A multi-scale investigation of the relationship between host diversity and Lyme disease

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ABSTRACT

This thesis investigates the relationship between host diversity and Lyme disease. There are two main hypotheses linking host diversity to Lyme disease incidence; the first, the "dilution effect" hypothesis predicts a negative relationship, while the second, the "amplification effect" hypothesis predicts a positive relationship. Research to date suggests that the diversity-Lyme disease relationship is more nuanced than either of these hypotheses, such that the observed relationship may be dependent upon the particular temporal or spatial scale of observation, among other factors. In my first chapter, I review previous theory, models, and empirical evidence relating to the host diversity-Lyme disease relationship. Chapter 2 is a statistical investigation, using generalized linear mixed models, of the relationship between tick host species richness and Lyme disease incidence in the United States from 1992 to 2011. We found an increasingly negative relationship between host diversity and disease incidence in time, indicating an increasing dilution effect. Chapter 3 is a statistical analysis, again using a generalized linear mixed model, of the relationship between small mammal diversity and the density of *Borrelia burgdorferi* infection, the pathogen responsible for Lyme disease in 27 forest sites in Southern Quebec in 2011, 2012, and 2013. We found a positive relationship between small mammal diversity and the density of *Borrelia burgdorferi*, indicating an amplification effect. We then explored the mechanisms driving this diversity-disease relationship in Southern Quebec using structural equation models. The contrasting findings of these two studies, which take place at different spatial and temporal scales, as well as at different degrees of Lyme disease emergence, reinforce previous findings that the diversity-Lyme disease relationship is highly context-dependent.

Ce mémoire a pour objectif d'étudier la relation entre l'incidence de maladie de Lyme et la diversité des hôtes porteurs de cette maladie. Il existe deux hypothèses reliant l'incidence de la maladie de Lyme à la diversité de ses hôtes. La première hypothèse, l'effet de dilution, prédit une relation négative entre l'incidence de la maladie et la diversité de ses hôtes tandis que la seconde hypothèse, l'effet d'amplification, prédit au contraire une relation positive. Les recherches antérieures suggèrent que la relation hôtes-Lyme est plus nuancée que l'une ou l'autre hypothèse prises individuellement. En effet, la relation observée dépendrait notamment des échelles spatiales et temporelles étudiées. Dans mon premier chapitre, je passe en revue les théories, modèles et études empiriques publiés sur la relation entre la maladie de Lyme et ses hôtes. Le chapitre 2 consiste en une étude statistique, basée sur les modèles linéaires mixes généralisés de la relation entre la richesse spécifique des tiques hôtes et l'incidence de la maladie de Lyme aux Etats-Unis de 1992 à 2011. Nous avons trouvé une relation de plus en plus négative entre la diversité des hôtes et l'incidence de la maladie pour la période de 1992 à 2011, ce qui indique un effet de dilution croissant dans le temps. Le chapitre 3 est une analyse statistique, utilisant à nouveau les modèles linéaires mixes généralisés, sur la relation entre la diversité des petits mammifères et la densité de l'infection par *Borrelia* burgdorferi, le pathogène responsable de la maladie de Lyme, dans 27 sites forestiers du sud du Québec en 2011, 2012 et 2013. Nous avons trouvé une relation positive entre la diversité des petits mammifères et la densité de *Borrelia burgdorferi*, indiquant un effet d'amplification. Nous avons ensuite exploré les mécanismes influençant les relations diversité des hôtes-maladie de Lyme dans le sud du Québec en utilisant des modèles d'équations structurales. Les conclusions contrastées de ces deux études, prenant place à différentes échelles spatiales et temporelles ainsi qu'à différents degrés d'émergence de la

maladie de Lyme, ce qui renforce l’hypothese que la relation diversité-maladie de Lyme dépend dans une large mesure du contexte d'étude.

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CONTRIBUTION OF AUTHORS

The content and objectives of the two manuscripts (Chapters 2 and 3) in this thesis were developed with the guidance of my co-supervisors Virginie Millien and Andrew Gonzalez, as well as my committee-member Nicholas Ogden. Chapter 2 is currently under review for the journal *Ecology* and is co-authored by S. Turney, A. Gonzalez and V. Millien. Chapter 3 is in the form of a manuscript co-authored by S. Turney, V. Millien, N. Ogden and A. Gonzalez. Contents of all chapters were written by me (Shaun Turney) and were edited and received intellectual input from Virginie Millien and Andrew Gonzalez. Nicholas Ogden contributed to the development and editing of the first manuscript (Chapter 2).

INTRODUCTION

General Introduction

Lyme disease is a zoonotic vector-borne disease, and so by definition a diversity of organisms is involved in its transmission cycle. It is caused by the bacteria *Borrelia burgdorferi* and when affecting humans, it can lead to neurological, arthritic, and cardiac symptoms (reviewed in Ogden et al., 2009). Lyme disease is transmitted to humans by *Ixodes* ticks, primarily *Ixodes scapularis* (Figure 1A) in eastern North America. The ticks, in turn, acquire *B. burgdorferi* when they take blood meals from a variety of host animals, including mammals and birds (Brunner et al., 2008; Figure 1B and Figure 2). The relationship between the diversity of the tick host species and the risk or incidence of Lyme disease is not yet clear (reviewed in Ostfeld and Keesing, 2012), although the perspective that the diversity-disease relationship is context-dependent is beginning to emerge (Randolph and Dobson, 2012). Models, theory, empirical evidence, and scientific opinion are contentiously split as to the hypothesized relationship between diversity and Lyme disease (positive or negative) which they support. This thesis investigates the diversity-Lyme disease relationship in general and in two specific case studies, while exploring the effects of temporal scale, spatial scale, and the stage of disease emergence on the diversity-Lyme disease relationship.

Rationale

An estimated 18.9% of deaths worldwide in 2010 were due to infectious diseases (Lozano et al, 2013). The burden of infectious diseases on global health may continue to increase as the number of emerging infectious diseases which originate each decade increases over time (Jones et al, 2008). An infectious disease is any disease caused by the invasion of the host organism's body

by pathogens. The majority of the infectious diseases in humans (60.3%) which have emerged since 1940 are zoonotic pathogens (Jones et al, 2008), meaning that non-human animals are involved in the transmission cycle of the pathogen. One of these emerging infectious zoonotic diseases, Lyme disease, is the most frequently-reported vector-borne disease in humans in temperate zones (Kurtenbach et al., 2006). If a non-human species, typically an arthropod, transmits the pathogen between infected hosts, the infectious disease is known as a vector-borne disease. Species belonging to the *Borrelia burgdorferi* species complex, the causative agent of Lyme disease, are present across North America, Europe, and Asia (Kurtenbach et al, 2006).

A number of authors have stated in a definitive manner that biodiversity protects human health, both in general (Chivian, 2001; Chivian and Bernstein, 2004; MEA, 2005a; McMichael, 2009) and in the case of Lyme disease (Ostfeld and Keesing, 2000b; MEA, 2005b). Certainly, the idea that protection from disease is an ecosystem service provided by biodiverse ecosystems (Cardinale et al., 2012) is appealing in that it provides a utilitarian motivation for biodiversity conservation to policy makers. It offers the optimistic possibility that biodiversity conservation is "win-win", presenting no or few trade-offs for human well-being (Randolph and Dobson, 2012). The evidence for a general and positive relationship between human health and biodiversity, however, is limited and far from definitive. Numerous studies have shown that biodiversity does not always protect human health, generally (Service, 1991; Hunyen et al., 2004; MEA, 2005b; Jones et al., 2008; Hough, 2013) or in the case of Lyme disease (Ogden and Tsao, 2009; Randolph and Dobson, 2012; Wood and Lafferty, 2013; Wood et al., 2014). This thesis tests the mechanisms that invoke a role for biodiversity in the incidence of Lyme disease.

Thesis Outline

In the first chapter of this thesis, I review and discuss the current research on biodiversity and Lyme disease. I discuss theory, models, and empirical evidence linking biodiversity and Lyme disease, as well as the generality of mechanisms driving the relationship between biodiversity and disease. In the second chapter, I used Lyme disease incidence data from 1992 to 2011 and tick host species richness in the United States, as well as a number of covariates, to investigate the diversity-disease relationship and how it has changed over time. In the third chapter, I used data collected from 27 forest sites in Southern Quebec in 2011, 2012, and 2013 to investigate the relationship between mammal host diversity and Lyme disease and the mechanisms driving that relationship. Southern Quebec is a particularly interesting location to study Lyme disease because it has been emerging here over the last decade.

Altogether, these chapters represent an integrated approach to investigate the relationship between biodiversity and Lyme disease, both theoretically and empirically, and at multiple spatial and temporal scales and at different stages of emergence.

Research Objectives

This thesis has three research objectives.

(1) **Chapter 1: To explore the relationship between biodiversity and Lyme disease.** This objective is carried out in the literature review, where previous research and theory pertaining to the biodiversity-Lyme disease relationship is discussed.

(2) **Chapter 2: To determine the relationship between biodiversity and Lyme disease at a large spatial scale over two decades.** Chapter 2 investigates the relationship between host biodiversity and Lyme disease incidence in the United States from 1992 to 2011.

(3) Chapter 3: To determine the relationship between biodiversity and Lyme disease in at a small spatial scale over three years. Chapter 3 investigates the relationship between host biodiversity and Lyme disease density in forest sites in Southern Quebec from 2011 to 2013.

Chapters two and three together represent two empirical case studies of the diversity-Lyme disease relationship. Together they investigate the effects of spatial and temporal scale and degree of Lyme disease emergence on the relationship between biodiversity and Lyme disease**.** The choice of contrasting case studies (Chapters 2 and 3), each at different spatial scales, temporal scales, and degrees of Lyme disease emergence, allows an exploration of the effects of these factors on the diversity-disease relationship.

CHAPTER 1: LITERATURE REVIEW

Diversity and disease

Biodiversity loss

Over the past generations, humans have had a marked effect on global ecosystems. Croplands, pastures, plantations, and urban areas have increased dramatically in total global surface area, driven by the needs of our growing human population for food, water, and shelter (Foley et al., 2005). Humans have caused global climate change, ozone depletion, chemical pollution, acid rain, species invasions, and habitat degradation, and fragmentation (Chivian, 2001, Barnosky et al., 2012). These global biogeochemical changes have led some to consider a new geological epoch to have begun – the Anthropocene (Steffen et al., 2007), in which humans are a global geophysical force. Biodiversity is the biotic variation within and between species and ecosystems (Mace et al, 2012), and is measured by many different metrics. One common metric is species richness – the number of different species. These global changes have not been without consequence for the biodiversity of this planet. According to one estimate (Pimm et al., 1995), current extinction rates are 100-1000 times pre-humans levels. The rate of biodiversity loss is predicted to accelerate (Pimm et al., 1995; MEA, 2005a; Bellard et al., 2012). The consequences of global change, especially biodiversity loss, for human health is an expanding area of research and much remains to be understood.

Biodiversity and disease regulation

The Millennium Ecosystem Assessment, initiated in 2001 by the UN, concludes that "biodiversity benefits people through more than just its contribution to material welfare and livelihoods. Biodiversity contributes to security, resiliency, social relations, health, good social relations, security, freedom of choice and action" (MEA, 2005a, Sec1:vi), which the MEA

consider to be the key measures of human well-being. On the other hand, high biodiversity has the potential to decrease human health and well-being. For instance, if there is more biodiversity it is more likely that there are species present that are beneficial to human health, but it is also more likely that there are species present that are harmful to human health (Hough, 2013; ex, disease vectors, agricultural pests). It is necessarily true, however, that if biodiversity is exploited to the point that it no longer provides necessary services, economic status and human health and well-being will decrease (Hough, 2013).

One area of particular interest to researchers has been the role of biodiversity in regulating disease incidence. Disease regulation is a potential mechanism through which biodiversity may mediate human health (MEA 2005a; Keesing and Ostfeld, 2012; Ostfeld and Keesing, 2012). If disease occurrence decreases with biodiversity, this is broadly termed a "dilution effect" (Keesing and Ostfeld, 2006). Conversely, if disease increases with biodiversity, it is termed an "amplification effect" (reviewed in Ostfeld and Keesing, 2012). A dilution effect has been proposed to occur for a number of human disease systems, including schistosomiasis (Johnson et al., 2009), malaria (Laporta et al., 2013), and West Nile virus (Swaddle and Calos, 2008). Lyme disease in particular has become the focus of much of the research concerning the relationship between biodiversity and disease. In this literature review, I discuss the relationship between biodiversity and Lyme disease using theory, models, and empirical evidence. I also discuss the effects of spatial and temporal scale, and the generality of mechanisms that may drive the diversity-Lyme disease relationship in space and time.

Lyme disease

Biodiversity and Lyme disease

The term "dilution effect" was first formally characterized in the context of Lyme disease ecology (Van Buskirk and Ostfeld, 1995; Ostfeld and Keesing, 2000b; Keesing and Ostfeld, 2006). Lyme disease has since then been a primary study system through which the investigation of dilution and amplification has taken place, and much of the debate concerning the diversity-disease relationship has centred on Lyme disease. It can be considered as a model system through which to understand the effects of biodiversity change on infectious disease in humans.

The debate about the nature of the diversity-Lyme disease relationship is ongoing and active. Wood and Lafferty (2013) have recently developed a synthesis model of Lyme disease which predicts a dilution effect at the within-forest scale but an amplification effect at broader scales. A further critique of the dilution effect, by Randolph and Dobson (2012), argued that the dilution effect is part of an overly-optimistic "Panglossian perspective" and that in reality the dilution effect only applies under very particular circumstances. Ostfeld and Keesing (Ostfeld, 2013; Ostfeld and Keesing, 2013) have responded to these critiques by agreeing that the dilution effect is not universal, but they argue that it is widespread.

Epidemiology of Lyme disease

Lyme disease is caused by the bacterium *Borrelia burgdorferi*, and is transmitted to humans via *Ixodes* ticks – primarily the black-legged tick (*Ixodes scapularis*; Figure 1A) in eastern North America. The tick acts as a disease vector for Lyme disease, transmitting the pathogen *B. burgdorferi* between hosts and humans. Black-legged ticks have three life stages (larva, nymph, adult), each of which requires a blood meal from a host in order to successfully molt into the next life stage or, in the case of adult females, to lay eggs (Figure 2). Larvae and nymphs typically take blood meals from small mammals while adults typically take blood meals from

white-tailed deer (*Odocoileus virginianus*) and other large mammals. *I. scapularis* are generalists and can take blood meals not only from mammals, but also from birds and reptiles. At least 54 mammalian, 57 avian, and 14 reptile hosts of *I. scapularis* have been identified (Keirans et al., 1996). Tick hosts, to varying degrees depending on the species, may also act as reservoirs of *B. burgdorferi*, with white-footed mice (*Peromyscus leucopus*; Figure 1B) being the most effective reservoir and white-tailed deer being reservoir incompetent (Brunner et al., 2008). Reservoir competence is defined as the probability that a vector (tick) feeding on a member of the host species will become infected (Brunner et al., 2008). Ticks can only acquire *B. burgdorferi* via blood meals because the bacteria is not transmitted transovarially from mother to offspring (Patrician, 1997).

The white-footed mouse is found throughout most of central and eastern United States and Mexico, and in southern Canada (Wilson and Reeder, 2013). They are highly generalist in terms of their habitat and dietary preferences (Wolff et al., 1985) and are very effective dispersers and colonizers (Cummings and Vessey, 1994). The range of the white-footed mouse is expanding northwards in Canada, likely due to global climate change, which may be contributing to the northward expansion of Lyme disease (Roy-Dufresne et al., 2013). Eastern chipmunks (*Tamias striatus*) and short-tailed shrews (*Blarina brevicauda*) are the second most effective reservoirs of *B. burgdorferi* in central and eastern Canada and the United states (Ostfeld and Keesing, 2012).

If a tick which has previously taken a blood meal from an infected host subsequently bites a human, the tick may transmit *B. burgdorferi* to the human which if left untreated may lead to neurological, cardiac, and arthritic symptoms (Ogden et al., 2009). Lyme disease is most often transmitted to humans by nymphal ticks (Barbour and Fish, 1993), and so the density of infected nymphs (DIN) is often used as a metric for disease risk (LoGuidice et al., 2008). Larvae have

low infection prevalence and adults are easily detected and removed by humans, while nymphs are more difficult to detect and have had the opportunity to become infected with *B. burgdorferi* from their larval blood meal. Human disease risk is determined in part by the ecological risk – which is determined by the infection density in a given area (Keesing and Ostfeld, 2012), as well as human behaviour that are increasing contact rates between individuals and infected vectors. Human disease incidence can be expected to be high in areas with a high density of infected nymphs, but the positive correlation between disease incidence in humans and ecological risk will not always hold, depending on human behaviour (Brownstein et al., 2005) and spatial scale.

Epidemiologically, Lyme disease ecology is an important area of research because of its large global burden and its potentially high sensitivity to ecological change (Leighton et al., 2012; Roy-Dufresne, 2013; Simon et al., 2014). Globally, Lyme disease is the most frequently-reported vector-borne disease of humans in temperate zones (Kurtenbach et al., 2006). It was first identified in 1975 when Steere and colleagues (1977) were investigating a cluster of childhood arthritis in Lyme, Connecticut. The disease had already existed in some form, perhaps for millenia, in North America and Europe. Genetic sequencing has found evidence that a 5300 year-old "ice man" found in Italy likely was infected with Lyme disease (Keller et al., 2012). Lyme disease only came to the attention of the medical community in the United States as the disease emerged to much greater prevalence (Barbour and Fish, 1993). Lyme disease may not be a new disease, but it is considered an emerging disease because it is increasing rapidly in incidence and geographic range (WHO, 2014; Figure 4). The cause of this emergence is generally attributed to the abandonment of farmland in the Northeastern United States, which quickly reverted to forest, and the accompanying increase in white-tailed deer population

(Barbour and Fish, 1993). White-tailed deer being the principle host for adult black-legged ticks, tick populations increased with deer populations. Lyme disease has continued to increase in prevalence and extent in the past four decades, and is now endemic in most of the north-central and northeastern United States (Steere et al., 2004) and is beginning to emerge in southern Canada (Ogden et al., 2009).

In Canada, Lyme disease is currently present in southern Manitoba, Ontario, Quebec, and parts of the Maritimes (Ogden et al., 2009). It was declared a nationally reportable disease in 2009 by the Public Health Agency of Canada, and Lyme disease has steadily increased since then. The expansion of Lyme disease into Canada is hypothesized to be due in part to global climate change. Warming temperatures may be allowing black-legged ticks (Ogden et al, 2006; Leighton et al., 2012) and white-footed mice (Roy-Dufresne et al., 2013) to extend their range northwards, bringing with them Lyme disease. The most effective reservoir, the white-footed mouse, and the vector, black-legged ticks are the same in Southern Canada and in the central and eastern endemic areas of the United States. But there are also differences, such as the mammal assemblage (Badgley and Fox, 2008) and the degree of disease emergence, and so Lyme disease ecology may be expected to behave differently in Canada than in the United States.

