Tick prevalence on the white-footed mouse, Peromyscus leucopus, in Bousson Environmental Research Reserve

By
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April, 2018



Allegheny College
Department of Biology

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements
for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

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Pledge

Approval by the Senior Thesis Committee:

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Dr. Matthew D. Venesky

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Dr. Becky Dawson

Abstract

Arthropod-borne diseases are transmitted via pathogens and can infect a wide range of hosts. Lyme disease (LD) is the most prevalent arthropod-borne disease in North America and Europe. The primary vector for LD, the blacklegged tick (*Ixodes scapularis*), and the primary reservoir for LD, the white-footed mouse (*Peromyscus leucopus*), were studied during this experiment. Factors such as temperature and acorn presence were tested to see if they had an effect on tick prevalence and mouse abundance. I hypothesized that as ambient temperature decreases, mouse abundance will increase. Tick prevalence would also increase as ambient temperature decreases due to mice being more active at cooler temperatures. I also hypothesized that transect 1 and transect 2, which had acorns present, would have higher catch rates than transect 3 and transect 4, which did not have acorns present. This research was conducted during the months of September and October at Bousson Environmental Research Reserve in Meadville, Pennsylvania. Sixteen traps between 4 different transects were set up at dusk and then checked the following morning. Mice that were caught were examined visually for ticks and then released at the site of capture. I found that as minimum ambient temperature decreased mouse abundance increased. As minimum ambient temperature decreased tick prevalence also increased. Transect 1 and transect 2 had the highest catch rate, which was expected due to the presence of acorns. These results were expected and are consistent with the literature. Future studies could be conducted by extending the trapping range and increasing the variability in habitats between transects. It would also be useful to remove ticks from captured mice and run quantitative polymerase chain reaction (qPCR) to test the prevalence of LD in Bousson.

Introduction

Arthropod-borne diseases are unique because they require transmission from an arthropod vector between primary and secondary hosts (Zouache and Failloux, 2015). There are many methods of transmission, but the most common form is taking a blood meal (Gubler, 1998). Many infectious diseases in the world are vectored by arthropods; one of the most common being dengue, which is transmitted primarily by the mosquito *Aedes aegypti* (Powell, 2018). There are approximately 100 million new cases of dengue every year, and up to 3.9 billion people are at risk of exposure to this pathogen.

An organism's risk for exposure to arthropod-borne diseases relies heavily on the population density of infected vectors (Gross, 2006). Climate change is one of the major factors on the population density of arthropod vectors (Gubler et al., 2001) Some of these climate factors include temperature, precipitation, and humidity. Another factor is outbreaks of a pathogen when they are introduced to a new environment (Magori and Drake, 2013). The final factor is pathogens that occur sporadically. These pathogens require an amplification of very specific habitats that lead to an outbreak of a pathogen (Magori and Drake, 2013). It is important to understand how these factors influence arthropod abundance, because the higher the abundance of vectors, the more likely that transmission to an uninfected host will occur.

Lyme Disease (LD) is currently the most prevalent arthropod-vectored pathogen in North America and Europe (Applegren and Kraus, 2017). The discussion of LD and its impact on human health has been a hot topic since its discovery in 1975. LD prevalence has been consistently increasing since 1991, and in the United States, data suggests that it is up to 10 times higher than levels estimated by the Centers for Disease Control with over 300,000 newly

infected individuals each year (Adetunji et al., 2016). In the United States, LD has been endemic to the northeastern and midwestern states, and is present in all 50 states (CDC, 2017).

The primary vector for LD in the northeastern endemic region of the United States is the black-legged tick, *Ixodes scapularis*, but is vectored across the globe by various species of *Ixodes* ticks (Herrmann et al., 2013). A closely related tick, *Ixodes ricinus*, is the primary vector for LD in Europe and studies have shown that there are many drivers that influence the complex ecology of these organisms (Ehrmann et al., 2017). These drivers that can affect the population density of ticks include climate, vegetation, soil, landscape, and habitat (Ehrmann et al., 2017).

LD is the result of the spirochete bacterium *Borrelia burgdorferi* that is transmitted by *Ixodes scapularis.* During the two-year life-cycle of this tick, reproduction occurs on a host, and the females oviposit off the host after approximately 14 days (Kocan et al., 2015). Approximately thirty-five days later, the eggs hatch into larvae. For ticks to progress in growth, a blood meal must occur during each stage of development (larvae, nymph, and adult) (Granter et al., 2014). Once the larva has found a host, it feeds for approximately 4 days, then drops off the host and molts into a nymph. It takes approximately 28 days for a nymph to become fully mature, which then requires it to have another blood meal. Nymphs feed for 4-6 days, then fall off the host and molt into their final adult stage. Adults find larger hosts for providing blood meal, and for finding mates. *I. scapularis* is polygamous and males copulate multiple times per day with the same or different female (Kocan et al., 2015). Reproduction occurs on the host, and 90% of female reproduction is facilitated by white-tail deer (Adetunji et al., 2016). White-tail deer overpopulation is a serious problem in endemic regions for LD. A common misconception is that white-tail deer also serve as a reservoir for the *B. burgdorferi* pathogen. However, white-tail deer are dead end hosts.

