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High resolution modeling of tick density and detection of Rickettsia spp. in Dermacentor spp. ticks at Turnbull National Wildlife Refuge, WA

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HIGH RESOLUTION MODELING OF TICK DENSITY AND DETECTION OF *RICKETTSIA SPP.* IN *DERMACENTOR SPP.* TICKS AT TURNBULL NATIONAL WILDLIFE REFUGE, WA

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By

Justin L. Donahue

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The tick species *Dermacentor andersoni* and *Dermacentor variabilis* are known vectors of pathogens. One such pathogen is the bacteria *Rickettsia rickettsii*, which causes Rocky Mountain spotted fever. The detection of this bacterium in ticks at Turnbull National Wildlife Refuge (TNWR) indicates a potential risk to human health. In order to follow up on this discovery, we had two separate objectives. First we developed a high resolution predictive map for *Dermacentor spp.* distribution across the public use area of TNWR. To do so, 50m transects (27 in total) were established across the public use area. Ticks were collected weekly within the transects from March 26th – June 5th, 2018. The transects were characterized by measuring percent vegetative cover, small mammal abundance, and large mammal usage (20 transects). Additionally, land cover class and 2017 burn status were determined for each transect. Generalized linear modeling (GLM) was used to evaluate which factors were the strongest predictors of *Dermacentor spp.* abundance. The strongest predictors included 2017 burn status, percent forb cover, percent shrub cover, and land cover class. All factors with the exception of forb cover were mapped across the public use area of TNWR at a 10m resolution using LiDAR and data from TNWR. These predictors were used with the corresponding GLM to map *Dermacentor spp.* density across the public use area of TNWR at a 10m resolution. The

highest predicted *Dermacentor* density occurred in open shrubland cover class with high shrub density, which was not burned in 2017. To confirm previous detections of *Rickettsia rickettsii* in ticks, 452 *Dermacentor* ticks collected in 2018 were tested for *Rickettsia spp.* by PCR amplification of the *rOmpB* gene fragment. Ticks that were putatively infected with *R. rickettsii* (positive for *rOmpB*) were further assessed by amplifying fragments of the *gltA* and *rOmpA* genes. Sequencing of the *rOmpB* gene fragment showed 21 ticks positive for *Rickettsia spp.* and 6 ticks positive for *R. rickettsii*. However, both gene fragments (*gltA* and *rOmpA*) were positive for *Rickettsia peacockii*, a non-pathogenic *Rickettsia spp.* that may also block the infection of *R. rickettsii* in ticks. While our results are inconclusive, they suggest that *Rickettsia rickettsii* is likely not present at TNWR.

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1.1 INTRODUCTION

Vector-borne diseases are of growing concern throughout the world. The two most threatening vectors to human populations are mosquitoes and ticks. In the United States, there is more than double the number of tick-borne disease cases relative to those transmitted by mosquitoes (Rosenberg et al. 2018). Tick-borne disease cases continue to rise, nearly doubling in the last 10 years to ~60,000 cases annually in the U.S. (CDC 2017). The ability of ticks to pass disease-causing pathogens through a single bite makes them effective vectors (Anderson and Magnarelli 2008). Because many tick-borne diseases begin with non-specific symptoms, it is likely that many infections are unreported (Schiffman et al. 2018). Common tick-borne diseases include Lyme disease, spotted fever rickettsiosis, babesiosis, and tularemia (Rosenburg et al. 2018).

The rising number of tick-borne disease cases can be attributed to two main reasons. One, as human populations continue to grow, urbanization is pushing communities into forested areas. This increases human crossover with the animal community, increasing human exposure to ticks and the pathogens they may carry (Uspensky 2014). Secondly, as overall climate warming trends continue, ticks and pathogens continue their expansion into once unsuitable regions. Host reservoirs' ranges are shifting with climate warming, facilitating tick and pathogen movement into these environments (Kilpatrick and Randolph 2012). Such is the case with Lyme disease as the

primary host, the white-footed mouse (*Peromyscus leucopus*), continues its northward expansion into Canada due to shorter and milder winters. With an estimated 80% of white-footed mice infected with the causative bacteria of Lyme disease (*Borrelia burgdorferi*), the uninfected ticks that reside in these locations are becoming infected and able to transmit the bacteria (Roy-Dufresne et al. 2013). This has resulted in an increase of Lyme disease cases along the northern expansion border of the white-footed mouse, creating an elevated public health risk (Bouchard et al. 2015).

Nearly all Ixodidae (hard-bodied) tick species of public health importance are characterized by a three-host life cycle (Figure 1.1). These include species in the genera *Amblyomma*, *Rhipicephalus*, *Dermacentor* and *Ixodes* (CDC 2017). The life cycle of all ticks begins when an engorged female mates, and drops off its' final host to lay thousands of eggs (Matheson 1950). After 2 to 6 weeks the eggs hatch into six-legged larvae and immediately seek out and attach to a first host, typically a small rodent. Once larvae become fully engorged they drop off of the host, digest their meal, and molt into eight-legged nymphs. Nymphs will then begin seeking a second host, generally another rodent or lagomorph. After attachment, nymphs will take over a week feeding on the host before becoming fully engorged (Loomis 1961). Following engorgement, nymphs drop off of the host, digest the blood meal, and molt into adults. Adults begin searching for a third and final host, typically a large mammal. Once an adult is attached to the third and final host it begins taking a final blood meal before searching for a mate on the host. When a mate is found, copulation occurs, and the female tick drops off of the host to lay eggs and restart the life cycle. During any stage of the life cycle ticks may go into a period of suspended development and energy conservancy known as diapause (Belozerov 1982).

While in diapause ticks can survive harsh climatic conditions such as extreme heat or overwintering with a survival rate over 60% (Brunner 2012, Cerny er al. 1982). Under ideal conditions Ixodidae tick species can complete their life-cycle in less than 170 days (Troughton and Levin 2007). When no hosts are present, the ability to enter diapause at any life stage can extend the life-cycle to greater than 4 years. During favorable conditions, ticks break diapause and begin searching for a host.

The process of ticks actively searching for a host is known as questing. Questing is accomplished by two different strategies that vary by species. In the hunter strategy ticks remain hidden, but once stimulated by host odorants or CO_2 they emerge and actively chase the host (Hess and De Castro 1986). The majority of Ixodidae tick species use the more common ambush strategy. Using this strategy, ticks crawl up emergent vegetation and extend their front legs waiting for a passing host. When an unsuspecting host brushes against the vegetation, the tick will latch on and move to a feeding location. Ticks can remain on vegetation for multiple hours at a time as long as they maintain appropriate water balance. Once this water balance threshold is met they will descend into a humid microenvironment where they reabsorb water before returning to vegetation to continue questing. This cycle is maintained until climate becomes too hot/dry or too cold. At this point they will die if they do not enter into diapause to sustain the conditions. When suitable conditions return the next year, ticks will reemerge and continue questing (Sonenshine and Roe 2014).

The seasonal periodicity of ticks is mediated by two main factors, photoperiod and temperature. Photoperiod is the hypothesized mechanism that cues the emergence of ticks from diapause (Belozerov et al. 2002). Through evolutionary time, ticks have

evolved adaptive behaviors that allow them to align questing activity to favorable times of the years when seasonal temperatures allow maximum questing efficiency. They avoid times of high temperature and low humidity (e.g. summer) or when temperatures are too low for questing (e.g. winter), to increase their survival (Burg 2001). Seasonal cues mediated by photoperiod allow ticks to enter diapause before natural occurring conditions become unfavorable for questing (Smith and Cole 1941). At a daily level, temperature is the important factor mediating questing behavior. Daily temperature affects a tick's ability to maintain water balance, determining times of activity throughout the day. Burg (2001) showed temperatures below 10°C significantly reduced the number of host-seeking *Dermacentor variabilis* adults. At the other end of the spectrum, surface temperatures over 24°C cease questing activity of *Dermacentor occidentalis* adults (Lane et al. 1995). This allows for two peaks of tick density in most areas of the United Sates, as ticks are active from early spring to late fall (Clark et al. 1998, Goddard and Paddock 2005). In the Western U.S., where high temperature and low humidity persists during the summer, there tends to be one peak of tick density. Ticks tend to be active from early spring to mid-summer, where they enter diapause and do not reemerge until the following year (Eisen 2007).

As ticks begin questing for passing hosts, vegetation becomes a crucial component. Tall vegetation such as grass, forbs and shrubs provides a rigid structure for adult ticks to ascend as they wait for a passing host. These types of vegetation support high questing tick densities in many regions (Shadix 2016, Micher and Rockett 1993, Dodds 1969). Shrubs can also be a primary and secondary food source of large mammal hosts (McCorquodale 1993, Campbell and Johnson 1983). Dense shrubs provide cover

for small mammals as they forage for food, creating higher small mammal densities (States 1976). Vegetation can create a favorable humid microenvironment, increasing tick survival and questing efficiency (Eisen et al. 2006). Therefore the presence of tall vegetation, such as shrubs, can be beneficial to ticks and mammals alike, potentially creating a hot spot of tick activity. Knowing the importance of these factors, it is reasonable to believe tick density could be predicted based off field-derived biotic measurements.

Many researchers in the past have attempted to predict habitat suitability for ticks. The majority have used maximum entropy algorithm (MaxEnt) to construct habitat suitability models (Warren and Seifert 2011). MaxEnt models are generally constructed in ArcGIS using land cover and climatic data from public sources such as Landsat and WorldClim.org. Data attributed to tick distribution, such as land cover class, elevation, temperature and humidity, are then extracted from these sources and used in the habitat suitability prediction. Presence only data is then collected from passive surveillance, such as ticks reported to local health departments from physicians or the public. Presence only data is incorporated with land cover and climatic variables to produce predicted habitat suitability maps at various scales. Passive surveillance data has many limitations and potential biases such as uncertainty of collection location, a bias towards populated areas, and variation in regional collection programs (Johnson et al. 2004, Ogden et al. 2015). This approach is commonly used to predict *Ixodes spp.* expanding habitat suitability due to climate change at a 1km resolution (Cheng et al. 2017, Brownstein et al. 2005). Researchers have also used MaxEnt to predicted *Dermacentor spp.* habitat at a 500m resolution (Atkinson et al. 2012). While these broad-scale predictive models are useful

for identifying larger areas (e.g. counties) where a vector may be present, they provide little usefulness for public health on a fine scale. In attempt to provide more localized information researchers have developed models to predict *Ixodes* ticks in state parks (Brownstein et al. 2003) and questing *Ixodes* ticks in small, forested areas (Khatchikian et al. 2012). To date, a high resolution habitat suitability model at a 15m resolution was accomplished by Soucy et al. (2018) to predict *Ixodes scapularis*, marking the highest resolution documented.

Recent advancements in remote sensing technologies give the potential to map habitat at a minute scale. LiDAR (Light Detection and Ranging) is a new remote sensing technology that is lacking in tick habitat suitability studies. LiDAR data is collected during LiDAR "flights." During flights the LiDAR instrument emits light pulses to the Earth's surface, which refract off of the surface structure back to the LiDAR instrument (Figure 1.2). The travel time of each light pulse is then used to calculate the height of the structure of which the pulse refracted from, resulting in point clouds (Dubayah and Drake 2000). Each pulse is within <1m of each other giving a high point density, with extremely accurate height for each point (NOAA 2012). LiDAR point clouds can then be used in a multitude of ways to look at different vegetative characteristics across a landscape (Martinuzzi et al. 2009). The ability to gather vegetative characteristics at a scale of 1m using LiDAR presents a unique opportunity to map habitat suitability at a higher resolution than previous research has achieved. LiDAR data is readily available for many areas in Washington State through the Puget Sound LiDAR Consortium (pugetsoundlidar.ess.washington.edu).

In Washington State there are 3 common Ixodidae tick species. These species include *Ixodes pacificus* (Western black-legged tick), *Dermacentor variabilis* (American dog tick), and *Dermacentor andersoni* (Rocky Mountain wood tick) (WDOH 2019). *Ixodes pacificus*, the state vector for *Borrelia burgdoferi,* the etiological agent for Lyme disease, is found primarily west of the Cascade Mountains (Eisen et al. 2016). Both *Dermacentor variabilis* and *Dermacentor andersoni* are found throughout Eastern Washington (James et al. 2006, Easton et al. 1977). These *Dermacentor* species are capable of vectoring pathogens that cause human diseases such as spotted fever rickettsiosis (including Rocky Mountain spotted fever) and tularemia (CDC 2019). Both *Dermacentor* species also vector important cattle diseases such as bovine Anaplasmosis (Kocan et al. 1981).