Mechanisms

Keesing and Ostfeld (2006) have produced a model of a vector-borne disease system which comprehensively lays out mechanisms through which biodiversity can increase or decrease disease. They identify six general types of mechanisms, through which biodiversity acts on the encounter rate, transmission rate, reservoir host abundance or health, or vector abundance to affect disease risk. None of the mechanisms relating diversity to disease risk are mutually

exclusive, and thus both dilution and amplification effects can occur in the same system. The question in this case becomes a matter of the net effect of competing mechanisms. Among the multiple possible mechanisms identified by Keesing et al., 2006, only a few have been focused on by researchers: frequency-dependent dilution, density-dependent dilution, and vector amplification.

Frequency-dependent dilution (Rudolf and Antonovics, 2005; Figure 3) can occur if additional hosts "dilute" the pool of hosts from which ticks are taking meals, such that the relative abundance (although not necessarily the absolute abundance) of the most competent reservoir species (in the case of Lyme disease, the white-footed mouse) is reduced. White-footed mice are present even in low diversity forests (Nupp and Swihart, 2000) and additional hosts are less effective reservoirs of *B. burgdorferi* than are white-footed mice (Giardina et al., 2000; LoGuidice et al., 2003; Brunner et al., 2008). The decreased relative abundance of the reservoir-competent host will decrease the disease transmission rate by this "wasted bite" mechanism if disease transmission is frequency-dependent (Rudolf and Antonovics, 2005). Confusingly, sometimes the term "dilution effect" is used to specifically refer to this particular type of dilution effect (Keesing et al., 2006).

Another potential dilution effect mechanism, density-dependent dilution (Figure 3), can occur if increased host diversity decreases the absolute abundance of the most competent reservoir species and the total community host abundance does not increase (Rudolf and Antonovics, 2005). This could happen if there is competition between the most competent reservoir and the additional host species (Ogden and Tsao, 2009). The transmission rate will be reduced due to the decreased absolute abundance of the reservoir-competent species.

Vector amplification is the increase in vector density caused by the presence of additional host species (Figure 3). It has been argued by some (Gilbert et al., 2001; Keesing et al., 2006; Ogden and Tsao, 2009) that if a greater number of host species are available for ticks from which to take blood meals, a greater population density of ticks could be supported and an amplification effect could occur. Similarly, alternate hosts could provide a "rescue effect" (Ostfeld and Keesing, 2000a; Ostfeld and Keesing, 2000b; Keesing et al., 2006), maintaining a constant population size and protecting the tick population from local extinction due to the population fluctuations that would occur with a single host population. Increased host density due to high host species-richness could increase the rate of disease transmission by increasing the contact rate between ticks and hosts.

Models

A series of models have found that infection prevalence inevitably decreases with host diversity (Giardina et al., 2000; Ostfeld and Keesing, 2000a; LoGuidice et al., 2003; Ostfeld and LoGuidice, 2003) under certain conditions that are demonstrably fulfilled in natural north-eastern United States Lyme disease systems (Patrician, 1997; Nupp and Swihart, 1998; Nupp and Swihart, 2000; LoGuidice et al., 2003; Brunner et al., 2008). These models, however, have been criticized for using nymphal infection prevalence (NIP) as the sole measure of disease risk. The models do not include the density of nymphs (DON), and are therefore only calculating half of the disease risk equation (Ogden and Tsao, 2009). A more appropriate measure for ecological Lyme disease risk is the density of infected nymphs (DIN; DIN = NIP x DON; Stafford et al. 1998).

Models which measure disease risk using DIN have predicted both a net dilution and a net amplification effect (Van Buskirk and Ostfeld, 1995; Norman et al., 1999; Gilbert et al., 2001; Schmidt and Ostfeld, 2001; Dobson, 2004; Ogden and Tsao, 2009). Dilution and amplification effects may both play a role in determining disease risk, and the net effect of the two forces depends on the parameterization of the model. One study has suggested that whether an amplification or dilution effect is observed depends on the scale of the observation (Wood and Lafferty, 2013). Ogden and Tsao (2009) have argued based on a detailed mechanistic simulation model that the amplification effect is the "default" relationship between disease risk and host diversity, but that under a few specific conditions a dilution effect can occur.

One factor which may determine whether an epidemiological model predicts a dilution or amplification effect is how the transmission term is modeled (Rudolf and Antonovics, 2005). Depending on the transmission mode, a model can produce completely different predictions of the disease's behaviour (Ryder et al., 2007; Rudolf and Antonovics, 2005). There are two commonly employed transmission models: (1) density-dependent transmission, which occurs when relevant contacts between hosts increase with host density, and (2) frequency-dependent transmission, which occurs when relevant contacts between hosts remain constant with host density. In a direct-contact two-host one-pathogen model, Rudolf and Antonovics (2005) found that whether a dilution or amplification effect is predicted depends on the type of transmission term used. Lyme disease is transmitted by vectors, rather than direct contact, and as a result its transmission dynamics differ from that of the disease modeled by Rudolf and Antonovics (2005). Transmission of vector-borne diseases could be thought of as having two components: transmission (1) from infected hosts to susceptible ticks and (2) from infected ticks to susceptible hosts. It is plausible that the transmission function of the two types of transmission could differ

from each other. Furthermore, there is no *a priori* reason to believe the relationship between contact rate and tick and host densities is linear, as the density-dependent transmission model assumes (Hochberg, 1991; Antonovics et al., 1995; Ryder et al., 2007; Smith et al., 2009; McCaig et al., 2011). The appropriate transmission term(s) for Lyme disease is unknown (Ostfeld and Keesing, 2012), and so epidemiological models of Lyme disease may provide limited or misguided insight into the diversity/disease relationship.

Empirical evidence

Are conditions for the dilution effect fulfilled?

Models and theory about diversity and Lyme disease, and diversity and infectious disease more generally, have led to predictions of conditions under which a dilution effect is expected (reviewed in Ostfeld and Keesing, 2012). When Ostfeld and Keesing (2000b) first proposed the dilution effect hypothesis in the context of Lyme disease, they argued that there are four conditions that are necessary but insufficient for a frequency-dependent dilution effect to occur in a given vector-borne zoonose. They argued that (1) the vector must be a generalist. It is well known that the black-legged tick parasitizes a wide variety of species (LoGuidice et al., 2003), including mammals, birds, and reptiles. In order for the host species composition to play a strong role in the pathogen prevalence, (2) the acquisition of the pathogen must be primarily oral. *Borrelia burgdorferi* is not passed from mother to offspring transovarially in the black-legged tick (Patrician, 1997), so the only way ticks can acquire the bacteria is through blood meals. (3) There must be variation in reservoir competence among hosts (Ostfeld and Keesing, 2000b). There is wide variety in the reservoir competence of host species, due to differences in prevalence, infectivity (Brunner et al., 2008), and tick survival (Brunner et al., 2008). Finally, (4)

there must be a positive correlation between reservoir competence of a host species and its numerical dominance in the community.

This final condition is related to the immuno-ecological trade-off hypothesis, which states that immune response and parasite resistance are costly, and so there are within and between species trade-offs between immune response and other traits such as reproduction and dispersal (Dowling and Simmons, 2009; Hawley and Altizer, 2011). If such a trade-off exists, one might expect a negative correlation between strength of immune response and numerical dominance in a community (condition 4). On the other hand, pathogen challenge in a species' evolutionary past may be more important than life-history trade-offs in determining immune response (Horrocks et al., 2011). Several studies find evidence for an immuno-ecological trade-off in bird species (Lee et al., 2006; Martin et al., 2007; Lee et al., 2008). Evidence is mixed for such a trade-off in mammals (Martin et al., 2007), but a positive correlation between reservoir competence and dominance seems to hold true for Lyme disease, at least in the northeastern United States (LoGuidice et al., 2003). White-footed mice thrive in both species-poor and species-rich communities, while less competent reservoirs, such as squirrels, are only present in high diversity communities (Nupp and Swihart, 2000). Contrarily, it may be that although white-footed mice may have the highest abundance, inconspicuous hosts such as shrews may be dominant over white-footed mice in terms of the proportion of ticks they feed (Brisson et al., 2008).

A dilution effect in its more general sense, as opposed to specifically a frequency-dependent dilution effect, is expected to occur under a number of conditions, but are not always necessary in order for a dilution effect to occur (Ostfeld and Keesing, 2012). These are that an increase in diversity (1) tends to add species that are relatively poor reservoirs or poor tick hosts,

(2) regulates population density of the most competent reservoir host or tick host, and (3) deflects tick meals away from most competent reservoir or tick host. As discussed above in the context of the immuno-ecological trade-off hypothesis, there is evidence that small mammal species have nested community occupancy, such that the species absent in all but the most diverse sites are also the poorest reservoirs. Loss of species in descending order of reservoir competence as biodiversity decreases (essentially a variation of condition 4, above) is a necessary condition in order for a dilution effect to occur in Lyme disease (Ostfeld and LoGuidice, 2003; Randolph and Dobson, 2012) and other disease systems (Johnson et al., 2013). The role of host diversity in regulating white-footed mouse population has received relatively little study, with some evidence indicating that mouse density is reduced by species richness or non-mouse abundance (Nupp and Swihart 1998; Nupp and Swihart, 2000; Loguidice et al., 2008) and some evidence indicating no such relationship (Werden et al., 2014). Evidence concerning the relationship between diversity and white-footed mouse tick burden is also limited, but a long-term field study of small mammals in New York found a negative relationship between chipmunk abundance and larval tick burden on white-footed mice (Brunner and Ostfeld, 2008).

In a complex mechanistic model of Lyme disease transmission, Ogden and Tsao (2009) identified ecological conditions under which their model predicts a dilution effect to occur. These are: (1) if diversity decreases white-footed mouse density via competition, (2) if the non-mouse community feeds proportionally more nymphs than larvae than the white-footed mice, and/or (3) if tick mortality is higher on non-mice. There is little direct evidence for the first condition, but a number of studies have found that non-mice have higher nymph:larva ratios than mice (Ogden and Tsao, 2009) and that larval tick mortality rates are much higher on non-mice than on white-footed mice due to behavioural defense of the host (Keesing et al., 2009). Ogden

and Tsao's model suggests that the "default" relationship between host diversity and Lyme disease is an amplification effect, but under certain conditions a dilution effect can occur. It has also been suggested that a dilution effect may occur if tick nymphs and larvae are preferentially feed on different host species, which would reduce transmission from infected hosts to susceptible nymphs (Bouchard et al., 2011).

Is a dilution effect observed in natural ecosystems?

Although theory and models about the diversity-Lyme disease relationship abound, there is a dearth of field studies on the topic. Many of the field studies which have been performed have investigated the relationship between landscape fragmentation and Lyme disease (Allan et al., 2003; Wilder and Meikle, 2004; Jackson et al., 2006). In many of the landscapes in which Lyme disease is present, forests are fragmented "islands" in an "ocean" of urban and agricultural area. These forest fragments vary in size, with lower diversity of mammals and a higher density of white-footed mice in the smaller patches than in the larger patches (Swihart et al., 2003). A gradient of forest fragment sizes therefore theoretically represents a gradient of host diversities, which presents an opportunity to indirectly test the dilution and amplification effect hypotheses.

In a field sampling study in New York, Allan and colleagues (2002) found that the density of nymphs, nymphal infection prevalence, and density of infected nymphs all decreased with forest patch area. If larger forest fragments are more diverse than small fragments, then Allan and colleague's findings are suggestive of a dilution effect. In Maryland, Jackson and colleagues (2006) found that in landscapes with higher percentages of land-cover edge represented by adjacent forest and herbaceous cover (ie, more fragmented landscapes) have higher incidences of Lyme disease among humans. Some studies of fragmented landscapes have

found evidence of an amplification effect, rather than a dilution effect. A field study in Ohio found that a lower proportion of white-footed mice are infected in small forest fragments than in large fragments (Wilder and Meikle, 2004). Brownstein and colleagues (2005) found that in the area surrounding Lyme, Connecticut, more highly fragmented landscapes had higher densities of infected ticks. Fragmentation was negatively correlated with human Lyme disease incidence, however, demonstrating the very important point that ecological risk does not always predict human disease incidence.

Empirical studies which use fragmentation as a means of studying the diversity-disease relationship are limited in that they do not necessarily demonstrate a direct relationship between diversity and disease and they do not identify a mechanism. At least two field studies have focused on demonstrating the mechanisms which could lead to a dilution effect, while not explicitly investigating the diversity-disease relationship. Keesing and colleagues (2009) subjected field-caught mammals hosts to black-legged ticks and found that some species, especially opossums and squirrels, kill 84-96% of ticks which attempt to take blood meals from them. They calculated from this that some species may be able to kill thousands of ticks per hectare, and therefore if these species are lost with biodiversity loss then vector abundance would increase. In southern Canada there is evidence of greater host partitioning between larval and nymphal ticks (nymphs and larvae taking blood meals from different species) in habitats with diverse host assemblages (Bouchard et al., 2011). Host partitioning could reduce transmission from infected hosts to susceptible nymphs, leading to a dilution effect. The Lyme disease transmission cycle is disrupted by ticks taking blood meals from different host species at different life stages.

A number of field studies have found direct or indirect evidence of an amplification effect, usually due to a positive relationship between host abundance and tick abundance—that is, vector amplification. It has been noted for several decades that there is a positive correlation between white-tailed deer abundance and tick abundance (Deblinger et al., 1993; Daniels et al., 1993; Stafford et al., 2003; Rand et al., 2004). Indeed, it is thought that Lyme disease emerged in the northeastern United States due to an increase in white-tailed deer abundance (Barbour and Fish, 1993). White-tailed deer are not the only species which may lead to vector amplification. An analysis of human Lyme disease incidence and species richness of hosts in the northeastern United States found negative correlations between Lyme incidence and species richness of small mammals and lizards, which is suggestive of a dilution effect (Ostfeld and Keesing, 2000a). There was a positive correlation between Lyme incidence and ground-dwelling bird species richness, which the authors suggest could be due to a rescue effect. Two separate field studies in southern Canada found a modulating effect of white-footed mouse abundance on the diversity-disease risk relationship (Bouchard et al., 2013; Werden et al., 2014). Field sampling in Quebec (Bouchard et al., 2013) found that although there was less nymphal infestation (fewer nymphs/host) where small mammal species richness was greatest, the overall nymphal abundance increased with species richness. Although vector amplification is suggested by these results, the authors found a negative correlation between nymphal infestation and tree species richness, which may indicate that a dilution effect is present when the wider community is considered. A study in the Thousand Islands region of Ontario, Canada found tick abundance is positively correlated with deer abundance (Werden et al., 2014). The authors find what they refer to as a "context-dependent role of biodiversity"; although there is an overall negative correlation

between the number of infected nymphs and species richness, species richness had little effect on infection prevalence at sites with a high relative abundance of mice.

Overall, field studies of the diversity-Lyme disease relationship confirm the conclusions from models; there is evidence that both amplification effects and dilution effects can occur, and the net effect depends on the conditions of the particular area under study. The diversity-Lyme disease relationship may also depend on spatial and temporal scale of study.

The role of temporal and spatial scale

Wood and Lafferty (2013) have developed a synthesis model of Lyme disease which addresses the diversity-Lyme disease relationship at the within-forest scale and at a landscape scale and finds that the relationship between biodiversity and disease is spatially-dependent (Figure 5). Wood and Lafferty contrast two models: the "traditional model" and the dilution effect model. In the traditional model, forestation is associated with both host diversity and competent host density. Thus, forested areas (forest-rich regions) will have high host diversity, high competent host density, and pathogen density. Non-forested areas (urban- or agriculture-rich regions) will have low host diversity and pathogen density. The positive relation between host diversity and pathogen density due to their shared correlation with forestation leads to a vector amplification effect. Wood and Lafferty (2013) review the evidence that human Lyme disease cases tend to be positively associated with forested regions in the United States (ex, Glass et al., 1995; Jackson et al., 2006). In the dilution effect model, as biodiversity increases, competent host density will decrease and non-competent host density will increase. Disease transmission will decrease and thus disease risk will decrease. The dilution effect takes place at the within-forest level. Under the assumption that forestation and biodiversity are roughly equivalent, Wood and Lafferty

synthesized the traditional and the dilution effect models into one model. They argue that the two models can be reconciled by considering spatial scale. An amplification effect should occur at the landscape scale because the degree of forestation will determine both the host diversity and the disease rate; in areas of low forestation there will be low host diversity and low Lyme disease rates. At the within-forest scale, the mechanisms of the dilution effect (via a reduced white-footed mouse abundance) can take place, and so a dilution effect can occur. Both a dilution and an amplification effect could be taking place within the same landscape: diverse forest fragments may have lower pathogen density than less diverse forest fragments, but regions with no forest (even lower host diversity) will have a lower pathogen density than forested regions.

Time may also play an important role in the diversity-Lyme disease relationship, particularly when the disease is emerging. As the *B. burgorferi* prevalence, transmission rates, and other factors affecting the diversity-Lyme disease relationship change and increase over time, the diversity-Lyme disease relationship likely also changes over time. Lyme disease has increased at different rates in different regions of the United States (Tuite et al., 2013, Chapter 1 of this thesis), which raises the possibility that Lyme disease could increase at different rates depending on host diversity. For instance, more diverse regions may have a lower density of white-footed mouse, which would lower transmission rates, and thus reduce the rate at which Lyme disease increases. Lyme disease would emerge slowly in diverse regions and quickly in non-diverse regions, leading to an increasing disparity between diverse and non-diverse regions in terms of Lyme disease incidence, and therefore an increasing host diversity-disease slope. If Lyme disease increases at different rates depending on host diversity, the relationship between diversity and disease is expected to vary in time. This could lead to a quantitative (strength) change in the diversity-disease relationship over time. A qualitative (direction) change in the

diversity-disease relationship over time is also plausible. A qualitative change could occur if the conditions which are correlated with the emergence of Lyme disease may differ from the variables correlated with high Lyme disease rates once Lyme disease has emerged. For instance, this may occur in a region where disease emergence is determined by tick immigration via birds and disease density at equilibrium is determined by white-footed mouse density. If bird migration is positively correlated with diversity and white-footed mouse density is negatively correlated with diversity, an amplification effect followed by a dilution effect could be expected.

Questions about the temporal dynamics of the diversity-Lyme disease relationship are particularly relevant in the Canadian context as Lyme disease is emerging in southern Canada (Ogden et al., 2006; Ogden et al., 2006; Ogden et al., 2009; Bouchard et al., 2011; Leighton et al., 2012; Bouchard et al., 2013; Ogden et al., 2013). The northward-moving invasion fronts of white-footed mice (Myers et al., 2009; Roy-Dufresne et al., 2013; Simon et al., 2014), black-legged ticks (Leighton et al., 2012; Simon et al., 2014), and *B. burgdorferi* (Simon et al., 2014) are all found in southern Canada.