The primary reservoir for LD is the white-footed mouse (Tsao et al., 2004). Many other small animals can serve as a reservoir for LD including pets such as dogs (Mather et al., 1994). There are up to 125 other small vertebrates that can serve as hosts for ticks, and it is important to recognize the importance of biodiversity and its relationship with LD (Granter et al., 2014). In some regions of the United States, it is found that the tick burden is higher in other small mammals such as squirrels, chipmunks, opossums, raccoons, skunks, and shrews compared to the white-footed mouse (LoGiuduce et al., 2003). The white-footed mouse has difficulty competing in areas with a high biodiversity, and the abundance of mice will be higher in areas with a low biodiversity (LoGiuduce et al., 2003).

The predictors of LD prevalence change between different locations. LD incidence was tested on factors of weather, mouse density, and acorn production (Schauber et al., 2005). Although there was no correlation for LD incidence resulting from temperature, there was a negative correlation for summer precipitation. An increase in precipitation resulted in a lower prevalence of LD. This could be due to an increase in predators and parasites that reduce the population of ticks, or because humans are known to be less active outdoors in wetter conditions. However, it is also found that that tick density of nymphs increased after higher spring precipitation 2 years earlier (Subak, 2003). Drought directly affects nymph tick survival as well. This is consistent with the knowledge that ticks cannot regulate evaporation, or take up water while they are feeding (Lees, 1946). Adults are likely more resistant to desiccation, which is why there is only a decline in nymph density due to drought. Mouse and acorn abundance can be a good predictor in certain locations, and in another study, it was found that as acorn density increased, mouse and chipmunk density increased (Ostfeld et al., 2006). With an increase in mouse and chipmunk density, there will be an increase in LD prevalence. Temperature does not

directly affect LD prevalence, but temperature could affect host density. If temperature is outside of an organism's optimal range, it will search for habitats that are inside its temperature range. Therefore, if there is a decrease in host density, there would be a decrease in LD prevalence. It is suggested that even a temporary effect of winter temperatures on mouse abundance could be important for the success of larval ticks in the following spring (Schauber et al., 2005). Although there still is no best predictor for LD, finding one would prove pivotal for future research and global health efforts.

In order for LD to be transmitted to a human, an infected tick must be attached for anywhere between 36-48 hours (CDC, 2017). If ticks are removed early enough, the pathogen cannot be transmitted. The most common early symptom of LD is erythema migrans, which is a large, bullseye shaped rash that appears at the site of the tick bite (Applegren and Kraus, 2017; CDC, 2017). Typically, this rash is neither itchy nor painful and can easily go unnoticed. Severe symptoms that can occur later include headaches, arthropathy, facial palsy, encephalitis, and carditis (Applegren and Kraus, 2017). LD is easy to treat and usually involves a 30-day antibiotic treatment duration (Johnson and Stricker, 2004). Doxycycline and Amoxicillin are the two most common forms of antibiotics that are used. There currently is no cure for LD and symptoms can reemerge over time even after full treatment (Johnson and Stricker, 2004).

During this research project, I studied the general ecology of the white-footed mouse, and the prevalence of hosts parasitized by ticks in the Bousson Environmental Research Reserve in Meadville, Pennsylvania owned by Allegheny College. The white-footed mouse is the primary reservoir for LD, which means that there should be a higher prevalence of LD in areas with a high abundance of mice (Tsao et al., 2004). The prevalence of hosts infected with ticks was studied by observing the abundance of ticks on all mice that were captured. Mice were trapped

using both Sherman box traps (1 door) and Hav-A-Hart traps (2 doors). Traps were placed in 4 separate transects depending on whether acorns were present or absent. Other variables that were observed include how well each trap performs, temperature, and precipitation. I hypothesized that as ambient temperature decreases, mouse abundance will increase. Tick prevalence would also increase as ambient temperature decreases due to mice being more active at cooler temperatures. As ambient temperature decreases, mouse abundance will increase. I also hypothesized that transect 1 and transect 2 would have higher catch rates than transect 3 and transect 4 due to the presence of acorns. Finally, the Hav-A-Hart traps should prove to be more successful than the Sherman box traps since they have more than one entrance. The implications of these variables could prove useful in understanding the risk of LD in Bousson. It will also prove useful for future researchers who are interested in studying mice or LD in this area.

Materials and Methods

Study sites

Field studies were conducted at the Bousson Environmental Research Reserve in Meadville, Pennsylvania (Figure 1). Bousson is a forested area with a mix of coniferous and deciduous trees. Bousson has varying levels of vegetation; however, for this study, all transects were located in areas with little to no vegetation. The ground floor primarily consisted of pine needles, and the primary source of water was a small stream that stretched through the study site.

