Lyme Disease & Indicators of Biodiversity

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ABSTRACT

Based on current statistics conducted by the CDC, annual incidences of Lyme disease have increased in Massachusetts since 2012 (CDC, 2015). This project used tick information from the UMass Amherst database to determine surrogates of biodiversity that best explain Lyme disease incidences in the state. Previous studies support the dilution effect, which hypothesizes that a loss of biodiversity can increase infectious disease prevalence. To test the dilution effect against indicators of biodiversity, we ran both correlation and Akaike Information Criterion (AIC_c) analyses. Our results demonstrated that the number of people influenced the percent of infected ticks and the dilution effect hypothesis was refuted.

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CHAPTER ONE: LITERATURE REVIEW

Problem Statement

Classified as an emerging infectious disease, reported incidences of positive Lyme disease have increased in the United States since 2001 (CDC, 2014; Petnicki-Ocwieja & Brissette, 2015). In 2015, approximately 95% of reported incidences of Lyme disease occurred in fourteen states, a majority of which were located in the Northeastern territories (CDC, 2015; Figure 1).

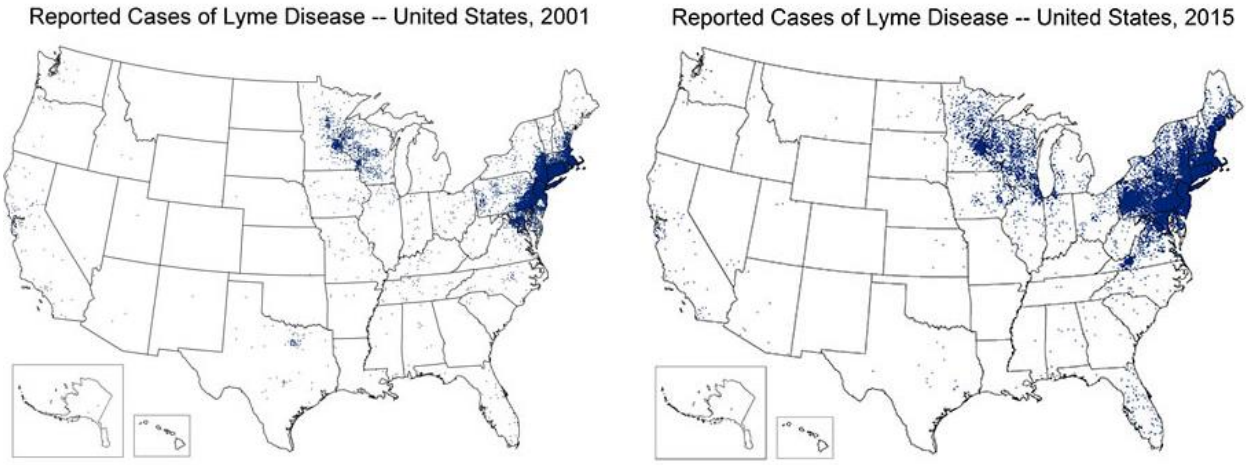

FIGURE 1: Reported cases of Lyme disease in the United States for 2001 & 2014 (CDC, 2015).

Although the Center of Disease Control (CDC) estimates that approximately 30,000 cases of Lyme disease are reported annually, there is still some skepticism regarding the surveillance of Lyme disease (CDC, 2015). According to several researchers, the statistical surveillance of Lyme disease is severely understated (Ostfeld, 2011; Petnicki-Ocwieja & Brissette, 2015). This can be due to the inaccuracy of the diagnostic tests and the difficulty diagnosing Lyme disease from the broad variety of possible symptoms linked to the disease (Ostfeld, 2011). In 2015, the state of Massachusetts reported 43 incidences of Lyme disease per every 100,000 individuals in

the state's' population (CDC, 2015). Overall, Massachusetts accounted for 14.4% of the 25,359 total cases recorded and was ranked as the second highest state for reported Lyme disease cases in 2014 (CDC, 2015). Figure 2 displays the incidence rates for the confirmed cases of Lyme disease in Massachusetts from 2010 to 2014.

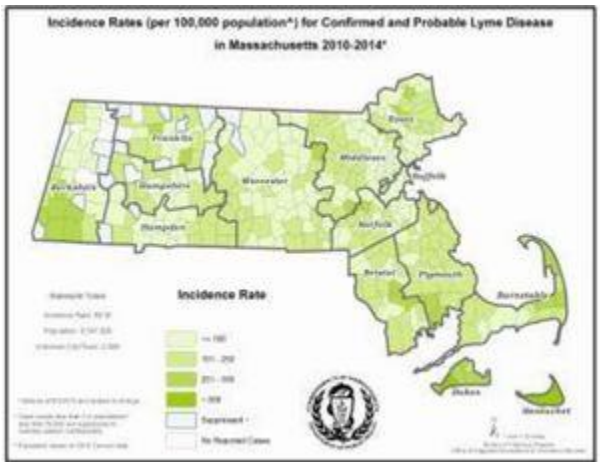

FIGURE 2: Incidence rates for population of confirmed reports for Lyme disease in Massachusetts from 2010- 2014 (MassGIS, 2016).

For this research project, our **goal** was to determine which abiotic and biotic indicators, associated with biodiversity, best explain the prevalence of Lyme disease in Massachusetts.

Lyme disease

Lyme disease is a vector-borne disease linked to transmission of *Borrelia burgdorferi*, a bacterium commonly carried by *Ixodes scapularis* ticks. Typically, ticks inhabit areas in the soil where they are able to gather nutrients to maintain homeostasis. The *B. burgdorferi* bacterium inhabits a tick as a vector which can transmit Lyme disease to a competent host. Pathogen transmission is more likely to occur if the tick attaches and feeds on a competent host for longer than twenty-four hours (Estrada-Peña, 2015; Petnicki-Ocwieja & Brissette, 2015).

Behavior of ticks have an effect on the transmission of Lyme disease (Arsnoe et al, 2015; Estrada-Peña, 2015). After hatching from larvae, ticks cannot sustain themselves on soil nutrients alone. They must travel out of the soil and leaf litter in search of a sustainable host for a blood meal (Estrada-Peña, 2015). The action of traveling to find a host to feed on is known as the questing period (Arsnoe et al, 2015; Estrada-Peña, 2015). In a recent study, it was hypothesized that the reason Lyme disease has become an epidemic is due to the differences in questing behaviors of southern and northern ticks (Arsnoe et al, 2015). In epidemic Northeastern regions, ticks are more likely to migrate out of leaf litter in search of a host, while in non-epidemic Southern regions ticks are less likely to move above the protection of the leaf litter. One source hypothesized that Southern ticks exhibit this behavior due to their ability to feed on hosts, such as lizards, that remain within the leaf litter (Arsnoe et al, 2015). The movement of the epidemic Northern ticks increases the probability of encountering a host that will increase pathogen transmission (Arsnoe et al, 2015).

Once feeding ends, the tick detaches itself from the host and falls to the ground to molt. Life stages of ticks include three parasitic stages: larva, nymph and adult tick (Figure 3). After feeding on a host, a tick will drop off and moult into the next stage. This continues until the tick becomes a mature adult tick and mates. Adult female ticks will fall off their hosts and instead of moulting, proceed to lay eggs (Arsnoe et al, 2015; Estrada-Peña, 2015).

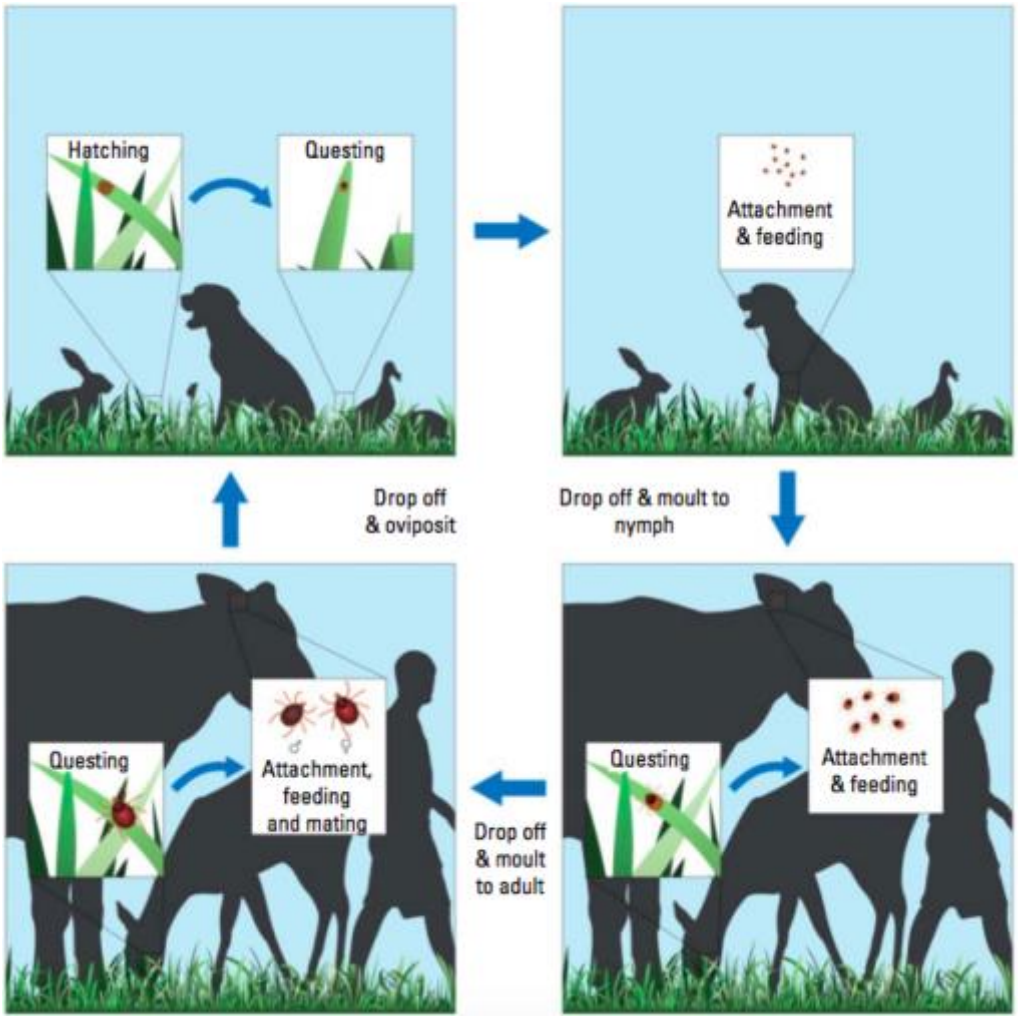

FIGURE 3: Tick life cycle (Estrada-Peña, 2015).

Ticks can only transmit Lyme disease to a host during their nymphal and adult life stages (Arsnoe et al, 2015; Estrada-Peña, 2015). Lyme disease transmission to a host is also reliant on a few additional variables including tick species, duration of attachment, and host competency. Although two families of ticks exist, only the *Ixodidae* family of ticks is capable of transmitting Lyme disease to a host due to their morphological features, namely their salivary glands (Estrada-Peña, 2015; Wilhelmsson et al, 2013). *Ixodidae* ticks secrete excess water derived from the blood meal back into their host during attachment. This allows for the bacterium to be passed

into the host's blood stream (Estrada-Peña, 2015). For pathogen transmission to occur, recent studies have suggested that the tick must be attached for at least twenty-four hours (Estrada-Peña, 2015; Wilhelmsson et al, 2013).

Reservoir competence is the ability of an infected host to reproduce and transmit the Lyme disease pathogen (Li et al, 2012; Wood et al, 2014). Although reservoir competence does vary between species, *Peromyscus leucopus* (white-footed mice) have been identified as the most competent hosts for *B. burgdorferi* transmission as shown in Figure 4 (LoGiudice et al, 2003; Wood et al, 2014). *Odocoileus virginianus* (white-tailed deer) populations were characterized as a secondary reservoir host for ticks during nymphal stage as well as raccoons and ground nesting birds (LoGiudice et al, 2003; Figure 4).

