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The effects of global climate change and habitat modification on the incidence of Lyme disease

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THE EFFECTS OF GLOBAL CLIMATE CHANGE AND HABITAT MODIFICATION ON THE INCIDENCE OF LYME DISEASE

by

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ABSTRACT

Lyme disease is one of the most common vector-borne diseases around the world, and the numbers of reported cases are quickly rising. *Ixodes* ticks are the principal vectors, while *Borrelia burgdorferi sensu lato* genospecies are the etiological agents of the disease. Climate change, namely global warming, and habitat modification, namely forest fragmentation, are hypothesized to play an active role in this rise in reported cases. An analysis of the primary literature, specifically of studies focused on North America and Europe, was conducted in order to investigate these hypotheses. These studies show that global warming has precipitated a growth in tick populations as well as a northward tick migration, thereby increasing the risk of Lyme disease in emergent and endemic areas alike, for *Borrelia* spirochetes quickly infect naïve tick populations. Furthermore, published studies support the idea that forest fragmentation near human population centers has also increased the risk of Lyme disease in North America, for edge habitats provide suitable conditions for ticks and provide edible vegetation for the animals on which ticks feed, animals which also serve as hosts for *B. burgdorferi sensu lato*. In contrast, a decrease in fragmentation was found to facilitate tick invasion and establishment in Europe. These studies demonstrate that anthropogenic habitat modifications of varying types can affect ticks and their host populations and increase the

risk of Lyme disease near human population centers. However, more research needs to be done to truly understand the different factors that are precipitating the rising number of cases of Lyme disease since there are significant interactions between climate change, habitat modification, and other drivers not examined here. Furthermore, understanding how these drivers function in specific geographic locations can help scientists and public officials tailor local public health measures appropriately. Finally, researchers and pharmaceutical companies must develop a safe, long-lasting, and effective vaccine against the Lyme disease spirochete, for there is not one currently available. Although easily treatable if diagnosed early, Lyme disease can progress to debilitating disease. Unfortunately, the risk of contracting this illness is currently rising and will continue to rise unless effective preventative measures are employed.

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LIST OF ABBREVIATIONS

CDC	Centers for Disease Control
CFC	Chlorofluorocarbons
CLD	Chronic Lyme Disease
ELISA	Enzyme Linked Immunosorbent Assay
EM	Erythema Migrans
EPA	Environmental Protection Agency
ERID	Emerging and Re-emerging Infectious Diseases
NLCD	National Land Cover Database
NOAA	National Oceanic and Atmospheric Administration
ORF	Open Reading Frame
OSP A-C	Outer Surface Protein A-C
PCR	Polymerase Chain Reaction
PLDS	Post Lyme Disease Syndrome
UK	United Kingdom
WHO	World Health Organization

INTRODUCTION

In the mid-1970s, between 50 and 60 cases of a rheumatoid arthritis-like condition were reported around the town of Lyme, Connecticut. Children in particular were developing rashes, fevers, swollen joints, and sometimes other more serious conditions after being bitten by deer ticks. In 1975, Lyme disease was recognized as a new form of inflammatory arthritis, taking its name after the town of Lyme, Connecticut where the condition was first described[1,2]. The clustering of cases in Connecticut, as well as reports of a similar condition in Europe starting at the beginning of the 20th century, focused academic attention on finding a cause for these symptoms. In the early 1980s, that search came to a close when a scientist named Dr. Wilhelm "Willy" Burgdorfer identified a spirochete as the etiological agent of Lyme disease. This bacterium, named in Dr. Burgdorfer's honor, is called *Borrelia burgdorferi*.

Lyme disease is a vector-borne illness, carried and transmitted to mammalian, avian, and even reptilian hosts by ticks of the *Ixodes* genus. *Ixodes scapularis* and *Ixodes pacificus* are the primary tick vectors in the United States, while *Ixodes ricinus* and *Ixodes persulcatus* are the primary vectors in Europe[3]. *Ixodes scapularis* is commonly known as the blacklegged deer tick. Ticks can attach themselves to any part of the human body, but commonly migrate to warm, moist areas where they might be hard to see such as the groin, armpit, and scalp[4]. Adult ticks are about the size of a sesame seed, while nymphs are about the size of a poppy seed, making them even harder to see. Most humans are infected through the bites of nymphal ticks due to their small size, relative greater numbers compared to adults, and their spring and summer feeding schedule.

During these warmer months of the year, humans spend more time outdoors and are therefore more likely to come into contact with questing (host-seeking) nymphs. Adult ticks by comparison are more active during the colder months when humans are less likely to spend time outdoors in tick habitat[4]. Centers for Disease Control and Prevention (CDC) statistics show that approximately 30,000 cases of Lyme disease are reported each year, making Lyme disease the most common vector-borne illness in the United States (Figure 1). Furthermore, the vast majority of these cases are reported in only 14 U.S. states (Figure 2). However, the CDC estimates that the true number of cases is likely 300,000 cases per year because only a fraction of cases are reported[5].

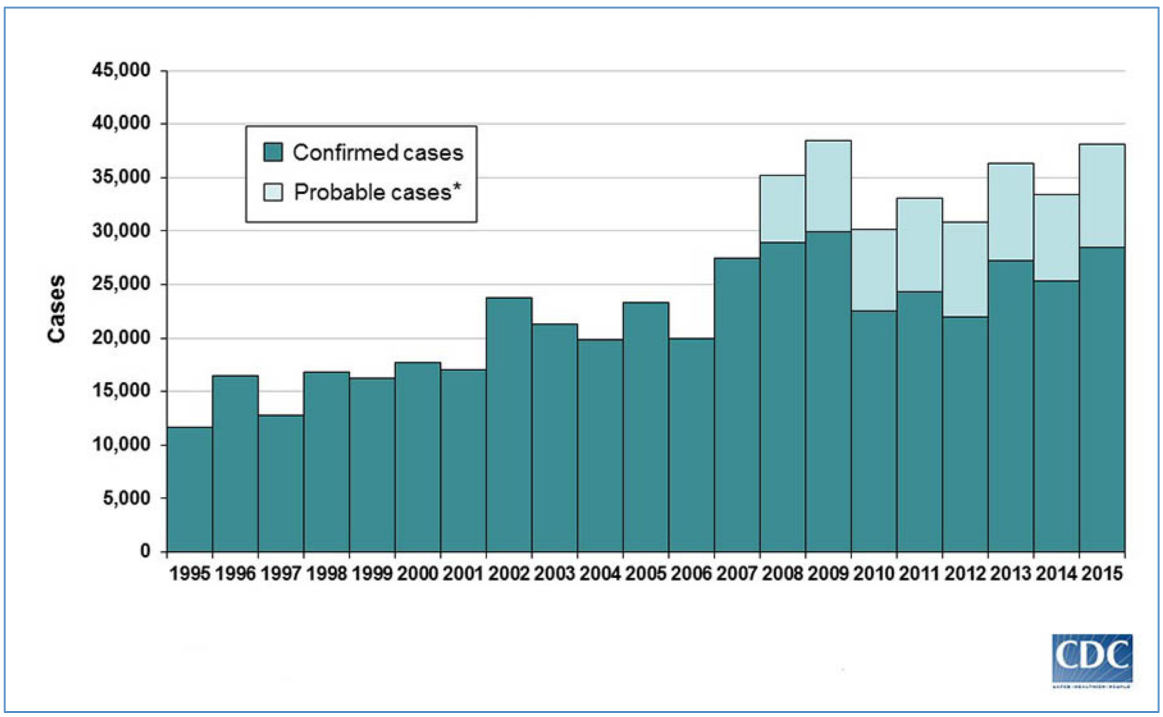

Figure 1: Reported cases of Lyme disease in the United States by year[6]

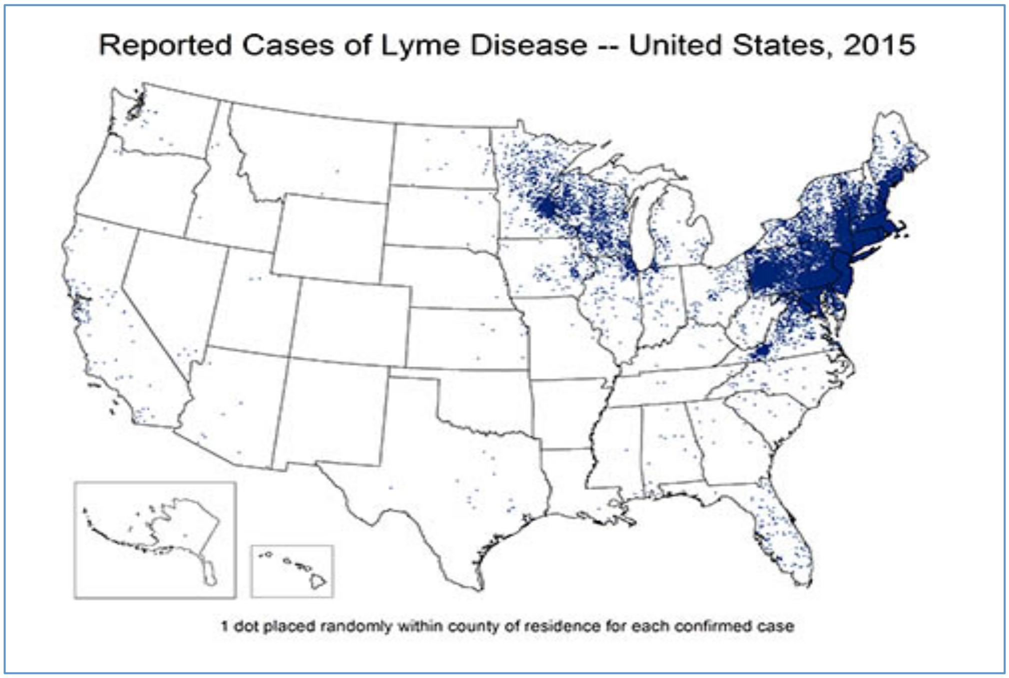

Figure 2: Reported cases of Lyme disease in the United States in 2015. 96 percent of reported cases come from 14 US states[6].

The life cycle of *Ixodes* ticks can take two years to complete as the ticks progress from egg to larvae, nymph, and adult (Figure 3). Ticks can become infected with *B. burgdorferi* spirochetes during any of these life stages, excluding the egg stage. Uninfected ticks ingest spirochetes along with the blood meal from an infected host. After ingestion, the spirochetes multiply in the midgut of the tick, where they persist through the molt to the next stage. During this period, the spirochetes are restricted to the midgut of the tick, and do not infect other tissues. During the next blood meal, the spirochetes migrate from the midgut to the salivary glands, where they can infect the next host through the tick saliva. This migration is a slow process, and efficient spirochete transmission to the mammalian host does not occur until 48 hours post-tick attachment[3].

