., F. P. Amerasinghe, J. M. Morgan, and T. W. Scott. 1994. Predicting *Ixodes scapularis* abundance on white-tailed deer using geographic information systems. *Am. J. Trop. Med. Hyg.* 51:538-544.
- Glass, G. E., B. S. Schwartz, J. M. Morgan, D. T. Johnson, P. M. Noy, and E. Israel. 1995. Environmental risk factors for Lyme disease identified with geographic information systems. *Am. J. Trop. Med. Hyg.* 85:944-948.
- Graham, A. J., P. M. Atkinson, and F. M. Danson. 2004. Spatial analysis for epidemiology. *Acta Tropica* 91:219-225.
- Guerra, M. A., E. Walker, C. Jones, S. Paskewitz, M. R. Cortinas, A. Stancil, L. Beck, M. Bobo, and U. Kitron. 2002. Predicting the Risk of Lyme Disease: Habitat Suitability for *Ixodes scapularis* in the North Central United States. *Emerging Infectious Diseases* 8: 289-297
- Hall, E. R. 1981. *The Mammals of North America*. Wiley, New York.
- Hess, G. R., S. E. Randolph, P. Arneberg, C. Chemini, C. Furlanello, J. Harwood, M. G. Roberts, and J. Swinton. 2002. Spatial aspects of disease dynamics. in P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. Dobson, editors. *The Ecology of Wildlife Diseases*. Oxford University Press, New York.
- Hoogstraal, H. 1985. Argasid and Nutalliellid ticks as parasites and vectors. *Advances in Parasitology* 24:135-238.

- Jackson, J. O., and G. R. Defoliart. 1970. *Ixodes scapularis* Say in northern Wisconsin. *Journal of Medical Entomology* 7:124-125.
- Johnson, B., C. Happ, L. Mayer, and J. Piesman. 1992. Detection of *Borrelia burgdorferi* in ticks by species specific amplification of the flagellin gene. *Am. J. Trop. Med. Hyg.* 47:730-741.
- Johnson, R. C., G. P. Schmid, F. W. Hyde, A. G. Steigerwalt, and D. J. Brenner. 1984. *Borrelia burgdorferi* sp. nov.: etiological agent of Lyme disease. *Internat. J. Syst. Bacteriol.* 34:496-497.
- Jones, C. J., and U. Kitron. 2000. Populations of *Ixodes scapularis* (Acari: Ixodidae) are modulated by drought at a Lyme disease focus in Illinois. *Journal of Medical Entomology* 37:408-415.
- Keirans, J.E., C.M. Clifford. 1978. The Genus *Ixodes* in the United States: A Scanning Electron Microscope Study and Key to the Adults. *Journal of Medical Entomology Supplement* 2: 1-149.
- Kitron, U. 2000. Risk Maps: Transmission and Burden of Vector-borne Diseases. *Parasitology Today* 16:324-325.
- Kitron, U., and J. J. Kazmierczak. 1997. Spatial Analysis of the Distribution of Lyme Disease in Wisconsin. *American Journal of Epidemiology* 145:558-566.
- Kitron, U., C. J. Jones, J. K. Bouseman, J. A. Nelson, and D. L. Baumgartner. 1992. Spatial analysis of the distribution of *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer in Ogle County, Illinois. *Journal of Medical Entomology* 29:259-266.
- Klich, M., M. W. Lankester, and K. W. Wu. 1996. Spring migratory birds (Aves) extend the Northern occurrence of the blacklegged tick (Acari: Ixodidae). *Journal of Medical Entomology* 33:581-585.
- Kolar, C. S., and D. M. Lodge. 2002. Ecological predictions and risk assessment for alien fishes in North America. *Science* 298:1233-1236.
- Levine, J. F., M. L. Wilson, and A. Spielman. 1985. Mice as Reservoirs of the Lyme Disease Spirochete. *Am. J. Trop. Med. Hyg.* 34:355-360.
- Mannelli, A., U. Kitron, C. J. Jones, and T. L. Slajchert. 1993. Role of eastern chipmunks as a host for immature *Ixodes dammini* (Acari: Ixodidae) in northwestern Illinois. *Journal of Medical Entomology* 30:87-93.
- Mather, T. N., M. L. Wilson, S. I. Moore, J. M. C. Ribeiro, and A. Spielman. 1989. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). *Journal of Parasitology* 75:66-69.

