2012

A SEARCH FOR AN ENDEMIC POPULATION OF IXODES SCAPULARIS IN SELECT AREAS OF PICTURED ROCKS NATIONAL LAKESHORE

Steven J. Schaar
Northern Michigan University

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A SEARCH FOR AN ENDEMIC POPULATION OF *Ixodes scapularis* IN SELECT AREAS OF PICTURED ROCKS NATIONAL LAKE SHORE

By

Steven J. Schaar

THESIS

Submitted to
Northern Michigan University
In partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE

Office of Graduate Education and Research

2012
This thesis by Steven J. Schaar is recommended for approval by the student’s thesis committee in the Department of Biology and by the Assistant Provost of Graduate Education and Research.

Committee Chair: Dr. Jackie Bird

First Reader: Dr. Donna M. Becker

Second Reader: Dr. Mason V. Reichard

Department Head: Dr. John Rebers

Dr. Brian D. Cherry
Assistant Provost of Graduate Education and Research
ABSTRACT
A SEARCH FOR AN ENDEMIC POPULATION OF *Ixodes scapularis* IN SELECT AREAS OF PICTURED ROCKS NATIONAL LAKESHORE

By

Steven John Schaar

A total of three female *Ixodes scapularis* ticks were removed from fishers live-trapped in Pictured Rocks National Lakeshore (PRNL) in 2002 and in 2005. In the current project, a tick survey using dragging and live trapping of rodent and mesocarnivores was conducted in this same area. In addition, serum collected from live-trapped mammals was screened for antibodies against *Borrelia burgdorferi* using a traditional ELISA and IDEXX® Laboratory’s Canine SNAP® 4Dx® tests. Adult *Dermacentor variabilis* were the only questing ticks recovered during the study. Collectively, close visual examination of raccoons, American marten, and *Peromyscus* species mice produced ticks representing five *Ixodes* species, including the first report of *Ixodes gregsoni* in Michigan. *Dermacentor variabilis* were recovered from raccoons and American marten. *Ixodes scapularis* were not recovered during the current project, and antibodies to *B. burgdorferi* were not detected in any serum samples collected from mammalian hosts.
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Steven John Schaar

October 29, 2012
DEDICATION

I dedicate this thesis to all the animals whose lives I interrupted during the summer of 2008. This project would not have been possible without their help.
ACKNOWLEDGEMENTS

I would like to thank the following people who made this project possible and my time at NMU an enjoyable and educational experience:

Dr. Jackie Bird: For serving as my thesis advisor and for all your help, understanding, and unbelievable patience during this project. You often went above-and-beyond your job responsibilities. I appreciate everything you have done for me more than I can express.

Drs. Donna Becker and Mason Reichard: For your insight, input, and expertise into my project. Again, your patience during the years it took me to finish is greatly appreciated.

Dr. Bird, Mike Peters, Grant Slusher, Bill Severud, Jessie Simmon, Andi Hales, Rachael Holman, and Trisha Sippel: For your help in the field.

Dr. Lindsay and Grant Slusher: For all your help in the lab.

Drs. Susan Little and Kelly Allen of Oklahoma State University: For performing my serology tests.

Susie Piziali: For all your help and encouragement during my time at NMU.

Fellow grad students at NMU including Bill Severud, Jessie Simmon, Grant Slusher, Mike Peters, Carla Serfas, Tiffany Opalka, Kari Farkas, Andi Hales, Julie Howard, Emily Durkin, and Kim Danielson: For the great times. All of you have made my time in Marquette memorable.

Kathy Germain from North Central Michigan College: For all your support and for providing me with a great knowledge base on which to build.
Employees of Pictured Rocks National Lakeshore including Jim Northup, Lora Loope, Leah Kainulainen, and Jerry Belant: For all the time, effort, and logistics you provided to my project.

National Park Service, NMU Excellence in Education Grant, NMU Biology Department Development Fund Grant, and Dr. Bird: For your contributions to funding of this project.

And finally to my family and friends: For all your support. I know you heard “I’m almost done” more times than I can count. And a special thanks to Dave Roth for reminding me how important finishing this project was to my late father.

This thesis follows the guidelines and format prescribed by The Journal of Parasitology and the Department of Biology.
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INTRODUCTION

Ticks as parasites

All ticks are hematophagous parasites belonging to the families Argasidae (soft ticks), Ixodidae (hard ticks), or Nuttalliellidae. Only members of Argasidae and Ixodidae are found in Michigan (Oliver, 1989). Argasid and ixodid ticks can be further defined in terms of their host seeking strategies. Nidicolous ticks find hosts by living in small, confined spaces (e.g., nests, caves, burrows) that are frequently visited by vertebrates. This strategy ensures that the ticks are protected from the climatic variability of open spaces, but readily exposed to potential hosts. In comparison, non-nidicolous ticks quest for a host outside of host burrows. They usually climb local vegetation and attach to a host animal as it passes. Non-nidicolous ticks descend their questing location to seek refuge under debris of the forest floor when climatic variables (e.g., temperature, humidity) become detrimental to their survival (Sonenshine, 1993).

Ixodidae

The family Ixodidae contains 13 genera and about 650 different species of ticks, nearly 500 more than that of Argasidae. A hard plate, the scutum, is found on the dorsum of members of the family. It covers the anterior dorsal portions of the body of immature and female hard ticks and the entire dorsal aspect of the body of males (Sonenshine, 1991). Hard ticks are globally widespread, and at least 35 species are present in the United States (Allan, 2001).

The life cycle of all hard ticks contains four stages that progress through the following sequence: egg, larva, nymph, and adult. Completion of the life cycle requires
one to three years, dependent on the species and geographic area involved. Non-nidicolous hard ticks spend the majority of their life on the forest floor, protected from climatic extremes by debris. A minimal portion of their life is spent attached to a host (Allan, 2001). Mating occurs on or off the host, depending on the species of tick (Sonenshine, 1991).

Most hard tick species are capable of feeding on a multitude of vertebrate hosts. Male hard ticks take multiple brief meals. All other post-embryonic life stages are dependent upon the tick having one sustained meal lasting 3-12 days. Following each feeding event, immature hard ticks detach from their host, enter a state of developmental diapause, and molt into the next life stage. Adult females feed, mate, and lay a large mass of eggs on the ground. The female dies following this single reproductive event (Oliver, 1989). Small vertebrates (often rodents, birds, and reptiles) serve as hosts for larval and nymphal hard ticks. Adult hard ticks most often feed on larger mammals, including deer, canids, and occasionally humans (Keirans et al., 1996).

Most hard ticks utilize a single host per feeding life stage (larva, nymph, adult) and are therefore known as “three-host ticks”. Following each feeding event, three-host ticks fall from their hosts and proceed through developmental diapause and molting on the ground. Exceptions to this three host generality exist, with a number of tick species using only one (“one-host ticks”) or two (“two-host ticks”) hosts during their lifetime.

One-host ticks quest for their only host as larvae and stay on that single animal throughout the remainder of their life, where they feed, undergo developmental diapause, and molt into the next life stage. Following each molt, the ticks take another blood meal (Oliver, 1989). *Dermacentor albipictus* (the winter tick) is a one-host tick found in
Michigan and is known to parasitize moose (*Alces alces*) (DelGiudice et al., 1997) and white-tailed deer (WTD) (*Odocoileus virginianus*) (Kellogg et al., 1971). Most two-host ticks feed from the same host during their larval and nymphal stages and from a second host while adults (Oliver, 1989).

**Argasidae**

The genera *Argas, Ornithodoros, Otobius*, and *Antricola* make up the family Argasidae, the soft ticks. Most members of the family are nidicolous (Oliver, 1989). The dorsal scutum characteristic of ixodid ticks is absent, resulting in this group being referred to as “soft ticks” (Sonenshine, 1991).

Male and female soft ticks generally mate while off their vertebrate host (Sonenshine, 1991). The subsequent life cycle entails a single egg, larval, and adult stage. Between the larval and adult stages are multiple (usually three to four) nymphal instars (Oliver, 1989). The entire life cycle of soft ticks ranges from a few months to greater than one year. Females are capable of multiple reproductive events, each involving the deposition of a single egg mass. Most argasid ticks in the United States are found in the southern and southwestern portion of the country (Allan, 2001).

Mammals, birds, and reptiles can all serve as host for soft ticks. Blood meals occur while the two animals share the same confined space, usually during host rest or sleep. Larval soft ticks generally take a single brief meal. Nymphal and adult soft ticks take numerous blood meals, each lasting minutes to hours. The short feedings of soft ticks result in the possibility of a number of individual hosts being parasitized by the same tick, while in the same life stage. In adults, each meal is followed by the production of gametes (Oliver, 1989).
Expansion of tick ranges

Recent rapid range expansion in some tick species has been predicted or observed. This phenomenon has been extensively studied in ticks responsible for human parasitism and disease transmission, including *Ixodes scapularis*.

Geographic range expansion of *I. scapularis* has been linked to climate change (Ogden et al., 2005) and habitat change, where high densities of deer followed regrowth of forest land previously cleared by logging or farming (Childs et al., 1998; Childs and Paddock, 2003; Paddock and Yabsley, 2007). Recent interest has been focused on the role of migratory birds in the long distance transport of immature deer ticks into previously uninfested northern locations (Scott et al., 2012). As the distribution of *I. scapularis* increases, so does the likelihood of human exposure to disease agents they carry, perhaps most notable *Borrelia burgdorferi*, causative agent of Lyme disease.