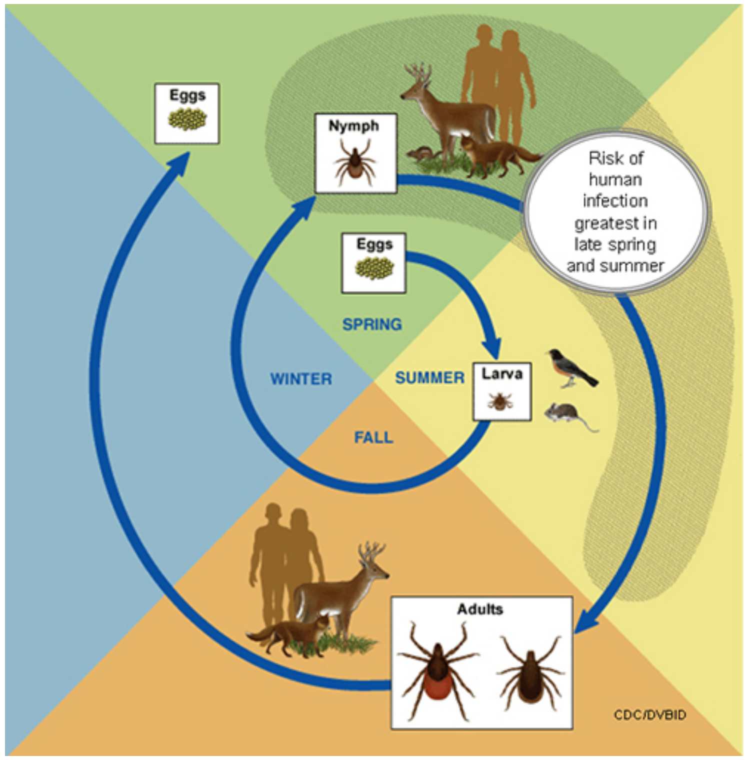

Figure 3: Life cycle of blacklegged ticks[4]

The progression of Lyme disease has customarily been divided into three stages: Early Localized, Early Disseminated, and Late Disseminated Lyme disease. The most common manifestation during the Early Localized stage is erythema migrans (EM), more commonly referred to as the "bulls-eye rash". EM typically presents within 7-14 days post-infection, appears in 80%-90% of cases, and is characterized by a migratory, ovoid or circular erythema (reddening of the skin)[7,8]. Other symptoms during the Early Localized stage include flu-like symptoms such as fever, fatigue, and stiffness. During the Early Disseminated stage, the *B. burgdorferi* spirochetes disseminate throughout the body via the blood or lymph, and infect multiple types of tissues including nervous, musculoskeletal, and cardiovascular tissues. Multiple EM lesions can also appear on the skin at sites distant from the initial site of infection[7]. Joint inflammation can cause arthritis-like symptoms, cardiac infection can lead to a prolonged PR interval (the time between the onset of atrial and ventricular depolarizations) and sometimes complete heart block, and nervous tissue infection can lead to meningitis and facial palsy, among other conditions[9,10]. If untreated, the disease can progress to the Late Disseminated stage, during which chronic arthritis is the primary symptom. However, additional neurological, cardiac, and dermatological symptoms can also occur, although rarely.

Lyme disease can be difficult to diagnose due to the variety of symptoms presented, and because the symptoms are similar to those seen in many other illnesses. EM is the only symptom specific to Lyme disease, but doesn't appear in all cases of Lyme disease. Furthermore, the EM rash may be confused with other skin conditions because only 19% of EM rashes are the characteristic "bulls-eye" rash[11]. The remaining 81% do not exhibit the target-like appearance, but appear as a solid, reddened patch of skin. The gold standard laboratory test involves a combination of spirochete culture followed by polymerase chain reaction (PCR) to directly confirm the presence of the spirochetes. However, these tests are not used frequently. Culturing the bacteria is expensive because it requires special media and laboratory expertise, and the concentration of spirochetes in the various bodily fluids (blood, lymph, cerebral spinal fluid, etc.) is not typically high enough to return a positive PCR result. Furthermore, *B. burgdorferi* is a slow growing organism, and results from culture can take 2-6 weeks to become available, limiting clinical usefulness[12]. Therefore, other tests for Lyme disease are employed clinically. In 1995, the CDC and other health agencies endorsed a twotiered testing algorithm[13]. The first tier involves an Enzyme-Linked Immunosorbent Assay (ELISA) that tests for the presence of antibodies in the patient that bind to antigens derived from *B. burgdorferi* whole-organism lysates. False positives can occur in patients with other borrelial diseases, some other spirochetal and bacterial infections, and with some autoimmune diseases. If this first test is positive, a confirmatory test is done. The second tier test involves a Western blot, also called an immunoblot. This assay tests for patient antibodies produced in response to borrelial antigens. However, these two tests can produce false positive results, so different studies have worked to improve the reliability of the ELISA and western blot testing algorithm[14]. In most cases though, Lyme disease can be diagnosed and treated based on a clinical exam and the patient history, including epidemiological risk factors.

The standard treatment for Lyme disease is a course of antibiotics, and tetracyclines, β-lactams, and macrolides are most effective[12]. Specifically, doxycycline, amoxicillin, oral cefuroxime axetil, and intravenous ceftriaxone are the first line agents, while macrolides are mostly used when theses first-line agents cannot be tolerated[15]. In general, oral antibiotics are recommended for early, localized infection, while intravenous antibiotics are reserved for patients experiencing neurologic symptoms, cardiac disease, or Lyme arthritis[11]. Due to the low rate of infection after a tick bite, primarily due to the length of time required for bacterial transfer, a full course of antibiotics is not typically recommended before symptoms appear. In fact, studies have shown that a single dose of doxycycline administered within 72 hours of tick removal is effective at reducing the rate of infection[12]. When a full course is required, older guidelines recommend a 20-day course of antibiotics, but more recent studies have shown that a 10-14 day course is just as effective as the longer treatment durations[15]. Most guidelines do not recommend treatment for longer than 2-4 weeks, but there is significant controversy surrounding this topic.

Some patients who have been diagnosed with Lyme disease report continuing symptoms, referred to as chronic Lyme disease (CLD) or post-Lyme disease syndrome (PLDS). These symptoms include pain, fatigue, neurologic disturbances, and cognitive slowing, and persist regardless of whether the patient received appropriate antibiotic treatment[16]. While the symptoms themselves and their impact on quality of life are not disputed, there is significant controversy surrounding the cause of the symptoms as well as the appropriate treatment. Some groups argue that a lingering, intracellular *B. burgdorferi* infection is to blame, and a prolonged course of antibiotics is the appropriate treatment regimen[11,17]. However, the scientific majority – including the American Academy of Pediatrics, the American College of Rheumatology, and the Infectious Diseases Society of America – rejects the theory of CLD/PLDS and protracted infection based on published studies and clinical trials. Instead, one of the prevailing hypotheses is that cross-reactive antibodies produced in response to the borrelial infection cause the symptoms described. Furthermore, the scientific majority does not recommend prolonged antibiotic therapy due to a lack of clinical evidence and the dangers of extended antibiotic use. Indeed, a number of clinical trials comparing the recommended treatment to extended treatment have shown no benefit of protracted antibiotic use[16,18–21].

As mentioned, *Borrelia burgdorferi* is the main etiological agent of Lyme disease in the United States. *Borrelia garinii* and *Borrelia afzelii* are the main genospecies that cause the disease in Europe. As a member of the phylum *Spirochaetaes*, *Borrelia* species are related to *Treponema pallidum* (the etiological agent of syphilis) and *Leptospira* species (the etiological agents of leptospirosis). The spirochete has both an inner and an outer cell membrane, and has a cell wall structure most similar to Gram-negative bacteria, although it cannot be Gram stained. *B. burgdorferi* also has a distinctive outer membrane composition. The spirochete's outer membrane consists of an abundance of lipoproteins, namely Outer Surface Proteins A-C (Osp A-C), but contains relatively few transmembrane proteins and lacks lipopolysaccharides and phosphatidylethanolamines completely[22,23]. Phosphatidylethanolamines are the principal phospholipids in Gram-negative bacterial outer-membranes. This distinctively different outer membrane precludes classification as Gram-negative, despite the characteristic double membrane. The *B. burgdorferi* spirochete is 10-30 μm long, 0.2-0.25 μm wide, and has a spiral shaped body consistent with other members of the phylum[24]. The organism also has numerous periplasmic flagella located between the inner and outer membrane at the ends of the organism that are used for motility, giving a characteristic corkscrew movement[3,24].

The genome of the *B. burgdorferi* spirochete is unusual in structure in that it is highly segmented. It has a small, ~911 kB linear chromosome with an average G-C content of 28.6%, and 853 open reading frames (ORFs) with an average size of 992 base pairs[3]. In addition to the linear chromosome, *B. burgdorferi* contains numerous linear and circular plasmids ranging from 5-56 kB in length, with some isolates containing greater than 20 plasmids [3]. There are a predicted 670 ORFs on the plasmids, but only 5.8% have convincing similarity to genes in other organisms with known function[25]. Research has shown that *in vitro* plasmid loss after long-term culture results in reduced infectivity in mice, indicating that virulence factors are encoded by the plasmids[26]. In comparison with other microorganisms, *B. burgdorferi* has few redundant gene sets, with the exception of a full set of motility genes that occupy 6% of the chromosome[25]. One hypothesis that might explain the lack of redundant genes is that they were lost during the transition to an obligate parasite life cycle[25]. What remains clear is that cell motility is crucial to spirochete proliferation.

Borrelial outer surface proteins play an important role in bacterial survival in the tick, as well as an important role for infection in humans. OspA is important for adherence to the tick midgut, while it is suspected that OspC plays a role in the spirochete migration from the tick midgut to the salivary glands as well as for the subsequent infection of the mammalian host[10]. Furthermore, other borrelial outer surface proteins have been linked to an ability to evade the immune system. Specifically, OspE has been shown to bind to human Factor H, a complement protein inhibitor[27]. Binding this inhibitor allows the bacteria to further evade the host immune response, specifically the innate immune response. Animal studies have shown that the innate immune system, including complement, is important for controlling Early Disseminated Lyme disease[10].

B. burgdorferi is an obligate parasite, meaning that it must obtain many of the nutrients necessary for life from its host. Interestingly, *B. burgdorferi* is one of the few pathogenic microorganisms that does not require iron (Fe^{2+}) to grow, nor does the organism encode any proteins that require iron for their function[25,28]. In fact, the spirochete genome does not have the necessary coding sequences for an iron transport system. The energy needs of the organism are met solely through glycolysis and substrate level phosphorylation. The genes coding the glycolytic enzymes have been identified, indicating that *B. burgdorferi* utilizes glucose for its energy needs. Maltose, mannose, n-acetyl glucosamine (GlcNac), chitobiose, and glycerol have also been recognized as additional carbohydrates that the spirochete may use in the glycolytic pathway[29]. However, the spirochete completely lacks the genes necessary for the tricarboxylic acid cycle and oxidative phosphorylation, indicating that glycolysis is the only energy-producing pathway available to the organism. Furthermore, *B. burgdorferi* does not encode the enzymes necessary for *de novo* biosynthesis of fatty acids, amino acids, or nucleotides[30]. Pyruvate, the product of glycolysis, is converted to lactate in order to regenerate NAD+, and all reducing power available to the organism comes from the pentose phosphate pathway[26].

The drastic changes in environment between the tick and the mammalian host – including changes in pH, temperature, nutrient availability, and cell density – are stressful for the organism. Predictably, these environmental changes induce changes in bacterial gene expression, most notably in the gene expression of the outer surface proteins. OspA is up regulated in the tick environment, while OspC is down regulated. During tick feeding, the expression of these proteins is reversed, and once the spirochete has entered the human environment, OspA is down regulated and OspC is up regulated[3,31,32]. The increase in OspC levels is also observed in the laboratory when cultures are shifted from

23°C (mimicking the tick host conditions) to 37°C (mimicking the mammalian host conditions)[32]. Interestingly and understandably, OspE expression also increases when the cultures are switched from 23°C to 37°C, for OspE is an important lipoprotein for bacterial dissemination and immune system evasion, as discussed[32]. Antibodies directed at these outer surface proteins have been shown to be effective at preventing *B. burgdorferi* infection, directing research endeavors towards an effective vaccine[31].