The dilution effect as a general phenomenon

The dilution effect is not general in the sense that a dilution effect will generally occur for all diseases or in all regions. Theory, models, and empirical evidence indicate that diseases are diluted by biodiversity under only specific circumstances. Instead, the dilution effect may be general in the sense that it can occur under the right circumstances in many different ecological systems. The dilution effect hypothesis is not the exclusive domain of Lyme disease. It has been proposed to occur in a number of other zoonotic disease systems, with some evidence indicating a negative relationship between diversity and disease in the cases of schistosomiasis (Johnson et

al., 2009), malaria (Laporta et al., 2013), and West Nile (Swaddle and Calos, 2008). Zooprophylaxis, the theory that human malaria prevalence can be reduced, under certain circumstances (Bouma and Rowland, 1995; Saul, 2003), by introducing non-human animals to divert mosquito bites on humans (WHO, 1982), can be considered a type of dilution effect. There is also evidence for a dilution effect in non-human disease systems (Mitchell et al., 2002). More generally, there is evidence a dilution effect or similar mechanism occurs in cases even outside the realm of disease ecology, although they have not necessarily been labeled as "dilution effects".

One example is the reduction of tree herbivory with increased tree biodiversity. A meta-analysis found that insect herbivory is reduced in mixed-forests as compared to single-species forests, but the effect varied with the host-specificity of the insects (Jactel and Brockerhoff, 2007). In mixed-stands the preferred tree host is relatively less abundant and its distribution is patchier so it is less available to specialized insect herbivores. Non-host tree can also provide physical or chemical barriers to foraging insects, in particular by blocking the movement of wind-dispersing species (reviewed in Jactel and Brockerhoff, 2007).

Another example from an entirely different field is the reduction of fish parasitism with increased fish biodiversity. A study of two related poeciliid fishes and their host-specific parasite, *Gyrodactylus spp.* found that both fish species had less parasitism in mixed-species groups (Dargent et al., 2012). The relative abundance of both species is reduced, which decreases the contact of parasites with susceptible hosts and increases the contact of parasites with non-susceptible hosts. The parallels to the dilution effect in disease ecology to both tree herbivory and fish parasitism are clear: the effective hosts are reduced in abundance so the vector/herbivore/parasite finds itself more often encountering ineffective hosts instead.

These cases provide evidence for the generality of the dilution effect. Most generally, one could say that a "dilution-like" mechanism can occur in any system where a "consumer" (pathogen, parasite, herbivore, or predator) has a preferred prey or host, and the consumer is less able to access that prey or host when its relative or absolute abundance is reduced by increased biodiversity, leading to a reduction in the abundance of the consumer. The conception of the dilution effect as a general phenomenon may allow greater collaboration between diverse fields and the sharing of theory and evidence of phenomena with analogous mechanisms.

Conclusions

Anthropogenic change since the Industrial Revolution has led to unprecedented levels of biodiversity loss. The impacts of this sustained biodiversity loss on human health and well-being are not yet fully understood. Lyme disease has been the centre of much of the research concerning the relationship between biodiversity and disease regulation. Despite an abundance of theory and models, it is not clear whether a dilution or amplification effect governs the relationship between host biodiversity and Lyme disease incidence. A consensus is beginning to emerge that the diversity-disease relationship depends on the particularities of the study system, including its temporal and spatial scale.

Research which expands the limited body of empirical studies of the diversity-Lyme disease relationship may shed light on the conditions under which a given diversity-disease relationship is expected. There is a need for research at a meso-spatial scale as well as a balanced approach which studies all mechanisms by which diversity mediates infection prevalence and disease risk. A more sophisticated understanding of the complex relationship between diversity

and Lyme disease may provide insight into the impacts of biodiversity loss on disease regulation and into "dilution-like" effects which are found in a wide variety of ecosystems.

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Figures

Figure 1. Photographs by Virginie Millien of (A) a nymphal black-legged tick (*Ixodes scapularis*) and (B) one of its hosts, the white-footed mouse (*Peromyscus leucopus*).

Figure 2. Illustration of the life cycle of black-legged ticks (*Ixodes scapularis*). Black-legged ticks have three life stages: larva, nymph, and adult. In order to molt into the next life stage or, in the case of the adult, to lay eggs, the tick must take a blood meal from a host. Larvae and nymphs typically take blood meals from small mammals and birds, while adults typically take blood meals from large mammals, in particular white-tailed deer (*Odocoileus virginianus*). Although humans are not target hosts of black-legged ticks, humans are sometimes incidentally fed on by the ticks and can be infected with *Borrelia burgdorferi* by infected ticks, leading to Lyme disease. *B. burgdorferi* infection cycles between larval and nymphal ticks and small mammal hosts.

Figure 3. Illustrations of the vector amplification, frequency-dependent dilution, and density-dependent dilution effects. In the low-diversity scenario, only the white-footed mouse (the most effective reservoir for *Borrelia burgdorferi*) is present. In the high-diversity scenario, other host species (illustrated here as short-tailed shrews and eastern chipmunks) are added. The red ticks represent infected ticks, while the black ticks represent uninfected ticks. The vector amplification effect predicts that the additional species will increase the total host abundance, which will increase the tick abundance, which will increase the density of infected ticks. The frequency-dependent dilution effect predicts that the additional species will decrease the relative abundance of white-footed mice, which will increase the tick infection prevalence, which will decrease the density of infected ticks. The density-dependent dilution effect predicts that the additional

species will decrease the absolute abundance of white-footed mice due to competition, which will decrease the tick infection prevalence and (if the white-footed mouse is also the most effective tick host) the tick abundance, which will decrease the density of infected ticks.

Figure 4. The total number of new human cases of Lyme disease in the contiguous United States for each year from 1992 to 2011. Data is from the United States Center for Disease Control (2012).

Figure 5. A schematic of Wood and Lafferty's (2012) synthesis model. Green circles represent forest fragments. The shade of green indicates the disease risk and the tick host diversity. At the forest scale, disease risk decreases with tick host diversity (dilution effect) because there is lower disease incidence in more diverse (larger) forest fragments. In the more diversity forests, the abundance of white-footed mice, the most effective *Borrelia burgdorferi* reservoirs, is reduced, which reduces Lyme disease incidence. At the broad scale, disease risk increases with tick host diversity (amplification effect) because both disease risk and diversity increase with forestation.

Figure 1

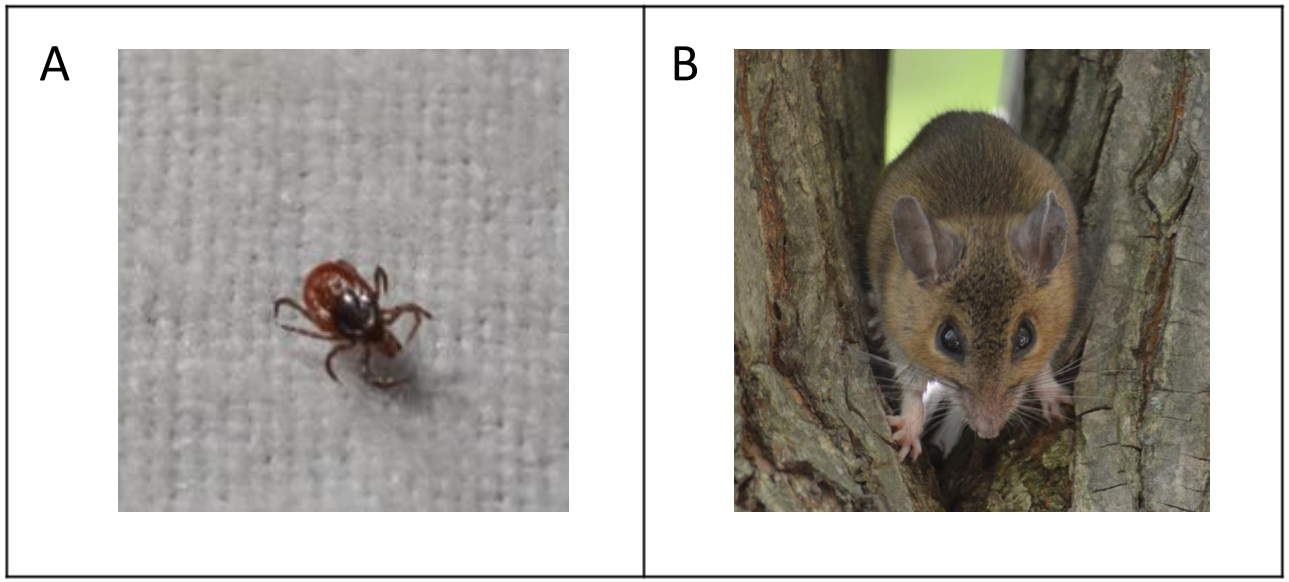


Figure 2

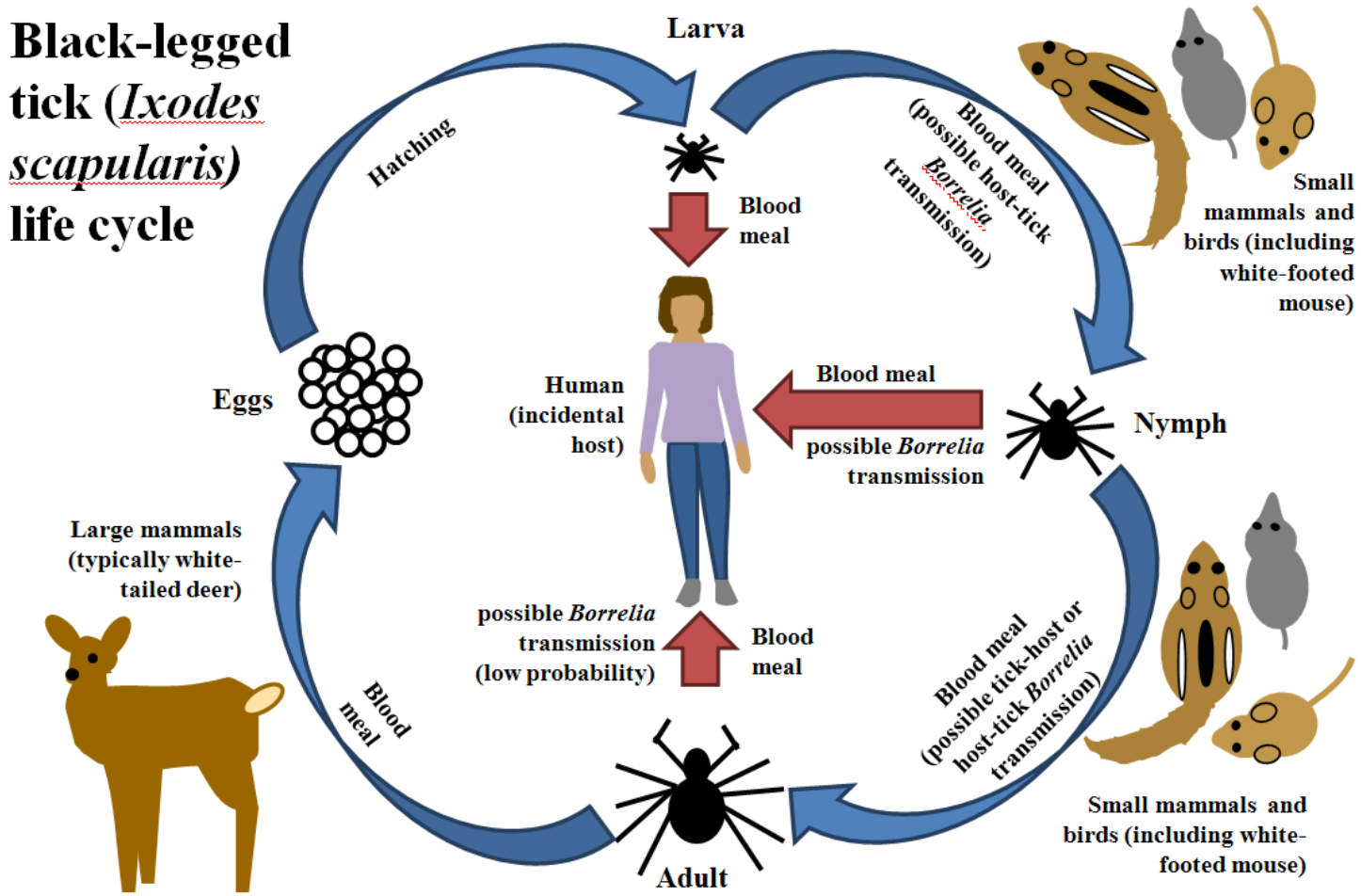

Black-legged tick (*Ixodes scapularis*) life cycle
Larva
Hatching
Blood meal
Blood meal (possible host-tick *Borrelia* transmission)
Small mammals and birds (including white-footed mouse)
Eggs
Human (incidental host)
Blood meal
possible *Borrelia* transmission
Nymph
Large mammals (typically white-tailed deer)
possible *Borrelia* transmission (low probability)
Blood meal
Blood meal
Blood meal (possible tick-host or host-tick *Borrelia* transmission)
Small mammals and birds (including white-footed mouse)
Adult

Figure 3

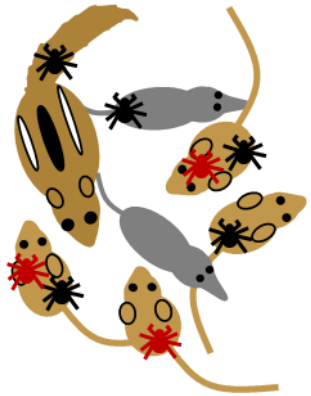
Diversity	Vector amplification effect	Frequency-dependent dilution effect	Density-dependent dilution effect
Low (White-footed mouse only)			
High (White-footed mouse plus other host species)			
Result of increased diversity	Tick abundance increases due to additional hosts, therefore **density of infected ticks increases.**	Infection prevalence decreases due to reduced white-footed mouse relative abundance, therefore **density of infected ticks decreases.**	Infection prevalence and tick abundance decrease due to reduced white-footed mouse absolute abundance, therefore **density of infected ticks decreases.**

Figure 4

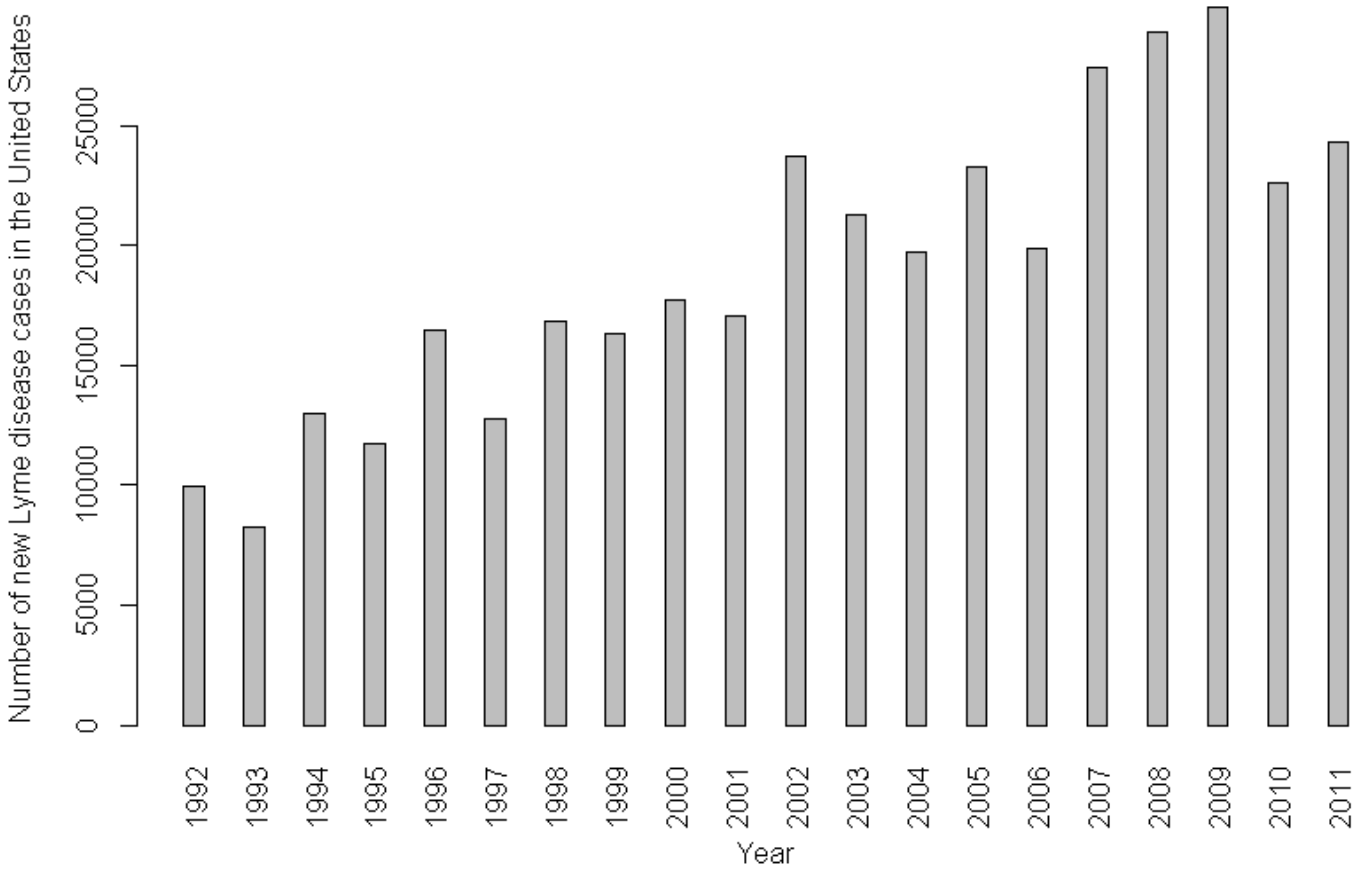
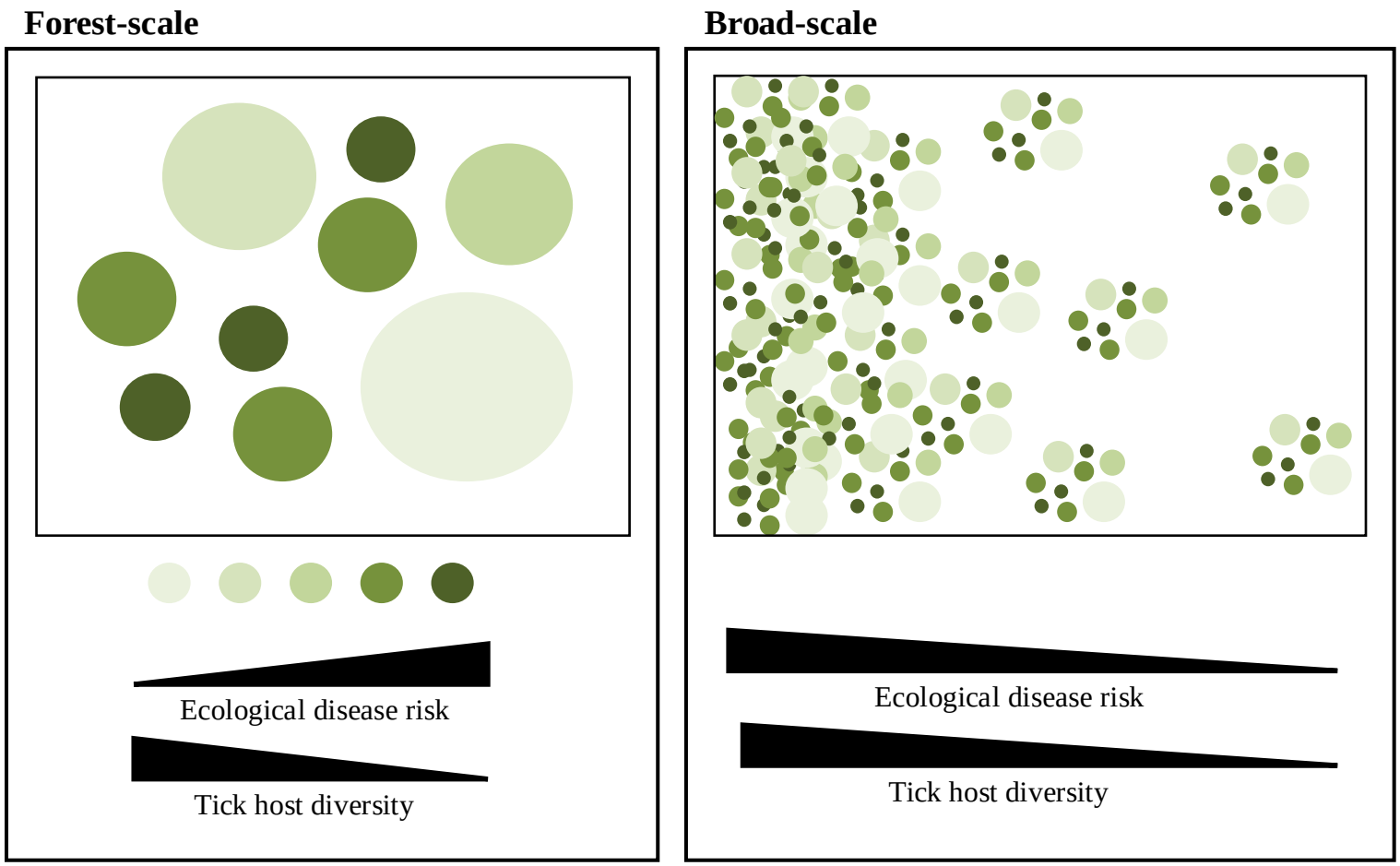

