Spring 2017

AN OBSERVATIONAL STUDY OF TICKS ON THE 30 ACRE LAKE TRAIL AT TURNBULL NATIONAL WILDLIFE REFUGE

Robert E. Shadix Jr.
Eastern Washington University

AN OBSERVATIONAL STUDY OF TICKS ON THE 30 ACRE LAKE TRAIL AT TURNBULL NATIONAL WILDLIFE REFUGE

A Thesis

Presented To

Eastern Washington University

Cheney, Washington

In Partial Fulfillment of the Requirements

for the Degree

Masters of Science in Biology

By

Robert E. Shadix Jr.

Spring 2017

THESIS OF ROBERT E. SHADIX JR.

_____DATE_____
Dr. KRISZTIAN MAGORI, Graduate study committee chair

_____DATE_____
Dr. MARGARET O'CONNELL, Graduate study committee

_____DATE_____
Dr. LUIS MATOS, Graduate study committee

_____DATE_____
Dr. JEB OWEN, Graduate study committee

_____DATE_____
Dr. STACY WARREN, Graduate study committee

MASTER'S THESIS

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Eastern Washington University, I agree that the JFK Library shall make copies freely available for inspection. I further agree that copying of this project in whole or in part is allowable only for scholarly purposes. It is understood, however that any copying or publication of this thesis for commercial purposes, or for financial gain, shall not be allowed without my written permission.

Signature_____

Date_____

Abstract

Ticks are known carriers of bacterial pathogens that cause diseases in humans and other mammals. Hosts (mice, chipmunks, rabbits, deer, and elk) must be in the tick's questing range to fulfill the life cycle. The questing range depends on the life stage of the tick, vegetation, and host accessibility. Mammal densities directly affect the number of questing ticks observed in the environment. The 30 Acre Lake Trail site was selected for this study due to the high density of *Dermacentor* species ticks observed in past studies and the only known site of *Rickettsia rickettsii* pathogen isolated from ticks on the Turnbull National Wildlife Refuge (TNWR) to date. I hypothesize that there is a risk of infection of Rickettsia at this location, given the large tick density as well as the large density of competent hosts. I trapped small mammals four days per week for six weeks from 30 March 2016 to 18 May 2016. Ticks were collected once per week in all zones off the 30 Acre Lake Trail. Tick DNA was extracted for sequencing to identify *Rickettsia* bacteria. There were 33 ticks that were positive for *Rickettsia spp*. bacteria of the 472 that were tested. Less than one percent were positive for *Rickettsia rickettsii*. A vegetation survey was performed and a percentage of cover was determined for each zone. More ticks were found in areas with more chipmunks and less deer mice and higher percentage of shrub vegetation. The possibility of a *Rickettsia* infection is present at the 30 Acre Lake Trail.

ACKNOWLEDGEMENTS

Dr. Krisztian Magori has provided me with spot on guidance throughout this process. He provided assistance with the process of designing my study, and in using his funds to complete this project and without his help this would not have been possible. I would also like to thank Dr. Luis Matos for sharing his expertise in molecular biology and the use of his lab and equipment, not to mention him talking me into continuing my education. His mentorship along with Dr. Peggy O'Connell have been invaluable throughout this journey. I would like to also thank Dr. Jeb Owen for his insight on ticks in the discussion we had. Dr. Stacey Warren was extremely helpful with the maps and instructing me on how to make them. Jarrett Celini's knowledge of plants and his ability to identify them was helpful in the vegetation study that he performed for us. I would like to thank Shelby Hunter for her help trapping the small mammals in the study. The many undergraduate students (490 Disease Ecology) who helped in the collection of ticks and other data used in this observational study. I would like to thank Naomi Matson who showed me the PCR process and helped in getting some of the ticks sequenced. I also thank everyone else who assisted me in my research. I would also like to thank the EWU Biology department for the mini-grant that they so graciously awarded to me to fund some of my research.

TABLE OF CONTENTS

FIGURES AND TABLES

Introduction

Ticks are known carriers of bacterial pathogens that cause many diseases in humans and other larger mammals (Sonenshine and Roe 2014). Rocky Mountain Spotted Fever (RMSF) and Lyme disease are two common tick-borne diseases. While Lyme disease tends to have long-term health effects (Klempner et al. 2001), RMSF can be fatal, with a 25% case-fatality rate if untreated (Jones et al. 1999). If RMSF is recognized early on during the infection, it can be treated relatively easily with the use of common antibacterial drugs (e.g. doxycycline). Rocky Mountain Spotted Fever can be asymptomatic until a rash appears, but many times this late diagnosis is too late to effectively treat patients (Childs et al. 2007, Raoult and Parola 2007). Therefore, awareness of the risk of infection from ticks is crucial among the public and in the healthcare community to insure timely diagnosis and to prevent unnecessary infections. The bacteria that causes RMSF is *Rickettsia rickettsii* and it can be transmitted by several species of ticks (Estrada-Pena et al. 2013). The primary vectors are *Dermacentor andersoni* (Rocky Mountain Wood Tick) that occurs in the Rocky Mountains and *Dermacentor variabilis* (American dog tick) that is primarily found east of the Rockies (Araya-Anchetta et al. 2013, Jongejan and Uilenberg 2004). The American dog tick has been increasing its range into western North America (Anderson and Magnarelli 2008) . Ticks can also pass the pathogen onto their offspring (transovarial) when the female lays her eggs (Lane 1994). While there are many species of bacteria in the Rickettsia genus that are transmitted by ticks, most of them are non-pathogenic, and can potentially block other Rickettsia species, including *Rickettsia rickettsii* from infecting the tick (Dergousoff et al. 2009).

Questing is the act of active searching, whether hunting or ambushing a host. When a questing tick finds and bites a host that is infected with the bacteria, the tick becomes infected and can transmit the bacteria onto other hosts (Sonenshine and Roe 2014). Different hosts are bitten in differing life stages of the tick, making the tick's life cycle integral in the transmission of pathogenic bacteria to hosts.

Dermacentor tick species (*Ixodidae*) have a three-stage life cycle: larvae, nymph, and the adult; and each stage requires a specific host (Jongejan and Uilenberg 2004). The cycle begins in fall when the adult female drops off of its host to lay her eggs. The adult female can lay about 5000 eggs (Sonenshine and Tigner 1969). The eggs hatch into six legged larvae and the larvae overwinter on the decomposing vegetation layer in a state of diapause; a period of suspended development during unfavorable environmental conditions. In the spring, the larvae quests for its first host, a small mammal (e.g. shrew or mouse) and attaches to consume a blood meal. The larvae fall off of the host in the summer months and molt to become nymphs. They then overwinter in diapause again. When spring days begin to lengthen, the nymphs break diapause and begin questing for a host that will be slightly larger than the previous year's host (e.g. chipmunk or rabbit). After the nymphs feed on this new host during spring or early summer, they fall off of the host and molt to the adult stage. The adults can attach to a host immediately if weather conditions are favorable or they can go into diapause again to overwinter. When the adults attach to the third, even larger host (e.g. dog, deer, or human), they will take a blood meal. The males will use this blood to produce sperm. After the blood meal, the males go in search of females on the same host. After the males find females and they mate, the mated females take a full blood meal, drop off the animal and lays eggs (Fig.

1). The eggs are deposited on the ground where it is humid so they can hatch. In a controlled laboratory the tick lifecycle can take between 88 and 134 days, but in nature it may take up to three years for the life cycle to be completed (Sonenshine and Roe 2014).

Tick movement during questing is a function of the environment: terrain, vegetation, microclimates, and host availability. A tick may not move at all, or its movements may be extremely short and can be measured in centimeters as it is questing for a host (Perret et al. 2003, Crooks and Randolph 2006). Tick movement varies with species. In *Ixodes scapularis*, for example, the nymph can travel up to 3 meters and adults more than 5 meters (Carroll and Schmidtmann 1996). These distances often require many weeks to accomplish. Ticks require a humid environment to live in while questing for hosts. If the conditions are too hot and dry, tick questing rate decreases and/or the tick dies (Perret et al. 2000). When ticks are questing, they can climb vegetation to encounter hosts. They will remain on the vegetation until the temperature increases and the humidity decreases, forcing them back to the cool and humid ground microclimate (Randolph and Storey 1999, Crooks and Randolph 2006). They will continue this questing behavior until they either find a host or use up their fat stores and die.

During the winter months, ticks will go into diapause, and wait until temperatures are warm enough for them to emerge in the spring (Randolph et al. 2002). There are two different types of diapause, behavioral and morphogenic. Behavioral diapause occurs when the ticks stop feeding to avoid adverse abiotic conditions (particularly low humidity). Morphogenic diapause delays the molting development of larvae and/or nymphs (Randolph 2009). Photoperiod plays a role in the tick's emergence from diapause, as the days begin to lengthen ticks break diapause (Belozerov and

Naumov 2002) and start questing. Belozerov and Naumov have shown that photoperiod may be the preferred indicator to break diapause (instead of temperature and humidity) to begin the search for a host.

Hosts must be in the ticks' questing range for the tick life cycle to be completed. Mammal densities directly affect the number of questing ticks observed in the environment. Ticks need small mammals to feed on when they are larvae. As a tick molts and develops into a nymph, there is a need for a larger host mammal (e.g. chipmunk, rabbit). Adult ticks require even larger mammals (e.g. dog, deer, elk) so that they can take a suitable blood meal to produce gametes and find a mate. When the density of these different sizes of mammals are reduced, the tick density in the area will be increased because of a lack of hosts that would export the ticks from the area after the tick attaches. If large mammal densities are low, then more adult ticks will be out questing (Ogden et al. 1997, Rand et al. 2003). These ticks will eventually find hosts, move, or die if unsuccessful in questing. This can cause a decline in the density of the ticks over time. This is true in the life cycle of hard shelled non-nidicolous (occupying open habitat) ticks from the family *Ixodidae*.

Turnbull National Wildlife Refuge (TNWR) is a federal wildlife refuge located about 32 km south southwest of Spokane Washington and approximately 8 km south of Cheney, Washington (headquarters Lat/long are 47°24'57.136"N 117°31'57.479"W, Fig. 2). The refuge contains about 73 km^2 of land and about 9 km^2 that are designated as public use areas (Fig.3). TNWR has approximately 30,000 visitors per year who visit to observe different species of wildlife in natural settings and enjoy the outdoors. Importantly, visitors can walk on many of the refuge's trails. This is where people can

come into contact with ticks that may be carrying *R. rickettsii.* Additionally, many of these people bring their pet dogs and some (though it is against the rules) will let their animals run off-leash. Some people will allow dogs to run on very long leashes that permit the dogs access to the areas bordering the trails.

Ticks and tick-borne pathogens pose a threat to people who frequent tick habitats. While documented cases of RMSF have been confirmed in eastern Washington since 1971, there were only 4 reported cases in 2015. Of the cases reported in 2015, only 2 resulted from a local tick (DOH Annual DC Surveillance Report, 2015). In the eastern United States, the infection prevalence (proportion of population that is infected) for *R. rickettsii* in of *D. variabilis* is one percent (Paddock 2009, Stromdahl et al. July 2011). If there are fewer ticks questing, there is a lower likelihood of humans being selected as a host, which decreases the chance of humans contracting RMSF. The majority of cases that have been documented in Washington were imported from the eastern United States where Washington residents who were travelling were bitten by ticks infected with *R. rickettsii*. Most people like to go into the outdoors during the spring and summer months. Unfortunately, these are the months when ticks are the most active and questing; and thus likely to encounter human hosts.

In 2014, 120 ticks were collected at TNWR by the drag method (Mills et al. 1995). The ticks were collected on the 30 Acre Lake Trail. Tick density was 1.4 ticks per 10 m^2. This was the highest tick density relative to other locations that she collected at on the TNWR (Fruscalzo et al., in prep), creating a potential threat of acquiring RMSF. Twenty-four of those ticks were infected with a *Rickettsia spp.* pathogen and two of the 24 ticks were positive for the *R. rickettsii* [unpublished data; tested by PCR (Simser et al.

2001)]. Therefore, in order to characterize the ecology of ticks and *Rickettsia rickettsii* at the 30 Acre Lake Trail, the current study was organized around five separate objectives.

1. Characterize the spatial and temporal distribution of *Dermacentor* species ticks.
2. Characterize the cover of different vegetation types along study area of the trail.
3. Estimate the population size of small mammals, investigate their spatial distribution, and make observations on large mammals.
4. Test for the presence of *R. rickettsia* and other Spotted Fever Group *Rickettsia* bacteria in collected ticks.
5. Investigate the relationship between tick density, the distribution of small mammals, and different vegetation types within the study site.

MATERIALS AND METHODS

Study site

The study site was the northern end of the 30 Acre Lake Trail (longitude: N 47 25.579', latitude: W 117 34.028') off of the main auto tour route on TNWR (Fig. 3). It is a popular trail where many visitors walk around ponds and view wildlife with their dogs (a host of *D. variabilis*). The trail is made of coarse gravel and is well marked. Surrounding the trail there are Ponderosa Pine trees and snow berry shrubs. Additionally, there are multiple species of grasses and sedges, down wood, bio crust, and barren rocks. There are small and large mammal trails that bisect the main trail. The northern part of the trail, is 220 meters long and ends at a bridge over an ephemeral pond (Figure 3). This area was divided into 88 zones that were 10 X 10 m^2. There were 44 zones on each side (East, West) of the trail. On each side, half of the zones were either closer (directly adjacent and noted as Inside zones) or farther (10 m away or noted as Outside zones) from the

trail. These divisions created 4 parallel transects along the trail. The area was grouped into three sections along each transect designated as Trailhead (zones 1-8), Middle (zones 9-16), and Waterside (zones 17-22).