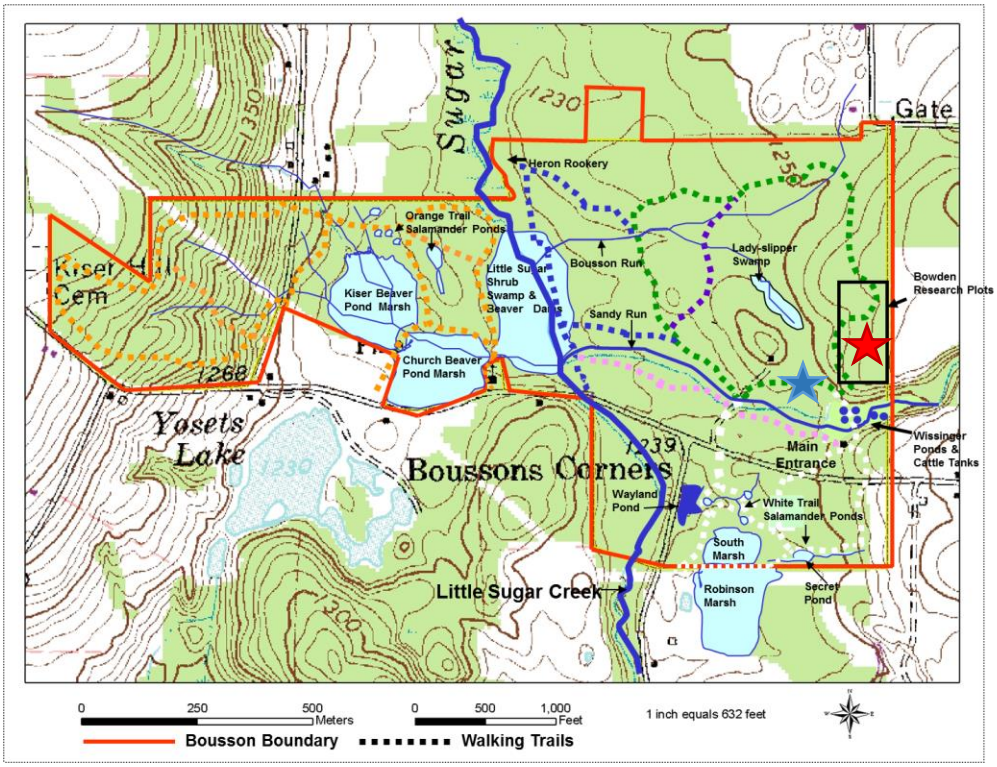

Figure 1. This is a map of the Bousson Environmental Research Reserve located in Meadville, Pennsylvania. All transects were located approximately 250 meters from the main entrance on the right side of the map. The red star indicates the location of transect 1 and transect 2. The blue star indicates the location of transect 3 and transect 4.

A recent study found that an increase in acorn density increased the abundance of mice (Ostfeld et al., 2006). Based on this finding, study sites were separated into 4 transects, with the primary difference being the presence or absence of acorns. Transect 1 and transect 2 were

located the closest to the main entrance of Bousson, and were approximately 20 meters apart from one another. These two transects both had acorns present. However, transect 1 was located deeper into the forest, whereas transect 2 was closer to a walking trail. Transect 3 was located approximately 50 meters from transect 2, and was very close to the walking trail. Transect 3 did not have acorns present. Transect 4 was located approximately 50 meters from transect 3, and 100 meters from transect 2. Transect 4 was located deeper into the forest than transect 3. Transect 4 did not have acorns present.

PPE Equipment

When working with the white-footed mouse, there is the risk of contracting hantavirus, which is transmitted through the feces, urine, and other airborne particles (Peters and Khan, 2002). Due to this, proper personal protective equipment was needed to conduct this study. At all times throughout this study, nitrile gloves, boots, and a p100 respirator were used to prevent the transmission of hantavirus. Protective equipment was worn before handling mice, and gloves were carefully discarded after manipulation. All equipment was stored on site at Bousson to prevent contamination to other people.

Trapping

All trapping, handling, and manipulation of animals was done so according to the Guidelines of the American Society of Mammalogists (Sikes et al., 2011). Traps were setup at approximately 19:00, and checked the next morning at approximately 9:00. Each transect contained 4 traps: 2 Sherman box traps (1 door) and 2 Hav-A-Hart traps (2 doors). Traps were spaced apart from one another with approximately 5-10 meters between each trap. Traps were placed in locations that would have the highest chance of catching mice: near logs, trees, and burrows. Traps would occasionally be moved within a transect if there was low success in

capturing mice. All traps were baited with the same mixture of oats and peanut butter throughout the duration of the experiment.

Triggered traps were opened at the site of capture, and mice were placed into a large, empty garbage can. Mice were then grabbed by the tail and scruffed at the neck with the thumb and index finger for manipulation, and to prevent escape. Each mouse was then examined for ticks on the head, ears, neck, and abdomen. In order to make ticks visible, combing through the fur with a finger helped to identify ticks. Ticks were not removed from the mouse during this study. After manipulation, each mouse was released at the exact location where it was captured.