Previous researchers have collected both *Dermacentor andersoni* and *Dermacentor variabilis* at Turnbull National Wildlife Refuge (TNWR) in Eastern Washington. In 2016, 829 *Dermacentor* ticks were collected on the 30-Acre Lake trail in the public use area of TNWR. Of these, 472 ticks were analyzed for the presence of *Rickettsia* species. *Rickettsia rickettsii*, the etiological agent for Rocky Mountain spotted fever, was detected in roughly 1% of these ticks (Shadix 2016). Rocky Mountain spotted fever has the highest fatality rate of any tick-borne disease (Chapman et al 2006), indicating a threat to visitors of TNWR. This presents a need to predict tick density for *Dermacentor* species within the public use area of TNWR.

The purpose of this research was to evaluate factors that influence *Dermacentor spp.* density within the public use area of TNWR through active field surveillance in order to create a high resolution predictive map for questing tick density. The

significance of these factors were determined using generalized linear modeling. GIS layers were created for significant factors using LiDAR and other data sources. These layers were then used in correspondence with a generalized linear model to create a predictive questing *Dermacentor spp.* density map at a 10m resolution within the public use area of TNWR.

1.2 METHODS

Study Site

TNWR is a federal wildlife refuge located in Spokane County, 20 miles southwest of Spokane, WA. The public use area is located in the southeast corner of TNWR and encompasses 3,276 acres of the 18,000 acre refuge (Figure 1.3). This area of Channeled Scabland is representative of much of the Inland Northwest as it contains basalt outcroppings, ponderosa pine forests, shrub-steppe habitat and aspen stands along many wetlands, marshes and lakes. The area is vital for many migratory nesting waterfowl and boasts local residents such as elk, moose, and deer. Each year the public use area attracts over 30,000 visitors to enjoy numerous hiking trails, and to photograph wildlife in their natural habitat (USFWS 2017).

Vegetation

Land cover classes were determined based on a classification system used by TNWR. A land cover shapefile from 1993 was acquired from TNWR and used in ArcGIS to preliminarily establish transects (Figure 1.4). In total, 30 transects were established among the 6 dominant vegetation classes (5 transects per class) present (Figure 1.5). Transects were 50m in length. Land cover classes consist of meadow (M), open

shrubland (S), closed pine (CP), open pine (OP), wetland vegetation (W) and aspen (A). Each transect was field-confirmed for land cover class prior to starting the study.

In 2017, TNWR burned approximately 170 acres of the public use area during a prescribed burn. This process removed all understory vegetation during our collection period. Two transects were established in the burned areas to determine how burning affected tick density. Each transect was given a value relative to its' 2017 burn status (0 = no burn, 1 = burn).

Within each transect, percent cover of each vegetation type was measured using the line interception method described by Canfield (1941). Vegetation cover measurements were carried out from April 20th, 2018 to June 4th, 2018. Areas of no vegetation were classified as "charred" (areas of burned ground), "litter" (areas of leaf litter), or "ground_other" (moss, rock, bare ground, etc.). Areas where vegetation was present were classified accordingly as either "grass", "forb" or "shrub." All wetland vegetation was combined into "wetland_veg." Percent cover for each vegetation class was calculated across each transects.

Tick Collections

Ticks were collected weekly at each transect during a 10 week period when ticks are most active in the area: March 26th, 2018 to June 5th, 2018. Each transect was sampled weekly over the 10 week period. The three transects where ticks were most abundant (S4, OP1, and CP1) were sampled an additional six times (once weekly for six weeks) until July 20th, 2018 to determine when tick activity ceased. All ticks were collected using standardized dragging techniques. With this technique, a 1m x 1m corduroy cloth is

dragged across the ground and vegetation, collecting any questing ticks. All collections were made between 1200 and 1430 hours. Prior to collection, both temperature and humidity were measured at the transect using a Lab Quest 2 Vernier Probe (www.vernier.com). During dragging the collector walked along the transect line, stopping every 10m to collect ticks present on the drag cloth and/or themselves. Genus and gender were determined for collected ticks before they were preserved in 70% ethanol for future DNA extraction. Within 24 hours of collection ticks were stored at -20°C.

Small Mammal Abundance

To investigate the relationship between small mammals and questing tick density, a trap line method (Malcolm 1988) was used to obtain overall small mammal abundance within each transect. To reduce the impact of seasonal activity, all trapping occurred during a 5 week period: April 30th, 2018 to June 1st, 2018. Trapping occurred for five subsequent days at each transect using standard sized (7.5 x 9 x 23 cm) Sherman live traps (www.shermantraps.com). Traps were arranged such that they encompassed the entire transect (Figure 1.6). A total of 18 traps were used at each transect. Each trap was baited with an oat and peanut butter mixture, and insulated with polyester fiber. Traps were initially set Monday morning prior to 0900. After initial set-up, traps were checked each afternoon by 1800 and each morning by 0900. During abnormal weather conditions (e.g. extreme heat or extreme cold), traps were checked an additional time during the day. Following trap check on Friday evening, traps were collected and placed at new locations the following Monday. Captured small mammals were identified to species, weighed and sex was determined. Small mammals were also examined for ectoparasites. All

ectoparasites were removed and stored in 70% ethanol. Each captured individual was marked with a non-toxic permanent marker so that subsequent recaptures could be noted. The total number of small mammals captured at each transect was used for relative abundance. A Spearman's rank correlation test was used to test for any significance between small mammal abundance and total tick numbers.

Large Mammal Usage

Motion detecting game cameras were used to observe large mammal usage within each transect. Four Stealth Cam Sniper Shadow (www.stealthcam.com) and two Moultrie Panoramic 150 Game Cameras (www.moutriefeeders.com) were used. Both types of game cameras have the ability to take daytime and nighttime images. The Moultrie game cameras were panoramic and therefore had a wider radius of capture. During the study period (March 26th – June 5th, 2018) 20 of our 30 transects were monitored for large mammal usage. The other 10 transects were in close proximity of trails or roads so camera observations were forgone. During monitoring, one game camera was affixed to a nearby tree in a way to best cover the entire transect. Of the 20 transects, 14 were observed for seven consecutive days, and six were observed for 14 days; two separate seven day periods. Usage of large mammals at each transect was calculated by dividing the total number of large mammals observed by the number of weeks in the observation period. A Spearman's rank correlation test was used to test for any significant correlation between large mammal usage and total tick numbers.

Statistical Model

A statistical model for tick abundance was created using the software package R (R Core Team 2013). A generalized linear model (GLM) was constructed with total tick abundance at each transect over the 10 week study period as the response variable, assuming a Poisson distribution. We used each of our field measurements within each transect as predictor variables. These included land cover class, 2017 burn status, small mammal abundance and large mammal usage. The percent cover for each vegetation type were also used as predictor variables. This included charred (CH), litter (L), ground other (G_OT), wetland vegetation (WL), shrubs (S), forbs (F), and grass (GR). Temperature and humidity data were excluded from the models because they were uniform within the transects across the study period. Three of our 30 transects remained flooded throughout the entire study period and were removed from the model. This included three transects without large mammal data. Only data collected between weeks 1-10 was used in the model. The inability to acquire large mammal data for all 27 transects required generating two sets of models. The first included large mammals but excluded the seven transects where no data was collected. The second excluded large mammals, but included all other data for each of the 27 transects. GLMs were established for each of the two sets of models. The dredge function in R package 'MumIn' (Barton 2012) was used to run all possible model combinations. A correction of the Akaike information criterion (AICc) values were used to determine the best combination of predictor variables (Zuur et al. 2007). An ANOVA was used to determine the significance of each predictor variable within the GLMs.

Mapping Tick Density

A geospatial model was created for tick density using the software package R and ArcGIS 10.6. The 'importance' function in R Package 'MumIn' was used to evaluate the relative importance of each predictor variable. Importance is calculated as the standardized sum of the AIC weights of all models including the predictor variable. Those predictors deemed important were evaluated for their ability to be mapped in ArcGIS. Mapping of those variables was completed where feasible.

LiDAR data was used to map shrub density across the public use area. LiDAR point clouds for the public use area of TNWR were retrieved from Puget Sound LiDAR Consortium (pugetsoundlidar.ess.washington.edu). This LiDAR flight was conducted by Watershed Sciences, Inc. (WSI) for TNWR between October 2012 and July 2013. The LiDAR survey utilized a Leica ALSS60 sensor mounted in a Cessna Caravan 208B. Vertical accuracy was reported by WSI to be 0.01m. Average LiDAR point spacing was 0.33m. LiDAR points were first separated into ground and non-ground points returns using the Multi-scale Curvature Classification (Evans and Hudak 2007). Ground points were removed from the surface. All non-ground points were classified by height into three separate categories; class code 3 (0-1.5m), 4 (1.5 – 5m), 5 (5 – 50m). Henceforth, class code 3 was altered to represent shrub points. A height of 1.5m represented our tallest shrub measurement. Points greater than this value were removed from surface. The remaining LiDAR points included a misleading high density in wetland vegetation, in rocky outcroppings, and along the bottoms of trees; likely points reflecting from branches <1.5m in height. To remove these from shrub points, the wetland polygons from the 1993 land cover shapefile was added to the map. Orthoimagery from 2013 at a 1m resolution

was obtained from TNWR and added to the surface. With the aid of the orthoimagery, wetland polygons were edited to best match the 2013 representation of wetland vegetation. The "Set LAS Class Codes Using Features" tool was used to reclassify all LiDAR points within the wetland polygon. Rock outcroppings were heads-up digitized by creating a polygon shapefile around the rock outcroppings using the orthoimagery. This shapefile was then used to reclassify all LiDAR points within the polygon, removing them from the shrub layer. To correct LiDAR points along the bottoms of trees, a polygon for trees was created with the LiDAR points in class codes 4 and 5. "Set LAS Codes Using Features" tool was used again to reclassify all points within the trees polygon at a buffer distance of 1m. Within the southern half of the refuge in open meadow areas, apparent banded artifacts were picking up extremely high density non-ground LiDAR points. Upon field-confirmation of extremely low to no shrub cover in these locations, all points within the meadow classification were also reclassified outside of class code 3. After modifying the dataset to exclude wetlands, rock outcroppings, and meadows, this resulted in all points within class code 3 representing LiDAR points where shrubs are present.

To map shrub density across the public use area of TNWR, LiDAR points within class code 3 were converted to rasters at a cell size of 1m. Each raster cell was reclassified to a value of 1 (data/shrubs present) or 0 (no data/shrubs present). A 10m x 10m fishnet grid was overlaid across the public use area of TNWR. The "Zonal Statistics" function was used to sum all raster values within each 10m x 10m fishnet cell, creating a shrub density raster at a 10m resolution. The shrub density raster was then exported as a TIFF file at 10m x 10m cell size.

A polygon layer for land cover classification was obtained from TNWR. Each polygon was classified in accordance with classification during preliminary transect establishment. After field determination for transects, two polygons were changed from meadow to open shrubland due to their high shrub density. The resulting layer was converted to a 10m x 10m raster at the same extent as the fishnet grid used for calculating tick density. The value of each cell represented one of the seven land cover classifications. The land cover raster was exported as a TIFF file at a 10m x 10m cell size.

A shapefile containing the locations of prescribed burns in 2017 was also obtained from TNWR. This indicated areas where no understory vegetation was present. First, the layer was clipped to the public use area to exclude burns outside of this area. The layer was converted to a raster with a cell size of 10m x 10m at the same extent as the fishnet grid. The raster was then reclassified into the values of 1 (burned) and 0 (no burn). The burn 2017 layer was exported as a TIFF file at a 10m x 10m cell size.

The construction of the predictive tick density layer was created using the ‘raster’ (Hijmans et al. 2011) and ‘rgdal’ (Bivand et al. 2015) packages in R. Each raster layer used in the final model creation was uploaded to R in TIFF format. Due to the limited ability to map all predictor variables across the landscape, only important variables feasible to map were used in the final GIS model (here after gisGLM). The predict function with type “response” was used to predict tick density across the public use area. With the predict function, the gisGLM is used to predict questing tick numbers. The function takes the value associated with each of the three predictor variables in each 10m cell and runs the model with the associated predictor values, resulting in a predicted

questing tick abundance map at a 10m resolution. Predicted questing tick numbers were divided by 500, giving us predicted questing tick density per $1m^2$. The value of 500 was used because we collected for 10 weeks at each of our 50m transects (10x50=500). The resulting predicted questing tick density map was exported as a TIFF file at a 10m resolution and can be used in ArcGIS.

Statistical analysis of predicted questing tick density was also carried out in R. An effects plot was created to show the relationship between each predictor and predicted questing tick numbers. The predict function was used with the gisGLM to predict questing tick numbers at each of our 27 transects. A linear model was used to compare the field collected total tick abundance vs. the predicted total tick abundance using the gisGLM.