Tick collections

Ticks were collected within each zone once per week beginning on March 30, 2016 and ending on May 18, 2016. The ticks were collected for three weeks, then there was a three week break between April 14, 2017 and May 3, 2017 for a planned prescribed controlled burn of the area. When the refuge decided to forego the burning, the collections began again for three more weeks from May 4, 2016 until May 18, 2016, at which time the collections were ended. The students in Dr. Magori's Biology 490 Disease Ecology Capstone class performed the collections using a tick drag. The tick drag is a one meter by one-meter piece of corduroy cloth that is attached to a broom handle at one end and a rope to pull behind the user. The rear end of the drag can have a weight attached to hold the material down on the vegetation. This collection method allowed the ticks to cling onto the cloth, and easily be seen and collected. Ticks were picked off of the drag using forceps, placed into 70% ethanol, and stored at -4°C until the DNA extraction (Estrada-Pena et al. 2013).

Vegetation survey

The 88 zones were assessed and estimates were made for percent of coverage of different vegetation types: shrubs, grass/forbs, wood, biocrust, bare ground. Additionally, areas of bare rock were estimated. Each 10 m^2 zone was examined to identify the major vegetation type that fit into the six categories that had been characterized. The 1 m^2

quadrat method was used for each zone. Within each zone we assigned a percentage of cover for the six major vegetation types that were characterized for this site.

Small mammal trapping

Small mammals were trapped using 88 Sherman traps in the same zones that were used to drag for ticks. The traps were placed in each 10 m^2 zone and were approximately 10 meters from other traps in other zones. Each trap contained bait made of peanut butter and oats, and some polyester fiberfill bedding. Trapping was conducted during the weeks of 30 March - 15 April - 10 May and 26 May of 2016 for four nights per week. During the first period, traps were open continually and were checked in the morning (8 am) and again in the evenings (5 pm) for a total of 2,112 trap "nights" (1 trap "night" = 1 trap checked 1 time). With increased temperatures during the second period, traps were closed during the day, opened in the evening (after 4 pm), and checked the next morning for a total of 1,056 trap nights.

Captured mammals were identified to species, examined for ticks, and given a unique numbered ear tag. Ticks were collected off the mammals and were stored in 70% ethanol at -4°C for future testing of *Rickettsia* parasites.

Large mammal activity survey

Game trail cameras were attached to trees along the 30 Acre Lake Trail and near game trails to identify the large mammals that were using the area. Four trail cameras were put onto ponderosa pine trees using a six foot cable lock, where game trails crossed the gravel trail. The coordinates for camera 1 was latitude 47°26'8.088"N longitude 117°32'24.622"W, for camera 2 it was 47°26'9.157"N 117°32'26.278"W, camera 3 was

placed at 47°26’10.115N 117°32’28.608”W, and camera 4 was located at 47°26’11.328”N 117°32’29.479”W. Moultrie A-7i cameras that could take pictures day or night were used. The cameras were placed on 30 March - 15 April 2016. Because of proposed prescribed burn at TNWR, the cameras were removed for three weeks and placed again on 10 May - 15 Jun 2016 for a total of 53 days/nights. This recorded animal and human traffic on the trail during this time. The photographs showed the different types of large mammals in the area and when they moved through the 30 Acre Lake Trail area.

Molecular testing for Rickettsia bacteria

DNA was extracted from the collected ticks using a previously developed protocol in preparation for PCR testing (Appendix 1). PCR was used to identify the presence or absence of the *Rickettsia* bacteria. This approach is the most effective and preferred method of identifying the pathogen (Estrada-Pena et al. 2013). A 431 base pair fragment of the 17kDa *ompB* gene was amplified with two primers: Rr17.61p and Rr17.492n (Simser et al. 2001). To prepare the samples for PCR, a mixture was prepared that contained 12.5 µl Master Mix, 1µl forward primer (Rf 17.492), 1µl reverse primer (Rf 17.61), 9.5 µl pure water, and 1µl of sample. The PCR amplification was performed by denaturing at 95º C for two minutes, then 60 cycles of denaturation (30 seconds at 95°C), followed by primer annealing (60 seconds at 55°C), and elongation (72°C for 60seconds). A final extension for five minutes at 72º C was conducted to finalize the reaction. The PCR products were electrophoresed in a 1% gel in 1XTAE and 0.35µg/mL of Ethidium Bromide (EtBr). The gel was electrophoresed for one hour at 80V and imaged (Fig. 4).

Samples positive for Rickettsia spp. were sent to Genewiz Inc. for Sanger sequencing (Fig. 5). Both the forward and reverse strands of the DNA were sequenced. The forward and reverse sequences were combined using free software from DNAbaser.com. Low quality bases at the extremities were trimmed and the resulting sequences were compared to the Genbank database using BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). The BLAST search returned many "hits" and the hit with the lowest E score was chosen as the best match for the respective sequence that was queried.

Statistical Analysis

To estimate tick density a Poisson regression was used in the statistical software R (R Core Team, 2012) with the number of ticks collected in each zone as a response variable. This method was selected because tick density was not normally distributed. To investigate the spatial distribution of ticks the predictor variables were the section, the side, and the position of each zone in relation to the trail. To investigate the temporal distribution of ticks the predictor variable was the week of collection. To estimate the population densities of small mammals Mark program was used. A closed model was used because of the small number of collected mammals. The small mammal captures per week were tabulated by capture and the model was run. Small mammal range was estimated by including all zones in between capture locations. For example, if a single chipmunk was captured in zone A4 and D6; that single chipmunk would be counted as present in all the zones in which it could have traveled (e.g. A4-A6, B4-B6, C4-C6, D4-D6). The number of animals using each zone was summed as a measure of habitat usage. To investigate the relationship between tick density and distribution of small mammal,

and vegetation cover we started with a full model including the following predictor variables (habitat usage of deer mice and chipmunks, cover percent of shrub, biocrust, wood, rock, grass-forb). Non-significant predictors were removed from the model one by one until all remaining predictors were significant.

Results

Tick collections

Two species of tick were captured, *Dermacentor andersoni* and *Dermacentor variabilis*. During the first week 393 ticks collected. During the second and third weeks, 171 and 113 ticks were collected, respectively. After the three week break for the prescribed burn, we began again with collection. The fourth week of tick collection netted 78 ticks, the fifth week netted 36 ticks, and during the final week of collection 38 ticks were collected. All of the ticks that were collected during the dragging were adult *Dermacentor spp*. ticks. Additionally, 47 nymph ticks were collected from captured chipmunk.

Tick density was significantly different across the weeks in which ticks were collected (p-value< 2.2e-16), between the different sections of trail (p-value=1.347e-11), and between the zones closer (inside) and further away (outside) to the trail (p-value< 2.2e-16), but not between the two sides of the trail (East vs. West) (p-value=0.602). Specifically, mean tick density on the first week of collection was significantly higher at 4.466 ticks per 10 m^2 compared to all other subsequent weeks (Fig. 6). Tick density during the second week, at 1.943 ticks per 10 m^2, was still significantly higher than all subsequent weeks. Tick density during the third and fourth weeks (at 1.284 and 0.886

ticks per 10 m^2, respectively) were still significantly higher compared to the subsequent weeks, but not significantly different from each other. Tick density did not significantly change from the fifth (at 4.091 ticks per 10 m^2) to the sixth week (at 0.432 ticks per m^2). Tick density in the Trailhead section was significantly higher at 2.04 ticks per 10 m^2 compared to both the Middle section at 1.141 ticks per 10 m^2, and to the Waterside section at 1.514 tick per m^2, which were also significantly different from each other (Fig. 7). Tick density was significantly higher closer to the trail at a mean tick density of 2.205 per 10 m^2 compared to a mean tick density of 0.936 per 10 m^2 (Fig. 8). Mean tick density on the East side of trail at 1.599 per 10 m^2 was not significantly different from mean tick density of 1.542 ticks per 10 m^2 on the West side (Fig. 9).

Small mammal trapping

The total trap effort of 3,168 trap nights during the six weeks of trapping yielded 193 captures of 48 individual yellow-pine chipmunks (*Tamias amoenus*), 110 captures of 36 individual deer mice (*Peromyscus maniculatus*), and 3 captures of 3 individual vagrant shrews (*Sorex vagrans*). Typically, chipmunks were caught during daylight hours and deer mice were caught when the traps were left out overnight. During the last week, all of the animals captured had been captured previously and marked with ear tags (Table 1).

A population size estimate analysis was conducted on the two small mammals (deer mice, chipmunks) that were trapped using the Mark online program (White and Burnham 1999). A closed model was used with a full likelihood of probability of capture and recapture on the weekly collections for both species of small mammals, assuming

that the two probabilities are equal. For the deer mice, the Mark program estimated the population at 47 animals per 880 m^2 with a 95% confidence interval of 42.3 and 61.9, the lower and upper limits respectively with an AIC of 65.4. The estimated chipmunk was 61 per 880 m^2 and a 95% confidence interval between 57.4 and 72.0 with an AIC of 84.1.

Habitat usage of deer mice was significantly different across different sections of zones (p-value=0.016), and between the two sides of the trail (p-value=4.331e-06), but not between closer and further away from the trail (p-value=0.871). Zones in the Trailhead section were used by 5.2 deer mice on average, while zones in the Middle section were used by 5.4 deer mice, which was significantly higher than the average habitat usage of zones in the Waterside section at 3.8 (p-value of 0.018). Zones on the west side of the trail have been used by significantly more deer mice at 6 compared to zones on the east side that were used on average by 3.8 deer mice (Fig 10).

Habitat usage of yellow-pine chipmunks was significantly different across different sections of zones (p-value= 0.0002), and between the two sides of the trail (p-value= 4.278e-10), but not between closer and further away from the trail (p-value= 0.0657). Zones in the Middle section were used by 13.2 chipmunks on average, significantly higher than zones in the Trailhead section at 10.7 chipmunks, as well as zones in the Waterside section at 9.5 chipmunks, which were not significantly different from each other. Zones on the west side of the trail have been used by significantly more chipmunks at 13.5 on average compared to zones on the east side that were used on average by 9.051 chipmunks (Fig 11).

Large mammal activity survey

The game cameras recorded qualitative evidence that larger mammals use the public use area at TNWR, typically when people were not present. Photographs indicated most of the people who used the trail did so on the weekends, holidays, or when weather was clear in the afternoon. The large mammals that were photographed included turkeys (*Meleagris gallopavo*), coyotes (*Canis latrans*), white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), elk (*Cervus canadensis*), and moose (*Alces alces*). The animals typically moved during late afternoons at dusk, and early evenings.

Vegetation

Percentage cover of shrub vegetation type was significantly different across different sections of zones (p-value< 2.2e-16), and between the two sides of the trail (p-value< 2.2e-16), as well as between closer and further away from the trail (p-value= 1.724e-13). Zones in the Trailhead section had the highest percentage of shrub cover at 33.75% on average, which was significantly higher than the percentage shrub cover in zones in the Middle section at 6.22% (p-value < 1e-05), as well as in zones in the Waterside section at 28.5% shrub cover (p-value=8.34e-05), which were also significantly different from each other (p-value<1e-05). Zones on the west side of the trail had significantly higher percentage of shrub cover at 29.48% on average compared to zones on the east side that had on average 15.14% shrub cover. Zones closer to the trail had significantly higher shrub cover on average at 25.29%, compared to an average shrub cover of 19.19% in zones further away from the trail (Fig 12).

Percentage cover of biocrust vegetation type was significantly different across different sections of zones (p-value< 2.2e-16), and between the two sides of the trail (p-value< 2.2e-16), as well as between closer and further away from the trail (p-value= 2.207e-15). Zones in the Trailhead section had the highest percentage of biocrust cover at 14% on average, which was significantly higher than the percentage biocrust cover in zones in the Middle section at 10.8125% (p-value= 0.000307), as well as in zones in the Waterside section at 7.33% biocrust cover (p-value<1e-04), which were also significantly different from each other (p-value<1e-04). Zones on the west side of the trail had significantly lower percentage of biocrust cover at 5.6% on average compared to zones on the east side that had on average 16.4% biocrust cover. Zones closer to the trail had significantly lower biocrust cover on average at 7.1%, compared to an average biocrust cover of 15.1% in zones further away from the trail.

Percentage cover of grass-forb vegetation type was significantly different across different sections of zones (p-value< 2.2e-16), and between closer and further away from the trail (p-value= 0.0002), but not between the two sides of the trail (p-value= 0.5852). Zones in the Middle section had the highest percentage of grass-forb cover at 77.4% on average, which was significantly higher than the percentage grass-forb cover in zones in the Trailhead section at 47.9% (p-value<1e-08), as well as in zones in the Waterside section at 55.8% biocrust cover (p-value<1e-08), which were also significantly different from each other (p-value= 2.05e-08). Zones closer to the trail had significantly lower higher grass-forb cover on average at 62.7%, compared to an average grass-forb cover of 58.8% in zones further away from the trail. Zones on the west and east side of the trail

did not have a significantly different percentage of grass-forb cover at 60.5% and 61.1% on average, respectively.

