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Ticks And Tick-Borne Pathogens In North Dakota

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TICKS AND TICK-BORNE PATHOGENS IN NORTH DAKOTA

by

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This thesis, submitted by Nathan M. Russart in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

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This thesis meets the standards for appearance, conforms to the style and format requirements of the Graduate School of the University of North Dakota, and is hereby approved.

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Title	Ticks and Tick-borne Pathogens in North Dakota
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ABSTRACT

In 2010 a statewide survey of ticks and tick-borne pathogens was conducted in North Dakota. Ticks were collected from the four eco-regions in the state by flagging for questing adults and by collecting feeding immature ticks from trapped small mammals. I collected 1762 individual ticks representing five species: *Dermacentor variabilis* (1449), *Ixodes scapularis* (307), *Ixodes woodi* (3), *Ixodes angustus* (2), and *Amblyomma americanum* (1). *Dermacentor variabilis* were collected in all areas of the state while *I. scapularis* were restricted to the northeast portion of the state. This provided sufficient evidence that *I. scapularis* have established populations within the state. *Ixodes scapularis* were tested for *Borrelia burgdorferi* (the agent of Lyme disease), *Anaplasma phagocytophilum* (agent of human granulocytic anaplasmosis), and *Babesia microti* (agent of human babesiosis). Of those tested, *A. phagocytophilum* was detected in 8.5%, *B. burgdorferi* was detected in 3.3%, and *B. microti* was not detected. These are the first reports of *A. phagocytophilum* and *B. burgdorferi* detected in the wild in North Dakota and provide evidence of westward range expansion of these organisms.

To determine the areas that the ticks were moving into, a study was conducted in Grand Forks County, ND in 2012 to determine the effects of forest patch size on the abundance of adult questing and immature host-feeding *I. scapularis* and the

prevalence of *A. phagocytophilum, B. burgdorferi,* and *B. microti* in those ticks. Increased forest patch size was significantly correlated with increased abundance of adult questing ticks and larval ticks collected from small mammals. Few *I. scapularis* were collected from the four smallest sites limiting the ability of pathogens to become established. Among the two largest sites there was not a significant difference in the prevalence of *B. burgdorferi* or *A. phagocytophilum* detected in questing adults or xeno-positive small mammals.

CHAPTER I

INTRODUCTION

Vector-borne pathogens are responsible for some of the most common and most debilitating diseases worldwide. From malaria in the tropics to West Nile Virus in the United States, humans across the globe are at risk of contracting vector-borne diseases (Gubler, 1991). These diseases range from acute infections caused by viruses and bacteria to chronic conditions caused by nematodes and protozoans (Gubler, 1991). In order to manage these pathogens, reduce the risk of human infection, and properly diagnose and treat patients that have acquired these pathogens we must fully understand their sometimes very complex lifecycles.

A vector is an organism, often an arthropod, which actively seeks out vertebrate hosts to feed on and may transmit an infectious agent from an infected vertebrate to an uninfected individual. Ticks are hematophagous ectoparasites that due to their unique extracellular digestion are able to serve as vector for a wide variety of pathogens including bacteria, viruses, protozoa, and nematodes (Jongejan & Uilenberg, 2004). This makes them especially important medical arthropods. Ticks, along with mites, make up the subclass Acari in the class Arachnida. Three families of ticks, including almost 900 species, comprise the order Ixodida: Ixodidae, the hard ticks; Argasidae, the soft ticks;

and Nuttalliellidae, which is comprised of a single species that has characteristics of both the hard and soft ticks (Nava et al., 2009). In this study, I focused on hard ticks because they are the primary carrier of human pathogens and are most prevalent in the study area. Certain aspects of the lifecycle of the hard tick make it an ideal vector.

The hard tick's lifecycle contains three stages: the larva, nymph, and adult. As a larva and nymph, the tick will take one bloodmeal then molt and emerge in the next lifestage (Sonenshine, 1991). As an adult, the tick will mate, take a bloodmeal, lay eggs (if female), then die. Most commonly the tick will feed on a different individual for each bloodmeal; however, some species remain on the host for two or all three bloodmeals. While many species of ticks are specialists on single or few species of hosts, those responsible for pathogens in humans are less specific in their host preference. In fact, they will often feed on different hosts specific to their lifestages, feeding on smaller hosts as larva and nymphs and larger hosts as adults (Sonenshine, 1991). This means that a tick feeding on a human has likely already fed on another animal and enhances the risk to humans of contracting zoonotic pathogens, or pathogens that originate in animals.

In the United States, hard ticks are vectors for the most common vector-borne disease, Lyme disease, as well as the notifiable diseases Ehrlichiosis, Anaplasmosis, spotted fever rickettsiosis, and Tularemia (Centers for Disease Control and Prevention, 2012). While vector-borne diseases are the greatest risk associated with ticks, they pose other threats as well. Large infestations on wildlife or livestock can lead to anemia, loss of fur through self-pruning, and malnutrition (Samuel & Welch, 1991). Tick paralysis is a

condition in which a tick releases a neurotoxin into it host. Although this rare condition is usually cured shortly after removal of the tick, it can lead to death if undiagnosed (Greenstein, 2002).

In order to assess the risk of tick-borne diseases to humans and livestock, it is necessary to have an understanding of: which tick species are present in the area under question, and pathogens present within the ticks. Tick-borne pathogens are often undiagnosed or misdiagnosed and reports of confirmed cases can be low (Parola & Raoult, 2001). Therefore, it is important to be aware of the presence of tick species that act as vectors of disease. The goal of this project was to develop a better understanding of the tick fauna in an area with little information and determine the presence of pathogens associated with those ticks.

CHAPTER II

A SURVEY OF TICKS AND TICK-BORNE PATHOGENS IN NORTH DAKOTA

INTRODUCTION

While ticks have been heavily studied throughout much of the United States, there is little concrete evidence of the tick fauna residing within North Dakota. A survey of ectoparasites of small mammals in Grand Forks County conducted in the mid 70's discovered only *Dermacentor variabilis* (Fellows, 1978). In fact, it has been generally accepted that *Ixodes scapularis* does not reside in the state. The state extension service published an AgAlert in 2010 stating this (Swenson, 2010). However, *Ixodes scapularis*, the vector of Lyme disease, is endemic to neighboring Minnesota (Drew et al., 1988; Sanders & Guilfoile, 2000) and evidence has suggested that the range of the black-legged tick has increased in other parts of the United States in recent years (Hamer et al., 2010). Starting around 2007, Grand Forks veterinarians began to collect *Ixodes scapularis* from dogs reported to not have left the state (Jefferson Vaughan, personal communication). The combination of these factors indicated that it was time to conduct a survey of ticks and tick-borne pathogens in the state.

Two objectives were outlined for this study. First, to determine the geographic range of tick species residing within the state of North Dakota with special emphasis on

I. scapularis and *D. variabilis*, the main vectors of zoonotic pathogens. The tick, serving as a vector, is a crucial component of pathogen lifecycles and presence of certain tick species will allow to us determine the potential of an area to sustain circulating pathogen populations. And secondly, to determine the prevalence of *B. burgdorferi*, *Anaplasma phagocytophilum,* and *Babesia microti* within any *I. scapularis* captured. This information will give us an accurate understanding of which tick species and which pathogens reside in the state and can be used to better diagnose diseases for persons that have not traveled outside of the state.

METHODS

Tick Collection

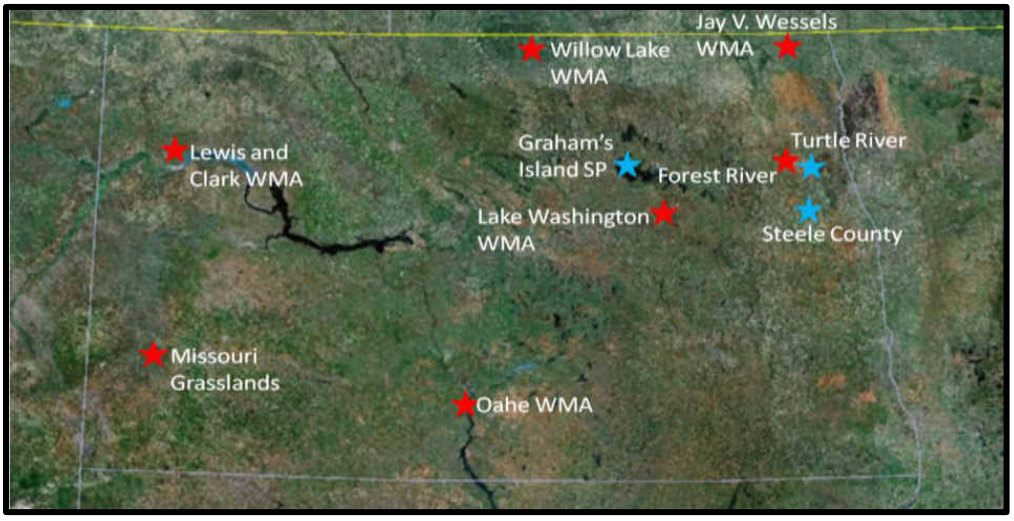
Three methods of collecting ticks were utilized: flagging, collection of feeding ticks from small mammals, and passive surveillance. Flagging, also termed dragging, consists of pulling a white cloth through the vegetation and is a widely used sampling method in the literature (Ginsberg & Ewing, 1989). Questing ticks, those that are seeking a host, attach to the cloth as it passes by and are collected and placed in a vial with ethanol. Specifically, I used a 27” x 36” crib sheet composed of a durable vinyl lining covered with polyester providing a surface the ticks can easily cling to. The flag was inspected every 10-20 meters and any ticks on the cloth, as well as any found on the individual sampling, were placed in vials containing 70% ethanol using forceps. Date and location of sample were recorded.