1.3 RESULTS

Tick Collections

Over the 10 week period a total of 410 adult ticks and 1 *Dermacentor* nymph tick were collected by dragging. An additional 83 adult *Dermacentor* ticks were collected in weeks 11 through 16 at transects S4, OP1, and CP1. The highest questing tick density was observed at transect S4 (0.198 ticks/m^2). Questing tick density for land cover classes ranked highest to lowest were: open shrubland (0.074 ticks/m^2), open pine (0.032 ticks/m^2), closed pine (0.03 ticks/m^2), meadow (0.016 ticks/m^2), aspen (0.011 ticks/m^2), and wetland vegetation (0.007 ticks/m^2). Within the burned transects (OP5 and CP5), one tick was collected. Questing ticks were active during our first collection week, the week of March 26th, 2018. Questing tick activity peaked during the week of May 28th (study

week nine, Figure 1.7). Questing tick numbers were much lower the following week. This concluded our collections at all 27 transects. In our three most abundant transects (OP1, CP1, and S4), we saw a late season peak of questing tick activity during the week of June 25. During the final week of collection, July 18th, there was minimal tick questing activity.

Vegetation

The percent cover for each vegetation type is presented in Table 1.1. Results were as expected. Transects established in wetland vegetation consisted of >90% wetland vegetation cover. Transects established in meadow vegetation tended to have higher values of grass cover. Open shrubland transects contained higher shrub values than most of the other transects. Both open and closed pine transects contained diverse understories, although there tended to be more litter in closed pine transects. Transects in aspen stands showed no distinguishable patterns of vegetation cover.

Small Mammal Abundance

Four separate species of small mammals were collected over the study period. Species ranked by individuals captured were: 30 deer mice (*Peromyscus maniculatus*), 17 yellow-pine chipmunks (*Tamias amoenus*), 2 meadow voles (*Microtus pennsylvanicus*), and 1 long-tailed weasel (*Mustela frenata*). The most small mammals were captured in transect S1. Trapping efforts yielded no captures at eight transects (Table 1.1). The land cover class with the highest weekly small mammal abundance was meadow (2.6 mammals/week). This was followed by aspen (2.33), open shrubland (2.2), open pine (2.0), closed pine (0.8) and wetland vegetation (0.5). Yellow-pine chipmunks had greater

larvae/nymph ticks present (14 total) than did deer mice (1) and meadow voles (1). No significant correlation was found between small mammal abundance and total questing tick numbers (p=0.5611).

Large Mammal Usage

Four large mammal species were present in our transects. These included white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), coyote (*Canis latrans*) and moose (*Alces alces*). White-tailed deer were the most captured species (13 captures) followed by elk (10 captures), coyotes (2 captures) and moose (1 capture). Transect S3 had the most large mammal usage with two deer captures and five elk captures over a two week period (Table 1.1). Wetland vegetation land cover class had a large mammal usage of 2.0 per week. This was followed by open shrubland (1.38), meadow (1.17), closed pine (1.0), aspen (0.67) and open pine (0.25). No significant correlation was found between large mammal usage and total tick numbers (p=0.1672).

Statistical Model

Generalized linear models were run with every possible predictor combination, excluding interactions for the 20 transects where large mammal usage data was collected. The dredge function in R evaluated 2,048 different GLM model combinations. The best model (AICc=143.16, pseudo-R^2=0.952) included the predictors percent forb cover, percent ground_other cover, land cover class, percent shrub cover and small mammal abundance (Table 1.2). Outside of percent ground other cover, all predictors had a positive relationship with tick abundance. Each predictor was a significant predictor of total tick abundance ([F, p= 0] [G_OT, p= 1.3e-11] [LC, p=0] [S, p=0] [SM, p=3.7e-07]).

The best model that included large mammal usage was ranked sixth (AICc=152.48, pseudo-R^2=0.910). A delta AICc value greater than 9.0 indicates it was far inferior to our best model. Therefore, large mammals were excluded from further analysis in order to incorporate data from all 27 transects.

Generalized linear models were run with data from all 27 transects, excluding large mammal usage. The dredge function in R evaluated 1,024 different GLM model combinations. The best model (AICc=189.95, pseudo-R^2=0.880) included the predictors 2017 burn status, percent forb cover, land cover class and percent shrub cover (Table 1.3). All predictors had a positive correlation with tick abundance, except 2017 burn status. Each of the predictors was a significant predictor of tick abundance ([Burn17, p=1.74e-13] [F, p=0] [LC, p=0] [S, p=0]).

Mapping Tick Density

The results for relative importance of each predictor are shown in Table 1.4. This function determined the four most important factors to be land cover class, percent forb cover, percent shrub cover and 2017 burn status. Due to the variability of forb cover from year to year, it is extremely difficult to map using remote sensing data. Therefore percent forb cover was excluded from predictive tick density mapping. A GLM with the predictors 2017 burn status, land cover class and shrub density was used to predict tick density across the public use area of TNWR, henceforth gisGLM. The gisGLM had an AICc of 254.37, explaining 74.4% (pseudo-R^2) of the variation in tick numbers between transects. Each predictor was significant in predicting tick abundance ([S, p=0] [LC, p=1.56e-14] [Burn17 p=3.96e-4]). Linear model results showed the gisGLM was

successful at predicting tick abundance within transects ($p=2.153e-09$, $R^2=0.6599$) (Figure 1.8). It predicted increasing tick abundance with increasing shrub cover (Figure 1.9). Areas within the 2017 prescribed burn had decreased predicted tick abundance (Figure 1.10). Within land cover class, open shrubland had the highest predicted tick abundance. Aspen and wetland vegetation had the lowest predicted tick abundance (Figure 1.11).

GIS raster layers were created for the predictors 2017 burn status, land cover class, and percent shrub cover using the previously stated methods (Figure 1.12). These layers were used with the gisGLM to create a predictive tick density map for the public use area of TNWR (Figure1.13). The values for tick density represent the weekly tick density per $1m^2$ averaged across the dates March 26th - June 5th. The highest predicted tick density was 1.01 ticks/m^2/week. The areas of high tick density were represented by open shrubland areas of high shrub cover and no recent burning. The lowest predicted tick density (0.0006 ticks/m^2/week) was the area represented by the 2017 prescribed burn. Generally, the model predicted greater tick density in the northern portion of the public use area compared to the southern portion.

1.4 DISCUSSION

Our results suggest that ticks emerge from diapause and begin questing prior to the last week in March at Turnbull National Wildlife Refuge. Their activity continues to increase until a point where questing becomes too costly and the number of questing ticks decreases. In 2018, questing peaked in late May. A second small peak in tick density during the week of June 25th may be attributed to *Dermacentor variabilis'* ability to

successfully quest in higher temperatures (McEnroe 1979). Questing activity became extremely reduced in mid-July, indicating that most *Dermacentor* ticks had either died after completing their life cycle, found a host, or entered diapause prior to or at this time. Tick abundance within transects of the same land cover classes varied greatly. This was expected because ticks tend to have clumped distributions (Ostfeld et al. 1996) and there is substantial variation of many factors between transects. However, land cover classes were useful to show an overall trend of habitats preferred by questing *Dermacentor* ticks.

Contrary to a similar study conducted by Shadix (2016) at TNWR, we did not find any significant relationship between small mammal abundance and total tick abundance. It is possible our study design was too limited, as trapping grids are generally employed in small mammal studies. However this method was chosen because we were not estimating small mammal densities. We used small mammal abundance because we wanted to ensure each mammal trapped was indeed using space within the transect, as not to include individuals outside of the transect within the predictive model. We were limited by our ability to successfully handle each captured small mammal. On a few occasions we were unable to successfully mark and examine individuals. While we attempted to account for these individuals by sex and weight observations, it is possible that our counts could be slightly overestimated. However, we do not believe these few miscounts would affect model output. The majority of larvae/nymph ticks on small mammals were present on yellow-pine chipmunks. This difference could be attributed to the diurnal nature of yellow-pine chipmunks as well as their sizeable home ranges (Broadbooks 1970), increasing the potential contact with immature tick stages. It is worthwhile to note that all small mammals using our transects were assumed to be using

areas outside of the transect. Therefore it is entirely possible that attached larvae/nymph ticks could have been contracted outside of the transect where the animal was trapped.

No significant correlation was found between large mammals and questing tick abundance. We were limited in two ways for this portion of the study. First, by only placing one camera at each transect we were unable to observe the full transect at many locations. Secondly, we were only able to observe transects for a one to two week period. An adequate number of game cameras would have allowed us to cover the entire transect, as well as expand the length of observation at each transect. This would have given us a more complete dataset, and could have affected the model results. Camera trapping was also foregone in seven transects. Alternative methods such as scat collection could have been used to collect data in these locations, although these alternative methods have their own limitations.

Although the inclusion of large mammals in the GLM evaluated more models (2,048) it was not a valuable predictor for tick abundance within our transects. If large mammal data was collected at all 27 transects this could have changed. Because large mammal usage was not present in the top ranked models, it was dropped for further GLM analysis. Dropping large mammal usage allowed us to evaluate data at all 27 transects. Although AICc values were greater after dropping large mammal usage (189.95 vs. 143.16), the top five models were capable of predicting total tick abundance within transects at >85%. It is important to note that AICc values between datasets excluding and including large mammals are not comparable because we included data at all 27 transects when excluding large mammals. Our best GLM model excluding large mammal usage included the predictors 2017 burn status, land cover class, percent forb cover and

percent shrub cover. Only two transects were within areas where the 2017 burn occurred. Only one tick was collected over both transects during the 10 week study period. Surprisingly our model predicted meadow land cover class to have the second highest impact on total tick abundance excluding all external factors. This was an interesting result as more ticks were collected in both open and closed pine land cover classes as compared to meadow. The model prediction may be because the value of shrub and forb cover is low within the meadow land cover class. Therefore, if values of shrub and forb cover are equal within each land cover, we would expect to find more ticks in the meadow land cover class. Due to low sample size, we were unable to include interactions within the GLM which would have accounted for these differences.

The gisGLM model represented only the 392nd ranked model. As expected by field observation, the gisGLM model was successful in predicting increasing tick abundance with increasing shrub cover. This result is similar to previous studies done on the refuge (Shadix 2016). Also expected was a predicted decrease in tick abundance from the 2017 burn status value 0 to 1. Likely, this is because prescribed burning in the previous year removes all ground vegetation, limiting the areas where ticks are able to quest. It is also possible that prescribed burning may effectively kill ticks in the area. Burning does reduce all tick life stages (Davidson et al. 1994), but the effects are short lived (1-2 years). Further research is required to determine the time after burning in which ticks are no longer affected at TNWR.

We were able to successfully predict *Dermacentor* tick density at a 10m resolution using ArcGIS and GLM functions in R. The predictive map shows average weekly tick density over the entire study period, March 26th to June 5th 2018. Our highest

predicted tick density was 1.01 ticks/m^2/week. This was greater than the highest tick density we observed during our collections in 2018. Our greatest tick density found was 0.198 ticks/m^2/week (S4). We know based on previous studies, that our tick densities are not the highest recorded within the public use area of TNWR. Shadix (2016) found an average tick density of 1.57 ticks/m^2 along the 30-Acre Lake Trail between the dates of March, 30th and May, 18th 2016. We expect our values for tick density to change depending on the time of the season. We would expect density values to be greatest during the last week of May. In contrast, these numbers would be reduced in both the beginning (late March – early April) and end (mid-June) of the tick season. Results of the predictive map show tick density tends to be greater in the northern half of the public use area around the auto tour loop. This is likely due to the presence of more open shrubland in that area, as the southern half of the public use area contains most of the meadow area. As we traveled from transect to transect, we subjectively found a trend of more ticks in the northern end of the public use area. Many highly used trails are located along the auto tour loop. It is possible that the higher presence of humans and dogs in this area could be contributing to increased tick density, by ticks moving on and off of dogs. Predicted tick density was greatest in open shrubland areas. There is the potential that areas of closed pine are creating a dense enough canopy to shade light from the understory. This would result in minimal understory growth, and less suitable questing locations. Ticks also have a tendency to be in higher abundance along trails (Carroll et al. 1991). Our predictive map did not include any data associated with trails. Higher tick density is expected adjacent to highly used trails.

The process of creating the shrub density layer in ArcGIS left room for error. All points within 1m of the created tree canopy layer were removed from the shrub layer. Therefore any shrubs present underneath trees were excluded from the shrub density layer. We also excluded any points within the meadow land cover class. While shrubs are not present in the vast majority of meadows, there are some areas of sagebrush. Therefore the predictive map is underestimating tick density in these few locations.

Future work is needed to validate the accuracy of our predictive tick density map. Specifically research is warranted on location of tick activity from year to year. If spatial activity varies from year to year, these changes will need to be further assessed in order to be incorporated into of our predictive map. The seasonal activity of ticks at TNWR also warrants further research. If ticks begin questing earlier or later in the year then that may hinder the ability of our map to successfully predict tick density. Collections within areas indicated by high tick density would allow for further evaluation, and fine tuning of our predictive map. This model has the capability to be expanded to areas outside of the public use area of TNWR, where habitat is similar.

Conclusions

While there are limitations, we have successfully created a predictive tick density map at a high resolution of 10m. With a high resolution map, visitors of the public use area of TNWR will now be able to avoid areas of high predicted tick density. This in turn will reduce visitors' risks to potential pathogens present within *Dermacentor* ticks. We predicted higher tick density in areas of open shrubland habitat, where shrub density is highest. Methods similar to ours could be employed in areas with higher pathogen

prevalence in ticks (e.g. areas of Lyme disease). To our knowledge this is the first successful predictive tick density map at a 10m resolution.