Number of new Lyme disease cases in the United States
0
5000
10000
15000
20000
25000
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
Year

Figure 5


Forest-scale
Broad-scale
Ecological disease risk
Tick host diversity
Ecological disease risk
Tick host diversity

CONNECTING STATEMENT

The previous chapter presented a review of theory, models, and research concerning the relationship between host diversity and Lyme disease incidence. Negative (a dilution effect) or positive (an amplification effect) diversity-disease relationships are both possible, and depends on the specifics of the study system, including spatial and temporal scale. In Chapters 2 and 3, I sought to determine the diversity-Lyme disease relationship in two study systems. In the following chapter, I investigated the relationship between mammal *Ixodes scapularis* host diversity and human Lyme disease incidence in states in the United States from 1992 to 2011. In this long temporal scale and broad spatial scale I found an increasing dilution effect of host diversity on disease incidence.

CHAPTER 2

The negative relationship between mammal host diversity and Lyme disease incidence strengthens through time

This is currently under revision for the journal *Ecology*.

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Abstract

Since its discovery in 1975 Lyme disease has spread and increased in much of central and eastern United States. Host diversity is thought to play a role in Lyme disease risk, and it has been suggested that the direction of the relationship between host diversity and disease risk may vary depending on the spatial scale of observation. Here we modelled the effect of mammal host species richness on the incidence of Lyme disease from 1992 to 2011 across all states in the United States with reported or established black-legged tick (*Ixodes scapularis*) populations. We tested two contrasting hypotheses: a positive versus a negative relationship between host species richness and Lyme disease incidence at the sub-continental scale. We also tested the hypothesis

that the strength of the diversity-disease risk relationship increased over time, as Lyme disease spread. We observed a strong negative relationship between mammal host species richness and Lyme disease incidence, and this relationship became more negative over time. Lyme disease increased over time more rapidly in host species-poor states than host species-rich states. Our findings support the importance of mammal host richness on Lyme disease risk at large spatial scales, and the importance of spatial and temporal scales on this diversity-disease relationship.

Introduction

Lyme disease is the most commonly reported vector-borne disease in the temperate zone and, if left untreated, can lead to neurological, cardiac and arthritic symptoms (reviewed in Ogden et al., 2009). It was first identified in 1975 in Lyme, Connecticut (Steer et al. 1977), although it had existed in North America at low levels for at least 50 years before (Barbour and Fish 1993). Lyme disease has increased in prevalence and spatial extent since 1975 (Bacon et al. 2008, CDC 2012), and is now endemic to most of the central and eastern United States (Steere et al. 2004). The disease is caused by the bacterium *Borrelia burgdorferi* and is transmitted to humans via ticks belonging to the genus *Ixodes*, primarily the black-legged tick (*I. scapularis*) in eastern and central North America. Larval and nymphal *I. scapularis* tend to take blood meals from small mammals and birds, while adults take blood meals from deer. Many of the small mammal and bird hosts are reservoirs of *B. burgdorferi* but they differ in their ability to transmit *B. burgdorferi* to feeding black-legged ticks, the most effective reservoir being the white-footed mouse (*Peromyscus leucopus;* Brunner et al. 2008).

A diversity of host species are involved in the Lyme disease system, and it is thought that this diversity mediates Lyme disease risk, but the nature of this relationship is a matter of ongoing debate (Dobson 2004, Keesing et al. 2006, Ogden and Tsao 2009, Randolph and Dobson 2012).

Previous studies have found that in areas of emergence of Lyme disease, the prevalence of infected ticks is driven by the interaction between host species richness and the relative abundance of the white-footed mouse, the most competent host for *B. burgdorferi* (Simon et al. 2014, Werden et al. 2014). Schmidt and Ostfeld (2001) hypothesized that Lyme disease risk decreases with biodiversity, suggesting that disease protection is an ecosystem service provided by biodiversity (the "dilution effect" hypothesis). They propose, among other possible mechanisms (Keesing et al. 2006), that increased host diversity reduces the relative abundance of white-footed mice, resulting in tick bites being diverted from the white-footed mouse. The white-footed mouse is amongst the most competent reservoirs for *B. burgdorferi*, and so this will result in a decrease in disease incidence via a decreased prevalence of infection in host-seeking ticks. An alternative hypothesis—the amplification effect—states that Lyme disease risk increases with biodiversity (Keesing et al. 2006); a mechanism that may also affect risk for other human infectious disease (Wood et al. 2014). Again, many possible mechanisms could lead to this relationship, but several authors (Gilbert et al. 2001, Keesing et al. 2006, Ogden and Tsao 2009) argue that increased host diversity may increase overall host abundance which would result in increased tick abundance, and therefore higher disease risk.

The ecology of Lyme disease is complex with many interacting variables, making it difficult to disentangle the relationship between Lyme disease incidence and host diversity. First, temporal scale should play a role in the diversity-disease relationship: in regions where Lyme disease is emerging, many of the key populations involved in the dynamics of Lyme disease transmission are unstable and increasing (most notably black-legged ticks, white-footed mouse, and *B. burgdorferi* populations), and so there is no reason to believe that the relationship between host diversity and disease risk should remain constant over time in these systems (e.g.

Tuite et al. 2013). Consequently, the relationship between Lyme disease incidence and any given regionally-dependent variable, including host diversity, may change over time. A change in the strength of the relationship between Lyme disease incidence and host diversity can be expected if host species-poor regions have some characteristics which allow Lyme disease to emerge more rapidly than in host-species-rich regions, or vice-versa.

Second, Wood and Lafferty (2013) proposed that the biodiversity-Lyme disease relationship may operate in different directions at the within-forest scale and at the landscape scale. They argue that historically, an amplification effect has occurred at the landscape scale because reforestation has driven an increase in host diversity resulting in an increase in competent host density; in areas with increased forest cover there is thus a higher host diversity and greater Lyme disease incidence. At the within-forest scale, a dilution effect may take place through the reduction in white-footed mouse relative abundance within host communities.

The relationship between host diversity and Lyme disease incidence may thus be dependent on both the temporal and spatial scales of observation. Here, we investigated the nature of the relationship between host species richness and Lyme disease incidence at the scale of the central and eastern United States, and how it has changed over the past two decades. We explored and controlled for several variables that may confound the relationship between host diversity and Lyme disease. Lyme disease cases have been reportable in the United States since 1992 and cases in each state are reported each year to the Centers for Disease Control (Bacon et al. 2008, CDC 2012). This database provides a record of the emergence of Lyme disease in the United States, allowing study of the relationship between disease cases and host species richness over time. Here, Lyme disease cases in the United States from 1992 to 2011 and the species richness of *I. scapularis* mammal hosts in each state with reported or established *I. scapularis* populations

were analysed, along with a number of covariates. The analyses were at a broad geographic scale, and we therefore hypothesized that (1) an amplification effect of host species richness is expected. In other words, a positive relationship between Lyme disease cases and host species richness should be observed across states of varying host species richness. Furthermore, we hypothesized that (2) the strength of the species richness-disease risk relationship changed significantly over time between 1992 and 2011, because the disease incidence has increased during this period. We expect an increase in the strength of the relationship between mammal host diversity and Lyme disease cases, as the overall number of cases of the disease is increasing in time.

Methods

Data

The number of cases of Lyme disease per 100,000 people for each of the 48 contiguous states from 1992 to 2011 was obtained from the United States Centers for Disease Control (CDC 2012). The *I. scapularis* mammal host species richness was obtained by tallying the number of *I. scapularis* mammal host species (provided by L. A. Durden) whose range overlaps with each of the 35 states (Smithsonian, 2013) with established or reported *I. scapularis* populations (Dennis et al. 1998). All of these states with reported or established *I. scapularis* are located in the east and central United States. All years from 1992 to 2011 were included in the analysis. The tick behaviour varies across its range and Stromdahl and Hickling (2012), identified states falling south of 35°N as having populations of ticks which differ from northern tick population (southern nymphs rarely feed from humans). We thus categorized the States into northern (27) and southern (8) States based on whether the state's mid-latitude fell north or south, respectively, of 35°N (United States Census Bureau 2010).

Six other variables which could affect Lyme disease incidence were also included in our model as covariates. The distance of the closest border of each State to the closest border of Connecticut and to the closest border of Wisconsin—the two areas recognized as the points of origin of Lyme disease in North America (Barbour and Fish 1993, Hoen et al. 2009) was measured using Google Earth (Google Inc. 2012). This variable aimed to capture how the spatial pattern of spread of *B. burgdorferi* from its likely source foci might have influenced Lyme disease incidence in any one State. We also included in our models the human population of each State (United States Census Bureau 2012) for each year from 1992 to 2011; as well as the total area of the State (United States Department of Agriculture 2007); and the area of each State covered by deciduous or coniferous forest (USDA 2007), the preferred habitat of *I. scapularis* (Ostfeld et al. 1995), and the average January temperature (US NOAA, 2013).

Data analysis

We investigated the relationship between host species richness and disease incidence with a non-linear mixed model of Lyme disease cases fit using *glmer* in the *lme4* (Bates et al. 2013) packages in R version 3.0.2 (R Core Team 2013). The outcome variable was the reported number of Lyme disease cases per year for each state from 1992-2011. We used a Poisson model with an observation-level random effect (also known as a log-normal Poisson model; Elston et al. 2001). The log of human population was included as an offset in the model. The model included host species richness, year, forest area, land area, distances from Connecticut and from Wisconsin, the interaction between host species richness and year and the interaction between host species richness and forest area as fixed effects and state as a random effect. We checked for co-linearity amongst the variables using the variance inflation factor (*vif* from the *HH* package in R version 3.0.2; Heiberger 2013); all variables had vifs <10 (Miles 2005), and so were kept in the model

(Appendix A). The full model was simplified by backwards selection with the function *drop1* (from the *stats* package in R version 3.0.2) which uses the Akaike Information Criterion (AIC) to rank the models based on both goodness-of-fit and complexity. The model with the lowest AIC after backwards selection was kept as the final model. The model was applied twice: to all the states with *I. scapularis* and to the northern states subset. Spatial autocorrelation in the number of Lyme disease cases was tested using the Moran Index (using the *Moran.I* function in the package *ape* in *R*; Venables and Ripley 2002). A Gaussian spatial correlation matrix was selected on the basis of a variogram (*variogram* function in the package *spatial* in *R;* Venables and Ripley 2002) and added to the final models (all states and northern subset) to account for spatial autocorrelation. The models with a spatial correlation matrix were built using *glmmPQL* (*MASS* package in *R*).

Results

Spatial autocorrelation was present with Moran's I ranging from 0.12 to 0.31 with a mean of 0.21 and a standard deviation of 0.061 ($P<0.0001$) for all years. The addition of the spatial correlation matrix controlled for autocorrelation in the residuals of the final models. Lyme disease cases decreased with mammalian host species richness and increased over time across the central and eastern USA (Appendix B, Table 1). The number of Lyme disease cases decreased with an increasing distance to Connecticut and the amount of forest area. There was no significant effect of the distance to Wisconsin, January temperature, or land area on Lyme disease incidence.

The model for the northern states subset was similar (Appendix B). Again, Lyme disease cases decreased with host species richness (Table 1). The relationship between Lyme disease

cases, the distance to Connecticut, and the distance to Wisconsin was negative, while all other variables were non-significant.

As indicated by the significant negative interaction between host species richness and year in the models above (Table 1), the slope of the relationship between Lyme disease cases and host species richness became more negative over time (Figure 1). In both the total and the northern models, the rate of increase in Lyme disease cases was most marked in States with lower host species richness (Table 1, Figure 2).

Discussion

A dilution effect

The negative relationship between tick host species richness and Lyme disease incidence is consistent with a dilution effect of host diversity from 1992 to 2011 in the United States. Both theory and observations suggest that a dilution effect will only occur under certain conditions and disease systems (Gilbert et al. 2001, Dobson 2004, Keesing et al. 2006, Begon 2008, Ogden and Tsao 2009, Wood and Lafferty 2012). Our observations at the scale of the central and eastern United States provide evidence of a dilution effect, with host species richness having the strongest effect on Lyme disease incidence at low levels of host species richness.

The dilution effect we observed across the United States could be due to regional mechanisms such as metacommunity processes in hosts (Leibold et al. 2004), since ticks have little capacity for movement other than on very mobile hosts. Furthermore, a positive local-regional species richness relationship has been found in field observations across many taxa (Caley and Schluter 1997), and a high-diversity state should be on average made up of high-diversity communities. If the overall Lyme disease risk in a region is determined by the disease

risk of the communities of which it is composed, then a dilution effect can result at the regional scale from mechanisms operating at the community-level.

Wood and Lafferty (2013) proposed that an amplification effect of biodiversity should occur at the regional scale, with a dilution effect occurring under specific conditions at the local scale. This proposition was based on the historical associations and observations of expansion of northern *I. scapularis* populations in response to re-forestation and the resulting expansion of key tick host populations (particularly white-tailed deer, *Odocoileus virginianus*), empirical observations relating the degree of forest cover and Lyme disease incidence at a broad regional scale (reviewed in Wood and Lafferty 2013), as well as local observations of dilution effects (Ostfeld and Keesing 2012).

Our observations do not support this hypothesis however, and we did not observe a positive relationship between host species richness and Lyme disease incidence for any year from 1992 to 2011. Instead, the incidence of Lyme disease decreased with the proportion of forest in the full model (Table1) and forest area had no effect in the northern states (Table 1). This suggests that while reforestation during the last century may have driven Lyme disease emergence (Wood and Lafferty 2013), this is no longer a significant driver of variations in Lyme disease risk and incidence.

Spatial heterogeneity in tick behavior may further modulate the relationship between Lyme disease and host diversity. Northern and southern *I. scapularis* represent two distinct clades (Duik-Wasser et al. 2006, Pepin et al. 2012, Diuk-Wasser et al. 2012), which also differ behaviorally. Southern *I. scapularis* ticks tend to take blood meals from reservoir-incompetent lizard hosts and rarely take blood meals from humans (Stromdahl and Hickling 2012). Lyme

disease cases are reported by the CDC based on state of residence, so an unknown proportion of Lyme disease cases reported in each state are travel-acquired, and this proportion is likely high in southern states. However, while ticks and human Lyme disease incidence may behave differently in the southern states, this did not affect our results, and a dilution effect was still observed when southern states were excluded from the analyses.

A strengthening effect over time

Another important finding is the increase in the strength of the dilution effect over time (Figure 1). The diversity-disease risk relationship became increasingly negative, providing empirical evidence that the diversity-disease risk relationship can indeed change through time as a zoonotic disease emerges. Management of Lyme disease may therefore become increasingly effective over time if it targets the diversity of host species within forest habitats.

While the widespread reforestation during the 20th century across a wide geographic area of the USA may have led to an increase in biodiversity to threshold levels needed to support tick and *B. burgdorferi* population expansion, the change in the diversity-disease relationship over time is mediated by a mechanism causing Lyme disease to increase more rapidly in low host species richness states than in high host species richness states. The change of Lyme disease incidence from 1992 to 2011 decreased with host species richness. If the absolute or relative abundance of white-footed mice, the most competent reservoir host for *B. burgdorferi*, is reduced by high host richness (Nupp and Swihart 2000), this could provide a plausible mechanism. Decreased white-footed mouse abundance would lead to a decreased transmission rate due to a lower encounter rate, leading to a slower emergence of Lyme disease in host species rich states. This hypothesis differs from the dilution effect hypothesis in that it predicts a decrease in the rate

of disease emergence with diversity, while the dilution effect predicts a decrease in disease incidence or risk with diversity.

The increasing dilution effect seen at the scale of the United States could also be due to a change in the relative importance of host dispersal and host community structure over time. Empirical modeling by Ogden and colleagues (2013) found that early in the emergence of Lyme disease, the tick immigration rate is more important in determining the speed of *B. burgdorferi* invasion in a community than the host diversity of the community (but see also Simon et al. 2014). This is supported by our observation that states with the highest incidence of Lyme disease are located closer to Connecticut, one of the sources of *Borrelia* in the USA. As Lyme disease emerges, there may then be an increasing dilution effect as host diversity becomes relatively more important in determining disease risk in communities.

