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A Molecular Ecological Survey of Tick (Ixodes scapularis) Population Density and Their Infection Rates with Borrelia burgdorferi at the New Jersey School of Conservation

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by

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A MOLECULAR ECOLOGICAL SURVEY OF TICK (*IXODES SCAPULARIS*) POPULATION DENSITY AND THEIR INFECTION RATES WITH *BORRELIA BURGDORFERI* AT THE NEW JERSEY SCHOOL OF CONSERVATION

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Abstract

Studying vector populations of the *Ixodes scapularis* tick is of major importance in New Jersey, an endemic area of Lyme disease. This species of tick is a vector for various disease pathogens including the bacterium that causes Lyme disease, *Borrelia burgdorferi.* This study focused on *Ixodes scapularis* tick surveillance at the New Jersey School of Conservation, in Sussex County, New Jersey. Collection of host-seeking ticks began in July 2009 and has continued until December of 2010, by drag-cloth sampling in order to monitor tick abundances of all life-cycle stages.

In 2010 relative abundances for larvae and nymphal stages declined as compared to the year 2009 data, as opposed to the adult stage where the relative abundances increased. In 2009, larvae *Ixodes scapularis* had a peak relative abundance in August of ~0.145 ticks/m^2 and in August of 2010 this decreased by 61% to ~0.056 ticks/m^2. Nymphal relative abundances decreased by 42%, in July 2009 the peak relative abundance was ~0.036 ticks/m^2 and in June 2010 peaked at ~0.021 ticks/m^2. In the fall, adult *Ixodes scapularis'* relative abundance peaked in November 2009 at ~0.007 ticks/m^2 and in October 2010 at ~0.034 ticks/m^2, showing a 486% increase. In the spring of 2010 adults from the 2009 fall that overwintered had an additional peak relative abundance at ~0.006 ticks/m^2. In addition, preliminary analyses of nymphal *Ixodes scapularis* infection with the bacterium *Borrelia burgdorferi* were conducted. An infection rate of ~54.5% was found from a sample size of 11 nymphs.

Although the infection rate of *B. burgdorferi* for the nymphal stage is the only available data for infection thus far, in the future, work will be conducted analyze infection for additional nymphs and adults as well as tests for other pathogens acquired

and transmitted by this tick vector. This information as well as future analysis of the population trends and abundances during activity periods will benefit our understanding of the public health risks that the *Ixodes scapularis* tick poses in New Jersey and in other endemic areas for tick-borne diseases.

Table of Contents

List of Figures

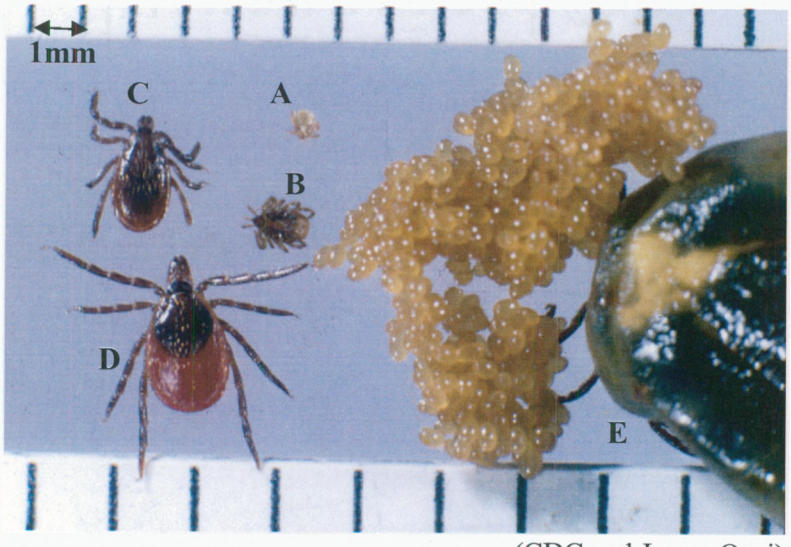
List of Tables

Introduction

Ixodes scapularis is an arthropod vector for various disease agents including the bacterium *Borrelia burgdorferi*, which causes Lyme disease. This species of tick is predominantly found in the northeast region of the United States in mixed woodlands and deciduous forests (Franz & Krause, 2003).

1.1 *Ixodes scapularis*

The *Ixodes scapularis* is a species of hard ticks in the subclass Acari, superoder Parasitiformes, and family Ixodidae. This species of tick is one of the primary vectors of the Lyme disease spirochete bacteria *Borrelia burgdorferi,* as well as other tick-borne disease pathogens. *I. scapularis,* commonly known as "blacklegged tick" has a two year life-cycle with three stages: larvae, nymph, and adult, and has one feeding during each of these stages (Fig.1). During any of these life-cycle stage feedings the tick can acquire disease pathogens if it feeds on a reservoir host.



(CDC and James Occi)

Figure 1- Life-cycle stages of *Ixodes scapularis*
(A) Larvae, (B) Nymph, (C) Adult Male, (D) Adult Female, and (E) Engorged Adult Female and eggs.

1.1.1 Life-cycle

During the late spring months, several hundred to thousand eggs are laid in the environment by an engorged female adult tick prior to its death. These eggs will hatch during the summer months and small six-legged larvae will emerge (Klompen, 2005). Newly emerged larvae ticks are the smallest size of the tick life stages, no bigger than half a millimeter and they do not carry any disease agents (Anderson & Magnarelli, 2008). Larvae will then remain close to the ground in moist/humid soil and climb small vegetation while questing for a host. This life-cycle stage feeding occurs mostly on small mammals and some birds, including the white footed mouse, *Peromyscus leucopus* (Wilder & Meikle, 2004). Feeding takes about 3-5 days until the larva becomes fully engorged, and then it drops-off the host into the environment (Klompen, 2005). This is the first feeding in which the larvae can become infected with the spirochete bacteria as well as other pathogens if feeding took place on a reservoir competent host. Engorged larvae can either molt into nymphs and overwinter or can overwinter with their blood meal and molt during the following spring. Unfed larvae will overwinter with a lower survival rate and continue their host-feeding activity during the end of spring the year after (Lindsay et al. 1999).

Ticks at their nymphal stage are most active during the summer months. During this activity period, nymphs will crawl onto vegetation and attach to passing hosts. Nymphs feed on a variety of hosts, including small mammals, birds and reptiles. Because nymphal activity is at its greatest during summer, there is a greater probability for tick-human encounters during these months (Paskewitz et al. 2001).

Unfed nymphs are about the size of a poppy seed, 1 to 1½ millimeter in length, have four pairs of legs and are light to dark brown color. After finding a host the nymphs will attach and feed for approximately 3-5 days, until fully engorged (Anderson & Magnarelli, 2008). At this stage, the tick can remain uninfected or become infected by feeding off of a reservoir host. If the nymph was previously infected at the larval stage, it can transmit disease pathogens to this second host during feeding. (Yuval & Spielman, 1990)

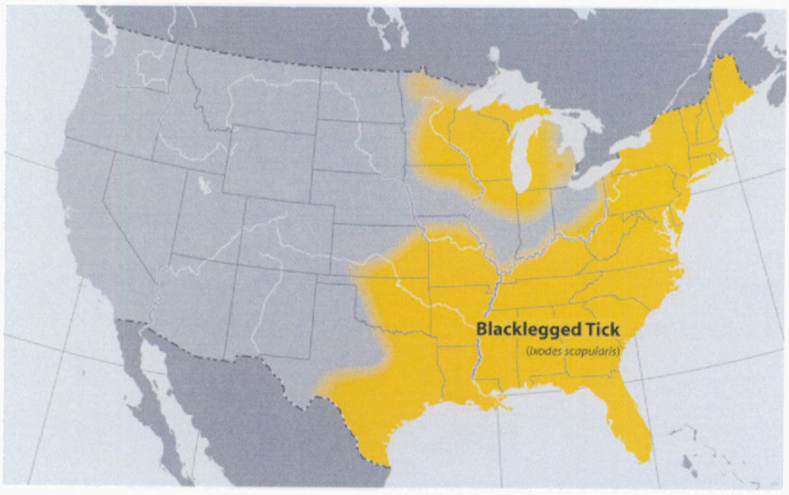
The engorged nymphs drop off the host into the environment and molt into mature adults either that same fall/winter season or early the following spring. At this stage of the life-cycle the tick will develop sexual dimorphism (Anderson & Magnarelli, 2008). Female adult *I. scapularis* are bigger in size than their male counterparts. Females are approximately the size of a sesame seed or 3-4mm in length; males are smaller measuring less than 2-3mm in length. Females have a black scutum and a reddish body; males have a black scutum that covers almost all of their body length.

Adult females can feed 5-7 days until engorgement and males feed sparingly if at all (Falco et al. 1999). Mating occurs either on vegetation but more commonly on the host animal during this last feeding. After feeding and mating females will drop off the host into the environment to develop and lays eggs. Unfed females can overwinter and continue to seek hosts during the spring in the subsequent year (Lindsay et al. 1998).

1.1.2 Geographical distribution

The *Ixodes scapularis* tick is distributed in the United States throughout the Northeast and upper Midwest (Fig. 2). This tick has adapted to diverse habitats with different temperature and precipitation regions as well as different vegetation zones

(Anderson, 2002). Although not pictured in the map, reported *I. scapularis* spans the southern regions of Canada (Dennis et al. 1998).



