Mark and recapture analysis of mammal density and its impact on tick prevalence and the spread of Lyme disease

Sofia Vaca and Scarlett Alvarez, Department of Biology, Hofstra University, Hempstead, NY, 11549
Russell Burke, Department of Biology, Hofstra University, Hempstead, NY 11549
Breann Ross, Department of Biology, Hofstra University, Hempstead, NY 11549
Timothy Green, Environmental Protection Division, BNL, Upton, NY, 11973

Abstract

Lyme disease (LD) is the most common vector borne disease in North America, it is transmitted to humans primarily by the nymphal stage of blacklegged ticks (*Ixodes scapularis).* In most of the U.S., persistence of Lyme disease depends mostly on white-footed mice *(Peromyscus leucopus)* as they are common hosts for larval and nymphal blacklegged ticks and are highly competent reservoirs of the LD agent, *Borrelia burgdorferi* (Levi et al. 2012). We attempted to test 3 hypotheses that follow from Levi et al. 2012: 1) that white-footed mouse densities are positively associated with *Ixodes* tick densities, 2) that white-tailed deer densities are only correlated with *Ixodes* tick population densities when deer densities are low and 3) that red fox densities are negatively associated with small mammal densities. We collected relevant data in the Long Island Pine Barrens ecosystem. Sherman live traps were set up in four sites for a total of 2,048 trap nights. We also collected ticks by flagging at each site. Ticks collected were categorized by species and life stage. Mice were marked so that population sizes could be estimated using mark-recapture analyses. Additionally, camera traps were set up at each site to monitor the abundance of other mammals such as deer and red foxes at those sites. We found that there was a significant positive linear relationship between the density of mice and amount

of nymphal *Ixodes* at each site. Therefore, if coyotes were to emerge in Long Island and prey on red foxes, it is expected that there would be an increase in the density of white-footed mice and in the amount of *Ixodes* ticks.

Introduction

Lyme disease (LD) is the most common tick-borne disease in the United States; the number of LD cases on the eastern coast has been increasing constantly since 1995 (CDC, 2015). LD is caused by the spirochete bacteria *Borrelia burgdorferi* and, in the eastern U.S., is transmitted by blacklegged ticks *Ixodes scapularis* (Maloney, 2015). LD is a serious public health problem because if patients are left untreated, the infection can spread to other parts of the body such as the heart or nervous system and symptoms can continue for several months and can become fatal (CDC, 2015). It is important to understand the dynamics of ecological systems that affect tick populations so that risk of Lyme disease can be properly assessed.

Host competence is a major factor in the spread of Lyme disease. Competent hosts are likely to maintain the bacteria and infect other ticks that subsequently feed on them (Hersh et al., 2012). *Borrelia burgdorferi* is found in a wide variety of mammals such as chipmunks, squirrels, and shrews, as well as, birds being that they are suitable hosts for the completion of the bacterium's enzootic life cycle (Wood and Lafferty, 2013). In the eastern U.S., *Ixodes scapularis* ticks typically acquire *Borrelia* from infected rodents while the larval ticks are feeding (Lane et al., 1991). During June and July, infected nymphs feed on other vertebrates prior to the larval feeding of the next generation in August (Frank et al. 1998). LD is transmitted to humans primarily by the nymphal stage of *I. scapularis* ticks which otherwise feed mostly on small mammals and birds in northeastern United States (Anderson and Magnarelli, 1984). Lyme disease may cause influenza-like symptoms and erythema migrans in infected humans, but many

small mammals have no response to this parasite and can become competent reservoir hosts for adult ticks (Tilly et al. 2008).

Unusually high tick reproduction rates occur during oak mast years because masts bring their hosts together when foraging for food. A strong correlation between white-footed mouse, chipmunk, and white-tailed deer population densities and acorn abundance suggests that as each increases it is easier for ticks to disperse from one host to the next (Ostfeld et al., 1996). Moreover, there is higher incidence of Lyme disease in humans the year after the mast year, where public health officials monitor oak trees in an effort to keep track of this disease because of its effects to humans (Ostfeld, 1997). This shows that density of the ticks is in part due to the interconnected relationships of the hosts' environment.

Recently, coyotes (*Canis latrans*) have spread into eastern and northeastern United States (Kays et al, 2008). Coyotes may reduce the numbers of red foxes through predation. Levi et al. (2012) suggested that without high fox abundance and their regulation of the numbers of small-mammals, especially white-footed mice, more infected *Ixodes* ticks would be expected where there are high small mammal densities. Thus Lyme disease may be increased indirectly due to the spread of coyotes.