The relationship between total ticks collected and small mammal habitat usage and vegetation cover

As a reminder, the full model used was a Poisson regression with the total number of adult ticks collected in each zone as the response variable, and the following predictor variables, including their interaction: the habitat usage for deer mice, the habitat usage for yellow-pine chipmunks, the percentage cover of shrub, biocrust, grass-forb and rock vegetation type. The overall model explained 71.7% of variation in the total number of tick collected in each zone, with an AIC of 901.8. However, the coefficient for the vegetation type "Rock" was not significant (p-value=0.507). In order to simplify the model, the vegetation type "Rock" was removed from the list of predictor variable, and the model was rerun. This reduced model still explained 51.76% of the variation in the total number of ticks collected, with an AIC of 711.05, which was much lower than the AIC of the full model. The direction of the effect of any of the remaining predictors in this reduced model were consistent with the direction of their effects in the full model, supporting our decision to remove the vegetation type "Rock" from the model. However, even in this model, the coefficient of vegetation type "Grass.Forb" was non-significant (p-value=0.06067). To further simplify the model, we also removed this vegetation type as a predictor. This final model still explained 34.15% of the variation in the total number of ticks collected, with an AIC of 754.1. All the coefficients of the remaining covariates were statistically significant (Table 2), with the direction of their effects the same as their direction in the full and reduced models. Several interaction terms were

also statistically significant, including three-way interactions between small mammal habitat usage and the remaining vegetation type percentages, supporting including the interaction terms in the model. The total number of ticks collected was significantly lower in zones used by higher number of deer mice, with a 23.3% reduction in total ticks collected with every additional deer mice using a zone (Fig. 13). In contrast, significantly more ticks were collected in zones used by higher numbers of yellow-pine chipmunks, with an 8.9% increase in the number of ticks collected with every additional yellow-pine chipmunk using a zone (Fig. 14). In addition, the number of ticks collected significantly increased with both increasing percentage of shrub vegetation type (Fig. 15) as well as with increasing percentage of biocrust vegetation type (Fig. 16). The number of ticks collected increased by 1.36% with every one percent increase in Shrub cover, and by 0.75% with every one percent increase in biocrust cover.

Molecular testing for Rickettsia bacteria

Molecular testing was performed on the ticks that were caught from 30 March 2016 to 18 May 2016. DNA was extracted and PCR was performed on the DNA of 472 ticks. This accounted for all the ticks captured in all the zones on the first sampling day and all the zones on the last two sampling days. Out of the 472 total ticks, 33 had a positive band which corresponds to a 7% infection rate with *Rickettsia spp*. (Fig. 4). There were 27 ticks positive for *R. rhipicephali*, which is a non-pathogenic bacteria. Of the other four ticks, three were positive for spotted fever group rickettsia (Fig. 17) bacteria and one was positively identified as *R. rickettsii*, the causative bacteria of Rocky Mountain Spotted Fever (Fig. 18).

Discussion

The distribution of tick density was heterogeneous within the site, with the number of ticks collected widely varying between sections of the trail at a small spatial scale (Fig. 10, 11). We observed that adult ticks are questing more in the early months of spring (March and April) relative to later in the year. There were more ticks collected in the first week than in the last three weeks combined. This relates to the importance of the photoperiod for the emergence of the ticks from their diapause (Belozerov and Naumov 2002). When the day lengthens and the sun reaches the proper angle in the sky, it signals the ticks to break their diapause. Their research shows that photoperiod is a stronger predictor as to when ticks will emerge from diapause. It is likely that that is why more ticks were collected in the early spring than later in the year. The reduction in the number of questing ticks is a result of the combination of increasing temperatures, decreasing humidity, and the attachment of ticks to hosts (Sonenshine and Roe 2014). While no ticks (larvae or nymphs) were seen on any of the deer mice, some nymphs were collected from some of the chipmunks. Likely, this is because deer mice are better groomers than chipmunks (Collinge and Ray 2006) and because the tick larvae are smaller and will only remain on the deer mice for a brief period (about a day). The tick nymphs may also prefer the chipmunks as better intermediate host. This might explain the positive relationship that was observed between the total number of ticks collected and the number of chipmunks using each zone, but a negative relationship with the deer mice.

There was a significant positive relationship between the number of ticks collected and shrub coverage in each zone, leading to higher tick density in zones with

high percent shrub cover. One potential explanation for this observation could be that adult ticks are questing in taller vegetation to increase their chance to latch onto a larger mammal to complete their three-year, three-stage life cycle (Sonenshine and Roe 2014). The taller shrub vegetation increases the probability of a larger mammal wandering by or foraging in the shrubs. Micro habitat conditions (temperature, humidity) might also be affected by vegetation cover, which could also impact tick density.

The small mammal host community of ticks at the 30 Acre Lake Trail study site consisted of chipmunks, deer mice and shrews. The density of chipmunks and deer mice at this location was comparable to other locations on the refuge (Mike Rule, pers. comm.), indicating that the increased tick density, as well as the presence of Rickettsia bacteria is likely not due to increased small mammal density. The cameras showed that larger mammals are passing through the area via the trail, providing the ticks with large mammal hosts to complete the adult life cycle. There was a variety of species of large animals on the trail and in the area as well, despite the large numbers of visitors to the trail. Large mammals can import engorged mated female ticks to the area, which drop off of them, and lay on average 5380 eggs (Sonenshine and Tigner 1969). Sufficient presence and transit of large mammals will sustain the local tick population year-to-year as long as at least one female tick can lay her eggs in this area each year. Studies have shown that elk and other game animals move into the public use area of TNWR to avoid hunters in the fall, when TNWR does allow draw hunting to manage the elk populations (Katherine Farrell MSc Thesis). Given that this particular trail is right at the edge of the public use area, it would make sense that it would be an important transit point for large mammals and the ticks being transported on them.

The molecular testing showed that some of the ticks are, in fact infected with *Rickettsia rickettsii*. Therefore, there is a chance of contracting the pathogen from ticks acquired on the 30 Acre Lake Trail. Most of the ticks that were positive for *Rickettsia* species contained *R. riphicephali*, which is a non-pathogenic bacterium in humans, although it has mild-to-moderate effect on small mammals (Sonenshine and Roe 2014). Less than 1% of the ticks tested were positive for *R. rickettsii*, the causative agent of Rocky Mountain Spotted Fever, a potentially lethal disease. This agrees with other studies that have shown infection prevalence in ticks around one percent. It was reported in 2009 that there was an infection prevalence of 0.6% for *D. andersoni* ticks collected in 1992 from western Montana. In *D. variabilis* ticks collected in Ohio in 1981, there was a 1.9% infection rate. (Paddock 2009, Stromdahl et al. July 2011). The detection of this pathogen in ticks in 2016 confirmed our previous detection in 2014 (unpublished results), indicating a persistent and low-level risk of infection for people using this trail at TNWR.

The objectives of my study were to characterize one location, the 30 Acre Lake Trail at the TNWR, looking at the densities of the small and large mammals and the ticks that can potentially transmit RMSF. Since this area is open to the public there is a possibility for there to be contact between visitors and wildlife even if it is not direct contact. As I have shown above, there is a small, but non-negligible threat to contract RMSF from the many ticks that are present during the spring months at this site. Since I only looked at this one site the 30 Acre Lake Trail, follow-up research should be done on more areas of TNWR in order to map tick densities and RMSF infection risk across the refuge.

I would like to see the refuge ask hunters to collect ticks and blood samples from animals that are harvested during the fall hunt so that they can be tested. This might help explain why the 30 Acre Lake Trail contains RMSF and high densities of ticks. If the larger animals are using this area during hunting seasons this could explain why there are so many ticks on this part of the trail.

I would like to recommend that TNWR put up signs and flyers to educate the public who use the public use area to raise awareness of ticks and tick-borne diseases specifically. The public should be informed that there is a small risk of contracting RMSF, and what the signs and symptoms are. While communicating this risk is important, we need to make sure to avoid causing unnecessary levels of concern by communicating the magnitude of the risk. TNWR could also put out a pamphlet to get this information out and what to do if you do go home with a tick and it is embedded in the skin. This would allow the public to better communicate with the health care community about their symptoms and the potential for RMSF as a differential diagnosis. Early detection and care are imperative to a sound diagnosis and treatment plan with the correct antibiotics. This also can serve to inform the WA Department of Health and CDC that there is a potential threat of RMSF in eastern Washington.

LITERATURE CITED

Anderson, J. F., and L. A. Magnarelli. 2008. Biology of ticks. Infectious disease clinics of North America 22 (2) :195-215.

Araya-Anchetta, A., G. A. Scoles, J. D. Busch, and D. M. Wagner. 2013. Hybridization in natural sympatric populations of *Dermacentor* ticks in northwestern North America. Ecology and Evolution 3 (3) :714-724.

Belozerov, V. N., and R. L. Naumov. 2002. Nymphal diapause and its photoperiodic control in the tick Ixodes scapularis (Acari: *Ixodidae*). Folia Parasitologica 49 (4) :314-318.

Carroll, J. F., and E. T. Schmidtmann. 1996. Dispersal of blacklegged tick (Acari: *Ixodidae*) nymphs and adults at the woods-pasture interface. Journal of Medical Entomology 33 (4) :554-558.

Childs, J. E., C. D. Paddock, P. Parola, and D. Raoult. 2007. Rocky Mountain spotted fever. Infectious Disease and Therapy Series 43:97-116.

Collinge, S. K., and C. Ray. 2006. Disease ecology: community structure and pathogen dynamics. Oxford University Press, Oxford, UK.

Crooks, E., and S. E. Randolph. 2006. Walking by *Ixodes ricinus* ticks: intrinsic and extrinsic factors determine the attraction of moisture or host odour. Journal of Experimental Biology 209 (11) :2138-2142.

Dergousoff, S., A. Gajadhar, and N. Chilton. 2009. Prevalence of Rickettsia Species in Canadian Populations of *Dermacentor andersoni* and *D. variabilis*. Applied and Environmental Microbiology 75 (6) :1786-1789.

Estrada-Pena, A., J. S. Gray, O. Kahl, R. S. Lane, and A. M. Nijhol. 2013. Research on the ecology of ticks and tick-borne pathogens-Methodological principles and caveats. Frontiers in Cellular and Infection Microbiology 3(29):1-12.

Jones, T. F., A. S. Craig, C. D. Paddock, D. B. McKechnie, J. E. Childs, S. R. Zaki, and W. Schaffner. 1999. Family Cluster of Rocky Mountain Spotted Fever. Clinical Infectious Diseases 28 (4) :853-859.

Jongejan, F., and G. Uilenberg. 2004. The global importance of ticks. Parasitology 129 (S1) :S3-S14.

Klempner, M. S., L. T. Hu, J. Evans, C. H. Schmid, G. M. Johnson, R. P. Trevino, D. Norton, L. Levy, D. Wall, J. McCall, M. Kosinski, and A. Weinstein. 2001. Two Controlled Trials of Antibiotic Treatment in Patients with Persistent Symptoms and a History of Lyme Disease. New England Journal Medicine 345 (2) :85-92.

Lane, R. S. 1994. Competence of ticks as vectors of microbial agents with an emphasis on Borrelia burgdorferi. In Ecological dynamics of tick-borne zoonoses pp. 45-67.

Mills, J., J. Childs, T. Ksiazek, C. Peters, and Velleca W. 1995. Methods for Trapping and Sampling Small Mammals for Virologic Testing. Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, NE MS G13 Atlanta, GA 30333.

Ogden, N. H., P. A. Nuttall, and S. E. Randolph. 1997. Natural Lyme disease cycles maintained via sheep by co-feeding ticks. Parasitology 115 (06) :591-599.

Paddock, C. D. 2009. The Science and Fiction of Emerging Rickettsioses. Annals of the New York Academy of Sciences 1166 (1) :133-143.

Perret, J., E. Guigoz, O. Rais, and L. Gern. 2000. Influence of saturation deficit and temperature on *Ixodes ricinus* tick questing activity in a Lyme borrelosis-endemic area (Switzerland). Journal of Parasitology Research 86 (7) :554-557.

Perret, J., P. M. Guerin, P. Diehl, M. Vlimant, and L. Gern. 2003. Darkness favours mobility and saturation deficit limits questing duration in *Ixodes ricinus*, the tick vector of Lyme disease in Europe. Journal of Experimental Biology 206 (11) :1809-1815

R Core Team. 2012. *R:* A language and environment for statistical computing. R Foundation for Statistical Computing.

Rand, P. w., C. Lubelczyk, G. R. Lavigne, S. Elias, Holman M.S., E. H. Lacombe, and R. P. J. Smith. 2003. Deer density and the abundance of Ixodes scapularis (Acari: *Ixodidae*). Journal of Medical Entomology 40 (2) :179-184.

Randolph, S. E. 2004. Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129 (S1) :S37-S65.

Randolph, S. E. 1997. Abiotic and biotic determinants of the seasonal dynamics of the tick *Rhipicephalus appendiculatus* in South Africa. Medical Veterinary Entomology 11 (1) :25-37.

Randolph, S. E., and K. Storey. 1999. Impact of microclimate on immature tick-rodent interactions (Acari; *Ixodidae*): implications for parasite transmission. Journal of Medical Entomology 36 (6) :741-748.

Randolph, S. E., R. M. Green, A. N. Hoodless, and M. F. Peacey. 2002. An empirical quantitative framework for the seasonal population dynamics of the tick *Ixodes ricinus*. International Journal of Parasitology 32 (8) :979-989.

Randolph, S. E. 2009. Chapter 6 Epidemiological Consequences of the Ecological Physiology of Ticks. Advances in Insect Physiology 37 :297-339.