GOALS

Studies conducted by the National Aeronautics and Space Administration (NASA) and other expert organizations and scientists have demonstrated that the earth's climate is changing, and agree that human activities are the root cause. Furthermore, human activities are changing and/or damaging many different natural ecosystems and endangering many different types of species in the process. The objective of this thesis is to investigate whether global climate change and environmental modification have had an effect on the incidence of Lyme disease. This thesis will also investigate whether the geographic distribution of *Ixodes* ticks has increased, or is predicted to increase, in response to global climate change. The primary focus of this work will be on Lyme disease and *Ixodes* ticks in North America, but data from Europe will also be examined. After reading this thesis, the reader will understand how global climate change and environmental modification may be driving the emergence of Lyme disease in new regions of North America and Europe. Additionally, the reader will acquire a greater understanding of Lyme disease in general. As the most common vector-borne illness in the United States, it is important to have a general knowledge of the disease to dispel misinformation.

DISCUSSION

Drivers for the Emergence of Lyme Disease:

Since the mid-1970s, the World Health Organization (WHO) has reported over 40 emerging and re-emerging infectious diseases (ERIDs) around the world[33]. Diseases such as dengue fever, malaria, HIV/AIDS, West Nile virus, and Lyme disease have all shown significant increases in the number of cases reported per year around the world. For these diseases and many others, human activities play an important role in their emergence or re-emergence. Factors such as the increasing growth and mobility of the world's population, poor sanitation in crowded areas, international distribution of food, overuse of antibiotics, and ecological variables and changes all contribute to ERIDs[34]. For Lyme disease in particular, climate change and land fragmentation have been investigated as important drivers for the emergence of the disease in North America and in Europe.

Climate Change:

Climate change has been investigated as a driver for emergence of Lyme disease, and it is therefore important to review climate change data before examining any changes in the rates of the disease. The evidence that the planet's climate is changing is almost overwhelming. Yet, the topic is still hotly debated in political arenas. However, most of the scientific community is in agreement that the planet's climate is changing, that human activities are the root cause, and that these changes will have dire consequences unless steps are taken to reduce anthropogenic impact. Perhaps the most publicly recognizable aspect of climate change is global warming. The global temperature has been carefully recorded since the 1880s and nine of the ten warmest years on record have all occurred since 2000 (Figure 4). Indeed, the warmest year recorded in recorded history was 2015[35]. 2015 was also the first year when global temperatures had increased by an average of 1 °C or 1.8 °F above the 1880 baseline average[36]. The cause for this increase in global temperature is attributed to a greenhouse effect caused by the sharp rise in carbon dioxide (CO_2) levels seen in recent decades.

Greenhouse gases, like CO_2, are called greenhouse gases because they trap heat in the earth's atmosphere. Radiation from the sun strikes the surface of the earth and warms it. As the surface heats up, some of this thermal energy is released back into the atmosphere. The greenhouse gases in the atmosphere absorb the infrared thermal energy that gets released from the surface, and then re-emit that thermal energy in all directions. Some of this energy is directed back toward the planet's surface, and this "downward" portion of the energy further warms the surface of the planet. Then the process begins again with even more energy than before. Furthermore, greenhouse gases also absorb some of the solar radiation entering the atmosphere for the first time. As the concentration of these gases increase, more of that energy will be absorbed, exacerbating the problem. This process is what the earth is experiencing right now.

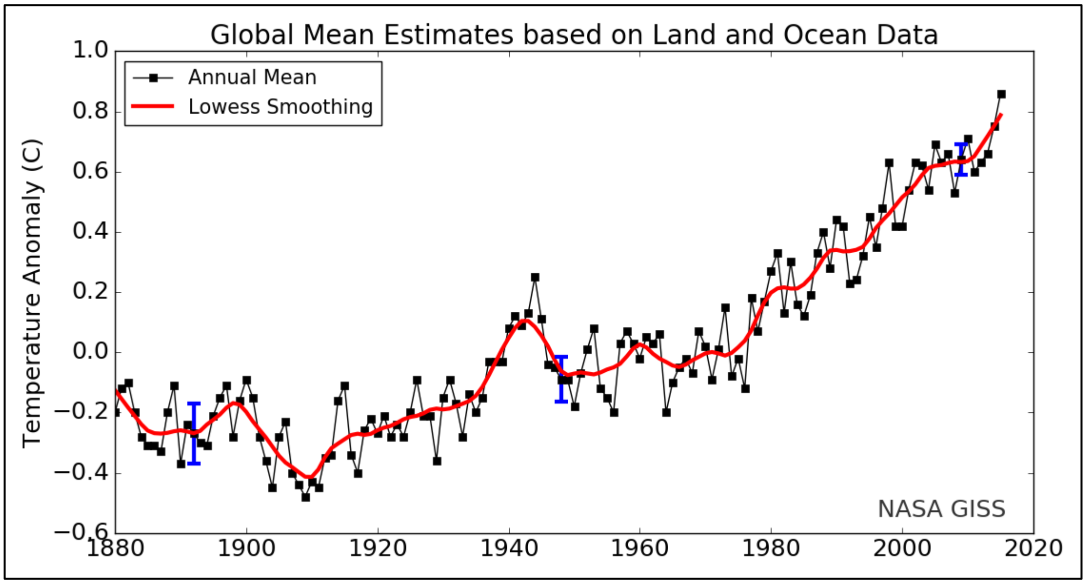

Figure 4: Global mean temperature recordings over time[37]

Human activities since the beginning of the industrial revolution have increased the concentration of CO_2 in earth's atmosphere by more than a third. In those short 150 years, CO_2 levels have risen from about 280 parts per million to just over 400 parts per million (Figure 5)[38]. However, CO_2 is not the only greenhouse gas present in the atmosphere. Nitrous oxide, methane, chlorofluorocarbons (CFCs), and even water vapor all function as greenhouse gases. Unfortunately, the problem of greenhouse gases doesn't stop there. The subarctic tundra, also called the permafrost, stores large amounts of CO_2, methane, and nitrous oxide in the frozen ground, and as global temperatures have risen, this ground has begun to thaw. As the ground thaws, the trapped greenhouse gases are released into the atmosphere and contribute to global warming, creating a positive feedback loop[39,40].

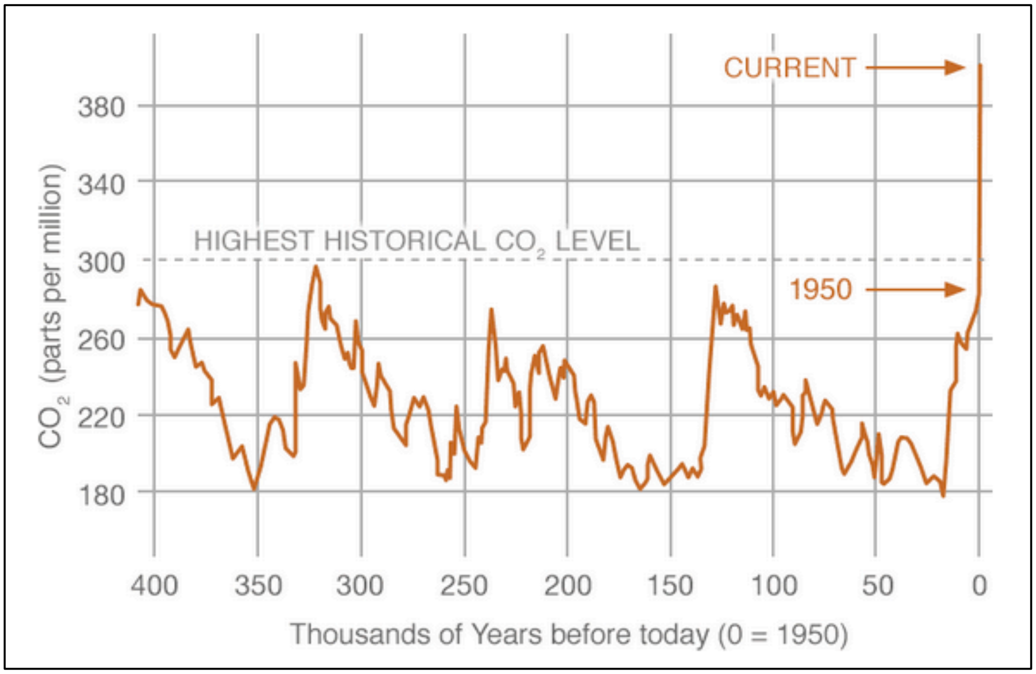

Figure 5: Atmospheric carbon dioxide levels over time[41]

Although an extra 120 parts per million of CO_2 may sound insignificant, its effects are already extreme. Sea levels are rising and threatening coastal populations and species; the oceans are warming and dissolved CO_2 levels are rising, slowing the deep ocean currents critical for a healthy ocean and destroying coral reefs respectively; land ice and sea ice are melting, threatening the species that rely on it; and extreme weather events are increasing around the world[36]. If the world's population does not unite to tackle the problem of climate change, the world of tomorrow may look very different from the world of today.

Land Fragmentation:

Land fragmentation, like climate change, has also been investigated as an important driver for the emergence of Lyme disease, land fragmentation refers to the mixture of human-maintained land, such as farmland, gardens, lawns, hedges, etc., and undeveloped land, like forests and wetlands. This type of landscape is common in Lyme endemic areas, and is usually prevalent around human population centers. Land fragmentation also refers to the active process of converting back and forth between undeveloped and developed land. This thesis will focus on the intersection between developed land and forests, rather than other types of natural habitats, because forests represent the ideal habitat for the mammal and avian species that serve as hosts for *Ixodes* ticks and reservoir hosts for the Lyme disease spirochete.

Forests cover 31% of the land on planet earth, producing the oxygen necessary for life and consuming the CO_2 in the atmosphere, thereby mitigating the progression of climate change. Unfortunately, deforestation – whether from fire, clear-cutting, logging, or degradation due to climate change – is threatening many of the forests around the world. Some 46,000-58,000 square miles (120,000-150,000 square kilometers) of forest are lost every year[42]. As an example, around 17% of the Amazon rainforest has been lost in the last 50 years, mainly due to clear-cutting for cattle ranching, oil drilling, mining, and logging[42]. The importance of the world's forests cannot be understated. As mentioned, forests help mitigate the effects of greenhouse gas emissions by acting as carbon sinks, but they become carbon sources when cut or burned. It is estimated that tropical forests alone hold more than 210 gigatons of carbon, and that deforestation in these areas contributes about 15% to global greenhouse gas emissions[42]. Furthermore, trees help regulate the water cycle, help provide clean drinking water, buffer the effects of natural disasters, and provide habitat for more than half of the world's land-based species[43]. Clearly, forests around the world are critical for a healthy planet and their survival is of paramount importance.

Lyme Disease in North America:

Lyme Disease and Climate Change:

The number of cases of Lyme disease in the United States has increased substantially over the past 30 years[6]. Part of this trend is attributed to higher rates of reporting and greater public awareness, but many researchers are now investigating whether global climate change has played a role in these increasing rates. As discussed, the world's climate is changing. The average concentrations of CO_2 and other greenhouse gases in the atmosphere are climbing, leading to a rise in average global temperature and a diverse array of other deleterious effects. These global changes have resulted in warmer temperatures at more northern latitudes and at higher altitudes. The hypothesis is that these conditions (particularly the milder winters that result from a warmer global temperature) will increase the number of ticks surviving to reproductive maturity at these northern latitudes and higher altitudes. As a result of this increased number of reproductively mature ticks, more ticks will become infected with the Lyme disease spirochete, and as the ticks spread northward, the number of cases of Lyme disease will increase.