The second method targeted larval and nymphal feeding ticks attached to host animals. Manpower constraints limited host collection to small mammals. Small mammals serve as hosts for immature lifestages that are not easily collected by flagging (Ginsberg & Ewing, 1989). Preliminary trapping was conducted using both Sherman live-traps and lethal snap-traps. Use of snap-traps was discontinued after discovering that larval and nymphal ticks detached themselves from deceased rodents. Traps were

arrayed in a grid with 25 meter intervals between traps. When possible, trapping areas covered both forested and non-agricultural field-type habitats in an effort to increase the host species diversity. Traps were set in the evening and baited with rolled oats and supplied with cotton for bedding. Traps were recovered in the morning and animals were collected and anesthetized by placing them in a bag containing a cottonball treated with isoflurane (Isothesia^{TM}, Butler Schein, Dublin, OH) as approved by the American Veterinary Medical Association and the UND Institutional Animal Care and Use Committee (#1005-1). Ticks were then collected from the animal and placed in a vial containing 70% ethanol. Vials were labeled with date, location, and species of mammal. Once fully recovered, animals were released into the area in which they were captured.

Lastly, a number of ticks were also supplied by passive surveillance, in which individuals other than the investigators collected ticks while conducting other activities and delivered them to the lab. These ticks were collected by other individuals in the Biology Department at the University of North Dakota who were aware of the study.

Figure 1. Locations of sample sites in North Dakota in 2010. Blue stars indicate areas that were sampled by flagging only while red stars indicated sites that were sampled by flagging and small mammal trapping.



Study Sites and Sampling Intervals

North Dakota can be divided into four Level III ecoregions (Bryce et al., 1996). Several study sites were identified in each of these ecoregions and one Level IV ecoregion (five total ecoregions) resulting in ten study sites that were actively sampled.

1. The *Lake Agassiz Plain* (i.e., Red River Valley) is a flat, ancient lakebed that runs along the eastern border of North Dakota. The historical tallgrass prairie has been largely replaced by intensive agriculture. Four sites were sampled in the northern portion of the Lake Agassiz Plain: The University of North Dakota's Forest River Biological Station located in the northwest corner of Grand Forks County, Turtle River State Park, a site in Steele County along the Goose River, and Jay V. Wessels Wildlife Management Area (WMA) situated 10 miles southeast of Walhalla in Pembina County.

2. The *Northern Glaciated Plains* lie west of the Lake Agassiz Plain and are characterized by rolling hills with countless wetland depressions. This study included one site on Lake Washington WMA in Eddy County and on Graham's Island State Park.

3. The *Turtle Mountains* is a Level IV ecoregion within the Northern Glaciated Plains and is a small but unique region in the north central part of the state that is characterized by an undulating landscape, abundant wetlands, and a forest cover of aspen, birch, elm, oak, and ash. One site on Willow Lake in Rolette County was sampled for this study.

4. The *Northwestern Glaciated Plains* occupies the area to the Northeast of the Missouri River and marks a transition from intensive agricultural land-use to the east to a predominance of ranching to the west. Two sites on the edge of the Northwest Glaciated Plains and the Northwestern Great Plains were sampled: one south of

Williston in the Lewis and Clark Wildlife Management Area in McKenzie County and one south of Bismarck in the Oahe Wildlife Management Area of Morton County.

5. The *Northwestern Great Plains,* and more specifically, the *Little Missouri Badlands* in western North Dakota lie along the Little Missouri River and are characterized by sparsely wooded ridges, bluffs, buttes, and pinnacles. One site on the North Dakota State Land Department's property near the southern unit of Theodore Roosevelt National Park in Billings County was sampled.

Most of these sites were sampled twice during the summer of 2010, once in June and once in July. Travel funds limited additional sampling; however sites nearer to Grand Forks were sampled more frequently. North Dakota's Department of Game and Fish's wildlife management areas or other public lands were selected in each of these ecoregions as our specific sampling sites (Figure 1).

Table 1. Sampling effort at each study site in a 2010 statewide survey of ticks in North Dakota.

Site	Flagging Effort (Min)	Trapping Periods	Trap Nights
Camp Grafton South	Passive Surveillance	0	0
Forest River	429	2	71
Graham's Island State Park	317	0	0
Icelandic State Park	Passive Surveillance	0	0
Jay V. Wessels WMA	160	2	61
Kelly's Slough	Passive Surveillance	0	0
Lake Tobiason	Passive Surveillance	0	0
Lake Washington WMA	170	2	61
Lewis and Clark WMA	242	1	36
Magpie Campground	262	2	81
Oahe WMA	330	2	81
Steele County	58	0	0
Turtle River State Park	382	0	0
Upham	Passive Surveillance	0	0
UND Campus	Passive Surveillance	0	0
Willow Lake WMA	340	2	82

Sample Processing

All ticks were returned to the lab in 70% ethanol where they were identified to species, sex, and lifestage (Clifford et al., 1961; Durden & Keirans, 1996;(Keirans & Litwak, 1989; Keirans & Clifford, 1978). Adults, nymphs, and engorged larvae were bisected sagitally and DNA was extracted using guanidine thiocyanate (Tkach & Pawlowski, 1999). The PCR for *Borrelia burgdorferi* and *Anaplasma phagocytophilum* was performed in 50 µl of solution containing 5µl of DNA extraction solution, 37µl ultrapure water, 0.8 µl of 10µmol/L forward and reverse pathogen-specific primers (Table 1), 0.8 µl of 10mM dNTP, 5µl 10x reaction buffer (BIOER), and 0.25µl of 5u/µl BioReady rTaq polymerase (BIOER). The PCR program for *A. phagocytophilum* consisted of initial denaturation for 4-min at 94°C followed by 40 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 1-min. For *B. burgdorferi,* 30 cycles of 94°C for 1-min, 37°C for 2-min, and 72°C for 3-min was followed by a final 7-min elongation at 72°C. Products were visualized on a 1.5% agarose gel stained with ethidium bromide in a 0.5X Tris-borate-EDTA buffer.

Real-time PCR was used to detect *Babesia microti*. 2.5µl of DNA extraction solution was combined with 7.5µl iTaq Universal SYBR Green Supermix (Bio-Rad), 1µl of 10µmol/L primers, and 3µl ultrapure water. After an initial denaturation step for 30s at 95°C, the sample was subjected to 40 cycles of denaturation at 95°C for 5s, and annealing/extension at 68°C for 25s. A melting curve analysis was conducted from 72°C to 90°C increasing the temperature 0.5°C every 3s. Melting points for positives were 81.5°C.

The DNA from PCR-positive samples were purified by combining 2µl of the PCR product with 5µl of ExoSap and heated to 80°C for 15-min then 37°C for 15-min. Sequencing reactions were performed using 2µl of the purified PCR product in a 10µl solution containing 1µl of BigDye Terminator ® v3.1, 2µl 5x buffer, 2µl of the forward primer, and 3µl ultrapure water in an Eppendorf Mastercycler®. Sequencing was conducted using an ABI PRISM® 3100 Genetic Analyzer. Sequences were edited using BioEdit Sequence Alignment Editor and compared to those in GenBank for the pathogens the primers specified.

Table 2. PCR primers used for pathogen detection.

Target Pathogen	Target Gene	Primer Names	Primer Sequences	Citation
Borrelia	flagellin	FL6	5'-TTCAGGGTCTCAAGCGTCTTGGACT-3'	Picken, 1992
		FL7	5'-GCATTTTCAATTTTAGCAAGTGAT-3'	
Anaplasma	p44	MSP3F	5'-CCAGCGTTTAGCAAGATAAGAG-3'	Zeidner et al., 2000
		MSP3R	5'-GCCCAGTAACAACATCATAAGC-3'	
Babesia	18S rDNA	smbaJF	5'-GCGTTCATAAAACGCAAGGAAGTGT-3'	Hersh et al., 2012
		smbaKR	5'-TGTAAGATTACCCGGACCCGACG-3'	

RESULTS

Flagging

A total of 1234 ticks were collected by flagging (87%) and passive surveillance (13%). A total of 42.3 hours was spent flagging resulting in the collection three tick species (Table 2). For *D. variabilis*, 885 (85%) were collected by flagging and the rest (157, 15%) were collected by passive surveillance. For *I. scapularis*, 185 (97%) were collected by flagging and the rest (6, 3%) were collected by passive surveillance.

The majority of the ticks collected by flagging were *Dermacentor variabilis* (84%). Only adult *D. variabilis* were collected by flagging. The remaining ticks consisted almost exclusively of *Ixodes scapularis* (15%). All three lifestages of *I. scapularis* were collected by flagging. All larval *I. scapularis* were collected during two sampling incidences at Graham's Island State Park in which the surveyor apparently encountered newly hatched egg masses. A single *Amblyomma americanum* adult was collected on a surveyor but the location of collection could not be determined.

Table 3. Species and lifestages of ticks collected by flagging and passive surveillance in North Dakota, 2010.

Species	*Dermacentor variabilis*	*Ixodes scapularis*	*Amblyomma americanum*	Total
Adult Male	456	22	0	478
Adult Female	586	16	1	603
Nymph	0	29	0	29
Larvae	0	124	0	124
TOTAL	**1042**	**191**	**1**	**1234**

Adult *D. variabilis* were collected from sample sites across the state except the Little Missouri Badlands. However, personal communication with park service employees indicated ticks were present in the area of our southwestern sample sites prior to our sampling. Abundances in the remainder of the state were greater in eastern sites and in the Turtle Mountains (Table 3). *Ixodes scapularis* were collected from three sites in the north-central Red River Valley (Turtle River, Forest River, and Steele County) and from Graham's Island State Park.

Table 4. Total number and species of ticks collected by flagging at each of 10 sample sites in North Dakota during 2010.