Lyme cases are likely underreported (by two thirds) during the early stages of emergence (Naleway et al. 2002) and are currently underreported by a factor of 10 in States with high Lyme disease incidence (http://www.cdc.gov/lyme/stats/humanCases.html). In some States, a decrease in the number of cases was observed. Rhode Island, Missouri, and New York all reported relatively large decreases (decrease of >100 cases from 1992 to 2011), likely due to changes in collections of surveillance data, as Lyme disease is still emerging in the United States. For example, officials at the Rhode Island Department of Health, the state with the largest per capita decrease in Lyme disease cases, attribute the decrease to a change in surveillance methods following a decrease in available funds in 2004-2005 (Rhode Island Department of Health, personal correspondence).

We assumed that the mammal host ranges remained constant over time, but climatic and human land use changes over the past decades may have resulted in range shifts (e.g. Roy-Dufresne et al. 2013) or contractions (Channell and Lomolino 2000). These range shifts could conceivably affect the diversity-disease relationship and could merit further study.

Given that the diversity-disease risk relationship can change over time, it may be important that researchers establish whether their focal system is at equilibrium. This could be tested by investigating whether some measure of disease incidence, such as reported incidences by clinics to a governmental agency, has increased over time in the study area. If the disease system has not reached equilibrium, there is no reason to expect that the relationship will remain constant. Ostfeld and Keesing (2000), concluded that a dilution effect occurred in the United States using CDC data from 1997 and 1998. In 1998, however, Lyme disease was still emerging in the United States (Bacon et al. 2008). Here, we also performed an analysis at the level of the United States, this time considering data covering a much greater span of time, as well as other variables. We also found a dilution effect, but demonstrated that the diversity-disease risk relationship had not remained constant.

Conclusions and future directions

When accounting for time, spatial scale, forest cover, tick phenotype, and spatial pattern of disease spread, we found a dilution effect of diversity on Lyme disease in the United States, and revealed that this effect was getting stronger over time. While adding to the body of evidence for a dilution effect (reviewed in Ostfeld and Keesing. 2012), our findings also have management implications for Lyme disease in the United States. We provide evidence that the strength of the relationship between Lyme disease and host diversity is dynamic. Our findings also have land

management and public health implications for other regions in which Lyme disease is currently emerging, including southern Canada. Future studies should focus on intermediate spatial scales; studies at the mesoscale may connect patterns at the very largest scales to the local process occurring within communities. Landscape studies offer the opportunity to examine within and between patch dynamics of Lyme disease emergence and spread (Simon et al. 2014). By bridging scales, we may reconcile the shifts in the strength and direction of the relationship between diversity and Lyme disease risk seen across studies.

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Appendix

Table 1. Log-normal Poisson mixed models of Lyme disease cases from 1992 to 2011 fit by maximum likelihood. The first model includes all 35 states in the United States with established or reported populations of *Ixodes scapularis*, while the northern states model includes the subset of states with a mid-latitude above 35 degrees.

Parameters	Estimate	Standard Error	z value	p
All states				
(Intercept)	7.262	6.408	1.133	0.2575
Host species richness	-4.614	1.991	-2.317	0.0271
Year	0.964	0.1314	7.338	<0.0001
Forest Area	-0.013	0.005	-2.559	0.0107
Distance to Connecticut	-0.002	0.0003	-6.451	<0.0001
Host species richness * Year	0.285	0.040	-7.092	<0.0001
Northern states				
(Intercept)	10.289	6.052	1.700	0.0897
Host species richness	-6.248	1.815	-3.442	0.0022
Year	0.884	0.152	5.819	<0.0001
Distance to Connecticut	-0.001	0.0004	-2.855	0.0090
Distance to Wisconsin	0.001	0.0006	2.160	0.0414
Host species richness * Year	-0.252	0.0465	-5.411	<0.0001

Figures

Figure 1. Lyme disease incidence (number of cases per 100 000 people) from 1992 to 2011 in all 35 states of the United States with established or reported *Ixodes scapularis* populations against tick host mammalian species richness. Trend lines were generated for two years: 1992 and 2011 by applying a locally weighted scatterplot smoothing function (*loess* in the *stats* package in R), with points weighted by number of states per host species richness level.

Figure 2. The number of Lyme disease cases in 1992 (A) and 2011 (B) in the 35 states of the United States with established or reported *Ixodes scapularis* populations, compared with the tick host mammalian species richness (C) and forest area (D) in these States. Lyme disease increased at a larger rate in host species-poor states than in host species-rich states (Table 1).

Figure 1

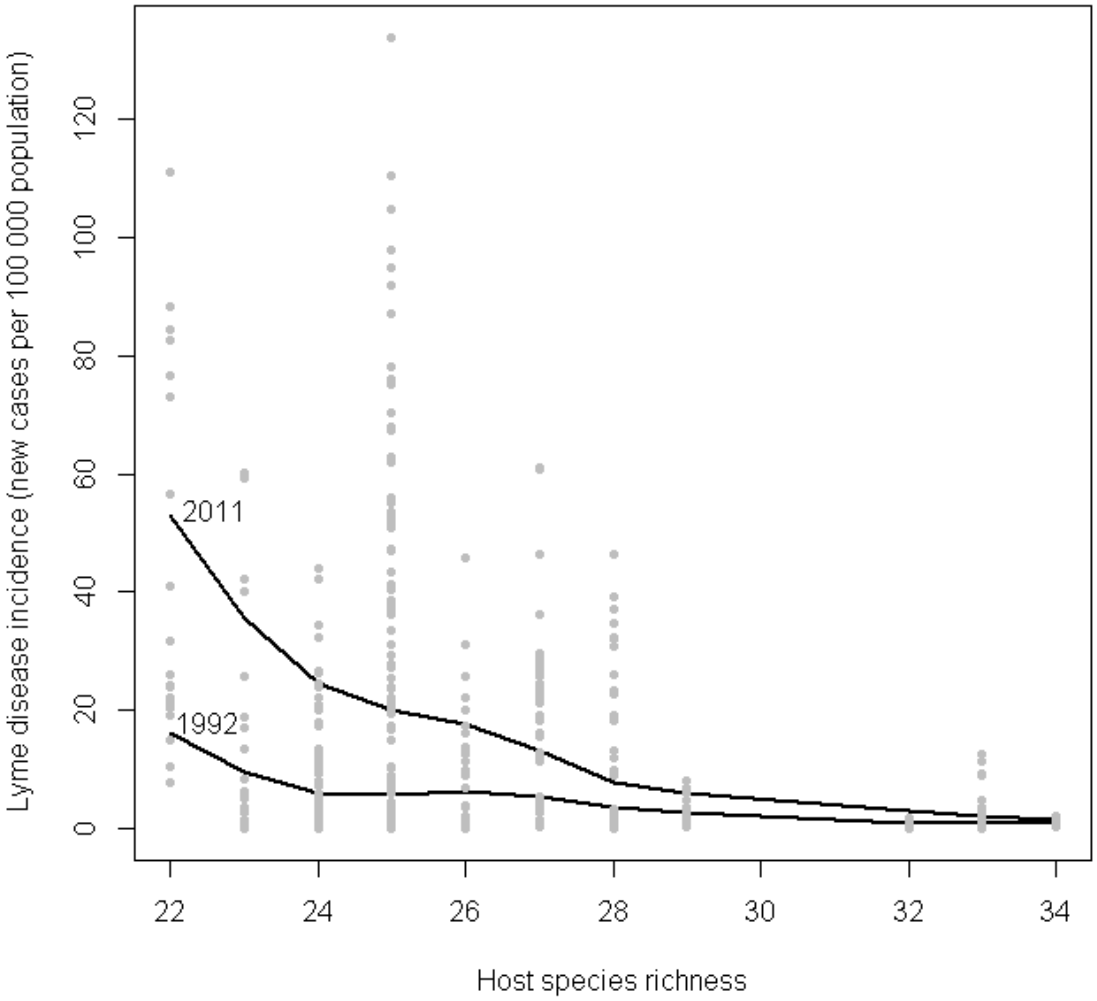
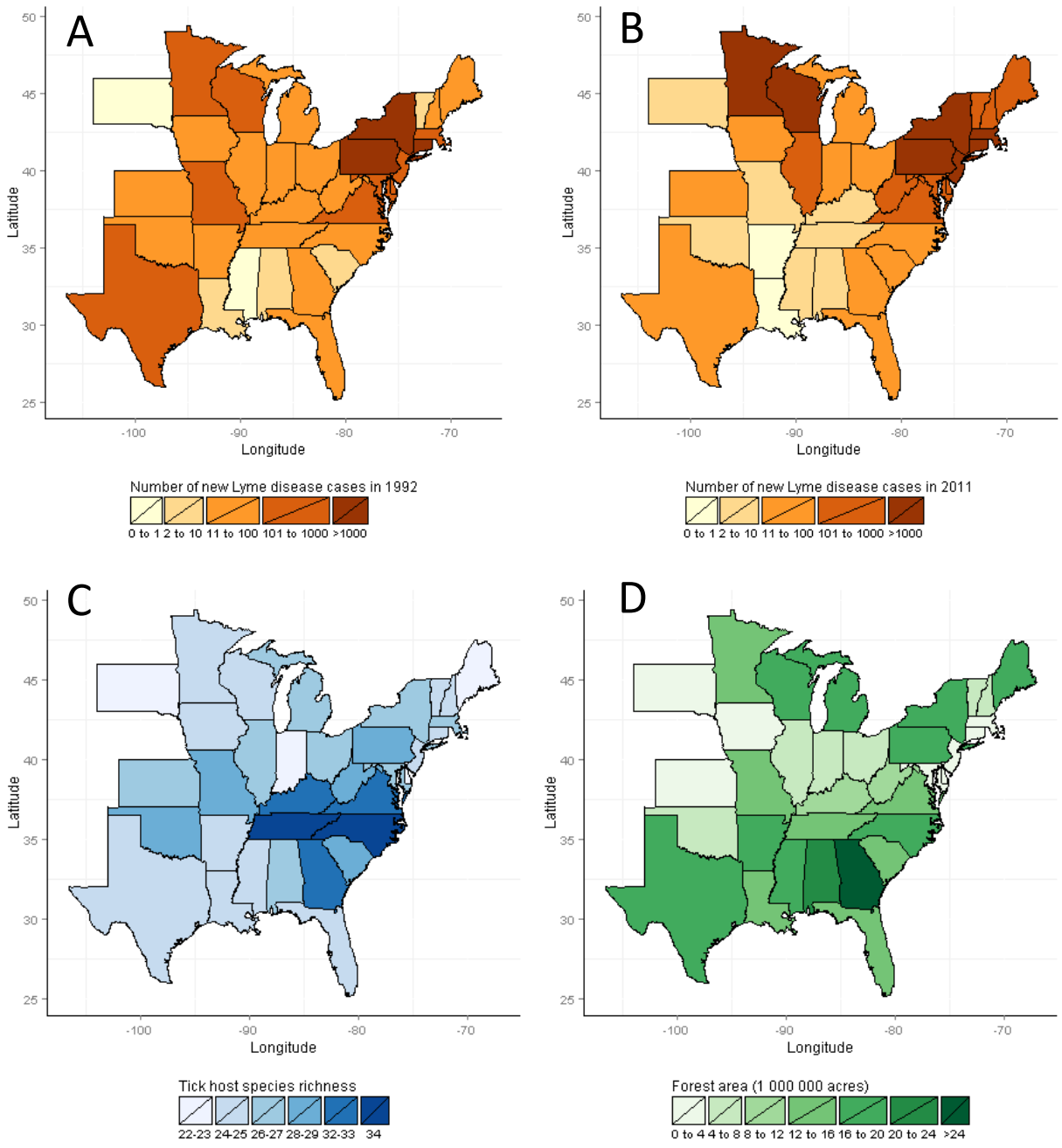

Lyme disease incidence (new cases per 100 000 population)
0
20
40
60
80
100
120
2011
1992
22
24
26
28
30
32
34
Host species richness

Figure 2


A
B
C
D
Latitude
Longitude
50
45
40
35
30
25
-100
-90
-80
-70
Number of new Lyme disease cases in 1992
0 to 1 2 to 10 11 to 100 101 to 1000 >1000
Number of new Lyme disease cases in 2011
0 to 1 2 to 10 11 to 100 101 to 1000 >1000
Tick host species richness
22-23 24-25 26-27 28-29 32-33 34
Forest area (1 000 000 acres)
0 to 4 4 to 8 8 to 12 12 to 16 16 to 20 20 to 24 >24

Supplemental Material

Appendix A

Variance inflation factors (VIF) of the variables used in the full model

Variable	All states VIF	Northern states subset VIF
Host species richness	2.882	1.587
Year	1.002	1.002
Distance to Connecticut	3.832	3.349
Distance to Wisconsin	2.690	1.916
Forest area	2.926	1.769
Land area	6.441	3.000
January temperature	2.134	2.135

Appendix B

All states model selection

Parameters	Estimate	Standard Error	z value	p
Full model:			AIC = 7799.978; BIC = 7850.040	
(Intercept)	6.683	6.652	1.305	0.1918
Host species richness	-5.134	2.067	-2.483	0.0130
Year	0.999	0.1607	6.214	<0.0001
Distance to Connecticut	-0.003	0.0005	-5.199	<0.0001
Distance to Wisconsin	0.00006	0.0005	0.120	0.9043
Forest area	-0.014	0.006	-2.340	0.0193
Land area	0.003	0.005	0.700	0.4839
January temperature	0.0002	0.007	0.030	0.9764
Host species richness * Year	-0.297	0.049	-6.051	<0.0001
Dropped: Distance to Wisconsin			AIC = 7797.994; BIC = 7843.505	
(Intercept)	8.911	6.586	1.353	0.1761
Host species richness	-5.186	2.064	-2.512	0.0120
Year	0.998	0.161	6.212	<0.0001
Distance to Connecticut	-0.003	0.0004	-5.271	<0.0001
Forest area	-0.014	0.006	-2.333	0.0196
Land area	0.003	0.005	0.693	0.4880
January temperature	0.0004	0.007	0.058	0.9549
Host species richness * Year	-0.297	0.049	-6.048	<0.0001
Dropped: January temperature			AIC = 7795.994; BIC =7836.953	

(Intercept)	8.532	6.543	1.305	0.1922
Host species richness	-5.065	2.044	-2.478	0.0132
Year	1.005	0.161	6.256	<0.0001
Distance to Connecticut	-0.003	0.0004	-5.320	<0.0001
Forest area	-0.014	0.006	-2.349	0.0188
Land area	0.003	0.005	0.687	0.4921
Host species richness * Year	-0.299	0.049	-6.092	<0.0001
Dropped: Land area (Final model)			AIC = 7794.466; BIC = 7830.875	
(Intercept)	8.156	6.510	1.253	0.2103
Host species richness	-4.906	2.027	-2.421	0.0155
Year	1.004	0.161	6.250	<0.0001
Distance to Connecticut	-0.002	0.0003	-6.906	<0.0001
Forest area	-0.012	0.005	-2.268	0.0234
Host species richness * Year	-0.299	0.049	-6.087	<0.0001

Table 3: Northern states subset model selection

Parameters	Estimate	Standard Error	z value	p
Full model:			AIC =6468.619; BIC = 6515.826	
(Intercept)	12.40	7.082	1.785	0.0743
Host species richness	-6.723	2.30	-2.921	0.0035
Year	1.386	0.160	8.683	<0.0001
Distance to Connecticut	-0.001	0.0006	-2.140	0.0324
Distance to Wisconsin	0.0007	0.0009	0.774	0.4388

Forest area	0.005	0.009	0.581	0.6510
Land area	-0.008	0.009	-0.852	0.3940
January temperature	0.006	0.007	0.771	0.4405
Host species richness * Year	-0.409	0.049	-8.396	<0.0001
Dropped: Forest area			AIC =6466.921; BIC = 6509.836	
(Intercept)	11.755	6.949	1.692	0.0907
Host species richness	-6.412	2.243	-2.858	0.0043
Year	1.384	0.159	8.686	<0.0001
Distance to Connecticut	-0.002	0.0005	-2.896	0.0038
Distance to Wisconsin	0.0006	0.0008	0.753	0.45146
Land area	-0.004	0.007	-0.589	0.5558
January temperature	0.005	0.007	0.945	0.4562
Host species richness * Year	-0.408	0.0486	-8.398	<0.0001
Dropped: Land area			AIC = 6465.303; BIC = 6503.927	
(Intercept)	12.451	6.617	1.882	0.0599
Host species richness	-6.845	1.990	-3.441	0.0006
Year	1.387	0.159	8.709	<0.0001
Distance to Connecticut	-0.002	0.0004	-3.608	0.0003
Distance to Wisconsin	0.001	0.0007	1.408	0.1592
January temperature	0.006	0.007	0.794	0.4275
Host species richness * Year	-0.409	0.049	-8.420	<0.0001
Dropped: January temperature (Final model)			AIC =6463.940; BIC = 6498.272	
(Intercept)	12.017	6.543	1.837	0.06627

Host species richness	-6.680	1.961	-3.407	0.0006
Year	1.392	0.159	8.733	<0.0001
Distance to Connecticut	-0.0017	0.0005	-3.576	0.0003
Distance to Wisconsin	0.001	0.0006	1.477	0.140
Host species richness * Year	-0.411	0.049	-8.447	<0.0001

Table 4: Mammal *Ixodes scapularis* hosts (compiled by L. A. Durden) used in this analysis

Blarina brevicauda	*Neotoma floridana*	*Sciurus niger*
Blarina carolinensis	*Ochrotomys nuttalli*	*Sigmodon hispidus*
Canis latrans	*Odocoileus hemionus*	*Sorex cinereus*
Clethrionomys gapperi	*Odocoileus virginianus*	*Sorex dispar*
Didelphis virginiana	*Oryzomys palustris*	*Sorex fumeus*
Felis concolor	*Peromyscus gossypinus*	*Sylvilagus aquaticus*
Lactarius rufus	*Peromyscus leucopus*	*Sylvilagus floridanus*
Marmota monax	*Peromyscus maniculatus*	*Tamias striatus*
Mephitis mephitis	*Peromyscus polionotus*	*Tamiasciurus hudsonicus*
Microtus breweri	*Podomys floridanus*	*Urocyon cinereoargenteus*
Microtus pennsylvanicus	*Procyon lotor*	*Ursus americanus*
Microtus pinetorum	*Reithrodontomys fulvescens*	*Vulpes vulpes*
Napaeozapus insignis	*Sciurus carolinensis*	*Zapus hudsonius*

CONNECTING STATEMENT

In the previous chapter, I found an increasingly negative relationship between host diversity and human Lyme disease incidence in states of the United States from 1992 to 2011. In the following chapter, I investigated the relationship between small mammal diversity and the density of infected ticks in a region of southern Quebec from 2011 to 2013. I find that the relationship is positive, providing an interesting contrast to the results of Chapter 2. As discussed in the literature review of Chapter 1, and as demonstrated by the results of Chapters 2 and 3, the relationship between host diversity and Lyme disease incidence depends on factors including temporal and spatial scale.