Chapter 2

Detection of *Rickettsia spp.* in *Dermacentor spp.* ticks at Turnbull National Wildlife Refuge, WA

2.1 INTRODUCTION

In the United States, ticks are the number one vector for disease causing pathogens (Rosenberg et al. 2018). Ticks can be infected with viruses, bacteria, or parasites (CDC 2011). Their ability to transmit these disease-causing pathogens through a single bite makes them effective vectors (Anderson and Magnarelli 2008). Of these infectious pathogens, bacteria are responsible for > 90% of the reported disease cases each year (CDC 2017). Common bacterial diseases transmitted by ticks include Lyme disease, anaplasmosis, spotted fever rickettsiosis, and tularemia (Rosenburg et al. 2018).

While Lyme disease is responsible for greater than 70% of tick-borne diseases in the U.S., Rocky Mountain spotted fever (RMSF) has the highest mortality rate. With appropriate and timely treatment the fatality rate of RMSF is 2% - 6% in the U.S. This rises to 23% if untreated (Chapman et al. 2006) making it the most fatal tick-borne disease. Cases of RMSF are reported with infections caused by other similar *Rickettsia* pathogens under spotted fever group rickettsiosis (SFGR). Common infectious SFGR in the United States include *R. rickettsii*, *R. parkeri*, *Rickettsia* species 364D, and *R. akari* (CDC 2019). Other species are classified as SFGR, although their pathogenesis to humans is currently unknown (Sahni et al. 2013, Zeringóta et al. 2017). The SFGR cases have increased dramatically from 495 cases in 2000 to more than 6,200 in 2017 (CDC 2019).

Rickettsia rickettsii is the bacterial causative agent of RMSF (Burgdorfer et al. 1966). Ticks are capable of becoming infected with *R. rickettsii* by two different modes. A tick may feed on a host infected with *R. rickettsii*, becoming infected and able to transmit the bacteria to other hosts (Sonenshine and Roe 2014). Infected females can pass the bacteria on to nearly 100% of their offspring via transovarial transmission (Burgdorfer 1963), creating a sustainable infected population. While ticks are easily infected, *R. rickettsii* has lethal effects on each life stage, resulting in <20% survival of infected individuals (Niebylski et al. 1999). Macaluso et al. (2008) has also demonstrated that ticks infected with non-pathogenic *Rickettsia spp.* may block the infection of other *Rickettsia spp.* (e.g. *R. rickettsii*). This contributes to the low *R. rickettsii* prevalence rate of ~1% within ticks in areas where *R. rickettsii* is known to occur (Stromdahl et al. 2011, Wikswo et al. 2008, Berrada et al. 2011).

Within the United States there are two main vectors for *R. rickettsii*. *Dermacentor andersoni* (Rocky Mountain wood tick), found primarily in Rocky Mountain states, and *Dermacentor variabilis* (American dog tick) found primarily east of the Rocky Mountains and along the California coast line (Burgdorfer 1975). A few cases have also shown *Rhipicephalus sanguineus* (brown dog tick) capable of vectoring the pathogen in the southwestern U.S. (Demma et al. 2005). In 2017 there were nearly 6,500 reported cases of SFGR in United States. Five states (North Carolina, Oklahoma, Arkansas, Tennessee, and Missouri) accounted for over 60% of SFGR cases (CDC 2019). In Washington State there were 90 cases of RMSF from 1920-1949, in contrast to only 10 cases from 2004-2016. In 2017 there were five reported cases of SFGR in the state, only one of which was contracted from a tick in the state (WDOH 2017). The last reported

cases of RMSF from in-state ticks were in 2011 when there were three such cases (WDOH 2011).

The timing of transmission of *R. rickettsii* to a human can vary greatly. On first contact, a tick will usually take ~24 hours to find a suitable spot and begin attachment. Transmission of *R. rickettsii* by *Dermacentor* adults takes an average of 10 hours, but can happen as quickly as 1 hour and 45 minutes after attachment (Ricketts 1909, Moore 1911). Early symptoms of RMSF include fever, headache and nausea and occur within 24 hours of infection. The most identifiable symptom is a petechial rash which forms 2-4 days post fever (Dantas-Torres 2007). The timing of rash development becomes crucial to assist in RMSF diagnosis. RMSF can be easily treated with common antibiotics such as doxycycline, but the timing of its administration is directly correlated to RMSF fatality rate. A case study by Regan et al. (2015) investigated RMSF infection in 205 patients. 15 cases were fatal. In each fatal case doxycycline treatment was not started until at least day 6 of symptom onset. Fatality occurred on average 9 days after symptom onset.

In 2014 and 2016 *Dermacentor spp.* ticks were collected by dragging at the public use area of Turnbull National Wildlife Refuge (TNWR) in Eastern Washington. *Dermacentor spp.* were tested to determine presence of *Rickettsia spp.* including *Rickettsia rickettsii*. In both cases presence of *Rickettsia spp.* was determined by using polymerase chain reaction (PCR) to amplify a fragment of the *rOmpB* gene (Simser et al. 2001). Between the two years a total of 594 *Dermacentor* ticks were collected and analyzed. Only *Dermacentor andersoni* and *Dermacentor variabilis* were identified. Of the 594 *Dermacentor* ticks, 56 (9.4%) were positive for *Rickettsia spp.* Sequencing results showed 42 ticks infected with *R. rhipicephali*, a SFGR of unknown pathogenesis

(Labruna et al. 2007). Five ticks were infected with *R. prowazekii*, the etiologic agent of epidemic typhus, and eight ticks were infected with *R. rickettsii* (Shadix 2016). The detection of multiple pathogenic bacteria in this location suggests a threat to the visitors of the refuge, and a need to further investigate their distribution.

The purpose of this research was to determine the distribution of SFGR within the public use area of TNWR. We also aimed to determine the identity of SFGR present, in order to assess the potential threat to visitors of the refuge. We report varying results with different primer sets, which may suggest there is no human risk to SFGR within the public use area of TNWR.

2.2 METHODS

Collection of ticks

TNWR is a federal wildlife refuge located in Spokane County, 20 miles southwest of Spokane, WA (see Figure 1.3). The public use area is located in the southeast corner of TNWR and encompasses 3,276 acres of the 18,000-acre refuge. Tick collections in 2018 occurred weekly in 27 separate 50m transects across the public use area (Figure 2.1). Collections occurred weekly at each transect from March 26th, 2018 to June 6th, 2018. Collections continued at our three most abundant transects (OP1, CP1, and S4) an additional six weeks until July 20th, 2018. Standard dragging techniques with a 1m by 1m corduroy cloth were used to collect questing ticks. Few nymph ticks were also collected from small mammals trapped within transects between the dates of April 30th, 2018 and June 1st, 2018. All collected ticks were immediately identified to genus. Ticks were then placed in 70% ethanol and stored at -20°C until DNA extraction.

PCR for Rickettsia presence

DNA was extracted from collected ticks using an established DNAzol (www.thermofisher.com), bead-beating protocol (Appendix B). In short, this method requires homogenization of each individual tick, followed by phase separation, DNA precipitation and DNA wash. Ending with DNA dissolved in 8mM NaOH which is stored in -20°C freezer. Following extraction, DNA quality and concentration were determined using a Thermo Scientific NanoDrop Lite spectrophotometer (www.thermofisher.com). Initial presence/absence of *Rickettsia spp.* was determined using polymerase chain reaction (PCR) and primer pair Rf17.61p-Rf17.492n (Table 2.1) to amplify a 431 base pair fragment of the *rOmpB* gene which encodes a *Rickettsia* genus-specific 17-kDa common antigen gene (Simser et al. 2001). The cycling conditions were initial denaturation at 95°C for two minutes, then 60 cycles of denaturation (30 seconds at 95°C), annealing (60 seconds at 55°C), and elongation (60 seconds at 72°C), and a final extension for 5 minutes at 72°C. PCR was performed in 25 µL reactions containing 12.5 µL Promega Master Mix (www.promega.com), 1 µL forward primer (Rf 17.492), 1 µL reverse primer (Rf 17.61), 9.5 µL pure water, and 1 µL template DNA or H_2O (negative control). PCR products were electrophoresed in a 1% agarose gel with 1X TAE buffer (40mM Tris-acetate and 1mM EDTA, pH 8.3) that contained 0.033 µg/mL of ethidium bromide. The gel was electrophoresed in 1X TAE at 80V for 45 minutes. Upon UV imaging, those samples with a band ~400 base pairs were determined positive for *Rickettsia*. Samples positive for *Rickettsia spp.*, including samples positive for *Rickettsia rickettsii* in 2014, were further analyzed using two alternate primer pairs. Primer pair RpCS.877p-RpCS.1258n was used to amplify a 381 base pair fragment of the *gltA* gene

which encodes the citrate synthase enzyme (Dergousoff et al. 2009). Primer pair Rr190.70p-Rr190.602n amplified a 532 base pair fragment of the *rOmpA* gene which encodes a 190-kDa outer membrane protein (Regnery et al. 1991). The cycling conditions for both primer pairs were an initial denaturation at 95°C for two minutes, then 40 cycles of denaturation (30 seconds at 95°C), annealing (20 seconds at 48°C), and elongation (60 seconds at 72°C), and a final extension for 5 minutes at 72°C. PCR was performed in 25 μL reactions in correspondence with the *rOmpB* primer pair. Gel electrophoresis was carried out as described above. Samples that were positive for *Rickettsia* showed bands at ~500 base pair (*rOmpA*) and ~400 base pair (*gltA*).

Determination of Rickettsia spp.

To determine *Rickettsia spp.*, positive PCR products for all three gene fragments (*rOmpB*, *rOmpA*, *gltA*) were sent to GENEWIZ LLC for Sanger sequencing. Forward and reverse sequencing was carried out for most samples; all samples for gene fragments *rOmpB* and *gltA*. For samples of the *rOmpA* gene, the results using forward sequencing were conclusive so reverse sequencing was forgone. Forward and reverse sequences were assembled into a contiguous DNA sequence using PRABI-Doua CAP3 Sequence Assembly Program (http://doua.prabi.fr/software/cap3). Low quality bases on the extremities of the DNA sequence were trimmed. The resulting DNA sequences were compared to homologous sequences using the National Center for Biotechnology Information (NCBI) BLASTn search tool. In GenBank, the closest identity match to our DNA sequence was used to determine the species of *Rickettsia* present.

2.3 RESULTS

In total 452 ticks were tested for *Rickettsia spp.* presence. This included five nymph ticks collected from small mammals (1.1%) as well as questing adults, 227 (50.2%) females and 220 (48.7%) males. All ticks collected were identified as either *Dermacentor andersoni* or *Dermacentor variabilis* but due to their equal importance as vectors, and the difficulty to distinguish between the two (Dergousoff and Chilton 2007), species was not confirmed.

Electrophoresis of the *rOmpB* gene fragment yielded 21 (4.6%) positive detections for *Rickettsia spp.* (Figure 2.2). Of these, six tick samples (501, 506, 507, 508, 510, and 511) produced extremely faint bands, although sequencing did yield results. All 21 positives were from adult questing ticks, of which 11 (52.4%) were female and 10 (47.6%) were male. Sequence comparison in GenBank showed 15 (3.3%) positives for *Rickettsia rhipicephali* (>98.2% identity), two (0.44%) positives for *Rickettsia rickettsii* (>99.8% identity), and four (0.88%) were 100% identical to *R. rickettsii*, *R. parkeri*, and *R. philipii* (Table 2.2).

Electrophoresis of the 190-kDa *rOmpA* gene fragment only yielded 15 (3.3%) positives for *Rickettsia spp.* Of these, eight (53.3%) were female and seven (46.7%) were male. This excluded six tick samples positive for *R. rhipicephali* under the *rOmpB* protocol (501, 506, 507, 508, 510 and 511). These samples all produced multiple non-specific bands during gel electrophoresis (Figure 2.3). Sequence comparison in GenBank showed nine ticks were positive for *Rickettsia rhipicephali* (>99.2% identity), in correspondence with the *rOmpB* results. The six positive samples for *Rickettsia rickettsii*

using the *rOmpB* gene fragment, showed positive for *Rickettsia peacockii* (>99.8% identity) with the *rOmpA* gene. The three samples positive for *R. rickettsii* in 2014 also showed positive for *R. peacockii* (>99.8% identity) with the *rOmpA* gene (Table 2.3).