Site	County	*Eco-Region*	*D. variabilis*	*I. scapularis*
Forest River	Grand Forks	Lake Agassiz Plain	365	32
Turtle River SP	Grand Forks	Lake Agassiz Plain	217	8
Steele County	Steele	Lake Agassiz Plain	32	3
Jay V. Wessels WMA	Pembina	Lake Agassiz Plain	44	0
Lake Washington WMA	Eddy	Northern Glaciated Plains	89	0
Graham's Island SP	Ramsey	Northern Glaciated Plains	20	146
Willow Lake WMA	Rolette	Turtle Mountains	174	0
Oahe WMA	Morton	Northwestern Glaciated Plains	18	0
Lewis and Clark WMA	McKenzie	Northwestern Glaciated Plains	2	0
Little Missouri Grassland	Billings	Northwestern Great Plains	0	0
TOTAL			**961**	**189**

Small Mammal Trapping

Seventy-one small mammals representing nine species were caught over the course of 473 trap nights for a 15.01% trap success rate (Table 4). Sherman live-traps were used for 407 of the trap-nights; snap traps were used for 66 trap-nights. Because of difficulty in accurate species identification, *Peromyscus* were identified only to genus.

Table 5. Species and numbers of mammals captured by site in North Dakota, 2010.

	Trap Nights	*Success Rate (%)*	*Blarina brevicauda*	*Myodes gapperi*	*Glaucomys sabrinus*	*Microtus montanus*	*Microtus pennslyvanicus*	*Mus musculus*	*Peromyscus sp.*	*Tamias striatus*	*Zapus hudsonius*	Total
Forest River	71	15.5	1	2	1	0	0	0	6	0	1	11
Jay V. Wessels WMA	61	16.4	0	9	0	0	0	1	0	0	0	10
Lake Washington WMA	61	18.0	0	4	0	0	0	2	3	0	2	11
Lewis and Clark WMA	36	5.6	1	0	0	0	0	0	1	0	0	2
Little Missouri Grassland	81	9.9	0	0	0	1	0	0	7	0	0	8
Oahe WMA	81	21.0	2	0	0	0	2	2	11	0	0	17
Willow Lake WMA	82	14.6	1	8	0	0	1	0	1	1	0	12
TOTAL	**473**	**15.0**	**5**	**23**	**1**	**1**	**3**	**5**	**29**	**1**	**3**	**71**

The majority (73%) of small mammals trapped were either *Peromyscus* (41%) or *M*. *gapperi* (32%) (Table 4). There was a significant correlation between the number of trap-nights and the total number of mammals trapped at each site (Pearson's correlation, p=0.002), however there was no correlation between the number of trap-nights and the number of species trapped at each site (Pearson's correlation, p=0.30), nor was there a significant correlation between the total number of mammals trapped and the number of species trapped (Pearson's correlation, p=0.14). This suggests that species diversity may have been greater at some sites than at others. The greatest number of species trapped was at Forest River and Willow Lake (n=5). *Peromyscus* was the most widely distributed species and was collected at all sites except the Jay V. Wessels site. *Blarina brevicauda* and *M. gapperi* were the second most widely-

distributed species and were trapped in four of the seven sites, although only two sites in common.

We collected 528 ticks from small mammals comprising four species (Table 5). *Ixodes scapularis* and *D. variabilis* accounted for all but five of the ticks collected. All ticks collected from small mammals were immature lifestages with the exception of *I. woodi,* in which only adults were collected. All *I. angustus* and *I. woodi* were collected in the Turtle Mountains region of the state. *Dermacentor variabilis* was found on small mammals at all trapping locations except the northwestern most study site and average tick burden was greater in northeastern study sites. *Ixodes scapularis* was found only in the northeast portion of the state.

Table 6. Total ticks collected from small mammal hosts at each sample site in North Dakota, 2010. Numbers in parentheses indicated number of small mammals collected.

	D. variabilis		*I. scapularis*		*I. woodi*	*I. angustus*
Study Site	Larvae	Nymphs	Larvae	Nymphs	Adults	Nymphs
Forest River (11)	161	2	74	11	0	0
Jay V. Wessels WMA (10)	63	41	11	0	0	0
Willow Lake WMA (12)	48	50	0	4	3	2
Oahe WMA (17)	14	15	0	0	0	0
Lake Washington WMA (11)	8	1	14	2	0	0
Little Missouri NG (8)	2	2	0	0	0	0
Lewis and Clark WMA (2)	0	0	0	0	0	0
TOTAL	**296**	**111**	**99**	**17**	**3**	**2**

Only one trapping session was conducted at Lewis and Clark WMA. Trap success was low resulting in only two mammals collected at this site, limiting tick collection. Average tick burden differed among sites, but with low number of hosts examined and different host species composition at each site, there is not enough evidence to attribute the difference to ecoregion.

Dermacentor variabilis was collected from six mammal species and *I. scapularis* was collected from five host species (Table 7). All *I. angustus* and *I. woodi* were collected from *M. gapperi*. Both *I. angustus* were collected from a single rodent while each *I. woodi* were collected from separate rodents. *M. gapperi* harbored more larval *D. variabilis* (Q_1=1, MED=2, Q_3=4.5) than *Peromyscus* (Q_1=0, MED=0, Q_3=1) (W=511, p=0.0006) and more nymphal *D. variabilis* (Q_1=0, MED=2, Q_3=4) than *Peromyscus* (Q_1=0, MED=0, Q_3=0) (W=522, $p=4.42e^{-5}$). There was no difference in the *I. scapularis* burdens for larva or nymphs. No ticks were collected from *Blarina brevicauda, Microtus montanus,* or *Tamias striatus.*

Table 7. Species and lifestages of ticks collected from small mammal species in North Dakota, 2010.

	D. variabilis		*I. scapularis*		*I. angustus*	*I. woodi*
Host Species (n)	Larvae	Nymphs	Larvae	Nymphs	Nymphs	Adult
Peromyscus sp. (29)	37	17	51	6	0	0
Myodes gapperi (23)	236	80	23	6	2	3
Mus musculus (5)	3	12	3	1	0	0
Zapus hudsonius (3)	19	0	20	3	0	0
Microtus pennslyvanicus (3)	0	2	0	0	0	0
Glaucomys sabrinus (1)	1	0	2	1	0	0
Blarina brevicauda (5)	0	0	0	0	0	0
Microtus montanus (1)	0	0	0	0	0	0
Tamias striatus (1)	0	0	0	0	0	0
TOTAL	**296**	**111**	**99**	**17**	**2**	**3**

Pathogen Detection

All *Ixodes scapularis* collected by flagging and engorged individuals from small mammals were tested for pathogens. Testing only larvae from small mammals allowed us to xeno-diagnose the host for the pathogen. In total, 94 *I. scapularis* were tested for

Anaplasma phagocytophilum and 92 were tested for *Borrelia burgdorferi*. Due to repeated PCR tests, extracted DNA in 71 of the samples was expended and therefore, the DNA from only 23 ticks was available to test for *Babesia microti*. *Anaplasma phagocytophilum* was detected in eight ticks (8.5%) from two locations; Forest River (n=6) and Turtle River (n=2). Six of those testing positive were adult questing ticks. Of the remaining two, both were nymphs attached to rodents; one on *Zapus hudsonius* and one on *Myodes gapperi,* both from the Forest River study site. *Borrelia burgdorferi* was detected in three ticks (3.3%); two questing adults from the Turtle River study site and a nymph collected from a *Z. hudsonius.* One nymph was positive for both *A. phagocytophilum* and *B. burgdorferi.* None of the samples were positive for *B. microti.*

DISCUSSION

Although it is common knowledge among sportsmen and outdoor enthusiasts that ticks are abundant in North Dakota, this research represents the first systematic survey for ticks in North Dakota. Indeed, I present here the first documented cases of several tick species and pathogens.

Ixodes angustus has been shown to be a competent *B. burgdorferi* vector in a laboratory setting (Peavey et al., 2000) and feeds mainly on small rodents and sometimes humans (Easton & Goulding, 1974). *Ixodes woodi* is primarily a parasite of wood rats (*Neotoma* spp.) but have been collected from other rodents (Keirans & Clifford, 1978). This is the first known collection of both of these species in North Dakota and the first collection of *I. woodi* on *M. gapperi*. These species rarely parasitize man and are of little medical importance.

The collection of a single *Amblyomma americanum* was unexpected. This tick species is an established tick in the southern United States. It has been reported on the east coast north to Maine (Keirans & Lacombe, 1998), west to Iowa and central Texas (Centers for Disease Control and Prevention, 2011). A single specimen is not sufficient to determine establishment of the species in North Dakota. *Amblyomma americanum* are generalists whose hosts include birds (Allan et al., 2010). In the case of migratory birds, it is possible for a tick to attach in an *A. americanum* endemic area and detach a long

distance away in unsuitable habitat. Anthropogenic movement may also introduce ticks into new areas where they may or may not establish populations.

Of greatest significance was the discovery of *I. scapularis* as well as the associated pathogens *B. burgdorferi* and *A. phagocytophilum.* This is the first documented report of these species naturally occurring in North Dakota and is supporting evidence that the range of *I. scapularis* is expanding (Hamer et al., 2010). The collection of all lifestages of *I. scapularis* from multiple study areas spanning a large geographic area provides sufficient evidence that the tick has become established in North Dakota. In order for a vector-borne pathogen to sustain itself in an environment, it is necessary to have both the vector and a reservoir host. *Peromyscus maniculatis* and *P. leucopus*, both known reservoirs of *B. burgdorferi* (Peavey & Lane, 1995; Anderson, 1989) are both present in North Dakota and were among the most commonly collected small mammal species in this study. These host species are also competent reservoirs of *A. phagocytophilum* (Walls et al., 1997; Rejmanek et al., 2011). The presence of the vector along with competent reservoirs provides the elements necessary to sustain these pathogens in North Dakota. Detection of both of these pathogens in multiple lifestages of field-collected *I. scapularis* indicates that *B. burgdorferi* and *A. phagocytophilum* have become established in certain areas of the state. Although *Babesia microti* was not detected in the small sample of ticks sampled in 2010, there is not sufficient evidence to declare its absence from the state. *Peromyscus* is also a reservoir for *B. microti* (Speilman et al., 1981) and with the presence of both a suitable

host and vector it may already occur in areas of North Dakota but escaped detection or may populate the area in the future.