Ixodes tick populations require specific environmental conditions to survive, including a limited temperature range and level of rainfall. It has been hypothesized that there is a northern boundary where the temperature in particular becomes too cold too early to permit tick survival. Perhaps most importantly, temperature affects the rate of development for *Ixodes* ticks. More specifically, colder temperatures increase the time for development for each life stage of the tick[44–46]. At this theorized northern boundary, temperatures become too cold too quickly for eggs to hatch, for larvae to molt into nymphs, and for nymphs to molt into adults. Under these conditions, tick populations become unsustainable because fewer individuals survive to reproductive maturity. Warmer temperatures earlier in the year, therefore, allow ticks to develop, find a suitable host, and mate more quickly. However, warmer temperatures can also affect ticks and tick behavior in a negative way. Tick questing (host-seeking) behavior peaks at 25°C, and this behavior decreases as the temperature increases[47]. Therefore, as the temperature increases beyond a certain point, fewer ticks will find a host and fewer will molt to the next life stage. Furthermore, fewer adult females will find a host and a mate, and fewer eggs will be laid. The end result is the same as when considering colder temperatures. The number of ticks surviving to reproductive maturity will fall, and tick populations will become unsustainable.

Numerous studies have modeled how climate change and global warming will affect tick populations in North America, and how those populations have already changed. Ogden et al. (2005) developed an *I. scapularis* population model in order to simulate the effects of temperature on the survival of tick populations and the seasonality of different tick life stages. The model was built using 12 different states, with each state representing a different tick life point. The authors used specific temperature data from Ontario, Canada and Maryland, USA as well as the known relationships between temperature and development rate, questing rate, and mortality rate as model parameters in order to investigate how temperature affects these different tick life points. The results generated from the model were validated through comparison to direct observations on tick questing activity in 1991 and 1992 made by Lindsay et al. (1999). The authors found that *I. scapularis* population numbers increased linearly with increasing temperature above a certain threshold. This threshold temperature varied between Ontario and Quebec, and was measured in degree-days >0°C (the sum of the differences between 0°C and the recorded average daily temperature over a given time period). The threshold was calculated to be 2826 degree-days > 0°C in Quebec, and 3063 degree-days > 0°C in Ontario. Overall, the study found that even a decade ago, the regions of Canada that were experiencing temperature conditions suitable for tick population establishment were more extensive than the distribution of ticks at the time. This result indicated a clear potential for the spread of ticks into other regions in Canada. Furthermore, this finding is in line with the observation that in the preceding decade (1995-2005), the number of areas in Canada in which *I. scapularis* was known to be found rose from one to seven[45]. The main criticisms of this study involve model validation. The first criticism is that the data used to validate the model were 13 and 14 years old at the time of this study's publication and may not accurately reflect environmental conditions in 2005. The second criticism is that the model was only validated using one study, although the authors did state that there was a shortage of appropriate field studies available for model validation.

Ogden et al. (2009) reviewed *I. scapularis* distribution surveillance data that had been submitted to Canadian federal and provincial health agencies and the Lyme Disease Association of Ontario between 1990 and 2006. The goal of this review paper was to discuss how the available surveillance data could relate to the diagnosis and prevention of Lyme disease. The *I. scapularis* surveillance data showed that the number of documented locations where *I. scapularis* was endemic had increased during that time period. In the early 1990s, there was only one known population of *I. scapularis*, located at Long Point on the Ontario shore of Lake Erie. Between the 1990s and 2003, distinct populations of *I. scapularis* had been found in southern Ontario, Nova Scotia, southeastern Manitoba, and New Brunswick. Furthermore, a potential emerging population had been identified in southern Quebec. On the other side of the country, British Columbia Center for Disease Control data indicated a wide distribution of *Ixodes ricinus* populations in southern British Columbia, and that these populations align with locations where *B. burgdorferi* is endemic in host animal populations. The authors correctly observed that the surveillance data indicated a spread of the ticks into Canada, and predicted that the projected increase in average temperature in Canada due to climate change is likely to accelerate this range expansion. Furthermore, the authors linked the documented range expansion of *I. scapularis* with the increasing number of reported cases of Lyme disease across Canada (Figure 6). The authors concluded that additional *I. scapularis* surveillance studies must be conducted in order to inform the public, the government, and clinicians about the local risk of contracting Lyme disease and the need for appropriate preventative measures. Understanding local risk could help achieve early diagnosis in more cases, preventing the potential progression to debilitating disease[48].

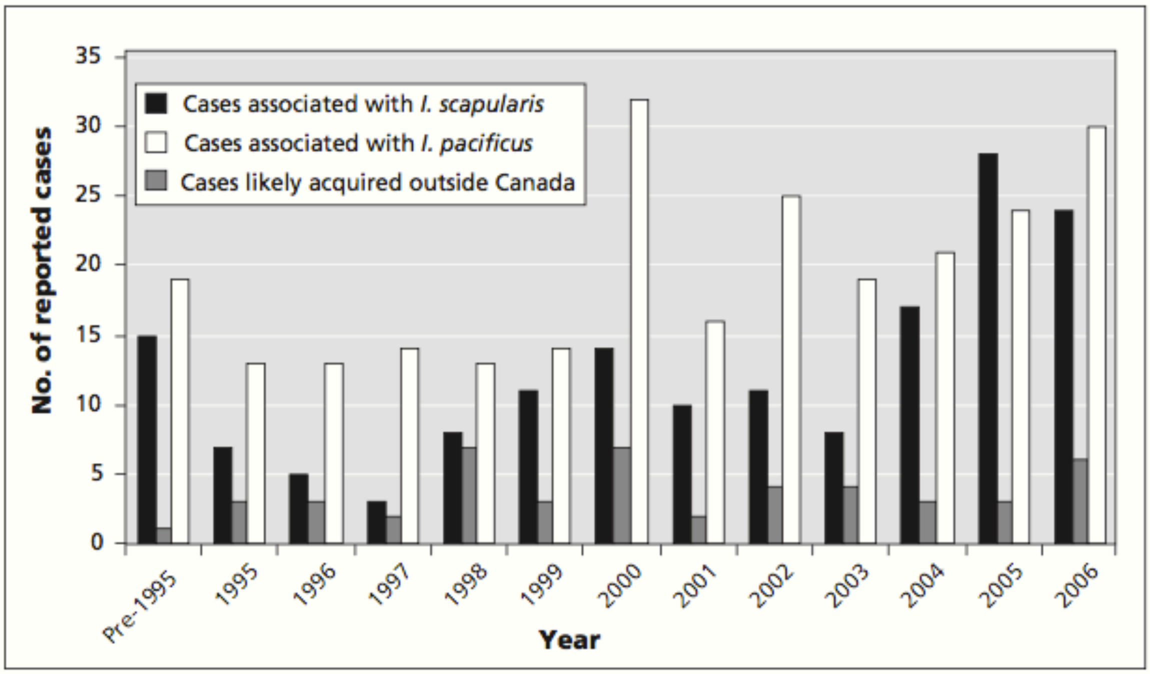

Figure 6: Annual reported cases of Lyme disease reported in Canada [48]

Based on existing data that showed that the incidence of Lyme disease (Figure 6) and the overall abundance of ticks in Canada are increasing, Leighton et al. (2012) developed a model to predict the speed of *I. scapularis* range expansion into Canada, with a goal to help inform public health measures. This model, based on 19 years of tick range surveillance data from the National Microbiology Lab in Winnipeg, Manitoba, was built to provide timeline projections for the northward range expansion of *I. scapularis* ticks in Canada. Using these data, the authors identified a threshold number of tick submissions in a given location that indicated a reproducing tick population. Using temperature data and factoring in short and long distance tick dispersal by birds and other animal hosts, the authors were able to model the rate of tick range expansion into new regions in Canada. Using long-term tick population data and temperature data, as well as validating the model by comparing its predictions to existing field studies, lends credibility to the study and its conclusions, discussed below.

Based on the model generated, Leighton et al. (2012) showed that the number of locations with probable established populations of ticks had increased exponentially since 1990. The criteria for a "probable established population" were determined by the number of tick submissions in a given area, and whether that number exceeded the establishment threshold for two consecutive years. The required number of submissions was determined by the number of submissions reported in areas with a pre-existing established tick population. The model showed that temperature was the strongest predictor of tick establishment, with a drop in time-to-establishment with an increasing number of degree-days > 0°C. Furthermore, tick populations established more quickly with increased annual rainfall, a neighboring established population, and a tick-endemic U.S. county within 425 km. The authors note that the model projects a 14-fold increase in range between 2010 and 2020, from 23,000 km^2 to 317,000 km^2. Finally, the model predicted that the northern suitable range front is advancing at 46 km per year. However, Simon et al. (2014) and the model used in that study calculated the rate of range expansion to be 3.5-11 km per year, although that study was limited to southern Quebec[49]. Either way, the consequence of this spread is that suitable tick habitats are rapidly encroaching on centers of human population. The authors do note, though, that this spread is unlikely to pose an immediate increase in Lyme disease risk, as *B. burgdorferi* will not spread concurrently. Rather, *B. burgdorferi* will spread after tick population establishment. Once the spirochete does eventually emerge, understanding where the existing tick populations are located and where new ones are likely to establish will help inform public health measures[50].

Wu et al. (2013) adapted a model from Ogden et al. (2005) in order to evaluate the effects of climate change on ticks. The authors explained that the difference between the model used in this study and the Ogden et al. (2005) model discussed previously was that this study used rates of development and questing activity rather than the duration of development as model parameters. These changes in model parameters produced modified model equations that the authors said more accurately fit their needs while maintaining accuracy when compared to existing field studies. All other data used to generate the model were the same as those used in the Ogden et al. (2005) study. The authors modeled the conditions under which ticks can survive by examining the basic reproductive number, which here generally refers to the conditions under which parasites can persist and propagate in nature. Specifically, the basic reproductive number for ticks is defined as the number of female offspring produced by a female when there are no density dependent constraints. According to the model, the threshold temperature conditions at which tick populations become sustainable were essentially identical to the Ogden et al. (2005) paper at 3100 degree days > 0 °C. These calculated values for both studies have been validated in the field against locations with endemic populations of *I.*

scapularis ticks in North America, lending them credibility. The map of the regions of Canada where the temperature conditions were already suitable for *I. scapularis* establishment or suitable for emergence was also similar to the map produced by Ogden et al. (2005). The model was also validated by its ability to accurately simulate the tick seasonality exhibited in northeastern North America where Lyme disease and ticks are already prevalent. The authors note that the generated model could be used to investigate the effects of a number of different environmental conditions on ticks, indicating its usefulness in future[51].

Ogden et al. (2014) used the model generated by Wu et al. (2013) that had been adapted from Ogden et al. (2005) in order to examine the effects of climate change on the basic reproductive number of ticks (defined above) in North America. The authors used this established model in order to examine 30 sites in Canada and two sites in northeastern United States with established tick populations. The model they generated was validated by accurately simulating the field conditions reported in southeast Canada where tick populations were already established. The model predicted that temperature increases in the examined locations to conditions that could support tick populations occurred before or during the establishment of the tick population, but never after. Furthermore, the authors found that temperature remained a statistically significant driver of the emergence of ticks in Canada in the locations tracked, consistent with the studies discussed previously. Perhaps worryingly, the authors also note that in regions currently suitable for *I. scapularis* establishment, further climate warming will accelerate the speed of tick invasion. As the number of established populations increases and tick abundance rises, the authors note that a *B. burgdorferi* invasion will follow, and that a "northward *I. scapularis* range expansion is synonymous with expansion in Lyme disease risk". Host population density and suitable habitat do not currently seem to be limiting the range expansion of the tick vector, so if the tick population grows large enough, the authors fear that current control methods may become ineffective[52].