Establishment of new tick populations

The establishment of new populations of ticks in non-endemic areas has also been studied in *I. scapularis*. Establishment is dependent on ticks being moved to new areas where climatic variables (e.g., temperature) (Ogden et al., 2004) and biotic variables (e.g., local habitat characteristics and host availability) are favorable. Frequency of introductions and number of individual ticks in each introduction also affect the success of a tick species establishing populations in a new area (Wilson, 1998). Ticks experience a high mortality rate as they mature from egg to adulthood (Carey et al., 1980). These high death rates dictate that large numbers of a tick species must be frequently moved into non-endemic areas with favorable climatic conditions, habitat, and host communities for an established population to develop.
Ixodid ticks in the Upper Peninsula of Michigan

Dermacentor variabilis, D. albipictus, Ixodes scapularis, Ixodes cookei, and Amblyomma americanum were found in the Upper Peninsula during a passive tick survey of Michigan (Walker et al., 1998). *Dermacentor variabilis* and *I. scapularis* are perhaps the most important of these tick species, due mostly to the commonality of *D. variabilis* and the disease potential associated with *I. scapularis*. *Dermacentor variabilis* has been collected from both peninsulas and nearly every county in Michigan (Walker et al., 1998). It is usually the most commonly collected tick during tick surveys performed in the state (Miedema, 2006; Opalka, 2011; Walker et al. 1998).

Established populations of *I. scapularis* in Michigan were first discovered in the Upper Peninsula’s Menominee County (Strand et al., 1992). Other established populations of the tick were not detected during a passive tick survey in the state (Walker et al., 1998). More recently, established *I. scapularis* populations have been found in the southwestern portion of the Lower Peninsula (Foster et al., 2005), and two individuals have been recovered in Marquette County (Opalka, 2011).

Substantial evidence supporting the expansion of established *I. scapularis* populations into north-central portions of Michigan’s Upper Peninsula has been lacking (Miedema, 2006; Opalka, 2011). A small number of ixodid ticks removed from mesocarnivores live-trapped in Pictured Rocks National Lakeshore (PRNL) from 2001-2005 were identified as *I. scapularis* at Northern Michigan University. All were adult females (J. Bird, pers. comm.), and all were removed from fishers (*Martes pennanti*) (Jerry Belant, pers. comm.). The ticks were collected in 2002 and 2005 and were the first *I. scapularis* collected in Alger County. No ticks collected from animals live-trapped in
2001, 2003, and 2004 were *I. scapularis*. Autologistic modeling performed by Brownstein et al. (2003) predicted that the environmental and climatic conditions near PRNL can support established populations of *I. scapularis*.

**Ixodes scapularis**

*Ixodes scapularis*, the deer tick or black-legged tick, is best known for its ability to transmit *B. burgdorferi* to its vertebrate hosts while feeding (Barbour et al., 1983; Burgdorfer et al., 1982; Johnson et al., 1984; Steere et al., 1983). The tick carries at least two other human and veterinary pathogens, *Anaplasma phagocytophilum* (Schulze et al., 2005) and *Babesia microti* (Adelson et al., 2004). A new *Ehrlichia* species bacterium was recently discovered in multiple human patients in Wisconsin and Minnesota. Deer ticks from the same region have since tested positive for the bacterium (Pritt et al., 2011).

**Life history**

*Ixodes scapularis* is a three-host, hard-bodied tick (Anderson, 1989). Its life cycle requires a minimum of two years, typically beginning each June or July when the adult female oviposits approximately 1000-3000 eggs onto the forest floor. Eggs hatch into larvae in the summer of the same year (Yuval and Spielman, 1990).

Larvae quest for a vertebrate host, usually small mammals or birds. Once successful, the questing ticks attach to their host and feed (Allan, 2001). Larvae that feed before September typically molt immediately and overwinter as unfed nymphs, while those that feed later in the year overwinter as fed larvae and molt the following spring (Yuval & Spielman, 1990).

Nymphs quest and feed on small to mid-sized mammals or birds (Allan, 2001) in spring or early summer of the tick’s second year of life (Yuval and Spielman, 1990).
Following this second bloodmeal, they fall from their host and molt into adults.

Although most adult deer ticks quest for their final host during that fall (Stafford, 1992; Ostfeld & Keesing, 2000), some have also been found questing (Sonenshine, 1993) and feeding (Yuval and Spielman, 1990) in the spring.

**Distribution**

Deer ticks have been reported to populate much of the eastern and midwestern United States through predictive logistic models, disease monitoring, and collection of the arthropod itself (Callister et al., 1988; Falco & Fish, 1989; Bouseman et al., 1990; Walker et al., 1994; Walker et al., 1998; Jackson et al., 2002; Adelson et al., 2004; Holman et al., 2004; Caporale et al., 2005; Schulze et al., 2005). Established populations have been found in Michigan’s Menominee County (Strand et al., 1992; Walker et al., 1998; Guerra et al., 2002) and in multiple counties in the southwest corner of the state (Foster et al., 2005). Populations of the tick have also been found throughout much of Wisconsin (Callister et al., 1988; Jackson et al., 2002; Guerra et al., 2002; Caporale et al., 2005), including Marinette County (Guerra et al., 2002), an immediate neighbor of the Upper Peninsula.

**Habitat**

The distribution of ticks is largely determined through a complex relationship between the arthropod and its local environment. Deer ticks in Maine have been associated with moist, heavily canopied deciduous forests containing shrubby undergrowth, ferns, deciduous litter, and forest grasses (Lubelczyk et al., 2004). In contrast, positive associations between the tick and dry/mesic to dry deciduous forests,
fertile soils with sand to loamy/sand texture, and sedimentary bedrock have been demonstrated in Wisconsin (Guerra et al., 2002).

Multiple studies have implicated deciduous forests, leaf litter, and logs as important factors in deer tick survival. Total leaf litter removal during each immature life stage’s peak activity season resulted in a greater than 70% reduction in the abundance of larvae and nymphs (Schulze et al., 1995). Siegel et al. (1991) found significantly more immature deer ticks along trail segments displaying a full hardwood canopy and a sizable layer of deciduous litter when compared to trail segments in open meadows with no canopy or in a mixture of the two. A higher abundance of all deer tick life stages was present within oak and maple forests of upstate New York when compared to neighboring dogwood, bluestem, and hayfield habitats (Ostfeld et al., 1995; van Buskirk and Ostfeld, 1998).

Increased presence of nymphal deer ticks on, or near, deciduous trees and logs has been documented. Carroll and Kramer (2001) found greater human risk for acquiring nymphs when sitting on logs than when walking, kneeling in leaf litter, and placing hands directly in leaf litter. They also recovered high densities of nymphs (2.6 nymphs/m²) by pressing a 0.25 m² piece of flannel to the upper surface of large logs. Lane et al. (2004) concluded that direct contact with wood (sitting against trees, gathering wood, and sitting on logs) posed a greater risk of acquiring nymphs of *Ixodes pacificus* (a close relative of the deer tick) than did contact with only leaf litter (walking, sitting on leaf litter, and stirring and sitting on leaf litter).
Hosts

Deer ticks are host generalists and have been documented parasitizing 125 vertebrate species in the United States. Forty-one species of mammals, 57 species of birds, and a minimum of 14 species of lizards are known to have served as host for immature life stages of the tick. Adult deer ticks have been documented feeding on 27 species of mammals and a single species of lizard.

The white-footed mouse (*Peromyscus leucopus*) and numerous other mammals serve as hosts for immature deer ticks, including Virginia opossum (*Didelphis virginiana*), raccoon (*Procyon lotor*), northern short-tailed shrew (*Blarina brevicauda*), eastern gray squirrel (*Sciurus carolinensis*), striped skunk (*Mephitis mephitis*), and deer mouse (*Peromyscus maniculatus*) (Keirans et al., 1996). Eastern chipmunks (*Tamias striatus*) are important hosts for immature stages in the absence of mice (Slajchert et al., 1997). Avian species such as gray catbird (*Dumetella carolinensis*), ovenbird (*Seiurus aurocapillus*), rose-breasted grosbeak (*Pheucticus ludovicianus*), blue jay (*Cyanocitta cristata*), common grackle (*Quiscalus quiscula*), and American robin (*Turdus migratorius*) are also parasitized by larvae and nymphs (Keirans et al., 1996).

White-tailed deer are an important host for adult deer ticks (Daniels et al., 1993; Deblinger et al., 1993; Stafford et al., 2003). Supplemental wild hosts of adults include Virginia opossum, raccoon, foxes (*Urocyon cinereoargenteus* and *Vulpes vulpes*), and bobcat (*Lynx rufus*). Domestic mammals such as cattle, horses, and dogs have also served in this role (Keirans et al., 1996).

*Dermacentor variabilis*

*Dermacentor variabilis*, the American dog tick, is a three-host tick abundant in the eastern United States (Sonenshine, 1991). It is perhaps best known for its widespread
distribution and its ability to transmit numerous rickettsial disease agents, including *Rickettsia rickettsia*, the bacterial agent of Rocky Mountain spotted fever (Burgdorfer, 1969).