After manipulations, traps were placed into a garbage bag and taken back to the Allegheny College campus where they were cleaned and disinfected with bleach. Traps were then air dried until the next trapping period.

Data collection

Data was collected on each individual white-footed mouse that was trapped during the research period between 8 September 2017 to the 21 October 2017. This data consists of what transect mice were trapped in, what trap they were caught with, how many ticks were found on each individual, the minimum-maximum ambient temperature, and how long it was since there has been precipitation.

Temperature was recorded for each night of trapping using a minimum-maximum thermometer. The thermometer was placed in between the 4 transects to get as accurate of a representation as possible with only using one thermometer. The maximum distance between the transects was approximately 200 meters; therefore, there should be no fluctuation in temperature between the traps.

During trapping, it was made sure that traps were not going to be active during any precipitation. The frequency of rainfall was determined by observing weather patterns and using online sources (USCD, 2018). Drought was found to negatively impact nymphal tick abundance due to desiccation (Ostfeld et al., 2006). Therefore, it is important to see if this also affects the tick burden on hosts.

Statistical analyses

The goal of this study was to evaluate the prevalence of ticks at Bousson with a secondary focus on mouse abundance. I used a linear regression analysis to test whether minimum ambient temperature had an effect on tick prevalence. Another linear regression analysis was conducted to test whether minimum ambient temperature had an effect on mouse abundance.

Results

Throughout this experiment I went on 11 trips to Bousson Environmental Research Reserve in the months of September and October of 2017. The number of mice that were caught per trip ranged between 0 and 5 with an average of 2.64 mice caught. The number of ticks found per mouse ranged from 0 to 1 with an average of 0.32 ticks found per mouse. The minimum ambient temperature ranged from 7.2^{o} C and 17.8^{o} C while the maximum ambient temperature ranged from 17.2^{o} C to 32.8^{o} C (Table 1; Figure 2).

Acorn presence had a strong positive effect on the total number of mice that were caught, but did not have an effect on the amount of ticks that were found on each individual mouse (Table 1; Figure 3). This is consistent with the presence of acorns since transects 1 and 2 had acorns present, whereas transects 3 and 4 had no acorns. There was a noticeable difference in the number of mice caught between the 4 transects, with transect 1 having the highest abundance

(Figure 4). As the minimum ambient temperature decreased the total number of mice increased (Figure 5). A linear regression analysis was conducted on this relationship showing that there is a significant difference ($P = 0.019$). As the minimum ambient temperature decreased the amount of ticks found per mouse increased (Figure 6). A linear regression analysis was also conducted on this relationship showing that there was a significant difference ($P = 0.016$). Sherman box traps were found to be far more successful than Hav-A-Hart traps due to the total number of mice caught between the two traps (Figure 7).

Trip	Min Temp (ºC)	Max Temp (ºC)	Ticks	Precipitation (days ago)	Mice caught with acorns present	Mice caught with acorns absent
1	7.2	17.2	1	1	0	1
2	8.3	21.1	4	3	3	1
3	12.2	27.8	1	2	2	0
4	16.7	28.9	0	4	1	0
5	17.8	32.8	0	8	0	0
6	11.1	31.1	0	13	1	1
7	12.8	27.8	0	3	2	0
8	11.7	23.9	0	1	3	0
9	10.0	24.4	1	2	3	1
10	8.9	20.0	2	3	4	1
11	7.8	21.1	2	5	4	1

Table 1. Data from mice caught at the Bousson Environmental Research Reserve during the months of September and October 2017.