In concurrence with the 190-kDa *rOmpA* results, electrophoresis of the *gltA* gene fragment yielded 15 (3.3%) positives for *Rickettsia spp.* (Figure 2.4). This again excluded tick samples 501, 506, 507, 508, 510 and 511 which tested positive using the *rOmpB* gene fragment. Sequence comparison in GenBank showed some matching results to the *rOmpA* gene fragment. Using only forward sequences the six samples positive for *Rickettsia peacockii* with the *rOmpA* gene were confirmed (>99.5% identity). Only two of the three samples from 2014 were positive for *Rickettsia spp.*, excluding sample 1-14. These two samples were positive for *Rickettsia peacockii*, also in correspondence with results from the *rOmpA* gene fragment. Forward sequencing initially revealed nine samples positive for *Rickettsia massiliae* (>99.7% identity). Reverse sequencing of these samples only yielded six quality reads. Once assembled into continuous DNA sequences, these resulted in six positives (1.3%) for *Rickettsia rhipicephali* (>98.7% identity), leaving only three *R. massiliae* positives (Table 2.4).

Rickettsia spp. were detected in ticks in eight different transects (CP1, OP1, OP2, OP3, OP4, S1, S3, and S4). This included six ticks in CP1; five ticks in OP1; three ticks in S4; two ticks in S1 and S3; one tick in OP2, OP3 and OP4. Tick samples 501, 510 and 511 were in CP1. Tick samples 506, 507 and 508 were in OP1.

2.4 DISCUSSION

All gene fragments (*rOmpB*, *rOmpA*, and *gltA*) yielded positive results for *Rickettsia rhipicephali* in tick samples 40, 67, 141, 171, 230, and 381 (Table 2.5). The *rOmpB* and *rOmpA* gene fragments were also in agreement for *R. rhipicephali* in samples 243, 325, and 335. The *gltA* fragment was closest in identity to *R. massiliae* for ticks 243, 325, and 335. These three tick samples only yielded quality forward sequences, and therefore their read length was shorter than the six samples positive for *R. rhipicephali*. With just the forward sequences we only saw a one base pair difference between *R. massiliae* and *R. rhipicephali*. *R. massiliae* is a pathogenic bacterium that has been detected in both California and Arizona but only in *Rhipicephalus sanguineus* ticks (Eremeeva et al. 2006, Beeler et al. 2011). *R. massiliae* has never been detected in our region, and is commonly found in non *Dermacentor* species in foreign countries (Matsumoto et al. 2005). *R. rhipicephali* is a common *Rickettsia* species found in *Dermacentor* ticks in the Western U.S. (Wikswo et al. 2014, Philip and Casper 1981). Therefore it is highly likely that all nine tick samples (40, 67, 141, 171, 230, 243, 325, 335, and 381) contain *R. rhipicephali*, giving us an infection rate of 2.0%. While some researchers classify *R. rhipicephali* as a SFGR (Sahni et al. 2013, Hayes and Burgdorfer 1979), its ability to cause human disease has never been definitively proven (Paddock et al. 2018). Therefore it is unknown whether this bacteria presents any threat to the public at TNWR.

Tick samples 501, 506, 507, 508, 510 and 511 were also positive for *R. rhipicephali* by sequence of the *rOmpB* gene fragment. When PCR was carried out using the *rOmpA* and *gltA* gene fragments, these same samples resulted in either multiple non-

specific bands or negative for *Rickettsia spp.* (see Figure 2.4). Previous students at Eastern Washington University have attempted to sequence the non-specific bands, concluding these are not *Rickettsia spp.* detections (unpublished results). It is possible there may have been some cross contamination or inadequate storage for these tick samples. We conclude that these samples are not positive for *Rickettsia spp.*

Collections from 2014 and 2016 suggested a low prevalence of *R. rickettsii* in *Dermacentor* ticks at TNWR. These detections were concluded by sequencing of the *rOmpB* gene fragment. Our preliminary results seemed to agree with previous results as six ticks (22, 23, 165, 239, 263, and 349) were positive for *R. rickettsii* by sequence of the *rOmpB* gene fragment. Upon further investigation, we could not confirm these results. Sequencing of the *rOmpA* and *gltA* gene fragments resulted in identification of *R. peacockii*. Previous studies have shown that PCR amplification and sequencing of the *rOmpA* gene fragment is best for distinguishing closely related *Rickettsia* species (Regnery et al. 1991). The sequences for *R. peacockii* only differed from published sequences by one base pair (*rOmpA*) and two base pairs (*gltA*). *R. rickettsii* was not on the queried list for any of the *rOmpA* sequences, indicating it had a greater than 10 base pair difference in the gene fragment. Sequencing of the *rOmpB* gene fragment showed closest relatedness to *R. rickettsii* from Yucatán, Mexico (Accession # DQ176856.1), while having only two base pair difference from *R. peacockii*. Therefore it appears that the *rOmpB* gene is a highly conserved region and difficult to use to distinguish *Rickettsia* species. Within our region, *R. peacockii* is also a common non-pathogenic bacterium found in *Dermacentor* ticks, where *R. rickettsii* is rare (Niebylski et al. 1997). A study by Burgdofer et al. (1981) suggests that *R. peacockii* may also prevent the infection of *R.*

rickettsii in ticks. Thus we propose that all previous detections for *R. rickettsii* are indeed *R. peacockii*.

A conversation with research entomologist Dr. Glen Scoles of the Agricultural Research Service (ARS) in Pullman, WA led to a potential alternative explanation. It was suggested that the process of PCR amplification may be amplifying multiple *Rickettsia spp.* in a single tick sample. This could result in multiple different detections upon sequencing. While uncommon, multiple *Rickettsia spp.* infections in a single *Dermacentor* tick have been documented. Carmichael and Fuerst (2006) found a single *Dermacentor variabilis* adult in Ohio infected with *R. bellii*, *R. montanensis*, and *R. rickettsii*. Wikswo et al. (2014) also detected *R. bellii* and *R. rhipicephali* in a *Dermacentor occidentalis* adult in California. Recent advances in microbiome analysis have also been used to detect multiple *Rickettsia spp.* in *Dermacentor andersoni* ticks collected from Oregon (Gall et al. 2017). If there are multiple *Rickettsia* infections in some of the ticks that we collected, this may suggest why we are obtaining unclear results.

All transects with ticks positive for *Rickettsia spp.* were located in the northern end of the public use area with the exception of OP4 and S3. These 2 transects yielded 3 of the 15 positive ticks. This indicates a higher infection rate in ticks within the northern half of the public use area of TNWR. The northern half contains the auto tour loop, as well as the majority of the hiking trail in the refuge. Thus if *Rickettsia rhipicephali* was proven to be a human pathogen, there would be elevated risk to visitors in this location, and likely across the region.

Conclusions

Due to the agreement between all three primer sets used in this study, we can conclude that nine of the 452 ticks (2.0%) collected were infected with *Rickettsia rhipicephali*. Agreement between the *gltA* gene fragment and our longest gene fragment (*rOmpA*) suggests that *R. rickettsii* detections using the *rOmpB* gene fragment are not reliable. The extremely low prevalence rate of *R. rickettsii* and the high prevalence rate of *R. peacockii* in our region suggest that six of the 452 ticks (1.3%) collected were infected with *R. peacockii*. Alternative methods such as vector cloning (Carmichael and Fuerst 2010) or microbiome analysis (Gall et al. 2017) could be used to determine if there is multiple *Rickettsia* infections in a single tick. Although our results are inconclusive, they suggest that *R. rickettsii* is not present at TNWR. Therefore, there is no risk of Rocky Mountain spotted fever to visitors of the refuge.

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TABLES AND FIGURES

Table 1.1. All data collected from March 26th – June 5th 2018 at each of the 27 transects. This data was used in all generalized linear models to predict total tick abundance within transects.

Transect ID	Land Cover Class	Total Ticks	Small Mammal Abundance	%GR	%F	%L	%S	%CH	%G_OT	%WL	Burn17	Large Mammal Usage
A2	A	11	1	5.6	37	21.6	20.6	0	15.2	0	0	1
A3	A	4	0	14.4	5.2	0	0.8	0	9.6	0	0	1
A5	A	2	6	5.2	6.8	56	0	0	32	0	0	0
CP1	CP	35	2	39.2	1.4	0	56.8	0	2.6	0	0	0
CP2	CP	9	0	37.2	14.6	41.8	4	0	2.4	0	0	0
CP3	CP	29	1	12.2	2	27.6	41.8	0	6.4	10	0	0
CP4	CP	1	0	5.2	0.4	75.4	5.2	0	13.8	0	0	3
CP5	CP	1	1	2.8	0.4	0	0	39.6	57.2	0	1	2
M1	M	1	2	77.6	6.2	0	0	0	16.2	0	0	1
M2	M	10	2	96.2	0	0	0	0	3.8	0	0	NA
M3	M	7	3	91	8	0	0	0	1	0	0	NA
M4	M	3	0	84.2	0.2	0	0	0	0	15.6	0	1
M5	M	19	6	90	2	0	0	0	2.4	5.6	0	1.5
OP1	OP	40	2	22.4	23.6	0	37.2	0	16.8	0	0	0.5
OP2	OP	20	1	52	25.4	7.6	7.2	0	7.8	0	0	0
OP3	OP	10	0	26	1.4	35.8	31.4	0	5.4	0	0	0
OP4	OP	11	3	48	13.8	7.6	30.6	0	0	0	0	NA
OP5	OP	0	4	39.4	0.6	0	0	5.8	54.2	0	1	0
S1	S	23	7	37.4	6.2	0	23	0	33.4	0	0	0
S2	S	9	0	85.8	5	0	0	0	9.2	0	0	NA

S3	S	27	1	58.2	6.4	3.8	27.2	0	0.8	3.6	0	3.5
S4	S	99	1	22.2	28.2	0	41.8	0	7.8	0	0	1
S5	S	26	2	42.2	0	0	41.6	0	16.2	0	0	1
W2	W	2	1	0	0	0	0	0	0	100	0	NA
W3	W	4	3	0	0	0	0.8	0	6.4	92.8	0	NA
W4	W	0	1	3.6	0	0	0	0	1	95.4	0	NA
W5	W	8	0	0	1.2	0	0	0	1	97.8	0	2

Table 1.2: Top 5 generalized linear models in ranked order for data including large mammals. Data in transects where large mammal data was not obtained were excluded from this analysis. Values represent coefficients of each predictor in the model for total tick abundance. A negative value represents a negative relationship with predicted total tick abundance. Land cover class is given as a plus if it is included in the model. Values of NA indicate predictor variable was not included in that GLM. Models are ranked according to AICc values.

%F	%G_OT	%GR	%L	%S	%WL	Land Cover Class	Small Mammal Abundance	df	AICc	ΔAICc
0.047	-0.049	NA	NA	0.032	NA	+	0.247	10	143.16	0
NA	-0.102	-0.048	-0.033	NA	NA	+	0.34	10	145.16	2.00
0.022	-0.092	-0.036	-0.028	NA	NA	+	0.33	11	146.08	2.92
0.051	-0.049	NA	NA	0.033	0.050	+	0.28	11	148.59	5.43
0.040	-0.064	-0.018	NA	0.027	NA	+	0.30	11	150.41	7.25

Table 1.3: Top 5 generalized linear models and gisGLM ranked in order. Data from all 27 transects are included. No large mammal data is included. Values represent coefficients of each predictor in the model for predicted total tick abundance. A negative value represents a negative relationship with predicted total tick abundance. Land cover class is given as a plus if it is included in the model. Values of NA indicate predictor variable was not included in that GLM. Models are ranked according to AICc values.

Burn17	**%F**	**%L**	**%S**	**%WL**	**Land Cover Class**	**Small Mammal Abundance**	**df**	**AICc**	**ΔAICc**
-2.191	0.043	NA	0.034	NA	+	NA	9	189.95	0
-2.550	0.043	-0.011	0.028	NA	+	NA	10	191.25	1.30
-2.222	0.046	NA	0.033	NA	+	0.053	10	192.42	2.47
-2.134	0.044	NA	0.034	0.025	+	NA	10	192.96	3.01
-2.540	0.044	-0.013	0.027	0.033	+	NA	11	193.61	3.66
-2.383	NA	NA	0.039	NA	+	NA	8	253.37	63.42

Table 1.4: Importance values for all predictor variables used in the full generalized linear model. Values closer to 1 indicate higher importance. Land cover class, % forb cover, % shrub cover, and 2017 burn status were the four most important predictors variables for tick abundance.