(CDC, 2010)

Figure 2- *Ixodes scapularis* in the United States
Map of the approximate distribution of *I. scapularis* "Blacklegged" tick.

1.1.3 Parasitic Vector

Prolonged attachment of an *I. scapularis* tick to its bloodmeal host can lead to the development of diseases. Transmission of pathogens can be to the host's blood, skin, joints, nervous system and even the heart (Wormser et al., 2006). In the United States, three major pathogens are of medical concern: *Borrelia burgdorferi, Babesia microti,* and *Anaplasma phagocytophilum.* These pathogens can lead to one of the following clinical manifestations: Lyme disease, human anaplasmosis and babesiosis. Infections are mostly presented by themselves or in rare cases as co-infections.

1.1.3.1 Lyme disease

Lyme disease is a bacterial vector-borne disease that is caused by *Borrelia burgdorferi* in the United States and both *Borrelia afzelii* and *Borrelia garinii* in Europe (Franz & Krause, 2003). The initial clinical studies in the United States occurred in 1975

after various cases of infantile arthritis occurred in a group of children in Lyme, Connecticut (Mahy, 2004). Initial symptoms such as the circular rash called Erythema chronicum migrans (EM) occurs at the site of the bite. However, in some clinical cases no EM occurs. The infection can manifest into other symptoms such as Bell's palsy, headaches, neck stiffness, and joint pain (Wormser et al., 2006).

1.1.3.2 Babesiosis

Babesiosis is a vector-borne disease that is also commonly transmitted by the *Ixodes scapularis* tick. It is also transmitted by infected blood transfusions. In North America the pathogen that causes this infection is *Babesia microti*. This is considered the second most common blood-borne parasite in mammals (Hunfeld et al. 2008). Other *Babesia* parasites have also been found to cause human disease, including *Babesia divergens*.

Most clinical manifestations are asymptomatic. In other cases, mild fever and anemia can go without notice. In severe cases, the symptoms can have similarity to malaria. In patients with a compromised immune systems or who have had a splenectomy, severe symptoms can occur (Hunfeld et al. 2008).

Asymptomatic patients do not require treatment, and many of the treatment decisions are made on an individual basis. If treatment is recommended, a combination of Atovaquone and Azithromycin or Clindamycin and Quinine is given for at least 7-10 days (Wormser et al., 2006).

1.1.3.3 Anaplasmosis

Anaplasmosis is another tick-borne disease that is transmitted by the *Ixodes scapularis* tick. It is caused by a species of bacterium called *Anaplasma*

phagocytophilum. This disease was formerly known as granulocytic ehrlichiosis (HGE) and human granulocytic anaplasmosis (HGA), the common term today is Human Anaplasmosis (Wormser et al., 2006).

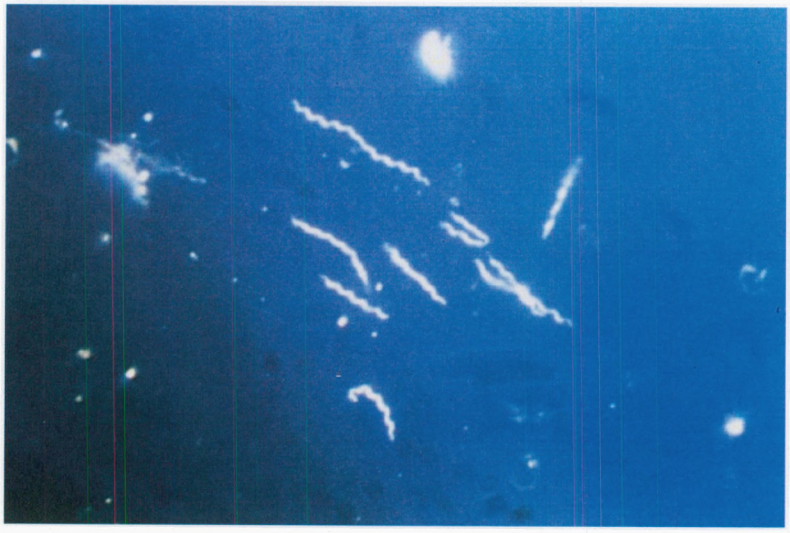
Symptoms can include fever, severe headache, muscle aches, and chills. These symptoms are usually similar to influenza. Less frequently, some patients can have nausea, vomiting and other gastrointestinal problems. Severity of the symptoms can occur if the patient has a compromised immune system. Treatment recommended is antibiotics such as Doxycycline for 10 days (Wormser et al., 2006).

1.2 *Borrelia burgdorferi*

The *Borrelia burgdorferi* is the pathogenic agent that causes Lyme disease, and belongs to the Eubacterial phylum of Spirochetes. It is very motile and has a corkscrew-shape (helical) and periplasmic flagella (Fig.3). The cell forms a configuration of about 3-10 loose coils that are 10-30μm in length and 0.2-0.5μm in width (Aguero-Rosenfeld et al. 2005).

1.2.1 Review of Species

Borrelia burgdorferi was the first spirochete whose genome was completely sequenced. Its entire genome is over 1½ megabases. The *B. burgdorferi* bacterium was also the first one to have a linear chromosomal topology and additional DNA plasmids (Fraser et al., 1997; Chen, 1996). The genome of *Borrelia burgdorferi* has been shown to include various chromosomal and plasmid encoding gene sequences for lipoproteins. The outer surface proteins A through F are some of these lipoproteins (Aguero-Rosenfeld et al. 2005). Very few proteins with biosynthetic activity are encoded in its genome, making it more dependent on its host (Tilly et al., 2008).



(CDC, 1993)

Figure 3- *Borrelia burgdorferi*
Darkfield photomicrograph (magnified 400x) of *B. burgdorferi* bacteria

1.2.2 Human infection

This bacterium infects a variety of vertebrate animals including mammals, lizards, and birds. The involvement of the *Ixodes* tick is as a vector of transmission between these types of hosts (Tilly et al. 2008). When a human becomes the blood host of an infected tick there is a possibility for transmission and infection. The time of attachment directly affects the probability of transmission of *Borrelia burgdorferi.* It has been found that at least a 36 hours attachment is necessary for spirochete transmission (Piesman et al. 1987).

Based on the clinical signs exhibited by Lyme diagnosis, the infection can be treated with antibiotics such as Doxycycline, Amoxicillin, and Azithromhycin during the different stages of the infection. Although much controversy lays with "chronic Lyme disease" and "posttreatment Lyme disease syndrome", conditions that have not been clearly defined. Some medical professionals and advocates suggest prolonged antibiotic treatment for several months and even years as a treatment for these chronic stages

(Baker, 2008). Clinical trials have shown positive results in patients with these conditions with the prolonged treatment, but many experts in infectious disease and health agencies continue to oppose the treatment due to unsafe side effects of long antibiotic use (Baker, 2008).

In 1998 a vaccine was approved by the Food and Drug Administration for use in persons older than 16 years. The vaccine consisted of a recombinant lapidated protein of *B. burgdorferi*, the outer surface protein A (OspA), and it was called LYMErix® (Franz & Krause, 2003). Despite positive results, the manufacturing company discontinued the vaccine in February 2002, due to low sales (Franz & Krause, 2003).

1.2.2.1 Incidence in the United States

In the United States the Centers for Disease Control and Prevention (CDC) compiles and publishes reported cases of Lyme disease. Reports are submitted by the health care providers, laboratories, or hospitals. The policies governing the reporting of this disease are determined by state laws.

In 2009 there were 29,959 confirmed cases of Lyme disease, additionally more than 8,000 probable cases (Centers for Disease Control and Prevention, 2010). Unfortunately, serious underreporting for this and other tick-borne diseases occur in the United States. A report found that in a sample of Connecticut physicians only 16% of cases of Lyme disease were reported (Young, 1998).

1.2.2.2 Incidence in the World

Lyme disease is not only prevalent in the United States but in Canada, Europe, and Asia (Franz & Krause, 2003). In analyzing the different spirochete strains around these regions a species complex, *Borrelia burgdorferi* sensu lato, was identified. The

complex is comprised of 12 species including the one prevalent in Lyme disease cases in the United States (Kurtenbach et al. 2006).

1.2.3 Prevention

Lyme disease can be prevented by both personal measures and by community-wide efforts. Personal measures include protective measures during outside activities and control of ticks around the home (Franz & Krause, 2003). These measures are recommended for endemic areas of Lyme disease.

These protective measures include: wearing long sleeves and long pants, tucking pants into socks and/or avoidance of wooded areas during peak seasonal vector activity. Frequent application of skin tick and insect repellents, such as those containing DEET, is also recommended. Permethrin repellents can also be applied to clothing and other fabrics, but not on skin. Daily screening for ticks and tick bites is most useful, as well as proper and prompt removal of ticks attached to skin. After a recognized tick bite, monitoring the appearance of symptoms is necessary, as well as consulting a physician for diagnosis and treatment if these occur (Talleklint & Jaenson, 1995).

In addition, endemic areas of Lyme disease can use landscape and host management to reduce the risk of infection. Around the home vegetative modifications to create an environment less suitable for ticks and its hosts can be used. Managing host abundance by reducing hosts and host habitat can be done by fencing off backyards and playgrounds (Stafford III, 2007).