Wood and Lafferty (2013) hypothesized that an amplification effect should take place at the regional level due to the effects of forestation, while a dilution effect can take place at the within-forest level. It is interesting to note that we find the opposite relationships in our two studies. In Chapter 2, we find a dilution effect at the level of the United States, while in Chapter 3, we find an amplification effect at the level of forest fragments in southern Quebec. In the case of Chapter 2, it may be that forestation no longer drives Lyme disease incidence in the United States although it could have contributed to its emergence. In the case of Chapter 3, it may be that host density is playing a larger role than white-footed mouse abundance in the emergence of Lyme disease in Quebec. It is not yet fully clear what drives the relationship between diversity and disease, but it is clear that the relationship is scale-dependent.

CHAPTER 3

Host diversity increases ecological disease risk across forest fragments in Southern Quebec from 2011 to 2013

Abstract

The relationship between biodiversity and Lyme disease has received considerable attention. Some have hypothesized that a "dilution" effect takes place: biodiversity should reduce the abundance of white-footed mice, the most effective Lyme disease reservoir, leading to a decrease in Lyme disease risk. Others have hypothesized that an "amplification" effect takes place: biodiversity should increase tick host abundance, which should increase infected tick abundance, leading to an increase in Lyme disease risk. In this study, the relationship between host diversity and Lyme disease is investigated in southern Quebec, where Lyme disease is currently in the early stages of emergence. We find a positive relationship between small mammal species richness and infected nymphal tick abundance. The mechanisms behind this relationship are investigated directly using path analysis and we find that a vector amplification effect model is supported by the data. The results of this study suggest that the small mammal assemblage modulates ecological Lyme disease risk in southern Quebec. An exploratory model suggests that latitude and resource availability may play a role in modulating Lyme disease risk. Continued study and monitoring of white-footed mice and other small mammals in Southern Quebec will be important in understanding and predicting the distribution of disease risk over the coming decades.

Introduction

Lyme disease is a vector-borne zoonotic disease caused by the bacteria *Borrelia burgdorferi* that, if left untreated, can lead to neurological, arthritic, and cardiovascular symptoms (Steere at al., 1987). It is endemic to much of the northern hemisphere, including North America, Europe and Asia (Lane at al., 1991). In eastern North America, the Lyme disease vector is the black-legged tick (*Ixodes scapularis*), which has three stages in its life cycle: larva, nymph, adult. Larvae and nymphs tend to take blood meals from small mammals while adults tend to take blood meals from large mammals, primarily the white-tailed deer (*Odocoileus virginianus*). Tick hosts differ in their efficiency as *B. burgdorferi* reservoirs, with white-footed mice (*Peromyscus leucopus*) being particularly efficient (Brunner at al., 2008).

Lyme disease was first identified in 1975 in Lyme, Connecticut (Steere at al., 1977) and has since spread to become endemic in much of north-central and north-east United States (Steere at al., 2004). By 1996, it had become the most common vector-borne disease in the United States (CDC, 1996). More recently, Lyme disease has begun to emerge in southern Canada (Ogden at al., 2009), particularly in southern Manitoba, Ontario, and Quebec, and regions of the Maritimes. It is hypothesized that climate warming may be allowing black-legged ticks to expand their range further northwards, bringing with them *B. burgdorferi* infection (Ogden at al., 2006). Lyme disease incidence has increased steadily since it was declared a nationally reportable disease by the public health Agency of Canada in 2009, and simulations indicate that the white-footed mouse (Roy-Dufresne et al. 2013; Simon et al., 2014), black-legged ticks (Leighton at al., 2012) and *B. burgdoferi* (Ogden at al., 2013; Simon et al., 2014) ranges will continue to expand into Canada in the coming years. The recent emergence of Lyme

disease in southern Canada provides an important window of opportunity to study how biodiversity mediates the emergence of zoonotic disease.

A range of organisms are involved in Lyme disease epidemiology, and the relationship between biodiversity and Lyme disease prevalence or risk is debated (Randolph and Dobson, 2012; Wood and Lafferty, 2013; Ostfeld, 2013; Ostfeld and Keesing, 2013). Ostfeld and Keesing (2000) hypothesized that increased host diversity could lead to a reduction in the relative abundance of white-footed mice (the most effective reservoir), which would lead to reduced infection prevalence, and therefore reduced disease risk (Van Buskirk and Ostfeld, 1995; Ostfeld and Keesing, 2000). They referred to this hypothesis as a "dilution effect", although the term has come to be used more generally to refer to any negative relationship between diversity and disease, regardless of the mechanism (Ostfeld and Keesing, 2006). More specifically, this reduction of infection prevalence via "wasted bites" of ticks on non-white-footed mice is called frequency-dependent dilution (Rudolf and Antonovics, 2005; Figure 1). Another potential dilution effect mechanism is density-dependent dilution (Figure 1). In this case, increased host diversity could lead to a decrease in the absolute abundance of white-footed mice via competition, leading to a decrease in tick abundance and *B. burgdorferi* prevalence in ticks, and therefore a decrease in disease risk.

Alternatively, increased host diversity may mean increased host density, which could lead to increased tick density, and therefore increased disease risk (Gilbert at al., 2001; Keesing at al., 2006; Ogden and Tsao, 2009). This mechanism of increased tick density is known as "vector amplification" (Figure 1). More generally, a positive relationship between biodiversity and disease is known as an "amplification effect" (reviewed in Ostfeld and Keesing, 2012). As with

the dilution effect, there are a number of possible mechanisms that could drive this relationship (Ostfeld and Keesing, 2006).

There is a dearth of empirical evidence for a dilution or amplification effect of Lyme disease in natural systems. A few studies have found a correlation between forest fragmentation and tick or human infection incidence (Allan at al., 2003; Brownstein at al., 2005; Jackson at al., 2006). Although theoretically biodiversity may be lower in small forest fragments, the link between forest fragmentation and host diversity was not explicitly tested in these studies. Furthermore, other studies have found a higher proportion of white-footed mice in large forest fragments than in small fragments (Wilder and Meikle, 2004), and a lack of correlation between ecological disease risk and human disease incidence (Brownstein at al., 2005).

A limited number of studies of the diversity-Lyme disease relationship have taken place in southern Canada where Lyme disease is emerging. A field study in Quebec found that the overall nymphal abundance increased with small mammal species richness, indicating a possible amplification effect, but a decrease in nymphal infestation with tree species richness may suggest that there is a dilution effect when a wider ecological community is considered (Bouchard at al., 2013). A field study in Ontario found an overall negative correlation between the number of infected nymphs and small mammal species richness, with a modulating effect of white-footed mouse abundance (Werden at al., 2014), which suggests a community context-dependent dilution effect. Simulation modeling of *B. burgdorferi* invasion in southern Canada suggested that the infected tick immigration rate plays a more important role in determining the rate of pathogen invasion than host density or diversity (Ogden at al., 2013), although Simon et al. (2014) found that the rate of expansion of Lyme disease risk in Southern Quebec was more likely constrained by the rate of expansion of the white-footed mouse in this region.

Here, we collected small mammals and ticks from 27 forest patches in southern Quebec in 2011, 2012, and 2013 and tested them for *B. burgdorferi*. We began by determining the relationship between small mammal richness and infection density (the abundance of infected ticks) in the forest fragments using a mixed effects model. We then further explored that relationship using path analysis models. The strength of path analysis is that it allows an exploration of the cause and effect relationships between variables. We investigated two main hypotheses: (1) the amplification effect model: as host species richness increases, host abundance increases, which causes an increase in tick abundance, which increases infection density, and (2) the dilution effect models: as species richness increases, white-footed mouse relative abundance (frequency-dependent dilution effect) and absolute abundance (density-dependent dilution effect) decrease, which decreases tick abundance and tick infection prevalence. It is possible that a number of mechanisms connecting richness to Lyme disease could be at play simultaneously resulting in a net effect that differs with context. In an exploratory model, we generated and evaluated hypotheses on the role of latitude and resource availability in modulating Lyme disease risk in Southern Quebec.

Methods

Field methods

We sampled twenty-seven forest patches of varying size in Southern Quebec (Figure 2) during the summers of 2011, 2012, and 2013. The data expands the data set used by Simon et al. (2014), but does not include sites for which there was only a single trap night. The forest patches were mixed deciduous-coniferous and ranged in size from small fragments of 0.4 km^2 to forests of 785.0 km^2. White-tailed deer are abundant in the region. The region of Southern Quebec in which the study sites are located stretched between two urban centres, Montreal and Sherbrooke,

and extended about 150 km to the North and South of the cities. The landscape is characterized by agriculture, urban development, and forest (Marrotte et al. 2014). The forest, which was at one time continuous, is fragmented by agriculture fields (dominated by soy, corn, and wheat), creating a landscape which is to some extent analogous to "islands" of forest in an "ocean" of agriculture (Laurance, 2004).

In each forest patch, grids of Sherman traps were set for two to three consecutive nights. Some of the sites were visited more than once so the number of trap nights per site ranged from 2 to 9 (Table 1). In 2011 and 2012, four grids of 28 traps (4x7) were set each night in a given forest, for a total of 112 traps, with 5 meters between traps. In 2013, one grid of 120 traps (6 x 20) was set each night in a given forest patch, with 10 meters between traps. In 2011 and 2012, the traps were closed during the days, while in 2013 the traps were kept open during the day at some sites. In all years, the traps were freshly baited each night with a peanut butter-oatmeal mixture. The traps were checked each morning following a trap night, and captured small mammals were euthanized. The mammals were then examined under a dissecting microscope and ticks were removed and preserved in ethanol. We trapped two mouse species, *Peromyscus leucopus* and *Peromyscus maniculatus*, that can be difficult to distinguish morphologically (Lindquist at al., 2003), and so were identified using a species-specific primers in multiplex PCR as described in Rogic et al. (2013).

In addition to collecting ticks from the trapped mammals, we sampled ticks during the summer by dragging $1m^2$ flannel sheets along the forest floor. The dragging transects followed the length of the trapping grids (a total of 480 m at each site in 2011 and 2012, and 1140 m at each site in 2013), and the sheets were examined thoroughly for the presences of ticks every 10 to 20m. All ticks collected were preserved in ethanol. Nymphs and adults collected from

dragging as well as all ticks from trapped mammals were later screened at the National Microbiology Laboratory of the Public Health Agency of Canada for the presence of *B. burgdorferi* using Real-time PCR targeting the 23S rRNA locus and *ospA* as described in Ogden at al. (2011).

In the fall of 2012 and the summer of 2013, samples of ground litter were collected to estimate the seed density in 15 of our forest patches. Litter from a grid of 1 m^2 quadrats were collected, and tree seeds known to be consumed by granivorous mammals were later sorted from the litter. In 2012, a 20 x 50 m grid of 18 quadrats was sampled, while in 2013 a 40 x 180 m grid of 9 quadrats was sampled at each site. In both years, the litter-sampled area covered the same zone of the forest patch as the small mammal sampled area.

The bird tick host species richness was estimated for each site using the Quebec Breeding Bird Atlas (Quebec Breeding Bird Atlas, 2014). We identified the Breeding Bird survey quadrant in which each site is located. We then tallied the number of breeding bird species which are known to be *I. scapularis* hosts (Provided by L. A. Durden) present in each quadrant.

All procedures performed were approved by the McGill center for animal research ethics (McGill AUP#5420) and the government of Quebec (SEG permits 2011-05-15-014-00-S-F, 2012-07-16-1417-16-17-SF, and 2013-07-04-1530-04-14-16-17-SF).

Statistical methods

To investigate the relative importance of the small mammal species in supporting tick populations, we tested a poisson family generalized linear model to compare the total number of ticks on each mammal species and the average number of ticks per individual for each species. The analyses were performed using *glmmPQL* in the *MASS* package (Venables and Ripley, 2002)

in R version 3.0.2 (R Core Team, 2013). Tukey tests from *glht* in the package *multcomp* (Hothorn et al., 2008) were then implemented to identify any significant differences between sites.

In order to determine the relationship between the number of infected ticks and the diversity of small mammals, we used a generalized mixed effects model using the *glmmADMB* package in R (Fournier et al., 2012). A zero-inflated Poisson family was selected to account for over-dispersion in the data. Small mammal diversity was measured by species richness. Species richness, year (standardized such that 2011 was year 0) and the distance over which the flannel sheets were dragged as fixed effects. The site was included as a random effect.

As in Simon et al. (2014), we used the number of ticks as a measure of tick density and the number of infected ticks as a measure of infection density, rather than the number of adult females and the number of infected nymphs, respectively. This is because the values are correlated, but also because the abundance of ticks or infected ticks may be low for a given life stage. The use of only one life stage rather than all life stages could results in some sites with a value of 0 where infection or ticks are present.

We used path analysis to more fully investigate the causal pathways connecting mammal diversity to the number of infected ticks at our study sites. The first step of path analysis is to test the d-separation (independence) claims which are implied by the hypothesized model. The claims were generated using the *Basiset* function in the *R* package *ggm* (Marchetti et al., 2014). If two given variables are d-separated as implied by the model, then they will be statistically independent. Therefore a p-value greater than 0.05 indicates that there is no evidence to reject the hypothesized model. The d-separation claims were tested using appropriate generalized linear mixed models (*R* packages *lme4*, Bates et al., 2014; and *nlme*, Pinheiro et al., 2013; Table 2). The

p-values of all the d-separation claims were combined into one summary p-value using Fisher's C statistic (Shipley, 2000), which indicates if there is sufficient evidence to reject the hypothesized model ($p < 0.05$) or not ($p > 0.05$).

If the model is not rejected, the next step in path analysis is to quantify the path coefficients, determine their p-values, and to calculate the amount of variation explained by the model for each variable (marginal R^2). These are models based on the direct effect relationships given in the hypothesized models. They are calculated using appropriate generalized linear mixed models (*lme4* and *nlme*). The Corrected Akaike Information Criterion (AICc), a measure of relative goodness-of-fit of a model, adjusted for small sample sizes, was calculated for each path analysis model (Shipley, 2013).

We first tested three models that capture the main mechanisms in this literature: the amplification, frequency-dependent dilution, and the density-dependent dilution effect models. Based on the results of these models and model exploration, we generated an exploratory model.

The number of white-footed mice and the total number of small mammals caught in the traps were used as proxies for white-footed mouse and small mammal host abundance, respectively. The sum of the ticks caught by dragging and from trapped mammals was used as an estimate of tick abundance. The proportion of the sampled ticks infected with *B. burgdorferi* and the total number of infected sampled ticks were used as estimates of tick infection prevalence and the abundance of infected ticks, respectively. In all d-claims tests and tests of the direct relationships, wherever the small mammal or white-footed mouse abundance was included, the number of trap nights was also included as a covariate. Wherever the tick abundance was included, the distance over which the sheets were dragged was also included as a covariate. In models with species richness as the response variable, general linear models were used. In

models with host abundance, white-footed mouse abundance, tick abundance, or abundance of infected ticks as response variables, Poisson generalized linear models were used. In the case of abundance of infected ticks as the response variable, an individual-level random effect was also included to account for over-dispersion (also known as a log-normal Poisson model; Elston at al., 2001). For tick infection prevalence and white-footed mouse relative abundance as response variables, a binomial generalized linear model was used.

We further explored and tested various models to generate new alternative hypotheses about the mechanisms connecting biodiversity to Lyme disease via pathways involving seed calorie density, seed genera richness, bird species richness, and latitude. The exploratory models included the two component variables of the abundance of infected ticks: tick abundance and tick infection prevalence (tick abundance * tick infection prevalence = abundance of infected ticks). The exploratory models were generated by non-exhaustively testing biologically-plausible path analysis models. The models were exploratory rather than being valid tests of hypotheses since the hypotheses were generated *post-hoc*, however they may indicate fruitful avenues of further research. The models tested were non-exhaustive, but instead were constrained by our knowledge of the system and understanding of plausible biological relationships between variables.

Results

A total of 9 different small mammal species were trapped at the 27 sites and the most frequently trapped species was *P. leucopus* (White-footed Mouse). The next species occurring at our sampling sites, in order from greatest to fewest number trapped were: *Blarina brevicauda* (Northern Short-tailed Shrew), *Sorex cinereus* (Cinereus Shrew), *P. maniculatus* (Deer Mouse), *Myodes gapperi* (Southern Red-backed Vole), *Tamias striatus* (Eastern Chipmunk), *Napaeozapus*

insignis (woodland jumping mouse), *Tamiasciurus hudsonicus* (Red Squirrel), and *Glaucomus volans* (Southern Flying Squirrel). The species richness per site ranged from 1 to 6 with an average of 3.65 (SD=1.34).

The seeds of a diversity of trees were identified in the leaf litter collected from sites in 2012 and 2013. Genera known to be consumed by small mammals (Grodzinski and Sawicka-Kapusta, 1970) were oak (*Quercus*), beech (*Fagus*), maple (*Acer*), ash (*Fraxinus*), hickory (*Carya*), and basswood (*Tilia*). The number of seed genera present at the site ranged from 0 to 5 and was 2.94 on average (SD=1.39).

The white-footed mouse was present at all sites in all years except for four sites in the Northeast area of the study region (Table 1; Figure 2). At sites where white-footed mouse was present (with the exception of one site in 2011), more white-footed mice were trapped than any other small mammal. At the three sites without white-footed mice, deer mice were the dominant captured species.

Ticks were found at 85% of the sites from either dragging or from trapped mammals in at least one year. The sites at which no ticks were found were all located in the Northern half of the study region (Table 1) and three of them were visited only once in 2011. The majority of sampled ticks were larvae (88%), nymphs made up 12% of the captured ticks, while adults were rare (<1%). No adult ticks were found on trapped mammals, as they tend to prefer large mammals as hosts. Only *I. scapularis* were included in this study, but we also occasionally found ticks of other species including *Haemaphysalis leporispalustris, Ixodes angustus,* and *Ixodes muris*.

More ticks were found in total on *P. leucopus* than any other species (β=5.85; df=289; $p<0.0001$; Figure 3). More ticks were found on *P. leucopus* per individual than all other species

(β=4.82; df=94; p=0.0004). except for *P. maniculatus* and *Myodes gapperi,* for which the difference in tick burden was insignificant .