Raoult, D., and P. Parola. 2007. Rickettsial diseases. CRC Press.

Simser, J., A. Palmer, U. Munderloh, and T. Kurtti. 2001. Isolation of a Spotted Fever Group Rickettsia, *Rickettsia peacockii*, in a Rocky Mountian Wood Tick, *Dermacentor andersoni*, Cell Line. Applied and Environmental Microbiology 67 (2) :546-552.

Sonenshine, D., and M. R. Roe. 2014. Biology of Ticks. Oxford University Press, 198 Madison Ave. New York, NY 10016.

Sonenshine, D., and J. Tigner. 1969. Oviposition and hatching in 2 species of ticks in relation to saturation deficit (Acarina: *Ixodidae*). Daniel E. Sonenshine, Joyce A. Tigner 1969. Annals of the Entomological Society of America 62 (3) :628-640.

Stromdahl, E. Y., J. Jiang, M. Vince, and A. L. Richards. July 2011. Infrequency of *Rickettsia rickettsii* in *Dermacentor variabilis* Removed from Humans, with Comments on the Role of Other Human-Biting Ticks Associated with Spotted Fever Group Rickettsiae in the United States. Vector-Borne and Zoonotic Diseases 11 (7) :969-977.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46 (Sup1):S120-S139.

FIGURES

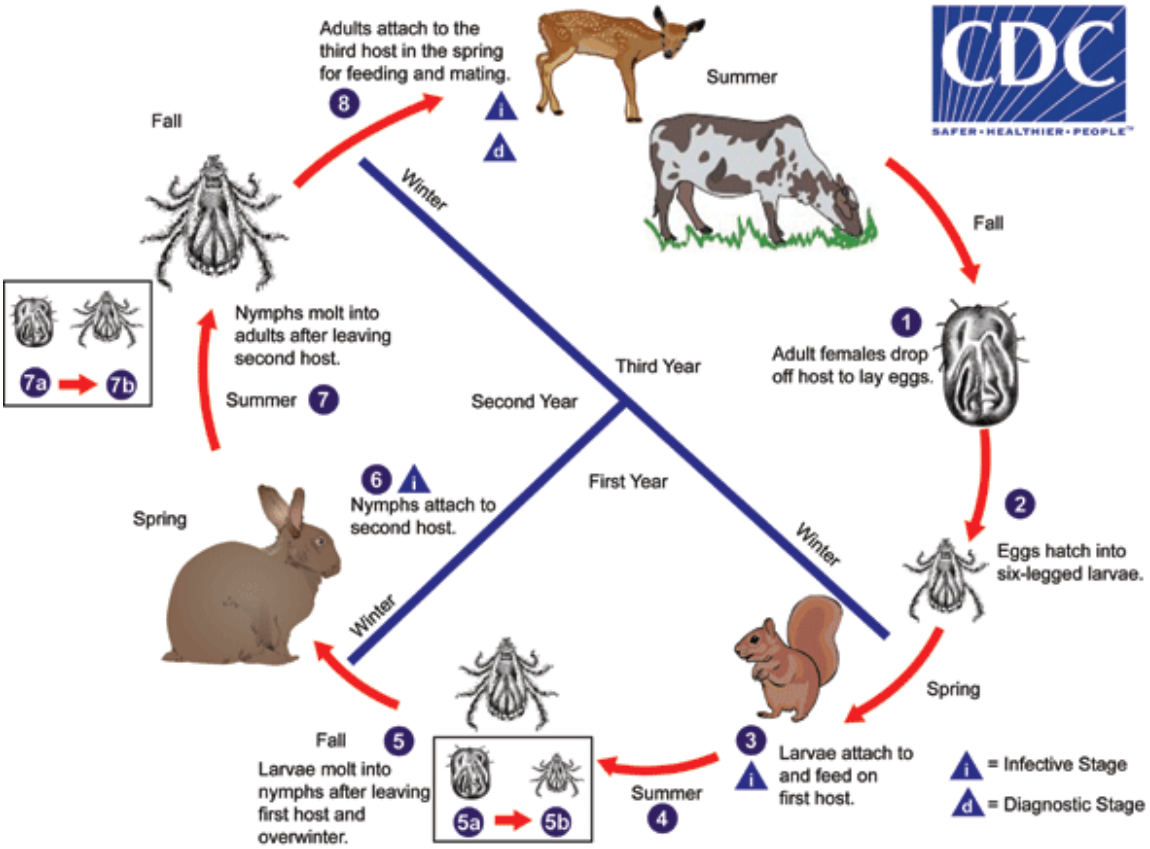


Figure 1: Life cycle of *Dermacentor spp.* ticks. It shows the timing of the different life stages with the most common time of year. It also shows an example of the size of host that particular life cycle is interested. This image was taken from the CDC website (www.cdc.gov/dpdx/ticks).

Figure 2: Location of Turnbull National Wildlife Refuge. The inset shows where TNWR is in relation to Spokane Wa.

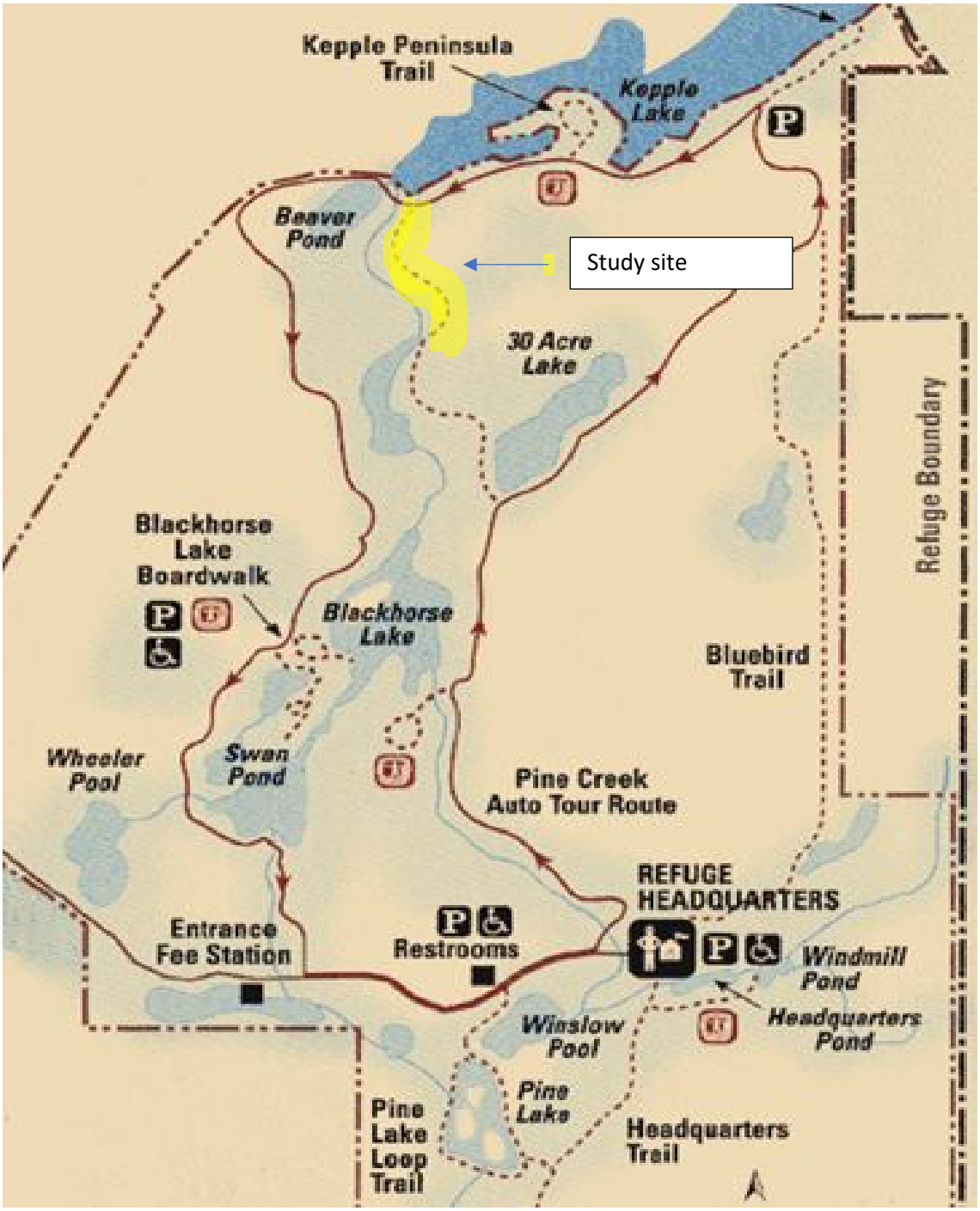


Figure 3: Map of the public use of area at Turnbull National Wildlife Refuge, with an arrow pointing at the study site.

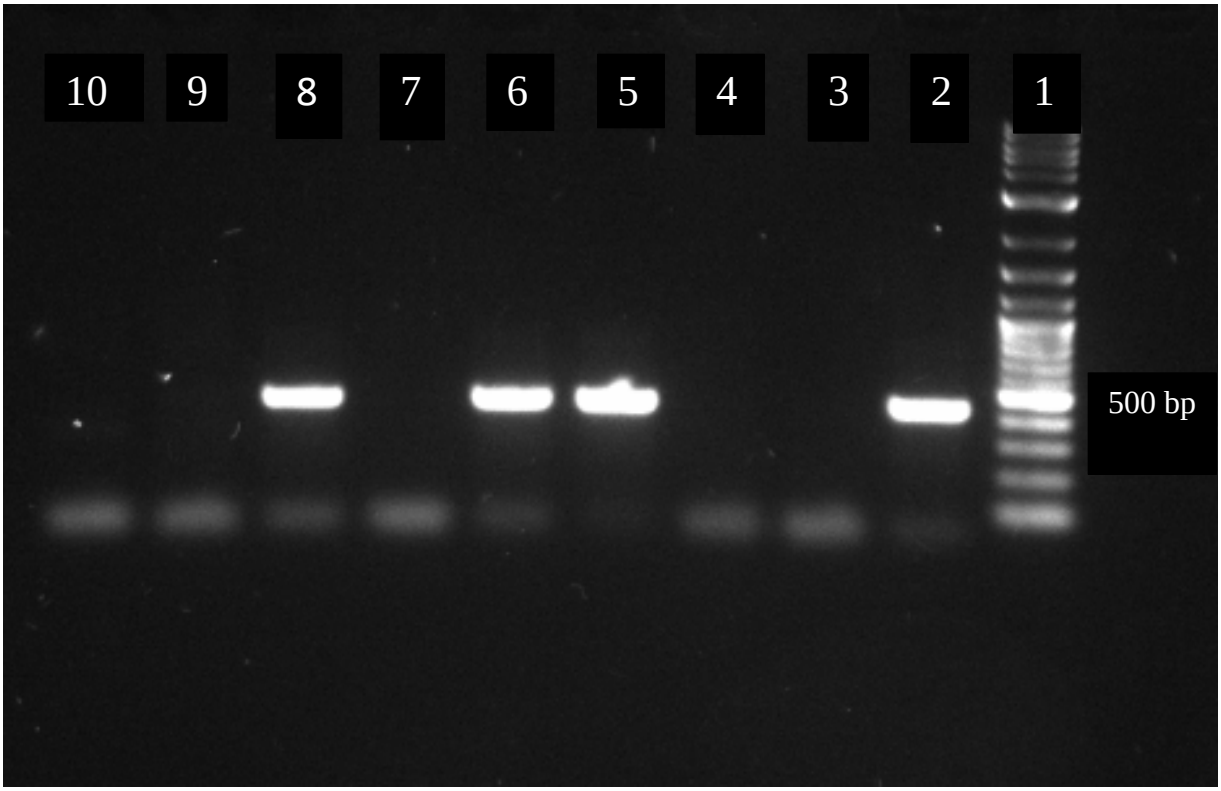


Figure 4: Gel electrophoresis of Positive *Rickettsia spp*. bands for Tick numbers 8 to 14. All of the bands are consistent with a 431bp amplicon, indicating a positive result of *Rickettsia spp*. Lane 1 is the DNA size standard, lane 2 a Positive control, lane 3 is a negative control, lane 4 is tick number 8, lane 5 is tick number 9, lane 6 is tick number 10, lane 7 is tick number 11, lane 8 is tick number 12, lane 9 is tick number 13, and lane 10 is tick number 14.

Figure 5: This chromatogram is the result of Sanger sequencing of Tick number 10 that was positive for *R. rickettsii*. All sequences were high quality with distinct peaks.