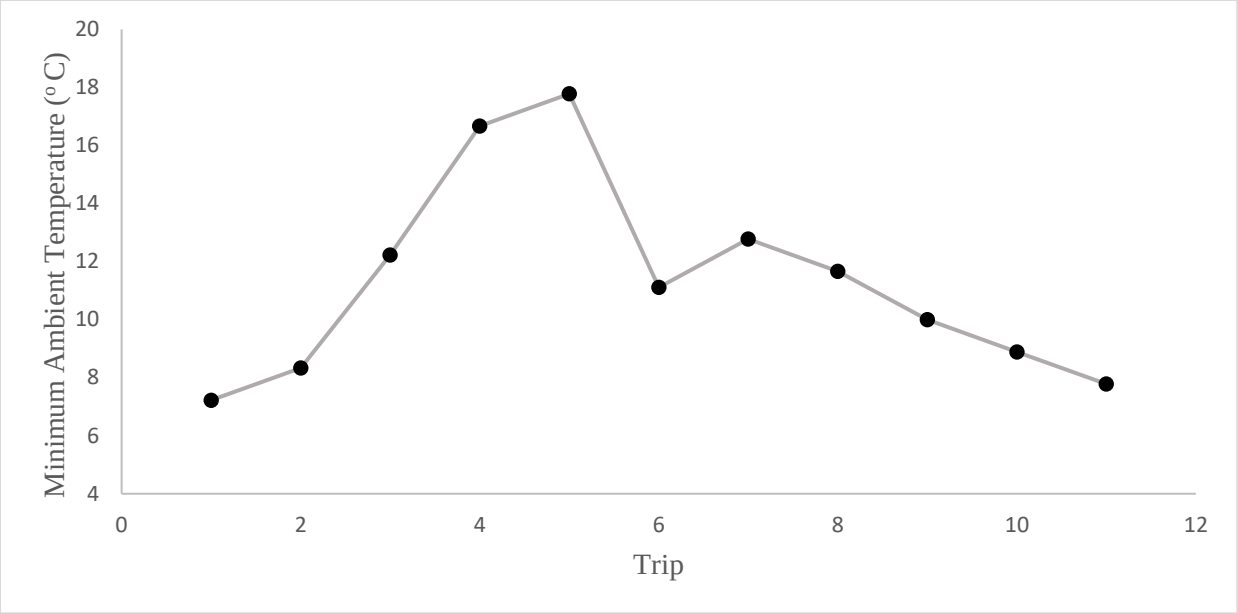

Figure 2. Graphical representation of the fluctuation in minimum ambient temperature (°C) for each trip at Bousson Environmental Research Reserve.

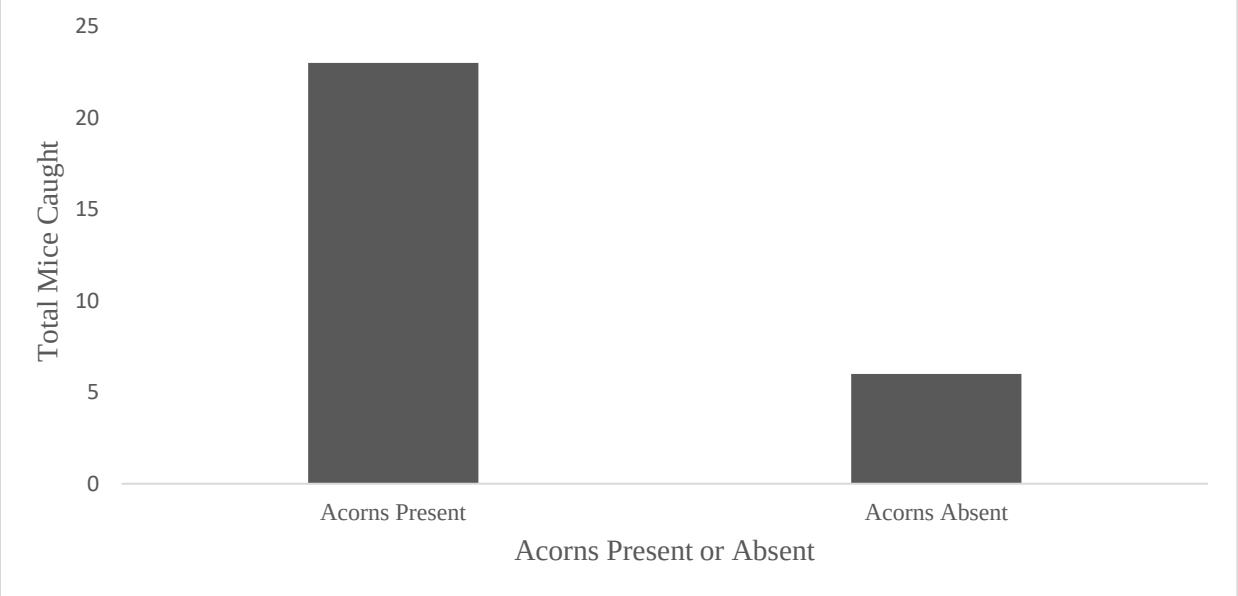

Figure 3. The effect that acorn presence has on mouse abundance was tested throughout this experiment. Four transects were used to trap mice, with two having acorns present and two having no acorns.