- Maxey, E. E. 1899. Some observations on the so-called "spotted fever" of Idaho. *Med. Sentinel* 7:433.
- MDA. 2003. *Ixodes scapularis*: Public submission from 1985-2002. Available at www.michigan.gov/emergingdiseases.
- MDCH. 1998. Final report on the Lyme disease active surveillance project.
- Murray, A. 1877. *Economic Entomology*. Aptera I, London.
- Nelson, J. A., J. K. Bouseman, U. Kitron, S. M. Callister, B. Harrison, M. J. Bankowski, M. E. Peeples, B. J. Newton, G. M. Trenholme, and J. F. Anderson. 1991. Isolation and characterization of *Borrelia burgdorferi* from Illinois *Ixodes dammini*. *Journal of Clinical Microbiology* 29:1732-1734.
- Novak, M. G., W. A. Rowley, K. B. Platt, D. M. Bartholomew, and M. D. Senne. 1991. *Ixodes dammini* (Acari: Ixodidae) and *Borrelia burgdorferi* in Iowa. *J. Iowa Acad. Sci.* 98:99-101.
- Obenchain, F. D., and R. Galun, editors. 1982. *Physiology of ticks*. Pergamon Press, Elmsford, New York.
- Oliver, J.H., M. R. Owsley, H. J. Hutcheson, A. M. James, C. Chen, W. S. Irby, E. M. Dotson, and D. K. McClain. 1993. Conspecificity of the ticks *Ixodes scapularis* and *I. dammini* (Acari: Ixodidae). *Journal of Medical Entomology* 30:54-63.
- Ostfeld, R. S., and F. Keesing. 2000. Biodiversity and Disease Risk: the Case of Lyme Disease. *Conservation Biology* 14:722-728.
- Parola, P., and D. Raoult. 2001. Ticks and Tickborne Bacterial Diseases in Humans: An Emerging Infectious Threat. *Clinical Infectious Diseases* 32:897-928.
- Piesman, J., and M. C. Dolan. 2002. Protection Against Lyme Disease Spirochete Transmission Provided by Prompt Removal of Nymphal *Ixodes scapularis* (Acari: Ixodidae). *Journal of Medical Entomology* 39:509-512.
- Piesman, J., J. G. Donahue, T. N. Mather, and A. Spielman. 1986. Transovarially acquired Lyme disease spirochetes (*Borrelia burgdorferi*) in field-collected larval *Ixodes dammini* (Acari: Ixodidae). *Journal of Medical Entomology* 23:219.
- Piesman, J., J. R. Oliver, and R. J. Sinsky. 1990. Growth kinetics of the Lyme disease spirochete (*Borrelia burgdorferi*) in vector ticks (*Ixodes dammini*). *Am. J. Trop. Med. Hyg.* 42:352-357.