**Life history**

The entire life cycle of *D. variabilis* entails 1-2 years, and begins when engorged females drop from their hosts and oviposit on the forest floor. Larvae hatch from the mass of up to 6500 eggs (Allan, 2001) and over-winter unfed (Burgdorfer, 1969). Upon emerging in the spring, larvae feed on small mammals. After feeding, they drop from their hosts and seek refuge under leaf litter on the forest floor. They molt into nymphs and feed on a second small mammal. Following this second bloodmeal, they drop from their host and molt into adults. A large mammal serves as their last vertebrate host (Alan, 2001).

**Distribution**

Established populations of *D. variabilis* are widespread throughout North America and extend from northern Mexico north into Canada. The tick can be found in most parts of the United States, with the exception being the Rocky Mountains (Burgdorfer, 1969). It is the most commonly encountered tick in Michigan and has been submitted from every county in Michigan’s Upper Peninsula (Walker et al., 1998).

**Habitat**

Adult *D. variabilis* are most frequently found along trails and edges in grassy and old field-forest ecotone habitats (Burg, 2001; Micher and Rockett, 1993; Sonenshine, 1991). Opalka (2011) found more adult *D. variabilis* in fields than in hardwood, coniferous, or mixed forests. Immature stages of the tick have been most frequently
found on hosts collected along powerlines and mixed wood habitats, including those with shrubs and trees. They are less common in open spaces (Allan, 2001).

**Hosts**

*Dermacentor variabilis* is a host generalist and has been documented feeding on raccoons, rabbits, and multiples species of cervids, rodents, mustelids, canids, felids, and bears (Allan, 2001). Red-backed vole (*Clethrionomys gapperi*) was the dominant host for immature life stages of the tick in Manitoba, Canada (Burachynsky, 1982). Gray squirrels and raccoons were the most important host for larvae and nymphs of *D. variabilis* in Tennesse; raccoons and opossums were the most important hosts for adults (Zimmerman et al., 1988).
BACKGROUND

The distribution of ticks and their associated diseases is expanding. This expansion is especially concerning when new areas of infestation increase human exposure to disease agents. *Borrelia burgdorferi* is the causative agent of Lyme disease and is vectored by deer ticks in the eastern and midwestern United States. Consistent presence of these ticks in the Upper Peninsula of Michigan has historically been limited to areas of Menominee County. However, three ticks recovered from fishers during a research project in Pictured Rocks National Lakeshore (PRNL) performed between 2001 and 2005 were later identified as deer ticks at Northern Michigan University (NMU) (J. Bird pers. comm.). Tick surveys have not been performed in PRNL, and these three deer ticks were the first record of the species in Alger County. In addition, two deer ticks were recovered during an unrelated tick survey performed in Alger County’s immediate neighbor, Marquette County, during 2008 and 2009 (Opalka, 2011). With hundreds of thousands of visitors to PRNL each year, the expansion of deer ticks into the park could elevate the risk of contracting *B. burgdorferi* in Michigan’s Upper Peninsula.

**Pictured Rocks National Lakeshore**

Pictured Rocks National Lakeshore is located in Michigan’s Alger County and was established as the country’s first national lakeshore on October 15, 1966 (U.S. Department of the Interior, National Park Service, 2005). The park is comprised of two distinct areas; a 13,557 ha lakeshore bordering 65 km of Lake Superior, and a 15,317 ha
inland zone which buffers the shoreline from significant anthropogenic effects. Annual visitation to the park in 2004 was 383,705 people.

The park’s surface geology and underlying sandstone bedrock were created through processes occurring in the Cambrian, Ordovician, and Quaternary Periods (U.S. Department of the Interior, National Park Service, 2007). A wide range of soils are present, with loam and sand being common (U.S. Department of the Interior, National Park Service, 2006). Seven ecological habitats have been identified within the park, including forests ranging from dry to saturated. Moist deciduous forests dominated by sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and an undergrowth rich in herbaceous plants and ferns are common in the western portion of the park (Chadde, 1996). Similar environmental characteristics have been linked to the presence of deer ticks (Guerra et al., 2002; Ostfeld et al., 1995; van Buskirk and Ostfeld, 1998).

**Collection of ticks**

Tick flagging, tick dragging, tick sweeping, walking, and the utilization of harnessed animals are all suitable for the collection of questing ticks (Sonenshine, 1993). Tick dragging and flagging are most commonly used. They are similar in function, and both allow for simple tick abundance calculations based on area, distance, or time.

Each tick drag is comprised of a large piece of fabric, weights, a pole, and cord. The pole is sewn into one end of the fabric and the cord is tied to both ends of the pole. The trailing end of the drag is weighted to facilitate direct contact with ground-level vegetation, as the user pulls it from the cord’s center. The drag is frequently examined and attached ticks collected (Sonenshine, 1993).
Each tick flag is constructed of a large piece of fabric secured to a long handle. The bottom of the fabric is weighted to promote the flag’s efficient travel through vegetation, as the user guides it by its handle. As with tick drags, tick flags must be examined frequently for attached ticks.

Feeding ticks are collected from wild vertebrate hosts through capture or killing. Small to medium-sized mammals are commonly live-trapped and anesthetized to facilitate removal of feeding ticks with forceps. High densities of appropriately sized live traps are baited with odorous food and placed at study sites. Ticks are removed from the host at its skin level to avoid damaging the mouth parts of the arthropods, which are necessary for taxonomic identification (Sonenshine, 1993). Removal of feeding ticks from vertebrate hosts is a sensitive sampling strategy in areas of low tick abundance (Ginsberg and Ewing, 1989; Kitron et al., 1991).

The efficiency of several collection methods in sampling deer ticks was evaluated by Ginsberg and Ewing (1989) and again by Falco and Fish (1992). Ginsberg and Ewing (1989) investigated the efficiency of four collection methods. After standardizing for collection effort, no significant difference was noted between the number of ticks/hr collected with flagging and walking. However, the high probability of immature deer ticks questing within leaf litter resulted in few larvae and nymphs being collected through walking.

Falco and Fish (1992) compared host examination, drag sampling, and CO₂ baited tick traps for their efficiency in collecting immature deer ticks. When standardized for time, they found that drag sampling (705 individuals) and tick trapping (593 individuals)
resulted in the largest number of nymphal ticks. All three methods resulted in similar larval yields.

**OBJECTIVE**

The objective of the present study was to search for *I. scapularis* in PRNL by performing a tick survey in the area that had previously produced deer tick infested fishers. Results of this project serve to develop a baseline inventory of tick species, along with their temporal questing and feeding patterns in this area of PRNL.

**MATERIALS AND METHODS**

**Study site**

Three fishers infested with deer ticks had been fitted with radiotelemetry collars by PRNL staff during an unrelated project (J. Bird pers. comm.). Global Positioning Satellite (GPS) coordinates tracking movements of the fishers over multiple years (2001-2005) were available (Fig 1). Based on these coordinates, a polygonal study site representing the outer limits of the home ranges of two of the fishers was produced and imposed onto a vegetation map of PRNL. Six areas of northern hardwoods forest within this polygon were repeatedly sampled throughout the study. These six areas represented areas of habitat favorable to deer ticks. The study sites included large portions of the Carmody and Miner’s areas of PRNL (Fig 2). Areas of movement of the third fisher were not included in the study site due to anticipated logging operations in much of its home range during the summer of 2008.

**Collection of questing ticks**

Questing ticks were collected from parallel transects within each sampling area by simultaneous walking and flagging between May 11 and August 16, 2008. Transects
were separated by 10 m, and all areas flagged were within hardwood forests. If possible, at least one transect was the edge of a road or trail. This allowed for collection of questing ticks from areas where they are most likely found. New transects were formed each week to survey greater portions of the study site throughout the timeframe of the project. Each flagging episode was done by 1-2 people. A flag constructed of a 1 m x 1 m flannel cloth was used. The cloth was examined following each 10 m traveled. All ticks attached to the flag or found on the clothing of researchers were removed and placed in labeled vials containing 70% ethanol.

The timeframe of this project was divided into seven equal two-week periods. Five to seven collection events totaling 1000-1700 m occurred during each period (Table I). Flagging was not done when temperatures exceeded 26° C and/or when vegetation was wet.

**Collection of Feeding Ticks**

*From mesocarnivores*

Twenty-four Tomahawk® live traps (80 cm x 25 cm x 30 cm) were placed in high density groups of 2-5 traps/group in wooded areas of the study site to capture medium-sized mammals. The traps were separated by at least 10 m. At least one group of traps was located in each of the six sampling areas. The traps were opened and partially covered with local forest debris and/or burlap three times per week from May 11 to July 30, 2008. Trap numbers were increased to 33 on July 30 due to recent decreased trapping success. Trapping continued until August 16, 2008. Traps were baited with raw meat and checked each morning. Traps not containing a captured animal remained open until the next morning. All traps were closed if there was no plan to check them the following
day. Commercially prepared marten lure was applied weekly to a tree in the immediate vicinity of each trap. The stick used to apply the lure was then placed inside the trap. Traps were not set if either freezing temperatures or a greater than 50% probability of precipitation was predicted. Traps remained in their same location throughout the study.

Captured raccoons were restrained in the trap using two commercially obtained trap dividers (Tomahawk®). The restrained raccoons were injected with 5mg/kg body weight of Telazol® into the musculature of the hind legs through the side of the trap (Gehrt et al., 2001). The trap dividers were removed, and the trap was covered to help facilitate onset of anesthesia. The animals were intermittently evaluated. The lack of response to physical stimuli was considered indicative of full anesthesia.

