NPS Staff: this proposal include sampling methods (trapping, mist-netting) not proposed for the April sampling of ticks from Indiana Dunes National Lakeshore

Preliminary Research Proposal

Sarah A. Hamer
Fisheries and Wildlife Department
Michigan State University
13 Natural Resources Building
East Lansing, MI 48824
(517)353-7981; fax (517)432-1699
yaremych@msu.edu

Graduate Research Committee:
Dr. Graham Hickling, Dept. of Fisheries and Wildlife, advisor
Dr. Jean Tsao, Dept. of Fisheries and Wildlife
Dr. Ned Walker, Dept. of Microbiology and Molecular Genetics
Dr. Linda Mansfield, Dept. of Microbiology and Molecular Genetics
Dr. Kelly Millenbah, Dept. of Fisheries and Wildlife

Exploring the role of vertebrate hosts in the invasion and emergence of Lyme borreliosis in southwest Michigan

The proposed work has been approved by the All University Committee on Animal Use and Care at Michigan State University in January 2004. A Scientific Collector's Permit has been approved and obtained from the State of Michigan Department of Natural Resources in October 2003. Bird work will be conducted with Sarah Yaremych as a subpermit of the master banding permit held by Dr. Joe Johnson, director of the bird sanctuary at Kellogg Biological Station.

Introduction

Emergence of infectious disease often results from interactions among wildlife, domestic animals, humans, and zoonotic pathogens. I aim to explore the ecological mechanisms of emerging Lyme disease in Michigan. The etiologic agents include spirochetes of the *Borrelia burgdorferi* complex, which are transmitted largely by the black-legged or deer tick *Ixodes scapularis.* I will investigate the role of vertebrate hosts in the expansion of *Ixodes* ticks from focal sites of recent invasion in southwest Michigan. In the United States, Lyme disease accounts for over 90% of all reported cases of vector-borne disease, with over 150,000 cases reported to the Centers for Disease Control and Prevention since national surveillance was initiated in 1982 (CDC 2003). In the Midwest, including Michigan (Fig. 1), *I. scapularis* appears to be expanding in range and invading new areas of suitable habitats. A better understanding of the geographic distribution of *I. scapularis* and other competent vectors of Lyme disease, combined with predicted rates of invasion into new areas and knowledge of the underlying ecological parameters permitting establishment, will allow for prediction of human risk and appropriate control action.

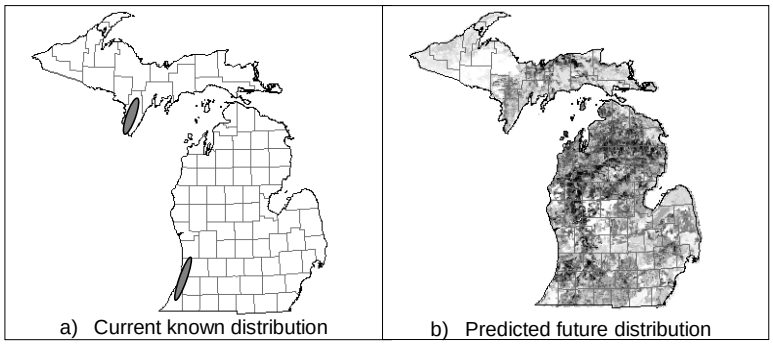

Fig. 1. a) The current (2003) detected distribution of *I. scapularis* in Michigan is comprised of an established population in the upper peninsula (Walker et al. 1998), and an invading population along the SW coast (E. Foster, pers. comm.). b) A model of habitat suitability for *I. scapularis* establishment (Guerra et al. 2002) applied to Michigan predicts the potential future distribution of the tick, with the darkest areas indicating highest risk for establishment upon introduction.

Though the hosts commonly associated with *I. scapularis* are the white-footed mouse (*Peromyscus leucopus*) for the larval and nymphal stages and the white-tailed deer (*Odocoileus virginianus*) for the adult stage, passive tick transport on these mammalian hosts may fail to account for the discontinuous distribution of *I. scapularis* (Smith et al. 1996). The means of tick dispersal into new areas is unknown. Because these ticks have a complex three-host life cycle, there are multiple opportunities for the ticks to become infected, to infect a new host, and to be carried to a new area. As ticks are not readily mobile, tick range expansion is due to the movement of the hosts upon which these arthropods feed. Migratory birds have been evaluated in their role as long distance dispersers of ticks, and hence, dispersers of *B. burgdorferi* (Anderson 1988; Battaly and Fish 1993; Smith et al 1996). Two alternate hypotheses may depict the predicted patterns of the spread of ticks from a focal site of invasion

(Fig. 2). Furthermore, the maintenance of *B. burgdorferi* in nature is not fully understood. The ecological implications of the exposure of naïve wildlife species to *B. burgdorferi* are unknown.