There is one main criticism of this study however. In the authors' description of their model, they stated that the impact of rainfall on off-host tick survival and host-seeking activity was accounted for in the model. They assumed that tick populations only become established in areas where the microclimate and amount of rainfall is suitable for tick survival, consistent with Lindsay et al. (1995). However, this 1995 study was nearly 20 years old at the time of this study's publication in 2014 and might not have been informative anymore. Furthermore, data from the Environmental Protection Agency (EPA) and the National Oceanic and Atmospheric Administration (NOAA) show that the annual level of rainfall is increasing in North America due to climate change (Figure 7). The authors did state that the projected increase in precipitation due to climate change was already occurring, so rainfall would be unlikely to limit the northward invasion of *I. scapularis*. However, they failed to consider that increasing rainfall might make currently suitable tick habitats unsuitable for future generations. Increased precipitation due to climate change could lead to flooding more frequently, which could kill existing tick populations. Additionally, with increasing global temperatures, there may be a greater amount of rainfall in the winter at increasingly northern latitudes, which could expose ticks to more freeze-thaw cycles and potentially kill over-wintering ticks of all life stages.

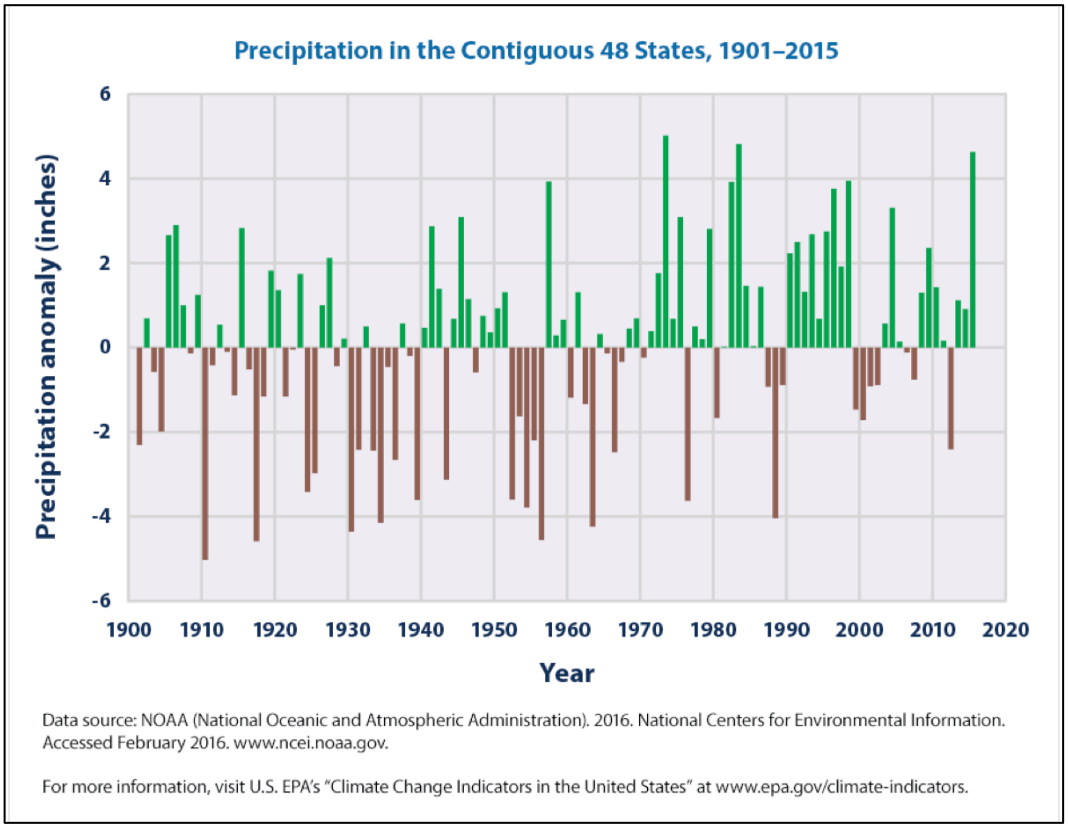

Figure 7: Level of precipitation in the United States between 1901-2015. The 1901-2000 average precipitation was used as the baseline.[53]

Finally, hotter temperatures beyond a certain threshold negatively impact ticks, as discussed. While northern latitudes are warming and becoming more suitable for ticks, southern latitudes in North America are becoming too hot, and are therefore becoming less suitable environments for ticks. Indeed, high temperatures and desiccation should lead to a further reduction in the rates of Lyme disease as the vector population diminishes in the south, even though the rates are not currently significant[50]. The effects that a change in environmental conditions, like what is predicted to happen at southern latitudes in North America, can be examined by looking at other regions around the world. In southern Italy, high temperatures and dry conditions have caused a decline in the questing activity of nymphal and adult ticks, which will likely lead to a decline in tick population as fewer survive to reproductive maturity[54]. Similarly, a temperature increase of 2°C in South Africa is predicted to decrease habitat suitability for four different tick species[54]. Increasing temperatures, then, may not lead to an overall expansion of tick range. Instead, the decreasing amount of suitable habitat in the south, combined with the expanding northern range, may result in a northward shift of suitable habitat rather than an overall range expansion[52]. However, what remains clear is that ticks and *B. burgdorferi* are invading new regions in Canada and represent a clear public health threat.

These studies and others seem to be in agreement that climate change and global warming have already facilitated the northward spread of *Ixodes* ticks into Canada, and that further warming will accelerate this spread. As Ogden et al. (2014) noted, tick range expansion is synonymous with an increase in Lyme disease risk, and the reported numbers of Lyme disease cases in Canada seem to suggest that to be true. According to the Canadian government, there were 144 cases of Lyme disease reported in 2009 (the earliest data available). In contrast, there were 917 cases of Lyme disease reported in 2015[55]. Although these numbers aren't as high as the 30,000 reported cases in the United States, they show a clear increase in a region where ticks and Lyme disease have not historically been endemic. However, the Lyme disease problem in North America is predicted to get worse. If global temperatures increase by the predicted average of 3.4°C by 2100, the basic reproductive number of ticks will increase 2.0-5.0 times and 1.5-2.0 times in Canada and the United States, respectively[44]. Furthermore, *B. burgdorferi* typically appears in tick populations 3-5 years after tick establishment[44], meaning that time is running out to prepare for the increased incidence of this infectious disease.

Lyme Disease and Land Fragmentation:

In many Lyme endemic areas, the landscapes contain a mixture of human-maintained land (farmland, gardens, lawns, hedges, etc.) and natural forests. Typically, the intersections of these different types of landscapes occur at population centers. Unfortunately, this mixed landscape leads to uncontrolled deer population growth and provides ideal habitats for many of the small mammal and avian species that serve as hosts for *Ixodes* ticks and reservoir hosts for the Lyme disease spirochete[56]. Deer in particular play an important role for they serve as hosts for great numbers of ticks at once, and facilitate tick mating by bringing adult males and females into close proximity. Therefore, studies have investigated whether land fragmentation near population centers increases the risk of humans contracting Lyme disease due to an increased number of infected vectors and reservoir hosts.

Brownstein et al. (2005) conducted a study around Lyme, Connecticut to investigate the relationship between landscape pattern and tick prevalence and human infection rate. The authors note that although the net amount of forest in the United States has not changed significantly, human population growth and expansion has increased the number of small forest patches in proximity to human populations. These small patches of forest serve as ideal habitats for white-tailed deer, the predominant hosts for adult ticks, and ideal habitats for small mammals, the main hosts for immature ticks and reservoir hosts for *B. burgdorferi*. Evidence shows that decreasing forest patch size increases the numbers of white-tailed deer and small mammal hosts, particularly the white-footed mouse (the primary reservoir host for *B. burgdorferi*), due to increased edible vegetation at the borders and reduced predator or hunter presence. As would be expected, the authors found a statistically significant relationship between landscape and *I. scapularis* density and infection rate. Even though white-tailed deer are not competent reservoirs for *B. burgdorferi*, their increasing numbers in fragmented forest habitats lead to a growth in the tick population as more adult ticks find mates on each deer. By contrast, the prevalence of infected ticks was found to be mediated by white-footed mouse population and by some reservoir competent birds rather than by deer. Interestingly though, the authors found that the greater risk of human infection associated with these fragmented forests did not translate to an actual greater number of human cases of Lyme disease. The authors explained this seemingly counterintuitive finding by arguing that while increasing land fragmentation drives up the number of infected ticks, that same fragmentation results in fewer residential properties immediately adjoining forest fragments that support tick populations, citing Maupin et al (1991) for these observations. Therefore, the average risk falls as fewer people are exposed to tick populations in the immediate area surrounding their homes. The authors conclude that forest fragmentation results in greater populations of infected ticks, but that this increased risk doesn't necessarily lead to an increased number of human cases of Lyme disease[57]. One limitation of this study was that the satellite imagery the authors used to delineate forest patches had a maximum resolution of 30 meters, which may have obscured the borders of forest patches and wouldn't have been able to distinguish between separate patches with less than 30 meters between them. Higher resolution aerial photography by drone or plane would have provided greater resolution and more precise results.

Jackson et al. (2006) also conducted a study to investigate how landscape pattern affects tick populations and Lyme disease risk, with the goal to help inform preventative landscape design. Research in the United States has shown that the primary location of exposure to infected ticks is around the home. The study location chosen was Maryland due to endemic Lyme disease there, the level of land development, and because the relevant health and environmental data was readily available. Within this study location, the authors found that landscapes containing fragmented forests and a large area of forest edge were statistically associated with the highest Lyme disease rates. With this result in mind, the authors then examined how future landscape development may be able to limit the risk associated with forest edges. Keeping the percentage of forest stable (50% in this model), the authors proposed limiting fragmentation and limiting the area of forest edge adjacent to residential areas so that fewer people are exposed to tick populations (Figure 7). These forest edges, the authors state, provide more protected foraging space for white-tailed deer and white-footed mice, the principal tick host and *B. burgdorferi* reservoir host, respectively. Therefore, the authors conclude that informed design may help to limit peridomestic exposure to infected ticks[58].

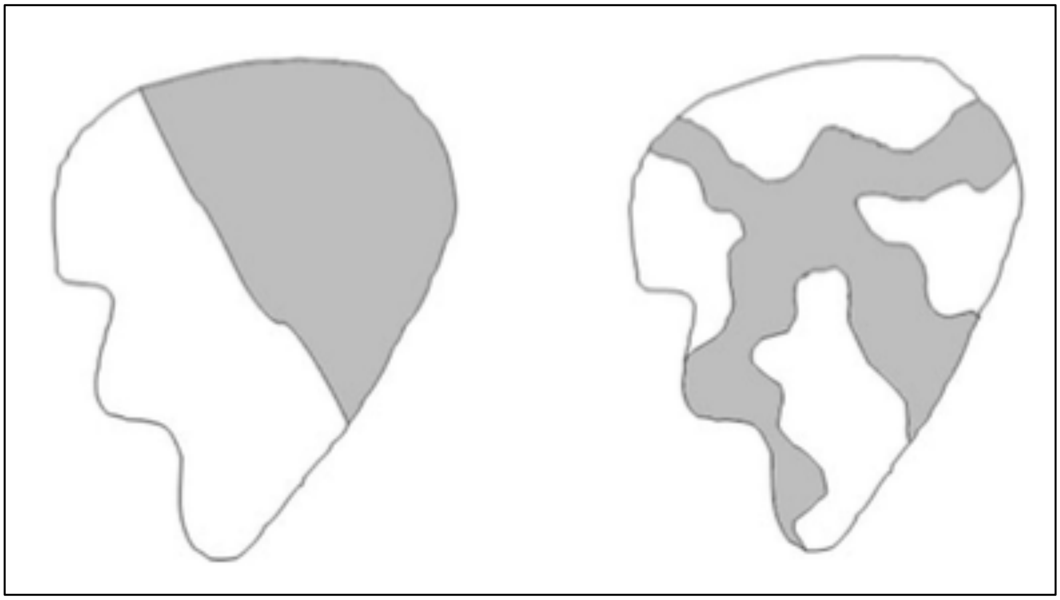

Figure 8: Jackson et al. (2006) hypothetical landscape designs to limit peridomestic Lyme disease risk. Dark shading represents forest, and covers 50% of the landscape in this model. Low area of intersection (left) is associated with lower risk, while high area of intersection (right) increases risk[58].