Trapping and handling of American martens (Martes americana) were similar to that of raccoons. Instead of restraining martens inside the trap, the animals were persuaded to enter a funnel-shaped sleeve placed at the trap’s door (Fig 3). The proximal (in relation to the trap) portion of the sleeve was constructed of canvas and the distal end of a heavy gauge wire cylinder, nearly the same size of a marten’s body. The trap door was opened, the marten entered the sleeve in an attempt to escape the trap, and the door was closed behind the marten. Escape from the sleeve was prevented by closing the sleeve behind the animal. The animal was forced to enter the distal portion of the sleeve, where it became wedged in the wire cylinder (Fig 4). The canvas portion of the sleeve was then unzipped to expose one hind leg of the marten, where 5 mg/kg body weight of Telazol® was injected (Bull et al., 1996). The sleeve was then covered with a cloth until the animal became anesthetized.
Each anesthetized animal was visually examined for ectoparasites. Special attention was devoted to the head, ears, and neck of the animals. Ticks from each animal were placed into a separate vial containing 70% ethanol. Blood samples were collected from each animal to test for antibodies against *B. burgdorferi*. A small, distinguishable pattern specific to each animal was clipped in its fur coat for future identification of animals as recaptures. The animals were weighed, sexed, and returned to the trap for recovery. Release occurred at the capture site following full recovery from anesthesia. The trap was reset in its original location. Because trapped mesocarnivores were only used to collect ticks that were active within the study sites, recaptured animals were processed the same as new captures.

**From small mammals**

Thirteen to 20 Sherman® live traps (25 cm x 7.5 cm x 7.5 cm) were set three afternoons per week between May 11 and July 13, 2008 and between July 20 and August 16, 2008, weather permitting. Traps were baited with a mixture of at least two of the following: peanut butter, oatmeal, and bacon grease. Polyester fiberfill was placed inside the traps to prevent hypothermia. Opened traps were placed near logs, trees, or other landscape features in the sampling areas that would provide favorable habitat for small mammals. The traps were checked the following morning. Traps were not set if either freezing temperatures or a greater than 50% probability of precipitation was predicted. Small mammal trapping was temporarily suspended on July 13 to reevaluate its importance due to recent shrew captures having high (100%) mortality rates. It was reinstated on July 20 and continued throughout the remainder of the project to help ensure the collection of immature ticks. Small mammal traps were rotated among
sampling areas each trap day so that every sampling area was surveyed at least once per two week period. Each time a sampling area was surveyed, the exact location of the traps was changed. This maximized the portion of the study site surveyed during the project. Approximately 10 m separated each trap from the next.

Captured small mammals were anesthetized in a sealed two compartment (500 mL total volume) chamber (Fig 5) using 25-30% v/v isoflurane in propylene glycol. A cotton cosmetic pad was placed in one compartment of the chamber, and 1 ml of the isoflurane/propylene glycol mixture was applied to it. Captured animals were transferred from the live trap to the second compartment, and the chamber was sealed. Wire mesh separated the compartments to prevent direct contact between the animal and the anesthetic. The mammal was monitored for onset of anesthesia and respiratory status.

Deep anesthesia was indicated by the loss of the animal’s righting reflex. The animal remained in the chamber for 10 secs following onset of deep anesthesia. It was then transferred to a clean work surface. A nose cone fashioned from a small syringe and a cotton cosmetic pad dampened with the isoflurane/propylene glycol mixture was used to maintain anesthesia during examination and tick removal. Respiratory rate and response to stimulus was monitored throughout the procedures. Depth of anesthesia was adjusted by changing the distance between the animal’s snout and the syringe opening (Itah et al., 2004).

All captured animals were carefully examined for ectoparasites, with special attention being devoted to the head, ears, and neck. Ticks from each animal were placed into a separate vial containing 70% ethanol. Release at the capture site followed
complete recovery from anesthesia. Small mammals were not marked when captured, and it is not known if any of them were recaptured during this study.

All vertebrate sampling methods were approved by NMU’s Institutional Animal Care and Use Committee (IACUC) under permit #063.

**Identification of ticks**

All ticks were transported to NMU and identified using light microscopy and taxonomic keys. Adult and nymphal ticks were identified to species; larvae were identified to genus due to difficulty in identification to species (Clifford et al., 1961; Durden and Keirans, 1996; Keirans and Clifford, 1978; Keirans and Litwak, 1989; Lindquist et al., 1999). Ticks identified as unexpected species were sent to Dr. Lorenza Beati, curator of the U.S. National Tick Collection at Georgia Southern University in Statesboro, Georgia, for species confirmation.

Additional individuals of these species were subjected to molecular analysis to aid in their identification (Poucher et al., 1999). Each tick was bisected longitudinally with a sterile scalpel. DNA was extracted from half of the tick using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). An approximately 900 bp long fragment of the second internal-transcribed spacer (ITS-2) of the ribosomal DNA gene was amplified using primer pair YTG CGA RAC TTG GTG TGA AT (forward) / TAT GCT TAA RTT YAG SGG GT (reverse) (Y = C/T; R = A/G; S = C/G) (Integrated DNA Technologies, Coralville, IA) and Bullseye HS Taq Polymerase, 2X Master Mix (MidSci, St. Louis, MO). The thermal profile for amplification consisted of 10 cycles of 92°C for 1 min, 50°C for 1 min, and 72°C for 2 min; followed by 30 cycles of 92°C for 1 min, 48°C for 35 sec, and 72°C for 2 min. Tubes were then held at 72°C for 7 min and then maintained
at 4°C. A negative control containing all reaction reagents and sterile water in place of template DNA was run with the PCR.

Amplicons were double digested using restriction enzymes (RE) \textit{CfoI} and \textit{MspI} (Promega, Madison, WI). Each digest consisted of 8 $\mu$L PCR product, 13 $\mu$L TE (pH = 8), 3 $\mu$L bovine serum albumin, 3 $\mu$L 10x Buffer B, and 1 $\mu$L of each RE. Digest conditions were 37°C for 3 hr. Products of digestion and a 1 kb molecular ladder (MidSci, St. Louis, MO) were electrophoresed through a 5.0% Super Fine Resolution (SFR) agarose (MidSci, St. Louis, MO) gel with 1X TBE and ethidium bromide. Photodocumentation using UV transillumination followed electrophoresis.

\textbf{Analyses}

Ticks collected by flagging were used to evaluate the temporal questing activity of each species. Each of seven two-week periods between May 11 and August 16, 2008 contained 5-7 flagging events totaling 1000-1700 m (Table I). Due to unequal distances flagged in each period, a weighted average of tick meters/m flagged was calculated for each two week period according to the formula: $\sum$(ticks collected in each flagging episode X m flagged in each episode)/(total m flagged in that 2 week period), and the weighted averages were plotted over time to evaluate tick questing activity.

Abundance of feeding ticks was calculated to evaluate feeding activity during each two week period. Abundance was defined as the number of ticks collected divided by the number of hosts examined (Bush et al., 1997). Tick abundances were plotted over time to evaluate feeding activity.
RESULTS

Questing ticks

A total of 9.2 km were flagged over 14 weeks, with 18 adult *D. variabilis* (6 males, 12 females) being collected. The weighted average of tick meters/m flagged peaked (1.25 tick x m/m flagged) during the two week period from June 22 to July 5 and decreased to zero by the end of the study (Fig 6). No other tick species or life stage was collected by flagging.

Seventeen more adult *D. variabilis* (12 males, 5 females) were removed from the clothing of researchers during this project. These were not used in examining the questing activity of *D. variabilis*, as the exact time and location they attached were not known, and it was impossible to standardize for distance.

Feeding ticks

A total of 1086 trap nights for mesocarnivores and 513 trap nights for small mammals occurred during this study. This effort resulted in 98 mammal captures (51 raccoon, 19 marten, five eastern chipmunk, 16 *Peromyscus* species mouse, two woodland jumping mouse (*Napaeozapus insignis*), four short-tailed shrew, and one pygmy shrew (*Sorex hoyi*)) and the collection of 232 ticks representing six species (Table II).

Two species (*D. variabilis* and *Ixodes gregsoni*) accounted for approximately 86% of all adult and nymphal feeding ticks collected. Feeding nymphs of *I. cookei* (n=7), *Ixodes marxi* (n=6), *Ixodes texanus* (n=3), and *Ixodes kingi* (n=3) were also collected, as were 72 *Ixodes* species larvae.

One adult *Ixodes* species female was destroyed while being removed from her host and could not be identified to species. Four larvae were destroyed beyond
identification to genus while attempting to clear them for identification to species. With
the exception of the four destroyed larvae, the possibility of any tick being a deer tick
was eliminated using taxonomic keys (Clifford et al., 1961; Durden and Keirans, 1996;

Raccoons were infested with *D. variabilis* throughout the study. Thirty of 51
(prevalence=58.9%) raccoons were infested, with feeding activity peaking at 2.7
ticks/raccoon (n=7) during the two week period from June 8 to June 21, 2008.
Abundance rapidly declined after the two week period of July 6 to July 19, but never fell
to zero. Overall mean abundance was 1.4 ticks/raccoon (n=51) (Fig. 7). Mean intensity
of infestation was 2.4 ticks/infested raccoon (n=30). Four of 19 (prevalence=21%)
marten captures resulted in recovery of *D. variabilis*. Overall mean abundance was 0.42
ticks/marten capture (n=19) and mean intensity of infestation was 2.0 ticks/infested
capture (n=5).