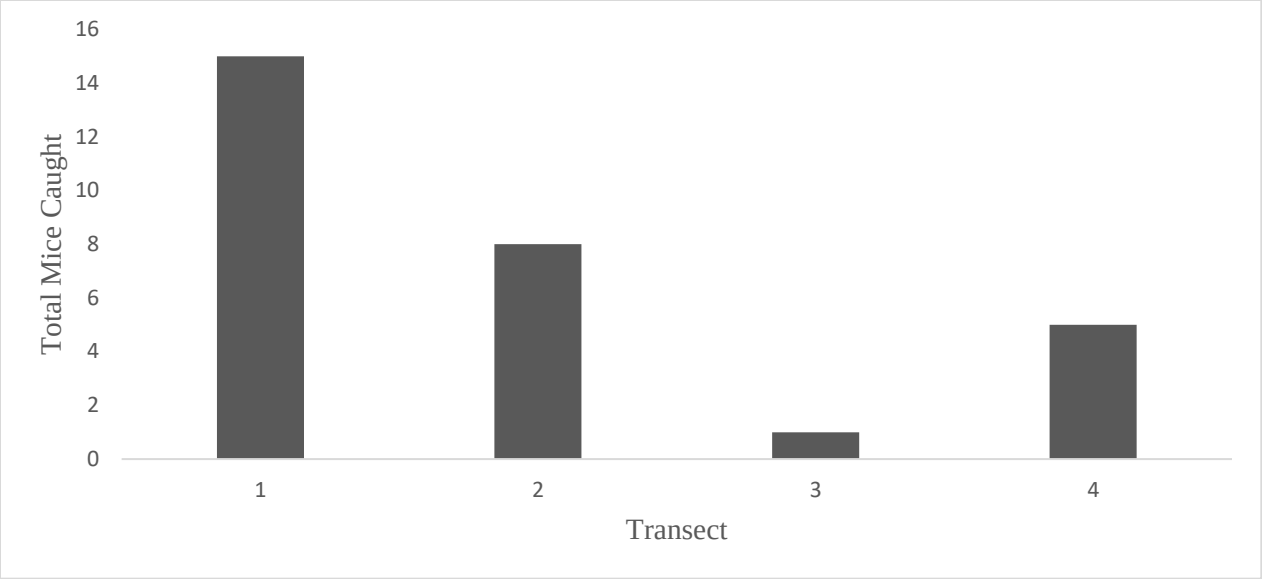

Figure 4. The total number of mice that were caught between each transect was recorded with the major difference between the transects is acorn presence. Transects 1 and 2 had acorns present, whereas transects 3 and 4 did not have any acorns. Transect 1 and transect 2 were approximately 20 meters apart; transect 3 was approximately 50 meters from transect 2; transect 4 was approximately 50 meters from transect 3, and approximately 100 meters from transect 2.

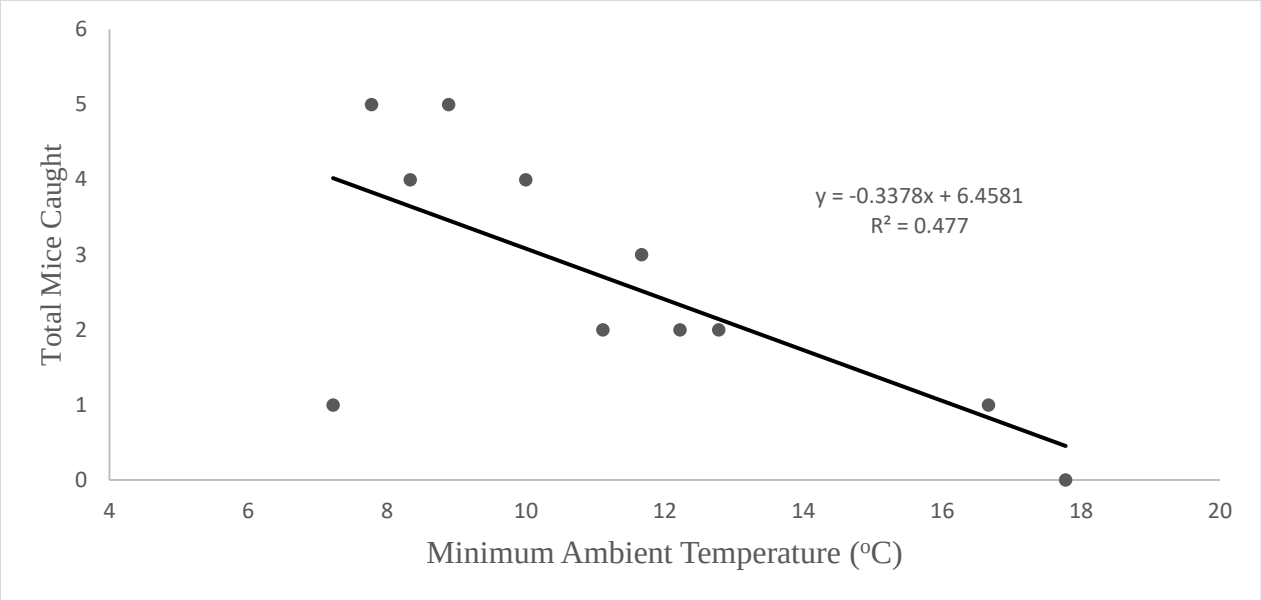

Figure 5. The effect of minimum ambient temperature (°C) was compared to the total number of mice that were caught for each trip at Bousson Environmental Research Reserve (P = 0.019).