This research project involves testing Levi et al. (2012)'s hypotheses. We did this by measuring the abundance of white-footed mice populations, *I. scapularis* ticks, white-tailed deer, and red fox at four plots at BNL. In particular, we examined the relationships between the distributions of *I. scapularis* ticks at different life cycle stages found on white footed mice populations. This project allowed us to examine what effects coyotes could have on the hosts' susceptibility to this disease prior to their arrival and thus provide vital information that would be useful when devising a plan to effectively control the spread of Lyme disease.

We hypothesized that:

H_1: White-footed mice populations are positively associated with *Ixodes* tick population densities.

H_2: White-tailed deer densities are only correlated with *Ixodes* tick population densities when deer densities are low.

H_3: Red fox densities are negatively associated with small mammal densities.

Methods

We conducted small mammal surveys in June and July 2016 at Brookhaven National Laboratory in Upton, NY. Four study plots were selected within BNL's campus. The two plots in the northeast habitat were densely wooded, herbaceous, and with shrubby land cover. The two plots in the southwest had scattered wooded and shrubby vegetation. At each of the four sites we established an 8x8 grid of Sherman traps spaced 5 meters apart, 64 traps per grid. Traps were baited with a peanut butter/oat mixture. Animals were trapped at each site over four consecutive nights during alternate weeks for a total of 8 days (or 512 trap nights/site, 2,048 total trap nights). Traps were checked each morning, captured animals were weighed, sexed, and marked with individual ear tags. Recaptured individuals were noted. All attached ticks were removed and preserved. We collected ticks directly by flagging twice at each corner of the four sites for one minute intervals, ticks collected from flagging were also preserved. The number and life stage of each tick species were later determined in the lab.

Program MARK Version 8.1, robust design model was used for a parameter estimate of white-footed mice abundance at each site. The robust design model considers the time interval between trapping sessions as "open" and the time interval during trapping as "closed" (Cooch

and White 2006). Additionally, Program DENSITY Version 5.0 was used to predict the size of white-footed mice population using the spatially explicit capture-recapture data collected (Efford 2012). Tick prevalence was assessed calculating the proportion of mice with at least one attached tick during the experimental period. Average number of ticks per mouse was also calculated. Additionally, four camera traps were set up at each site to monitor the appearance of other mammals such as red foxes and deer at those sites. To attract mammals to the camera traps a scented disc was placed under the vegetation surrounding each camera.

Results

In the northeastern sites, 153 mice were captured throughout 495 encounters. In the southwestern sites, 51 mice were captured throughout 157 encounters. A total of 663 ticks were collected from mice, with the majority consisting of *Ixodes* ticks (Figure 1). On the other hand, the majority of ticks collected from flagging were *Amblyomma*, and only adult female Ixodes were found (Figure 2).

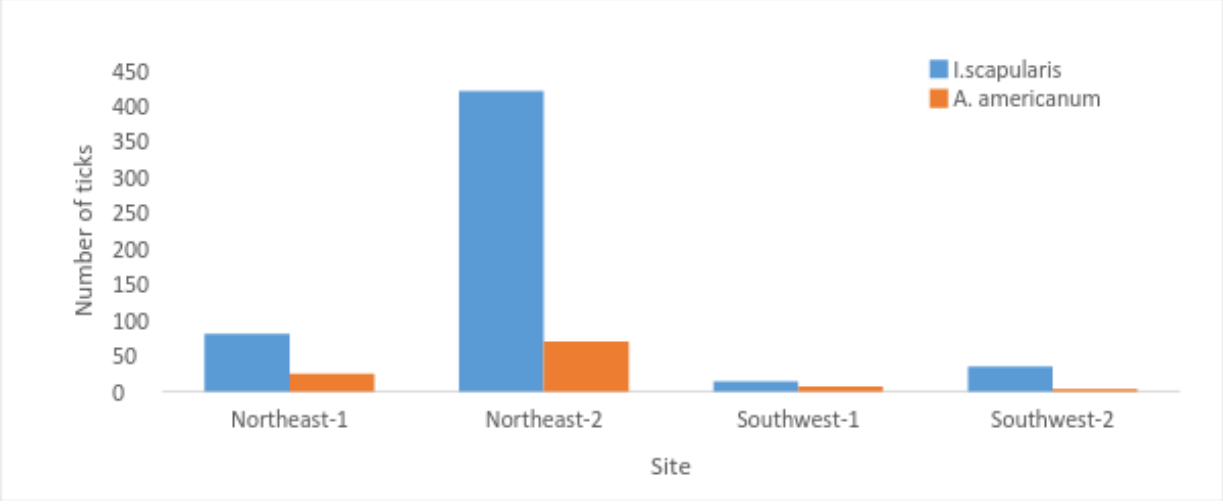

Figure 1 Total number of ticks collected from captured mice by categorized by tick species (n=663).