1.3 Tick surveillance

It is of great importance to maintain tick surveillance efforts in endemic areas of Lyme disease. Benefits from surveillance of vectors can help understand the dynamics of

the disease and disease transmission in any given area. Various methods have been developed for tick surveillance: drag-cloth surveillance, tick flagging, tick walks, dry ice (CO_2) traps, and tick collections from captured small animals, etc. (Evans, 1998).

Long term monitoring at sites of collection can be used finding statistically comparable data. In addition different methods of collection are not always able to collect all of the life-cycle stages equally, thus a variety of methods should be used for collection (Ginsberg & Ewing, 1989).

In the United States various tick surveillance programs occur either through effort by research laboratories or by public health agencies. Some of these programs are in the states where it is recognized that Lyme disease is endemic, for example Connecticut, Delaware, Maryland, Massachusetts, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island, and Wisconsin (Centers for Disease Control and Prevention, 2007). Tick surveillance has increased in New York and New Jersey since the 1980s. All of the counties in both of these states have been recognized as areas with risk of Lyme disease (Dennis et al. 1998).

1.4 Detection of *Borrelia burgdorferi*

Several methods for detection of *Borrelia burgdorferi* are currently used. Samples investigated range from infected vectors, host reservoirs, and clinical samples from patients. Some of the methods used are: dark-field microscopy, antigen detection, cultivation of the bacteria, and polymerase chain reaction of gene regions such as flagellin (Wang et al. 2010).

1.4.1 Molecular detection methods

Amplification of nucleic acids by PCR-assay is used for detecting *B. burgdorferi* in tick vectors, host reservoirs specimens, and clinical specimens. PCR efficiency is established by the selection of a suitable gene region as target and an appropriate set of primers (Wang et al. 2010).

Many gene regions have been used as target for PCR amplification in research settings, but only a few have been able to be used for PCR-based detection. Genes including: ribosomal RNA, flagellin and OpsA have been successfully used as PCR targets for detection (Schmidt, 1997).

1.4.2 Direct microscopic methods

Both ticks and animal tissues can be prepared and used in dark-field microscopy for detection of *Borrelia burgdorferi*. Dark-field microscopy is a more useful technique for samples in which many spirochetes are expected to be present, ruling out its use for detection in tissues or fluids of patients (Wang et al. 2010). Immunoflourescence (DFA) methods are also sensitive for detection of the spirochetes.

1.5 Purpose of the study

This study was conducted to understand the population of the *Ixodes scapularis* tick at the New Jersey School of Conservation. This field site gave us an area of New Jersey that had not been studied in this field of interest. We conducted survey of ticks both for the purpose of collection of specimens and for observing the population characteristics and dynamics throughout the year. These observations will help us see trends of activity of each of the tick's stages of life-cycle and the overall density of host-

seeking ticks. In addition we will also focus on factors that may influence this population of *I. scapularis* tick.

With the tick samples collected at this site of surveillance, we will also test for the infection of the pathogenic bacterium *Borrelia burgdorferi*. Ticks will be subjected to nucleic acid extraction for the use in the molecular analyses. We will use a PCR-assay based analysis to test for optimal extraction of tick DNA and for the presence of the bacterial flagella DNA.

Infection rate of the tested tick samples will help us increase our understanding of the risk of infection by visualizing the enzootic prevalence of the *Borrelia burgdorferi* bacterium in its primary arthropod vector. Future and additional work can help other efforts in the scientific and public health community that can help reduce the transmission of the disease pathogen by tick attachments.

Materials and Methods

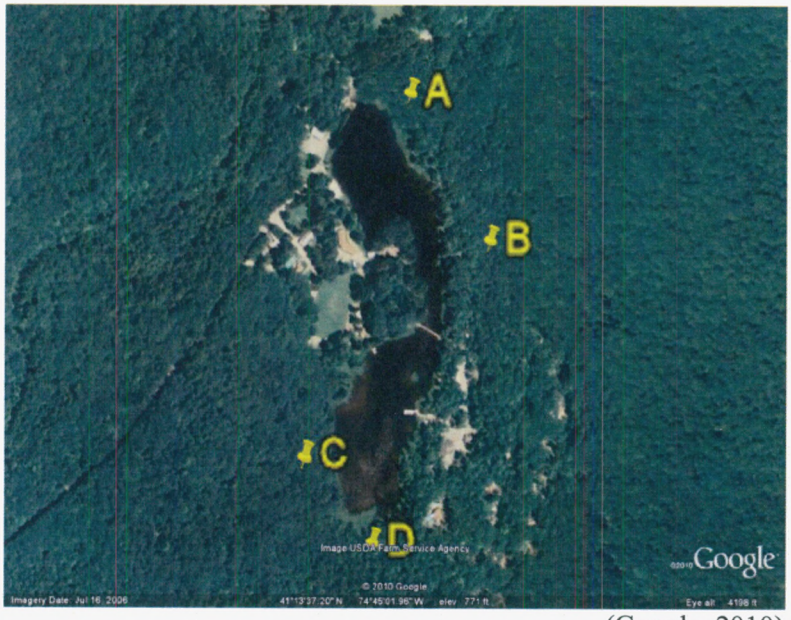
2.1 Fieldwork

Fieldwork was conducted at the New Jersey School of Conservation located in Sussex County, New Jersey (Fig.4). This is a research station and environmental field campus for Montclair State University. It is located in Stokes State Forest which is a recently reforested, mixed deciduous forest site that contains suitable habitat and host for *I. scapularis* to complete its life cycle, including an abundance of deer (National Audubon Society, 2011).

2.1.2 Sample collection

Tick surveillance began in July 2009 and continued through December 2009, then started again during March of 2010 and continued until December 2010. Collection between December 2009 and March 2010 was not conducted due to winter weather conditions and snow covering on the forest floor on most of these days.

The frequency of collection was dependent on weather conditions. On average collections occurred once a week, with a higher frequency during projected peak periods of activity. Surveillance was focused on selected locations of the NJSOC grounds: Northeast Lakeshore, East of Lake, South of Lake, and West of Lake. Areas of collection are shown on Fig. 4. During collection, ticks encountered from each area of surveillance were kept in separate collection vials for comparisons among sites at the NJSOC.



(Google, 2010)

Figure 4- NJSOC sites of tick surveillance
Northeast of lake (A), East of Lake (B), West of Lake (C), and South of Lake (D).

2.1.2 Collection method

The collection method used was drag sampling (Fig. 5). The drag cloth panel measured 1 meter by 1 meter. It was corduroy fabric with drapery bead-weighted cord on the bottom hem and a pocket on the top hem for a 1 inch by 2 inch wooden stick. A rope was attached to the sides of the wood and used as a handle while dragging across vegetation (Falco & Fish, 1992).

(Egan, 2009)

Figure 5- Collection of ticks by drag sampling
Collection of ticks using drag sampling method. The author shown with protective clothing.

Each collector calibrated his/her stride length and counted the number of steps while collecting in order to calculate the distance traversed. This way, tick abundances could be later on calculated as the number of ticks encountered per square meter of distance surveyed. Collectors wore protective clothing such as long sleeve pants, long socks used for tucking in pants, white cotton coveralls on top of clothing, hiking boots and tape to cover all zippers/openings of the coveralls as well as the boot-coverall gap (Fig. 5). Host-seeking ticks clung to the cloth as it was dragged on top of the vegetation and forest floor. The cloth was checked after having surveyed an approximate area of $20m^2$. Clinging ticks were removed off of the drag cloth with the use of forceps. Ticks were collected in glass vials; adult male and female ticks were collected into separate vials. Due to their small size, and high abundance, larvae were collected by rolling a lint-

roller over the drag cloth at 100 meter intervals. In some cases if visualized by the collector, larvae were removed with forceps and collected in glass vials. Tapes with larvae were stored in zip-lock bags with the label of the site of collection and the area covered, these were brought back to the lab for counting and removal from the tape.

2.1.3 Field journal

Field notebooks were kept during each collection. The details incorporated in each entry included: temperature, locality within the NJSOC grounds, time of day, visual observations (i.e. encounter of deer and other animals, approximate location from a known trail or landmark, etc.), number of ticks encountered, other ticks if encountered (i.e. *Dermacentor variabilis*), and at which point were larvae collected.

At the time of sample storage, additions to the journal notebooks were marked with their appropriate date. These additions could include: the total area covered by all collectors, the total number of each tick, lifecycle stages, and gender collected, storage temperatures and dates of storage. Each journal entry was also: photocopied to keep a hard- copy file and the data entered into a digital workbook.

2.1.4 Sample Storage

Tick samples that were collected at NJSOC were temporarily held in storage vials and transported to the laboratory facilities at MSU in a small lunch bag with ice pack to maintain alive. In addition, a blade of grass was kept in the vials to maintain a minimum level of moisture and to keep the ticks alive longer.

Ticks were temporarily stored at 4°C until they were able to be properly named and identified. The naming system consists of the date of collection, the field site name, the site of collection, and a number assigned to each tick (Table 1). For example a tick

collected on December 25, 2010 in the West Hillside at NJSOC will be named: 122510SC1.