Tran et al. (2013) examined the simultaneous effects of climate change and landscape variables on the incidence of Lyme disease. The authors note that forest fragmentation creates an intersection between human population densities, large deer populations, and large populations of *B. burgdorferi* reservoir hosts, such as the white-footed mouse. However, the existing literature has not come to a definitive conclusion on the effect that forest fragmentation has on the incidence of Lyme disease, so a goal of this study was to clarify this relationship. The study area included 13 states in northeastern United States where Lyme disease is endemic, and the authors found that high Lyme disease incidence occurs near forested areas with high deer populations, even with low population density. Edge habitat provides ample edible vegetation for white-tailed deer, the predominant host for adult ticks. Forest fragmentation generates this type of edge habitat, and when this habitat borders residential property, the risk of Lyme disease increases. Indeed, of the landscape designs examined, the model showed that “developed land” (i.e. residential property) bordering “open space” (i.e. small forest habitat) had the strongest connection with Lyme disease incidence. Previous studies had found that forest fragmentation increases the risk of Lyme disease but not the incidence. However, the authors explain that while overall landscape fragmentation is not a significant factor in Lyme disease incidence, fragmentation of residential areas in particular does have a strong influence on Lyme disease incidence. The study concludes that land fragmentation increases both the risk and the incidence of Lyme disease, and that the true impact that factors like land fragmentation have on Lyme disease can only be “elucidated at the appropriate spatial and temporal scales”. The authors mean that examining an entire state or province as a whole for only a short period of time may not reveal a relationship between forest fragmentation and Lyme disease risk, whereas studying individual counties or towns over a longer period of time could reveal a significant relationship[56].

Simon et al. (2014) developed a model to investigate how climate change and habitat change affect the incidence of Lyme disease, specifically by investigating the effects on the white-footed mouse and blacklegged ticks. The study focused on southern Quebec, as a region where ticks, *B. burgdorferi*, and Lyme disease are all emerging. The findings and conclusions of this study regarding the impact of climate change on the incidence of Lyme disease were similar to those of the studies previously discussed, so only their findings on the effects of habitat change will be discussed here. The authors state that extensive agricultural land expansion and deforestation have led to the fragmentation of landscapes. The study found that land use, land patch area, and connectivity between land fragments were significant predictors of tick abundance. The authors found greater tick densities in fragmented landscapes compared to continuous forest landscape. Furthermore, they found that fragmented landscapes also had a higher abundance of hosts for ticks, including white-tailed deer and white-footed mice. Although the results from this study are similar to those of the studies discussed previously, the authors drew a different conclusion. Instead of contributing to the spread of ticks and Lyme disease in Canada, the authors concluded that forest fragmentation in southern Quebec is actually limiting the distribution of ticks and mice. The authors state that fragmentation also generates unfavorable habitats for ticks and mice, creating a barrier to movement and therefore limiting the distribution. However, the authors also found that landscape variables were weaker predictors of the presence of *B. burgdorferi* in tick populations compared to climatic variables[49].

Finally, Messier et al. (2015) conducted a study to investigate landscape risk factors for Lyme disease in the Hudson River valley of New York state. Specifically, the authors sought to investigate how the categorization of a landscape influences its association with Lyme disease incidence. The goal of this study was to help determine whether existing landscape guidelines and recommendations to reduce the risk of Lyme disease, as provided by previous studies cited by the authors, and existing models remained accurate upon clarification of landscape variables. For example, the authors sought to distinguish deciduous forests, evergreen forests, and mixed forests from the aggregate "forest", as well as investigate the association between each independent type of forest and Lyme disease incidence. After examining the National Landcover Database

(NLCD) variables for classifying landscapes, the authors found that the individual variables, e.g. evergreen forest vs. forest, predict the incidence of Lyme disease better than the aggregate variables. By considering the individual variables, the authors note that models will be better able to predict which part(s) of the aggregate data is associated with Lyme disease incidence. Furthermore, refining the landscape variables will help health officials assess their specific landscapes for Lyme disease risk. Using these refined variables, the model generated in this study found that landscapes with either a low percentage of deciduous forest or landscapes where deciduous forests dominated are associated with a decreased risk of Lyme disease. The model also found that the percent of edge habitat, as defined previously, had the strongest association with Lyme disease incidence. Together, these findings suggest that the conditions suitable for a high incidence rate of Lyme disease occur where residential land (lawns, gardens, etc.) or farmland (crop rows, pastures, etc.) border a forest fragment, consistent with previous studies. In these locations, humans are more likely to come into contact with infected ticks due to increased numbers of tick hosts and *B. burgdorferi* hosts. The authors concluded that the examined regional-scale models for Lyme disease incidence rates, notably the one generated by Jackson et al. (2006), as well as the model generated in this study in order to examine NLCD data are robust for the Hudson River valley. Furthermore, they suggested that future analyses in any region must use the most refined landscape variables specific to that region in order to produce the most informative models and most reliable landscape guidelines to limit the incidence of Lyme disease[59].

These studies seem to agree that landscape fragmentation is a strong driver of Lyme disease risk, particularly when that fragmentation results in residential land bordering deciduous forest edge. Whether that fragmentation leads to a greater incidence of Lyme disease is debated, but Tran et al. (2013) found that fragmentation is positively correlated with incidence at the appropriate spatial scales. Although landscape variables were not found to be as strong of a driver compared to climate change, future development in regions were ticks and *B. burgdorferi* are emerging should attempt to limit the amount of residential land directly bordering forest fragments in order to reduce risk as much as possible. Furthermore, reforestation efforts should be made with caution, for the initial emergence of Lyme disease in the United States has been linked to reforestation and repopulation of white-tailed deer, according to Brownstein et al. (2006)[57].

Mechanisms for the Spread and Establishment of Tick Populations:

Climate change and forest fragmentation are creating ideal habitats for ticks at increasingly northward latitudes, as discussed. However, the mechanisms of that spread have yet to be examined. The first important mechanism is animal host migration. Over long distances, birds are the primary transport system for ticks. During the spring migration north, birds transport primarily nymphal ticks an average distance of 425 km and deposit them into new habitats[50]. In locations conducive to tick establishment (i.e. appropriate climate conditions, landscape conditions, and host density), ticks deposited by birds may start a new population of ticks. Even at the edge of a suitable habitat for ticks where they may not be expected to survive, constant re-introduction by birds and other hosts may help sustain tick populations[51]. Birds even help deliver *B. burgdorferi* into new habitats. It has been estimated that 10% of the ticks transported by birds are infected with *B. burgdorferi*[48]. With an appropriate density of hosts that double as *B. burgdorferi* reservoir hosts, infection can spread through tick and host populations, as well as threaten human populations. Over shorter distances (less than 5 km), mammalian hosts play a larger role in transporting ticks. Deer in particular carry large numbers of adult ticks into new habitats, including pregnant female ticks[50]. As discussed, reforestation efforts are actually contributing to white-tailed deer population growth, and have been linked to the emergence of Lyme disease in the United States[57]. Above a relatively low threshold though, increases in deer population have a negligible effect on tick abundance, meaning that other factors like the abundance of hosts for immature ticks limit tick population size[60]. This growing deer population facilitates the dispersal of ticks and the introduction of *B. burgdorferi* into new habitats.

The white-footed mouse is another important mammalian host that contributes to the local spread of ticks and *B. burgdorferi*. Roy-Dufresne et al. (2013) showed that the range of the white-footed mouse is growing northward as northern latitudes warm and become increasingly suitable for this species. The mouse's range has been expanding northward at a rate of 15 km/year in northern Michigan, and at 10 km/year in southern Quebec. Furthermore, the authors predict that the range will grow by 300 km in the coming four decades, representing a rate of 8 km/year. As the principal reservoir host, the occurrence of the white-footed mouse is associated with the occurrence of *B. burgdorferi*.

The mouse is highly susceptible to lifelong infection and highly efficient at transmitting *B. burgdorferi* to uninfected ticks, with 90% of ticks acquiring a new infection after feeding on the infected mouse[49]. Therefore, as the mice move further north, they bring ticks and *B. burgdorferi* with them. When these new populations of mice and ticks encounter human population centers, the risk and the incidence of Lyme disease goes up[61].

As discussed, climate change and forest fragmentation are changing the landscape at increasingly northern latitudes, and as a consequence, these changes are driving biodiversity loss in habitats suitable for tick establishment. In fact, the decline in biodiversity is predicted to worsen in the coming decades as the effects of climate change worsen, with the worst-case scenario being a global mass extinction[54]. It has been hypothesized that high biodiversity can actually help reduce the risk of Lyme disease and other infectious diseases. This hypothesis, termed the dilution effect, proposed that the relative abundance of *B. burgdorferi* competent reservoir hosts is lower in biodiverse ecosystems than in non-diverse systems. Assuming that ticks feed on both competent and non-competent hosts at the same rate, fewer ticks in a given population will feed on a *B. burgdorferi*-infected host and fewer ticks will become infected themselves. Therefore, increasing biodiversity functions to limit the transmission of spirochetes between ticks and hosts, and reduces the risk that humans will encounter an infected tick[54,62]. However, climate change and forest fragmentation are driving a reduction in biodiversity in these habitats, creating optimal conditions for the white-footed mouse to establish a dominant population. In fact, small forest patches like the ones created through forest fragmentation have been shown to contain lower vertebrate diversity compared to large forest patches[62]. Fragmentation and climate change also directly facilitate the establishment of mouse and deer populations. These hosts in particular are critical for the tick and *B. burgdorferi* life cycles, as discussed. Therefore, declining biodiversity, as a consequence of climate change and forest fragmentation, may be facilitating the establishment of tick populations at increasingly northern latitudes.

Finally, changing predator-prey dynamics have also facilitated the establishment of small mammal hosts, *Ixodes* ticks, and *B. burgdorferi* spirochetes in new northern habitats. Levi et al. (2012) conducted a study to investigate these changes, and how they have affected the emergence of Lyme disease in the United States. The authors began by explaining that over the last 50 years, the coyote population and coyote habitat range have grown significantly. As a result, the coyote has become the new top predator in the United States due to the disappearance of the grey wolf. Unfortunately, this coyote population growth has altered the abundance of several other small-mammal predators. Specifically, coincident with coyote population growth has been a decline in the abundance of the red fox, and the authors cite interference competition as the likely mechanism for this decline. Interference competition is when one species population interferes with another species populations' ability to exploit the local resources via aggressive behavior. Small mammals represent the majority of the red fox diet, whereas coyotes rely far more on deer, of which there are plenty after the deer population growth of the 20th century. Therefore, the expansion of the coyote population has actually led to a decrease in predation on small mammals, the predominant hosts for nymphal ticks and reservoir hosts for *B. burgdorferi*. In fact, the authors of this study found that the increase in Lyme disease incidence is more closely tied to changing predator-prey dynamics than to changes in deer population numbers. Although this result seems surprising, changes in deer population above a certain threshold have little effect on the abundance of ticks, as discussed. It then makes sense that changing predator-prey dynamics are more closely tied to the currently increasing incidence since deer populations have remained above that threshold. The authors conclude that increases in coyote populations, declines in red fox populations, and a relatively stable deer population are contributing to the increasing incidence of Lyme disease in North America, due to reduced predation on small mammals that serve as tick and *B. burgdorferi* hosts[60].