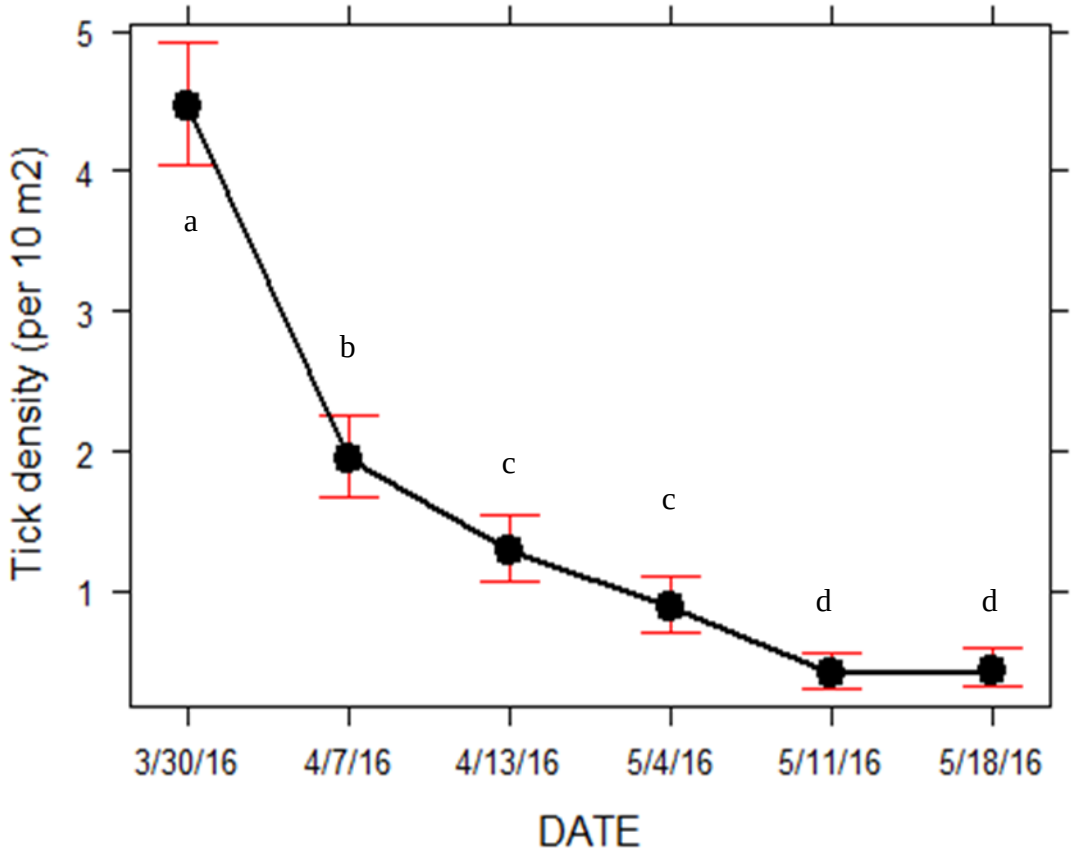


Figure 6: Mean tick density across the study area as a function of the date of collection based on 10 m^2. Error bars show 95% confidence intervals based on Poisson regression model. Letters differentiate weeks in which tick density was significantly different.

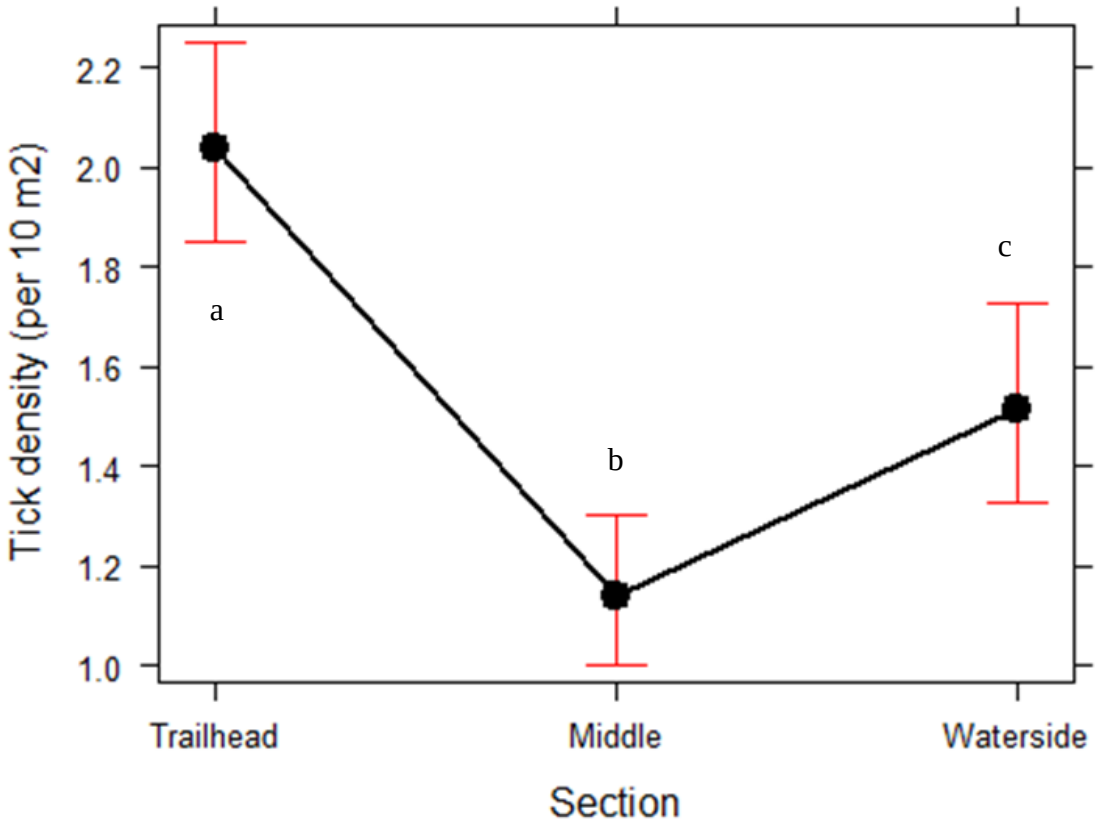


Figure 7: Mean tick density with in the different sections of the study site. Error bars show 95% confidence interval based on the Poisson regression. Letters differentiate sections in which tick density are significantly different.

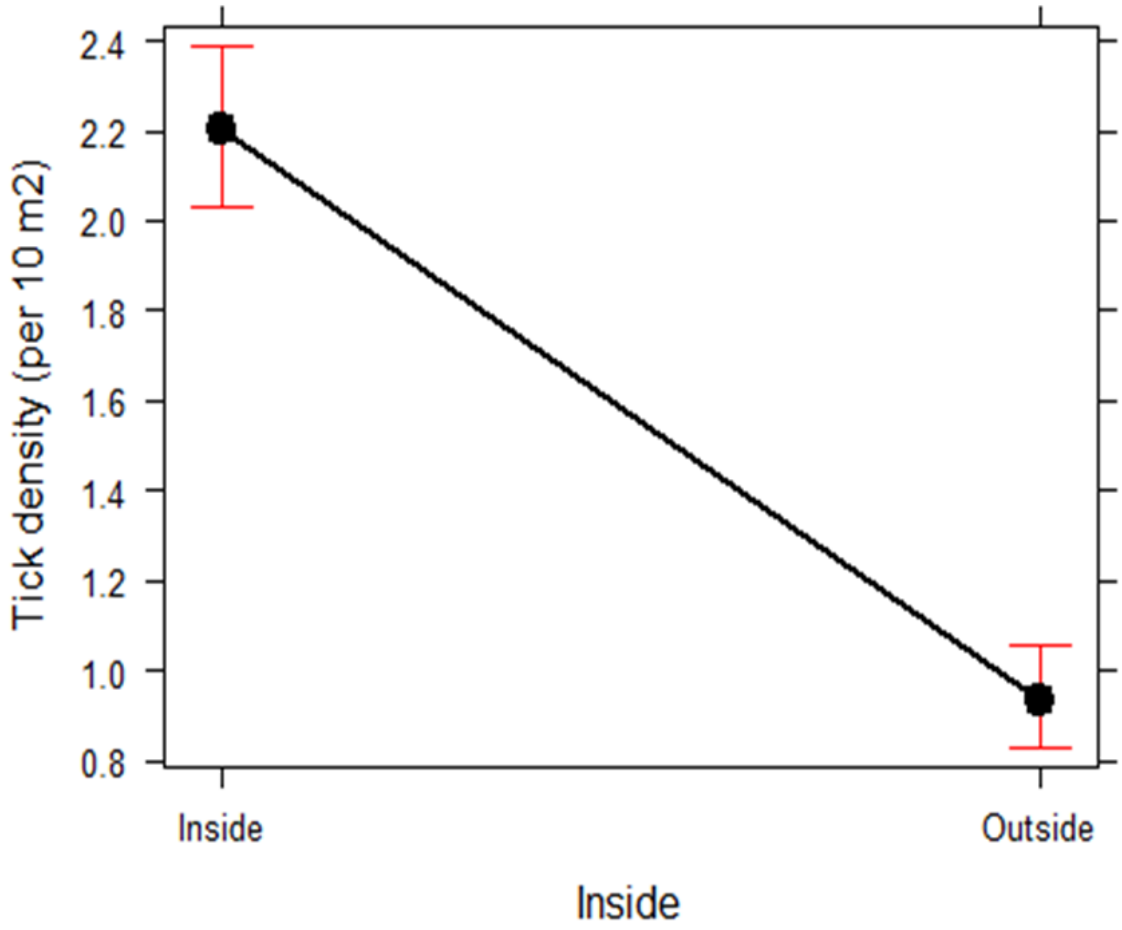


Figure 8: Mean tick density within the zones closer to the trail (inside), and further away from the trail (outside). Error bars show 95% confidence interval based on Poisson regression. Tick density is significantly different between the inside and outside zones.

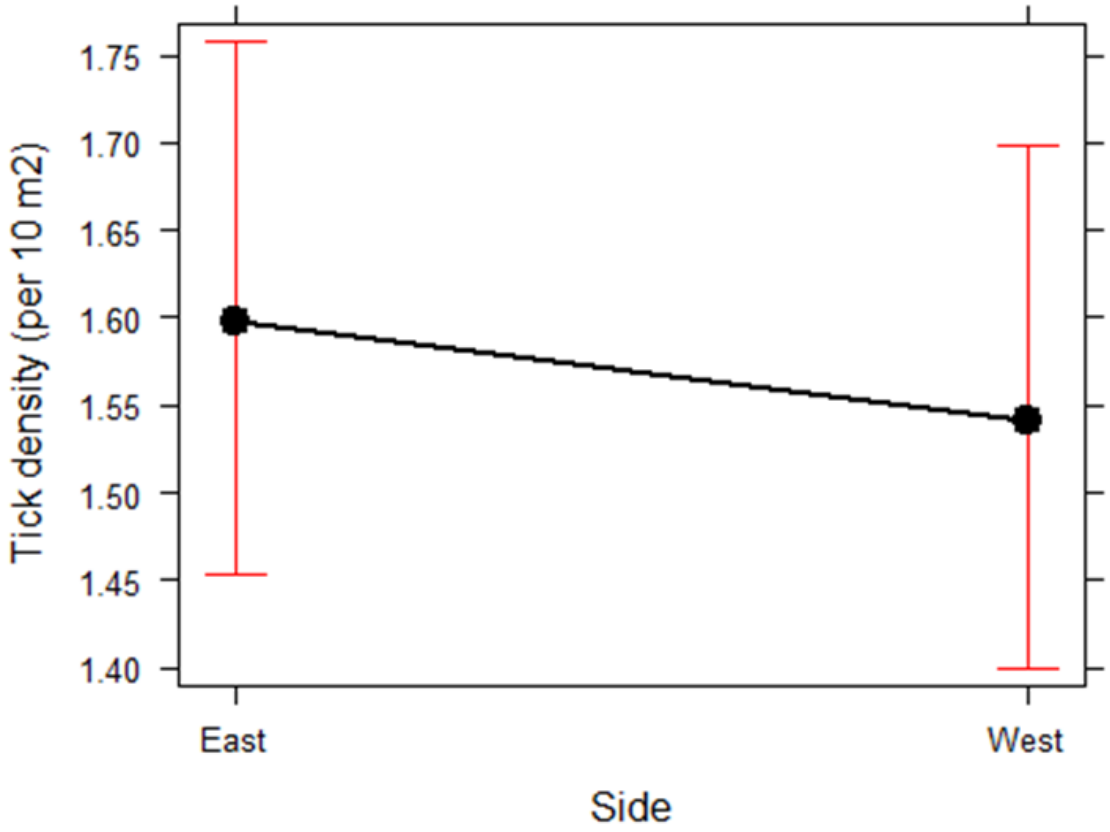


Figure 9: Mean tick density in the zones based on cardinal directions of East or West of the 30 Acre Lake Trail. Error bars show 95% confidence interval based on Poisson regression. Tick density is not significantly different between the two sides of the trail.