Table 1- Collection sites at NJSOC	
Site Name	*Letter*
Northeast Lakeshore	A
East Lake Hillside	B
West Hillside	C
South of Lake	D

Adult and nymphs were transferred to a temperature of -80°C after being labeled and assigned to 1.5ml microcentrifuge tubes. Larvae were either counted on the tape of collection or if possible picked-off and placed in a 1.5ml microcentrifuge tube with 70% Ethanol in RNase, DNase-Free Water and stored at -20°C. The larvae naming also followed the same system previously described. If larvae were originally collected off of the drag-cloth, using forceps, these were stored without ethanol in a 1.5ml microcentrifuge tube at a temperature of -80°C.

2.1.5 *Dermacentor variabilis* and donated ticks

In addition to the field survey for *Ixodes scapularis*, some encountered *Dermacentor variabilis* ticks were also collected. If collected, these ticks were stored at -80°C. Other *D. variabilis* ticks were also donated by staff and other research teams working at the New Jersey School of Conservation. Some collected ticks were temporarily stored at room temperature until they were donated to our laboratory. Donated ticks were kept in their donated storage containers and any solutions that were used for storage and are being stored at -20°C.

2.1.6 Seasonal activity

The total number of *I. scapularis* ticks collected for each of the life-cycle stages was used for determining the seasonal activity through the year. Using the values calculated from the relative abundance calculations, seasonal activity observations were made.

2.1.7 Relative abundance

The relative abundances for each tick life cycle stage were calculated as the number of encountered ticks per area surveyed. This data also describes the density of host-seeking *Ixodes scapularis* tick in the area surveyed. The average abundances for a weekly period were calculated starting with July 2009. Values for relative abundance calculated were graphed for each of the life-cycle stage of the *I. scapularis* tick.

2.1.8 Climate

Using the "Records of River and Climatological Observations" obtained from the weather station Sussex 2 NW available online at the National Oceanic and Atmospheric Administration National Weather Service, we gathered daily temperature and precipitation data for the months of collection (July2009-Present). Sussex 2NW is located about 7km north from the NJSOC (Fig. 6). Data utilized from these resource documents were both daily maximum temperatures and the amount in inches of rainfall or melted snow accumulated. Values were used to calculate the mean maximum temperatures and mean precipitation accumulation bi-weekly for each month of collection in order to examine environmental conditions and their potential correlation with tick abundances.

(Google, 2010)

Figure 6- Reference NJSOC in New Jersey
NJSOC, Sussex 2NW Weather Station and Montclair State University are shown.

2.2 Molecular Analysis

The following molecular methods are the final optimized ones that were found to give optimal results. Much of the molecular work of this project involved the optimization of these methods, which is discussed below in the results section.

2.2.1 Sample Pre-treatment

All consumables, tips, micropipettes, and solutions were UV treated for 10 minutes before use. Aliquots of solutions that were UV treated were kept at small volume within a 1.5mL microcentrifuge tube for UV light to penetrate. Pre-treatment of tick samples was conducted before the DNA extraction to clean the surface of the tick from any contamination. All pre-treatment was performed atop ice packs to minimize the thawing and freezing of the tissue and maximize DNA recovery and under a dissecting microscope to prevent from damaging any of the tick parts.

One tick of the selected group was pre-treated at a time. The remaining ticks were kept in the freezer to minimize thawing. The tick selected for extraction was then placed

on a clean UV treated 60 x 15 mm petri dish. A volume of 20µL of UV treated-1% sodium hypochlorite (i.e. a 10% Clorox solution) was added to the tick. Due to surface tension, the same tip was used as to move the tick around the solution to clean the surface as much as possible. The solution was discarded and a 100µl volume of UV treated RNase - DNase free water was added onto the tick. This water was used to rinse the tick surface; the surface tension also required manual movement of the tick with the help of a pipette tip. The water is then discarded and the rinsing step is repeated once more. The tick is then placed back in the freezer until all ticks are pre-treated.

2.2.2 Dissection of tick samples

(Egan, 2010)

Figure 7- Nymph *Ixodes scapularis* dissection prior to DNA extraction. Nymph *I. scapularis* visualized with a dissecting microscope and cut lengthwise for DNA extraction.

After pre-treatment, ticks were taken one at a time out of the freezer for dissection. The petri dish containing the pre-treated tick was placed on top of the ice pack

and under the dissecting microscope. With a UV treated razor blade the tick was cut lengthwise (Fig.7). One half of the tick is returned to a UV treated 1.5mL microcentrifuge tube labeled with the sample name as a tissue voucher. The tissue vouchers were stored at -80°C. The other half of the tick was then placed in a UV treated 1.5mL microcentrifuge tube for DNA extraction. The same procedure was utilized for the remaining ticks.

2.2.3 Total DNA Extraction

For total DNA extraction from the tick samples, the DNeasy Blood and Tissue Kit by QIAGEN was used as well as the adjusted protocol "Purification of total DNA from ticks" provided by the manufacturer.

After the ticks were pre-treated and dissected, the one half of the tick was used for DNA extraction. The lysing process began with the addition of warm 100µL Buffer ATL to each tick half. This was done both to keep precipitates from forming in Buffer ATL and also to heat shock the cold tick tissue and help rupture cells in the exoskeleton. With a UV-treated 1.5mL pestle the half tick was crushed, making sure the tissue is cleaned off the pestle tip it was rinsed inside the microcentrifuge tube with warm 80µL of Buffer ATL. After that, 20µL of Proteinase K were added, vortexed to mix and incubated overnight at 56°C on a multi-tube vortexer to continue movement during lysis.

Following the overnight incubation, tubes were removed from the incubator and vortexed for 10 seconds, and 200µL of Buffer AL were added and mixed immediately by vortexing. Incubation at 70°C in a water bath for 10 minutes followed, then 230µL of ethanol (100%) were added and mixed thoroughly. The mixture was pipetted, including the tick exoskeleton, into the DNeasy mini spin column provided by the kit, then

centrifuged at 6000 x g (8000 rpm) for 1 minute. All flow-through and collection tubes were kept until all extraction steps and verification of extraction was completed.

The spin column was placed into a new 2mL collection tube provided by the kit. 500µL of Buffer AW1 were added and centrifuged for 1 minute at 6000 x g (8000 rpm). The spin column was again placed into a new 2mL tube provided by the kit. 500µL of Buffer AW2 were then added and centrifuged for 1 minute at 16000 x g (13,000rpm) for 3 minutes.

In order to elute the DNA from the spin column, the column was taken out carefully from the flow-through collection tube and placed into a new UV-treated 1.5 mL microcentrifuge tube (not provided by the kit). 35 µL of Buffer AE were then added to the spin column and left to incubate at room temperature for 1 minute, then centrifuged at 6000 x g (8000 rpm) for 1 minute. Another 30 µL of Buffer AE were pipetted to the spin column and left to incubate for an additional minute at room temperature. After this, the columns were centrifuged at 6000 x g (8000 rpm) for 1 minute. The eluted DNA was then labeled and stored at -20°C.

2.2.4 Polymerase Chain Reaction primers

Primers and PCR conditions that were optimized for both the CO1 and the flagellin amplifications are described in Table 2.

Table 2- Primers and cycling conditions			
PCR Reaction	*Primer Sequence*	*Cycling conditions*	*Reference*
CO1	LCO1490 5'GGTCAACAAATCATAAAGATATTGG3'	94°C/5min., 38 cycles of: 94°C/45sec., 51°C/45 sec., 72°C/45 sec., and 72°C/7min	Folmer et al. 1994
	HC02198 5'TAAACTTCAGGGTGACCAAAAAATCA3'		
B. burgdorferi Flagellin	X14841forward 5'-ATTTCGTCTGTAAGTTGCTCTATTTCAA-3'	94°/5min., 36 cycles of: 94°C/20sec., 55°C/30sec., 72°C/30sec., and 72°C/7min	Daniel's Lab, pers. comm.2009
	X14841reverse 5'-TTAATCGAGCTTCTGATGATGCTGC-3'		
B. burgdorferi Flagellin Re-Amp	X14841forward 5'-ATTTCGTCTGTAAGTTGCTCTATTTCAA-3'	94°/5min., 20 cycles of: 94°C/45sec., 60°C/45sec., 72°C/45sec; and 72°C/7min	Daniel's Lab, pers. comm.2009
	X14841reverse 5'-TTAATCGAGCTTCTGATGATGCTGC-3'		

2.2.5 Polymerase Chain Reaction – Confirm *Ixodes scapularis* DNA extraction

As a test for the quality of the DNA extracts prior to attempting *Borrelia* amplifications, we used the "universal" primers for amplifications of the tick mitochondrial cytochrome c oxidase subunit I gene (COI). The PCR reaction was performed using 1µL DNA extract as a template in a 50µL total reaction volume. The following reagent volumes and final concentrations were in the PCR reaction: 5µL GeneAmp 10X PCR Buffer (50mM KCl, 10mM Tris-HCL [ph 8.3], 1.5mM $MgCl_2$), 5µL of GeneAmp dNTP mix (1mM of each dNTP), 0.1µL of each of the primers (0.2mM), 0.2 µL of Taq polymerase (0.5 units), and 38.6 µL of UV-DNase, RNase-free water. For a positive control a previous successful tick extraction was used. Reactions were amplified by the cycling parameters stated in Table 2.