Two of three female ticks submitted to Dr. Beati were confirmed as *I. gregsoni*. The third was identified as *I. texanus*. Three of 31 other female ticks identified as *I.
gregsoni* during this project were subjected to molecular analysis, as was a single adult
deer tick collected in an unrelated study. Electrophoretic bands following endonuclease
digest of DNA from ITS-2 of ribosomal DNA from the three ticks identified as *I.
gregsoni* were located as follows: single band immediately above 298, two bands
between 201 and 154, single band between 154 and 134. Electrophoretic band pattern
following endonuclease digest of DNA from ITS-2 of ribosomal DNA from the deer tick
was consistent with that previously described (Fig. 8) (Poucher et al., 1999).
Eleven *Ixodes* species ticks not analyzed by Poucher et al. (1999) have been documented east of the Mississippi River. Based on morphology, the ticks identified as *I. gregsoni* in the current study were not individuals of any of these 11 species (Keirans and Litwak, 1989).

Adult *I. gregsoni* (1 male, 30 females) were recovered during nine of 19 marten captures (prevalence=47.4%). Twenty-seven nymphs were recovered during the same number of captures (prevalence=47%). Adult activity peaked during the two week period of June 22-July 5, when the single marten capture resulted in recovery of 20 adult *I. gregsoni*. Nymphal *I. gregsoni* activity displayed a bimodal distribution. One peak of activity occurred during the two week period of May 25-June 7, and a second, taller peak appeared to occur during the two week period of July 6-July 19 (Fig 9). Overall mean abundance for adult *I. gregsoni* was 1.6 ticks/marten (n=19) and mean intensity was 3.4 ticks/infested marten (n=9). Overall mean abundance for *I. gregsoni* nymphs was 1.4 ticks/marten (n=19) and mean intensity was 3 ticks/infested marten (n=9). No marten captures occurred during the two week period of July 20 through August 2, and no data are available for that timeframe.

*Ixodes cookei* nymphs were recovered from both marten (n=3) and raccoons (n=4), as were nymphs of *I. marxi* (American marten, n=5; raccoons, n=1). *Ixodes texanus* nymphs (n=3) were recovered from raccoons only, and *I. kingi* nymphs (n=3) were recovered from marten only. *Ixodes* species larvae were recovered from *Peromyscus* species mice (n=2), marten (n=69) and raccoon (n=1).
DISCUSSION

*Dermacentor variabilis*

Adult *D. variabilis* was the only questing tick and most commonly recovered adult feeding tick collected during this study. No immature life stages of the tick were recovered during a time when they are known to be active (Burgdorfer, 1969). Opalka (2011) also failed to recover questing immature *D. variabilis* during her two year study of ticks in Marquette County, but at least one other study in the Upper Peninsula has recovered adults, nymphs, and larvae while flagging (Miedema, 2006).

At least two other tick surveys performed in the last six years have estimated *D. variabilis* abundance in the Upper Peninsula. The number of adult *D. variabilis* per km flagged (2.7-3.3 ticks/km) collected by Miedema (2006) was more similar to that collected in this study (3.8 ticks/km) when compared to Opalka (8.6 ticks/km average) (2011). Both Miedema (2006) and the current study concentrated search efforts on areas favorable to deer ticks and not *D. variabilis*. This may explain the low numbers of *D. variabilis* collected when compared to Opalka (2011). Assuming the ticks collected by flagging in this study are representative of the non-nidiculous tick community within the study site, the risk of exposure to questing ticks during tick season is low and essentially limited to adult *D. variabilis*.

Throughout its geographic distribution, seasonal activity of adult *D. variabilis* begins in spring and ends by early autumn. The actual months of activity vary by location in North America, as does the number of peaks. Activity of questing *D. variabilis* has displayed a unimodal pattern peaking in May or June in northern portions of its distribution, including Nova Scotia, Massachusetts (Burg, 2001), Marquette County,
MI (Opalka, 2011), and Lucas County, OH (Micher and Rockett, 1993). Activity of *D. variabilis* during this project was similar to that found by Micher and Rockett (1993) and Opalka (2011), but the single peak in activity was later in the summer (late June/early July).

Like all three-host ticks, *D. variabilis* is reliant on taking a blood meal from a vertebrate host during each feeding life stage. During this study, adult *D. variabilis* were collected from raccoons and martens, but no larvae or nymphs of the species were recovered from any host. Immature *D. variabilis* have been recovered from deer mice (Burachynsky, 1982) and raccoons (Zimmerman et al., 1988). Numerous individuals of raccoons and *Peromyscus* sp. mice were surveyed in this study, but immature *D. variabilis* were not recovered. Trapping of small mammals was conducted in areas favorable to the survival of deer ticks, but not necessarily favorable to the survival of *D. variabilis*. Martens and raccoons have larger home ranges than all small mammal species captured in the present study. Travel by these mesocarnivores in their larger home ranges might have exposed them to questing adult *D. variabilis* in areas outside of the study site of this project. Infestation of raccoons with immature *D. variabilis* might have gone undetected due to the dark, thick hair coat of the animal, as has been previously seen with immature deer ticks (Fish and Daniels, 1990).

The ability of a vertebrate to serve as an efficient host for ticks is based on the host immune response to infestation, host grooming (Kaufman, 1989), and host travel. Raccoons and marten experienced a similar intensity of infestation with adult *D. variabilis* during this study. This suggests that both mammals are equally efficient hosts for the tick, once the host is infested. However, success in feeding to repletion and
subsequent egg production in female ticks can vary based on the host from which a tick feeds (Wikel, 1996). The degree to which *D. variabilis* successfully feeds and produces offspring following a blood meal from raccoons or martens is unknown.

**Ixodes gregsoni**

Several individuals of an unrecognized tick species were collected from mustelids of western Ontario Canada during the 1990’s. The ticks were subsequently described by Lindquist et al. (1999) and assigned the species name *Ixodes gregsoni*. The new species is believed to be a sister species of *I. texanus*, the most morphologically similar ixodid tick previously described. Genetic analysis comparing the two species has not been performed (Lindquist et al., 1999; Lubelczyk et al., 2007).

Studies of *I. gregsoni* are few, and knowledge of the species is introductory. It was believed the distribution of the tick was limited to boreal forests in Ontario (Lindquist et al., 1999), but it was later collected from mustelids trapped in wetlands of the eastern United States (Lubelczyk et al., 2007).

Morphological differences between *I. gregsoni* and *I. texanus* are limited to minor differences in the basis capituli and scutum. A more noticeable difference in the two species is seen in the porose areas of the female, with those of *I. gregsoni* being relatively large and sub-triangular, and those of *I. texanus* being small and sub-circular. Although *I. texanus* has been collected from both raccoons and mustelids, *I. gregsoni* has not been recovered from raccoons (Lindquist et al., 1999; Lubelczyk et al., 2007).

The two ticks confirmed to be *I. gregsoni* by Dr. Beati were described as “not typical” for the species, having a more roughened scutum than the single voucher specimen in her possession. Although these two ticks were not exactly representative of
the species, she believes they are *I. gregsoni* based on other morphological characteristics (porose areas and basis capituli) and the host species from which they were collected (American marten) (L. Beati, pers. comm.). Variation in physical characteristics in individuals of the same tick species collected from different geographic areas have been noted in other ixodid species, such as *I. scapularis* (Oliver et al., 1993; Wesson et al., 1993).

The electrophoretic pattern following endonuclease digest of DNA from the ITS-2 of ribosomal DNA from ticks identified as *I. gregsoni* is not characteristic of *I. texanus* or any of the other 16 *Ixodes* species previously analyzed. The band pattern following endonuclease digest of DNA from the ITS-2 of ribosomal DNA from the deer tick was consistent with that previously seen (Poucher et al., 1999). Dr. Beati’s species confirmation, molecular analysis performed during the current study, and morphological differences from other *Ixodes* species ticks previously documented east of the Mississippi River strengthen the assertion that ticks collected in PRNL during 2008 and identified as *I. gregsoni* are indeed individuals of that species.

Life history traits of *I. gregsoni* are largely unknown. Thirty females and a single male of *I. gregsoni* were collected from American marten during this study. The lone male was found attached to a female and was not feeding. A similar female:male ratio and non-feeding adult males is characteristic of hard-bodied nidicolous ticks, including *I. texanus* (Bishopp and Trembley, 1945). Multiple flagging events on numerous days throughout the summer of 2008 occurred in the immediate area where feeding *I. gregsoni* were removed from American marten. No *I. gregsoni* were collected while flagging, again suggesting the species is nidicolous.
A sex ratio severely biased towards females as seen in *I. gregsoni* of this study could be explained by a number of factors, including a result of heavy inbreeding and development of males from unfertilized eggs (Hamilton, 1967), frequent infection with a male-killing bacterium such as *Wolbachia* (Stouthammer et al., 1999), or a mating strategy that does not require the male to attach to a host (e.g., mating occurs off the host, allowing the male to remain in the host burrow). All of these conditions have been seen in acarines (Hamilton, 1967; Kiszewski et al., 2001; Plantard et al., 2012; Sonenshine, 1993; Stouthammer et al., 1999).