Although the range of *I. scapularis* is currently expanding, I believe it is unlikely that it will continue to move west. *I. scapularis* presence is associated with forested areas (Guerra et al., 2002). As one moves west from Minnesota into North Dakota the landscape transitions from a densely forested area to the Great Plains. As such, forested areas dwindle, hanging on along river bottoms, then altogether disappear. There is very little suitable habitat in which *I. scapularis* could survive and western invasion of the tick will be halted. Nonetheless, medical practitioners must be made aware of the presence of *I. scapularis, B. burgdorferi,* and *A. phagocytophilum* in North Dakota and be familiar with their symptoms. With this knowledge, they will include these diseases in routine tests resulting in properly diagnosed patients and healthier North Dakotans.

CHAPTER III

EFFECT OF FOREST PATCH SIZE ON ABUNDANCE OF TICKS IN GRAND FORKS COUNTY, ND

INTRODUCTION

Results of my 2010 statewide survey demonstrated that *Ixodes scapularis* had expanded its range into eastern North Dakota (see Chapter II). Because of the medical importance of this tick species, I wanted to examine what areas of North Dakota were being colonized and develop some hypotheses as to why this was. To do this, I can compare the distribution of *I. scapularis* (i.e., a species first colonizing North Dakota) with that of *Dermacentor variabilis* (i.e., a species endemic to North Dakota) across Grand Forks County in forested patch areas of varying sizes.

Forested patches were examined because *I. scapularis* displays an affinity for forested areas (Ginsberg & Ewing, 1989). Immature *Dermacentor variabilis* also are found in greater abundances in forested areas, presumable because of higher moisture content in these areas (Sonenshine & Stout, 1968). The agricultural land surrounding the forest patches has the additional hazard of plowing that may expose ticks to the elements.

Examining forested areas of varying sizes may provide insight into what areas are first being colonized by *I. scapularis.* The forested patches examined in this study may

act as islands in a sea of unsuitable agricultural habitat. It has been widely discussed in the literature that colonization rates of species increase with increased island size (e.g., Lomolino, 1990; Connor & McCoy, 1979). This target area hypothesis, as it is called, theorizes that there is a greater chance of transient individuals encountering a large area as opposed to a small one. Once encountered, large areas are more likely to have sufficient resources to sustain a population of the colonizing species. The propensity of large islands to have additional resources also makes them more resistant to extinction (MacArthur & Wilson, 1967). In the case of *I. scapularis* invading North Dakota, we would expect to see large islands more frequently colonized by and sustaining populations of the tick.

I can further explore the effects of forest patch size on the abundance of ticks. Allan et al. (2003) found questing *I. scapularis* nymph density increased with decreasing forested patch size. This result was attributed to a greater density of *Peromyscus leucopus* in smaller patches (Nupp & Swihart, 1996). Because of the importance and efficiency of *P. leucopus* as a reservoir of *Borrelia burgdorferi* (Mather et al., 1989), an increased density of *P. leucopus* relative to other host species can lead to higher infection prevalences in ticks in what Ostfeld and Keesing (2000) call the dilution effect.

My objective for this study was to determine if the distribution of *I. scapularis* and *D. variabilis* was driven by the size of forest patches in eastern North Dakota. Tick abundance may also be influenced by abundance of small mammals (Schmidt et al., 1999; Ostfeld et al., 2001). Therefore, to attribute differences in tick abundance to patch size, it was also necessary to determine differences in host abundance.

METHODS

Study Sites and Sampling Intervals

Six forested study sites of varying sizes were identified in Grand Forks County ranging from 7 to 349 hectares (Table 1, Figure 1). Area for study sites was determined using aerial imagery on ACME labs Google Planimeter (Poskanzer, 2000). These study sites were surrounded by farmland, effectively creating the islands necessary to explore the objectives. Each site was sampled once a week from late May through mid August 2012. Flagging and small mammal trapping techniques were used as described in Chapter I with some exceptions. Traps were located only in forested areas to target suitable *I. scapularis* habitat. Traps were spaced at 10 meter intervals instead of 25 meters for ease of sampling. Each sampling session consisted of approximately 25 traps. Traps were arrayed in a grid starting near the forest edge.

Flagging was conducted for one hour at each site. At sites that consistently produced few to no ticks, flagging time was reduced to one half hour. This was done so that more effort could be directed towards sites that were producing a large number of ticks, increasing the number of samples for pathogen detection. Time was used as a measure of sampling effort to determine relative abundance of ticks because of the

difficulty in sampling a fixed-size area in forested habitats and difficulty in measuring long transects in the large areas sampled.

Table 8. Size of six study sites that were surveyed to determine effects of forest patch size on tick abundance and on the prevalence of *Borrelia burgdorferi, Anaplasma phagocytophilum,* and *Babesia microti* pathogens within *I. scapularis* ticks in Grand Forks Co., ND, 2012.

Site	Size (Hectares)
Oslo	7
Manvel	31
Northwood	103
Janice	204
Turtle River	254
Forest River	349

Figure 2. Location of the six study sites that were surveyed to determine effects of forest patch size on tick abundance and on the prevalence of *Borrelia burgdorferi, Anaplasma phagocytophilum,* and *Babesia microti* pathogens within *I. scapularis* ticks in Grand Forks County, ND, 2012.

Data Analysis

Count data were tested for normality by plotting histograms and found not normally distributed. Logarithmic transformation did not normalize count data. Therefore non-parametric Mann-Whitney tests were used to compare count data among sites. Proportional data were analyzed by Chi square or Fisher's exact tests when expected frequencies were less than five. Linear regressions were used to analyze relationships between patch size (independent variable) and tick abundance (dependent variable). Statistical analyses were performed using the software, R (R Core Team, 2012).

Questing Ticks. To determine the effect of patch size on the abundance of questing ticks, data points were plotted using patch size as the explanatory variable and ticks collected per hour for each sampling period as the response variable. A linear regression was fitted to the data. Beginning with a first-order model, higher order terms were added until the model reached significance.

Small Mammals. Differences among sites were compared for overall small mammal abundance (the number of mammals collected per trap-night) and composition of *Peromyscus* and *Myodes gapperi* using Pearson's chi-squared test of independence. Within sites, binomial tests were used to determine if abundance of *Peromyscus* and *M. gapperi* differed.

Tick Infestation on Small Mammals. Two measures of tick burden were used to determine differences in host tick burden and effects of increasing patch size on tick abundance: infestation prevalence, and infestation intensity. Infestation prevalence is

the proportion of hosts harboring ticks. Infestation intensity calculates the average number of ticks per infested host. Measures of infestation prevalence included hosts that were infested with either larval or nymphal ticks. The overall number of nymphs collected during this study was much lower than that of larvae (i.e., less than 1/10), so only larval ticks were included in infestation intensity calculations. Infestation intensity data was not normally distributed so data were transformed ($\log_{10}+1$) prior to statistical analysis. A linear regression model was developed to examine the effects of increasing patch size on infestation intensity.

Host Utilization by Immature Ticks. For comparison of host utilization by each tick species, only data from the Forest River study site was used. This site had the highest abundance of both tick species allowing for more robust results. However, this limits any conclusions drawn to this one site as well. Infestation prevalence and intensity were examined for *Peromyscus* and *M. gapperi.* Infestation prevalence was examined using Pearson's Chi-squared test. Infestation prevalence was examined using Mann-Whitney tests.

Temporal Dynamics. All figures of temporal abundance were created using data from only the Forest River study site. Ticks collected by flagging and from small mammals were grouped by lifestage and the week they were collected. All flagging events during each one week period were averaged to determine a relative abundance for each week. The number of larvae and nymphs collected from all small mammals during a given week were divided by the number of hosts examined that week to determine average ticks per host. To visualize temporal trends more easily, data for

each lifestage were normalized. This was accomplished by multiplying the greatest abundance observation for each lifestage by a constant so that it would equal 100. The rest of the data points were then multiplied by their lifestage specific constant and plotted by week.

RESULTS

Abundance of Immature Ticks on Small Mammals

A total of 749 immature *I. scapularis* were collected from small mammals (Table 9). Over 97% of the ticks were collected from the Turtle River (55% of total) and Forest River study sites (42% of total); the two largest study areas. Similar numbers of mammals were trapped at the four smallest sites (Table 9) but tick infestations on small mammals at these sites were sparse. No *I. scapularis* were collected from small mammals at the smallest site (Oslo) and less than 10 ticks each were collected off small mammals at the Manvel, Northwood and Janice sites.

A total of 999 immature *D. variabilis* were collected off of hosts (Table 9). Larvae made up 79% of *D. variabilis* collected while nymphs accounted for the rest of the ticks collected from hosts. The majority of this species were collected at the Forest River study site (56.3%), however *D. variabilis* had a greater distribution over the sites than *I. scapularis*: Oslo (3.2%), Manvel (5.9%), Northwood (17.1%), Janice (3.8%), and Turtle River (13.7%).

Table 9. Total (average) immature ticks collected from small mammals in forest patches of varying sizes in Grand Forks County, ND, 2012.

	Size (Hectares)	Mammals Trapped	*Dermacentor variabilis*		*Ixodes scapularis*	
			Larvae	Nymphs	Larvae	Nymphs
Oslo	7	57	24 (0.42)	8 (0.14)	0 (0.00)	0 (0.00)
Manvel	31	60	55 (0.92)	4 (0.07)	2 (0.03)	6 (0.10)
Northwood	103	48	154 (3.21)	17 (0.35)	2 (0.04)	0 (0.00)
Janice	204	37	34 (0.92)	4 (0.11)	9 (0.24)	0 (0.00)
Turtle River	254	81	107 (1.32)	30 (0.37)	397 (4.90)	19 (0.23)
Forest River	349	69	419 (6.07)	143 (2.07)	285 (4.13)	29 (0.42)
TOTAL		**352**	**793 (2.25)**	**206 (0.59)**	**695 (1.97)**	**54 (0.15)**

There was a significant positive correlation between the transformed larval *Ixodes scapularis* tick infestation on hosts and increasing forested patch size (adjusted R^2=0.3522, p<0.0001, $F_{1, 350}$=191.8, Figure 3). There was also a significant positive correlation between larval *Dermacentor variabilis* tick infestation on hosts and increasing forested patch size (adjusted R^2=0.0973, p<0.0001, $F_{1, 350}$=38.84, Figure 3).