- Pinger, R. R., L. Timmons, and K. Karris. 1996. Spread of *Ixodes scapularis* (Acari: Ixodidae) in Indiana: Collections of Adults in 1991-1994 and Description of a *Borrelia burgdorferi*-Infected Population. *Journal of Medical Entomology* 33:852-855.
- Platt, K. B., M. G. Novak, and W. A. Rowley. 1992. Studies on the biology of *Ixodes dammini* in the upper Midwest of the United States. *Ann. NY Acad. Sci.* 653:78-87.
- Rand, P. W., C. Lubelczyk, G. R. Lavigne, S. Elias, M. S. Holman, E. H. Lacombe, and R. P. Smith. 2003. Deer density and the abundance of *Ixodes scapularis* (Acari: Ixodidae). *Journal of Medical Entomology* 40:179-184.
- Randolph, S. E., C. Chemini, C. Furlanello, C. Genchi, R. S. Hails, P. J. Hudson, L. D. Jones, G. Medley, R. A. Norman, A. Rizzoli, G. Smith, and M. E. J. Woolhouse. 2002. The ecology of tick-borne infections in wildlife reservoirs. *in* P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. Dobson, editors. *The Ecology of Wildlife Diseases*. Oxford University Press, New York.
- Reineke, A., P. Karlovsky, and C. P. W. Zebitz. 1998. Preparation and purification of DNA from insects for AFLP analysis. *Insect Molecular Biology* 7:95-99.
- Schwan, T. G., and J. Piesman. 2002. Vector Interactions and Molecular Adaptations of Lyme Disease and Relapsing Fever Spirochetes Associated with Transmission by Ticks. *Emerging Infectious Diseases* 8:115-121.
- Scrimenti, R. J. 1970. Erythema chronicum migrans. *Archives of Dermatology* 102:104-105.
- Slajchert, T. L., U. Kitron, C. J. Jones, and A. Mannelli. 1997. Role of the eastern chipmunk (*Tamias striatus*) in the epizootiology of Lyme borreliosis in northwestern Illinois, USA. *Journal of Wildlife Diseases* 33:40-46.
- Slocum, T.A. 1999. *Thematic Cartography and Design*. Prentice-Hall, New Jersey.
- Smith, R. P., P. W. Rand, E. H. Lacombe, S. R. Morris, D. W. Holmes, and D. A. Caporale. 1996. Role of bird migration in the long-distance dispersal of *Ixodes dammini*, the vector of Lyme disease. *Journal of Infectious Diseases* 174:221-224.
- Smith, T., and F. L. Kilbourne. 1893. Investigations into the nature, causation, and prevention of Texas or southern cattle fever. *Bull. Bur. Anim. Ind., US Dept. Agric.* 1:301.
- Sonenshine, D. E. 1991. *Biology of Ticks, Vol I*. Oxford University Press, New York.
- Sonenshine, D. E. 1993. *Biology of Ticks, Vol II*. Oxford University Press, New York.

- Sonenshine, D. E., T. Adams, S.A. Allan, J. McLaughlin, F.X. Webster. 2003. Chemical composition of some components of the arrestment pheromone of the black-legged tick, *Ixodes scapularis* (Acari: Ixodidae) and their use in tick control. *Journal of Medical Entomology* 40: 849-859.
- Spielman, A., M. L. Wilson, J. F. Levine, and J. Piesman. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annu. Rev. Entomol.* 30:439-460.
- Steere, A. C. 1989. Medical progress: Lyme disease. *New England Journal of Medicine* 311:586-596.
- Steere, A. C., J. Coburn, and L. Glickstein. 2004. The emergence of Lyme disease. *Journal of Clinical Investigation* 113:1093-1101.
- Steere, A. C., S. E. Malawista, D. R. Snyderman, R. E. Shope, W. A. Andiman, M. R. Ross, and F. M. Steele. 1977. Lyme arthritis; an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. *Arthritis Rheum.* 20:7-17.
- Stobierski, M. G., W. N. Hall, B. Robinson-Dunn, H. Stiefel, S. Shiflett, and V. Carlson. 1994. Isolation of *Borrelia burgdorferi* from two patients in Michigan. *Clinical Infectious Diseases* 5:944-946.
- Strand, R. M., E. Walker, and R. W. Merritt. 1992. Field studies on *Ixodes dammini* in the upper peninsula of Michigan. *Vector Control Bull. North Central States* 1:111-118.
- Telford, S. W., J. E. Dawson, and K. Halupka. 1997. Emergence of tickborne diseases. *Sci. Med.* 4:24-33.
- Telford, S. W., T. Mather, S. Moore, M. L. Wilson, and A. Spielman. 1988. Incompetence of deer as reservoirs of the Lyme disease spirochete. *Am. J. Trop. Med. Hyg.* 39:105-109.
- Walker, E., M. G. Stobierski, M. L. Poplar, T. W. Smith, A. J. Murphy, P. C. Smith, S. M. Schmitt, T. M. Cooley, and C. M. Kramer. 1998. Geographic distribution of ticks (Acari: Ixodidae) in Michigan, with emphasis on *Ixodes scapularis* and *Borrelia burgdorferi*. *Journal of Medical Entomology* 35:872-882.
- Walker, E. D., T. W. Smith, J. DeWitt, D. C. Beaudou, and R. G. McLean. 1994. Prevalence of *Borrelia burgdorferi* in Host-Seeking Ticks (Acari: Ixodidae) from a Lyme Disease Endemic Area in Northern Michigan. *Journal of Medical Entomology* 31:524-528.