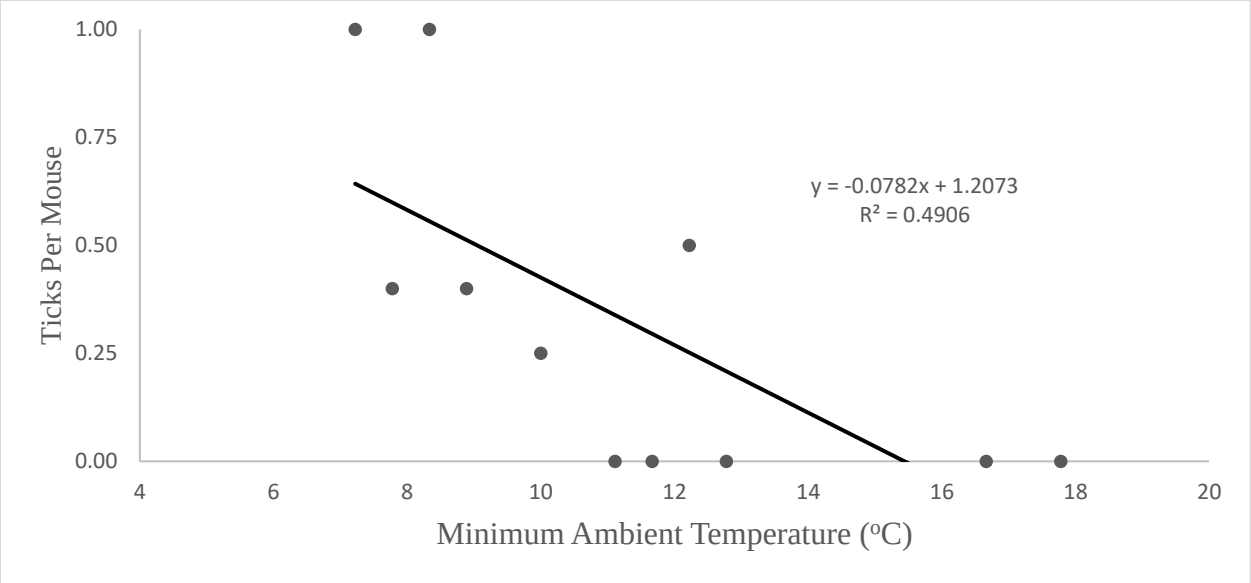

Figure 6. The effect of minimum ambient temperature (°C) was compared to the total number of ticks that were found on each individual mouse for each trip at Bousson Environmental Research Reserve (P = 0.016).

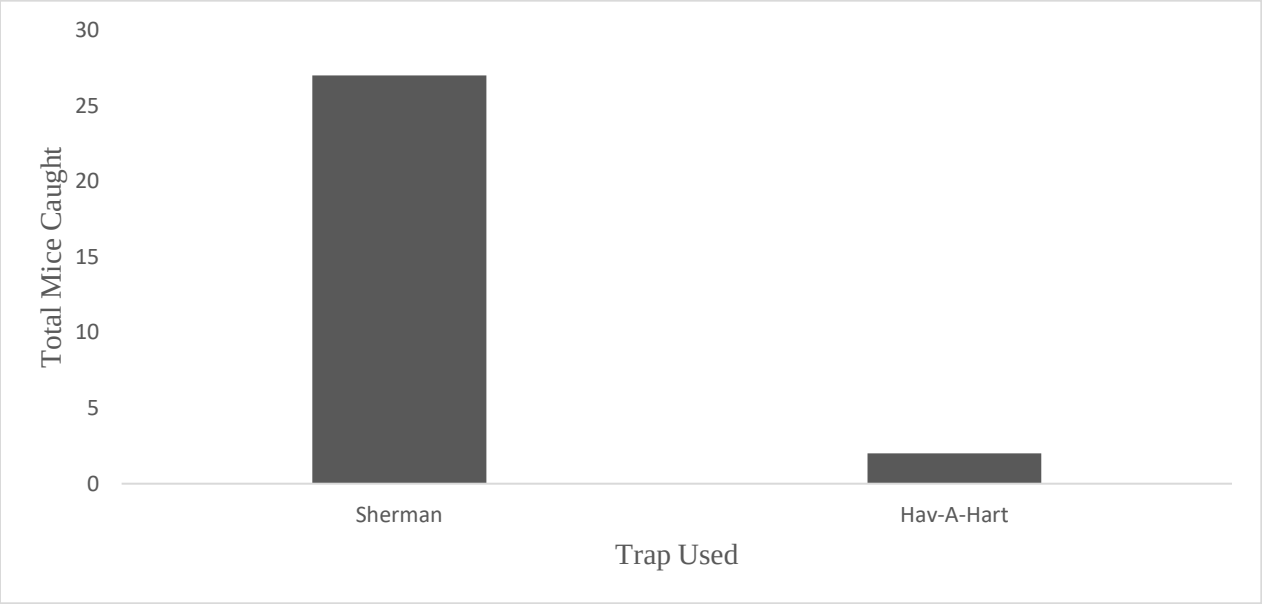

Figure 7. The success of each trap was examined throughout this experiment. Traps were evenly distributed between the four transects and consisted of Sherman box traps (1 door) and Hav-A-Hart traps (2 doors).

Discussion

This study was primarily conducted to survey tick prevalence on the white-footed mouse. The white-footed mouse is the primary reservoir for LD, and it is important to understand the factors that influence mouse abundance, which can be related to tick prevalence (Tsao et al., 2004). These factors include acorn presence and temperature (Shauber et al., 2005). However, tick prevalence is directly influenced by desiccation (Lees, 1946).