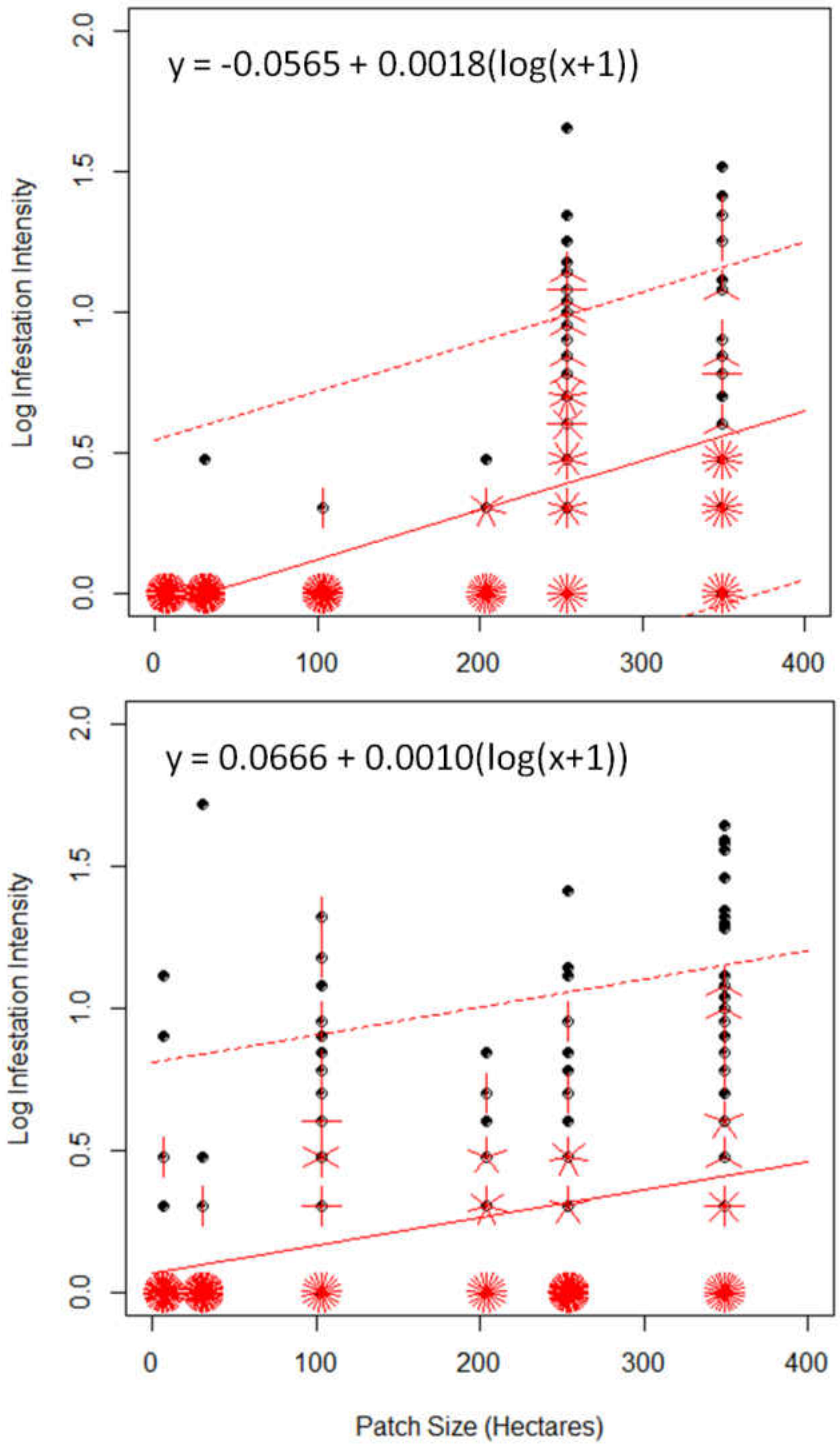


Figure 3. Infestation intensity of both *Ixodes scapularis* (top) and *Dermacentor variabilis* (bottom) increased with increasing forested patch size in Grand Forks Co., ND. 2012. Each point represents larval tick infestation intensity of an individual mammal. Each spoke indicates a mammal with identical infestation. Solid lines represents the fitted linear regression and dotted lines are 95% confidence envelope.

Abundance of Questing Ticks

Overall, 287 questing *I. scapularis* ticks were collected (Table 10). The Turtle River and Forest River study sites, the two largest study sites, contained all but two of the *I. scapularis* collected. The Oslo, Manvel, and Janice study sites did not produce any ticks *I. scapularis*. The majority of *I. scapularis* collected (98%) were adults. There was a significant relationship between forested patch size and *I. scapularis* collected per hour using a second order linear regression model (Adjusted R^2=0.4754, $F_{2, 80}$=38.15, p<0.0001, Figure 4).

Dermacentor variabilis were collected at all study sites by flagging (Table 10). Only the adult lifestage was collected. The largest two study sites, Turtle River and Forest River, contained 98% of the *D. variabilis* collected. A second order linear regression model plotting patch size against ticks collected per hour flagging found a significant positive correlation (adjusted R^2=0.544 $F_{2,80}$=49.91, p<0.0001; Figure 4).

Table 10. Questing ticks collected by flagging (ticks per hour) within forest islands of varying sizes. Grand Forks Co., ND. 2012.

	Size (Hectares)	Sampling Periods	Effort (minutes)	*Dermacentor variabilis*	*Ixodes scapularis*
Oslo	7	12	490	1 (0.1)	0 (0.0)
Manvel	31	12	490	2 (0.2)	0 (0.0)
Northwood	103	12	495	7 (0.8)	2 (0.2)
Janice	204	12	480	4 (0.5)	0 (0.0)
Turtle River	254	18	1150	191 (10.0)	49 (2.6)
Forest River	349	17	1105	603 (32.7)	236 (12.8)
TOTAL		**83**	**4210**	**808 (11.5)**	**285 (4.1)**

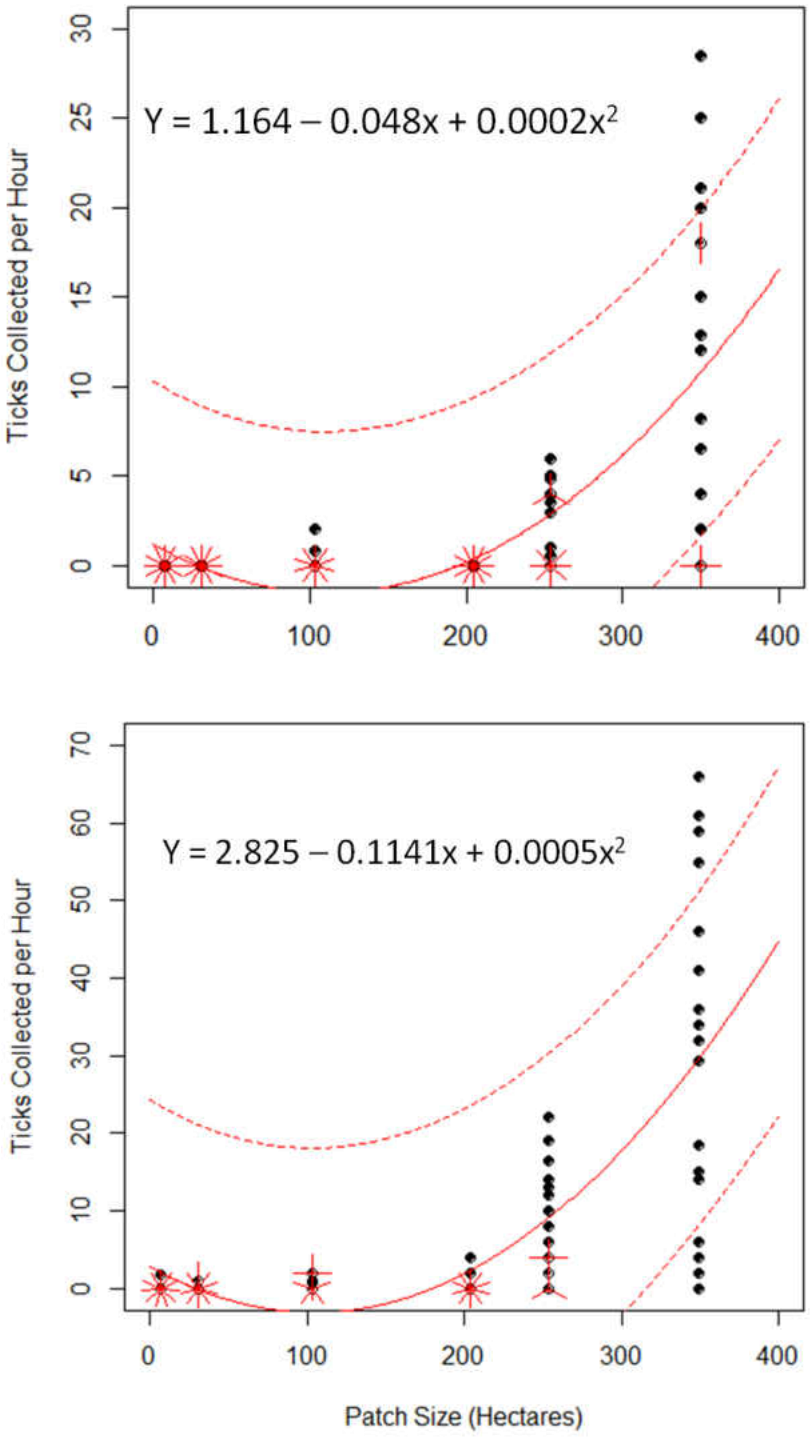


Figure 4. ***Ixodes scapularis*** **(top) and** ***Dermacentor variabilis*** **(bottom) collected per hour increased with forest patch size in Grand Forks County, ND, 2012. Each point represents one flagging event. Spokes indicate flagging events with identical number of ticks collected per hour. Solid lines represents the fitted linear regression and dotted lines are 95% confidence envelope.**

Host Utilization by Immature Ticks

I collected 352 small mammals over 1425 trap-nights. Of eight species captured, over 95% consisted of *Peromyscus spp.* (63.1%) and *Myodes gapperi* (32.4%; Table 11). No trend was observed that would indicate forested patch size had an effect on species richness or diversity. However, *M. gapperi* and *Peromyscus* were not distributed evenly across study sites (Pearson's Chi-squared = 73.7, DF=5, $p<0.0001$). *Myodes gapperi* and *Peromyscus* were collected evenly at the Oslo (Binomial Test $p = 1$), Manvel ($p=1$), and Forest River ($p = 0.11$) study sites. However, *Peromyscus* was the dominant mammal species captured at the Northwood ($p<0.0001$), Janice ($p<0.0001$), and Turtle River sites ($p<0.0001$; Figure 5).