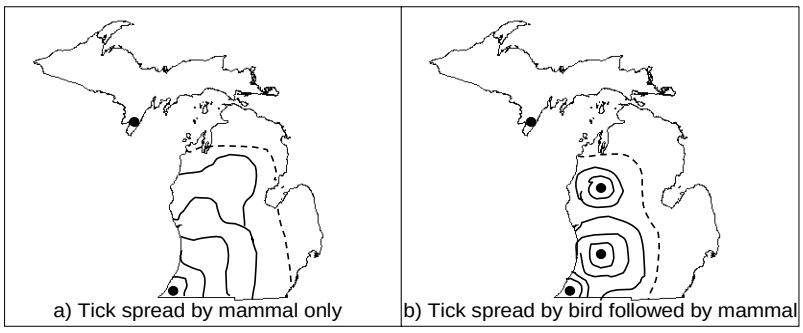

Fig. 2. Alternative hypotheses predicting the spread of Lyme Disease across Michigan. a) The gradual, concentric expansion from the site of initial tick invasion at the southwest coast may indicate spread by mammals with adjacent home ranges. b) The multiple patchy focal sites of invasion along the coast may indicate spread by migratory birds, in which case ticks may drop off birds, establish, and subsequently be spread by mammals.

Proposed Research Questions

1. Did the presence of *B. burgdorferi* in southwest Michigan precede the establishment of *I. scapularis* (in 'silent' sylvatic cycles), or did the pathogen arrive with invading *I. scapularis*?
2. What are the differential roles of small mammals, medium-sized mammals, and birds in the invasion and expansion of *B. burgdorferi* and competent vectors; what is the tick parasite distribution on available avian and mammalian hosts?

Ixodes dentatus

I aim to determine if the presence of *B. burgdorferi* precedes the invasion or presence of *I. scapularis*, perhaps in 'silent' cycles of transmission involving other tick species and their avian and mammalian hosts. If borreliae are cycling between non-*I. scapularis* ticks and hosts, a situation may exist where the arrival and establishment of *I. scapularis* in the system and the feeding of *I. scapularis* on previously infected hosts will create Lyme disease risk to humans. One initial focus will be in determining the presence and pathogen status of *I. dentatus* ticks, commonly called the bird-rabbit tick. This species occurs in Michigan, though its host-seeking phenology, abundance, and distribution are unknown. I will begin with a detailed investigation of birds and eastern cottontail rabbits, the preferred hosts for the juvenile and adult stages of *I. dentatus*, respectively, in southwest Michigan. *I. dentatus* has been implicated as a competent vector for *B. burgdorferi* (Tedford and Spielman 1989). Though it does not regularly bite humans, there are a number of documented instances of *I. dentatus* on humans. Therefore, *I. dentatus* may serve as a bridge vector, opportunistically passing *B. burgdorferi* from a natural cycle to a human host.

Literature Review

Few studies document the ecology of *I. dentatus*, and this species has yet to be explored in Michigan. This tick is found predominantly in the eastern United States, especially the Middle Atlantic

States. In a study of parasites of birds and rabbits in Virginia and North Carolina, adult stages of *I. dentatus* were found only on rabbits, whereas immatures were encountered most frequently on members of the bird families Fringillidae, Mimidae, Troglodytidae, Turdidae, and Parulidae, with the most important host species being the White-throated Sparrow and the Carolina Wren, year-round residents (Sonenshine and Stout 1970). Based on the sampling, in Virginia, the host-seeking activity of *I. dentatus* included presence of adults on rabbits from March through May and again in November. Nymphs infested hosts in April and May, with a small peak in November. The heaviest infestations larvae were in February and March, with a smaller peak in October and November. In North Carolina, feeding phenology was similar with nymphs on hosts in March-May, and larvae on hosts in peak numbers in November, with a smaller spring peak in April. The host selection and phenology of *I. dentatus* seems to parallel that of *Haemaphysalis leporispalustris*, also a bird-rabbit tick and a natural vector of Rocky Mountin Spotted Fever. However, unlike *I. dentatus*, *H. leporispalustris* has not been shown to be vector competent for Lyme spirochetes.