Burn17	%F	%G_OT	%L	%S	%WL	%CH	%GR	Land Cover Class	Small Mammal Abundance
0.86	1.00	0.10	0.31	0.98	0.20	0.15	0.10	1.00	0.26

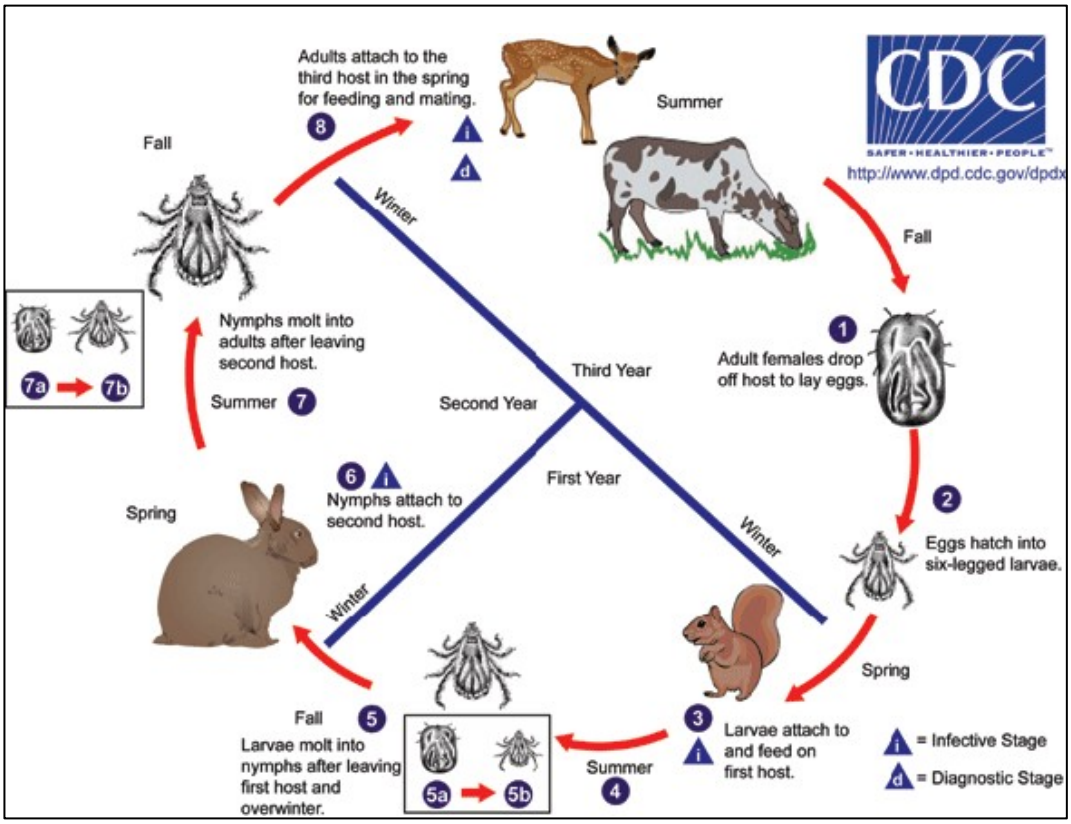

Figure 1.1: The three-host life cycle of many Ixodidae tick species including *Dermacentor* species. This shows the different life stages of ticks, and a potential host for each of those life stages. Courtesy of CDC (www.cdc.gov)

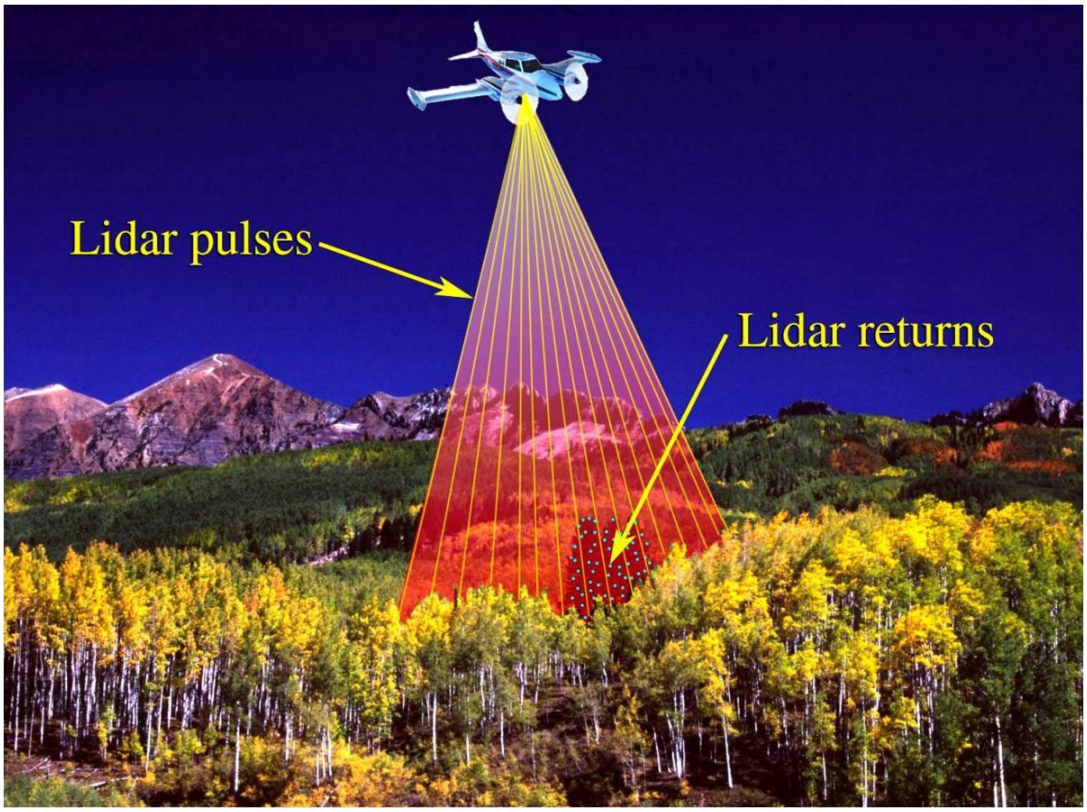

Figure 1.2: An example of a LiDAR flight. Pulses are emitted from the plane towards the ground surface. Pulses refract off of surface objects and return to the plane allowing height of each pulse to be determined.

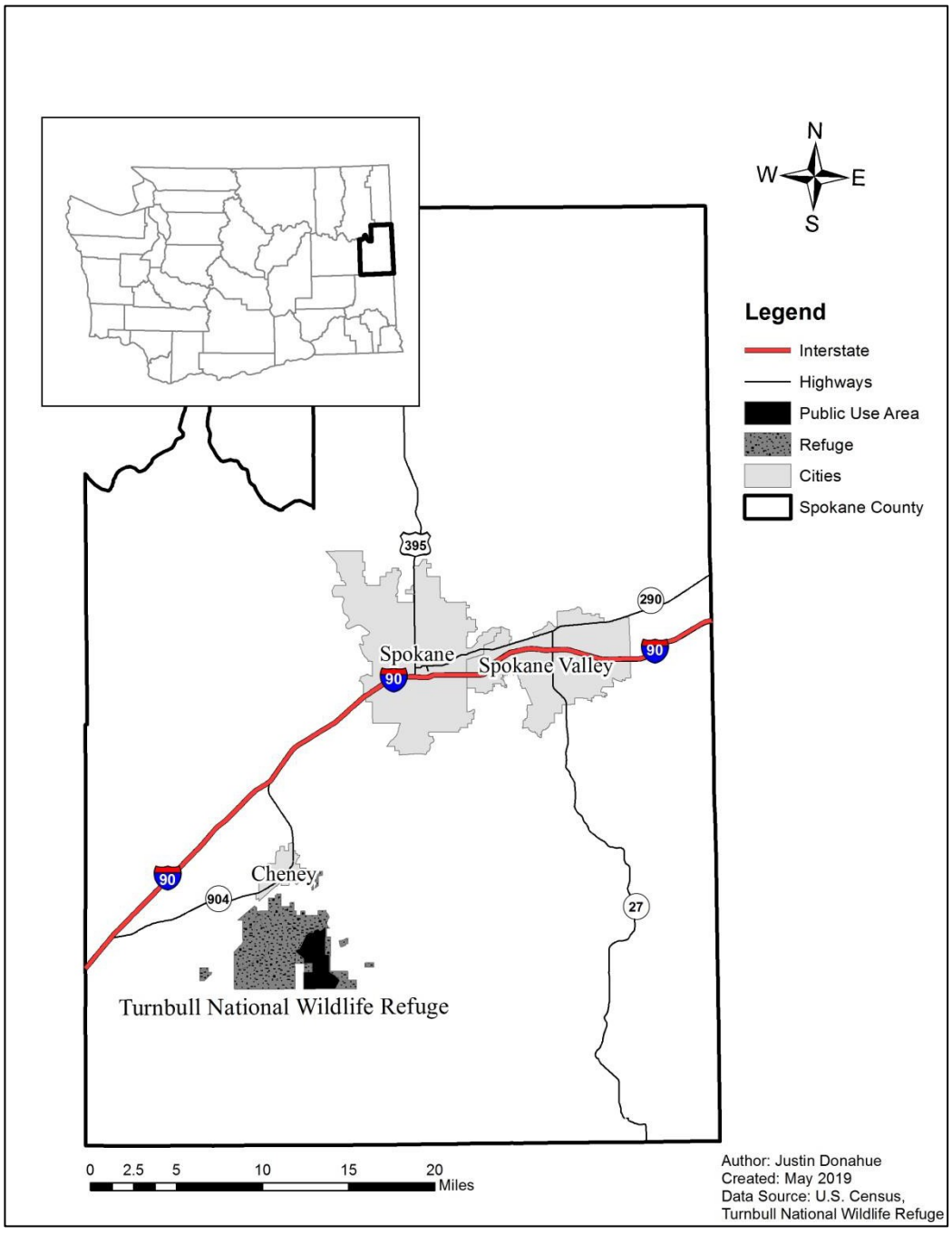

Figure 1.3: The location of the public use area inside Turnbull National Wildlife Refuge. Turnbull National Wildlife Refuge is located in Spokane County, 20 miles southwest of Spokane, WA.

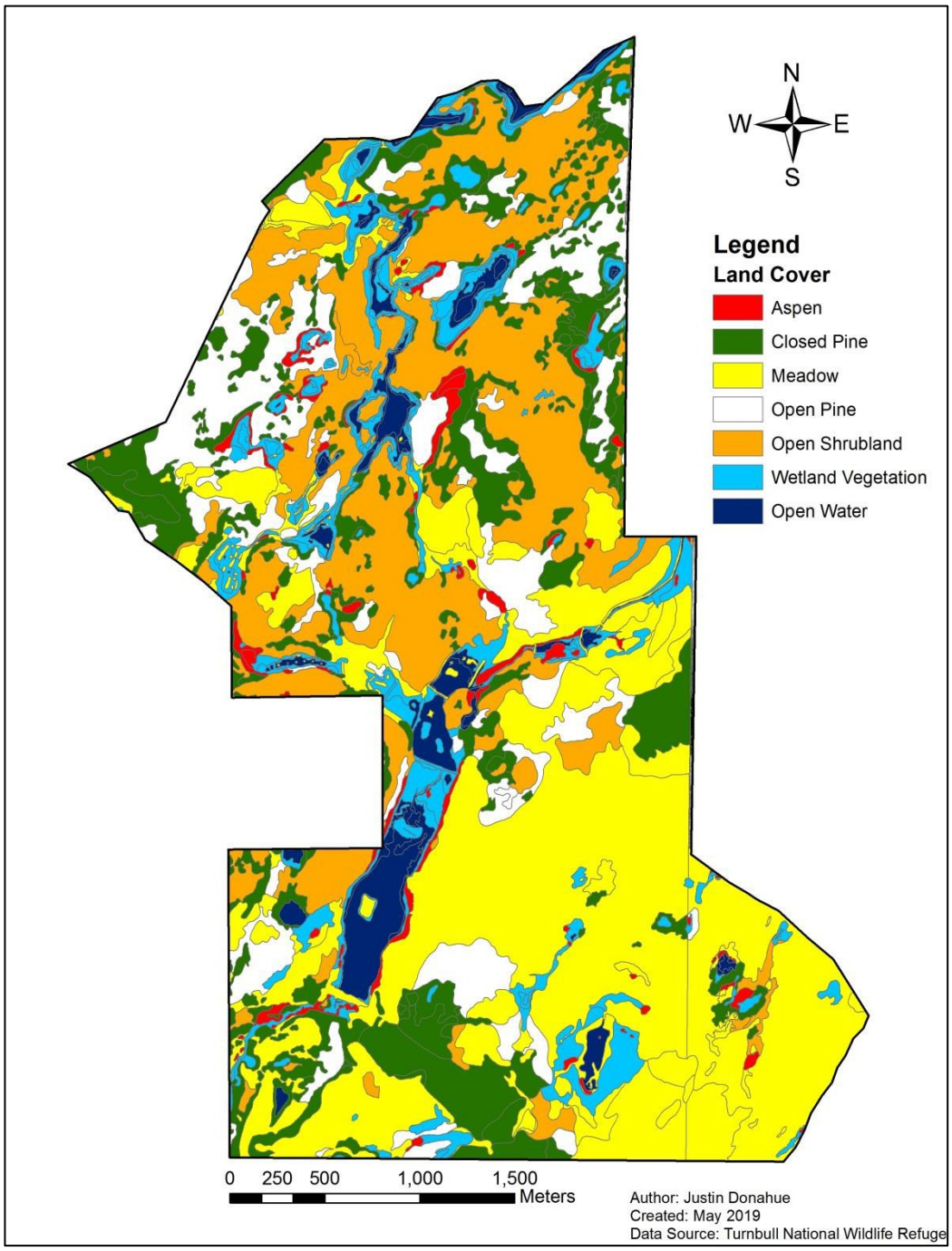

Figure 1.4: Land cover classes present in the public use area of Turnbull National Wildlife Refuge.