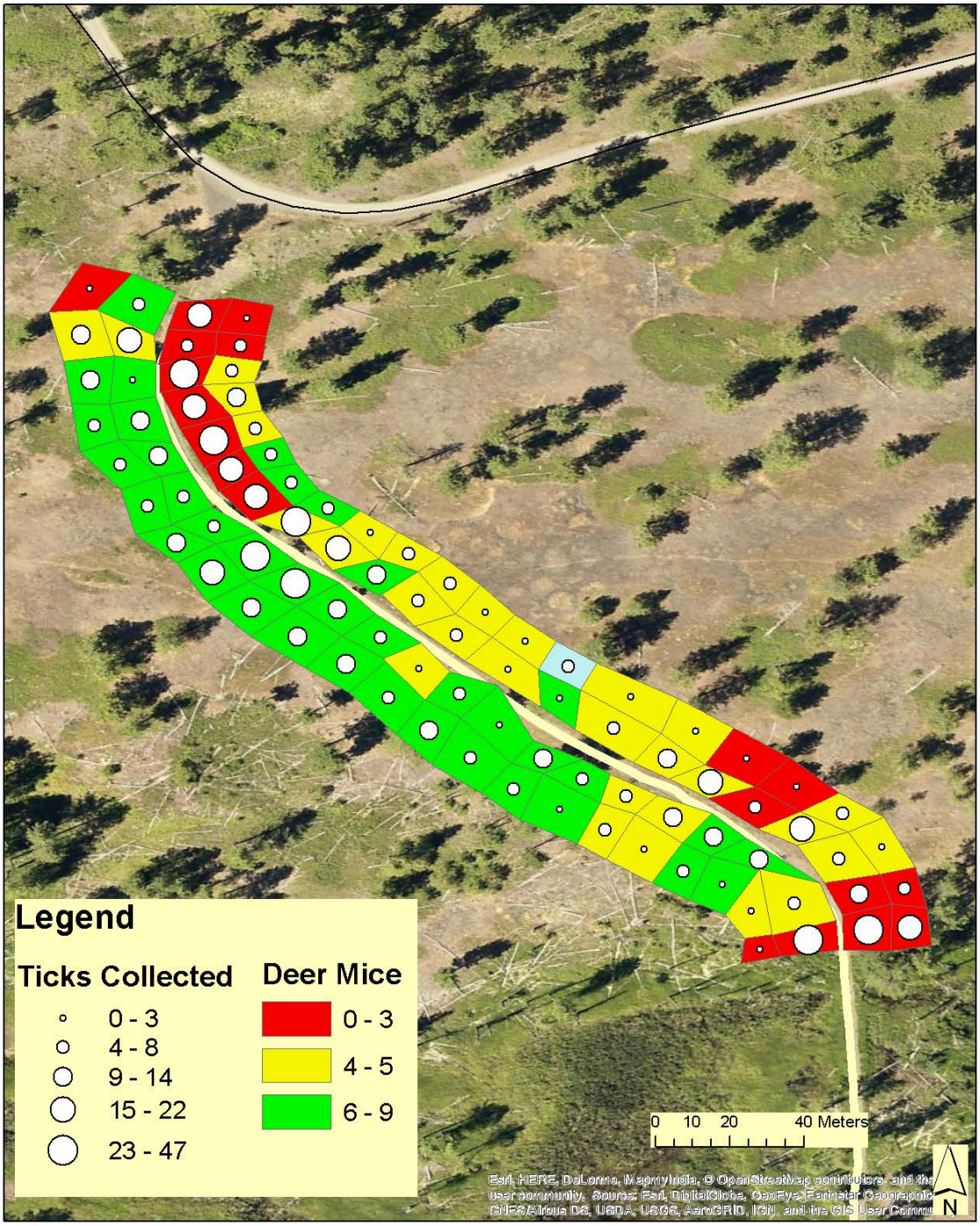


Figure 10: Habitat usage map of Deer Mice across the study site in relation to the total number of ticks collected in each zone.

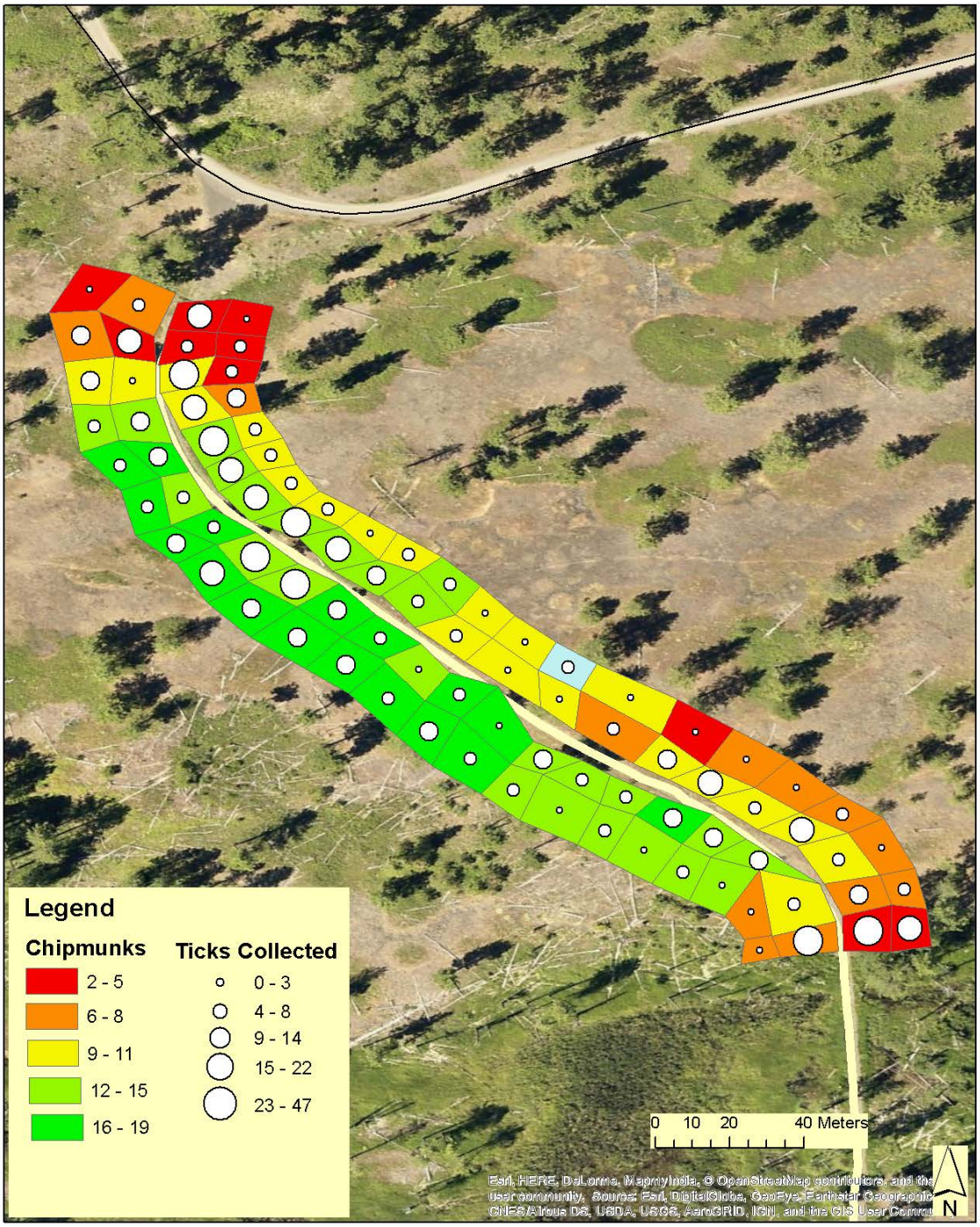


Figure 11: Habitat usage of Chipmunks across the study site in relation to the total number of ticks collected in each zone.

.

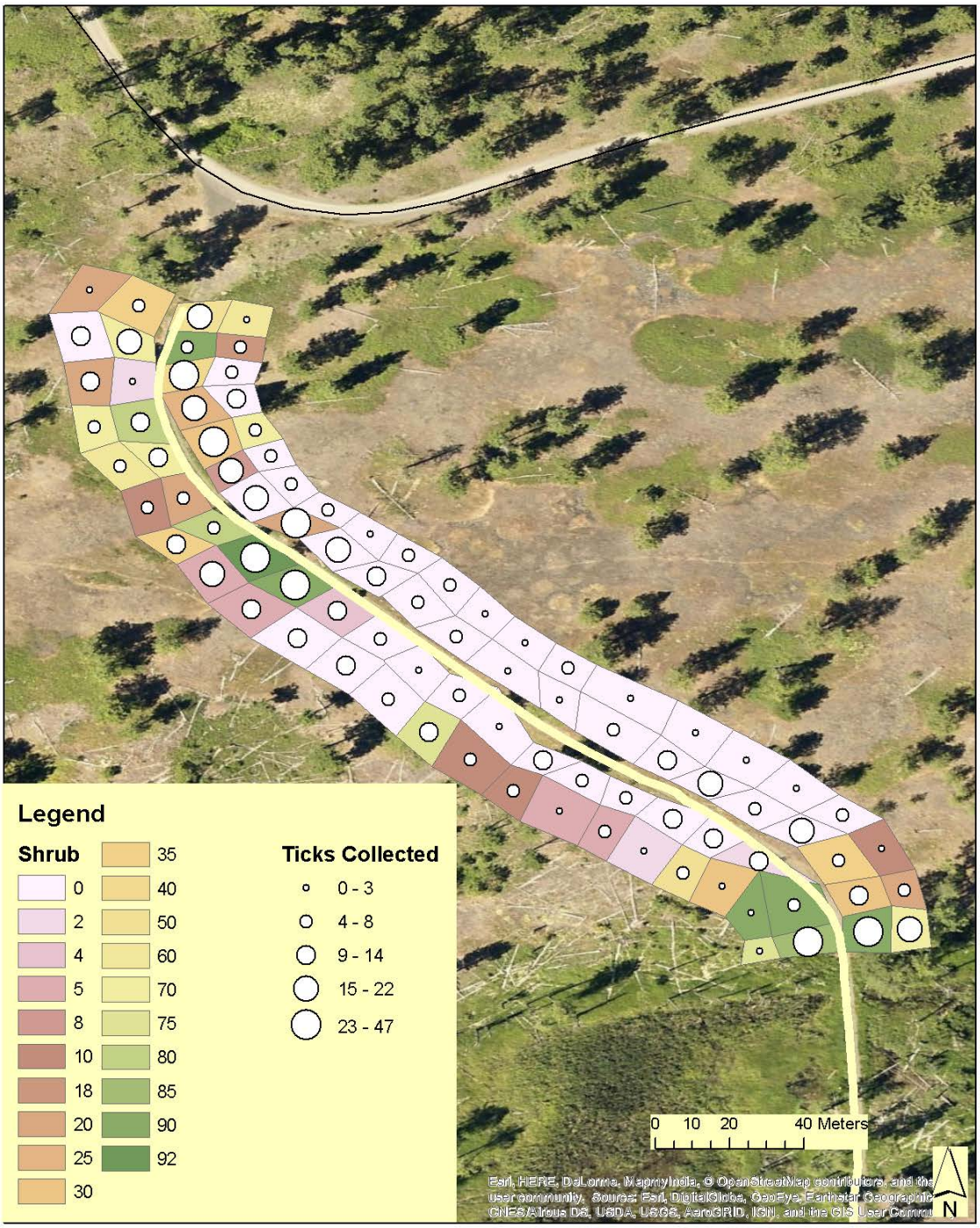


Figure 12: Map showing shrub vegetation percentages within the study area in relation to the total number of ticks collected in each zone.

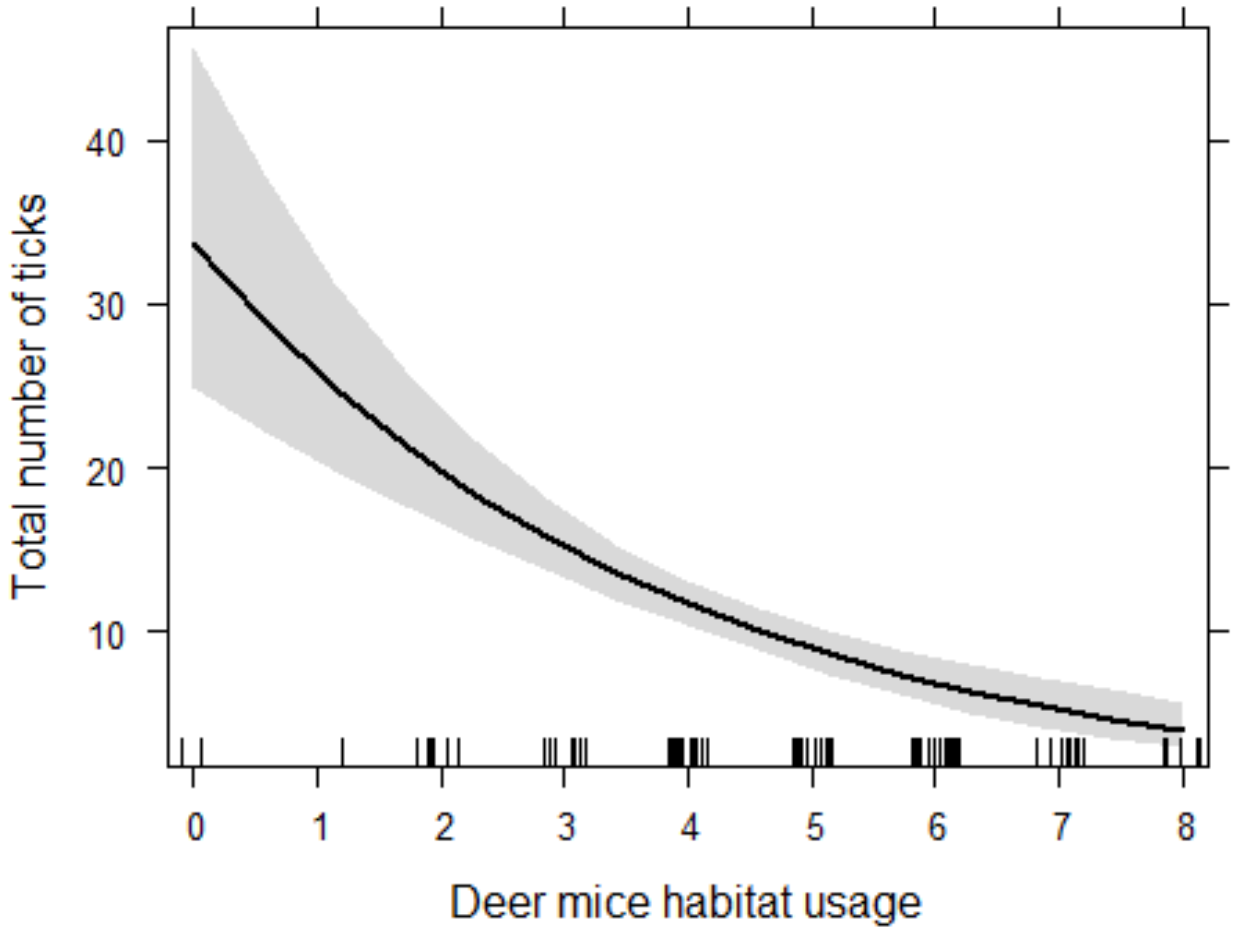


Figure 13: Total number of ticks collected per zone as a function of deer mice habitat usage. The gray band indicates the 95% confidence interval based on Poisson regression.