Battaly et al. (1987) examined the role of birds as dispersal agents of ticks, and consequently *B. burgdorferi*, in a Lyme disease endemic area in New York. On an average of 5 days per month for 1 year, birds were captured with 11 mist nets and 6 ground traps. Of 251 birds examined, 88 birds representing 19 species yielded 231 ticks representing 4 species, including I. *scapularis, I. dentatus, H. leporispalustris, and Dermocenter variabilis*. The majority of the tick collections were larval and nymphal *I. scapularis* and *I. dentatus*; no adults of any species were encountered. *I. dentatus* larvae parasitized birds from March through May and September through December, with the largest peak in the fall. Nymphal *I. dentatus* was found in May and June. The phenology of *I. scapularis* was found to differ from that of *I. dentatus* in that *I. scapularis* larvae were active in May through September, and nymphs were active in May through July, which parallels reports of occurrence of *I. scapularis* on mammals in the east. The authors captured birds that were hosting both *I. scapularis* and *I. dentatus* simultaneously. A small number of ticks collected from the birds were examined for spirochetes using dark-field microscopy; 6 of 36 *I. scapularis* and 0 of 6 *I. dentatus* contained spirochetes. The authors note, however, that spirochetes were found in an *I. dentatus* nymph questing on site.

Telford and Spielman (1989a) collected ticks from rabbits in April through May in Massachusetts to determine if rabbits maintain an enzootic transmission cycle of *B. burgdorferi*. Larval *I. dentatus* was present at a low level in April, with peak occurrence in August through October. Nymphs were present throughout the sampling season, with the highest numbers recovered in October. Adults were most abundant in April, and trailed off through August. This ‘inversion in developmental stages’ would permit larvae to feed on rabbits previously infected by the elder life stages. As rabbits are susceptible to infection and infectious to ticks in a laboratory setting, this species is reservoir competent. Rabbits were

shot and blood samples were obtained, after which rabbit carcasses were stored in a manner to allow for retrieval of ticks as they dropped off the host. Larval ticks were allowed to molt, and the resulting nymphs were then examined for the presence of *B. burgdorferi*. As transovarial transmission of *B. burgdorferi* is thought to be rare in ticks, the occurrence of spirochetes in fed larvae implicate their host as being reservoir competent, in a natural xenodiagnosis technique. The authors concluded that spirochetes infected most of the rabbits on the study site, as only 2 of 50 rabbits were not seropositive for antibodies to *B. burgdorferi*, and that only *I. dentatus* (not *H. leporispalustris*) may become infected after feeding on the rabbits. In April through October, nearly all rabbits were infested with numerous ticks. Though four species of ticks were present in all three life stages on rabbits including *I. scapularis, I. dentatus, H. leporispalustris, and D. variabilis,* the authors state that the only candidates for tick-borne infections of rabbits at their site are *I. dentatus* and *H. leporispalustris* due to their relative abundance.

I. scapularis will feed on birds and rabbits as well, and through feeding on humans, this vector that is believed to cause the majority of human Lyme disease cases. A system may exist whereby a 'silent' (with respect to human disease) enzootic cycle of transmission of *B. burgdorferi* among *I. dentatus*, birds, and rabbits is present prior to the arrival of *I. scapularis*. Invasion of *I. scapularis*, perhaps as a consequence of habitat or climate change, may allow this tick to enter the cycle, pick up the spirochetes, and begin transmission to humans. Knowledge of the occurrence and location of enzootic cycles involving *I. dentatus*, combined with existing knowledge of suitable habitat for establishment of *I. scapularis*, should enhance our ability to map human risk and spatial spread of disease. The interactions among the following series of observations have been considered in the motivation for this research:

Observations

1. Discovered in the summer of 2002, there is a Lyme disease system involving *I. scapularis* and *B. burgdorferi* in fragmented habitats of southwest Michigan (E. Foster and E. Walker, pers. comm.). The tick and the pathogen are invading and establishing in a way that appears to conform to a recently published *I. scapularis* habitat model, which predicts the spatial risk of human Lyme disease (Guerra et al, 2002).