This is the first documented collection of *I. gregsoni* in Michigan, but the presence of the tick in PRNL was not totally unexpected. After habitat destruction and over-harvesting of American marten led to extirpation of the mustelid from the state, Michigan’s Department of Natural Resources relocated martens from Canada to the Upper Peninsula. Nearly 100 animals from Ontario were released into Alger and Delta counties (Earle et al., 2001, Williams et al., 2007). The introduction of *I. gregsoni* transported on martens relocated to the PRNL area could have occurred during this time. Alternatively, populations of *I. gregsoni* may have always been present in the Upper Peninsula, but have gone undetected, as tick surveys which include mustelids are uncommon.

*Ixodes scapularis*

**Range expansion**

Large numbers of a tick species must be moved into non-infested areas with favorable climatic and environmental conditions in order for new populations to develop. Until recently, Menominee County has been the sole well-documented stronghold for
established populations of deer ticks in Michigan’s Upper Peninsula (Walker et al., 1998). The tick was not found during the current project, despite the study site encompassing portions of PRNL where it previously had been recovered.

The introduction of ticks into new areas usually occurs while they feed, attached to a host traveling long distances. Because they can support high tick burdens while ranging over long distances, WTD can be important in the expansion of the geographic range of deer ticks, as large numbers of individuals are moved to new areas while feeding on this important host (Madhav et al., 2004). The home range of WTD in the area of PRNL ranges from 730 ha to 3037 ha dependent on the season of year and the year surveyed. Approximately one-half of deer display migratory behavior between summer and winter home ranges, with a median distance of 1.66-5.51 km separating the two. The majority of these deer returned to the same summer and winter ranges (Van Deelen et al., 1998). If these patterns of movement are typical of WTD in PRNL, the movement of a deer from PRNL would not extend to known deer tick endemic areas.

The movements of WTD have also been studied in other parts of the United States. In Minnesota, WTD occupy home ranges of 204-522 ha (Nelson and Mech, 1984; Rongstad and Tester, 1969). Distances between winter and summer home ranges of WTD in the state were 17-30 km (Nelson and Mech, 1981; Rongstad and Tester 1969), and average distance between birth and adult home range was 7 km (Nelson and Mech, 1984). Again, none of these home ranges or travel distances would connect PRNL to Menominee County, if WTD in the Upper Peninsula behave similarly.

Some variation in home range size and movement of WTD has been linked to habitat type. This project occurred in an area of PRNL dominated by hardwood forests
with occasional coniferous stands. Tierson et al. (1985) investigated the movements of 371 deer in a similar habitat in the Adirondacks for eight years. They found a home range of 132 to 233 ha, dispersal distances of less than 28 km, and a high fidelity to home range from year to year. At over 160 km, the distance separating PRNL from Menominee, MI is far greater than these dispersal distances and maximum possible length of these home ranges. High fidelity to home ranges would prevent movement of WTD from the Menominee area into PRNL over time.

Deer ticks are host generalists and have been documented feeding on multiple mammalian species in addition to WTD. Other mammals in the PRNL area that occupy a large home range and could serve as host to the tick include American marten, raccoon, fisher, and black bear. Home ranges of these animals are 1-4 km², 0.5-3 km², 15-35 km², and 5-150 km², respectively (Kurta, 2005). Even assuming the shape of these home ranges is a narrow (two km wide) rectangular shape, none of these home ranges is large enough to extend into Menominee County, where questing deer ticks might have been acquired and subsequently transported back to PRNL. A dispersing male black bear might be seen as the exception to this list. Dispersal distances in these animals have been documented to exceed that necessary to travel from Menominee County to PRNL. However, this long dispersal took the bear months (Rogers, 1987); far longer than the feeding time of any deer ticks it might be carrying.

Forested areas along the shoreline of the Great Lakes are important stopover locations for migrating birds (Bonter et al., 2008; Diehl et al., 2003). Tick surveys along these same shorelines have resulted in the intermittent collection of small numbers of
deer ticks, including those by Opalka (2011) near Marquette, MI and Callister et al. (1991) near Chicago, IL.

Migratory patterns of birds have been implicated in the geographic spread of multiple diseases in both the United States (Brinkerhoff et al., 2009) and Europe (Vorour et al., 2007). Migration has also been shown to expand the distribution of ectoparasites, as birds travel between wintering grounds in the south and breeding grounds farther north (Scott et al., 2001). Long distance translocation of ectoparasites during bird migration can be especially important in parasites such as ticks, which remain attached to their host for days acquiring a bloodmeal (Brinkerhoff et al., 2009).

Birds have been found to be infested with deer ticks (Keirans et al., 1996), including in areas where established population of the tick do not exist (Brinkerhoff et al., 2009; Ogden et al., 2008; Rand et al., 2004). Avian species such as rose-breasted grosbeaks that summer in northern Michigan pass through areas of the country with established deer tick populations during their spring migration (Graber, 1968; Lincoln et al., 1998). They can become infested with immature deer ticks and subsequently transport them to non-endemic areas. The intermittent presence of deer ticks in PRNL (and other areas along Michigan's Great Lakes shorelines which harbor high densities of migrating birds in the springtime) might be due to migrating avian hosts transporting small numbers of the tick into important stopover locations.

**Host availability**

Although deer ticks have been documented feeding on multiple mammalian species, WTD remain as an important host (Daniels et al., 1993; Deblinger et al., 1993; Ginsberg and Zhioua, 1999; Rand et al., 2004). Exclosure of WTD using fences that
allowed passage of other hosts such as raccoons and mice resulted in drastic reductions in
deer tick populations (Daniels et al., 1993).

The ability of WTD to exploit disturbed landscapes has become important in the
ecology of human tick-borne diseases (Paddock and Yabsley, 2007). Cases of human
Lyme disease and populations of deer ticks have declined when high deer densities were
decreased by culling. A reduction in WTD density from approximately 15/km² to less
than 2.3 km² coincided with a decrease of annual Lyme disease cases from 16% to
approximately 1% in one area of Massachusetts (Telford, 2002). Similarly, Stafford et al.
(2003) noted a decline in juvenile *I. scapularis* after WTD herds were culled from 90/km²
to approximately 15/km². Recovery of questing adult ticks on Monhegan Island, ME
decreased from 6-17 ticks/hr to 0.67 ticks/hr when deer densities were decreased from
37/km² to zero (Rand et al., 2004). In comparison to these densities, WTD densities in
PRNL during 2004 were approximated at 3/km² (Belant et al., 2007), markedly lower
than areas where *I. scapularis* have thrived and below the threshold of 7/km² previously
demonstrated to be an important density to support detectable populations of *I. scapularis*
(Rand et al., 2003).

**Environmental parameters**

Established populations of deer ticks have been associated with deciduous forests
(Guerra et al., 2002; Lubelczyk et al., 2004; Schulze et al., 1995), specific leaf litter
depth, proximity to water (French, 1995), and specific soil characteristics (Guerra et al.,
2002). The area of PRNL studied in this project was largely comprised of northern
hardwood forests with multiple rivers and creeks, but the environmental parameters listed
above were not measured. Although it appeared portions of the study site were suitable
for deer tick survival, lack of habitat cannot be ruled out as an explanation for the absence of deer ticks from PRNL without more specific measurements.

**SUMMARY AND CONCLUSION**

This study surveyed the tick community in areas of PRNL where adult deer ticks were recovered from fishers. Six tick species, including a first state record of *I. gregsoni*, were collected during this study spanning May to August 2008. Activity of adult *D. variabilis* was similar to that seen in other studies.

Intermittent presence of deer ticks has previously been documented in forested areas near Great Lakes shorelines. These same areas have also been shown to be important stopover locations for migratory birds. The small number of adult deer ticks recovered from fishers in PRNL during 2002 and 2005 were likely acquired as immatures by migrating birds, as they passed through endemic areas of the United States. They were subsequently transported to PRNL, an area with low deer densities located far from established populations of deer ticks. The ticks progressed through their maturation process, eventually finding fishers of PRNL as their final host. This infrequent introduction of small numbers of the tick into an area of low host availability appears insufficient to have resulted in established populations.

Future research should focus on monitoring the tick community in PRNL for signs of established deer tick populations, a scenario which could significantly impact the level of human exposure to tick-borne disease in the Upper Peninsula. Improvements in study design include a longer tick survey that spans multiple years, expansion of the study site to include areas of travel of the third deer tick infested fisher live-trapped by PRNL staff, increased search effort of both feeding and questing ticks, and evaluation of
migratory birds for their contribution to tick introductions into PRNL. More intensive trapping of small mammals and confinement of mesocarnivores over water could help explain the absence of an obvious host of immature *D. variabilis* seen in this study.

*Ixodes gregsoni* appears to be well established in the study site of this project. Future tick surveys of mustelids in the area could provide further insight into the ecology of this recently described species of tick.
BACKGROUND

*Borrelia burgdorferi* and Lyme disease

The long, Gram negative (Johnson, et al., 1984) spirochete *Borrelia burgdorferi* is the causative agent of Lyme disease (Barbour et al., 1983; Burgdorfer et al., 1982; Johnson et al., 1984; Steere et al., 1983) and the most commonly reported tick-borne pathogen in the United States (Holden et al., 2005). Tick species belonging to the *Ixodes ricinus* complex (including *Ixodes scapularis*) are the only known vectors capable of transmitting the bacterium to humans (Barbour et al., 1983; Burgdorfer et al., 1982; Johnson et al., 1984; Steere et al., 1983).