B. burgdorferi infection of ticks was detected in at least one year at 11 sites (41% of the sites). The majority of infected ticks were nymphs (76% of all infected ticks). All of the infected ticks (except for a tick in 2011 and another in 2012) were sampled in 2013. At sites with infected ticks, the average nymphal infection prevalence was 0.225 (SD=0.120). *B. burgdorferi* was not present at any of the sites where white-footed mice were absent. The sites with *B. burgdorferi* infection were located in the Southern half of the study region (below a latitude of 45.43; Figure 2).

There was a significant positive relationship between small mammal species richness and the total number of infected ticks (Table 2). No other variable included in the model was significantly related with the total number of infected ticks (Table 2).

None of the models – the amplification, frequency-dependent dilution, and density-dependent dilution effect models, were rejected (C-statistic p-values of 0.286, 0.450, and 0.282, respectively; Table 3). The AIC and AICc values varied little between the three models, although the frequency-dependent dilution effect model had the lowest AIC and AICc values (Table 3).

In the amplification effect model (Figure 4A), small mammal host abundance increased significantly with small mammal species richness. The abundance of infected ticks increased significantly with small mammal host abundance. The relationships between the variables are therefore as hypothesized for the amplification effect model. A large proportion of the variation in small mammal abundance (marginal R^2=0.704; Table 3) and a moderate proportion of the variation in abundance of infected ticks (marginal R^2=0.301; Table 3) is explained by the model.

In the frequency-dependent dilution effect model (Figure 4B) there was a negative relationship between small mammal species richness and white-footed mouse relative abundance, as hypothesized. The frequency-dependent dilution effect hypothesizes a positive relationship between white-footed mouse relative abundance and abundance of infected ticks, but we did not detect such a significant relationship between these two variables. Only a small proportion of the variation in white-footed mouse relative abundance and abundance of infected ticks is explained by the model (marginal R^2 of 0.114 and 0.206, respectively; Table 3).

The density-dependent dilution effect hypothesizes a negative relationship between small mammal species richness and white-footed mouse absolute abundance. It also hypothesizes a positive relationship between white-footed mouse absolute abundance and abundance of infected ticks. Neither relationship was found to be statistically significant. A moderate amount of the variation in white-footed mouse absolute abundance is explained by the model (marginal R^2 = 0.388). Only a small amount of the variation in abundance of infected ticks is explained by the model (marginal R^2 = 0.163; Table 3).

The amplification effect model was chosen for further exploration and expansion due to its explanatory power of the observed positive relationship between species richness and abundance of infected ticks (Table 2) and its ability to explain variation in the data (high marginal R^2). The expanded amplification effect model (Figure 5) broke the abundance of infected ticks into its component variables: abundance of ticks and tick infection prevalence. This model had an AIC and AICc higher than the previous models (Figure 4) but a much higher C-statistic p-value (p=0.677; Table 3). As with the amplification effect model, the expanded amplification effect model detected a positive relationship between small mammal species richness and small mammal host abundance. The expanded amplification effect model also found

a significant positive relationship between small mammal host abundance and tick abundance, but no significant relationship between small mammal host abundance and tick infection prevalence.

The amplification effect model was then further expanded into an exploratory model (Figure 6). In this model, we found a positive relationship between seed species richness and small mammal host abundance. We also found a negative relationship between latitude and both tick abundance and tick infection prevalence. The exploratory model had the highest AIC of all the models but the lowest AICc (Table 3). The p-value of the exploratory model was equal to that of the expanded amplification effect model (p=0.677; Table 3).

Discussion

Positive relationship between diversity and infection density

In Southern Quebec from 2011 to 2013, we found that there was an amplification effect of small mammal diversity on infected tick density (Table 2, Figure 4). This observation is consistent with models which predict that an amplification effect will occur under certain conditions (Van Buskirk and Ostfeld, 1995; Norman at al., 1999; Gilbert at al., 2001; Schmidt and Ostfeld, 2001; Dobson, 2004; Ogden and Tsao, 2009). The amplification effect only became apparent in 2013; in 2011 and 2012 there were too few sites with infected ticks to observe any relationship between host diversity and infected tick density. It may be that as time passes and *B. burgdorferi* abundance continues to increase in southern Quebec, the amplification effect may continue to strengthen or some other relationship between diversity and disease may emerge. In such a highly dynamic, emerging system, it is unlikely that the diversity-disease relationship will remain unchanged.

To know the relationship between host diversity and disease is not enough information to know the mechanisms driving that relationship. Given that there is a positive relationship between small mammal host diversity and the number of infected ticks, we can hypothesize that vector amplification (Figure 4A) could be taking place. In order to determine if vector amplification, or any other mechanism, is taking place, the pathways of that mechanism must be investigated directly. Few empirical studies have tested for a relationship between biodiversity and *B. burgdorferi* prevalence (Ostfeld and Keesing, 2000; Bouchard at al., 2013; Werden at al., 2014), whilst no studies have subsequently directly investigated the mechanisms driving the observed relationship in this system.

Our models focused on the role of *P. leucopus* in particular, because this species is considered the most effective reservoir for *B. burgdorferi* in northeastern North America (Brunner at al., 2008), and because it fed more ticks than other host species in our study (Figure 2). *P. leucopus* may thus play a central role in the transmission and spread of *B. burgdorferi* in southern Quebec (Simon et al., 2014).

Mechanisms connecting host diversity to infection density

Numerous mechanisms have been proposed which could lead to an amplification effect of biodiversity on Lyme disease (Keesing at al., 2006) although many authors have suggested that vector amplification could play an important role (Gilbert at al., 2001; Keesing at al., 2006; Ogden and Tsao, 2009). The observed positive relationship between biodiversity and *B. burgdorferi* prevalence does not preclude the possibility that some diluting mechanisms could also be taking place along with amplifying mechanisms, but that the net effect is an amplification effect. The amplification, frequency-dependent dilution, and density-dependent dilution were tested and not rejected (Figure 4). Many of the paths were significant with the coefficients in the

hypothesized direction, while other hypothesized links were non-significant, such as the lack of significant relationship between small mammal host abundance and tick abundance (Figure 4A).

The amplification effect model was the only model for which all the hypothesized relationships were supported by our data. The amplification effect model, which models the vector amplification hypothesis, accounts for the observed positive relationship between small mammal host species richness and abundance of infected ticks. The dilution effect models were not rejected but the hypothesized relationships in these models were not all supported by our data. Our data thus support the hypothesis that a vector amplification effect is taking place at the regional level in southern Quebec.

The expanded amplification effect model disentangled the effect of small mammal abundance on the two components of abundance of infected ticks: tick abundance and tick infection prevalence. There was a significant positive relationship between small mammal host abundance and tick abundance but no signification relationship between small mammal host abundance and tick infection prevalence. This is expected if a vector amplification effect is occurring: as small mammal host abundance increases, the number of hosts available to ticks increases, and so the abundance of ticks increases.

B. burgdorferi ecology in an emerging system

The fact that southern Quebec is currently in early-stage emergence may explain why certain hypothesized pathways were non-significant, especially the pathways between white-footed mouse abundance and the abundance of infected ticks. The observation of low tick and infected tick abundance in 2011 and 2012, with increased abundance in 2013 is consistent with early-stage emergence of Lyme disease. A number of other authors have noted that Lyme disease is at

the beginning of its emergence in southern Quebec (Ogden at al., 2006; Ogden at al., 2009; Leighton at al., 2012; Milford et al., 2013). As an emergent system, it may behave differently relative to an established system, such as in the northeastern United States. Much of the previous work on the diversity-Lyme disease relationship has taken place in the northeast United States, and findings and theory based on that work may not be applicable in other regions. For instance, in areas where Lyme disease is only beginning to emerge, the dispersal of tick-carrying migrating birds into a given forest may play a larger role than the host biodiversity present in that forest in determining the prevalence of *B. burgdorferi* (Ogden at al., 2013). As Lyme disease becomes more established in southern Quebec it may be that the relationships between small mammal communities, ticks and *B. burgdorferi* communities become closer to those relationships hypothesized for regions where Lyme disease is established.

Early in the emergence of Lyme disease, temporal effects may play a larger role in determining pathogen density than host diversity. If *B. burgdorferi* density is increasing rapidly over time, as it is the case in southern Quebec, the year in which a person is exposed to a forest may determine their disease risk to a greater extent than the host diversity in that forest. The pathogen must be present in the first place before its density can be affected by variables such as host diversity. We can thus predict that any host diversity-disease relationship will increase in strength over time as Lyme disease continues to emerge in southern Quebec.

Although not enough sites were absent of white-footed mice to draw any statistical conclusions, *B. burgdorferi* was absent at the four sites where white-footed mice were absent. This suggests that certain aspects of a host community are necessary in order for the pathogen to occur. As the range of white-footed mice moves northward (Roy-Dufresne et al., 2013), this may allow Lyme disease to move further northward (Simon et al., 2014).

The role of latitude and resource diversity

The exploratory model (Figure 5), generated through model exploration, suggests that the mammal community, the *I. scapularis* and *B. burgdorferi* are strongly affected by environmental factors such as the seed assemblage. In addition to small mammal species richness, white-footed mouse abundance is also affected by seed species richness. The greater the number of seed species available to the granivorous white-footed mouse, the greater the population size. Previous research has linked climate to food resource availability to white-footed mouse abundance (Jones at al., 1998; Ostfeld at al., 1996; Ostfeld at al., 2001). The relationship between seed species richness and white-footed mouse abundance suggests a role of biodiversity in the Lyme disease system that goes beyond the diversity of tick hosts, and extends to the resources of the host community.

The exploratory model also suggests that tick abundance and the prevalence of infected ticks are affected by latitude. The plausible mechanisms involving latitude are twofold and interrelated. First, the ranges of ticks and *B. burgdorferi* are moving northwards due to climate change (Leighton et al., 2012; Ogden et al., 2013; Simon et al., 2014). We would thus expect that the abundance of ticks and *B. burgdorferi* are greater at more southern latitudes. Second, latitude is essentially a proxy for climate. At low latitudes the warmer temperatures may generate habitats with conditions more amenable to white-footed mice (Roy-Dufresne et al., 2013) and other small mammals. Latitude probably plays a lesser role than dispersal from the south given the small geographic range of this study. At low latitudes, where *B. burgdorgeri* has had time to disperse, white-footed mice and other small mammal species may flourish due to climate-related variables. Regardless of whether latitude links host community and tick and *B. burgdorferi* communities through northern range shifts or climatic effects, the function of latitude in the

exploratory model suggests a role of climate change in Lyme disease emergence in Southern Quebec, as a number of other authors have also suggested (Ogden et al., 2006; Roy-Dufresne et al., 2013; Simon et al., 2014). Climate warming may exacerbate the increase of Lyme disease in Canada and Quebec.

The exploratory model was generated post-hoc to investigate other explanatory factors. It cannot be considered a rigorous statistical test of the role of latitude in this system. Instead, it suggests the importance of continued study of the role of regional processes (e.g. climate-related variables) and northern range shifts in understanding and predicting Lyme disease risk in Southern Quebec. We were not able to generate any biologically-plausible models which included bird host species richness. This may be because the abundance of the migratory birds is more important than their diversity in determining Lyme disease presence and density, or that the bird dispersal is uniform over the study region such that it does not produce a detectible effect. It is likely that dispersal via birds of infected ticks into the forests does play a role in determining disease risk in this emerging system (Ogden at al., 2013), and so the question may merit further study.

Conclusions

The exploratory and hypothesis-testing models together suggest that the richness of the small mammal communities is an important modulator of ecological Lyme disease risk in Southern Quebec. We observed an amplification effect of small mammal species richness on infected tick density. Path analysis suggests a vector amplification effect is driving the abundance of infected tick through small mammal host species richness. Model exploration additionally suggests an important role of regional processes and resource availability. Lyme disease is a highly dynamic and emerging system in southern Quebec. The monitoring of climate and small mammals,

especially white-footed mice, will continue to play an important role in understanding and predicting the ecological disease risk posed across the landscape. Furthermore, this study demonstrates that an observed relationship between host diversity and disease are not sufficient to determine the mechanisms driving that relationship. Further work may determine the role of competing mechanisms in determining the role of diversity in determining the rate of spread of Lyme disease.

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Appendix

Table 1. The number of trapped *Peromyscus leucopus* and *Peromyscus maniculatus*, the species richness and total number of trapped small mammal hosts, the number of trapping nights, total number of trapped ticks and infected ticks (dragging and removed from trapped hosts), and latitude of all sites in 2011, 2012, and 2013.

Site	Year	*Peromyscus leucopus*	*Peromyscus maniculatus*	Species richness	Total trapped hosts	Trap nights	Total ticks	Total infected ticks	Latitude
	2011	0	7	4	15	2	7	0	
1	2013	0	6	4	12	3	0	0	45.20
2	2013	4	4	4	10	3	521	33	45.30
3	2011	7	0	3	11	3	0	0	45.48
6	2013	13	0	5	36	3	92	5	45.87
7	2011	27	0	4	39	8	67	0	45.38
8	2013	10	8	5	22	3	61	10	45.12
	2011	31	0	6	92	8	60	1	
	2012	1	0	5	8	3	158	1	
9	2013	5	0	3	7	3	129	3	45.49
	2011	35	0	1	35	8	5	0	
10	2012	8	0	5	13	3	1	0	45.52
	2011	19	0	3	24	5	1	0	
11	2012	12	0	2	13	3	22	0	45.48
	2011	34	1	6	51	8	36	0	
14	2012	NA	NA	NA	NA	NA	NA	NA	45.45
	2011	18	0	4	30	8	2	0	
17	2012	9	1	5	13	3	9	0	45.49

	2011	13	13	3	29	7	4	0	
18	2012	NA	NA	NA	NA	NA	NA	NA	45.46
19	2011	0	4	3	21	2	3	0	46.18
20	2013	20	5	5	29	2	123	12	45.06
	2011	35	0	4	50	9	6	0	
	2012	2	0	2	3	3	13	0	
21	2013	8	0	1	8	3	11	1	45.42
25	2013	0	3	6	22	3	4	0	45.99
26	2013	13	4	3	19	3	8	1	45.21
27	2013	20	4	5	31	2	13	0	45.25
32	2013	5	1	5	23	3	283	11	45.18
33	2013	4	0	3	6	2	20	3	45.04
	2011	6	1	3	10	2	1	0	
34	2012	9	0	2	11	3	6	0	45.48
	2012	12	0	3	16	3	204	0	
35	2013	12	0	4	16	3	438	3	45.53
	2011	NA	NA	NA	NA	NA	1	0	
36	2012	7	0	3	9	3	0	0	45.54
37	2013	21	0	4	26	4	2	0	46.30
38	2013	0	2	3	4	3	0	0	46.57
39	2011	3	0	2	4	2	0	0	45.52
40	2011	3	0	2	4	2	0	0	45.51

Table 2. Zero-inflated Poisson generalized mixed models of the total number of ticks infected with *Borrelia burgdorferi* at 27 forest sites in Southern Quebec in 2011, 2012, and 2013. Small mammal species richness, year, dragging distance, and trap nights were included as fixed effects and site was included as a random effect.

Coefficient	Estimate	Standard Error	z value	P value
(Intercept)	-8.13	5.73+06	0	1.0000
Species richness	0.768	0.178	4.31	<0.0001
Year	1.73	2.10e+06	0	1.0000
Dragging distance	2.79e-03	3.19e+03	0	1.0000

Table 3: The C-statistic, p-value of the C-statistics, AIC and AICc of each model and the R^2 of the variables in the model.

Model	Variable	R^2	C-statistic	p-value of C-statistic	AIC	AICc
A) Amplification effect	Small mammal host abundance	0.704	5.253	0.512	30.512	54.512
	Tick abundance	0.038				
	Abundance of infected ticks	0.442				
B) Frequency-dependent dilution effect	White-footed mouse relative abundance	0.099	3.114	0.794	22.794	33.794
	Tick abundance	0.083				
	Tick infection prevalence	0.001				
C) Density-dependent dilution effect	White-footed mouse absolute abundance	0.011	3.157	0.789	28.789	48.789
	Tick abundance	0.024				
	Tick infection prevalence	0.040				
Exploratory model	White-footed mouse relative abundance	0.233	6.395	0.998	72.998	-2591.002
	Tick infection prevalence	0.205				
	Tick abundance	0.263				

Figures

Figure 1. Illustrations of the vector amplification, frequency-dependent dilution, and density-dependent dilution effects. In the low-diversity scenario, only the white-footed mouse (the most effective reservoir for *Borrelia burgdorferi*) is present. In the high-diversity scenario, other host species (illustrated here as short-tailed shrews and eastern chipmunks) are added. The red ticks represent infected ticks, while the black ticks represent uninfected ticks. The vector amplification effect predicts that the additional species will increase the total host abundance, which will increase the tick abundance, which will increase the density of infected ticks. The frequency-dependent dilution effect predicts that the additional species will decrease the relative abundance of white-footed mice, which will increase the tick infection prevalence, which will decrease the density of infected ticks. The density-dependent dilution effect predicts that the additional species will decrease the absolute abundance of white-footed mice due to competition, which will decrease the tick infection prevalence and (if the white-footed mouse is also the most effective tick host) the tick abundance, which will decrease the density of infected ticks.

Figure 2. The location of the 27 forest sites in Southern Quebec. Data was collected from these sites during the summers of 2011, 2012, and 2013. Map was prepared using Google Earth (Google Inc., 2012) with a World Physical Map basemap by the US National Park Service.

Figure 3. The total number of *Ixodes scapularis* ticks found on each host species trapped at each of the 27 sites in 2011, 2012, and 2013 in Southern Quebec.

Figure 4. The parameterized amplification (A), frequency-dependent dilution (B), and density-dependent dilution (C) models. The coefficients and p-values are given for each path are given. Non-significant paths are shown as dotted grey arrows and significant paths are shown as solid black arrows.

Figure 6. A parameterized model generated through model exploration. The coefficients and p-values are given for each path are given.