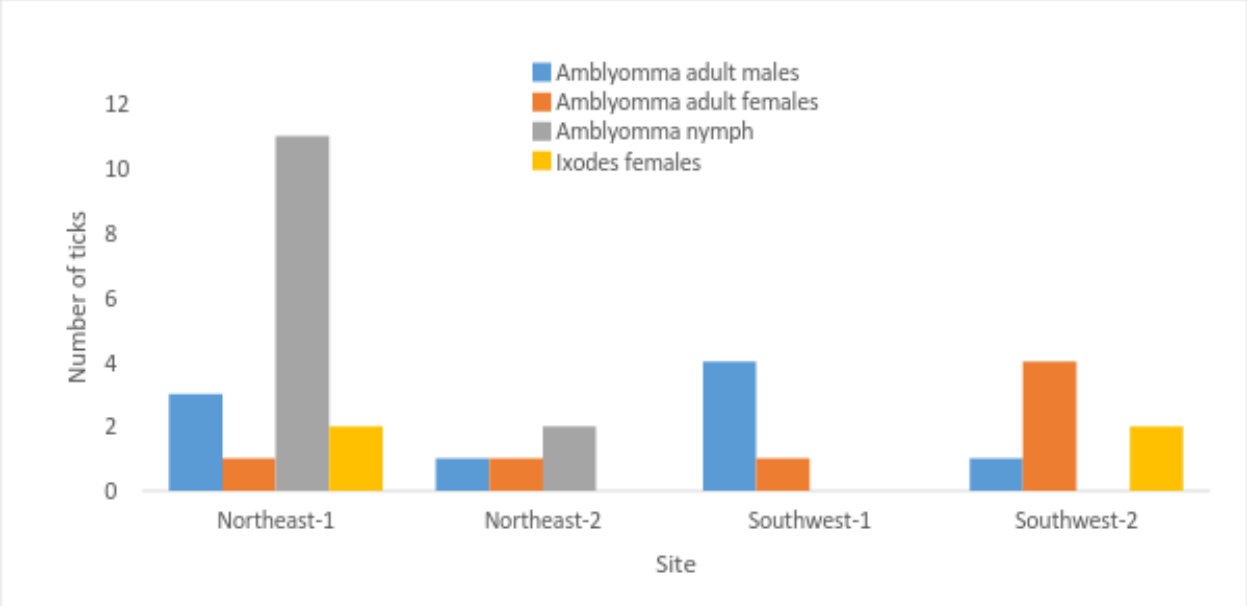

Figure 2 Number of ticks collected by tick flagging categorized by tick species and tick life stage (n=8).

Adult females were the found the most over the other life stages for *Ixodes* ticks in most sites (Figure 3A). However, in Northeast-2, where the most mice were captured there was a dramatic increase in the amount of larva collected. There was also an overall decrease in the amount of *Ixodes* ticks collected in the southwestern sites (Figure 3A). On the other hand, larva *Amblyomma* ticks were found the most than other life stages in all sites expect Southwest-1 where the least number of mice were collected (Figure 3B).