Table 11. Numbers and species of small mammals trapped within forest islands of varying sizes. Grand Forks Co., ND, 2012.

	Oslo	Manvel	Northwood	Janice	Turtle River	Forest River	Total
Forest Size (hectares)	7	31	103	204	254	349	
Trap Nights	201	199	203	205	310	307	1425
Mammal Species							
Mus musculus	0	0	0	1	0	0	1
Sciurus niger	0	1	0	0	0	0	1
Tamias striatus	0	0	0	3	0	0	3
Tamiasciurus hudsonicus	0	2	0	0	1	0	3
Blarina brevicauda	0	1	0	0	1	2	4
Zapus hudsonias	0	0	0	2	1	1	4
Myodes gapperi	29	28	4	1	12	40	114
Peromyscus spp.	28	28	44	30	66	26	222
TOTAL	**57**	**60**	**48**	**37**	**81**	**69**	**352**
Total per trap-night	**0.28**	**0.30**	**0.24**	**0.18**	**0.26**	**0.22**	**0.25**

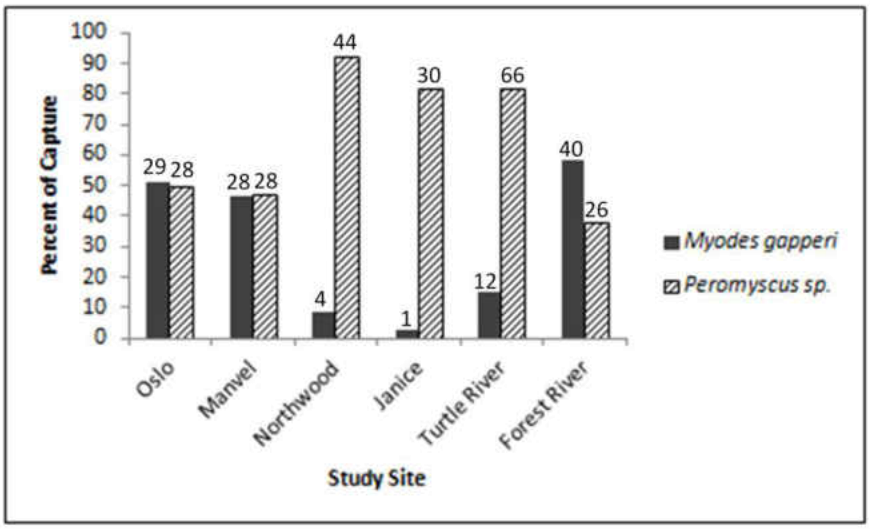


Figure 5. Percent of *Myodes gapperi* and *Peromyscus* of total capture collected at each study in Grand Forks County, ND, 2012. Numbers on bars represent number collected at each site. These two species were not evenly distributed across study sites.

Most of the immature *I. scapularis* ticks (97.9%) were collected from either *Peromyscus* (76.0%) or *M. gapperi* (21.9%), with smaller percentages of ticks collected from *Zapus hudsonias* (0.9%), *Sciurus niger* (0.8%), *Blarina brevicauda* (0.3%), and *Tamiasciurus hudsonicus* (0.1%). Most immature *D. variabilis* were collected from *M. gapperi* (58.0%) and *Peromyscus* (41.5%), with the remainder collected from *Mus musculus* (0.4%) and *Tamias striatus* (0.1%). Infestation prevalence of *I. scapularis* significantly differed between *M. gapperi* and *Peromyscus* at the Forest River study site (Table 12). Infestation prevalence of *D. variabilis* did not differ between *M. gapperi* and *Peromyscus* at the Forest River study site (Table 12). Larval infestation intensity did not differ among *Peromyscus* or *M. gapperi* (Table 13). The nymphal infestation intensity was not different for *Peromyscus,* however the greater infestation intensity of *M. gapperi* by *D. variabilis* than *I. scapularis* approached significance (Table 13).

Table 12. Infestation prevalence of the dominant small mammal species with immature ticks at the Forest River study site in Grand Forks Co., ND. 2012. Pearson's chi-squared test was used. Numbers in parentheses indicate number of hosts examined.

	Peromyscus	***Myodes gapperi***	**X^2**	**p-value**
Ixodes scapularis	88.5%(26)	60.0%(40)	6.2265	0.0126
Dermacentor variabilis	80.8%(26)	82.5%(40)	0.0317	0.86

Table 13. Infestation intensity of immature *Ixodes scapularis* and *Dermacentor variabilis* on small mammals at the Forest River study site, Grand Forks Co., ND. 2012. Intensity values are given as the median values and interquartile range.

	Ixodes scapularis	***Dermacentor variabilis***	**Mann- Whitney U**	**p-value**
Larvae				
Peromyscus	2.5 (1.0, 6.25)	3 (1.0, 3.75)	132	0.69
Myodes gapperi	2 (2.0, 6.0)	4 (1.5, 6.5)	203.5	0.66
Nymphs				
Peromyscus	1.0 (1.0, 1.0)	1.0 (1.0, 1.25)	30	0.80
Myodes gapperi	1.0 (1.0, 3.0)	4.0 (2.0, 6.0)	118.5	0.06

Temporal Dynamics

The abundance of adult *I. scapularis* was greatest during the initial sampling period of 2012 (Figure 6). It remained relatively high until mid-June, and then decreased through mid-July, after which no additional adults were collected. Nymphal abundance was high from the initial sampling period and peaked in early June. Once peaked, the abundance quickly dropped and remained low through our last nymphal detections in late July. Larval *I. scapularis* peaked in late May and at lower abundances through the last sampling period in mid August.

Adult and larval *D. variabilis* peaked in mid May (Figure 7). Adult abundance increased to this point but this was the first small mammal trapping period. Both adults

and larvae decreased throughout the season. Nymphs were detected at low levels until early July, peaking in Mid July.

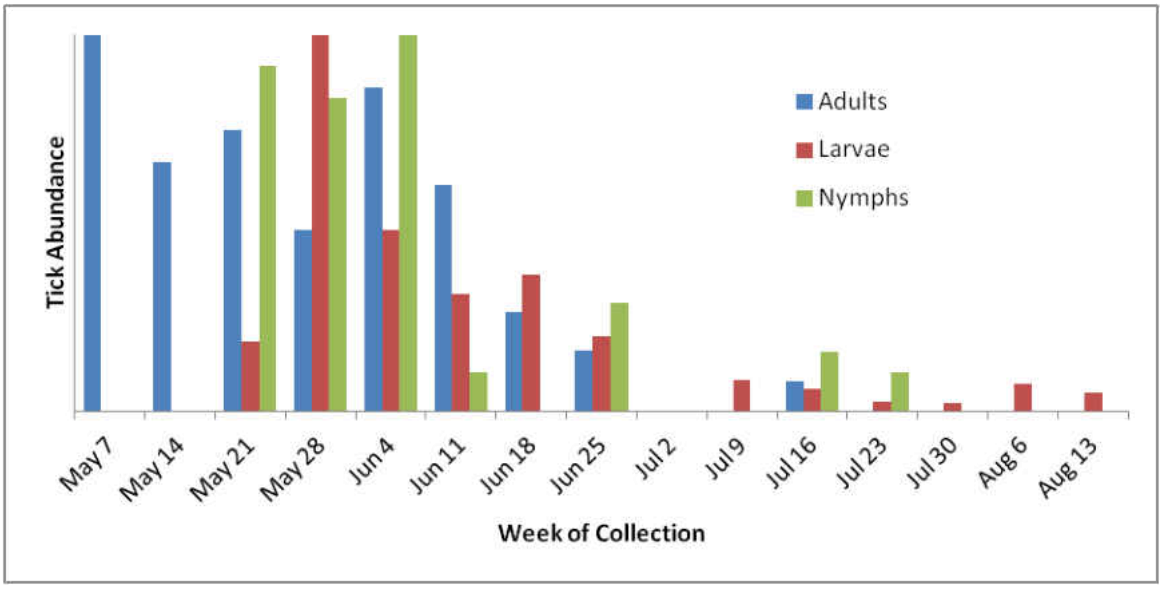


Figure 6. Temporal dynamics of *Ixodes scapularis.* Lifestage abundances were standardized for ease of comparison. Adults were collected by flagging the weeks of May 7 through August 6. Small mammals were examined for larvae and nymphs from May 21 through August 13. No sampling occurred the week of July 2.