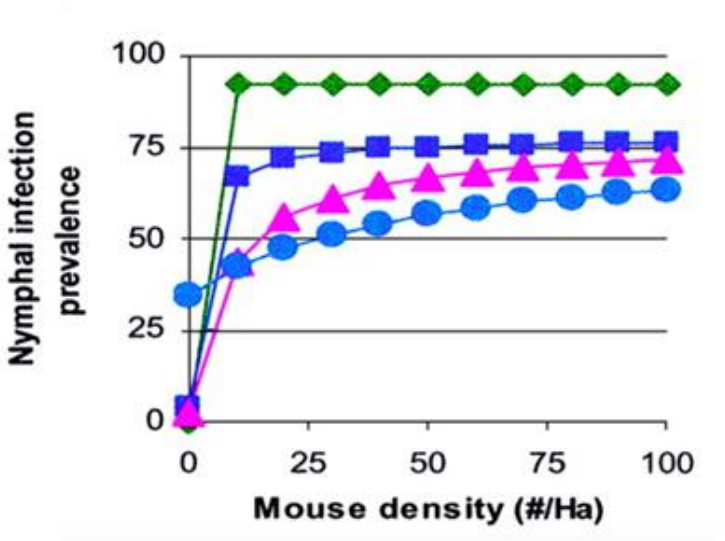

FIGURE 4: Reservoir competent hosts for Lyme disease pathogen vector with respect to nymphal infection prevalence and mouse density (Legend: green diamonds = white-footed mouse; blue squares = white-tailed deer; purple triangles = raccoons; blue circles = ground nesting birds) (LoGiudice et al, 2003).

Host competence may be dependent on both host behavior and host immunological response to the pathogen (Barron et al, 2015; Dizney & Dearing, 2016). After a host has been

exposed to the *B. burgdorferi* pathogen, the host's immune system will either be susceptible to the pathogen or reject the pathogen. If the host is susceptible to the pathogen, then the organism will support proliferation of the parasitic disease and will be infected with Lyme disease (Li et al, 2012). The duration of the infection can increase the probability of spreading the pathogen when the infected organisms encounters other susceptible organisms, thus transmitting Lyme disease (Barron et al, 2015). For vector-borne diseases such as Lyme disease, competent host population density and susceptibility of infection play an important role in the continued transmission of the disease (Wood et al, 2014).

Biodiversity

Recently, researchers proposed that an increase in the amount of biodiversity present in an ecosystem can have adverse effects on pathogenic transmission of a disease (Johnson et al, 2015). For the purpose of this paper, biodiversity will be defined as all the species richness present in a particular area or habitat. Species richness is inclusive of the number of different species present in an ecosystem (Laurila-Pant et al, 2015).

Biodiversity can be measured both directly and indirectly (Johnson et al, 2015; Laurila-Pant et al, 2015). Direct measurements of biodiversity include quantifying diversity measures by using the Shannon diversity index (Laurila-Pant et al, 2015). Large measurements of biodiversity are usually indicative of high species richness (Laurila-Pant et al, 2015). However, biodiversity is difficult to quantify because of the multitude of variables needed to adequately determine it. Although biodiversity is difficult to measure, indicators are used to estimate biodiversity indirectly (Laurila-Pant et al, 2015). In one research article, researchers used a "top-down taxonomic" method to estimate species richness and biodiversity (Williams & Gaston, 1994). A

species of a higher taxonomic group was measured to predict the species richness of an organism from a lower taxonomic level (Williams & Gaston, 1994). Other indicators of biodiversity can be related to the area of habitat or specific land covers. A mixture of different types of land cover is usually indicative of more biodiversity (Mittermeier et al, 1998).

The Dilution Effect Controversy

Interactions between abiotic and biotic factors can influence biodiversity present, which may or may not have an effect on the transmission of Lyme disease. Several researchers have hypothesized that loss of biodiversity in a habitat increases the risk of pathogen transmission (LoGuidice et al, 2003; Ostfeld, 2011). This hypothesis, known as the dilution effect, is reliant on three conditions: (1) the host species must differ in host reservoir competence, (2) low competent hosts can disrupt vector distribution, and (3) competent hosts are not vulnerable to species loss (Ostfeld, 2011). The dilution effect proposes that a decrease in biodiversity can be responsible for an increase in pathogen transmission due to an increase in interactions between the pathogen and competent hosts (Johnson et al, 2015).In addition, fragmented landscapes can decrease biodiversity in a habitat, especially if the separated habitats are approximately two hundred meters apart (Li et al, 2012; Zolnik et al, 2015). Thus, landscape fragmentation should increase incidence of Lyme disease.

However, some research has discredited the dilution hypothesis showing that land fragmentation has no effect on Lyme disease specifically (Zolnik et al, 2014). A counter argument supports that Lyme disease prevalence does not follow the proposed conditions outlined by the dilution effect (Wood et al, 2014; Zolnik et al, 2015). This theory, the amplification effect, states that an increase in biodiversity would facilitate an increase in

pathogen transmission of Lyme disease through the variability of competent hosts (Ogden & Tsao, 2009; Wood et al, 2014; Zolnik et al, 2015).

Biotic Factors

Biotic factors such as deer populations, land cover, and leaf litter were examined as possible indicators that may affect the prevalence of Lyme disease. In previous studies, white-tailed deer were characterized as the second most common competent host for nymphal infection prevalence (LoGiudice et al, 2003). Although the white-footed mouse is the most competent host for spreading Lyme disease, it is often difficult to directly quantify their population density. Larger species, such as the white-tailed deer, are easier to track and roughly estimate their population size (LoGiudice et al, 2003). Massachusetts deer population data was gathered from the Massachusetts Division of Fisheries & Wildlife to better determine if Lyme disease prevalence is directly associated with deer density.

When evaluating land cover, it is important to quantify the amount of cover an area has, as well as determine the type of forest cover. Types of forest cover, such as coniferous or deciduous forest, and land cover can alter the biodiversity of the environment. Massachusetts has a broad spectrum in both types of land cover and developed land (Defenders of Wildlife, 2016). Grasslands consist of relatively low species richness when compared to other forest types, both coniferous and deciduous (Guerra et al, 2002). Although coniferous forests have a higher species richness than grasslands, deciduous forests have the greatest species richness and thus the largest range of biodiversity (Guerra et al, 2002). Specifically, deciduous forests have supported several different species including ticks, with a broad range of food supply (Ostfeld et al, 2006).

Land cover also plays a large role in the amount of biodiversity of an environment. Land cover classifications include urban, suburban, and rural areas. Urban areas, such as large cities, have a lower amount of biodiversity due to development of infrastructure and removal of habitats. Urban areas often experience a shift in biodiversity from a large species richness to only a few species with higher population densities (McKinney, 2002; Goddard et al, 2010). Suburban areas are environments that have some infrastructure in place, but not to the extent of cities. Suburban areas do experience loss of habitat and land fragmentation due to a lower rate of land development, which could decrease the amount of biodiversity present (McKinney, 2002; Goddard et al, 2010). In rural areas a majority of land is not developed which can lead to high species richness and biodiversity (McKinney, 2002). Land cover varies throughout Massachusetts; as land cover transitions from urban to suburban and suburban to rural, the amount of biodiversity increases (McKinney, 2002).

Leaf litter, dying organic matter above the soil, is another biotic factor that can affect biodiversity present in an ecosystem (Swan, 2012). As the organic matter of leaf litter breaks down, the nutrients and minerals are reabsorbed into the environment through soil uptake. This nutrient rich soil supports a broader range of diverse organisms (Swan, 2012). High amounts of plant diversity can stimulate a high amount of organismal diversity (McKinney, 2002; Goddard et al, 2010). In addition, leaf litter aids in water conservation in the soil which can influence tick questing behavior (USDA, 2016).

Abiotic Factors

Abiotic factors, such as temperature and precipitation, affect species richness and the amount of biodiversity present in an environment (Costanza et al, 2007; Wang et al, 2009). Both

temperature and precipitation influence the vegetation and organisms that can be supported in a particular environment. For example, organisms that inhabit ecosystems such as deserts and rainforests vary (Costanza et al, 2007). In this study, both indicators of seasonal temperature and seasonal precipitation were evaluated to determine if either of these factors indirectly have a positive or negative correlation to tick database research.

Environments with extremely high or extremely low temperatures rarely contain an abundant amount of biodiversity since species must adapt to survive in these conditions (Wang et al, 2009). Overall, higher temperatures are conducive to an increase in species richness until an upper limit in temperature is reached (Wang et al, 2009). Recent studies found a direct correlation between temperature and questing duration of ticks (Greenfield, 2011). A tick will not search for a blood meal when temperatures are below 42 degrees Fahrenheit (Greenfield, 2011). As the temperature increases, the amount of time a tick spends questing also increases, which leads to a higher probability of Lyme disease transmission (Greenfield, 2011). Seasonal temperatures in Massachusetts can affect tick behavior patterns. High temperatures in the spring and summer months can promote an increase in tick questing, while low temperatures during fall and winter decrease the probability of pathogen transmission and tick activity (Greenfield, 2011). In addition, during the colder temperatures, general biodiversity loss occurs (Shimadzu et al 2013). During winter months, available vegetation decreases and several species of animals hibernate which further decreases the likelihood of transmitting Lyme disease (Shimadzu et al 2013).

Precipitation can also influence the amount of biodiversity present in an ecosystem. From research presented, environments with greater amounts of precipitation and humidity typically

exhibit higher abundances of biodiversity (Costanza et al, 2007). Water is an essential nutrient living organisms need to survive and encourage growth (Greenfield, 2011). With respect to tick behavior, soil moisture may be an important factor because ticks reside in the soil before and after questing (Gilbert, 2014). Unfortunately, for our research purposes, it was difficult to quantify soil moisture. Instead, we decided to use precipitation to estimate moisture that may be available in the soil and thus promote an increase in tick populations. Ticks' survival increases in areas where there is higher humidity and soil moisture that allows ticks to store water while questing for a host (Gilbert, 2014).

Ethical Considerations

For the scope of this study, we researched ethical considerations surrounding animal rights and human health. Our first ethical consideration is on the topic of animal rights since opposition has arisen revolving around the use of live organisms for the purpose of research (Mika, 2006). Tick specimens were submitted to the surveillance database where they were tested for *B. burgdorferi*, and other infectious diseases (University of Massachusetts, Amherst, 2016). Although ticks were submitted directly to the University of Massachusetts, Amherst *Tick-borne Diseases Passive Surveillance Database*, it should still be considered whether using these species for testing outweighed the cost of the organism's life (UMass, Amherst, 2016). Tick samples were individually removed and presumably killed during the removal process before being sent to the database, therefore ticks were not killed specifically for scientific purposes.

Our second ethical consideration is the scientist's' obligation to inform the public of the risks of infectious diseases. The prevalence of Lyme disease has been debated over the past several years (Ostfeld, 2011). Questions have arisen as to whether disease surveillance is

increasing or overestimated due to difficulties in diagnosis (Auwaerter et al., 2011; Johnson et al., 2010). Data from the UMass Amherst database supports that the number of infected Lyme disease ticks did increase in the last several years (CDC, 2014; University of Massachusetts, Amherst, 2016). From this inquiry, research surrounding this topic has recently spiked due to an increase in the number of ticks submitted and, of those ticks submitted, an increase in the number of ticks tested positive for Lyme disease (CDC, 2014; UMass Amherst, 2016). Given the risk to human health, there is an argument as to whether or not researchers are obligated to share information pertaining to Lyme disease (Nelson & Vucetich, 2009). From our ethical standpoint, we support that scientists should clearly communicate information that has been thoroughly researched to the public (Halliday, 2009).