Lyme Disease in Europe

Lyme disease, or Lyme borreliosis, is also a common disease in many European countries. However, there are a few key differences between Lyme disease in Europe and the United States that warrant a brief review. In Europe, *Borrelia afzelii* and *Borrelia garini* are the primary species responsible for Lyme disease, although *B. burgdorferi* is also identified but less commonly. Interestingly, the symptoms these two species elicit are different than the symptoms caused by *B. burgdorferi*. Whereas *B. burgdorferi* is particularly arthritogenic, *B. afzelii* mainly causes skin infections and *B. garini* is especially neurotropic[63]. Furthermore, the tick species that transmit the disease are also different in Europe. Whereas *Ixodes scapularis* and *Ixodes pacificus* are the primary tick vectors in the United States, *Ixodes ricinus* and *Ixodes persulcatus* are the primary vectors in Europe[3]. Although the rates of Lyme disease aren't as high as rates in North America, incidence has been increasing over the past decade with approximately 65,000 cases reported annually across Europe[64]. In terms of tick and *Borrelia* reservoir hosts in Europe, roe deer, small mammals, and birds are important hosts for ticks and important hosts for *Borrelia* species (excluding deer). Interestingly, *B. afzelii* is more commonly associated with rodents while *B. garini* is more commonly associated with birds[65].

Numerous studies have investigated ticks and Lyme disease in Europe over the past few decades. Before examining those papers though, it's important to review some of the factors that have been identified as drivers for the emergence of ticks and spirochetes in new regions of Europe. Medlock et al. (2013) sought to investigate the drivers of tick range expansion and thereby Lyme disease in Europe. *I. ricinus* has been found across Europe, but reports have indicated that these ticks are spreading into new areas and increasing in abundance in endemic areas. Climate change, specifically global warming, has been identified as one of the factors driving this expansion. The authors elaborate that warmer temperatures benefit ticks directly by shortening development time and indirectly by creating suitable conditions for ticks to survive at higher altitudes and latitudes. Furthermore, the authors state that global warming will facilitate deer, bird, and small mammal population growth, potentially increasing the number of suitable hosts for ticks, *B. afzelii*, and *B. garini*. Changes in forests and wildlife management have also been identified as important drivers for the spread of ticks and Lyme disease. Interestingly though, the authors of this study state that previous studies in Europe have shown that greater connectivity between forest patches leads to a greater abundance of *I.*

ricinus, and they postulate that continued de-fragmentation of habitats will further facilitate tick invasion and establishment because it allows for easier animal migration. However, similar to the studies discussed in relation to North America, the authors note that ticks exploit the margins of these forested areas where vegetation provides food for host species[66].

Lindgren et al. (2000) investigated whether climate change had influenced the documented northward range expansion and population growth of *I. ricinus* in Sweden between the early 1980s through the mid-1990s. During this time period, winters in Sweden became somewhat milder with fewer days reaching temperatures below -12°C (10.4°F). The authors focused on 20 districts in central and northern Sweden, in which the appropriate climatic and tick population data were available. These districts were divided into five zones, and minimum temperature data for these five zones was examined alongside tick population data in order to determine whether climate change may have had an effect on the northward migration of ticks in Sweden. In agreement with studies that demonstrated the effects of climatic factors on ticks, the authors found that the northward expansion of tick range was indeed related to the mild winters of the 1980s and 1990s. Furthermore, the authors found that warming spring and autumn temperatures in central and southern Sweden during this same period contributed to the population growth observed. When considering other factors that could have contributed to the tick population growth and range expansion if present, the authors identified land-use changes such as de- and reforestation. However, the authors discounted these factors as no major land-use changes occurred within the region encompassed by the study. The authors concluded that climatic factors, particularly warmer average temperatures, were positively correlated with the observed range of expansion of *I. ricinus*, and that ticks may continue to spread into higher latitudes and altitudes in the coming decades. Furthermore, the authors hypothesized that this spread may influence the incidence of tick-borne diseases, like Lyme disease and Tick-Borne Encephalitis[67].

A decade later, Jaenson et al. (2011) conducted a study to investigate the relationship between climate and the northern distribution limit of *Ixodes* ticks in Sweden and predict the future risk of Lyme disease in this region. The authors postulated that the documented tick range expansion is due to a warming climate that has shortened winters, facilitated tick development, and increased the abundance of hosts critical for tick and borrelial species. The authors reviewed previous studies and the same tick population and temperature data used by Lindgren et al. (2000), and used current climate data and the known effects of temperature on *I. ricinus* occurrence in order to model the future distribution of ticks in Sweden. Their results suggest that by the end of this century, the distribution of *I. ricinus* will expand to include all of Sweden, Norway, and Finland. Additionally, the authors predicted that the incidence of Lyme disease will rise at increasingly higher northern latitudes. As an explanation, the authors state that the vegetation period, in which tick activity can start, will begin 75 days earlier in southern Sweden and 30 days earlier in central Sweden. However, the authors also note that Lyme disease may actually become less prevalent in southern Sweden as the amount of rainfall drops, for ticks are especially susceptible to desiccation. A warmer climate is also predicted to support deer and small mammal populations, as well as their northward migration, by encouraging plant growth. Furthermore, evidence has shown that birds are migrating further north and to higher altitudes, potentially carrying ticks and/or *B. garini* with them. The study concludes that climate change is likely to continue to facilitate northern range expansion for *I. ricinus*, and an increase in the incidence of Lyme disease in northern Scandinavia[68].

Medlock et al. (2015) examined the effects of climate change on Lyme disease and *I. ricinus* by reviewing existing studies and field data focused on the United Kingdom (UK). The authors note that Lyme disease cases are increasing in the UK every year, with an average of 1000 cases reported each year. Additionally, *I. ricinus* already exists as far north as northern Scotland and has been found up to an altitude of 700 meters. A review of existing studies led the authors to conclude climate change and wildlife factors are likely to increase the incidence of Lyme disease in the UK. The authors speculate that warmer winters may extend tick activity, and that warmer springs may allow ticks to develop earlier and quicker. In fact, warmer temperatures will affect human behavior as well, since people will likely spend more time outdoors in environments that bring them into closer contact with infected ticks. Furthermore, partially as a result of a warmer climate, roe and red deer populations in the UK are growing and contributing to the spread of ticks. However, areas with high tick density are not necessarily associated with a high infection rate in ticks. The authors explain that large animals like deer that serve as reproductive hosts for *I. ricinus* ticks can actually act to dilute the infection rate in ticks since deer are not competent *Borrelia* hosts. With cases of Lyme disease on the rise throughout the UK, the authors conclude that authorities need to develop plans to deal with the increasing populations of ticks, and control the population of deer that are contributing to the tick range expansion[69].

A few studies have examined how forest and land-use factors affect the incidence of Lyme disease in Europe, but not nearly to the same extent as studies focused on North America. As mentioned, Medlock et al. (2013) investigated forest-related drivers for the emergence of Lyme disease and ticks in Europe. Previous studies have shown that habitat connectivity is positively correlated with the abundance of ticks and tick hosts, unlike in North America. The authors speculate that because greater connectivity between forest patches is correlated with an increased abundance of ticks, any reduction in the distance between patches might increase the likelihood of successful tick invasion and establishment. Furthermore, increased connectivity between patches facilitates animal dispersal, and thereby the dispersal of ticks. Perhaps differences between *I. scapularis* and *I. ricinus*, or differences between the tick hosts in North America and Europe could explain the differing conclusions about the effects of habitat fragmentation of tick abundance. However, more studies would need to be conducted to determine the true reason for this difference. Similar to studies in North America though, data from the UK suggest that ticks are more prevalent at the edges of forested land, where forest meets developed land. These edges provide vegetation for tick hosts, most importantly deer and small mammals. The authors note that there are many habitat connectivity and re-forestation projects in progress throughout Europe, and that these projects may be contributing to the increasing *I. ricinus* population. They also state that any increase in tick population also increases the potential for *B. garini* or *B. afzelii* transmission. Based on their review of field studies in Europe, the authors conclude that increasing connectivity between forest fragments increases the abundance of ticks and contributes to an increasing rick of Lyme disease in Europe[66]. However, more field studies need to be conducted in Europe to understand whether these conclusions are accurate.

Hubálek et al. (2006) examined the effects of forest clearing on the abundance and infection rate of ticks. The goal of this study was to evaluate the potential risk of Lyme disease following a forest habitat change in the South Moravia region of the Czech Republic. Tick populations had been monitored along four forest trails in this region since 1991. However in 2002, one of these trails was cleared of small trees, shrubs, and grassy vegetation, leaving only tall trees. In order to evaluate the effects of habitat change on the risk of Lyme disease, the authors compared the abundance of ticks on the cleared trail to the abundance of ticks on the control (un-cleared) trails. The results of the study showed that in the spring after the clearing, the abundance of ticks on the cleared trail differed from the control trails. However, this difference became smaller over time. Specifically, the frequency of encountering a nymphal tick on the cleared trail was 71%, 47%, and 18% lower than the frequency on the control trails in 2003, 2004, and 2005 respectively. Furthermore, the frequency of encountering an adult tick on the cleared trail was 96%, 75%, and 54% lower than the frequency on the control trails over the same respective years (Table 1). However, no P values were provided so it is unclear whether these results show statistical significance. The authors did not have frequency data on the individual control trails from before the trail clearing because the collected ticks from all four trails were previously pooled together prior to examination and testing.

The frequency of infected nymphs did not differ statistically significantly between cleared and control trails over the three years following the clearing. The frequency of infected adults also did not differ statistically significantly between the cleared and control trails in 2004 and 2005 (Table 1). The authors state that statistical analysis on the infection rate in adult ticks between the cleared and control trails was not possible in 2003 due to insufficient numbers of adults ticks caught on the cleared trail. Within individual trails, the infection rate for both nymphs and adults did not differ statistically significantly between years, although no exact values were provided. *B. garini* was the principal *Borrelia* species found, and the authors speculate that birds were responsible for the ticks reintroduced into the cleared habitat. As mentioned, *B. garini* is particularly associated with birds. Although limited by the precise location, this study has implications for the suitability of forest edge habitat for ticks and host species, and may also represent an option for controlling tick populations and thereby Lyme disease risk. Indeed, the authors note that controlled burning in Florida resulted in a 96%, 53%, and 67% reduction in adult *I. scapularis* populations over a three-year time period. The authors concluded that the habitat modification examined reduced the populations of adult and nymphal ticks in the Czech Republic, and that simple forest edge clearing could be a viable option to reduce the abundance of *I. ricinus* ticks in Europe[65].