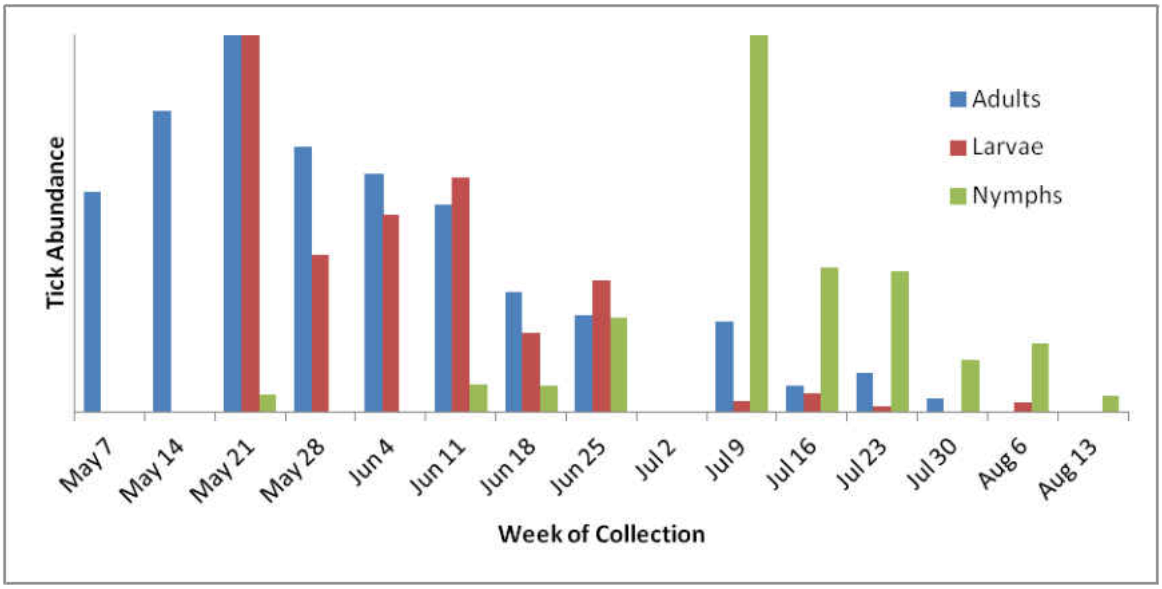


Figure 7. Temporal dynamics of *Dermacentor variabilis.* Lifestage abundances were standardized for ease of comparison. Adults were collected by flagging the weeks of May 7 through August 6. Small mammals were examined for larvae and nymphs from May 21 through August 13. No sampling occurred the week of July 2.

DISCUSSION

Abundance of Ticks

The abundance of adult questing ticks and immature ticks feeding on hosts increased with increasing forest patch size for both tick species. This can indicate that either larger forest patches can sustain a greater density of ticks or that the ticks have inhabited these large patches for a longer time and have been able to maximize their population densities towards their carrying capacity. The abundance of small mammal hosts was not influenced by forested patch size in North Dakota. Because *I. scapularis* populations are well-established at two of the study sites I can infer that small mammal hosts are sufficiently abundant at each study site to sustain the immature tick lifestages. Deer are also abundantly present at each site (personal observation) to sustain the adult lifestage. Therefore, in the absence of some unknown factor unrelated to patch size, all study sites examined in this project have the theoretical ability to sustain *I. scapularis* populations. Mobility of ticks themselves is limited to areas less than 10 meters (Carrol & Schmidtmann, 1996) so the movement of ticks from one area to another over large distances is dependent on host movement. Immature ticks regularly parasitize birds (Smith Jr. et al., 1996; Klich et al., 1996; Scott et al., 2012). I believe that the large

patches were found to have greater densities of ticks because they are more frequently encountered by birds carrying ticks.

Host Utilization

Ixodes scapularis had a higher infestation prevalence on *Peromyscus* than *M. gapperi*. *Dermacentor variabilis,* on the other hand, infested both mammal species equally. This indicates that *I. scapularis* may be showing some kind of preferential feeding patterns. Larval intensity did not differ between tick species for either host meaning those individuals that are infested are infested by larvae equally. Finally, *M. gapperi* had higher infestation intensity by nymphal *D. variabilis* than *I. scapularis* while *Peromyscus* infestation intensity with both tick species was the same. *Myodes gapperi* were more heavily infested by *D. variabilis* than by *I. scapularis.*

There are competing hypotheses to explain these phenomena. First, the ticks may be utilizing hosts not collected using Sherman traps. For example, *Ixodes scapularis* nymphs or *D. variabilis* larvae may be feeding on host species not collected. Alternately, survival rates may differ between species. More *D. variabilis* larvae surviving to become nymphs than *I. scapularis* would account for the increased abundance of *D. variabilis* observed in the study. I am inclined to believe that there is a greater abundance of *D. variabilis* larvae as well that were not detected in this study because both adult and nymphal abundance of *I. scapularis* were less than *D. variabilis*. Collection of additional host types (i.e., birds or medium-sized mammals) would provide insight into this theory.

On the topic of host utilization, the association of tick species and host species turned out to be rather interesting. I propose two hypotheses to explain why larval tick species would not be evenly distributed between the two most common hosts: (1) differential encounter rates, and (2) host preference. In order for the differential encounter scenario to hold true, the tick and host species must utilize different microhabitats. In our case, *I. scapularis* would more closely share a microhabitat with *Peromyscus* than *M. gapperi. Dermacentor variabilis* may inhabit multiple microhabitats thus allowing it to encounter both mammal species equally. In this scenario, we postulate that there is no difference in tick or host species after-encounter attachment rate. The second hypothesis, host preference, postulates that encounter rates are equivalent for all host and tick species but the after-encounter attachment rate differs. In this study, *I. scapularis* would pass over a possible *M. gapperi* host for the opportunity to feed on *Peromyscus.* In 2010 I observed *I. scapularis* readily cling to a flag made of synthetic material in Graham's Island State Park. This observation only exhibits the tendency of *I. scapularis* to cling to non-specific hosts (as opposed to actually attaching and feeding). However, in this study *I. scapularis* were found on six of eight mammal species. They were not collected on *Mus musculus* and *Tamias striatus,* caught only one and three times and all at the Janice study site where few *I. scapularis* were collected. This leads me to believe that *I. scapularis* at least, does not exhibit host preference. Thus, the differential encounter rate hypothesis seems to be the more likely scenario.

Temporal Dynamics

Adult *D. variabilis* abundance peak coincided with larval peak. Adult abundance increased from the first sampling period to the peak then declined throughout the season. Unfortunately, this was the first sampling period for immature ticks and I was not able to get a good representation of the rate of emergence. Nymphs peaked later in the summer. The trends observed for each lifestage indicated a two-year lifecycle. Adult ticks emerge in the spring to seek a host and obtain a bloodmeal. Eggs are laid and larvae hatch but do not seek hosts until the following spring. Larvae seek hosts and feed in the spring then molt into nymphs. Nymphs feed later that same year molt into adults to overwinter. This lifecycle is in agreement with other studies (Burachynsky & Galloway, 1985; Garvie et al., 1978). A unimodal peak of larval abundance was observed, in agreement with patterns in other *D. variabilis* populations in northern latitude (Campbell, 1979; McEnroe, 1979).

The greatest abundance of adult *I. scapularis* was observed during the first sampling period of the season and thus, the date at which they first emerge and rate at which the emergence increases is not evident. Nymphal abundance was also near its peak during the first sampling period for immature ticks and has the same limitations that applied to adults. Larvae peaked during the second collection period and declined throughout the season. The observed peak abundance of all three life stages early in the season suggests a three-year lifecycle in which ticks of each lifestage emerge in the spring to take a bloodmeal then molt or lay eggs. Although the two-year lifecycle is

generally accepted as a standard, annual variation may contribute to longer lifecycles (Yuval & Spielman, 1990).

Seasonal dynamics in the northeast United States typically have a peak nymphal abundance prior to larvae (Ostfeld et al., 1996; Main et al., 1982). It is this delayed larval abundance that allows for pathogen infection prevalence to compound and flourish over a relatively few transmission seasons (Wilson & Spielman, 1985). In this pattern, infected nymphs can infect small mammals which then serve to infect the subsequent generation of larvae. With larval peak occurring before or simultaneously to nymphal peak, as observed in Grand Forks County, the rate at which pathogen infection prevalence can amplify in both tick and small mammal populations is severely decreased.

CHAPTER IV

MOLECULAR DETECTION OF PATHOGENS IN *IXODES SCAPULARIS* IN GRAND FORKS COUNTY

INTRODUCTION

The black-legged tick, *Ixodes scapularis*, is the most important tick vector of human disease in the United States (Centers for Disease Control and Prevention, 2012). This tick is known to transmit *Borrelia burgdorferi, Anaplasma phagocytophilum, Babesia microti,* and Powassan virus (Jongejan & Uilenberg, 2004). Over the last decade, this tick has been expanding its range (Hamer et al., 2010). In the previous two chapters I provided evidence of breeding populations of *I. scapularis* in Grand Forks County, ND. Examination of archived material in the UND Biology collection has revealed two adult female *I. scapularis* collected in Grand Forks County in 1988. However, this is not conclusive evidence that an established population was present in the area at that time. In my study, I found all lifestages of the tick and in sufficient numbers to determine that indeed, *I. scapularis* has become established in Grand Forks County. Because of its importance as a vector of various diseases, I tested all *I. scapularis* adults as well as pools of larvae collected in Grand Forks County in 2012 for *B. burgdorferi, A. phagocytophilum,* and *B. microti.*

METHODS

All *I. scapularis* collected by flagging were assayed for *Borrelia burgdorferi, Anaplasma phagocytophilum,* and *Babesia microti* using methods described in Chapter I. As a type of xenodiagnosis, engorged larval ticks collected from individual hosts were assayed to determine presence of pathogens in the host. Pathogen detection in engorged, attached larvae would indicate that the host mammal was the source of the pathogen. None of these pathogens are transovarially transmitted, meaning they must be acquired from a host. Engorged nymphal ticks were not included in xenodiagnoses because of the possibility that infected nymphs may have acquired their pathogens from their larval bloodmeal and not necessarily from the nymphal bloodmeal. Therefore, only in larval ticks is a direct route of infection source present.

Pathogen prevalence was analyzed for differences between host species. Fisher's exact test was used because of low expected values. Fisher's exact test was used to determine difference in infected hosts between the two largest sites.