2. Immature stages of *I. dentatus* have been found on migratory as well as local birds in southwest Michigan (E. Walker; B. and R. Keith, pers. comm.). This tick has been found in all life stages on its preferred adult host, the eastern cottontail (*Sylvilagus floridanus*), in Kalamazoo county in southwest Michigan (E. Walker, pers. comm.). *I. dentatus* has been implicated as a competent enzootic vector of Lyme disease (Tedford and Spielman, 1989b). *B. burgdorferi* has been found in *I. dentatus* removed from a number of bird species (Levine et al., 1991), suggesting the importance of bird movement patterns (migratory and post-fledgling dispersals) in encouraging the spread of vector

competent ticks and spirochetes. The eastern cottontail has been shown to be a reservoir host for *B. burgdorferi* (Sonenshine 1991). *I. dentatus* has fed on humans on occasion (Anderson et al 1996, Walker et al 1992). However, the borreliae cycling between *I. dentatus* and eastern cottontails in some areas in the eastern United States is different than the classic B31-type strains that have been isolated from humans (Anderson 1988; Anderson et al, 1996). The pathogenicity of this bird/rabbit *Borrelia* to humans is unknown.

3. Birds of many species may serve as hosts to the juvenile stages of *I. scapularis* (Anderson 1988; Battaly and Fish 1993; Anderson and Magnarelli 1980) and some birds species, including the American Robin, have been shown to be reservoir competent through xenodiagnosis (Richter et al, 2000). Furthermore, birds may carry the Lyme disease as a latent infection which may reactivate under periods of stress (Gylfe et al, 2000).

With an interest in the *I. dentatus* system and its overlap with the invading *I. scapularis* system, I plan to conduct a field survey of birds and mammals in southwest Michigan for recovery of ticks parasitizing these hosts. The current tick community composition of SW Michigan is unknown. Previous studies in this location involved tick collection through dragging vegetation, small mammal trapping, surveys of hunter-killed deer at check stations, and recoveries of ticks from canines (E. Foster, pers. comm.). Though much study abounds the role of small mammals (largely the white-footed mouse) as reservoirs for *B. burgdorferi*, multiple medium-sized mammals and bird species serve as alternate hosts for *I. scapularis* and other tick vectors, and are competent reservoir hosts for *B. burgdorferi*. Raccoons and opossums have been implicated as reservoirs of the agent of Lyme disease through positive blood cultures or serology (Anderson et al. 1983). Because virtually all *I. scapularis* larvae are hatched uninfected, the recovery of *B. burgdorferi*-positive larvae that have just fed on a host will implicate that host as a competent reservoir of the spirochetes. Fish and Daniels (1990) investigated the ability of medium-sized mammals (raccoons, opossums, and skunks) to infect *I. scapularis* larvae and found that these mammals can serve as reservoirs of infection, with, on average, a single raccoon producing as many infected nymphs as six mice. The role of bird and mammal species, aside from the white-footed mouse, in the maintenance of Lyme disease in nature has been underestimated, and the epizootiology of Lyme disease is likely more complex than previously thought (Fish and Daniels 1990).

Methods

Tick collections and vertebrate investigations in Michigan

To determine the occurrence of *I. dentatus*, *I. scapularis*, and other vectors of importance and presence of *B. burgdorferi* transmission cycles in particular localities, I will conduct an ecological study of ticks in their natural environment. Sonenshine (1993) divides sampling methods into the following

three categories: (1) those that collect passive, questing ticks by direct contact; (2) those that use attractants to collect ticks from a distance; and (3) those that collect ticks that are attached to host animals. I will employ a combination of methods to collect questing ticks from the vegetation and to survey wild vertebrate hosts for collection of parasitizing ticks. The selection of field sites will be based on the following criteria: (1) previous collections of *I. scapularis* from a core area in Berrien, Van Buren, and Allegan Counties of SW Michigan (E. Foster and E. Walker, pers. comm.) in 2001-2003 suggesting that an invasion is taking place from NE Indiana along the Lake Michigan coast; (2) discoveries of ticks in areas beyond the core invaded area of SW MI in fall of 2003 on hunter harvested deer in Kalamazoo County and on vegetation in Muskegon County; (3) the goal of determining the current boundary of distribution of *I. scapularis* populations and *B. burgdorferi* infections; and (4) the habitat suitability model of Guerra et al. (2002). Through dragging vegetation and small mammal trapping, Guerra et al. (2002) studied tick distribution in portions of Wisconsin, Illinois, and the upper peninsula of Michigan. Environmental data was collected at field sites and combined with existing coverages of environmental data (i.e. topography, soil type) and portrayed in a Geographic Information System (GIS). Tick presence was found to be positively associated with deciduous, dry to mesic forests and sandy soils overlaying sedimentary rock; tick absence was associated, in part, with grasslands, conifer forests, wet forests, and acidic soils. This map indicates suitable habitats where this tick is already established, as well as areas of high probability that the tick will become established if introduced. Assuming that the risk of a human contracting Lyme disease is proportional to the suitability of the habitat in which the human resides for tick establishment, this map can be used to predict the risk of human Lyme disease.