As additional information is collected, new testing strategies allow for more analysis on predicting the risk and prevalence associated with Lyme disease (Ozdenerol, 2015). The Geographic Information Systems (GIS) can be used to test new variables, such as soil composition, predator populations, humidity, or other disease incidences and their correlations to gather more information about Lyme disease risk (Ozdenerol, 2015).

Akaike Information Criterion (AIC)

Akaike Information Criterion (AIC) is a type of analysis that allows for ranking of models from a given data set. Although there are several different models of statistical analysis that can be used, AIC modeling compares output of each model and then determines which model best explains the data closest to the true relationship (Posada et al, 2004).

The analysis can determine which variables best represent the relationship between an indicator and one of two response variables: percent infected ticks and number of ticks

submitted. Corrected AIC (AIC_c) scores take small sample size into account and correct the relationship or correlation based on the expected data for a larger sample size. AIC weighting was used to determine which data set, out of each indicator being tested, would have the greatest likelihood of being the best model from the set. Delta AIC determines the difference between the likelihood of each indicator being the best model with the best fit data (Posada et al, 2004).

CHAPTER TWO: BIOTIC & ABIOTIC INDICATORS OF BIODIVERSITY AND THEIR CORRELATION TO LYME DISEASE TRANSMISSION

Abstract

Incidences of Lyme disease have increased steadily in Massachusetts since 2012. The University of Massachusetts has used a self-submission based tick testing database to document the number of ticks submitted and the number of infected ticks tested positive for tick-borne diseases. This project's goal was to determine which potential surrogates of biodiversity best explain the incidence of Lyme disease in Massachusetts. Loss of biodiversity across landscapes may play an important role in the increased spread of infectious diseases. Based on the dilution effect high levels of biodiversity can reduce the spread of diseases. Using correlation and AIC_c analyses, we examined the influences of forest cover, number of people, deer density, seasonal temperature, and seasonal precipitation have on tick submissions and infection of ticks. Our results demonstrated that the number of people best explained the percent of infected ticks and was negatively correlated. The dilution effect was not supported by these analyses. However, based on our findings that number of people influences percent infected ticks, awareness efforts can be implemented to curb pathogen transmission.

Introduction

Lyme disease is a vector-borne disease transmitted by an *Ixodidae scapularis* tick that is infected with the pathogenic bacterium *Borrelia burgdorferi* (Estrada-Peña, 2015). During a blood meal, *B. burgdorferi* is passed from the tick to a competent reservoir host such as the *Peromyscus leucopus* (white-footed mice) or *Odocoileus virginianus* (white-tailed deer). These species allow maintenance of the parasitic bacterium to thus increase transmission of the disease (Fiset et al, 2015). Although ticks may encounter an incompetent host, a host that cannot support

the *B. burgdorferi* pathogen, these species facilitate tick survival throughout their larvae, nymph, and adult life stages by providing a blood meal (Estrada-Peña, 2015).

Biodiversity, or abundance of different species present in a designated area, can influence the transmission and maintenance of pathogens (Ostfeld, 2011; Li et al, 2012). A proposed hypothesis, called the dilution effect, suggests that a decrease in host species diversity will result in an increase in the prevalence of Lyme disease (Ostfeld, 2011; Li et al, 2012). In addition to the host species diversity present, the hypothesis states that three other conditions have an effect on the infection rate. The three conditions include that (1) host species differ in reservoir competence of the disease, (2) a low number of competent hosts disrupt the distribution of the pathogen, and (3) competent hosts are not susceptible to species loss (Ostfeld, 2011). Well known competent hosts for maintaining and transmitting Lyme disease include the white-footed mouse and the white-tailed deer. Humans are affected by Lyme disease as low competence hosts (LoGuidice et al, 2003; Ostfeld, 2011). Overall, the dilution effect suggests that a general loss in biodiversity would increase the risk of pathogen transmission (LoGuidice et al, 2003; Ostfeld, 2011). Alternative research using simulation data challenges that the dilution effect is not plausible (Zolnik et al, 2015).

Climate, soil moisture, and land cover can have an effect on the species diversity that is present in a habitat, which in turn may affect pathogen transmission (Costanza et al, 2007; Greenfield, 2011; Wang et al, 2009). Areas with high temperatures and high precipitation have been known to have the greatest amount of biodiversity because of the diverse species richness in the environment (Costanza et al, 2007; Wang et al, 2009). Soil moisture influences tick behavior that may lead to an increase in the number of ticks that can infect a competent host (Greenfield, 2011; Gilbert, 2014). Moreover, areas of land that are well developed may lead to a

decrease in biodiversity present (McKinney, 2002). This paper will examine the role of these biodiversity indicators and their influence on the prevalence of Lyme disease in areas across Massachusetts.

Goals & Hypotheses

The goal of our research was to determine which indicators of biodiversity best explain the prevalence of Lyme disease in Massachusetts. This project used data compiled from the University of Massachusetts, Amherst *Tick-borne Diseases Passive Surveillance Database* in conjunction with data pertaining to number of people, land use, and seasonal weather to determine if these identified indicators have a positive or negative correlation to the prevalence of Lyme disease in selected study areas of the state. From our research, we hypothesized that deer density, average seasonal temperature, number of people, and percent composition of forest cover would significantly affect the prevalence of Lyme disease. We predicted that the first three indicators (deer density, average seasonal temperature, and number of people) would show a positive correlation to Lyme disease prevalence, while percent composition forest cover would have a negative correlation relationship.

In previous research, white-tailed deer are competent hosts that can maintain and transmit the *B. burgdorferi* pathogen (Ostfeld et al, 2006; Ostfeld, 2011; Fiset, 2015). It was proposed that an area with high deer populations will result in a higher percentage of recorded positive cases of Lyme disease due to high host competency (Ostfeld, 2011; Fiset, 2015). Average seasonal temperature was also hypothesized to exhibit a positive relationship in Lyme disease prevalence. We hypothesize that higher average summer and winter temperatures would result in an increase in percent infected Lyme disease cases (Ostfeld et al, 2006; Greenfield, 2011; Levi, 2016). Tick survival decreases in colder temperatures since questing behavior is reduced

(Estrada-Peña, 2015). Warmer temperatures increase the amount of ticks questing which may increase the prevalence of Lyme disease if an infected tick feeds on a competent host and spreads the disease (Estrada-Peña, 2015; Greenfield, 2011). Number of people was hypothesized to have a positive correlation relationship with Lyme disease prevalence. Urban areas with high numbers of people are usually indicative of less forest cover (Johnson et al, 2015). By coupling number of people and land cover data with the dilution effect, we propose that Lyme disease is expected to be greatest in urban areas and lowest in rural areas due to the amount of biodiversity present in each area (Johnson et al, 2015). Based on prior research, it was hypothesized that higher percent composition of forest cover may negatively affect the prevalence of Lyme disease (Dobson, 2012; Estrada-Peña, 2015; Levi, 2016). This infers that high forest cover and high quantities of biodiversity are usually directly related, and will have a direct correlation to the decrease in pathogen transmission as proposed by the dilution effect (Dobson, 2012; Ostfeld, 2011; Levi, 2016).

Methods

Study Areas in Massachusetts

ArcMap 10.4.1 (ESRI, 2011) was used to determine ten or twelve spatially independent study areas. Study areas are defined as an individual zip code or an aggregation of zip codes. Zip codes were categorized based on the 2010 human population census. The number of people from the census ranged from 0 people per square mile (depicted by the dark green color) to 620,000 people per square mile (depicted by the dark red regions; Figure 5). After separating zip codes by population, zip codes were selected based on classification of rural or urban regions. Three towns were selected from urban, highly populated regions and four towns were selected

from rural, sparsely populated regions. The remainder (n = 12) of selected zip codes were chosen based on distribution of population densities in between the extremes. Furthermore, zip code areas in square miles were collected to ensure the sizes for each location were relatively comparable. If a selected study area was determined to have an inadequate area, localized zip codes were aggregated together to increase the area being tested. We obtained the total number of ticks submitted for each study area in Massachusetts from the University of Massachusetts, Amherst *Tick-borne Diseases Passive Surveillance* public database (LMZ, 2016). The information for the total number of ticks submitted for each zip code in Massachusetts were compiled for ticks submitted for testing from January 1st, 2006 until November 8th, 2016. Inadequate number of ticks submitted to the database restricted some zip codes from being selected for our research. Final study areas were distributed across the state (Figure 6).

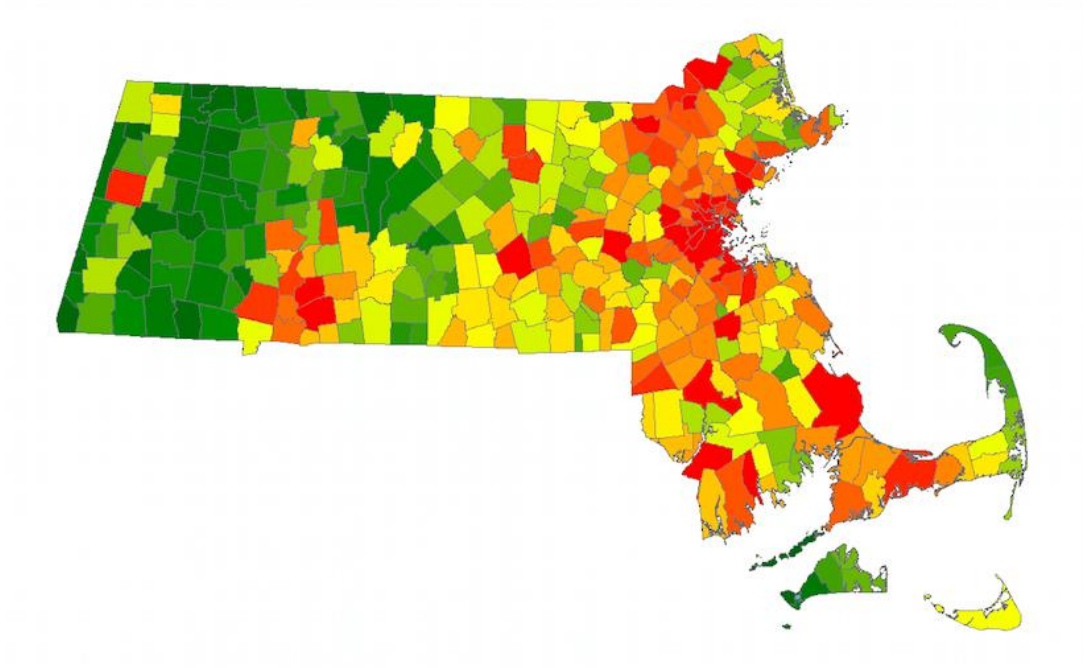

FIGURE 5: Visual representation of number of people from the 2010 census across Massachusetts.