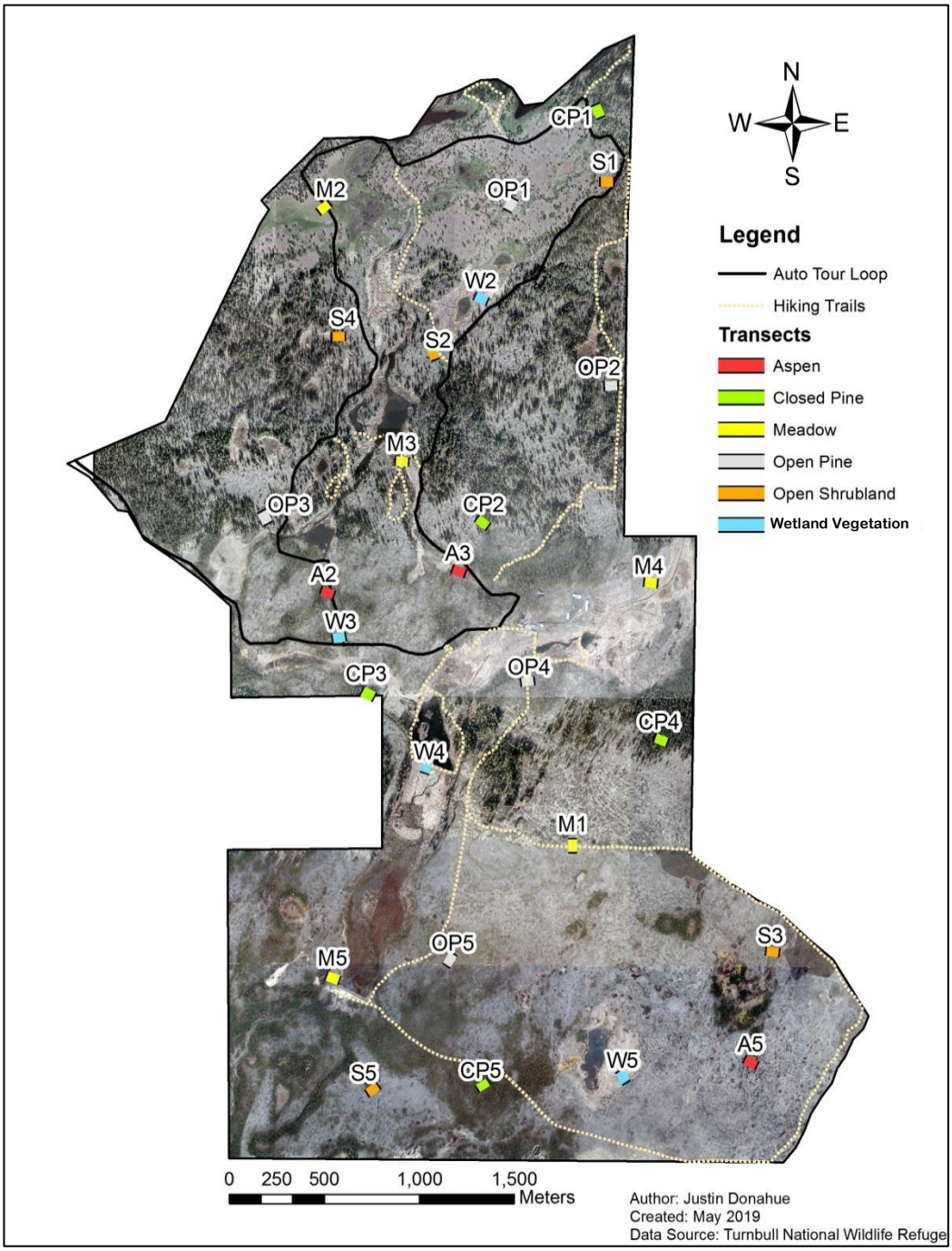

Figure 1.5: Location of 27 transects across the public use area of Turnbull National Wildlife Refuge. Colors of transects indicate which land cover class they reside in. All transects are 50m in length. Transects are enlarged here for visibility.

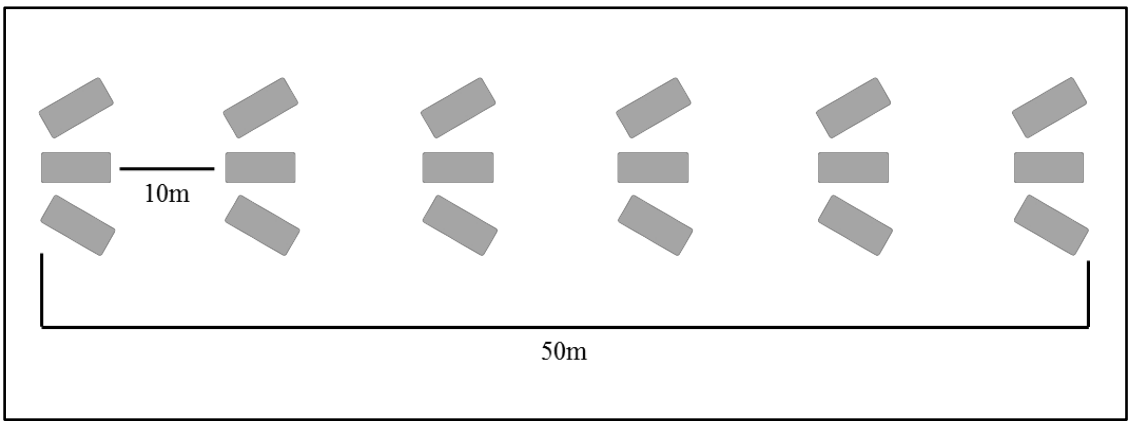

Figure 1.6: Small mammal trap configuration at each transect. Traps were placed in groups of three, 10m apart from each other starting at the beginning of the transect. A total of 18 traps were placed at each transect.

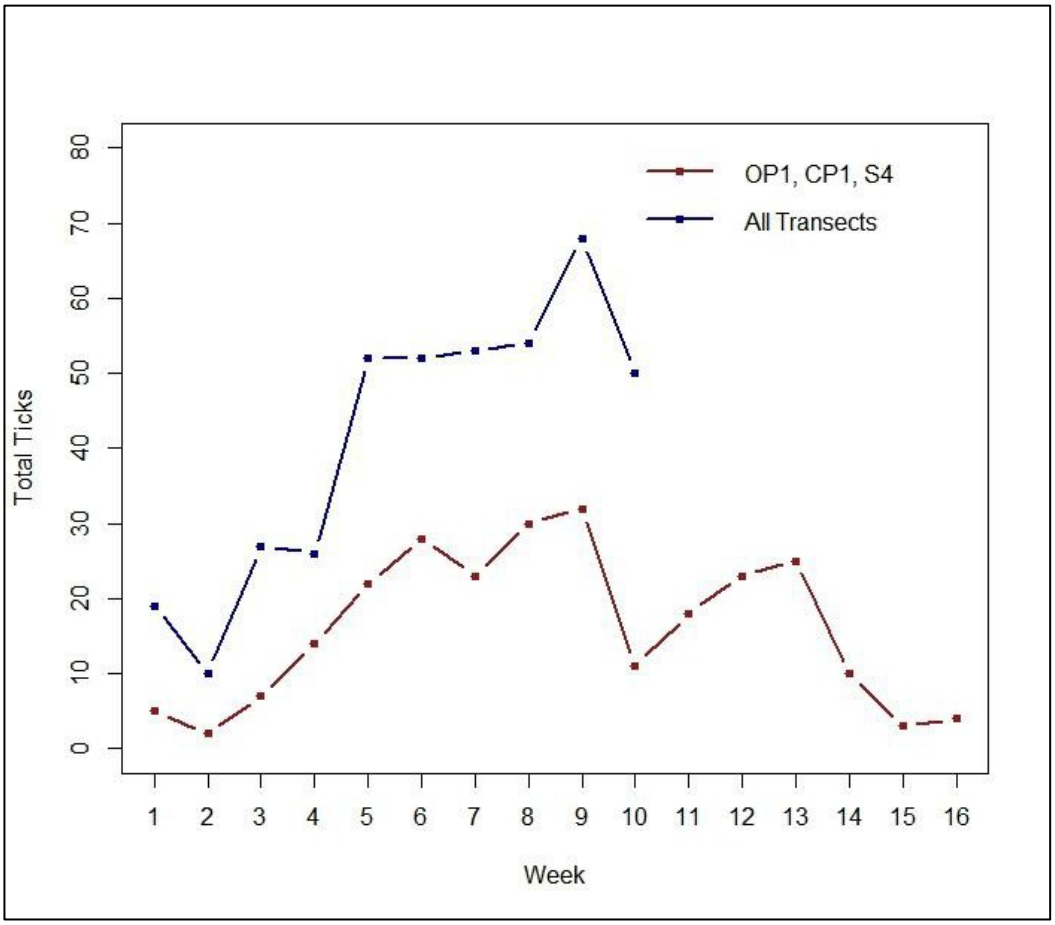

Figure 1.7: All ticks collected across the entire study period in the spring/summer of 2018. Ticks collected in all transects is shown in blue. All three transects collected for 16 weeks (OP1, CP1, and S4) are shown in red.

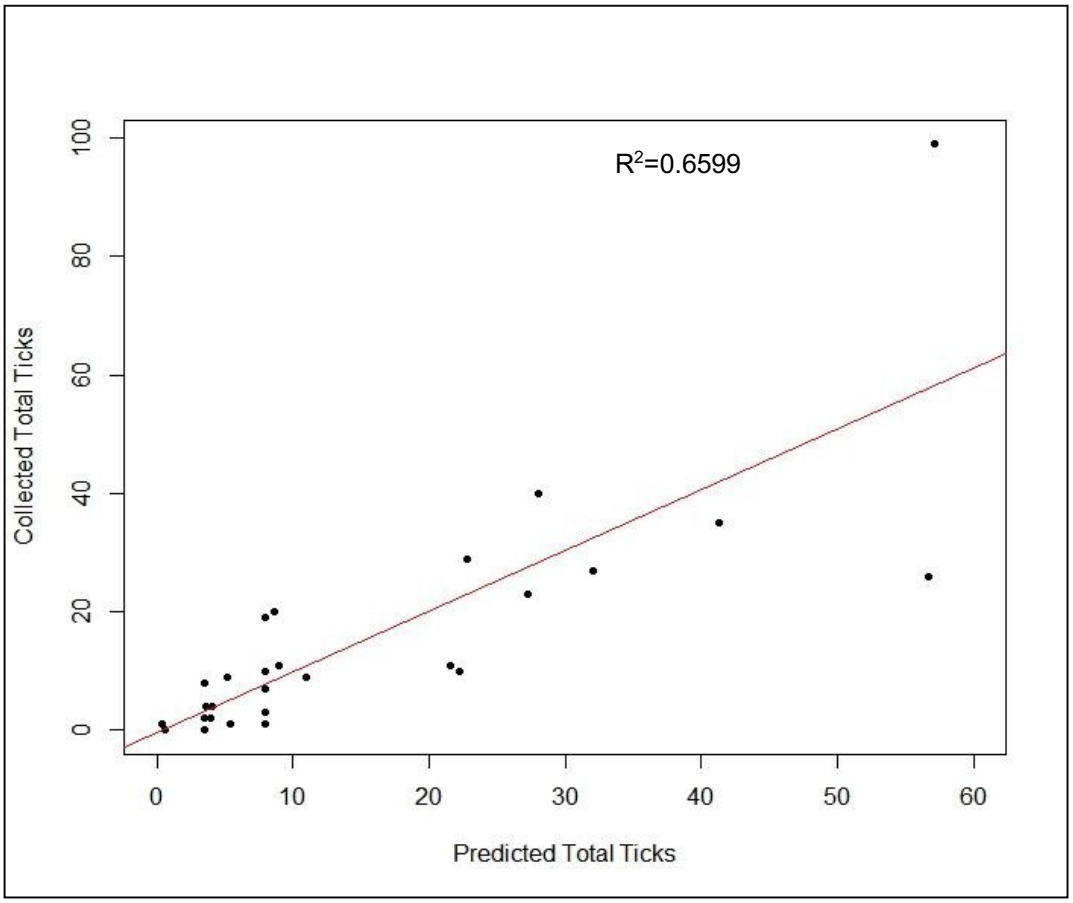

Figure 1.8: The gisGLM model was successful at predicting total tick numbers across the study season in the 27 collection transects (p=2.66e-07).