Out of the 29 total mice that were caught, only 11 ticks were found. One possible reason for this is related to the life cycle of *Ixodes scapularis*. Nymphal ticks are most active during the late spring to early summer months (Kocan et al., 2015). It is possible that during the research period most of the ticks have already molted into their final adult stage, which would result in those ticks questing to find larger hosts to facilitate feeding and reproduction (Adetunji et al., 2016; Kilpatrick et al., 2014; Kocan et al., 2015). However, it is still possible that nymphs could have been found during the research period since ticks are still active above freezing temperatures (Torina et al., 2018). Results showed that as the minimum ambient temperature decreased, the amount of ticks per mouse increased (Figure 5). Although this is supported by a linear regression analysis ($P = 0.016$), I hypothesize that other factors played an important role. As minimum ambient temperature decreased, mouse abundance increased (Figure 4). It is logical to believe that more ticks will be found on trips that caught the most mice. Hence, why lower temperatures resulted in more ticks found.

One subjective observation that I made was based on how recent precipitation was (days ago), and temperatures were highest when drought was at its peak (Table 1). Therefore, it is possible that the absence of ticks during the hottest periods was due to drought rather than temperature (Lees, 1946).

Mice are known to be meticulous groomers, and 10 of the 11 ticks found were located on the face or ears. I was unable to observe the dorsal side of each individual mouse due to limitations with handling. If most ticks were found on areas where mice cannot groom, it is possible that many of the ticks were missed on regions such as the back of the neck and base of the tail. In order to fully examine each individual mouse, it would be helpful to have a partner or use sedation.

The second study point of this research was to observe the activity of the white-footed mouse. As minimum ambient temperature decreased, the number of mice caught increased (Figure 4). This is consistent with prior research stating that mice are most active between the temperatures of 4.4-10°C (Orr, 1959). In this study, the most mice were caught between the temperatures of 7.8-10°C. Studies on colder days were not attempted due to a fear of hypothermia on the mice, but it would be predicted that as temperatures got closer to freezing, there would be less mice caught. Four transects were utilized during this study with the primary difference being the presence of acorns (Figure 3). Data suggests that as acorns are present, the abundance of mice increased (Figure 1). This is consistent with previous findings on small rodents and acorn presence (Ostfeld et al., 2006; Shauber et al., 2005). Both transect 1 and transect 2 had a high abundance of acorns and were relatively close to one another. The only difference between the two transects were the amount of burrow holes that were observed. Transect 1 had noticeably more burrow holes than transect 2. Therefore, placing traps in between acorn sites and burrow holes proved to be successful in catching the most mice. Transect 3 and transect 4 were located in the absence of acorns and caught noticeably less mice. I believe that transect 4 performed better than transect 3 because of the habitat. Transect 3 was very open,

whereas transect 4 was in a grassier location. However, I am not positive if other factors could have influenced the difference in mice caught between transect 3 and transect 4.

A third focus of this study was to look at the success rates between Sherman box traps and Hav-A-Hart traps. Sherman box traps proved to be significantly better at catching mice than the Hav-A-Hart traps (Figure 2). This is not consistent with my prediction that the Hav-A-Hart traps would perform better because it had 2 doors as opposed to only 1 door. One possibility for this finding was the trigger for each trap. It is possible that the Hav-A-Hart traps are more difficult to trigger than the Sherman box traps. Another possibility is trap placement. The Sherman box traps may have been placed more strategically in areas that were known for catching more mice. For future studies, I would recommend only using Sherman box traps as opposed to Hav-A-Hart traps. The Sherman box traps are lightweight, can fold and are much easier to transport between locations.

This was a general study that surveyed the abundance and activity of mice, and the tick prevalence on mice at Bousson Environmental Research Reserve. There are many future studies that can be conducted from this research at Bousson. The main issue with ticks is the threat of LD. If ticks can be removed from mice, quantitative polymerase chain reaction (qPCR) can be conducted to test whether the *Borrelia burgdorferi* pathogen is present or not. This will also give insight towards how many mice are infected with LD at Bousson. Understanding the prevalence of LD in Bousson will provide information about human risk for contracting the disease.

Another future research project is extending the trapping range and adding increased variability in habitats between transects. The primary difference between transects in this study was the presence of acorns; however, there are many other factors that can be observed, such as

water presence, grass height, or burrow density (Schauber et al., 2005). This would give more precise information about which habitats are preferred by mice.

Moreover, this research was unique because it is the first of its kind to observe tick prevalence and mouse abundance at the Bousson Environmental Research Reserve. *Ixodes scapularis* was chosen as the primary research organism, because it is the primary vector for LD, whereas the white-footed mouse was chosen as the secondary research organism, because it is the primary reservoir for the *Borrelia burgdorferi* pathogen (Tsao et al., 2004). LD is the most prevalent arthropod vectored disease in North America and Europe, and understanding more about how this disease is spread can prove pivotal in preventing its spread to humans.

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