Ticks are infected while they feed on an infected host, and infection is passed transstadially (Burgdorfer et al., 1989). Transovarial transmission of *B. burgdorferi* in ticks is extremely rare (Nefedova et al., 2004). Infected ticks are capable of transmitting the pathogen to their future hosts, including humans, while feeding (Center for Disease Control and Prevention, 2011).

Although white-footed mice (*Peromyscus leucopus*) have been identified as the primary reservoir for *B. burgdorferi* in the United States (Adelson et al., 2004), reservoir competency has also been demonstrated in other animals, including eastern chipmunk (*Tamias striatus*) (Ostfeld and Keesing, 2000; Slajchert et al., 1997), deer mouse (*Peromyscus maniculatus*) (Rand et al., 1993), and American robin (*Turdus migratorius*) (Ostfeld and Keesing, 2000). *Ixodes scapularis* and *B. burgdorferi* have both been found
in Michigan (Golde et al., 1998; Stobierski et al., 1994; Strand et al., 1992; Walker et al., 1994; Walker et al., 1998) and Wisconsin (Caporale et al., 2005; Callister et al., 1988; Jackson et al., 2002).

The number of Lyme disease cases in the United States climbed from 16,273 in 1999 to 38,468 in 2009, a greater than two-fold increase in 10 years. In total, over 250,000 cases of Lyme disease were reported to Centers for Disease Control and Prevention (CDC) during this timeframe. The increase in the number of Lyme disease cases could be due to an actual increase in the number of people infected with *B. burgdorferi*, problems in diagnosis of Lyme disease including misdiagnosis and over-diagnosis (Steere et al., 1993), increased awareness in the public and healthcare community, and better surveillance programs (Bacon et al., 2008). Ninety-five percent of all reported Lyme disease cases came from disease foci in 12 northeastern and north-central states in 2009 (Center for Disease Control and Prevention, 2011).

The number of counties in Minnesota, Pennsylvania, and Wisconsin reporting Lyme disease increased between 1992 and 2006, suggesting the spread of *I. scapularis* and competent hosts into previously unaffected areas (Bacon et al., 2008). The estimated annual economic impact of human Lyme disease is in the hundreds of million dollars (Zhang et al., 2006).

In 2009, 103 cases of Lyme disease were reported by Michigan healthcare professionals, an increase from just 11 a decade earlier. Sixty-seven of these cases were believed to be linked to in-state exposure to *B. burgdorferi*, 33 were believed to be travel-associated, and the source of three infections were unknown (Michigan Department of Community Health, 2009). A single case of human Lyme disease was reported to Luce-
Mackinac-Alger-Schoolcraft District Health Department in 2004 and again in 2005. One of these reported cases was an employee of Pictured Rocks National Lakeshore (PRNL). No cases of Lyme disease have been reported in Alger County since that time (J. R. Lussman, pers. comm.).

**Immunologic response to B. burgdorferi infection**

Immunologic response to *B. burgdorferi* infection has been described in numerous mammalian species, including white-tailed deer (WTD), dogs, and raccoons. Antibodies to the bacterium were detected in WTD by means of enzyme linked immunoassay (ELISA) and Western blot within three weeks of infection via *B. burgdorferi* infected tick bites and remained detectable in the deer for at least 10 weeks (Luttrell et al., 1994).

Similar immunologic responses were found in dogs infected with *B. burgdorferi* by tick bite. Dogs seroconverted in approximately 4-8 weeks following exposure, and antibodies remained detectable through ELISA and Western blot analysis until the studies ended months later (Appel et al., 1993; Straubinger et al., 1997).

Five raccoons challenged with *B. burgdorferi* via tick bite displayed varied immunologic responses. Only one of the five raccoons seroconverted after being fed on by a single cohort (20 individuals) of *B. burgdorferi* infected nymphs. Antibodies to the bacterium in this raccoon were detected via ELISA four weeks post-infection. The raccoon remained positive for these antibodies for seven weeks, at which time the study ended. The four remaining raccoons seroconverted 4-6 weeks after being fed on by a second cohort of 20 infected nymphs. Once detectable, antibodies to *B. burgdorferi* persisted in these raccoons for 2-15 weeks (Norris et al., 1996).
Numerous other mammalian species have been screened for antibodies to *B. burgdorferi* during studies investigating *I. scapularis* and Lyme disease, including white-footed mice (Magnarelli et al., 2006), rabbits (Lane and Regnery, 1989), striped skunks, and opossums (Fish and Daniels, 1990). Gill et al. (1994) suggested that the natural travel of animals and their susceptibility to infection with *B. burgdorferi* makes screening for antibodies in local fauna a more sensitive detector of small or neighboring *I. scapularis* populations than collection of the arthropod itself.

**Serologic testing for antibodies to *B. burgdorferi***

The antigenicity of invariable regions 1-6 (IR1, 2, 3, 4, 5, 6) of *B. burgdorferi*’s variable surface antigen has been analyzed in mice, humans, non-human primates (Liang and Phillip, 1999), and dogs (Liang et al., 2000). Although it was found that immunodominance of IR1-6 varied in these species, antibodies to *B. burgdorferi* in all four groups reacted to IR6 when tested through ELISA.

A 26-mer synthetic peptide (C6) based on IR6 is commercially available. Assays based on this synthetic antigen have been useful in detecting antibodies to *B. burgdorferi* in serum of humans (Liang et al., 1999) and dogs (Liang et al., 2000). The sensitivity and specificity of these tests are equivalent to those of Western blot assays, traditionally considered the confirmatory test of serum samples positive for *B. burgdorferi* antibodies detected during ELISA tests (Mogilyansky et al., 2004).

The Lyme disease portion of IDEXX® Laboratory’s Canine SNAP®4Dx® test uses C6 as its primary antigen, making it a highly sensitive and specific test in detecting antibodies to *B. burgdorferi* in dogs (Chandrashekar et al., 2010). The ability of the test to accurately detect antibodies to *B. burgdorferi* in non-canid species including cats
(Levy et al., 2003), WTD (Murdock et al., 2009), and horses (Johnson et al., 2008) has been validated. In addition to antibodies to *B. burgdorferi*, the Canine SNAP®4Dx® cassettes also test for antibodies to two other tick-borne pathogens, *Anaplasma phagocytophilum* and *Ehrlichia canis*.

**OBJECTIVE**

*Ixodes scapularis* is the only known vector of *B. burgdorferi* in Michigan. Antibodies against the bacterium would be detected in Michigan wildlife only if it had previously been exposed via a feeding *I. scapularis* tick. The objective of the current study was to screen serum samples collected from mammals live-trapped in PRNL for antibodies to *B. burgdorferi*, thereby indirectly detecting populations of *I. scapularis* in the area.

**MATERIALS AND METHODS**

**Study site**

Three fishers infested with *I. scapularis* had been fitted with radiotelemetry collars by PRNL staff during an unrelated project. Global Positioning Satellite (GPS) coordinates tracking movements of the fishers over multiple years were available. Based on these coordinates, a polygon representing the outer limits of two of the animals’ travel was produced and imposed onto a vegetation map of PRNL. This polygonal area was the study site of this project. Six areas of northern hardwoods forest within the study site were designated sampling areas and represented areas of habitat favorable to *I. scapularis*, likely visited by infested fishers (Fig 2). The study site included large portions of the Carmody and Miner’s areas of PRNL. Areas of travel of the third fisher
were not included in the study site due to anticipated logging operations during the summer of 2008.

**Source of Samples**

Serum samples tested in this study were collected from mesocarnivores live-trapped during a tick survey in the study site spanning spring and summer of 2008. Following anesthesia, the foreleg or neck of each animal was soaked with 70% alcohol. A maximum of 3 mL of blood was drawn from either the cephalic vein or external jugular vein using a 5 ml syringe and a 22 gauge needle. Collected blood was immediately transferred to individual non-heparinized blood tubes and placed in an ice-filled cooler. All syringes, needles, and blood tubes were sterile upon use. Samples were transported to Northern Michigan University at the end of each day and centrifuged to separate serum. Individual serum samples were stored at -20°C until they were shipped in ice-filled containers to Dr. Mason Reichard at Oklahoma State University for screening.

**Screening of samples**

Serum samples were screened for antibodies to *B. burgdorferi* using a traditional ELISA targeting the C-terminal invariable domain of the bacterium’s variable surface antigen. Each well of a 96 well microplate was coated with 100 μL of Streptavidin (Affinity, Rockford, IL) diluted to 4 μg/mL in carbonate coating buffer. Following overnight incubation at 4°C, the plate was washed three times with PBS/0.05% TWEEN 20 detergent using a plate washer (Nunc ImmunoWash, Denmark), and each well was filled with 100 μL of synthetic peptide Cт (diluted with BSA diluent (KPL) to a final concentration of 5 μg/mL). The plate was incubated for two hours at room temperature,
washed twice with PBS/0.05% TWEEN 20, and blocked with 200 μL per well of 5% Isomil soy formula (diluted in PBS/0.05% TWEEN 20). Following a two hour incubation at room temperature, the plate was washed twice with PBS/0.05% TWEEN 20, and an individual serum sample (0.75 μL sample diluted with BSA diluent to final volume of 200 μL) was added to each well. The plate was then incubated for one hour at room temperature. After five washings with PBS/0.05% TWEEN 20, 100 μL of peroxidase labeled anti-raccoon conjugate (diluted to 1:3000 in BSA diluent) was dispensed into each well. The plate was incubated for one hour at room temperature and washed five times with PBS/0.05% TWEEN 20. One hundred microliters of TMP 2-component substrate (KPL) (1:1 ratio) was added to each well. Final incubation occurred at room temperature for six minutes. The reaction was stopped using 100 μL 2N sulfuric acid. The optical density was read at 450 nm (Molecular Devices vMax plate reader, Sunnyvale, CA). All washings were followed by paper towel blotting of the plate. Room temperature incubations occurred on a plate shaker (LabLine Instruments, Melrose Park, IL) at 150 RPM. Positive control serum came from a raccoon with a known *B. burgdorferi* infection and high antibody titers to the bacterium. Negative controls were phosphate buffered saline. All samples were also tested with Canine SNAP® 4Dx® cassettes as directed by the manufacturer for dogs.