In a systematic sampling scheme, we will set up one coastal and one inland transect in SW MI, each approximately 193121 m (120 mi) in length. Both transects will begin at the Lake Michigan coast in Van Buren County, a site known to be positive for *I. scapularis* presence. From this origin, the transects will extend into habitat that was previously determined to be negative for *I. scapularis* presence and habitat that has not been investigated. The coastal transect will extend northward from the origin and curve along the coast through Oceana County. The inland transect will run directly eastward from the origin through Jackson County. Each transect will be divided into eight equidistant segments of 24140 m (15 mi). The Guerra et al. (2002) risk model will guide the selection of sampling sites within each segment, as the habitat predicted to be most suitable for tick establishment is identified in the model. One of the most important criteria used in selecting sampling sites for field studies is the extent to which the site is representative of typical tick-infested areas (Sonenshine 1993). As our goal is to find ticks if they are present, we will search the most suitable habitats within specified sampling segments. Within each segment, four sites of suitable habitat will be randomly chosen. These sites will include forested or edge habitat in private or state-owned land. The first of these sites will be sampled, and if this site is found to

be inaccessible, the second randomly chosen site will be sampled, and so on. We will sample one site per segment, for a total of eight sites per transect, or 16 total sites. Each transect will be sampled simultaneously by a pair of field workers, and the sampling methods to be used at each site will follow a rotation.

Rotation 1

Rotation 1 will start in early May and will consist of sampling all sites by dragging vegetation and small mammal trapping. In the afternoon of day 1, the vegetation at site 1 will be dragged. The most widely-used device for sampling unfed ticks of a species that quest in the vegetation is the tick drag (Sonenshine 1993). A drag is a 1 m^2 heavy cloth with a pole attached to the border of one side. A rope is attached to both ends of the pole, and the cloth is dragged over the low-lying vegetation and leaf litter by pulling it from the center with the rope for a fixed distance or time. Draggers will stop after a fixed distance or amount of time to check the cloth for attached ticks and remove them. Ticks that have attached to worker clothing will also be removed and placed in vials. With two workers simultaneously dragging the same site for one hour, two person-hours of drag time will be acquired and we will document number of ticks collected per unit time. Knowledge of tick activity periods, both seasonally and diurnally, would aid in guiding proper dates and times of day to drag.

After the dragging, 40 small mammal traps will be set out at site 1. Traps will be placed at roughly 10 m apart to cover a distance of 400 m. Traps will be baited with peanut butter and/or sunflower seeds and disguised with vegetation. After site 1 has been dragged and set with small mammal traps, the pair of workers will travel to site 2, roughly 15 miles away, to conduct the same dragging and trap setting process in the late afternoon. On the morning of day 2, the traps at site 1 will be checked and all captured mammals will be processed. Mammals will be removed from the traps and anesthetized with either an inhalant (methoxyflurane or isoflurane) or via injection with a ketamine/xylazine combination. Each mammal will be examined for ticks by combing fur with forceps. Ticks will be removed and placed in vials for identification and testing for presence of *B. burgdorferi*. Two ear punch biopsies will be taken with a sterile 2mm puncher, and a blood sample will be obtained via the pedal bleed technique. A portion of the blood samples will be inoculated into modified Barbour-Stoenner-Kelly (BSK) media for growth of *B. burgdorferi* isolates, and the remainder of the blood and the biopsies will be assayed for detection of *B. burgdorferi* though polymerase chain reaction. Serum antibodies to *B. burgdorferi*, indicating past exposure, will be detected with an enzyme linked immunosorbent assay. All mammals will be subcutaneously injected with a microchip (AVID microchips) to allow for individual identification and obtaining recapture status of the animals. Upon recovery from the anesthetic, mammals will be released. Following the processing of mammals at site 1 on the morning of day 2, mammals from site 2 will be processed. On the afternoon of day 2, the vegetation will be dragged and small mammal traps will be set

at sites 3 and 4. This process will continue until the mammals have been processed and released at site 8, and will be completed in 5 days (Figure 3).

Figure 3. Rotations 1 and 2 through field sites 1-8 to drag and sample mammals and birds.