Table 1: Differences in encounter frequency and infection rate of ticks on the cleared trail compared to the control trails (Hubálek et. al 2006)[65]

Encounter Frequency (Cleared vs. Control)	2003	2004	2005
Nymph	-71%	-47%	-18%
Adult	-96%	-75%	-54%
Infection Rate (Cleared vs. Control)	2003	2004	2005
Nymph	0%	-4%	+1%
Adult	-10%	-6%	-2%

Lauterbach et al. (2012) conducted a study in order to understand how climate and habitat conditions impact tick density in different forests in Germany. Since climate has already been addressed, and the authors' conclusions were consistent with the previously discussed studies, only the findings on the impact of habitat conditions will be examined here. Specifically, the authors sought to investigate how tree species composition, forest stand type, and forest successional stage affect questing nymph density. The authors collected ticks by dragging a section of woolen cloth across the forest floor and ground vegetation, an established method for collecting ticks, in experimental plots. Each plot was 100 meters x 100 meters and the authors sampled 49 plots. Tree species composition, stand type, and successional stage of each plot were assessed on site. Successional stage, ranging from bare ground to mature forest, was found to be an important predictor for the density of questing nymphs. The authors found that younger forest stands in particular are associated with increased nymph densities when compared to older forest stands. Furthermore, the authors found that herbaceous cover was associated with a reduced density of nymphs. The authors speculate that this herbaceous cover may be an unsuitable habitat for rodents, the primary hosts for nymphal ticks. The authors also note that tick questing behavior is strongly linked to temperature and humidity. In younger forests stands, where the authors found a greater population of ticks, increased levels of solar radiation reach the ground, keeping the temperature warmer. The authors then speculated that under thick herbaceous cover, less of this radiation reaches the ground and tick questing behavior declines. With a reduced questing rate, fewer nymphal ticks are likely to find a suitable host, and fewer therefore are likely to survive to reproductive maturity. Although climate factors were not discussed here, the authors concluded that habitat composition and microclimate are intimately linked when considering suitable tick conditions, consistent with the conclusions of previous studies. The authors recommended further study on the interplay between these two factors, as understanding both together could better inform public health measures[70]. The authors noted that they did not collect data on the abundance of host species in these habitats, a limitation of the study. Another limitation of this study was that not all experimental plots were sampled at once. Data collection occurred over three years, and tick populations may have changed over that time. However, the authors did note that tick activity and density varies throughout the year, and dragging at two separate times within one year could have affected the results of the study. Collecting during the same time period over successive years was the best way the authors could limit this effect since they did not have the resources to sample each plot simultaneously.

Beyond climate change and forest/wildlife management, various other anthropogenic and natural factors have been identified as potential drivers of tick population growth and range expansion in Europe. Medlock et al. (2013) reviewed some of these factors. In central and eastern Europe, the area of land used for grazing herds of cattle and sheep, as well as the area of land used for crops, have both declined. These factors have led to the regrowth and re-invasion of herbaceous plants, and subsequently the re-invasion of deer and rodents. Furthermore, woody vegetation has also regrown, potentially providing suitable habitats for birds. As discussed, when deer, rodent, and bird populations grow, tick populations have the potential to grow as well. Furthermore, as deer, rodent, bird, tick populations grow, the infection rate of reservoir hosts and ticks is also likely to rise, and when these vectors come into proximity with human population centers, the risk and incidence of Lyme disease rises. Additionally, in these same areas, pesticide use has fallen, likely due to the reduced area of cropland, and has allowed for better tick population establishment and survival. Finally, as in North America, changing predator-prey dynamics have also been investigated as an indirect driver for the expansion of tick range and population, and were discussed in this review. In the 1970s, an outbreak of sarcoptic mange dramatically reduced the population of foxes in Europe, allowing for a rapid growth of the deer population. Foxes are a predator of young deer, and the reduced fox population in the 1970s allowed for greater numbers of deer to survive to adulthood. Although the populations of roe deer have since declined, they remain much higher than before. As discussed, deer are the primary reproductive hosts for ticks and greater numbers of deer favor tick population growth[66].

CONCLUSION

Lyme disease rates are increasing in both endemic and non-endemic areas around the world, and tick populations are growing. This thesis sought to assess some of the causes of these observations, and hypothesized that climate change and environmental modification could be key factors. Accordingly, this thesis examined the published literature regarding climate change and environmental modification and their effects on tick populations and the incidence of Lyme disease. The existing literature seems in agreement that climate change, particularly global warming, is facilitating a growth in tick population numbers, a northward invasion of ticks and *Borrelia* species in both North America and Europe, and an increasing incidence of Lyme disease. Environmental modification, particularly growing forest fragmentation, is also generally agreed upon to be a contributing factor to the spread and establishment of tick populations and the increase in Lyme disease incidence in North America. Meanwhile, the studies focused on habitat factors in Europe, while limited in number, suggest that reduced forest fragmentation is facilitating the spread and establishment of tick populations and thereby increasing the risk and incidence of Lyme disease. This discrepancy could possibly be due to some difference between *I. scapularis* and *I. ricinus*, possible differences between the host species in North America and Europe, or perhaps differences between experimental designs. Regardless of the direction of fragmentation though, studies in both North America and Europe show that environmental modification has effects on ticks and Lyme disease. Therefore, this thesis is able to conclude that both climate change and environmental modification have been and will continue to be contributing factors to the spread and establishment of ticks and the increasing incidence of Lyme disease in North America and Europe.

Understanding how these environmental variables affect tick populations, both infected and uninfected, and Lyme disease could help inform public health measures. In particular, understanding which habitat structures favor tick populations and reservoir hosts could help reduce risk and incidence of Lyme disease because landscape variables can be controlled. Accordingly, further, more refined studies need to be conducted to determine specifically which habitats support tick populations and *Borrelia* reservoir hosts, and which control measures are most effective for these individual types of habitat. One type of intervention may effectively control tick populations in one type of habitat, but have a minimal effect in another based on the specific host populations and/or specific vegetation composition. For example, Eisen et al. (2012) discussed the possibility of host-targeted interventions in small forest fragments, since fragmentation reduces bio-diversity and therefore the number of viable *Borrelia* reservoir hosts. Specifically, the authors discussed vaccination of specific host species to reduce the number of infected nymphs. However, this control method may not work in areas with high bio-diversity because ticks would presumably have other *Borrelia*-competent hosts readily available, and it may not be feasible to vaccinate each one[71].

On the other hand, global climate change is a harder issue to tackle because it cannot be controlled locally and therefore cannot inform immediate public health measures. A coordinated global effort to move away from fossil fuels and toward sustainable clean energy sources like wind, ocean current, and solar energy would be required to slow and eventually reverse anthropogenic climate change. However, educational efforts regarding climate change and global warming and their effects on ticks, Lyme disease, and overall health could help encourage people to adopt environmentally friendly practices in their daily lives. As temperatures warm, humans are more likely to spend time outdoors in areas likely to contain infected ticks, and with prolonged spring and fall seasons, people are also more likely to encounter an infected tick due to increased tick and *Borrelia* reservoir host population numbers. Simple measures like avoiding areas with high grass and leaf litter, applying insect repellents before engaging in outdoor activities, wearing long pants, socks, and shoes, and checking oneself or another for ticks on and/or under clothing after returning inside could all help reduce the likelihood of successful infection.

Along the same line of thought, additional tick and *Borrelia* surveillance studies must be conducted in order to gain a greater understanding of their geographic distribution. Physicians, veterinarians, and the general public could help achieve this goal by increasing their efforts to report tick bites. Furthermore, if people could save the ticks they pull off of themselves or their pets, and submit them to these surveillance studies for testing, along with the location where the ticks were most likely picked up, a clearer picture of the *Borrelia* distribution could be generated. This information could be useful in order to understand where tick populations are emerging, where tick populations are already established, and/or where established tick populations are growing. Furthermore, understanding the true distribution of ticks could help inform which locations need public health attention, and what kind of control measures would be most appropriate based on the surrounding landscape.

Finally, a safe and effective Lyme disease vaccine must be developed as the annual number of cases continues to rise around the world. There was previously a vaccine available but the manufacturer discontinued production in 2002 due reportedly to an insufficient demand, and a public perception that the vaccine was associated with serious side effects. Unfortunately, those who received this vaccine when it was available are unlikely to have remained protected to this point. As discussed previously, Lyme disease can present with a variety of symptoms similar to other illnesses and therefore isn't always reliably diagnosed. Without early diagnosis and treatment, Lyme disease can progress to a debilitating neurological and musculoskeletal disease in some patients, and even when treated and the active infection is cleared, persistent and unexplained symptoms plague some patients. Preventing infection rather than treating it would be preferable for patients and would cost considerably less. Therefore, developing a safe, effective, and long-lasting vaccine should be of paramount importance because of the increasing incidence of Lyme disease around the world and the potentially debilitating symptoms this disease can cause.

An estimated 300,000 people per year in the United States, a reported 1000 people per year in Canada, and an estimated 65,000 people per year in Europe contract Lyme disease. Although not investigated in this thesis, Lyme disease is also prevalent in many Asian countries. Therefore, Lyme is truly a global disease, and as this thesis has shown, the number of cases is predicted to increase dramatically as the effects of climate change and other anthropogenic factors, like forest fragmentation, intensify. Given the increasing public health threat that Lyme disease represents, the general public needs to be educated about the disease and understand how to take the appropriate precautions to prevent tick bites and borrelial infection. Furthermore, the scientific community needs to continue research on the bacterium and its hosts and vectors to develop effective mechanisms to manage ticks and their habitats as well as develop an effective vaccine. Finally, governments around the world must adopt environmentally friendly policies to slow and eventually reverse the effects of global climate change before infectious disease rates rise and before the damage to the planet becomes irreparable.

LIST OF JOURNAL ABBREVIATIONS

Am. Fam. Physician	American Family Physician
Ann. N.Y. Acad. Sci.	Annals of the New York Academy of Sciences
Can. Med. Assoc. J.	Canadian Medical Association Journal
Clin. Infect. Dis.	Clinical Infectious Diseases
Clin. Lab. Med.	Clinics in Laboratory Medicine
Curr. Allergy Asthma Rep.	Current Allergy and Asthma Reports
Emerg. Infect. Dis.	Emerging Infectious Diseases
Environ. Health Perspect.	Environmental Health Perspectives
Euro Surveill. Bull. Eur. Sur Mal. Transm. Eur. Commun. Dis. Bull	Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin
Evol. Appl.	Evolutionary Applications
FEMS Immunol. Med. Microbiol.	FEMS Immunology and Medical Microbiology
FEMS Microbiol. Lett.	FEMS Microbiology Letters
Front. Cell. Infect. Microbiol	Frontiers in Cellular and Infection Microbiology
Glob. Change Biol.	Global Change Biology
Infect. Dis. Clin. North. Am.	Infectious Disease Clinics of North America
Infect. Immun.	Infection and Immunity
Int. J. Epidemiol	International Journal of Epidemiology
Int. J. Parasitol. Parasites Wildl.	International Journal of Parasitology: Parasites and Wildlife

Int. J. Paratisol.	International Journal of Parasitology
J. Appl. Ecol.	Journal of Applied Ecology
J. Autoimmun.	Journal of Autoimmunity
J. Clin. Invest.	Journal of Clinical Investigation
J. Clin. Microbiol.	Journal of Clinical Microbiology
J. Immunol.	Journal of Immunology
J. Med. Entomol.	Journal of Medical Entomology
J. Mol. Microbiol. Biotechnol.	Journal of Molecular Microbiology and Biotechnology
J. Theor. Biol.	Journal of Theoretical Biology
JAMA	Journal of the American Medical Association
Lancet Infect. Dis.	The Lancet Infectious Diseases
Med. Vet. Entomol	Medical and Veterinary Entomology
Microbiol. Spectr.	Microbiology Spectrum
N. Engl. J. Med.	New England Journal of Medicine
Nat. Rev. Dis. Primer	Nature Reviews Disease Primers
Nat. Rev. Microbiol.	Nature Review Microbiology
Parasit. Vectors	Parasites and Vectors
Proc. Natl. Acad. Sci. U. S. A.	Proceedings of the National Academy of Sciences of the United States of America
Spat. Spatio-Temporal Epidemiol.	Spatial and Spatio-Temporal Epidemiology
Ticks Tick-Borne Dis.	Ticks and Tick-Borne Diseases
Trends Ecol. Evol.	Trends in Ecology and Evolution

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CURRICULUM VITAE

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