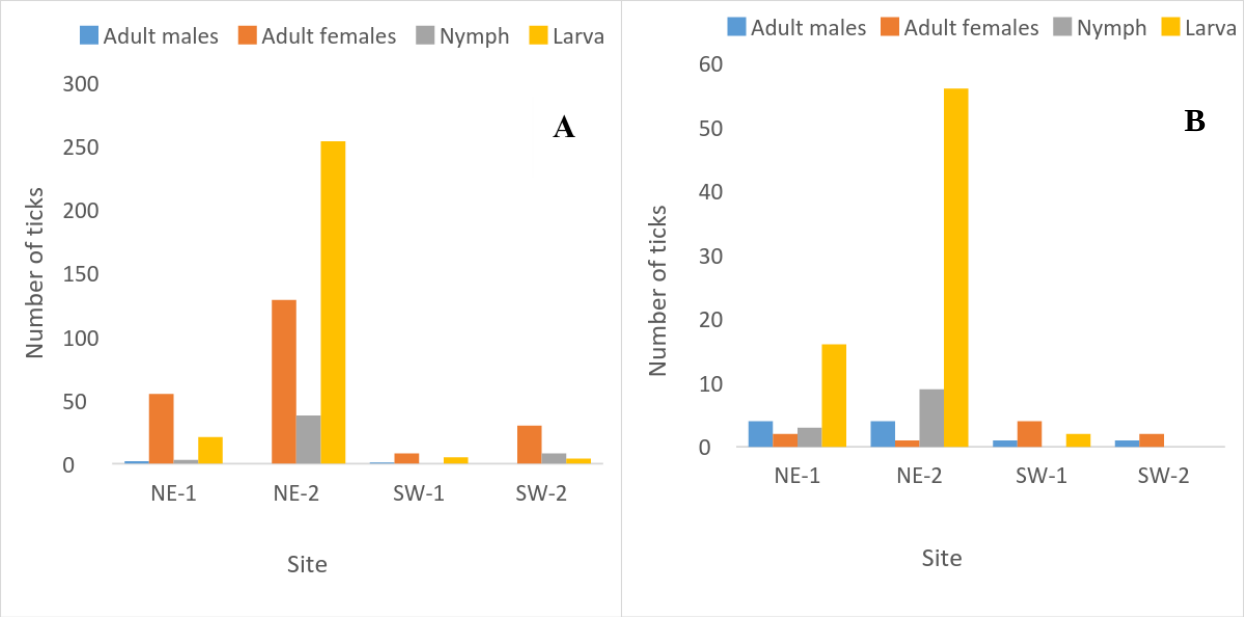

Figure 3 Number of *Ixodes* ticks collected from captured mice categorized by tick life stage (A) (n=558). Number of *Amblyomma* ticks collected from captured mice categorized by tick life stage (B) (n=105).

The best fit line shows a positive exponential relationship with the density of mice at each site with its amount larva collected. (Figure 4A). The basic linear regression showed there was no significant difference found between the density (acquired from program DENSITY) of mice at each site with its amount larva collected (R^2= 0.93, t(3) = 3.21, p = 0.08, n= 663). However, the high R squared value indicates that testing in more sites may have led to a lower p-value.

The best fit line shows a positive linear relationship with the density of mice at each site with its amount nymphs collected. (Figure 4B). The basic linear regression showed there was a significant difference found between the density of mice at each site with its amount nymphs collected (R^2= 0.99, t(3) = 12.19, p = 0.007, n=663).