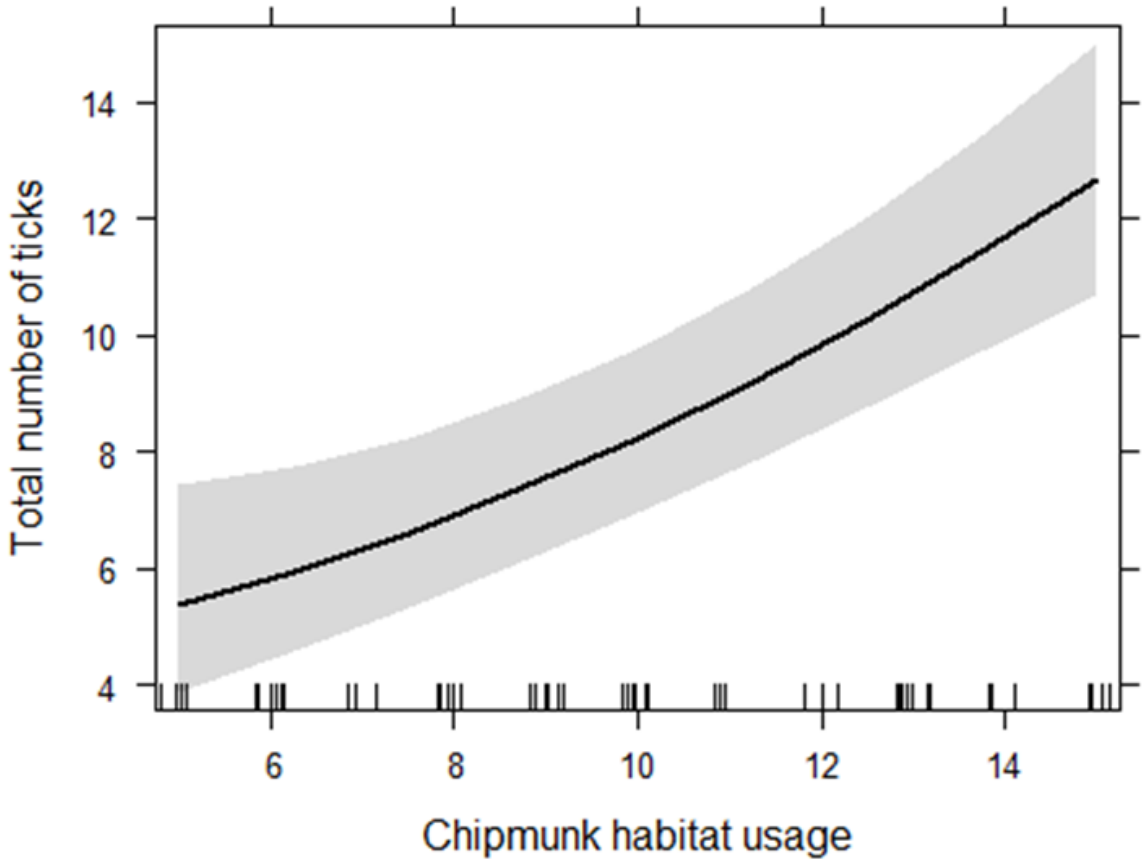


Figure 14: Total number of ticks collected per zone as a function of chipmunk habitat usage. The gray band indicates the 95% confidence interval based on Poisson regression

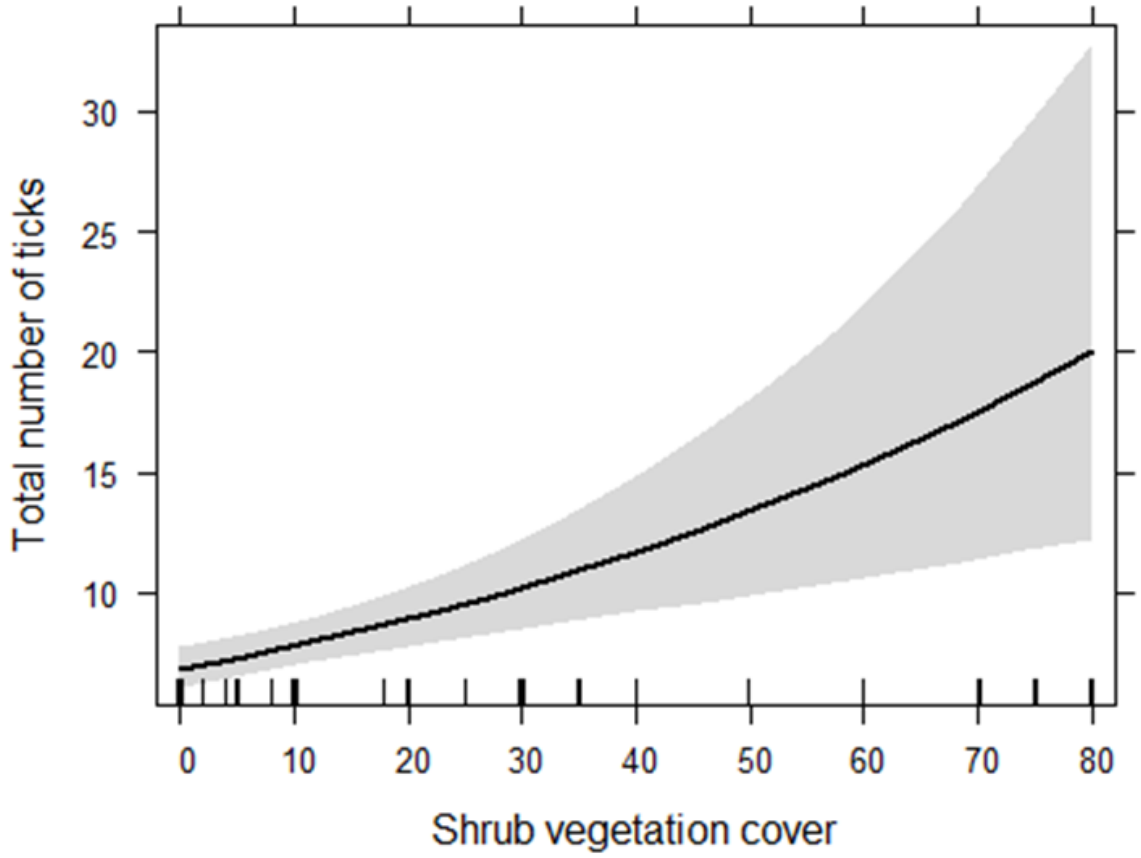


Figure 15: Total number of ticks collected per zone as a function of percent of shrub cover. The gray band indicates the 95% confidence interval based on Poisson regression.

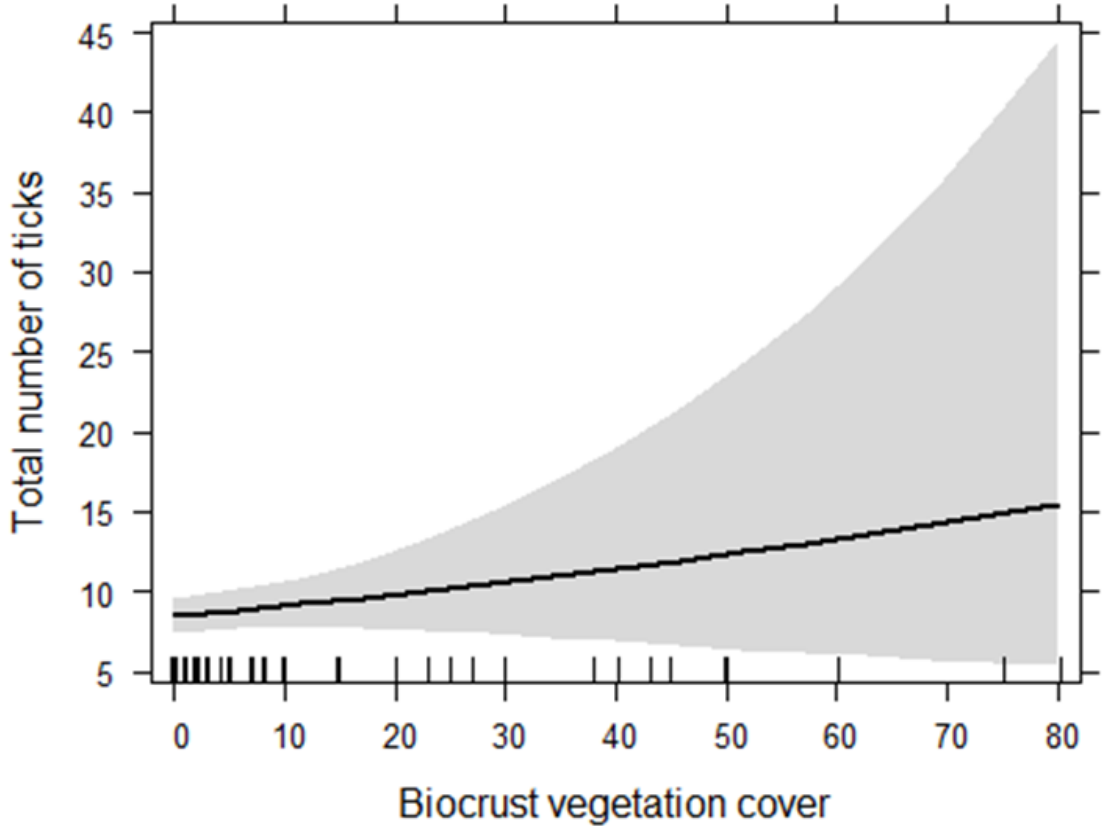
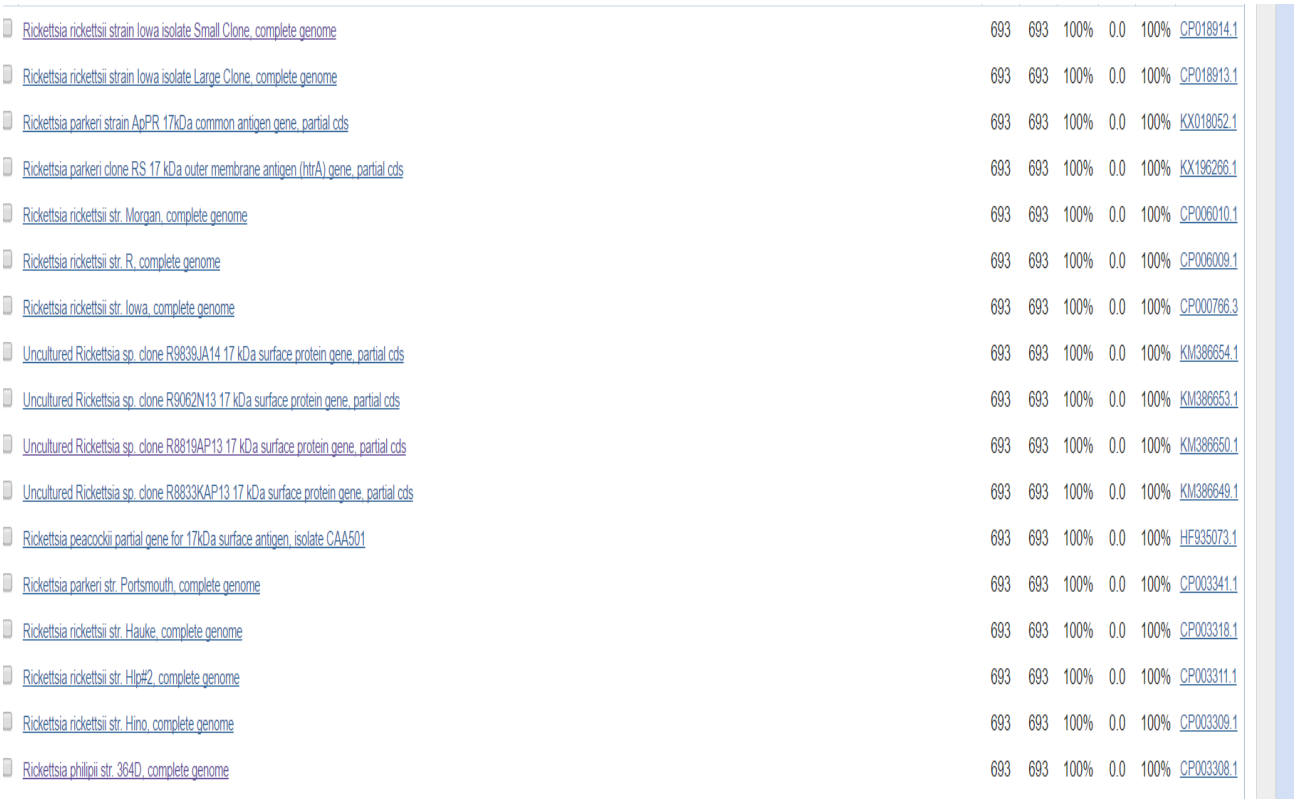
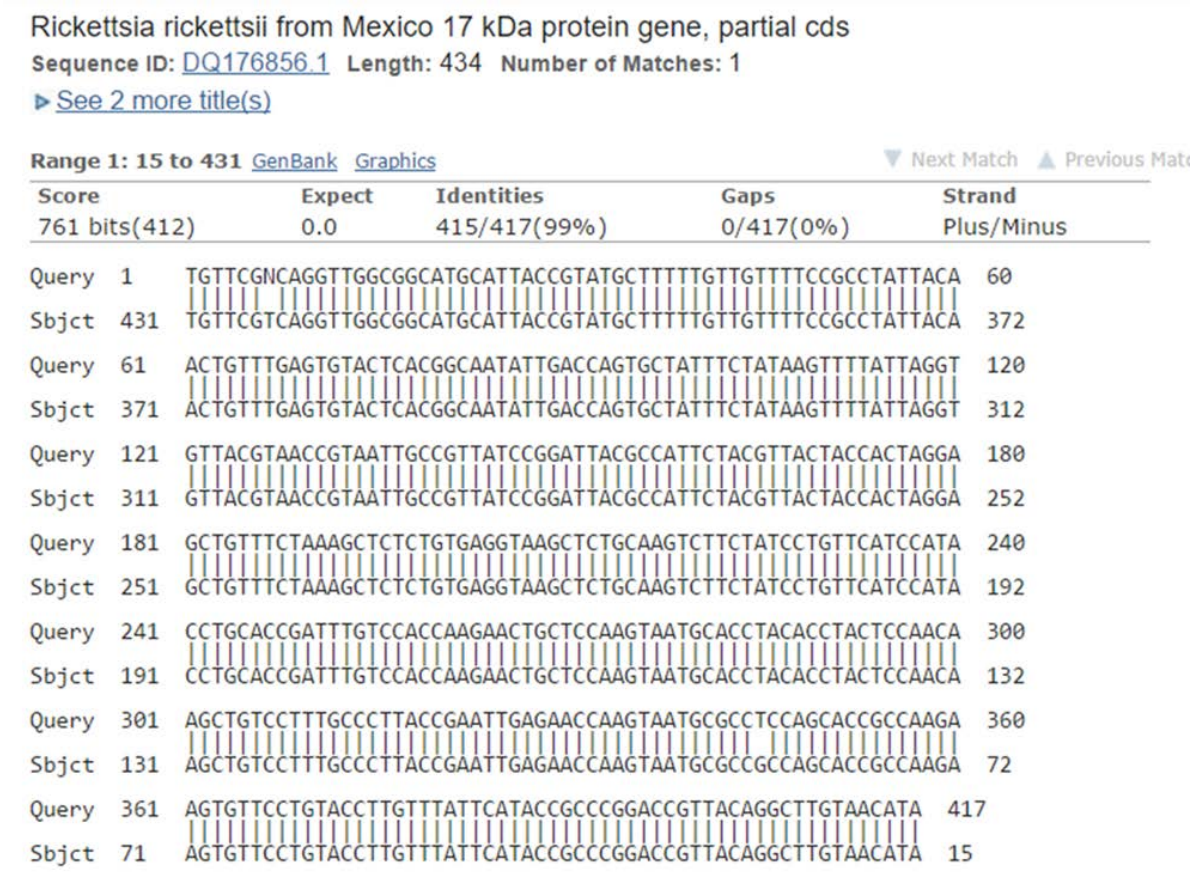