- Wilson, M. L. 1998. Distribution and abundance of *Ixodes scapularis* (Acari: Ixodidae) in North America: Ecological processes and spatial analysis. *Journal of Medical Entomology* 35:446-457.
- Wilson, M. L., S. W. Telford, J. Piesman, and A. Spielman. 1988. Reduced abundance of immature *Ixodes dammini* (Acari: Ixodidae) following elimination of deer. *Journal of Medical Entomology* 4:224-228.
- Wilson, M. L., G. H. Adler, and A. Spielman. 1985^A. Correlation between abundance of deer and that of the deer tick *Ixodes dammini* (Acari: Ixodidae). *Ann. Entomol. Soc. Am.* 78:172-176.
- Wilson, M. L., and A. Spielman. 1985^B. Seasonal activity of immature *Ixodes dammini* (Acari: Ixodidae). *Journal of Medical Entomology* 22:408-414.
- Wormser, G. P., R. B. Nadelman, R. J. Dattwyler, D. T. Dennis, E. D. Shapiro, A. C. Steere, T. J. Rush, D. W. Rahn, P. K. Coyle, D. H. Persing, D. Fish, and B. J. Luft. 2000. Practice guidelines for the treatment of Lyme disease. *Clinical Infectious Diseases* 31:S1-14.

APPENDIX 1

Record of Deposition of Voucher Specimens

Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2004-006

Title of thesis or dissertation (or other research projects):

IXODES SCAPULARIS (ACARI: IXODIDAE) AND *BORRELIA BURGDORFERI* IN
SOUTHWEST MICHIGAN: POPULATION ECOLOGY AND VERIFICATION OF A
GEOGRAPHIC RISK MODEL

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s):

Erik S. Foster

Date: August 16, 2004

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.
Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.

Museum(s) files.

Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1-1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:							Museum where deposited
		Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	
<i>Ixodes scapularis</i>	Bridgman, MI (Berrien Co.) - 11/15/03						2		MSU
<i>Ixodes scapularis</i>	Plainwell, MI (Allegan Co.) - 11/16/03					1			MSU
<i>Ixodes scapularis</i>	Bangor Twp, MI (Van Buren Co.) - 11/15/03					1	1		MSU
<i>Ixodes scapularis</i>	Richland, MI (Kalamazoo Co.) - 11/15/03						1		MSU
<i>Ixodes scapularis</i>	Niles, MI (Berrien Co.) - 11/15/03						7		MSU
<i>Ixodes scapularis</i>	Yanket Springs Rec. Area (Barry Co.) - 11/15/03					1			MSU
<i>Ixodes scapularis</i>	Fennville, MI (Allegan Co.) - 08/07/03		13						MSU
<i>Dermacentor variabilis</i>	Bridgman, MI (Berrien Co.) - 07/23/03		6						MSU
<i>Dermacentor variabilis</i>	Covert Twp, MI (Van Buren Co.) - 09/03/03		5						MSU
<i>Dermacentor variabilis</i>	Van Buren St. Pk. (Van Buren Co.) - 06/07/02					15	17		MSU

(Use additional sheets if necessary)

Investigator's Name(s)

Erik S. Foster

Voucher No. 2004-006

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Date August 16, 2004

Curator

Date

MICHIGAN STATE UNIVERSITY LIBRARIES



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