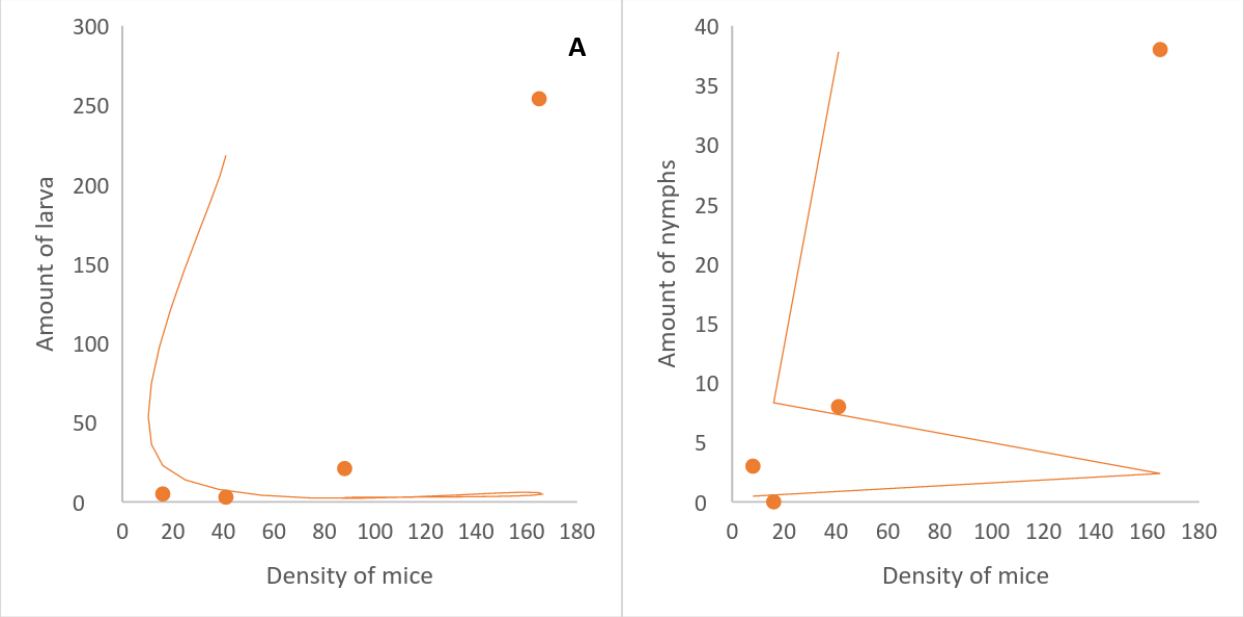

Figure 4 Amount of larva in relation to the density of mice at each site (A) (R^2= 0.93, t(3) = 3.21, p = 0.08, n= 663). Amount of nymphs in relation to the density of mice at each site (B) (R^2= 0.99, t(3) = 12.19, p = 0.007, n=663).

The MARK recapture analysis gave the abundance of mice as 48, 75, 10, and 26 mice for sites NE-1, NE-2, SW-1, and SW-2 respectively. Additionally, tick prevalence in relation to the percentage of mice captured that had at least one tick on them was calculated as 27.45%, 47.88%, 21.74%, and 38.32%, for the respective sites. Lastly, the camera traps that were set did not give much results. Interestingly, only 3 deer were found on various days in Northeast-1, 3 deer in Southwest-2, and 1 deer was found in Southwest-1. There were no other mammals captured on footage, thus we were unable to test the relationship between the density of deer and red foxes in respect to the population of larval and nymphal *Ixodes* ticks.

Discussion

White-footed mice are commonly considered competent hosts for Lyme disease (Tsao et al., 2004). Therefore, understanding the ecological relationships that affect their population is

crucial to assessing Lyme disease risks in any area. Interestingly, the primary species of ticks found on mice (*Ixodes*) did not compliment the primary species found while tick flagging (*Amblyomma)*. Instead, *Ixodes* ticks were found in much more abundance than *Amblyomma* ticks from captured mice, and vise-versa for tick flagging (Figure 1; Figure 2). This indicates that the primary tick population may not necessarily reflect the ticks that are using mammals as hosts in the respective environment.

Additionally, there is a same general trend of obtained life stages for both *Ixodes* and *Amblyomma* ticks, with the exception of the large amount of adult female *Ixodes* (Figure 3). Therefore, the difference in found tick populations was not due to skewed results. This further shows that taking direct data from prospective hosts and their surroundings is important in obtaining information on accurate ecological relationships.

Lyme disease is already a serious health issue in northeastern United States (Maloney, 2015). The spread of coyote populations is also increasing to this part of the country (Kays et al, 2008). Coyotes prey on smaller mammals such as red foxes, which regulates the population of small mammals in the area (Levi et al. 2012). If fox abundance were to be lowered, then small mammal populations, which include white-footed mice, would be expected to increase. As the data suggests, at least 21% or more white-footed mice in an area can be found with ticks on them. Therefore, increasing the density of white-footed mice can lead to a large increase of ticks in an area. Additionally, with the white-footed mice increases larva *Ixodes* ticks will have more hosts available to them that will most likely transmit the bacteria to them, which will lead to future hosts being infected when nymphs feed in the upcoming August (Lane et al., 1991; Frank et al. 1998). Thus, Lyme disease rates may be increased indirectly due to the spread of coyotes in the northeast.

We hypothesized three statements, (1) white-footed mice populations are positively associated with *Ixodes* tick population densities, (2) white-tailed deer densities are only correlated with *Ixodes* tick population densities when deer densities are low, and (3) red fox densities are negatively associated with small mammal densities. The results allowed us to only test the first hypothesis in which white-footed mice populations are positively associated with *Ixodes* tick population densities. The results supported this hypothesis in which there was a significant increase in the amount of nymphal *Ixodes* ticks (Figure 4B; $R2= 0.99$, $t(3) = 12.19$, $p = 0.007$, $n=663$). Since nymphs are the primary transmission of Lyme disease to humans, an increase in white footed mice density may lead to an increase in the transmission of LD to humans (Anderson and Magnarelli, 1984). As mentioned a significant positive relationship may have been found between white-footed mice and *Ixodes* densities if more sites were tested since the p-value is almost on the boarder of being significant (Figure 4A; $R2= 0.93$, $t(3) = 3.21$, $p = 0.08$, $n= 663$). More data is necessary to understand relationship with mice density in respect to *Ixodes* larva (Figure 4A).