	Rotation 1					
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	
am		PSM 1 PSM 2	PSM 3 PSM 4	PSM 5 PSM 6	PSM 7 PSM 8	
pm	D, SSMT 1 D, SSMT 2	D, SSMT 3 D, SSMT 4	D, SSMT 5 D, SSMT 6	D, SSMT 7 D, SSMT 8		
	Rotation 2					
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
am		MN 1 PMM 1	MN 2 PMM 2	MN 3 PMM 3	MN 4 PMM 4	
pm	D, SMMT 1	D, SMMT 2	D, SSMT 3	D, SSMT 4		D, SMMT 5

Day 7	**Day 8**	**Day 9**	**Day 10**	
MN 5 PMM 5	MN 6 PMM 6	MN 7 PMM 7	MN 8 PMM 8	
D, SMMT 6	D, SMMT 7	D, SMMT 8		

D = drag
SSMT = set small mammal traps
PSM = process small mammals
SMMT = set medium mammal traps
MN = mist net
PMM = process medium mammals

Rotation 2

Following completion of rotation 1, rotation 2 will begin and will consist of dragging vegetation, mist-netting, and medium-sized mammal trapping at all sites. On the afternoon of day 1, the vegetation will be dragged for 2 person/hours, and a series of medium-sized mammal traps will be set. The series of traps will consist of 25 wooden box traps for rabbits, 5 large live traps, 5 medium live traps. Target species include eastern cottontail rabbits (*Sylvilagus floridanus*), raccoons (*Procyon lotor*), opossums (*Didelphis virginiana*), skunks (*Mephitis mephitis*), and squirrels (*Sciuridae* spp.). Traps will be baited with species-specific bait putties, bread and peanut butter, sunflower seeds, etc. In the early morning of day 2, a series of 6 mist-nets will be set at site 1. Mist nets will run for 5 hours, and will be constantly monitored for presence of birds. Simultaneously, 5 bait traps will be placed on the ground near the nets to target ground-dwelling species as these species will be likely candidates for harboring ticks that quest just above ground level. Bird populations sampled will include year-round residents as well as summer breeders and migrants. Captured birds will be examined for ticks using a head-mounted magnifying loupe by blowing or combing feathers on the head and neck, including inside the ears. All ticks will be removed with forceps, placed in vials, and transported to the Medical Entomology Laboratory at Michigan State University for identification and determination of infection status. A blood sample will be taken through jugular venipuncture for detection of spirochetes, though the duration of spirochetemia is unknown. Blood will be inoculated into modified Barbour-Stoenner-Kelly (BSK) media for growth of *B. burgdorferi* isolates, or *B. burgdorferi* will be detected thought polymerase chain reaction. Antibodies to *B. burgdorferi*, indicating past exposure, will be detected with an enzyme linked immunosorbent assay. All captured birds will be banded and released, with holding time not to exceed 15 minutes. Recorded data will include the bird species, band number, number of ticks on each bird, proportion of infested birds among those captured, and date of collection. Bands will allow for notification of recaptured birds, and repeated tick checks and blood sampling of these birds will provide insight to seroconversion, the

chronology of tick feeding in the local area or that area from which the birds have migrated. Likely candidates for harboring ticks would be the bird species that forage and/or on the ground in forested habitats or edge habitats. Studies have implicated the following inexhaustive list of species as hosts for *I. scapularis* (Klich et al. 1996; Anderson and Magnarelli 1983; Weisbrod and Johnson 1989; Battaly and Fish 1993):

Tyrannidae- Eastern Phoebe

Corvidae- Blue Jay

Paridae- Black-capped Chickadee

Sittidate- White-breasted Nuthatch

Troglodytidae- House Wren, Carolina Wren

Mimidae- Gray Catbird

Turdidae- American Robin, Swainson's Thrush, Hermit Thrush, Veery

Vireonidae- White-eyed Vireo

Parulidae- Blue-winged Warbler, Pine Warbler, Northern Waterthrush, Louisana Waterthrush, Common Yellowthroat, Yellow-breasted Chat, Ovenbird

Icteridae- Red-winged blackbird, Common Grackle, Brown-headed Cowbird *Cardinalidae*- Northern Cardinal, Rose-breasted Grosbeak

Fringillidae- Purple Finch

Emberizidae- Eastern Towhee, Chipping Sparrow, Field Sparrow, White-throated Sparrow, Swamp Sparrow

While running the mist-nets, the medium-sized mammal traps at the site will be checked and retrieved. Captured mammals will be anesthetized with a ketamine/xylazine combination through insertion of the syringe through the cage. Proper dosing will be calculated after weighing the trap containing the animal with a spring balance scale. Medium mammals will be processed in the same manner as the small mammals.