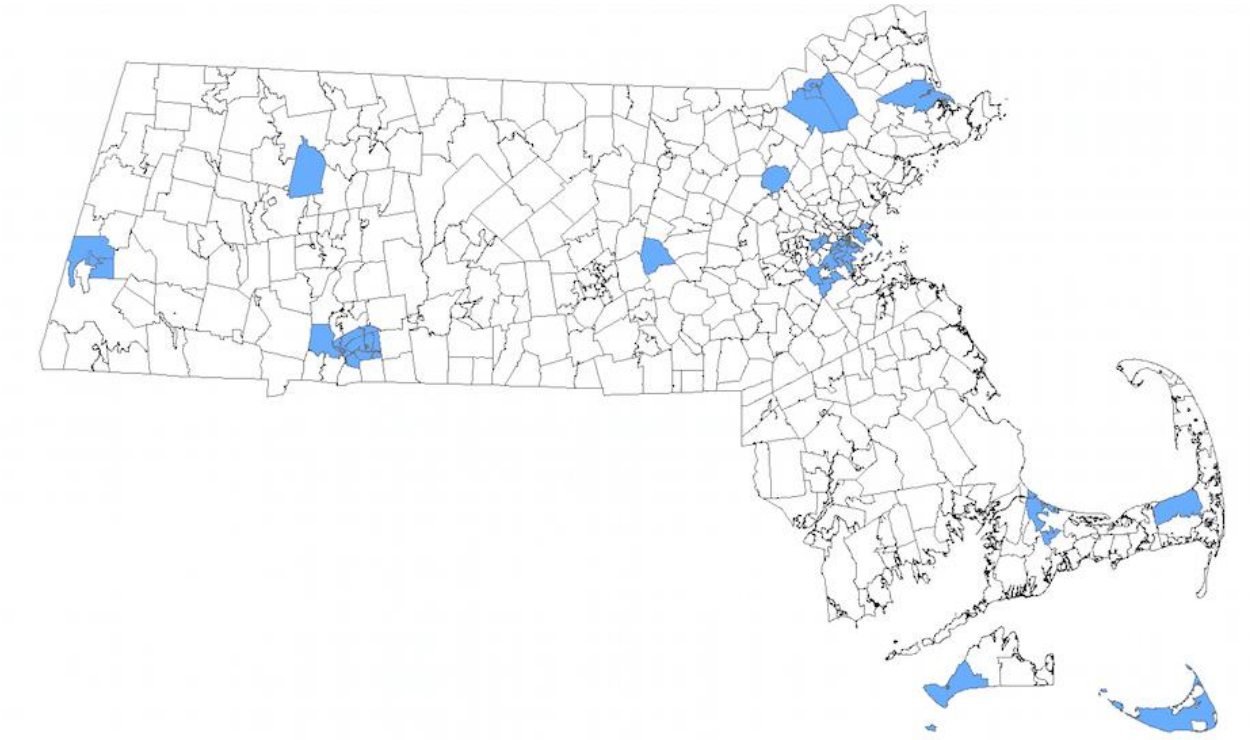

FIGURE 6: Distribution of study areas in Massachusetts.

Land Cover

MassGIS Land Use 2005 datalayer (MassGIS, 2016) was clipped to selected study areas in ArcMap 10.4.1 to quantify land use and land cover. The aspects of use and cover examined for this project's purposes were forest (combined coniferous and deciduous), open land, brushland/successional, powerline/utility, transitional, commercial, population density, and water. For our statistical analysis, we combined powerline/utility with brushland/successional to limit the variables tested. We calculated the percentage of each land cover type for each selected study area in ArcMap (Figure 7).

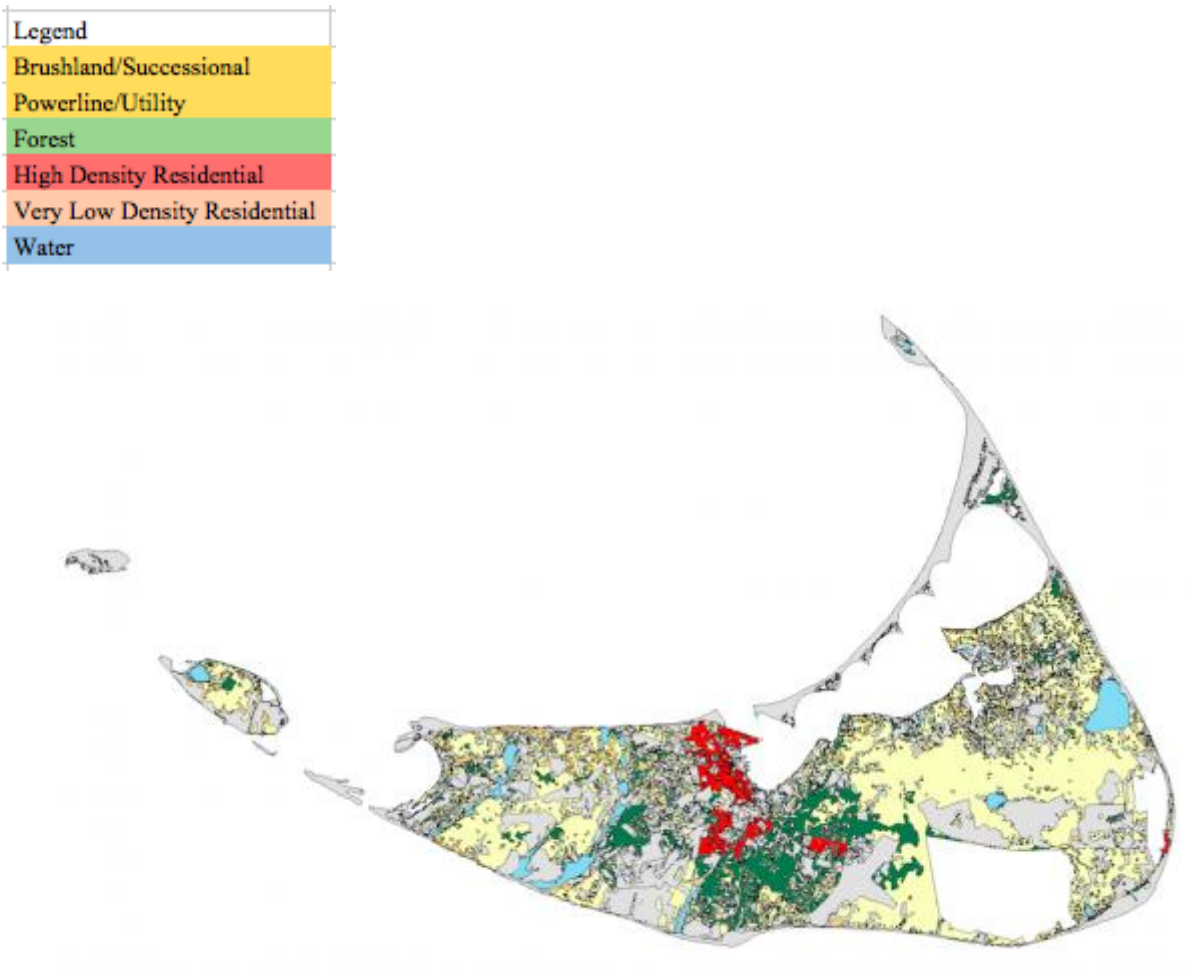

FIGURE 7: Land use for the Nantucket study area.

Temperature and Precipitation

Temperature and precipitation records from 2005 to 2016 for each of the sample study areas were obtained through the use of Weather Underground, an online weather station (Weather Underground, 2016). For each study area selected in Massachusetts, the monthly average temperature and amount of precipitation were collected. Based on the monthly average records, the seasonal temperature and amount of precipitation were also determined. Seasonal temperature was calculated by averaging the recorded data for the months of each season as follows: Winter (December, January, and February), Spring (March, April, and May), Summer

(June, July, and August), and Fall (September, October, and November). The recorded monthly and seasonal temperatures and amounts of precipitation are found in Appendix C.

Deer Population

High and low estimated deer population densities were acquired through the Deer and Moose Project Leader, David Stainbrook, at the Massachusetts Division of Fisheries and Wildlife (MassWildlife) for each of the selected study area. Deer population densities were then calculated by multiplying the density recorded by the square miles of forest present (Stainbrook, personal communication, 2016). MassWildlife determines the deer population estimates by surveying areas during hunting seasons (Stainbrook, personal communication, 2016).We used this upper deer density limit calculation to run our statistical and correlation data.

Study Area (Town Name)	Lower Limit Deer Density (deer/sq. mile)	Upper Limit Deer Density (deer/sq. mile)	Calculated Deer Population
Boston	0	80	268
Andover, N Andover & Lawrence	20	50	1309
Springfield & West Springfield	12	25	308
Bedford	30	60	268
Brewster	15	30	411
Ipswich	30	60	681
Nantucket	30	60	237
Northborough	15	30	252
Conway	10	20	608
Chilmark	30	60	954
Stockbridge/West Stockbridge	12	25	602
Sandwich	15	30	264

TABLE 1: Deer density and deer population estimates

Data Analysis

To remove redundancy and reduce the number of predictor variables, we used a correlation analysis (SAS version 9.4; SAS, 2013). For any pairs of variables that were highly correlated (p-value 0.05) we retained variables that were of greatest interest in terms of management. We tested for correlation in the following variables: high deer density, low deer density, average winter temperature, average fall temperature, average spring temperature, average summer temperature, average winter precipitation, average fall precipitation, average spring precipitation, average summer precipitation, percent successional/brushland cover, percent open land cover, percent forest cover, percent very low density residential cover, percent

high density residential cover, percent commercial cover, percent transitional cover, and number of people from 2010 census.

Additionally, we ran two Akaike's Information Criterion (AIC) analyses to determine explanatory variables that would best represent each response variable. We developed a set of models to examine the relationship between environmental predictor variables (deer density, human population number, land cover, average seasonal temperature, average seasonal rrecipitation) and response variables (percent of infected ticks & number of ticks submitted; see Tables 4 & 5 for all models). A null model with no predictor variables and a full model with all predictor values were included in analyses.

We used AIC_c analyses to compare and rank eleven models while correcting for small sample size. K is the number of indicators used in running the model including the intercept. Delta AIC_c is the difference between the model's AIC_c value and the model with the smallest AIC_c value. Ex is the expectancy of the model having a direct impact on the response variable. The model weight is listed under Akaike weight, which explains which indicator is the most important in determining which factor may affect the percent infected ticks and the number of ticks submitted. Lastly, the log likelihood value for each model is based on generalized linear mixed models. We then graphed relationships between response and explanatory variables from top-ranked models.

Results

The UMass Amherst database recorded information for each individual tick submitted to the database from 2006 to 2016. The number of ticks tested specifically in Massachusetts steeply increased from 2013 to 2014 and then remains relatively consistent from 2014 to 2016 (Figure

8). The number of ticks tested annually in Massachusetts ranged from 1-3792 and averaged 1049 (±462.22SE). This was inclusive of ticks that carry Lyme disease (*B. burgdorferi sensu lato*) and generic ticks (*Borrelia general species*) (UMass, 2016).

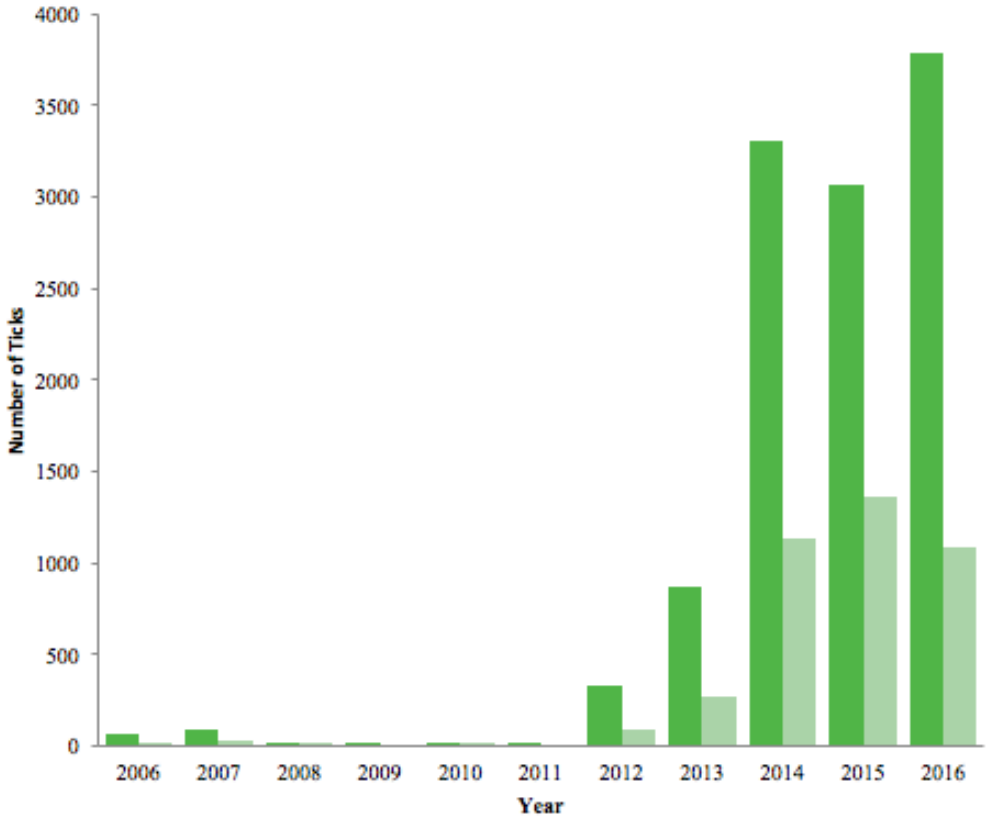

FIGURE 8: Number of ticks tested in Massachusetts state from 2006-2016 (UMass, 2016). The number of ticks submitted to the database drastically increased in 2014. Dark blue represents the number of ticks the UMass database tested, while the light blue accounts for the number of ticks tested positive for Lyme disease.