2.2.6 Visualization of CO1 PCR

Confirmation of the CO1 PCR reaction was performed by visualizing positive PCR amplification using agarose gel electrophoresis with SYBR Safe staining. In order

to separate the 710-bp DNA fragment we used a 1% Agarose LE/1X TBE gel with 2.5μL of SYBR Safe stain (per 50mL of gel mixture). The gels are submerged in 1x TBE buffer and a current of about 130V is applied for approximately 20 minutes to allow sufficient separation of DNA fragments. Positive confirmation is determined by taking a digital image.

2.2.7 Testing: minimum amount of *Borrelia burgdorferi* DNA for positive control for PCR

Although the use of a positive control DNA is necessary for assessing the success of the PCR reaction, it also poses a risk for introducing contaminating DNA. In order to minimize this potential risk, PCR positive DNAs were aliquoted and stored in another lab. In addition, in order to minimize the amount of positive control DNA used, experiments were conducted to determine the minimum amount required that would give a weakly positive signal. These efforts were particularly important because the detection of *Borrelia burgdorferi* from field caught specimens required a second round of PCR to re-amplify first round PCR products.

Borrelia burgdorferi genomic DNA was purchased from ATCC in a dry form (CAT#35210D-5). DNA was re-suspended in UV-DNase, RNase-free water to give a stock concentration of 10ng/μL. In order for this DNA to be used for a positive control for the PCR reactions of tick extracts, a smaller concentration was needed. The dried form, re-suspension and aliquots were performed and stored in another laboratory to prevent cross-contamination.

The quantities tested to find the minimum concentration were: 20ng, 10ng, 5ng, 1ng, 0.5ng, 0.1ng, 0.05ng, 0.01ng, 0.005ng, 0.001ng, $5x10^{-4}$ng, $1x10^{-4}$ng, $5x10^{-5}$ng, $1x10^{-}$

^{5}ng, $5x10^{-6}$ng, $1x10^{-6}$ng, $5x10^{-7}$ng, $1x10^{-7}$ng, $5x10^{-8}$ng, and $1x10^{-8}$ng. For the PCR reaction volume of 50µL, a 2µL template DNA was used. For the reaction the following components and volumes were used: 5µL GeneAmp 10X PCR Buffer (50mM KCl, 10mM Tris-HCL [ph 8.3], 1.5mM $MgCl_2$), 5µL of dNTP mix (1mM of each dNTP), 37.6µL of UV-DNase, RNase-free water, 0.1µL of each forward and reverse X14841 primer (0.2mM), and 0.2 µL of Taq polymerase (0.5 units). The cycling parameters are described in Table 2.

An additional PCR assay was conducted using 1µL of the PCR product from the first *B.b.* PCR amplification. In this reaction the components and volumes were similar to the previous reaction with the only difference of 38.6µL UV-DNase, RNase-free water. Cycling parameters are listed in Table 2.

Visualization of both PCR assays were confirmed using 2% Agarose LE with 1X TBE gel electrophoresis and 2.5µL SYBR Safe stain (per 50mL of agarose gel mixture). A digital photograph was taken to confirm which amount of *Borrelia burgdorferi* DNA was the minimum (Fig. 8).

2.2.8 Amplification of *Borrelia burgdorferi* flagellin DNA

In order to test for *Borrelia burgdorferi* infection, all tick extracts that showed a good extraction based on the COI amplification of tick DNA were used for a PCR assay with the flagellin primers X14841. Total DNA extracts from the ticks were used as template for the following assays. For the initial PCR 10µL of template DNA were used in 50µL reaction volume containing: The following reagent volumes and final concentrations were in the PCR reaction: 5µL GeneAmp 10X PCR Buffer (50mM KCl, 10mM Tris-HCL [ph 8.3], 1.5mM $MgCl_2$), 5µL of GeneAmp dNTP mix (1mM of each

dNTP), 0.1µL of each of the primers (0.2mM), 0.2 µL of Taq polymerase (0.5 units), and 29.6µL of UV-DNase, RNase-free water. The cycling parameters are described in Table 2.

A re-amp PCR assay was also performed following the following pecifications: 1µL of the PCR product from the first *B.b.* PCR amplification, 5µL GeneAmp 10X PCR Buffer (50mM KCl, 10mM Tris-HCL [ph 8.3], 1.5mM $MgCl_2$), 5µL of GeneAmp dNTP mix (1mM of each dNTP), 0.1µL of each of the primers (0.2mM), 0.2 µL of Taq polymerase (0.5 units), and 38.6 µL of UV-DNase, RNase-free water. The new cycling parameters are described in Table 2. In all re-ampification PCR reactions, the original PCR and extraction negatives were also re-amplified in order to monitor for potential contamination.

Both PCR assays were visualized to confirm an amplification of the region of the flagellin gene. We used a 2% Agarose LE gel with 1X TBE buffer and 2.5µL of SYBR Safe DNA stain. A picture was taken of the gels to confirm amplification.

2.2.9 Visualization of *B. burgdorferi* PCR

Both PCR assays were visualized to confirm an amplification of the region of the flagellin gene. We used a 2% Agarose LE gel with 1X TBE buffer and 2.5µL of SYBR Safe DNA stain. The gels were submerged in 1X TBE buffer under a current of about 130Volts. A picture was taken of the gels to confirm amplification.

2.3 Nymphal tests for *B. burgdorferi* infection.

All of the tick specimens followed similar DNA extraction protocols, and PCR-assays to confirm a proper extraction (CO1 primers), and infection of *Borrelia burgdorferi* (X1841 flagellin primers). A molecular result spreadsheet was organized to

maintain records of which samples were infected. The rate of infection is a result of the number of samples that positively tested for infection of *B. burgdorferi* per the total amount of samples tested.

Results

3.1 Seasonal Activity

Tick collection for 2009 began in July, when the seasonal host-seeking activity period of larvae and nymphs had already begun. Because surveillance 2009 data for the spring and beginning of summer are not available for comparison, only some patterns of seasonal activity can be observed.

In 2009 some questing activity of larvae was observed during August and September, similarly for the year 2010, there was a questing activity in addition a peak of activity during August through the beginning September (Fig. 8). Nymphal-stage *I. scapularis* were also host-seeking during the summer months in both years of surveillance. In 2009 nymphs showed constant activity from the beginning of surveillance (July) until the beginning of October. A clearer pattern of activity was observed in 2010, where nymphs had a steady activity from June to August and a slow decline in activity until November. (Fig. 8)

Host-seeking adult ticks were encountered during the fall and beginning of winter in 2009, during the spring of 2010, and during the fall and beginning of winter of 2010 (Fig. 8). Since collection lapsed late December to the beginning of March (2009-2010), no observations for the *I. scapularis* tick were made.

3.2 Relative abundance

The relative abundance of the *Ixodes scapularis* tick was calculated as the number of ticks encountered per unit effort, meter squared (Fig. 8).

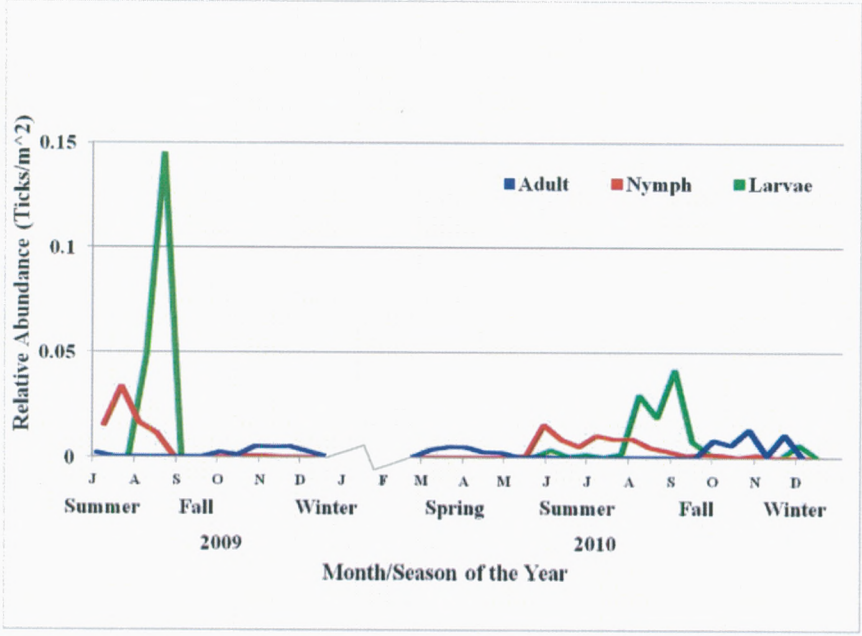


Figure 8- Relative abundance of *Ixodes scapularis* ticks at the NJSOC (2009-2010)

Relative abundance (ticks/m^2) vs. Month of collection from July 2009 until December 2010 [Sampling did not occur during winter months]. Adult *I. scapularis* is shown in blue, nymphs in red and larvae in green.

Larvae and nymphal stages of the *I. scapularis* tick were observed to be questing for hosts during the summers for both years of surveillance data. Peak relative abundances can be observed for 2010 and partially for 2009.

When comparing relative abundances for the nymphal and larval stages of the *I. scapularis* tick, a decrease in the year 2010 from the 2009 year is seen. On the other hand, the adult life-cycle stage shows an increase in 2010 as compared to 2009. Overwintered adult ticks cannot be compared due to lack of 2009 surveillance data (Table 3).