Other experiments may replicate this study using more sites in order to obtain a better understanding of the relationship between the ecological relationships that affect Lyme disease. Additionally, being able to obtain substantial data on the deer and red fox population would be important to further knowledge of this relationship. Some potential modifications to this experiments replications may include sites located in other areas besides BNL and testing the ticks for the presence of *B.burgdorferi*. However, if *Canis latrans* is to spread into eastern Long Island, including BNL, then this experiment may be repeated to attain the post effects of this ecological change.

Acknowledgments

We would like to give a special thanks to Jennifer Higbie for all of her support and guidance on this project. We would also like to thank the rest of the environmental department interns for their help and insight. This project was supported in part by the U.S. Department of Energy, Office of Science, Office of Workforce Development for Teachers and Scientists (WDTS) under the Visiting Faculty Program (VFP).

Citations

Anderson, J. F., & Magnarelli, L. A. (1984). Avian and mammalian hosts for spirochete-infected ticks and insects in a Lyme disease focus in Connecticut.*The Yale journal of biology and medicine*, *57*(4), 627.

Cooch, E., & White, G. (2006). Program MARK: a gentle introduction. *Available in. pdf format for free download at http://www. phidot. org/software/mark/docs/book.*

Efford, M. G. (2012). DENSITY 5.0: software for spatially explicit capture–recapture. *Department of Mathematics and Statistics, University of Otago, Dunedin, New Zealand http://www. otago. ac. nz/density*.

Frank, D. H., Fish, D., & Moy, F. H. (1998). Landscape features associated with Lyme disease risk in a suburban residential environment. *Landscape Ecology*, *13*(1), 27-36.

Hersh, Michelle H., Michael Tibbetts, Mia Strauss, Richard S. Ostfeld, & Felicia Keesing (2012) "Reservoir Competence of Wildlife Host Species for Babesia Microti."*Emerging Infectious Diseases* 18(12), 1951-1957.

Kays, R. W., Gompper, M. E., & Ray, J. C. (2008). LANDSCAPE ECOLOGY OF EASTERN COYOTES BASED ON LARGE-SCALE ESTIMATES OF ABUNDANCE. *Ecological Applications*, *18*(4), 1014-1027.

Lane, R. S., Piesman, J., & Burgdorfer, W. (1991). Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe.*Annual review of entomology*, *36*(1), 587-609.

Levi, T., Kilpatrick, A. M., Mangel, M., & Wilmers, C. C. (2012). Deer, predators, and the emergence of Lyme disease. *Proceedings of the National Academy of Sciences*, *109*(27), 10942-10947.

Maloney, E. (2015). What Every Primary Care Clinician Should Know About the Diagnosis of Lyme Disease. *The Taproot*, *24*(2).

Ostfeld, R. S., Jones, C. G., & Wolff, J. O. (1996). Of mice and mast.*BioScience*, *46*(5), 323-330.

Ostfeld, R. (1997). The Ecology of Lyme-Disease Risk Complex interactions between seemingly unconnected phenomena determine risk of exposure to this expanding disease. *American Scientist*, *85*(4), 338-346.

Tsao, J. I., J. T. Wootton, J. Bunikis, M. G. Luna, D. Fish, & A. G. Barbour (2004). "An Ecological Approach to Preventing Human Infection: Vaccinating Wild Mouse Reservoirs Intervenes in the Lyme Disease Cycle." *Proceedings of the National Academy of Sciences* 101(52), 18159-18164.

Tilly, K., Rosa, P. A., & Stewart, P. E. (2008). Biology of infection with Borrelia burgdorferi. *Infectious disease clinics of North America*, *22*(2), 217-234.

Way, J. G., & White, B. N. (2013). Coyotes, red foxes, and the prevalence of Lyme disease. *Northeastern Naturalist*, *20*(4), 655-665.

Wood, C. L., & Lafferty, K. D. (2013). Biodiversity and disease: a synthesis of ecological perspectives on Lyme disease transmission. *Trends in ecology & evolution*, *28*(4), 239-247.