We compiled 1,021 entries of ticks from 12 spatially independent study areas throughout Massachusetts from 2006-2016 from the UMass Amherst database. Study areas were defined by the town name and zip code or aggregation of zip codes (shown in Table 2). We classified study areas into rural, suburban or urban locations based on the number of people present and land

cover. The number of ticks tested for our study areas ranged from 14-284 and averaged 85 (± 23.14SE). The lowest recorded percent infected ticks was 7.14% for Boston, while the highest recorded percent infected ticks was 38.89% for Northborough.

Study Area (Town Name)	Zip Code(s)	Square Miles	# Ticks	Percentage of Positive Ticks	Classification of Area (Rural, suburban, urban)	Number of People (Census 2010)
Boston	Aggregated List	20.64	14	7.14%	Urban	617,594
Andover, N. Andover & Lawrence	Aggregated List	66.83	94	23.40%	Urban	137,930
Springfield & W. Springfield	Aggregated List	47.83	14	21.40%	Urban	181,451
Bedford	1730	13.85	138	28.99%	Suburban	13,320
Brewster	2631	24.85	284	32.04%	Suburban	9,820
Ipswich	1938	30.07	26	19.23%	Suburban	13,175
Nantucket	2554	44.42	108	32.41%	Suburban	10,172
Northborough	1532	18.71	36	38.89%	Suburban	14,155
Conway	1341	36.47	156	30.77%	Rural	1,897
Chilmark	2535	28.21	32	21.88%	Rural	866
Stockbridge/W. Stockbridge	01262, 01266	37.77	24	20.83%	Rural	3,523
Sandwich	2563	43.79	95	28.42%	Suburban	20,675

TABLE 2: Summary table of each of the selected study areas with associated town name, zip code, number of ticks submitted, area, and the number of people.

Data Analysis Results

Based on the correlation analysis results for our explanatory variables, we retained seven variables: high deer density, average winter temperature, average spring precipitation, percent successional/brushland cover, percent forest cover, percent very low residential cover, and

number of people from 2010 census. The correlation table with p-values (0.05) for our original indicators is located in Appendix D. Variables were excluded from the retained indicators if they were highly correlated to the other indicators measured (shown in Table 3).

Retained Indicators	Highly Correlated Indicators
High Deer Density	Low Deer Density
Average Winter Temperature	Average Fall Temperature & Average Summer Precipitation
Average Spring Precipitation	Average Winter Precipitation
Percent Successional/Brushland Cover	Percent Open Land Cover
Percent Forest Cover	Percent Transitional Cover
Percent Very Low Density Residential Cover	Percent High Density Residential Cover
Number of People (2010 Census)	Percent Commercial Cover, Percent High Density Residential Cover, Average Summer Temperature & Average Spring Temperature

TABLE 3: Retained explanatory indicators determined for response variables from correlation data analysis. The correlation between indicators was used to determine which indicators would be used to run AICc analysis against the number of ticks submitted and the percent of infected ticks.

Percent Infected Ticks Predicting Factors

Results from AIC_c modeling demonstrated that number of people best explained variation in the percent infected ticks that tested positive for Lyme disease (Table 4). Number of people was shown to be negatively related to percent infected ticks based on the 95% confidence interval that is significant since the range is not inclusive of zero (Table 5; Figure 9). The top model with number of people was 20.8x more supported than the next model that did not contain this explanatory variable. Although the number of people predictor variable best explains the percent infected ticks response, the second model (number of people + high deer population) was

ranked with a delta AIC_c less than 2 (Table 4).. However, we suggested that the number of people variable is the most important. This was especially true since the 95% confidence interval for high deer population was not significant and included zero (Table 5).

Model	K	AIC_c	delta AIC_c	Ex	Akaike Weight
NumofPeople	2	-27.4301	0.000	1.000	0.664
NumofPeople + HighDeerPop	3	-25.5888	1.842	0.398	0.264
Null	1	-21.3764	6.054	0.048	0.032
Forest	2	-18.5749	8.855	0.012	0.008
Successional	2	-18.3493	9.081	0.011	0.007
HighDeerPop	2	-18.2371	9.193	0.010	0.007
VeryLowResDen	2	-17.9211	9.509	0.009	0.006
SpringPrec	2	-17.7779	9.652	0.008	0.005
AveWinter	2	-17.7457	9.685	0.008	0.005
Forest + Successional	3	-15.6512	11.779	0.003	0.002
Full Model	8	60.7652	88.196	0.000	0.000
				1.507	1.000

TABLE 4: Models run in Akaike's Information Criterion response to percent infected ticks. The delta AICc explains the model that best explains percent infected ticks. The models that best explain percent infected ticks were number of people and combined number of people and high deer density.

Explanatory Variable	Estimate	Standard Error	95% Confidence Interval Lower Limit	95% Confidence Interval Upper Limit
NumofPeople	0.0000	0.0000	0.0000	0.0000
HighDeerDen	-0.0001	0.0001	-0.0002	0.0001

TABLE 5: Parameter estimates and 95% Confidence intervals of number of people and high deer density.

We used graphs to visually display the relationships between top ranked models for the percent infected ticks response variable. As shown in Figure 9, the relationship between percent of infected ticks and number of people was negative ($R^2 = 0.551$). Additionally, the relationship between second ranked model (high deer density and percent infected ticks) was graphed to show the variability between the 12 study areas ($R^2 = 0.0438$; Figure 10).

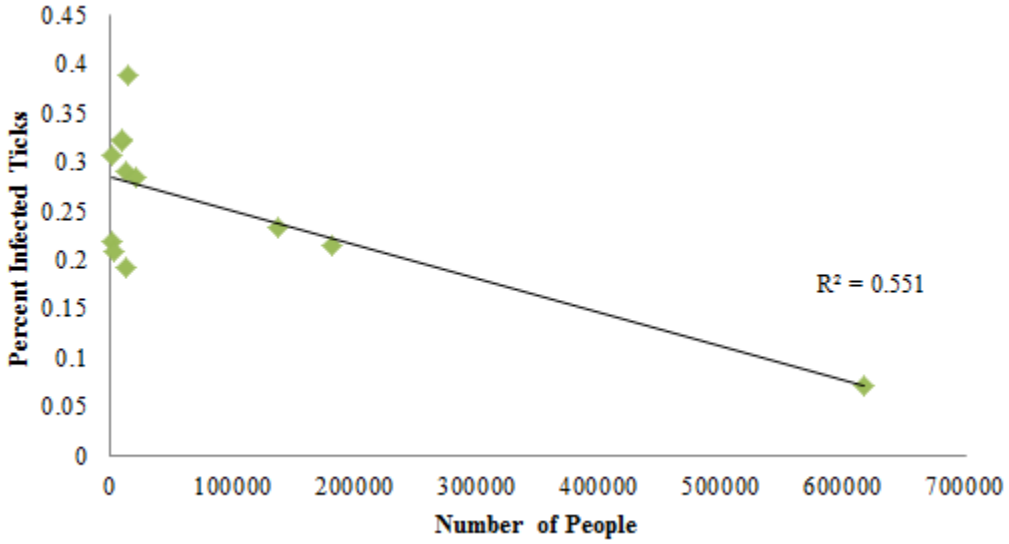

Figure 9: Graph of the relationship between the percent infected ticks and number of people. The data of infected ticks and number of people were retrieved from 12 sample locations.

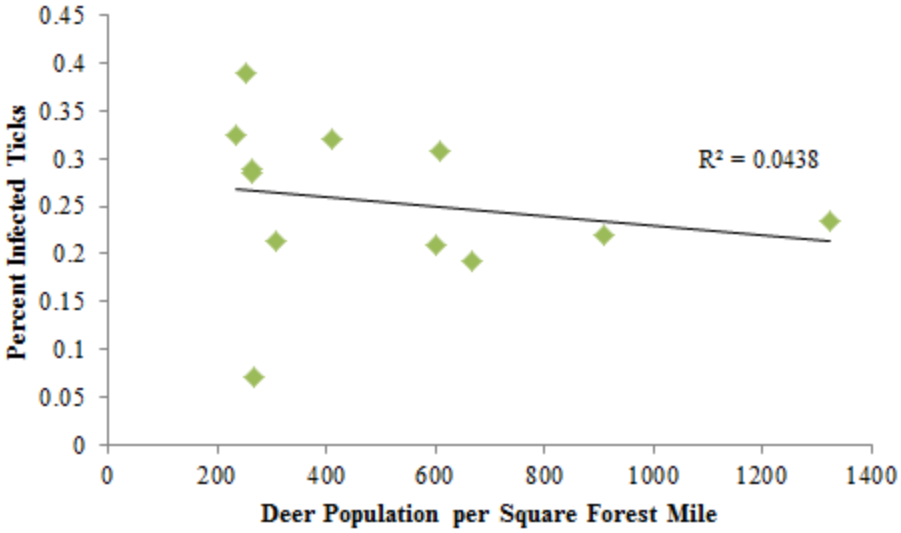

FIGURE 10: Graph of the relationship between percent infected ticks and the high deer density in a sample area. The data for each variable was collected in 12 study areas.

Number of Ticks Submitted Predicting Factors

Results from the AIC_c modeling demonstrated that the null model best explained the variation in the number of ticks submitted to the UMass Amherst database (Table 6). The null model was 2.9x more supported than the next model. The second ranked model (the number of people indicator) was not ranked with a delta AIC_c less than 2 and therefore was not considered important. In addition, the 95% confidence interval for the number of people was not significant because it included zero (Table 7).

Model	K	AIC_c	delta AIC_c	Ex	Akaike Weight
Null	1	143.5557	0.000	1.000	0.366
NumofPeople	2	145.699	2.143	0.342	0.125
AveWinter	2	146.0267	2.471	0.291	0.106
SpringPrec	2	146.1493	2.594	0.273	0.100
VeryLowResDen	2	146.3653	2.810	0.245	0.090
Forest	2	146.8232	3.268	0.195	0.071
HighDeerPop	2	147.1703	3.615	0.164	0.060
Successional	2	147.1842	3.629	0.163	0.060
NumofPeople + HighDeerPop	3	150.2409	6.685	0.035	0.013
Forest + Successional	3	151.2995	7.744	0.021	0.008
Full Model	8	230.9502	87.395	0.000	0.000
				2.730	1.000

TABLE 6: Models run in Akaike's Information Criterion response to number of ticks submitted. The delta AICc and Akaike weight shows the models that best explain the number of ticks submitted. The resulting models that best explain the number of ticks submitted was the null.