Table 3- Summary of Seasonal Activity and Relative Abundance of *I. scapularis*

I. scapularis Life-Cycle Stage	Period of Seasonal Activity	2009 Peak of Relative Abundance (tick/m^2)	2010 Peak of Relative Abundance (tick/m^2)
Larvae	August-September	0.145	0.048
Nymph	June-July	0.036	0.016
Adult	October-December	0.0066	0.0168
Adult (overwintered)	March-May	N/A	0.0055

3.3 Climate patterns

Average monthly temperatures for 2010 were higher than 2009. The average maximum temperature for the months of July-August 2010 was higher ~88°F and in 2009 it was ~83°F (Fig.9).

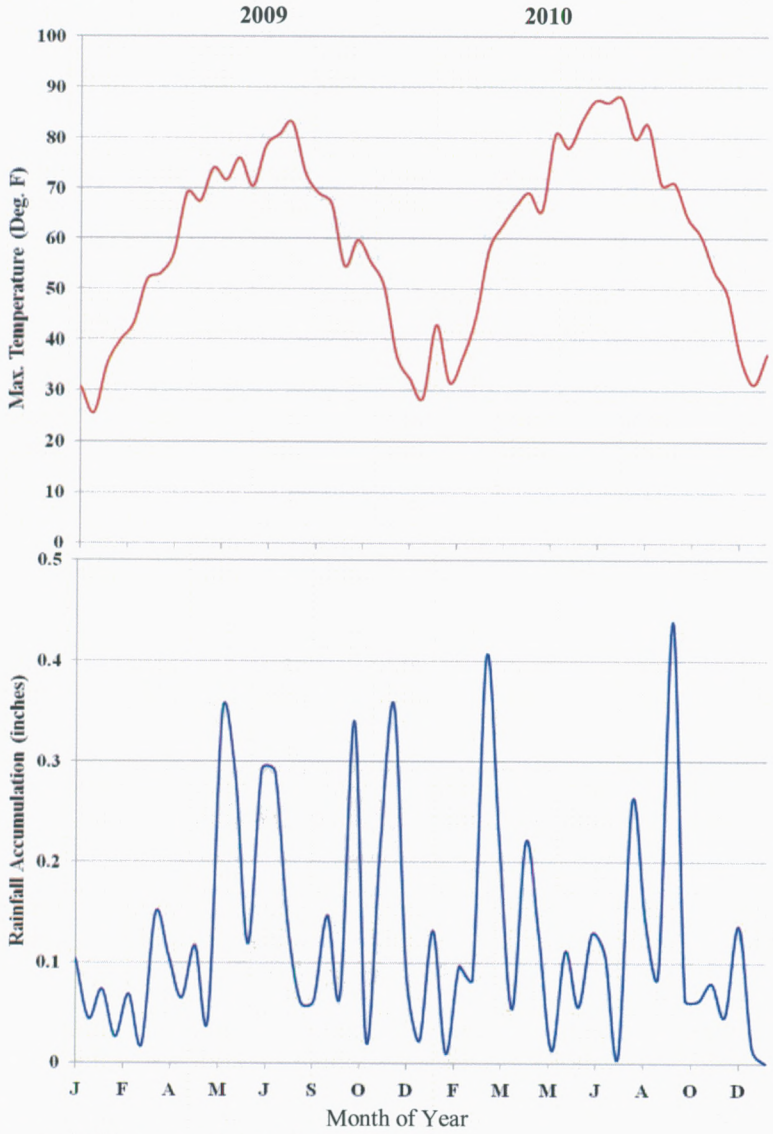


Figure 9- Climate patterns for 2009-2010
Daily data for maximum temperature (°F) and rainfall accumulation (in.) from Sussex 2NW Weather Station.

Average rainfall accumulation for the summer months for 2010 was less than 2009 (Fig.9). For July-August 2010 the mean rainfall accumulation was around 0.16

inches, and for 2009 it was around 0.21 inches. The average precipitation accumulation for these months suffered a decrease of about a 25%. Climate factors such as temperature and rainfall accumulation can be show a trend that correlates with the abundances patterns of nymphal ticks (Fig. 10).

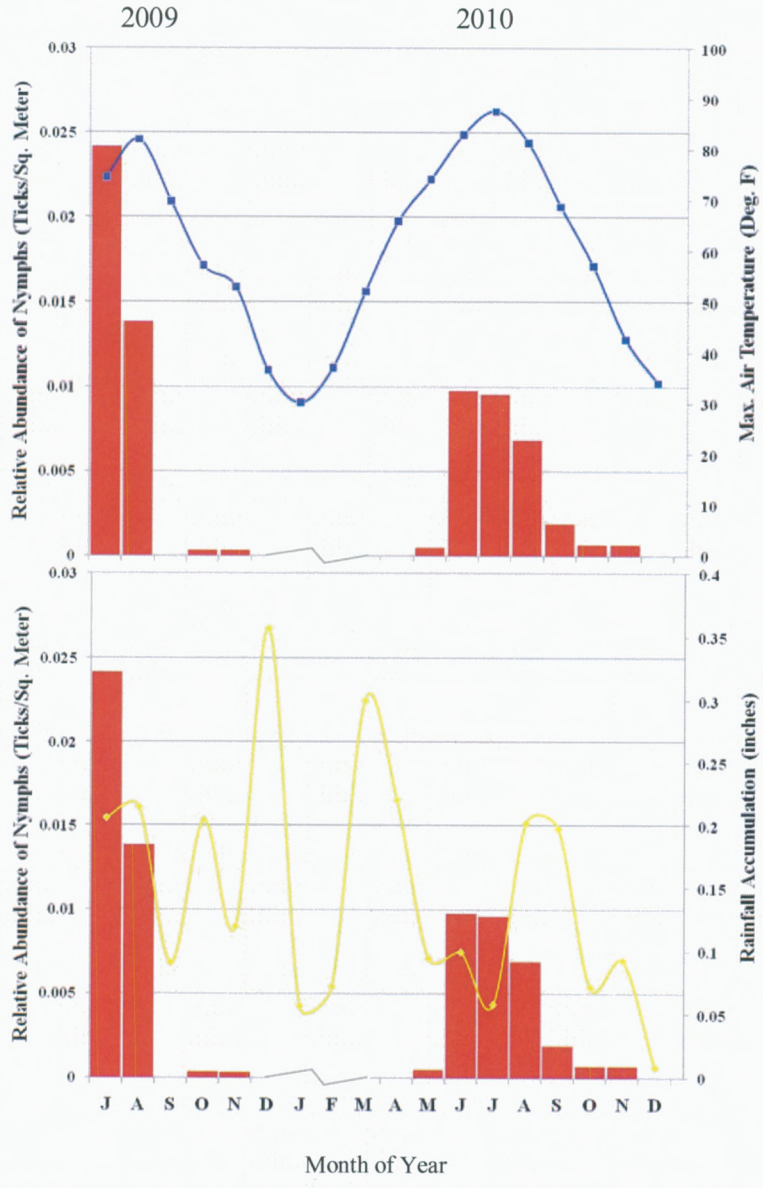


Figure 10- Relative abundance of nymphs vs. Max. Air Temp. and Rainfall Accumulation

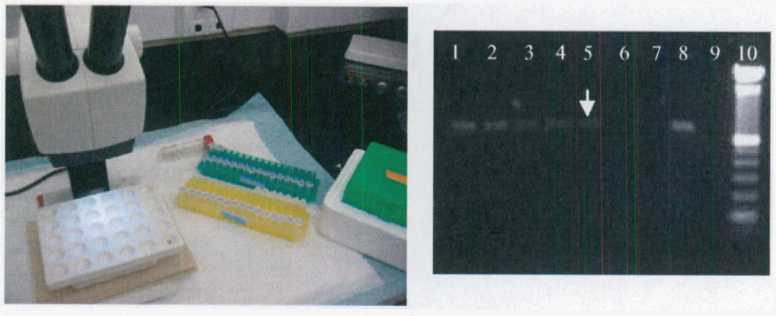
Relative abundance for nymphs from July 2009 until December 2010 and the trends of Maximum Air Temperatures (°F) and Rainfall Accumulation (in.) for the same dates. Nymphal relative abundance are red bars, maximum air temperatures is the blue line and rainfall accumulation is the yellow line.

We are able to see the impact of both these factors on the tick relative abundance. Nymphal abundances were higher in the 2009 season of activity than in 2010. In 2009 the summer months between June-August had a lower air temperature as compared to the same months in 2010. Similarly in 2009 these months show more rainfall accumulation as opposed to 2010. Overall the 2010 season of activity for nymphs was hotter and drier than in 2009.

3.4 Methods optimization

Due to the larger quantity of collected nymphs as opposed to adults and the possibility of their infection with the *Borrelia burgdorferi* bacterium, nymphs were used for optimizing the various methods for nucleic acid extraction and for molecular detection. Optimizing methods to have reliable results also proved to be more efficient and cost-effective for the analysis of *Borrelia burgdorferi* infection. Controls for contamination or error were performed at various steps throughout the different methods and help when trying to pin-point what needed adjusting.