Removing ticks by hand from mammals, especially large animals, is time-consuming and runs the risk that some ticks may be overlooked, especially the tiny life stages (Sonenshine 1993). Fish and Daniels (1990) demonstrated that the initial counts of ticks on hosts in the field consistently underestimated the total number of ticks feeding on the host, as determined by placing animals in wire cages elevated over water for a number of days. In this time, attached hosts will feed to repletion and drop off naturally, were they will be trapped in the water and collected. Therefore, a subsample of mammals, not including skunks, will be transported to the Kellogg Biological Field Station in Kalamazoo County for holding in cages over trays of water for up to 72 hours to recover engorged ticks. The number

of mammals transported to holding will depend on the number captured. If it is decided that a mammal will be transported, no anesthesia will be delivered in the field, as all processing will occur once at the holding room. After this time, mammals will be released at the site of capture. As the process of transporting animals for holding, processing animals at the holding site, and caring for these animals is time-consuming, flexibility has been built in to the rotation schedule. Rotation 2 will be completed within 2 weeks. Once the initial rotations help to identify the areas of interest, intensive sampling including holding of mammals for tick recovery will occur regularly.

As the transport of mammals and associated ticks from possible Lyme disease endemic areas to the Kellogg Biological Field Station for housing raises concerns of biosecurity, precautions will be taken to ensure tick containment. Mammals will be transported in their traps from the site of capture to the field station. During the transport, traps will be in the bed of a trunk on top of a tarp. The tarp will be lined with double-sided tape, as to inhibit movement of any ticks that happen to dislodge from hosts while in transport. Once at the field station, animals will be housed in a stainless steel, six-unit bank of caging. The tarp and traps will be checked for ticks. The bottom of each cage will contain a tray of shallow water. As in Fish and Daniels (1990), ticks that feed to repletion and dislodge from hosts during the period of confinement will drop into the tray of water, from which they will not be able to move. We will line the rim of the tray with vasoline to further inhibit mobility of the ticks. The perimeter of the caging unit, as well as a border around the caging unit on the floor, will be lined with double-sided tape to capture and escaped ticks. Signs will be posted on all doors to the animal holding room alerting the presence of animals and ticks. Worker clothing will be thoroughly checked for tick presence when exiting the animal holding room.

There are many unknown factors associated with this work that can potentially slow the rotations, including the number of animals captured at each site, weather conditions, transport time, etc. Accordingly this sampling scheme will be adaptive. Additionally, the number of medium-sized mammal traps may be increased to enhance capture success.

Tick abundance and relative occurrence on hosts and vegetation may distinguish established populations from recent invasions.

Hypotheses 1: A gradient of *I. scapularis* density will be detected along each transect with highest density in the SW corner of MI in year-1. As the invasion continues, populations of ticks will expand from focal sites and establish in new areas.

Hypothesis 2: *B. burgdorferi* is maintained in cycles involving non-*I. scapularis* ticks in MI, thereby creating an environment in which the arrival and establishment of *I. scapularis* will create Lyme disease risk to humans.

Conclusion

A more comprehensive understanding of the spatial distribution and spread of competent tick vectors is fundamental to assessing human risk for Lyme disease. Throughout my work, I will build upon existing climate- and habitat-based risk maps that predict the occurrence of *I scapularis* (Guerra et al. 2002, Brownstein et al 2003). I plan to add a time dimension to these risk models through determination of the rate of invasion from a focal site. This will be accomplished through first building upon baseline tick collection data to determine the boundary of the current invasion. I will collect birds, mammals, and ticks along series of transects to include the focal site of invasion, and habitat clearly beyond the invasion site. Repeat sampling during questing season each year will allow for determination of the rate of spread. In investigating the possible existence of silent cycles of *B. burgdorferi*, the ecological parameters constituting a receptive site for *I. scapularis* invasion and increased *B. burgdorferi* transmission may further be defined.

In this pathogen-vector-host system, environmental determinants control the distribution and abundance of all members of the triad. The dynamics of transmission is a function of a successful combination of abiotic and biotic processes that affect the ecosystem and permit inhabitation by birds and mammals and establishment of the vector. We must account for anthropogenic alteration of the environment as a risk factor for disease emergence and/or resurgence; for example, the emergence of Lyme disease is likely a consequence of the deforestation and reforestation of the northeastern United States, with a subsequent rise in deer population numbers (deer are a preferred host for adult stages of *I. scapularis* ticks) (Barbour and Fish 1993). With this planned research, I hope to improve the scientific basis for management decisions, improve human health risk assessment, and provide guidelines for effective surveillance and control measures. Accordingly, communicating results of this research to the scientific community and land managers, as well as the public will be a priority. This research will synthesize the emergence of infectious disease with invasion biology, and modeling of this system will provide early warnings for health practitioners as well as outdoor enthusiasts.