Explanatory Variable	Estimate	Standard Error	95% Confidence Interval Lower Limit	95% Confidence Interval Upper Limit
Null (Intercept Only)	85.0833	22.1508	41.6685	128.4982
NumofPeople	-0.0002	0.0001	-0.0004	0.0001

TABLE 7: 95% Confidence interval of null and number of people models for number of ticks submitted response variable.

The model that best represents the number of ticks submitted is the null model. The null model predicts that the variables that we explored did not influence the prediction of the number of ticks submitted. The second best model to predict the number of ticks submitted was the number of people model (Figure 11). However, the R^2 value (0.1192) and the 95% confidence

interval suggested that the relationship between the number of people and the number of ticks submitted was not important.

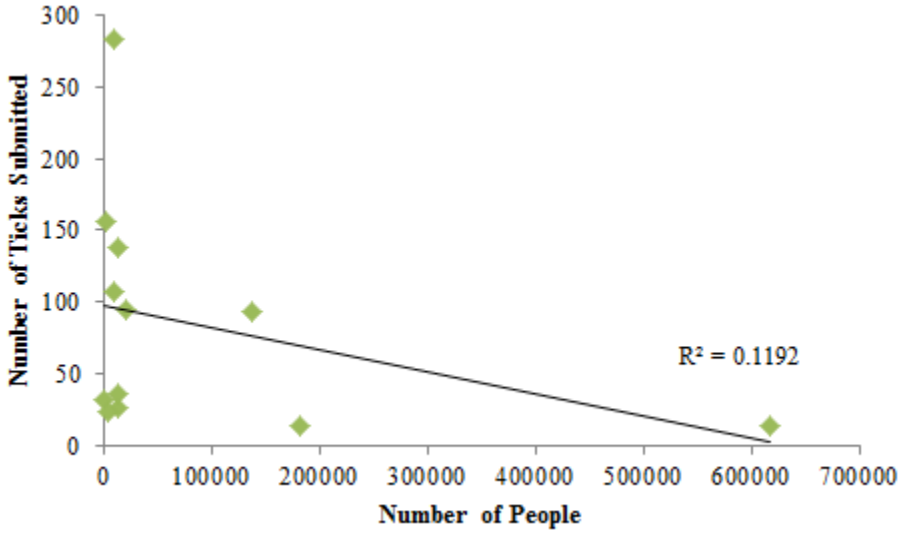

FIGURE 11: Graph of the relationship between the number of ticks submitted to the number of people for each sample location. The R^2 value was 0.1192, indicating this is not as strong as a relationship compared to the previous response model.

Discussion

Percent Infected Ticks

Surprisingly, we found that the number of people model was negatively correlated to the the percent of infected ticks response variable. Thus, our results do not support the dilution effect hypothesis. The dilution effect proposes that a higher number of species richness would lower the chance of an individual coming into contact with an infected tick and decrease the incidence of Lyme disease. Although the larger number of people influences the percent of infected ticks, this is not indicative of species richness in a study area. Therefore, we cannot conclude that the dilution effect would be supported.

In addition, our hypothesis for number of people was refuted. Originally, we hypothesized the opposite effect would occur because a higher number of people should lower the percent forest cover through urbanization that can lead to a decrease biodiversity in an area studied, thus increasing Lyme disease incidence rates (McKinney, 2002; Goddard et al, 2010; Guerra et al, 2002). However, from our collected data we realize that number of people ranging from approximately 200,000 to 600,000 were not tested (shown in Figure 9). If we considered data points within this range, our results may change. Other indicators that had a high correlation to number of people were percent commercial cover and percent high density residential cover (shown in Table 4). It could be inferred that high numbers of people and high percent commercial composition could result in a low percent of infected ticks because of decreased amount of forest habitat present (Guerra et al, 2002). Insufficient amount of forest habitat could decrease species richness supported in an area and thus decrease the amount of ticks that inhabit the area (Guerra et al, 2002; Ostfeld, 2011). Previous studies investigated that land fragmentation caused by urbanization had no effect on infection rates and thus did not support the dilution effect hypothesis (Zolink et al, 2015). In addition, research supports that a negative relationship exists between tick infection prevalence and species richness in fragmented habitats (LoGiudice et al, 2008; Zolink et al, 2015).

The second explanation for the percent infected ticks response variable was the combined number of people and high deer density indicators (delta AIC_c = 1.842). Although this combined result showed a high AIC_c value (-25.5888), we did not believe the number of people + high deer density was an appropriate explanation on its own. From our analysis, it was suggested that the number of people indicator may have influenced the AIC_c value when in combination with high deer density. This may be especially true since the high deer density indicator alone did not have

a high AIC_c value or a well-represented 95% confidence interval (shown in Table 6 & Table 7). Since this interval included zero, the interval is not significant, therefore deer density is not a representative model for percent infected ticks. High deer density was strongly correlated with low deer density. This was expected because we received ranges from the Massachusetts Division of Fisheries and Wildlife (MassWildlife), which predicted the density based on square forest mile (Stainbrook, personal communication). We omitted low deer density because, for some study areas the low estimate was zero, which may not have been appropriate representation of the deer density present.

The data retrieved from the MassWildlife on deer population densities limited our results. After conversing with a project leader at this organization, we recognized that deer densities vary greatly even within a two-mile radius due to deer movement (Stainbrook, personal communication). Thus, it is challenging to estimate the population of deer within a specific study area. In addition, deer population estimates are calculated during hunting seasons which may overestimate or underestimate the population size (Stainbrook, personal communication, 2016). Therefore, both low and high deer population estimates were recorded for the range the MassWildlife provided (Stainbrook, personal communication, 2016). Other research further suggests that deer populations exhibit variable and weak interactions with ticks (Ostfeld et al, 2006). Observed interactions between ticks and deer did not effect nymphal abundance in subsequent seasons regardless of the deer population size (Ostfeld et al, 2006).

Number of Ticks Submitted

Based on the analysis, the null hypothesis was the best explanation for the response variable number of ticks submitted (delta $AIC_c = 0.000$). None of the models containing the explanatory variables we chose explained the number of ticks submitted. However, we expected

this result because the number of ticks submitted to the UMass Amherst database should not be dependent on any biodiversity indicator tested. From our data collection, we relied heavily on self-submitted ticks to the UMass Amherst database which may not have been an appropriate representation of tick populations in Massachusetts. We inferred that distance from the tick submission laboratory and low amount of tick submissions could have affected our data. Therefore, we did not include Amherst in our study area to eliminate any potential bias. In addition, study ares were selected based on the number of ticks submitted.

Limitations

Due to the scale and scope of our project, we would like to address some additional limitations that are evident in this report. These limitations include potential biasing due to selection of our sample study areas, sample size, and available datalayers.

Hand-picking the Sample

When determining the selection of potential areas for our study, we were unableto perform a random sampling method to counteract biases due to limitations in data submitted to the tick database. To establish a control for our project, selected study areas were within similar land sizes, spatially separated, contained a broad range in human population size, and were from different quantifications of land development. Since random sampling did not occur, potential sample biasing may have occurred.

Small Sample Size

Our sample size consisted of 12 study areas. A small sample size runs the risk of bias, especially since we hand-picked our sample. In addition, 12 study areas may not be representative of the entirety of Massachusetts. A larger sample size would negate the potential

bias from selected study areas and would be more representative of Massachusetts state as a whole.

Land Cover

Although we were able to separate different types of land cover in each of the selected study areas, we encountered a limitation within the Land Cover datalayer from MassGIS. Forest cover could not be filtered into different forest types: deciduous and coniferous covers. This differentiation of forest types could allow for specificity in type of forest cover that best correlates with Lyme disease prevalence (Guerra et al, 2002; Ostfeld et al, 2006). In addition, a secondary limitation occurred with the MassGIS Land Cover datalayer. The land cover datalayer used for this analysis was collected in 2005. This datalayer may not have been representative of the land cover present during the submission of ticks to the database, which were recorded from 2005 through 2016.

Future Recommendations

In future studies, additional indicators, such as elevation, soil moisture, and leaf litter should be tested against our response variables. Due to global increases in temperature, some ticks are able to move to higher elevations that they were not previously able to survive (Brownstein et al, 2005). Changes in suitable habitats for ticks may affect the prevalence of Lyme disease in new habitats (Brownstein et al, 2005). Previous research hypothesized that the amount of leaf litter and moisture in the soil could have a positive effect on tick survival (Guerra et al., 2005). However, we could not collect quantitative data for the amount of moisture in the soil or the amount of leaf litter in any selected study area to use in our analysis. In previous studies soil moisture was quantified from data collected on daily temperature and humidity

readings (Medlock et al, 2008). We hypothesize that soil moisture and degree of leaf litter in a study area are indicators of Lyme disease prevalence in Massachusetts due to their association with temperature and humidity (Estrada-Peña, 2015; Greenfield, 2011; Fiset, 2015).

In summary, our research suggested that human population numbers, a potential indicator of biodiversity, best explained the percent of infected ticks in Massachusetts. Incidences of Lyme disease may be correlated to the proportion of ticks that are infected, however this would require additional experimentation. From our research, the number of people was also positively correlated with percent high density residential cover, percent commercial cover, average spring temperature, and average summer temperature. Although there is no definitive explanation for incidences of Lyme disease, public awareness of biotic and abiotic influences in general could help to curb pathogen transmission.

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Appendices

Appendix A: Steps taken in ArcMap 10.4.1 with Datalayers

Layer: Zip Code (5-digit codes)

1. MassGIS Datalayers (http://www.mass.gov/anf/research-and-tech/it-serv-and-support/application-serv/office-of-geographic-information-massgis/datalayers/layerslist.html)
2. Find Datalayer 'ZIP Codes (5-Digit) from HERE' under the Political/Administrative Boundaries Section.
3. Download the shapefile and save in a specified folder.
4. Open ArcMap 10.4.1 and open a blank map.
5. Navigate to Add Data.
6. In the Add Data window, select Connect to Folder and find the folder the files are saved in. Select Okay.
7. Once again, in the Add Data window, select the shapefile for the data and select Add. The shapefile will be added as a layer in the Table of Contents window.
8. Select the zip codes needed.
    a. Select the Zip code layer under Table of Contents
    b. Select Open Attribute Table
    c. The Table will open and select the City_Town heading. Click Sort Ascending.
    d. Select the zip codes and towns being used for the study.
        i. While holding the Control key on the keyboard, click on each zip code/ town to be displayed.
        ii. The selected zip codes/ towns will be displayed after exiting the attribute table.
        iii. Make selected zip codes/ towns layer.

Layer: Selected Zip Codes/ Towns

1. Zip codes/ towns were selected and highlighted from the selected zip codes/ towns from Layer: Zip Codes, #8, d.
2. Right click on the Zipcodes layer.
3. Click Selection.
4. Select 'Create Layer From Selected Features'
5. A new layer will be produced with only the selected zip codes/ towns. Rename the layer Selected Zip Codes.

Layer: Datalayers from the 2010 U. S. Census

1. MassGIS Datalayers (http://www.mass.gov/anf/research-and-tech/it-serv-and-support/application-serv/office-of-geographic-information-massgis/datalayers/layerslist.html)
2. Find Datalayer 'Datalayers from the 2010 U. S. Census' under the Census/Demographic Data
3. Find the category Other Geography. Download the Census 2010 Town data Shapefile.
4. Copy the files to your own folder.

5. In ArcMap 10.4.1, navigate to Add Data.
6. In the Add Data window, select Connect to Folder and find the folder the files are saved in. Select Okay.
7. Once again, in the Add Data window, select the shapefile for the data and select Add. The shapefile will be added as a layer in the Table of Contents window.
8. To view the population data visually, select the variables and the color ramp.
    a. Right click on the CENSUS2010TOWNS_POLY layer
    b. Select Properties
    c. Select the Symbology tab
    d. Select Categories under the 'Show:' box on the left hand side of the tab
    e. Select Unique values, many fields
    f. Under Value Fields, select POP2010.
    g. Select the green to red color ramp
    h. Add All Values.
    i. Select Okay
    j. The attributes for population will be shown under the Census 2010 layer in the Table of Contents.