3.4.1 Optimization of extraction methods



(Egan, 2010)

Figure 11- Contamination by use of depression plate.
Set up for DNA extraction showing depression plate. PCR Amplifications for CO1 DNA. (1)-(4):070709S1-4, (5) Depression Plate Negative Control, (6) DNeasy Kit Negative Control, (7) PCR Negative Control, (8) PCR Positive Control, (9) BLANK,(10) Ladder

Both pre-treatment and extraction methods were optimized for obtaining better results when analyzing for tick and pathogen DNA. Pre-treatment was used for minimizing contamination and extraction of foreign nucleic acids that might have been acquired by the tick before and during collection and also during handling and storage.

Initially, a high-throughput approach was intended and a ceramic depression plate was used for pre-treatment and dissection of the ticks (Fig.11). This method was later found to introduce a greater chance for contamination, either due to improper cleaning before and after the protocols or by contamination during the pre-treatment and tick dissection.

In order to maximize the DNA yield from extraction methods the incubation time with Proteinase K was extended for overnight. In addition, dissection was followed by grinding of the tick tissues with a clean and UV-treated pestle. At this point not the entire buffer for lysing (Buffer ATL) is added, but a remainder is left for rinsing off any tissue from the pestle.

3.4.2 Optimization of molecular detection methods

Molecular methods for amplifying *Ixodes scapularis* and *Borrelia burgdorferi* DNA were optimized for easy detection. A summary of the molecular PCR-assays is detailed in Table 4.

Table 4- Summary of Molecular PCR-Assays								
Name of Specimen	*Year*	*Species Name*	*Life Stage*	*Date Collected*	*Location of Collection*	*CO1 PCR*	*B.b. PCR*	*B.b. Re-Amp*
052510AB2	2010	*I. scapularis*	Nymph	5/15/2010	East Lake Hillside	Neg.	N/A	N/A
052510AB1	2010	*I. scapularis*	Nymph	5/25/2010	East Lake Hillside	Neg.	N/A	N/A
080709SC1	2009	*I. scapularis*	Nymph	8/7/2009	West Hillside	Pos.	No bands	Pos.
080709SC2	2009	*I. scapularis*	Nymph	8/7/2009	West Hillside	Pos.	No bands	Pos.
080709SC3	2009	*I. scapularis*	Nymph	8/7/2009	West Hillside	Pos.	No bands	Neg.
080709SC4	2009	*I. scapularis*	Nymph	8/7/2009	West Hillside	Pos.	No bands	Pos.
081909SB1	2009	*I. scapularis*	Nymph	8/19/2009	East Lake Hillside	Pos.	No bands	Pos.
081909SB2	2009	*I. scapularis*	Nymph	8/19/2009	East Lake Hillside	Pos.	No bands	Neg.
081909SB3	2009	*I. scapularis*	Nymph	8/19/2009	East Lake Hillside	Pos.	No bands	Neg.
081909SB4	2009	*I. scapularis*	Nymph	8/19/2009	East Lake Hillside	Pos.	No bands	Neg.
070709S1	2009	*I. scapularis*	Nymph	7/7/2009	Not Specified	N/A	N/A	N/A
070709S2	2009	*I. scapularis*	Nymph	7/7/2009	Not Specified	N/A	N/A	N/A
070709S3	2009	*I. scapularis*	Nymph	7/7/2009	Not Specified	N/A	N/A	N/A
070709S4	2009	*I. scapularis*	Nymph	7/7/2009	Not Specified	N/A	N/A	N/A
082709SB4	2009	*I. scapularis*	Nymph	8/27/2009	East Lake Hillside	Neg.	No bands	N/A
082709SB5	2009	*I. scapularis*	Nymph	8/27/2009	East Lake Hillside	Pos.	No bands	Pos.
082709SB6	2009	*I. scapularis*	Nymph	8/27/2009	East Lake Hillside	Pos.	No bands	Neg.
082709SB7	2009	*I. scapularis*	Nymph	8/27/2009	East Lake Hillside	Pos.	No bands	Pos.
051910SD1	2010	*I. scapularis*	Nymph	5/19/2010	Bottom of Lake	Pos.	N/A	N/A
051910SD2	2010	*I. scapularis*	Nymph	5/19/2010	Bottom of Lake	Pos.	N/A	N/A
070210SA1	2010	*I. scapularis*	Nymph	7/2/2010	NE Lakeshore	Pos.	N/A	N/A
070210SA2	2010	*I. scapularis*	Nymph	7/2/2010	NE Lakeshore	Pos.	N/A	N/A
070210SA3	2010	*I. scapularis*	Nymph	7/2/2010	NE Lakeshore	Pos.	N/A	N/A
070210SA4	2010	*I. scapularis*	Nymph	7/2/2010	NE Lakeshore	Pos.	N/A	N/A
070210SA5	2010	*I. scapularis*	Nymph	7/2/2010	NE Lakeshore	Pos.	N/A	N/A
070210SA6	2010	*I. scapularis*	Nymph	7/2/2010	NE Lakeshore	Pos.	N/A	N/A
070210SB1	2010	*I. scapularis*	Nymph	7/2/2010	East Lake Hillside	Pos.	N/A	N/A
070210SB2	2010	*I. scapularis*	Nymph	7/2/2010	East Lake Hillside	Pos.	N/A	N/A
070210SB3	2010	*I. scapularis*	Nymph	7/2/2010	East Lake Hillside	Pos.	N/A	N/A
070210SB4	2010	*I. scapularis*	Nymph	7/2/2010	East Lake Hillside	Pos.	N/A	N/A
070210SB5	2010	*I. scapularis*	Nymph	7/2/2010	East Lake Hillside	Pos.	N/A	N/A
070210SB6	2010	*I. scapularis*	Nymph	7/2/2010	East Lake Hillside	Pos.	N/A	N/A

3.4.2.1 Amplification of tick genomic DNA

PCR primers selected for confirming *I. scapularis* total DNA extraction, the insect CO1 primers (LCO1490, HCO2198) were selected because these gave positive extraction results even when comparing with primers specific for *I. scapularis* (Egan, pers. comm.). Confirmation of positive DNA extraction was primarily visualized by

electrophoresis and SYBR Safe DNA stain (Fig. 12). The summary of all of the PCR-assays for CO1 are compiled in Table 4. Additionally, DNA sequencing, where *I. scapularis* sequences were shown to be amplified (Egan, pers. comm.).

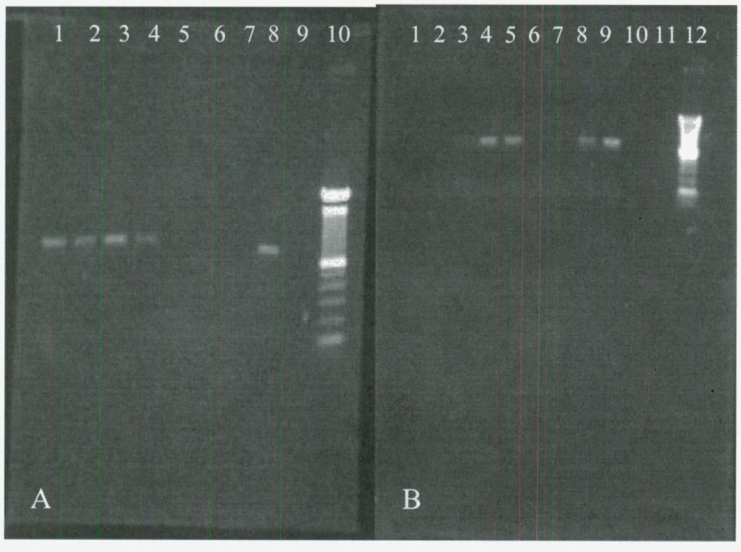


Figure 12- Representative gels of PCR-Assays for CO1 Amplification
A: (1-4) 081909SB1-4, (5) Xtr. Neg.1, (6)Xtr. Neg. 2, (7) PCR Neg, (8) PCR Pos. (9) Blank, (10) Ladder
B: (1) Xtr. Neg., (2-5) 082709SB4-7, (6) Xtr. Neg. 2, (7) PCR Neg., (8) PCR Pos., (9) PCR Pos., (10-11) Blank, (12) Ladder

3.4.2.2 Amplification of pathogen DNA

Borrelia burgdorferi DNA was amplified using primers X14841 forward and reverse (Falco, per. Com). Standard PCR does not yield any visible results through gel electrophoresis. This is probably due to small amount of DNA amplified. Re-amplification of the PCR products was performed to increase this amount of amplified DNA for detection. Gel electrophoresis at this point should was able to visualize amplified DNA products (Fig. 13).