**RESULTS**

Including recaptured animals, 46 serum samples (41 from raccoons and five from an American marten) from 34 individual mesocarnivores (33 raccoons, one American marten) were tested for antibodies to the tick-borne pathogens *B. burgdorferi*,
A. phagocytophilum, and E. canis. Antibodies to any of the bacteria were not detected in any of the samples.

DISCUSSION

All serum samples tested during this study were obtained from two sources; numerous individual raccoons and a single American marten captured multiple times throughout the summer. The ability of raccoons to develop antibodies to B. burgdorferi, to transmit the bacterium to naïve ticks (Norris et al., 1996), and even to serve as host for I. scapularis has been questioned (Craig et al., 1996). Despite this, studies have reported raccoons to be infested with the tick and seropositive for antibodies to B. burgdorferi (Hamer et al., 2010; Ouellette et al., 1997; Magnarelli et al., 1991) and other pathogens for which the tick serves as vector (Dugan et al., 2005; Yabsley et al., 2008). In addition, Fish and Daniels (1990) found a mean abundance of 136 larval I. scapularis on raccoons, compared to a mean abundance of eight larvae on white-footed mice. Fourteen percent of ticks removed from raccoons as larvae and tested for B. burgdorferi after they molted into nymphs were positive for the bacterium.

Studies performed in the eastern and midwestern United States have demonstrated that I. scapularis and/or local populations of mammalian hosts are usually infected with B. burgdorferi, even when the populations of the tick are small or undetectable (Callister et al., 1991). Wildlife (e.g., WTD) positive for antibodies to B. burgdorferi in areas without established I. scapularis populations can also be indirect evidence of a very low number of the tick in an area, or the presence of established populations in adjacent locales (Gill et al., 1994).
Antibodies to *B. burgdorferi* were not detected in any serum sample tested during this study, including samples that were collected from the same animals recaptured on multiple dates throughout the summer. Included in these recaptures were a raccoon processed four times between May 13 and July 1, a second raccoon processed four times between June 11 and August 10, and an American marten processed five times between May 29 and August 13.

Raccoons in rural settings have been shown to travel approximately 700 m/hr (Hodges et al., 2000) and remain active in a home range of 50-300 ha (Kurta, 2005) for nine hrs/day (Turkowski and Mech, 1968). Male American martens have been shown to travel 600-900 m/day in a home range of 1 km² (Kurta, 2005). If the recaptured animals listed above display similar movements, these three animals would have collectively traveled approximately 700 km throughout areas of PRNL that previously produced *I. scapularis*, without being exposed to *B. burgdorferi*. In addition, antibodies to the bacterium in numerous mammal species (including raccoons) have been shown to develop in approximately 4-8 weeks post exposure. The current study spanned from May to August 2008, sufficient time for detectable antibodies to develop in animals exposed in May, June, and possibly July.

*Ixodes scapularis* collected from PRNL in 2002 and 2005 were recovered from fishers. No fishers were captured or tested for antibodies to *B. burgdorferi* during this study. However, *I. scapularis* are host generalists, and serum from individuals representing two mammalian species that could serve as host for the tick were tested. Antibodies to the bacterium were not detected in any of the serum samples. In addition, the single American marten and the majority of raccoons tested were adults at the onset
of this study in April 2008. Antibodies to *B. burgdorferi* have been shown to persist for months in dogs (Appel et al., 1993; Straubinger et al., 1997) and deer (Magnarelli et al., 2010). If this is also true in raccoons and American marten, exposures to the bacterium in 2007 would have been detectable in spring/summer of 2008.

Improvements to future studies investigating the presence of *I. scapularis* and *B. burgdorferi* in PRNL might benefit from adjustments in study design. White-tailed deer in Michigan’s Upper Peninsula are readily harvested by hunters in the Munising area. Due to the documented longevity of antibodies to *B. burgdorferi* in this species, serosurveys of deer harvested by hunters in autumn would be effective in detecting local *I. scapularis* populations, regardless of the time of year the deer were infected with the bacterium (Magnarelli et al., 2010).

With hunter cooperation, collection of blood from harvested WTD would be less time consuming and more cost effective than the extensive labor and travelling necessary for live-trapping. Screening these deer would also eliminate the need to capture a single target host species (in this case, fishers) that likely have low populations in the area.

The immune response to *B. burgdorferi* is well described in WTD, and the ability of the IDEXX® C6-based Canine SNAP®4Dx® test to accurately detect the presence of antibodies to *B. burgdorferi* in WTD has been validated (Murdock et al., 2009). These two facts would eliminate the question of host response that has been raised with raccoons and allow for the detection of antibodies to *B. burgdorferi* with virtually no laboratory investment other than the purchase of the SNAP® tests themselves.

Screening mammalian serum for antibodies to tick-borne disease agents is a sensitive method for detecting small populations of vector ticks. Testing for antibodies
against *B. burgdorferi* in a larger number of serum samples collected in PRNL over multiple years would increase the likelihood of indirectly detecting a very low number of *I. scapularis* in the area.

**SUMMARY AND CONCLUSION**

Antibodies to *B. burgdorferi* were not detected in animals live-trapped in PRNL during this study. Despite its limitations (e.g., the capture of mostly raccoons), this study used a sensitive method for indirectly detecting *I. scapularis* and found no evidence of its presence. The lack of antibodies to *B. burgdorferi* in local fauna of PRNL supports the suggestion that populations of *I. scapularis* are not established in the area, and collection of individuals of this species during the summers of 2002 and 2005 were likely isolated introductions, followed by local extinctions.


TABLE I. Number of flagging events and distance flagged for each two week period from May 11-August 16, 2008.

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of flagging events</th>
<th>Total distance flagged (m)</th>
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<tr>
<td>May 11-May 24</td>
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</tr>
<tr>
<td>May 25-June 7</td>
<td>6</td>
<td>1500</td>
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<tr>
<td>June 8-June 21</td>
<td>5</td>
<td>1100</td>
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<tr>
<td>June 22-July 5</td>
<td>5</td>
<td>1200</td>
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<tr>
<td>July 6-July 19</td>
<td>5</td>
<td>1500</td>
</tr>
<tr>
<td>July 20-Aug 2</td>
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<td>1200</td>
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<tr>
<td>Aug 3-Aug 16</td>
<td>5</td>
<td>1000</td>
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TABLE II. Ticks collected during 1086 trap nights for mesocarnivores (raccoons and American marten) and 513 trap nights for small mammals (eastern chipmunk, *Peromyscus* spp. mice, jumping mice, and shrews) during summer 2008. Abbreviations: "L" = larva(e); "N" = nymph(s); "F" = female(s); "M" = male(s); "-" = 0.

<table>
<thead>
<tr>
<th></th>
<th><em>Ixodes</em></th>
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<th>Dermacentor</th>
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<td></td>
<td><em>gregsoni</em></td>
<td><em>texus</em></td>
<td><em>cookei</em></td>
<td><em>marxi</em></td>
<td><em>kingi</em></td>
<td>spp.</td>
<td><em>variabilis</em></td>
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<td><em>Peromyscus</em> spp.</td>
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<tr>
<td>Jumping mice</td>
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Figure 1. Polygons representing borders of movement of three fishers infested with deer ticks in Pictured Rocks National Lakeshore. Each color represents a different fisher.
Figure 2. Study site based on movements of three deer tick infested fishers in Pictured Rocks National Lakeshore. Each "+" indicates a sampling area where search effort was concentrated.
Figure 3. Transfer of American marten to immobilization sleeve for administration of anesthetic.
Figure 4. American marten wedged in wire cylinder of immobilization sleeve.
Figure 5. Anesthesia chamber used for inhalation administration of isoflurane to small mammals.
Figure 6. Questing activity of adult *Dermacentor variabilis* in Pictured Rocks National Lakeshore during spring/summer 2008.
Figure 7. Abundance of *Dermacentor variabilis* infesting raccoons.
Figure 8. Electrophoretic band pattern of DNA from the ITS-2 of ribosomal DNA from three *Ixodes gregsoni* ticks following restriction enzyme digest. Lane assignment is (from left to right): Lane 1 - 1 kb ladder, Lane 2 - digested *I. gregsoni* DNA, Lanes 3 and 4 - undigested *I. gregsoni* DNA, Lane 5 - digested *I. gregsoni* DNA, Lane 6 - 1 kb ladder, Lane 7 - digested *I. gregsoni* DNA, Lane 8 - undigested *I. gregsoni* DNA, Lane 9 - digested *Ixodes scapularis* DNA, Lane 10 - undigested *I. scapularis* DNA.
Figure 9. Mean abundance of adult and nymphal *Ixodes gregsoni* infesting American marten.