Figure 1

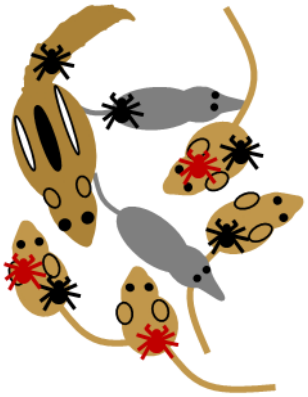
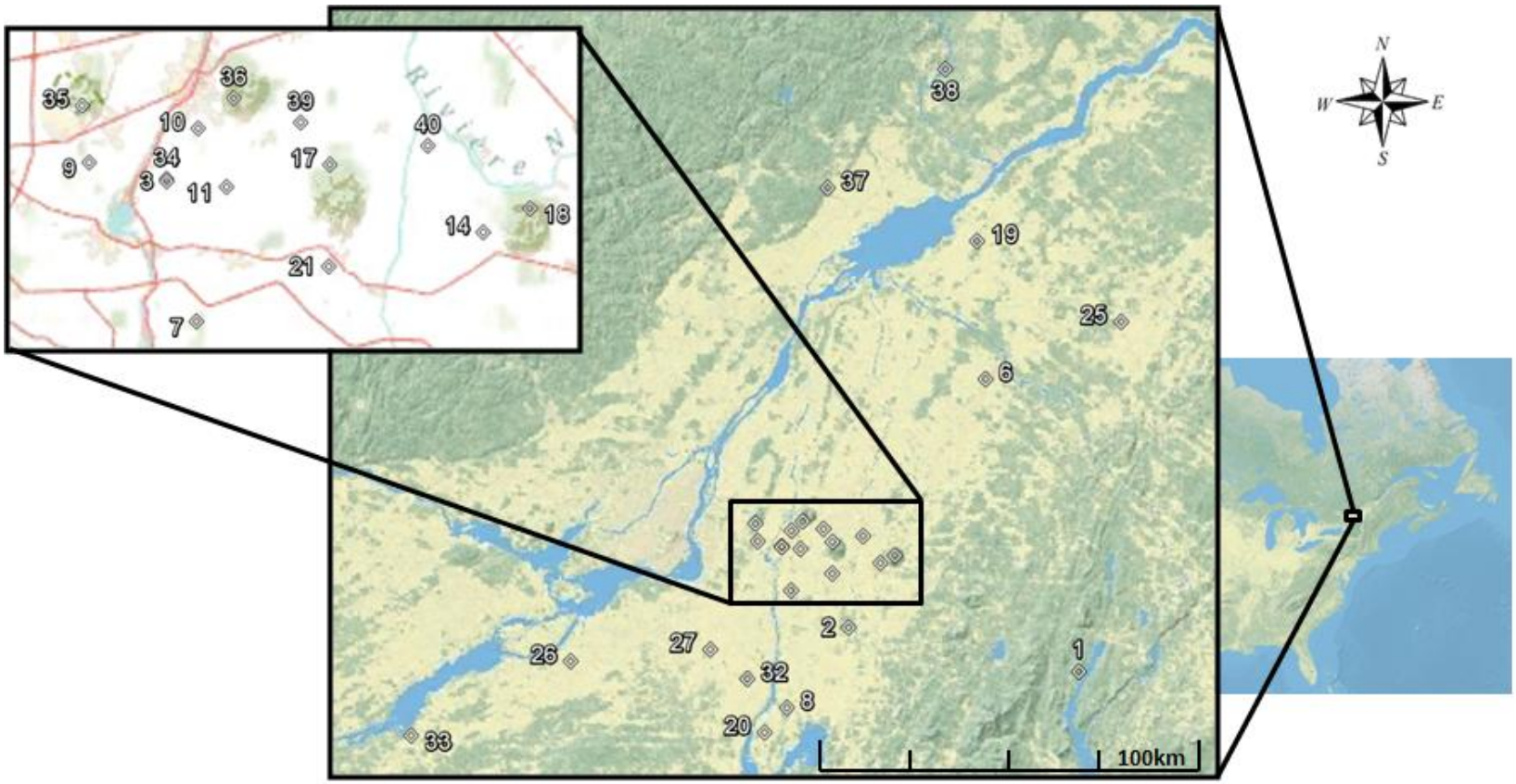
Diversity	Vector amplification effect	Frequency-dependent dilution effect	Density-dependent dilution effect
Low (White-footed mouse only)			
High (White-footed mouse plus other host species)			
Result of increased diversity	Tick abundance increases due to additional hosts, therefore **density of infected ticks increases.**	Infection prevalence decreases due to reduced white-footed mouse relative abundance, therefore **density of infected ticks decreases.**	Infection prevalence and tick abundance decrease due to reduced white-footed mouse absolute abundance, therefore **density of infected ticks decreases.**

Figure 2


N
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6
2
27
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8
20
33
100km

Figure 3

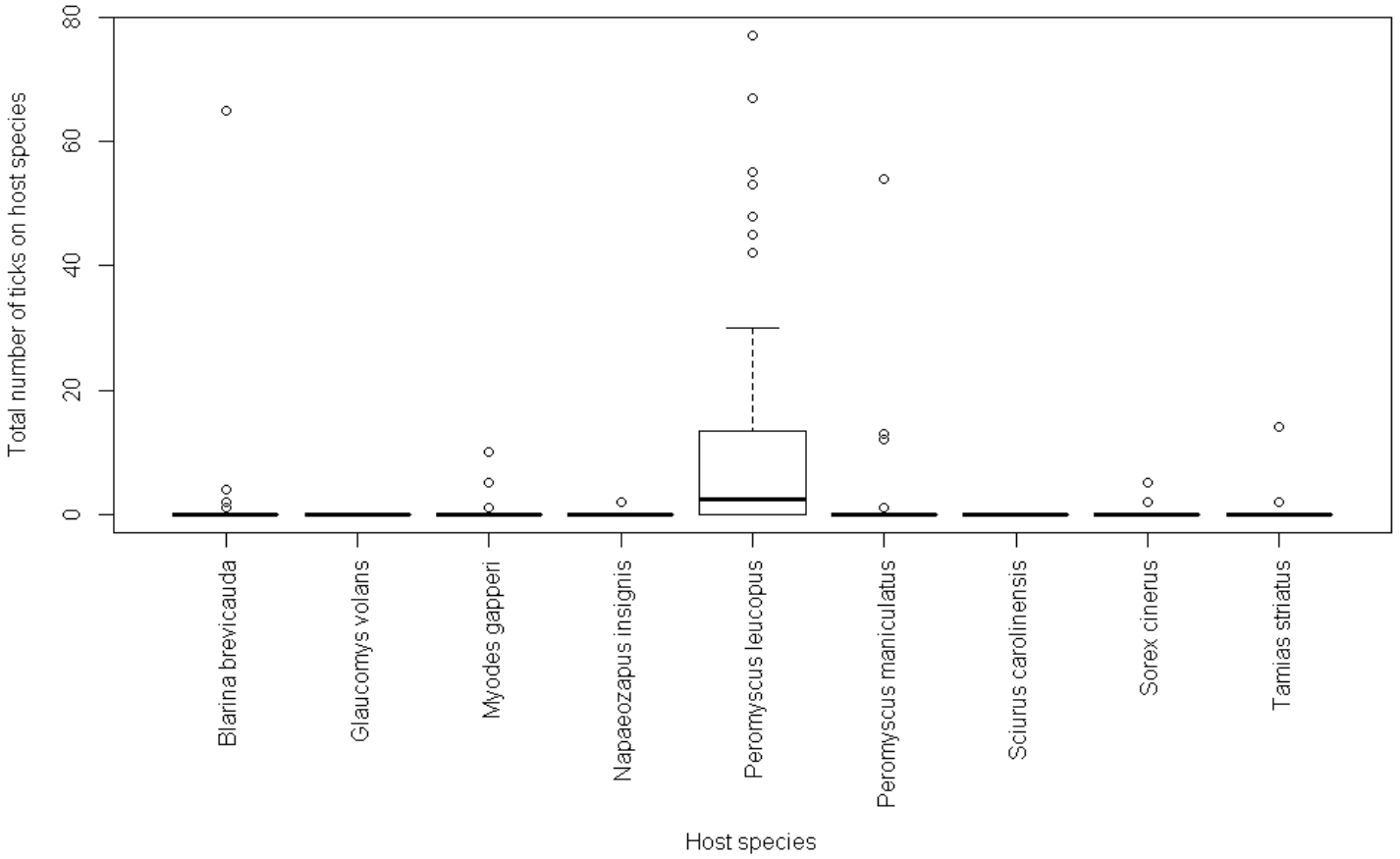

Total number of ticks on host species
0
20
40
60
80
Blarina brevicauda
Glaucomys volans
Myodes gapperi
Napaeozapus insignis
Peromyscus leucopus
Peromyscus maniculatus
Sciurus carolinensis
Sorex cinerus
Tamias striatus
Host species

Figure 4

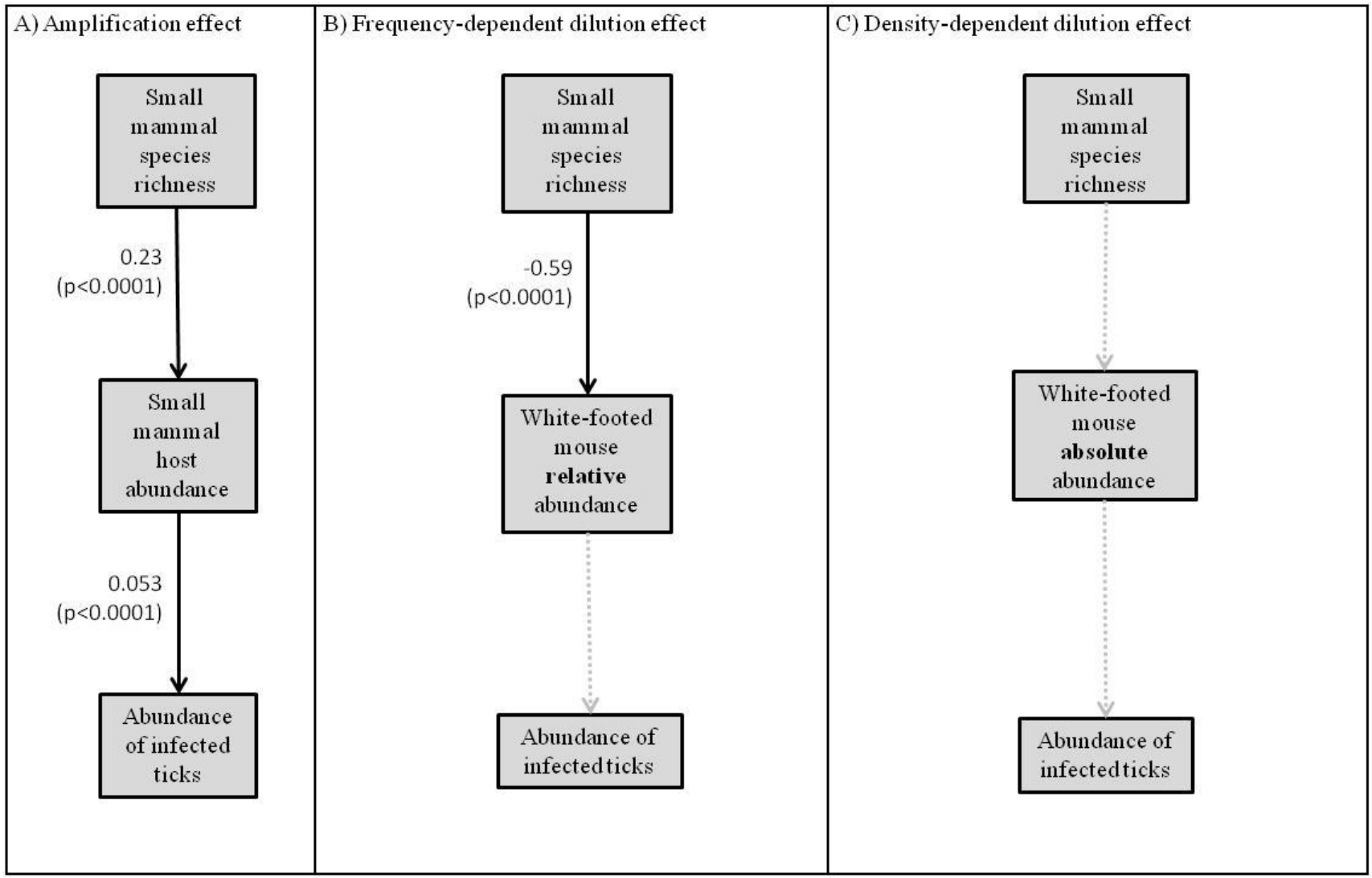

A) Amplification effect
Small mammal species richness
0.23 (p<0.0001)
Small mammal host abundance
0.053 (p<0.0001)
Abundance of infected ticks
B) Frequency-dependent dilution effect
Small mammal species richness
-0.59 (p<0.0001)
White-footed mouse relative abundance
Abundance of infected ticks
C) Density-dependent dilution effect
Small mammal species richness
White-footed mouse absolute abundance
Abundance of infected ticks

Figure 5

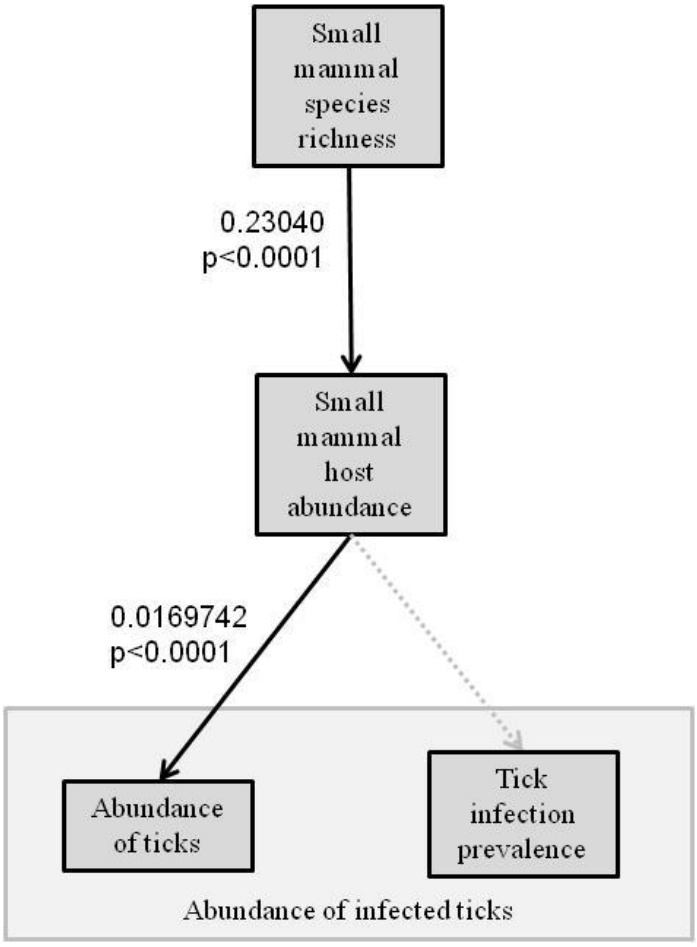
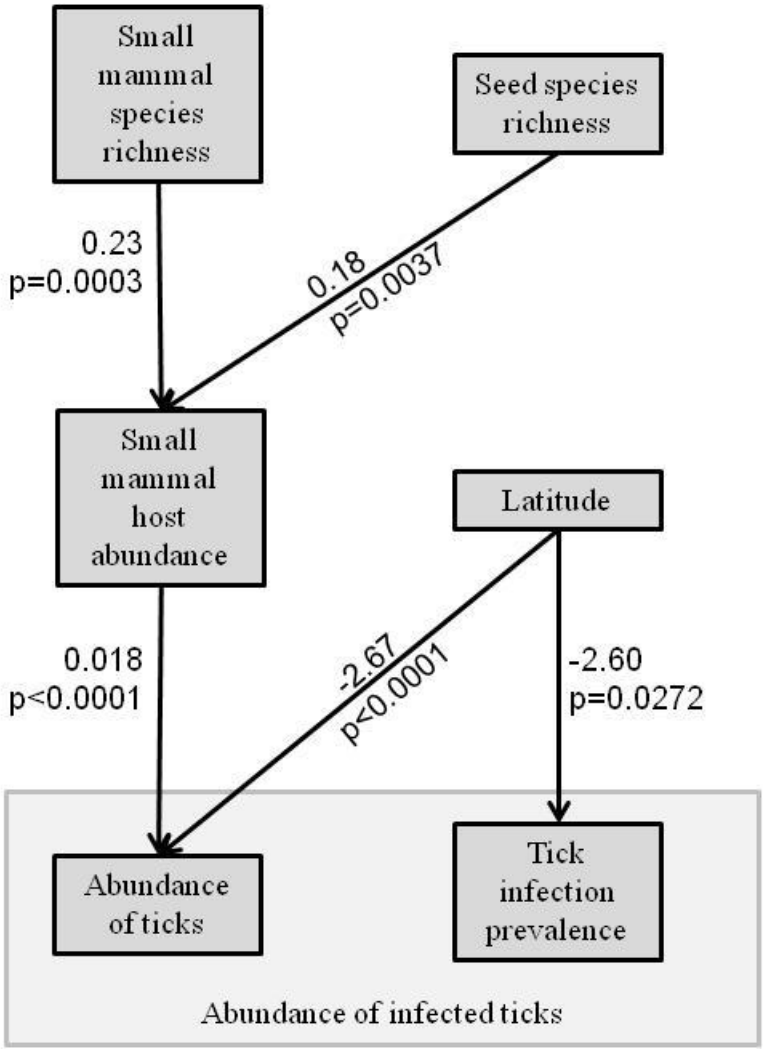

Small mammal species richness
0.23040
p<0.0001
Small mammal host abundance
0.0169742
p<0.0001
Abundance of ticks
Tick infection prevalence
Abundance of infected ticks

Figure 6


Small mammal species richness
Seed species richness
0.23
p=0.0003
0.18
p=0.0037
Small mammal host abundance
Latitude
0.018
p<0.0001
-2.67
p<0.0001
-2.60
p=0.0272
Abundance of ticks
Tick infection prevalence
Abundance of infected ticks

FINAL CONCLUSIONS

Although the relationship between biodiversity and Lyme disease is a contentious question, there is an emerging consensus that the sign and strength of the relationship is highly context-dependent. Other authors have suggested that the diversity-Lyme disease relationship may depend on spatial scale (Wood and Lafferty, 2013), host community dynamics (ex, Ogden and Tsao, 2009), measure of Lyme disease (NIP, DIN, human disease incidence, etc.; Brownstein et al, 2005), or transmission dynamics (Rudolf and Antonovics, 2005). Our investigation of diversity and Lyme disease in the United States and Southern Quebec allows us to add to the list of possible factors affecting the diversity-disease relationship: temporal scale, index of diversity, measure of Lyme disease, degree of disease emergence, and geographical location.

In the United States, where Lyme disease is relatively well-established, we found that at a sub-continental spatial scale over two decades there has been an increasing dilution effect of small mammal species richness on human Lyme disease incidence (Chapter 2). In Southern Quebec, where Lyme disease is in the early stages of emergence, we found that at the regional scale over three years there has been an amplification effect of small mammal diversity (Shannon diversity and species richness) on the density of infected ticks (Chapter 3). The reasons for these contrasting relationship between our two studies is as of yet unclear; future field studies at the landscape scales combined with process-based spatial models may allow better understanding of the diversity-disease relationship under a given set of conditions. What is clear from our work, and previous studies, is that there is no one single relationship between diversity and Lyme disease. Instead, the diversity-Lyme disease relationship is variable over time and space and more research is needed to determine how biodiversity mediates the incidence of this important zoonotic disease at relevant spatial and temporal scales.