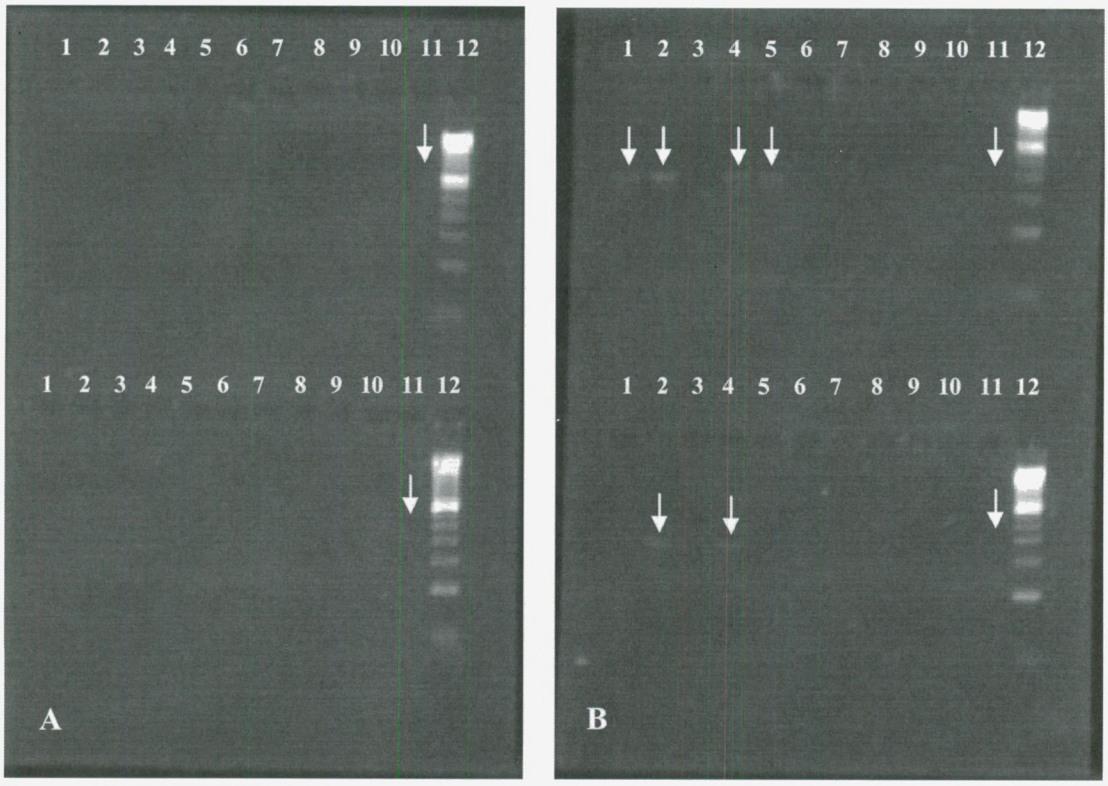


Figure 13- PCR Reactions for Amplification and Re-Amplification of *Borrelia burgdorferi* flagellin.
(A) PCR amplification using X14841 primers, (B) PCR Re-Amp using X14841 primers. For both gels TOP: (1-4) 080709SC1-4, (5-8) 081909SB1-4, (9) PCR NegI, (10) PCR NegII, (11) PCR Pos., (12) Ladder. BOTTORM: (1-4) 082709SB4-7, (5-8) Blank, (9) PCR NegI, (10)PCR NegII, (11) PCR Pos. , (12)Ladder.

3.4.2.3 Stability of PCR positive control

A major concern during the PCR-assays for detection of *Borrelia burgdorferi* was the availability and stability of our PCR positive control. Initially positive controls used were previously recognized samples with the presence of *B. burgdorferi.* In order to discontinue using DNA extracts, *Borrelia burgdorferi* genomic DNA was purchased from ATCC.

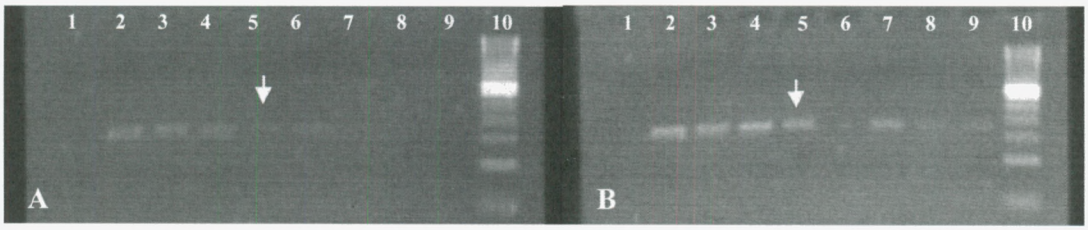


Figure 14- PCR Reaction of *Borrelia burgdorferi* genomic DNA and Re-Amp PCR

(A) PCR amplification of *B. burgdorferi* ATCC genomic DNA and (B) Re-Amplification of PCR. DNA concentrations for both gels: (1) Negative control, (2) 0.05 ng, (3) 0.01 ng, (4) 0.005 ng, (5) 0.001 ng, (6) $5x10^{-4}$ ng, (7) $1x10^{-4}$ ng, (8) $5x10^{-5}$ ng, (9) $1x10^{-5}$ ng, and (10) Ladder.

In order to have a small amount of *B. burgdorferi* as our PCR control, tests to find this amount were carried out and a dilution of genomic DNA that gave of 0.001ng/µL was found to give a faint visualization during the first amplification and a stronger amplification during the second re-amplification (Fig.14). After finding a good volume of dilution, aliquots were kept at -20°C for PCR assays. These PCR positive controls did not amplify constantly after a round of thawing and freezing (Fig. 13). The PCR control DNA at this concentration of 0.001ng/µL does not seem to be stable. Future tests will need to be carried out or fresh dilutions prepared prior to each PCR-assay.

3.5 Rates of infection of *Borrelia burgdorferi*

Thirty two nymph *I. scapularis* collected on 2009 and 2010 were used for DNA extraction. From the samples studied only 81% showed a good quality of DNA extraction (amplification of CO1 fragment of DNA). Most of the samples that did not have an optimal extraction result were not utilized for PCR-assays for detection of *B. burgdorferi.*

From these positive DNA extraction samples, 56% did not have reliable results for detection of the pathogen. This was due to either contamination during PCR re-amplification or during troubleshooting of both PCR assays. The results from this group were not taken into consideration for infection rate calculations.

The infection rate of the nymphs that had reliable PCR-assay results is 54.5%. This infection rate is from a total sample size of eleven nymphs.

Discussion

This study is the first study focusing on tick surveillance and the infection rates of ticks in northwestern New Jersey. Other areas in New Jersey, mostly central and southern NJ, have been studied including: Atlantic, Burlington, Camden, Cumberland, Essex, Gloucester, Hunterdon, Mercer, Monmouth, Morris, Ocean Salem, Somerset, Sussex, Union, and Warren Counties (Schulze et al. 2003). However previous studies in NJ have focussed on adult ticks and in some cases had very limited sample sizes in order to broadly sample across the state. In addition, the study by Schulze and co-workers did not determine seasonal activity patterns at any of these sites. Rather, collections made at each site were of as few as one to several ticks per site. In Sussex County, the sites previously focused on were the central and southern regions of the county. The NJSOC is in northwest of Sussex County (Fig. 6).

Populations of the *Ixodes scapularis* tick have been shown to be present in northeast United States for many years. Some reasons attributed to this change are the manner land usage changed as reforestation occurred and the increase in white-tail deer populations (Barbour & Fish, 1993). In order to understand the population's dynamics of *I. scapularis* and other ticks, various methods of collection have been used (Ginsberg & Ewing, 1989). The method of collection used in this study gave only a relative population abundance of the *Ixodes scapularis* tick, but was an efficient method for collection of all life-cycle stages of the tick.

The relative abundance of the nymph and larvae decreased in 2010 seasons from the year 2009, and increased for adults. According to a study by Schulze (2009), abiotic factors such as air temperature and rainfall have not been useful in predictions of

abundances of ticks. However in a smaller scale rainfall and air temperature may have a role in regulating the dynamics of questing for hosts, since ticks need sufficient humidity for water regulation during long periods of questing and survival between bloodmeals (Nieto et al. 2010). Our results likewise suggest that immature stages of the *I. scapularis* tick, the larvae and nymph, may have been affected by the increased temperatures and decreased rainfall that occurred in 2010. Future work on the influence of air temperature and rainfall data on the questing dynamics of the tick can be enhanced by measurements at the site of collection rather than record data from a weather station, as well as by monitoring cohorts of ticks across seasons and life-cycle stages to explore whether short term weather patterns affect tick survival rates to the next life-cycle stage or whether the patterns observed merely reflect decreased host-seeking activity in the short term.

Nymph tick samples were used in a PCR-assay to detect the presence of the *Borrelia burgdorferi* pathogen. A rate of infection of 54.5% was found from a sample size of 11 nymphs. This rate of infection is much higher than other studies in nearby areas, for example in Westchester NY a 19% of *Borrelia burgdorferi* infection of collected nymphs was seen (Schwartz et al. 1997). If true, this would suggest a higher risk for contracting Lyme disease. However, the sample size tested is still very small and may not be a reliable indicator of the actual infection rate. A bigger sample size is needed for a more accurate rate of infection. In addition adults *I. scapularis* should also be tested for *Borrelia burgdorferi*. For adults in southern and central areas of Sussex County a rate of infection of 52.2% and in Westechester NY an infection rate 52% were observed (Schwartz et al. 1009) (Schulze et al. 2003). If our preliminary infection rate for

nymphs remains higher than other areas in this region, then it is possible that the adult *I. scapularis* ticks the NJSOC have a higher rate of infection as well.

Future analysis of the tick samples collected will utilize the optimized methods for confirming good nucleic acid extraction and detection of pathogens such as the *Borrelia burgdorferi* bacterium from this study. Data derived from these future analyses can be of great importance in understanding population dynamics for the *Ixodes scapularis* tick and the infection rate of such populations in New Jersey. Studying the primary vector for Lyme disease can help other work that focuses on public health approaches for prevention of this disease.

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