RESULTS

Three different tick-borne pathogens were detected in questing *I*. *scapularis* ticks at the Forest River and Turtle River study sites; *Borrelia burgdorferi* (spirochetal agent of Lyme disease)*, Anaplasma phagocytophilum* (rickettsial-like agent of human anaplasmosis)*,* and *Babesia microti* (protozoal agent of human babesiosis) (Table 14). Few ticks were collected at the four smallest study sites for pathogen testing (Manvel n=1, Janice n=3, Northwood n=1, Oslo n=0) so pathogen prevalence was compared only amongst the largest two sites (Turtle River [TR] and Forest River [FR]). The infection prevalence for questing ticks did not differ significantly between these two sites for *B. burgdorferi* (TR=6.1%, FR=6.0%; Fisher's Exact test p=0.73), *A. phagocytophilum* (TR=0%, FR=5.5%; p=0.13), or *B. microti* (TR=2%, FR=0.4%; p=0.30). In questing ticks, *Borrelia burgdorferi* (5.9%) and *A. phagocytophilum* (4.5%) were more prevalent than *B. microti* (0.7%) (Fisher's Exact test p = 0.001).

Two of the three tick-borne pathogens found in questing ticks (*i.e., B. burgdorferi* and *A. phagocytophilum*) were also detected in engorged larval ticks attached to small mammals. For *B*. *burgdorferi*, the prevalence of xeno-positive mammals did not differ between *Peromyscus* and *M. gapperi* (Fisher's Exact Test, p=1.0), with both species combined having a xeno-positive prevalence of 5.7% (Table 15). For *A*. *phagocytophilum*, the prevalence of xeno-positive *Myodes gapperi* (15%) was five times

higher than that of *Peromyscus* (3%). This difference was statistically significant at the 90% confidence level but not the 95% confidence level (Fishers exact test, p=0.08). The overall prevalence for xeno-positive small mammals did not differ significantly between the Forest River and Turtle River sites for either *B. burgdorferi* (TR=10.4%, FR=2.7%; Fisher's Exact Test p=0.23) or *A. phagocytophilum* (TR=4.2%, FR=8.1%; Fisher's exact test p=0.65). The prevalence of *B. burgdorferi* in questing ticks (5.9%) did not differ from xeno-positive mammals (5.7%) (Pearson's Chi-squared test p=0.93). The prevalence of *A. phagocytophilum* in questing ticks (4.5%) did not differ from xeno-positive mammals (5.7%) (Pearson's Chi-squared test p=0.66).

Table 14. Molecular detection of pathogens in questing *Ixodes scapularis* ticks collected in Grand Forks Co., ND. 2012.

Site	Tick Lifestage	No. Ticks Collected	Ticks tested	*Borrelia burgdorferi*	*Anaplasma phagocytophilum*	*Babesia microti*
Northwood	Adult	2	2	0 (0.0%)	0 (0.0%)	0 (0.0%)
Turtle River	Nymph	3	3	0 (0.0%)	0 (0.0%)	1 (33.3%)
	Adult	46	46	3 (6.5%)	0 (0.0%)	0 (0.0%)
Forest River	Nymph	4	4	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Adult	234	231	14 (6.1%)	13 (5.6%)	1 (0.4%)
TOTAL		**289**	**286**	**17 (5.9%)**	**13 (4.5%)**	**2 (0.7%)**

Table 15. Number of small mammals (%) xeno-positive for tick-borne pathogens as determined by testing engorged larvae collected from the hosts. Grand Forks Co., ND. 2012.

	Number sampled	*Borrelia burgdorferi*	*Anaplasma phagocytophilum*
Peromyscus	66	4 (6.1%)	2 (3.0%)
M. gapperi	20	1 (5.0%)	3 (15.0%)
TOTAL	**88**	**5 (5.7%)**	**5 (5.7%)**

DISCUSSION

This brings us to the effect of forested patch size on the prevalence of pathogens. I tested for pathogens only in the ticks and therefore am limited in my analysis of current pathogen prevalence to the sites in which ticks were collected. However, I have data for *I. scapularis* populations and small mammal communities for all of the study sites and, naturally, the presence of the pathogen relies greatly upon the presence of the primary vector and reservoir hosts. Just as I proposed future *I. scapularis* distribution, I can do the same for the pathogens based on small mammal (reservoir) communities and abundance of adult questing ticks. I will address each pathogen individually starting with *Borrelia burgdorferi.*

Allan et al. (2003) found a higher prevalence of *B. burgdorferi* in questing nymphs from smaller forest patches due to the higher density of *P. leucopus*, a highly competent reservoir. In this study the small mammal community consisted primarily of *Peromyscus* (*maniculatis* and/or *leucopus*) and *M. gapperi*. While the high reservoir competency of *Peromyscus* has been widely established, little is known of the ability of *M. gapperi* to transmit *B. burgdorferi* to ticks. Bey et al. (1995) experimentally infected *M. gapperi* (then *Clethrionomys gapperi)* with *B. burgdorferi* and were able to detect the spirochete in tissue samples from the rodent. This demonstrated the ability of *B. burgdorferi* to persist in the vole, yet did not demonstrate the ability to acquire the

pathogen from, or transmit it to the tick. The detection of *B. burgdorferi* from *I. scapularis* larvae feeding from *M. gapperi* in the current study proves without a doubt that indeed, *M. gapperi* is a competent reservoir. Infection prevalence of *B. burgdorferi* was similar in *Peromyscus* and *M. gapperi* indicating that the competency of *M. gapperi* may be similar to that of *Peromyscus*. To strengthen this assertion, I looked at the prevalence of questing adults. Although patch size had no effect on the relative abundance of the two mammal species, the two sites in this study in which I found pathogens had significantly different compositions of these reservoirs. The fact that infection prevalence of adult ticks did not differ among the two sites is another indicator that *M. gapperi* is of similar reservoir competency as *Peromyscus.*

With patch size and reservoir abundance ruled out, abundance of the vector, *I. scapularis,* is left as the limiting factor affecting *B. burgdorferi* prevalence. I predict that as the pathogen and vector become better established in North Dakota, *Borrelia burgdorferi* will occur in roughly equal prevalences among tick populations in different size patches.

For *Anaplasma phagocytophilum*, the difference in infection prevalence approached significance with greater prevalence of *M. gapperi* than *Peromyscus* harboring the pathogen. This is in contrast to a 1997 study (Walls et al., 1997) that found no difference in infection prevalence of *M. gapperi* and *P. leucopus* in Minnesota (Fisher's p=0.5281). Just as was the case with *B. burgdorferi*, both sites had equal prevalence among questing adults indicating that both mammal species act as competent reservoirs. Because of the difference in prevalence between the two primary

reservoirs, it is difficult to determine relative competency. Therefore, *A. phagocytophilum* will be found in ticks inhabiting different patch sizes but prevalence may slightly differ depending on composition of reservoir host communities.

Lastly, *Babesia microti* was discovered in two questing ticks in this study. My ability to draw any conclusions on the effect of the size of forested patches on the prevalence of *B. microti* is once again limited due to the low number of ticks collected from the smallest study sites. Even among the largest two study sites, prevalence of infected questing ticks was too low to determine an effect of reservoir abundances. *Babesia microti* has been detected in both of our most abundant small mammals in wild-caught individuals in Maine (Goethert et al., 2003). That being said, the necessary reservoir hosts and vectors are present in North Dakota to sustain the pathogen. More importantly, the pathogen was detected in the state, meaning the acquisition of this pathogen by humans is possible in North Dakota.

CHAPTER V

SUMMARY

In 2010 two statewide surveys were conducted for ticks in North Dakota. *Dermacentor variabilis* were found throughout the state and *Ixodes scapularis* were restricted to the Northwest region of the state. Using PCR to assay *I. scapularis,* I detected the agents of Lyme disease and human granulocytic anaplasmosis. *Ixodes scapularis* was collected in sufficient numbers, in all three lifestages, and across a large geographic area to confidently conclude that they have become established within the state of North Dakota. This was the first records of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* naturally circulating in the state.

Borrelia burgdorferi is the most common vector-borne disease in the United States (Centers for Disease Control and Prevention, 2012). In the midwest and northeast U.S., *Ixodes scapularis* is the responsible vector for this pathogen as well as *A. phagocytophilum* and *Babesia microti* (Sonenshine, 1991). In order to better understand the distribution of the organisms across the North Dakota landscape I tested the hypothesis that newly invading *I. scapularis* would adhere to the concepts of island biogeography as they colonized North Dakota woodlands. To test this hypothesis, my second field season was conducted in 2012 and was restricted to forested areas of

varying sizes in Grand Forks County. The hypothesis was confirmed; larger numbers of both adult and subadult *I. scapularis* were present in forested patches of increasing size. This trend was found to be true for *D. variabilis* as well, indicating that indeed, the forested patches are acting as islands in a matrix of inhospitable land under agricultural production. In this study, the abundance of ticks dropped off sharply from our 254 hectare study site to the 204 hectare site. This suggests that there is a minimum size threshold in which ticks will establish abundant, stable populations.

More important than the discovery of *I. scapularis* in North Dakota is the discovery of the causative agents of Lyme disease, human granulocytic anaplasmosis, and human babesiosis. Even though tick density in our largest site was relatively low compared to levels in Minnesota, Wisconsin, or in the Northeastern United States, the risk of contracting these tick-borne pathogens is possible in North Dakota.

Myodes gapperi was the second most abundant mammal collected in this study and was host for a large portion of the immature ticks collected. By detecting *B. burgdorferi* in engorged larval ticks collected from *M. gapperi,* we determined this mammal to be a competent reservoir host for the pathogen. *Myodes gapperi* was a prominent host in forested areas of North Dakota, and thus may play a substantial role in the transmission cycle of *B. burgdorferi* in the state. Further study is required to determine the efficiency of this reservoir and its impact on pathogen prevalence.

Finally, although the temporal dynamics portion of this study was limited to one field season, we observed a co-emergence of larval and nymphal *I. scapularis* in spring.

If this lifecycle is in fact true, it would severely limit the ability of pathogens to flourish. North Dakota would continue to see a low prevalence of pathogens within its ticks.

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