Layer: Land Use (2005)

1. MassGIS Datalayers (http://www.mass.gov/anf/research-and-tech/it-serv-and-support/application-serv/office-of-geographic-information-massgis/datalayers/layerslist.html)
2. Find Datalayer "Land Use (2005)" under Physical Resources: Land Use/Land Cover, Geological/Geophysical, Atmospheric
3. Download the shapefile.
4. Copy the downloaded files into specified folder
5. In ArcMap 10.4.1, navigate to Add Data.
6. In the Add Data window, select Connect to Folder and find the folder the files are saved in. Select Okay.
7. Once again, in the Add Data window, select the shapefile for the data and select Add. The shapefile will be added as a layer in the Table of Contents window.
8. Select attributes to be displayed to show and change the data being examined.
    a. Right click on the layer
    b. Select Properties
    c. Select the Symbology tab
    d. Select Categories on the left hand window under 'Show'.
    e. Select Unique values, many fields
    f. Select the data to be shown in the Value Field required box. In this case, it is (LU05_DESC).
    g. Select Add All Values, which will extract the data
    h. Select Okay
    i. Change the colors of the layers:
        i. The attributes will be displayed under the layer in the Table of Contents window.

ii. Right click the color block next to the attribute and select the color wanted for each attribute.
iii. If attribute is not wanted to be examined, color the attribute grey.
iv. All unwanted attributes will be the same color so the wanted attributes can be examined based on their color.
v. Select Okay.

Indicators of Land Use selected in Layer: Land Use (2005), steps f-i:

- Transitional and Commercial (Combined)
- Power lines and Brush/Successional (Combined)
- Densities (Low and High → the two extremes or comparison)
- Forest
- Open Land
- Water

Land Use and Zip Codes Clipped Data:

1. Using Land Use 2005 layer and the Selected Zip Codes layer, the two layers can be clipped together so land use data will only show for the selected zip codes.
2. Select 'Selected Zip Codes'
3. Select Geoprocessing
4. Select Clip
5. In Input Features, add the Selected Zip Codes file.
6. In Clip Features, add the Land Use 2005 file.
7. In Output Feature Class, label the clipped file specifically.
    a. Example: LandUse_SelectedZips_Clipped.
8. Select Okay. A new layer will appear with the clipped Land Use and Selected Zip Code

Appendix B: Summary Table of Weather for Selected Study Areas

Town Name	Winter Avg. Temp	Spring Avg. Temp	Summer Avg. Temp	Fall Avg. Temp.	Winter Total Precipitation	Spring Total Precipitation	Summer Total Precipitation	Fall Total Precipitation
Boston	32.97	49.22	72.25	56.05	0.138	0.129	0.143	0.126
Andover, N Andover & Lawrence	29.94	48	70.97	53.61	0.088	0.110	0.115	0.117
Springfield & West Springfield	27.89	47.89	70.36	52.11	0.093	0.103	0.144	0.115
Bedford	28.92	47.28	69.97	52.08	0.097	0.108	0.114	0.118
Brewster	35.28	46.36	68.89	55.33	0.128	0.113	0.093	0.130
Ipswich	30.11	46.56	69.17	52.92	0.109	0.123	0.117	0.126
Nantucket	35.33	45.92	66.89	55.72	0.099	0.099	0.088	0.160
Northborough	27.97	46.89	69.42	52.17	0.156	0.143	0.163	0.161
Conway	25.86	45.7	68.6	50.11	0.099	0.094	0.127	0.132
Chilmark	34.05	46.53	68.28	54.39	0.101	0.103	0.088	0.130
Stockbridge/West Stockbridge	25.45	44.78	66.92	49.45	0.113	0.107	0.153	0.140
Sandwich	32.53	46.42	68.92	53.75	0.128	0.097	0.088	0.100

Appendix C: Summary Table of Land Cover for Selected Study Areas

Study Area (Town Name)	Brushland/ Successional	Powerline/ Utility	Commerial	Transitional	Forest	Open Land	High Density Residential	Very Low Density Residential	Water
Boston	0.4		10.84	0.39	7.92	1.79	12.96	0.06	2.47
Andover, N Andover & Lawrence	0.15	0.5	2.56	0.29	39.18	1.09	4.56	1.12	5.12
Springfield & West Springfield	0.01	0.23	7	0.23	34.47	1.09	22.86	0.24	3.8
Bedford	0.04		2.7	0.39	4.58	0.88	1.97	0.46	0.12
Brewster	0.08	0.65	0.75	0.13	55.07	0.47	0.48	1.28	9.08
Ipswich	0.14	0.05	0.67	0.09	37.74	0.98	1.68	3.47	1.91
Nantucket	36.03		0.65	0.16	8.9	11.21	2.52	2.98	3.23
Northborough	0.08	0.33	1.76	0.36	44.92	1.15	0.46	3.15	1.26
Conway	0.08	0.48	0.03	0	83.33	1.03	0.03	1.72	0.94
Chilmark	5.51		0.17	0.03	56.34	3.96	0	6.76	9.24
Stockbridge/West Stockbridge	0.05	0.38	0.36	0.06	63.8	2.43	0.05	3.1	3.04
Sandwich	0.04	0.94	1.64	0.12	53.16	0.93	2.05	1.03	1.89