Figure 16: Total number of ticks collected per zone as a function of percentage of biocrust. The gray band indicates the 95% confidence interval based on Poisson regression.



Rickettsia rickettsii strain Iowa isolate Small Clone, complete genome	693	693	100%	0.0	100%	CP018914.1
Rickettsia rickettsii strain Iowa isolate Large Clone, complete genome	693	693	100%	0.0	100%	CP018913.1
Rickettsia parkeri strain ApPR 17kDa common antigen gene, partial cds	693	693	100%	0.0	100%	KX018052.1
Rickettsia parkeri clone RS 17 kDa outer membrane antigen (htrA) gene, partial cds	693	693	100%	0.0	100%	KX196266.1
Rickettsia rickettsii str. Morgan, complete genome	693	693	100%	0.0	100%	CP006010.1
Rickettsia rickettsii str. R, complete genome	693	693	100%	0.0	100%	CP006009.1
Rickettsia rickettsii str. Iowa, complete genome	693	693	100%	0.0	100%	CP000766.3
Uncultured Rickettsia sp. clone R9839JA14 17 kDa surface protein gene, partial cds	693	693	100%	0.0	100%	KM386654.1
Uncultured Rickettsia sp. clone R9062N13 17 kDa surface protein gene, partial cds	693	693	100%	0.0	100%	KM386653.1
Uncultured Rickettsia sp. clone R8819AP13 17 kDa surface protein gene, partial cds	693	693	100%	0.0	100%	KM386650.1
Uncultured Rickettsia sp. clone R8833KAP13 17 kDa surface protein gene, partial cds	693	693	100%	0.0	100%	KM386649.1
Rickettsia peacockii partial gene for 17kDa surface antigen, isolate CAA501	693	693	100%	0.0	100%	HF935073.1
Rickettsia parkeri str. Portsmouth, complete genome	693	693	100%	0.0	100%	CP003341.1
Rickettsia rickettsii str. Hauke, complete genome	693	693	100%	0.0	100%	CP003318.1
Rickettsia rickettsii str. Hlp#2, complete genome	693	693	100%	0.0	100%	CP003311.1
Rickettsia rickettsii str. Hino, complete genome	693	693	100%	0.0	100%	CP003309.1
Rickettsia philipii str. 364D, complete genome	693	693	100%	0.0	100%	CP003308.1

Figure 17: BLASTN result for tick number 81 showing hits to the Spotted Fever group (SFG) rickettsia species. The e score (693) and the percent match (100%) is the same for these SFG showing that this may be a conserved area of the *ompB* gene shared by these bacteria. Some of these bacteria are *R. rickettsii*, *R. parkerii*, and *R. philipii*.



Rickettsia rickettsii from Mexico 17 kDa protein gene, partial cds

Sequence ID: DQ176856.1 **Length:** 434 **Number of Matches:** 1

▸ See 2 more title(s)

Range 1: 15 to 431 GenBank Graphics ▼ Next Match ▲ Previous Mat

Score	Expect	Identities	Gaps	Strand
761 bits(412)	0.0	415/417(99%)	0/417(0%)	Plus/Minus

```
Query  1    TGTTCGNCAGGTTGGCGGCATGCATTACCGTATGCTTTTTGTTGTTTTCCGCCTATTACA  60
            |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  431  TGTTCGTCAGGTTGGCGGCATGCATTACCGTATGCTTTTTGTTGTTTTCCGCCTATTACA  372

Query  61   ACTGTTTGAGTGTACTCACGGCAATATTGACCAGTGCTATTTCTATAAGTTTTATTAGGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  371  ACTGTTTGAGTGTACTCACGGCAATATTGACCAGTGCTATTTCTATAAGTTTTATTAGGT  312

Query  121  GTTACGTAACCGTAATTGCCGTTATCCGGATTACGCCATTCTACGTTACTACCACTAGGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  311  GTTACGTAACCGTAATTGCCGTTATCCGGATTACGCCATTCTACGTTACTACCACTAGGA  252

Query  181  GCTGTTTCTAAAGCTCTCTGTGAGGTAAGCTCTGCAAGTCTTCTATCCTGTTCATCCATA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  251  GCTGTTTCTAAAGCTCTCTGTGAGGTAAGCTCTGCAAGTCTTCTATCCTGTTCATCCATA  192

Query  241  CCTGCACCGATTTGTCCACCAAGAACTGCTCCAAGTAATGCACCTACACCTACTCCAACA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  191  CCTGCACCGATTTGTCCACCAAGAACTGCTCCAAGTAATGCACCTACACCTACTCCAACA  132

Query  301  AGCTGTCCTTTGCCCTTACCGAATTGAGAACCAAGTAATGCGCCTCCAGCACCGCCAAGA  360
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  131  AGCTGTCCTTTGCCCTTACCGAATTGAGAACCAAGTAATGCGCCGCCAGCACCGCCAAGA  72

Query  361  AGTGTTCCTGTACCTTGTTTATTCATACCGCCCGGACCGTTACAGGCTTGTAACATA  417
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  71   AGTGTTCCTGTACCTTGTTTATTCATACCGCCCGGACCGTTACAGGCTTGTAACATA  15
```

Figure18: BLASTN result for Tick number 10. It shows that the sequence is a 99% match to the 17kDa *ompB* gene on file for *R. rickettsia* from Mexico.

Table 1: Total captures of small mammals. **Table 1-A** This table shows **all** the animals captured in each of the 6 weeks of trapping. PEMA is the *Peromyscus maniculatus* commonly called the Deer Mouse. TAAM is the *Tamias amoenus* commonly called the Yellow Pine Chipmunk. **Table 1-B** This table shows all the **first time** captured animals in each of the 6 weeks of trapping. The abbreviations are the same as in Table 1-A.

Table 1-A

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PEMA	7	22	16	17	12	36
TAAM	56	62	41	13	7	14

Table 1-B

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PEMA	7	6	6	8	9	0
TAAM	34	8	2	2	1	0

Table 2: Analysis of deviance table for the final Poisson regression model for the total number of ticks collected in each zone. P-values test the null hypothesis that the addition of the listed predictor variable or interaction does not reduce the deviance significantly, using a Chi-square approximation. Bolded p-values are lower than the significance level of 0.05.

Predictor variable	p-value
Deer mice habitat usage	0.0001304
Yellow-pine chipmunk habitat usage	3.436e-13
Shrub vegetation cover	< 2.2e-16
Biocrust vegetation cover	0.0124468
Deer mice habitat usage x Yellow-pine chipmunk habitat usage	0.0019148
Deer mice habitat usage x Shrub vegetation cover	0.7273643
Yellow-pine chipmunk habitat usage x Shrub vegetation cover	0.1775907
Deer mice habitat usage x Biocrust vegetation cover	0.7478732
Yellow-pine chipmunk habitat usage x Biocrust vegetation cover	0.3200438
Shrub vegetation cover x Biocrust vegetation cover	0.0617408
Deer mice habitat usage x Yellow-pine chipmunk habitat usage x Shrub vegetation cover	0.4160123
Deer mice habitat usage x Yellow-pine chipmunk habitat usage x Biocrust vegetation cover	0.0014025
Deer mice habitat usage x Shrub vegetation cover x Biocrust vegetation cover	0.0021315
Yellow-pine habitat usage x Shrub vegetation cover x Biocrust vegetation cover	0.5707964
Deer mice habitat usage x Yellow-pine chipmunk habitat usage x Shrub vegetation cover x Biocrust vegetation cover	0.1027415

APPENDIX

DNA Isolation Protocol with DNAzol (For Ticks)

1. HOMOGENIZATION
    a. Homogenize ticks for 10 seconds with 5-10 Zirconium beads (depending on tick size) in .500mL of DNAZOL reagent.
        i. It may be necessary to do multiple homogenizations at 10 second intervals. In 10 second intervals, homogenize until the abdomens are visibly opened. Try to minimize this as excessive heat can denature the DNA.
        ii. If necessary, use a flame sterilized scalpel to longitudinally cut the tick in half to expose the tick's gut.
    b. Incubate the homogenized samples for 10 minutes at room temperature
2. PHASE SEPARATION
    a. Centrifuge the samples for 10 minutes at >5,000g at 4°C
    b. Following centrifugation, transfer the resulting viscous supernatant to a fresh tube, careful to not transfer exoskeleton remains.
3. DNA PRECIPITATION
    a. Add 0.5mL of 100% ethanol per 1mL of DNAzol used to the tube containing the fresh supernatant
    b. Mix samples to form a homogenous solution by inverting tubes 5-8 times
    c. Incubate samples for 3 minutes at room temperature
        i. DNA should quickly become visible as a cloudy precipitate
    d. Centrifuge the precipitated DNA at >5,000g for 5 minutes at 4°C
        i. This should produce a gel-like whitish pellet on the side and bottom of the tube
        ii. Remove supernatant and discard
4. DNA WASH
    a. Add 1.0mL of 75% ethanol
    b. Mix the samples by vortexing then centrifuge at 5,000g for 2 minutes at 4°C
    c. Discard the ethanol
    d. Repeat steps 4a-4c
    e. Quick spin the tubes and use a pipette to discard extra ethanol at the bottom of the tubes
5. DNA SOLUBILIZATION
    a. Dissolve DNA
        i. Add 0.05mL TE
        ii. Agitate sample by flicking
        iii. Store samples in -20° freezer

VITA

Author: Robert E. Shadix Jr.

Place of Birth: San Pablo, Seville Spain

Undergraduate Schools Attended:

Eastern Washington University
Community College of the Air Force

Degrees Awarded:

Bachelor of Science, 2015, Eastern Washington University
Associates Degree, 2002, Community College of the Air Force

Honors and Awards:

Graduate Assistantship, Biology Department, 2015-2017, Eastern Washington University

Eastern Washington Biology Department Mini Grant, 2015

Professional Experience:

TATE, Inc, Survival Evasion Resistance and Escape Instructor, Spokane Wa 2006-2012

United States Air Force, Survival Evasion Resistance and Escape Instructor, Fairchild AFB. WA, 1992-2006