Literature Cited

Anderson, J. F. 1988. Mammalian and avian reservoirs for *Borrelia burgdorferi*. Ann. N.Y. Acad. Sci. 539:180-181.

Anderson, J. F., R. A. Flavell, L. A. Magnarelli, S. W. Barthold, F. S. Kantor, R. Wallich, D. H. Persing, D. Mathiesen, and E. Fikrig. 1996. Novel *Borrelia burgdorferi* isolates from *Ixodes scapularis* and *I. dentatus* ticks feeding on humans. J. Clin.Microbiol.34:524-529.

Anderson, J. F. and L. A. Magnarelli. 1980. Vertebrate host relationships and

distribution of ixodid ticks (Acari: Ixodidae) in Connecticut, USA. J. Med. Ent. 17:314-323.

Anderson, J. F., L. A. Magnarelli, W. Burgdorfer, and A. G. Barbour. 1983. Spirochetes in *Ixodes dammini* and mammals in Connecticut. American Journal of Tropical Medicine and Hygiene. 32:818-824.

Battaly, G. R. and D. Fish. 1993. Relative importance of bird species as hosts for immature *Ixodes dammini* (Acari: Ixodidae) in a suburban residential landscape of southern New. J. Med. Ent. 30:740-747.

Barbour, A. G. and D. Fish. 1993. The biological and social phenomenon of Lyme disease. Science 260:1610-1616.

Brownstein, J. S., T. R. Holford, and D. Fish. 2003. A climate-based model predicts the spatial distribution of the Lyme disease vector Ixodes scapularis in the United States. Environ. Health Perspect. 111:1152-1157.

CDC. Centers for Disease Control and Prevention. 2003. Lyme Disease. Available: http://www.cdc.gov/ncidod/dvbid/lyme/index.htm. Accessed 10/2003.

Fish, D. and T. J. Daniels. 1990. The role of medium-sized mammals as reservoirs of *Borrelia burgdorferi* in southern New York. J. Wildl. Dis. 26:339-345.

Guerra, M., 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. Emerg. Infect. Dis. 8:289-297.

Gylfe, A., S. Bergstrom, J. Lungstrom, and B. Olsen. 2000. Epidemiology: Reactivation of *Borrelia* infection in birds. Nature. 403:724-725.

Klich, M., , M. W. Lankester, , and K. W. wu. 1996. Spring migratory birds (Aves) extend the Northern occurrence of blacklegged tick (Acari: Ixodidae). Journal of Medical Entomology 33:581-585.

Levine, J. F., D. E. Sonenshine, W. L. Nicholson, and R. T. Turner. 1991. *Borrelia burgdorferi* in ticks (Acari: Ixodidae) in coastal Virginia. J. Med. Entomol. 28:668-674.

Richter, D., A. Spielman, N. Komar, and F. Matuschka. 2000. Competence of American Robins as reservoir hosts for Lyme disease spirochetes. Emerg. Infect. Dis. 6:133-138.

Smith, R. P., P. W. Rand Jr., 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.

Sonenshine, D. E. Biology of Ticks, Volume 2. 1993. Oxford University Press, New York, USA.

Sonenshine, D. E. and I. J. Stout. 1970. A contribution to the ecology of ticks infesting wild

birds and rabbits in the Virginia-North Carolina piedmont. J. Med. Ent. 7:645-654.

Tedford, S. R. III and A. Spielman. 1989a. Enzootic transmission of the agent of Lyme disease in rabbits. Am. J. trop. Med. Hyg. 41:482-490.

Tedford, S. R. III and A. Spielman. 1989b. Competence of a rabbit-feeding *Ixodes* (Acari: Ixodidae) as a vector of the Lyme disease spirochete. J. Med. Entomol. 26:118-121.

Walker, E. D., M. L. Poplar, and H. L. Russell. 1992. *Ixodes dentatus* (Acari: Ixodidae) in Michigan: first state records and occurrence on a human. Gt. Lakes Entomol. 25:303-304.

Wesibrod, A. R. and R. C. Johnson. 1989. Lyme disease and migrating birds in the Saint Croix River Valley. Applied and Environmental Microbiology 55:1921-1924.