Appendix D: Correlation Analysis for P-values <0.05

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	SqMiles	NoTicks	NoPositiveTicks	PercentPositiveTicks	HumanPopNo	LowDeerDen	HighDeerDen	AveWintTem	AveSpringTem	AveSummerTem	AveFallTem	WinterPrecip	SpringPrecip	SummerPrecip	FallPrecip	Successional	Utility	Commercial	Transitional	Forest	Openland	HighDenRes	LowDenRes	Water
SqMiles	1.00000	-0.21459 0.5030 12	-0.25221 0.4290 12	-0.38661 0.2144 12	0.34966 0.2652 12	0.37881 0.2246 12	0.49428 0.1024 12	-0.09124 0.7779 12	0.27708 0.3833 12	0.21604 0.5001 12	0.06099 0.8506 12	-0.51852 0.0841 12	-0.17931 0.5771 12	0.14278 0.6580 12	-0.01152 0.9717 12	0.19957 0.5340 12	-0.28466 0.4944 8	0.28279 0.3731 12	0.09221 0.7874 11	-0.06222 0.8477 12	0.23170 0.4687 12	0.45163 0.1632 11	-0.16278 0.6132 12	0.16403 0.6105 12
NoTicks	-0.21459 0.5030 12	1.00000	0.99488 <.0001 12	0.52403 0.0803 12	-0.34529 0.2717 12	-0.00223 0.9945 12	-0.02140 0.9474 12	0.30795 0.3302 12	-0.24598 0.4409 12	-0.16391 0.6107 12	0.17370 0.5893 12	-0.04602 0.8871 12	-0.29248 0.3563 12	-0.50091 0.0971 12	-0.04909 0.8796 12	0.05378 0.8682 12	0.55725 0.1513 8	-0.37505 0.2296 12	-0.15145 0.6567 11	0.16978 0.5978 12	-0.09805 0.7618 12	-0.43966 0.1760 11	-0.26425 0.4066 12	0.31116 0.3249 12
NoPositiveTicks	-0.25221 0.4290 12	0.99488 <.0001 12	1.00000	0.57021 0.0529 12	-0.36247 0.2469 12	-0.05575 0.8634 12	-0.08398 0.7953 12	0.31077 0.3255 12	-0.27531 0.3864 12	-0.20219 0.5286 12	0.17252 0.5918 12	0.00917 0.9774 12	-0.25922 0.4159 12	-0.47261 0.1208 12	0.02147 0.9472 12	0.08504 0.7927 12	0.53606 0.1708 8	-0.38370 0.2182 12	-0.15802 0.6426 11	0.17484 0.5868 12	-0.06884 0.8317 12	-0.44165 0.1738 11	-0.24103 0.4505 12	0.30411 0.3365 12
PercentPositiveTicks	-0.38661 0.2144 12	0.52403 0.0803 12	0.57021 0.0529 12	1.00000	-0.74509 0.0054 12	-0.02246 0.9448 12	-0.09213 0.7758 12	-0.05472 0.8659 12	-0.49983 0.0980 12	-0.47911 0.1150 12	-0.19722 0.5390 12	0.13078 0.6854 12	-0.07530 0.8161 12	-0.16468 0.6090 12	0.41000 0.1856 12	0.22143 0.4892 12	0.37312 0.3626 8	-0.64553 0.0234 12	-0.33150 0.3193 11	0.23300 0.4661 12	0.06439 0.8424 12	-0.53081 0.0930 11	0.12907 0.6893 12	0.03503 0.9139 12
HumanPopNo	0.34966 0.2652 12	-0.34529 0.2717 12	-0.36247 0.2469 12	-0.74509 0.0054 12	1.00000	-0.40547 0.1910 12	-0.27380 0.3892 12	0.14785 0.6465 12	0.79791 0.0019 12	0.75695 0.0044 12	0.41292 0.1822 12	0.23590 0.4604 12	0.35239 0.2613 12	0.33879 0.2814 12	-0.19144 0.5512 12	-0.13917 0.6662 12	-0.19694 0.6402 8	0.92896 <.0001 12	0.81358 0.0023 11	-0.42409 0.1695 12	-0.02555 0.9372 12	0.61502 0.0440 11	-0.46794 0.1250 12	-0.21869 0.4947 12
LowDeerDens	0.37881 0.2246 12	-0.00223 0.9945 12	-0.05575 0.8634 12	-0.02246 0.9448 12	-0.40547 0.1910 12	1.00000	0.98039 <.0001 12	-0.14428 0.6546 12	-0.24864 0.4358 12	-0.18251 0.5702 12	-0.23601 0.4602 12	-0.53380 0.0739 12	-0.25429 0.4251 12	-0.22531 0.4814 12	-0.12603 0.6963 12	-0.16878 0.6000 12	-0.19992 0.6350 8	-0.52560 0.0793 12	-0.54169 0.0852 11	0.48522 0.1098 12	-0.15257 0.6360 12	-0.29435 0.3796 11	0.51186 0.0889 12	0.50492 0.0941 12
HighDeerDen	0.49428 0.1024 12	-0.02140 0.9474 12	-0.08398 0.7953 12	-0.09213 0.7758 12	-0.27380 0.3892 12	0.98059 <.0001 12	1.00000	-0.13528 0.6751 12	-0.11280 0.7271 12	-0.04836 0.8814 12	-0.17555 0.5852 12	-0.52766 0.0779 12	-0.20391 0.5250 12	-0.18027 0.5750 12	-0.16329 0.6121 12	-0.18098 0.5735 12	-0.14006 0.7408 8	-0.40433 0.1924 12	-0.40550 0.2160 11	0.40039 0.1971 12	-0.16111 0.6169 12	-0.21494 0.5256 11	0.39337 0.2058 12	0.46766 0.1253 12
AveWintTemp	-0.09124 0.7779 12	0.30795 0.3302 12	0.31077 0.3255 12	-0.05472 0.8659 12	0.14785 0.6465 12	-0.14428 0.6546 12	-0.13528 0.6751 12	1.00000	0.19574 0.5421 12	-0.00726 0.9821 12	0.94263 <.0001 12	0.13002 0.6871 12	-0.03838 0.9057 12	-0.74316 0.0056 12	0.00443 0.9891 12	0.49150 0.1046 12	0.49634 0.2109 8	0.64403 <.0001 12	-0.04723 0.8903 11	-0.35939 0.2512 12	0.46969 0.1234 12	-0.01624 0.9622 11	0.15547 0.6295 12	0.51733 0.0850 12
AveSpringTemp	0.27708 0.3833 12	-0.24598 0.4409 12	-0.27531 0.3864 12	-0.49983 0.0980 12	0.79791 0.0019 12	-0.24864 0.4358 12	-0.11280 0.7271 12	0.19574 0.5421 12	1.00000	0.94576 <.0001 12	0.47735 0.1166 12	0.09091 0.7787 12	0.40834 0.1876 12	0.18361 0.5678 12	-0.35421 0.2586 12	-0.23359 0.4650 12	-0.13994 0.7519 8	0.85405 0.0004 12	0.81123 0.0024 11	-0.57139 0.0523 12	-0.20688 0.5188 12	0.65250 0.0295 11	-0.48617 0.1090 12	-0.12487 0.6990 12
AveSummerTemp	0.21604 0.5001 12	-0.16391 0.6107 12	-0.20219 0.5286 12	-0.47911 0.1150 12	0.75695 0.0044 12	-0.18251 0.5702 12	-0.04836 0.8814 12	-0.00726 0.9821 12	0.94576 <.0001 12	1.00000	0.27442 0.3881 12	0.13887 0.6669 12	0.43268 0.1601 12	0.29589 0.3504 12	-0.46466 0.1280 12	-0.49324 0.1032 12	-0.10509 0.8044 8	0.80099 0.0017 12	0.79049 0.0038 11	-0.37624 0.2281 12	-0.47764 0.1163 12	0.55339 0.0774 11	-0.58559 0.0454 12	-0.21473 0.5027 12
AveFallTemp	0.06099 0.8506 12	0.17370 0.5893 12	0.17252 0.5918 12	-0.19722 0.5390 12	0.41292 0.1822 12	-0.23601 0.4602 12	-0.17555 0.5852 12	0.94263 <.0001 12	0.47735 0.1166 12	0.27442 0.3881 12	1.00000	0.19050 0.5531 12	0.15355 0.6338 12	-0.54822 0.0650 12	-0.00647 0.9841 12	0.42772 0.1654 12	0.36349 0.3761 8	0.31482 0.3196 12	0.27097 0.4203 11	-0.51558 0.0862 12	0.41695 0.1775 12	0.16965 0.6180 11	-0.01979 0.9513 12	0.37213 0.2336 12
WinterPrecip	-0.51852 0.0841 12	-0.04602 0.8871 12	0.00917 0.9774 12	0.13078 0.6854 12	0.23590 0.4604 12	-0.53380 0.0739 12	-0.52766 0.0779 12	0.13002 0.6871 12	0.09091 0.7787 12	0.13887 0.6669 12	0.19050 0.5531 12	1.00000	0.70845 0.0099 12	0.32874 0.2968 12	0.28843 0.3633 12	-0.22339 0.4852 12	0.22554 0.5912 8	0.17846 0.5789 12	0.33660 0.3115 11	-0.22339 0.4852 12	0.22554 0.5912 8	0.17846 0.5789 12	0.33660 0.3115 11	-0.19226 0.5494 12	-0.19966 0.5561 11	-0.03237 0.9204 12	-0.17217 0.5928 12
SpringPrecip	-0.17931 0.5771 12	-0.29248 0.3563 12	-0.25922 0.4159 12	-0.07530 0.8161 12	0.35239 0.2613 12	-0.25429 0.4251 12	-0.20391 0.5250 12	-0.03838 0.9057 12	0.40834 0.1876 12	0.43268 0.1601 12	0.15355 0.6338 12	0.70845 0.0099 12	1.00000	0.55569 0.0607 12	0.36678 0.2409 12	-0.27527 0.3865 12	-0.45676 0.2552 8	0.32096 0.3091 12	0.53971 0.0866 11	-0.25578 0.4223 12	-0.23998 0.4525 12	-0.01641 0.9618 11	0.00880 0.9783 12	-0.19942 0.5344 12
SummerPrecip	0.14278 0.6580 12	-0.50091 0.0971 12	-0.47261 0.1208 12	-0.16468 0.6090 12	0.33879 0.2814 12	-0.22531 0.4814 12	-0.18027 0.5750 12	-0.74316 0.0056 12	0.18361 0.5678 12	0.29589 0.3504 12	-0.54822 0.0650 12	0.32874 0.2968 12	0.55569 0.0607 12	1.00000	0.26230 0.4102 12	-0.42051 0.1735 12	-0.63295 0.0921 8	0.40752 0.1885 12	0.45219 0.1626 11	0.05819 0.8574 12	-0.34002 0.2795 12	0.29459 0.3792 11	-0.22380 0.4844 12	-0.48942 0.1063 12
FallPrecip	-0.01152 0.9717 12	-0.04909 0.8796 12	0.02147 0.9472 12	0.41000 0.1856 12	-0.19144 0.5512 12	-0.12603 0.6963 12	-0.16329 0.6121 12	0.00443 0.9891 12	-0.35421 0.2586 12	-0.46466 0.1280 12	-0.00647 0.9841 12	0.28843 0.3633 12	0.36678 0.2409 12	0.26230 0.4102 12	1.00000	0.54493 0.0669 12	-0.43197 0.2852 8	-0.28224 0.3741 12	-0.01301 0.9697 11	-0.04377 0.8926 12	-0.53705 0.1788 12	-0.44165 0.1738 11	-0.24267 0.4505 12	-0.13080 0.9660 12
Successional	0.19957 0.5340 12	0.05378 0.8682 12	0.08504 0.7927 12	0.22143 0.4892 12	-0.13917 0.6662 12	-0.16878 0.6000 12	-0.18098 0.5735 12	0.49150 0.1046 12	-0.23359 0.4650 12	-0.49324 0.1032 12	0.42772 0.1654 12	-0.22339 0.4852 12	-0.27527 0.3865 12	-0.42051 0.1735 12	0.54493 0.0669 12	1.00000	-0.25138 0.5481 8	-0.39379 0.5475 12	-0.16166 0.6349 11	-0.40691 0.1893 12	0.97147 <.0001 12	-0.08611 0.8012 11	0.25554 0.4228 12	0.07238 0.8231 12
Utility	-0.28466 0.4944 8	0.55725 0.1513 8	0.53606 0.1708 8	0.37312 0.3626 8	-0.19694 0.6402 8	-0.19992 0.6350 8	-0.14006 0.7408 8	0.49634 0.2109 8	-0.13994 0.7519 8	-0.10509 0.8044 8	0.36349 0.3761 8	0.22554 0.5912 8	-0.45676 0.2552 8	-0.63295 0.0921 8	-0.43197 0.2852 8	-0.25138 0.5481 8	1.00000	-0.20900 0.6194 8	-0.11162 0.8117 7	-0.53705 0.1788 8	-0.24379 0.5607 8	-0.29081 0.4847 8	-0.48308 0.2253 8	0.23552 0.5745 8
Commercial	0.28279 0.3731 12	-0.37505 0.2296 12	-0.38370 0.2182 12	-0.64553 0.0234 12	0.92896 <.0001 12	-0.52560 0.0793 12	-0.40433 0.1924 12	0.64403 <.0001 12	0.85405 0.0004 12	0.80099 0.0017 12	0.31442 0.3196 12	0.17846 0.5789 12	0.32096 0.3091 12	0.40752 0.1885 12	-0.28224 0.3741 12	-0.39379 0.5475 12	-0.20900 0.6194 8	1.00000	0.81747 0.0021 11	-0.51736 0.0850 12	-0.11490 0.7222 12	0.81244 0.0024 11	-0.59351 0.0419 12	-0.27995 0.3782 12
Transitional	0.09221 0.7874 11	-0.15145 0.6567 11	-0.15802 0.6426 11	-0.33150 0.3193 11	0.81358 0.0023 11	-0.54169 0.0852 11	-0.40550 0.2160 11	-0.04723 0.8903 11	0.81123 0.0024 11	0.79049 0.0038 11	0.27097 0.4203 11	0.33660 0.3115 11	0.53971 0.0866 11	0.45219 0.1626 11	-0.01301 0.9697 11	-0.16166 0.6349 11	-0.11162 0.8117 7	0.81747 0.0021 11	1.00000	-0.64422 0.0324 11	-0.11610 0.7339 11	0.36667 0.2973 10	-0.52672 0.0960 11	0.44601 0.1461 11
Forest	-0.06222 0.8477 12	0.16978 0.5978 12	0.17484 0.5868 12	0.23300 0.4661 12	-0.42409 0.1695 12	0.48522 0.1098 12	0.40039 0.1971 12	-0.35939 0.2512 12	-0.57139 0.0523 12	-0.37624 0.2281 12	-0.51558 0.0862 12	0.05533 0.8664 12	-0.25578 0.4223 12	0.05819 0.8574 12	-0.04377 0.8926 12	-0.40691 0.1893 12	0.35705 0.3852 8	-0.51736 0.0850 12	-0.64422 0.0324 11	1.00000	0.39409 0.2049 12	-0.33606 0.3123 11	0.31799 0.3138 12	0.28606 0.3674 12
Openland	0.23170 0.4687 12	-0.09805 0.7618 12	-0.06884 0.8317 12	0.06439 0.8424 12	-0.02555 0.9372 12	-0.15257 0.6360 12	-0.16111 0.6169 12	0.46969 0.1234 12	-0.20688 0.5188 12	-0.47764 0.1163 12	0.41695 0.1775 12	-0.19226 0.5494 12	-0.23998 0.4525 12	-0.34002 0.2795 12	0.56432 0.0560 12	0.97147 <.0001 12	-0.24379 0.5607 8	-0.11490 0.7222 12	-0.11610 0.7339 11	-0.39409 0.2049 12	1.00000	-0.04033 0.9063 11	0.33825 0.2822 12	0.08318 0.7972 12
HighDenRes	0.45163 0.1632 11	-0.03966 0.1760 11	-0.44165 0.1738 11	-0.53081 0.0930 11	0.61502 0.0440 11	-0.29435 0.3796 11	-0.21494 0.5256 11	-0.01624 0.9622 11	0.65250 0.0295 11	0.55339 0.0774 11	0.16965 0.6180 11	-0.19966 0.5561 11	-0.01641 0.9618 11	0.29459 0.3792 11	-0.33065 0.3206 11	-0.08611 0.8012 11	-0.29081 0.4847 8	0.81244 0.0024 11	0.36667 0.2973 10	-0.33606 0.3123 11	-0.04033 0.9063 11	1.00000	-0.59156 0.0552 11	0.01260 0.9707 11
LowDenRes	-0.16278 0.6132 12	-0.26425 0.4066 12	-0.24103 0.4505 12	0.12907 0.6893 12	-0.46794 0.1250 12	0.51186 0.0889 12	0.39337 0.2058 12	0.15547 0.6295 12	-0.48617 0.1090 12	-0.58559 0.0454 12	-0.01979 0.9513 12	-0.03237 0.9204 12	0.00880 0.9783 12	-0.22380 0.4844 12	0.44267 0.1496 12	0.25554 0.4228 12	-0.48308 0.2253 8	-0.59351 0.0419 12	-0.58906 0.0565 11	0.31799 0.3138 12	0.33825 0.2822 12	-0.59156 0.0552 11	1.00000	0.44601 0.1461 12
Water	0.16403 0.6105 12	0.31116 0.3249 12	0.30411 0.3365 12	0.03503 0.9139 12	-0.21869 0.4947 12	0.50492 0.0941 12	0.46766 0.1253 12	0.51733 0.0850 12	-0.12487 0.6990 12	-0.21473 0.5027 12	0.37213 0.2336 12	-0.17217 0.5928 12	-0.19942 0.5344 12	-0.48942 0.1063 12	-0.01380 0.9660 12	0.07238 0.8231 12	0.23552 0.5745 8	-0.27995 0.3782 12	-0.52672 0.0960 11	0.28606 0.3674 12	0.08318 0.7972 12	0.01260 0.9707 11	0.44601 0.1461 12	1.00000