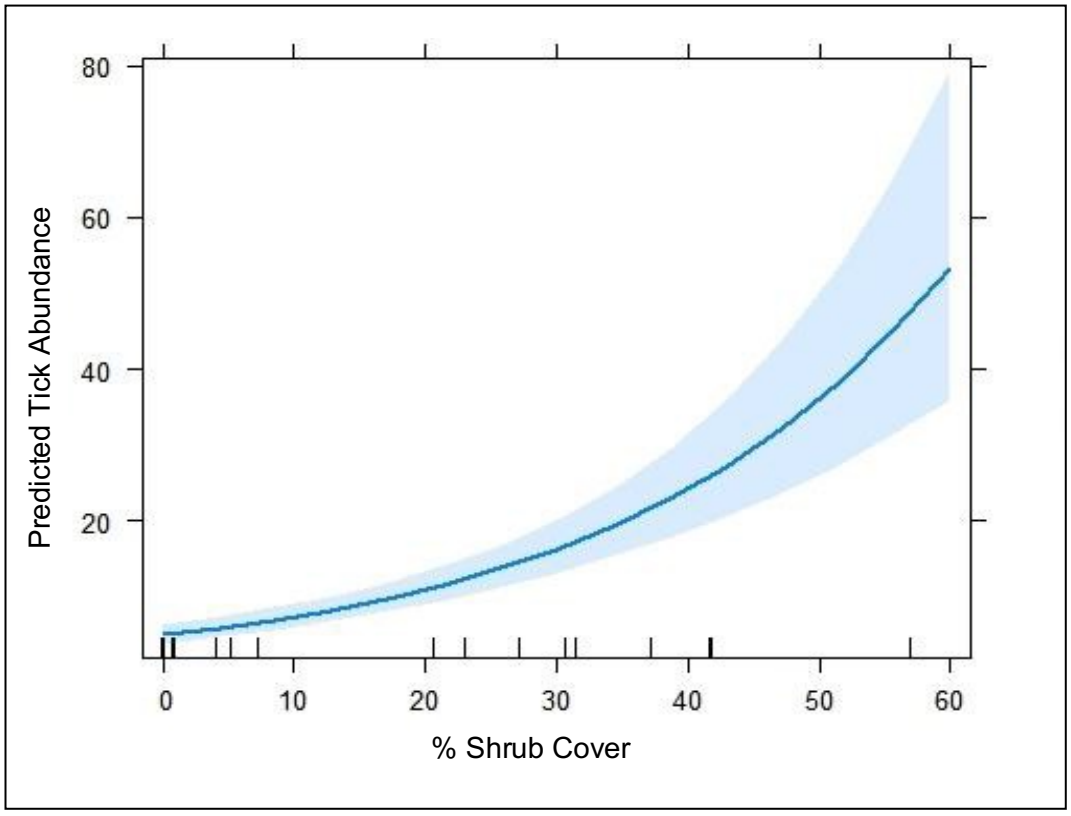

Figure 1.9: Predicted questing tick abundance as a function of percent shrub cover. The blue band indicates the 95% confidence interval based on a Poisson distribution.

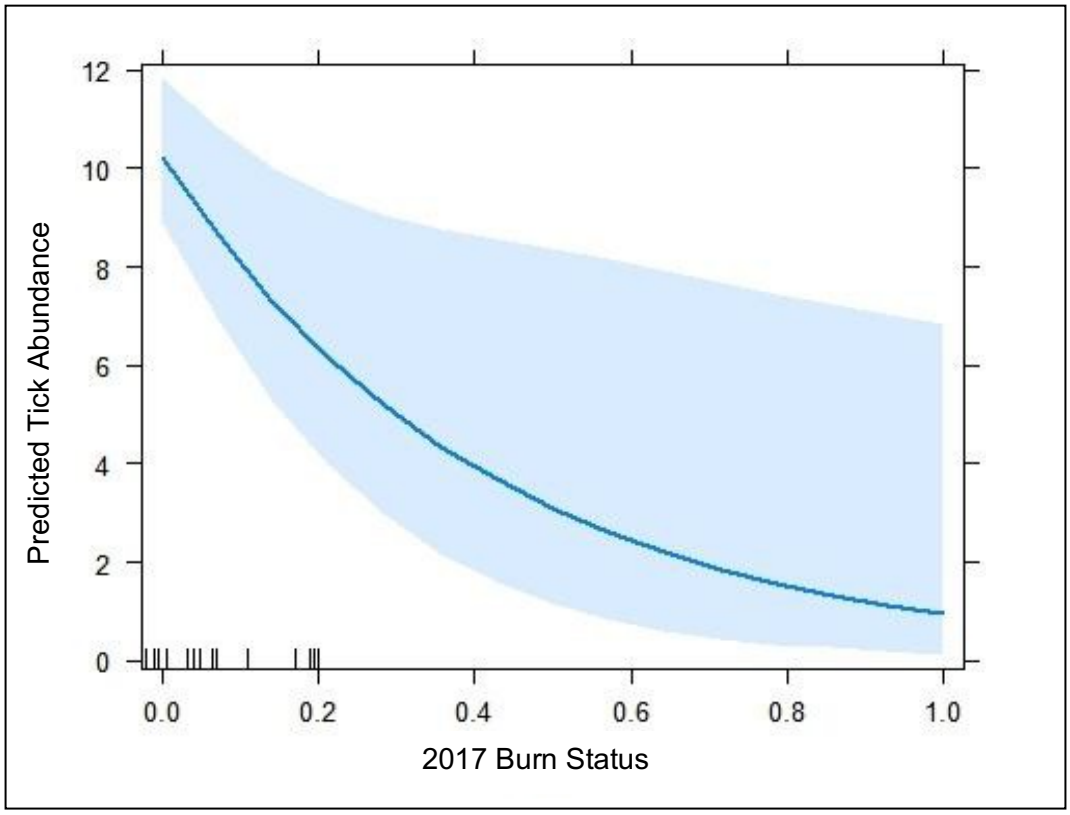

Figure 1.10: Predicted questing tick abundance as a function of 2017 burn status. The blue band indicates the 95% confidence interval based on a Poisson distribution.

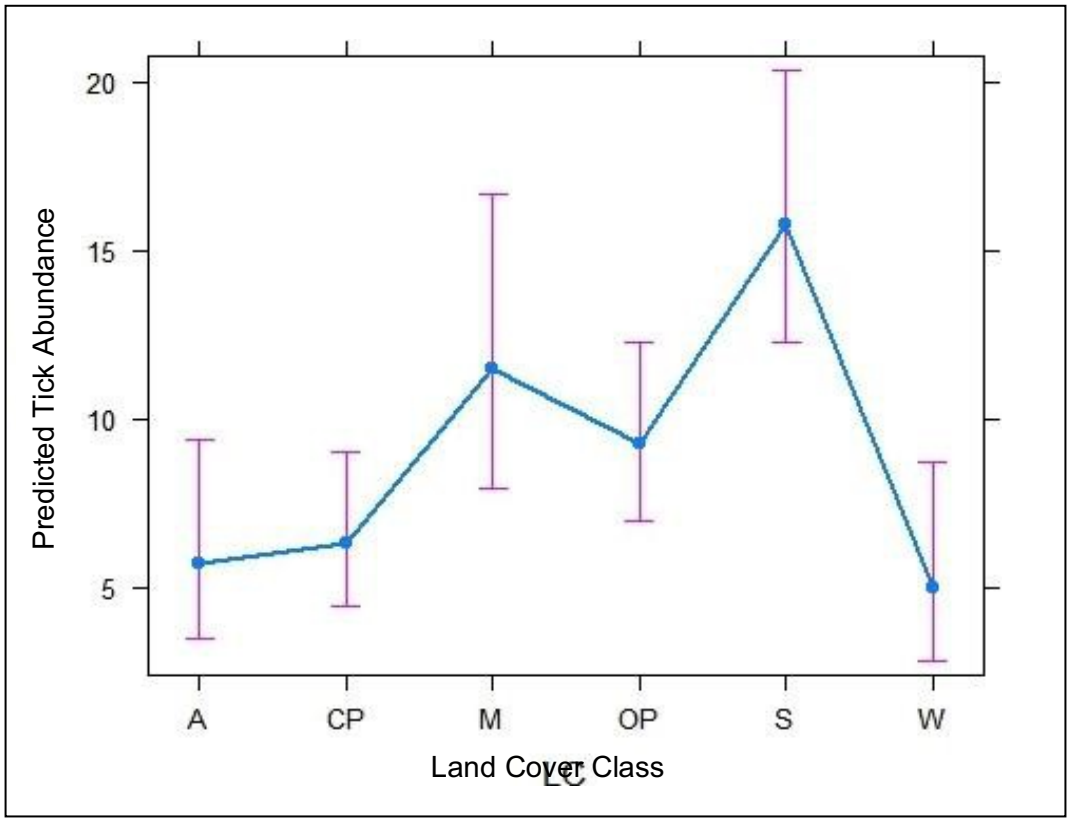

Figure 1.11: Predicted questing tick abundance as a function of land cover class. The blue band indicates the 95% confidence interval based on a Poisson distribution.

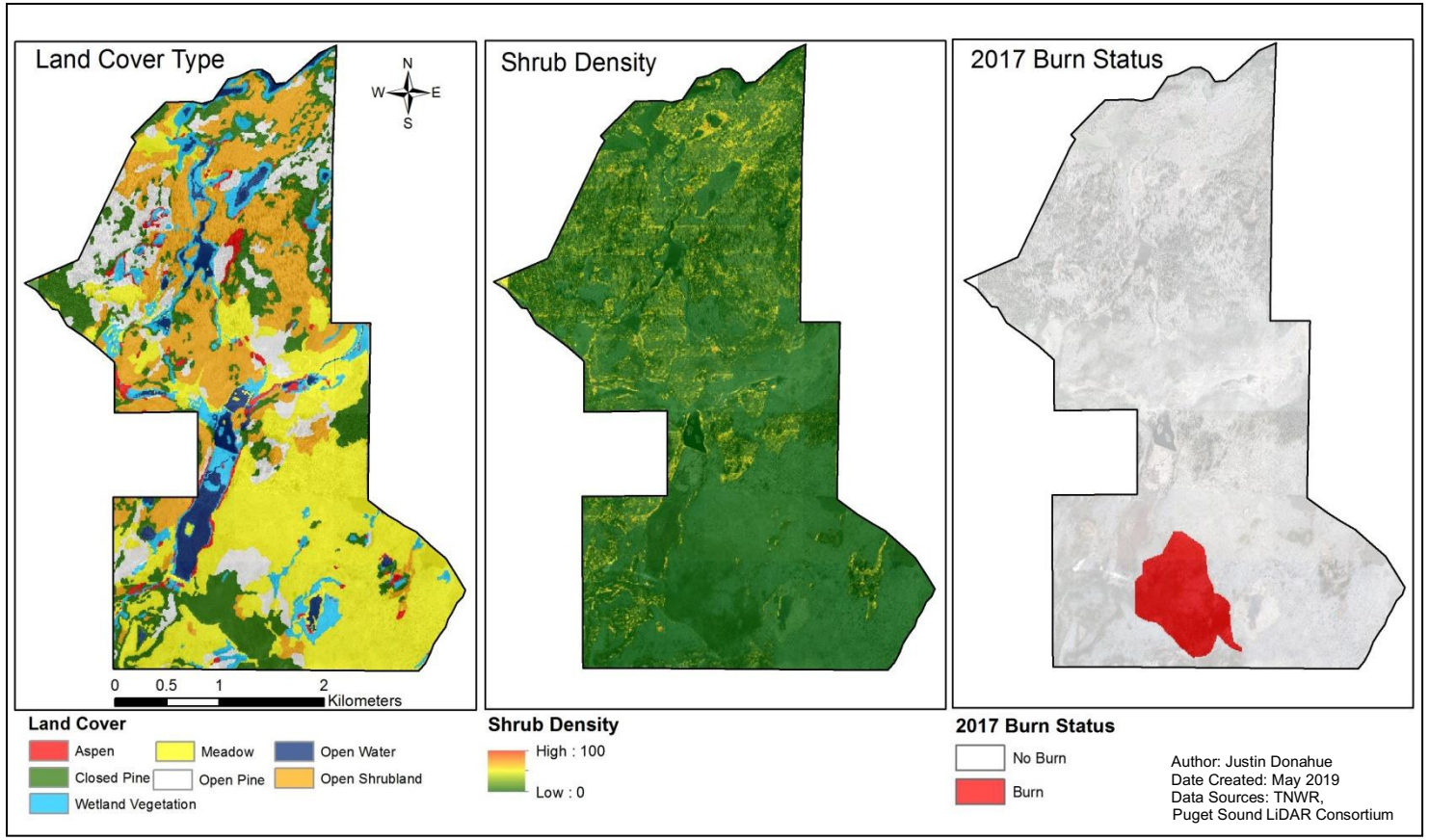

Figure 1.12: All raster layers used to predictive tick density within the public use area of Turnbull National Wildlife Refuge. Each raster was created at a cell size of 10m by 10m.

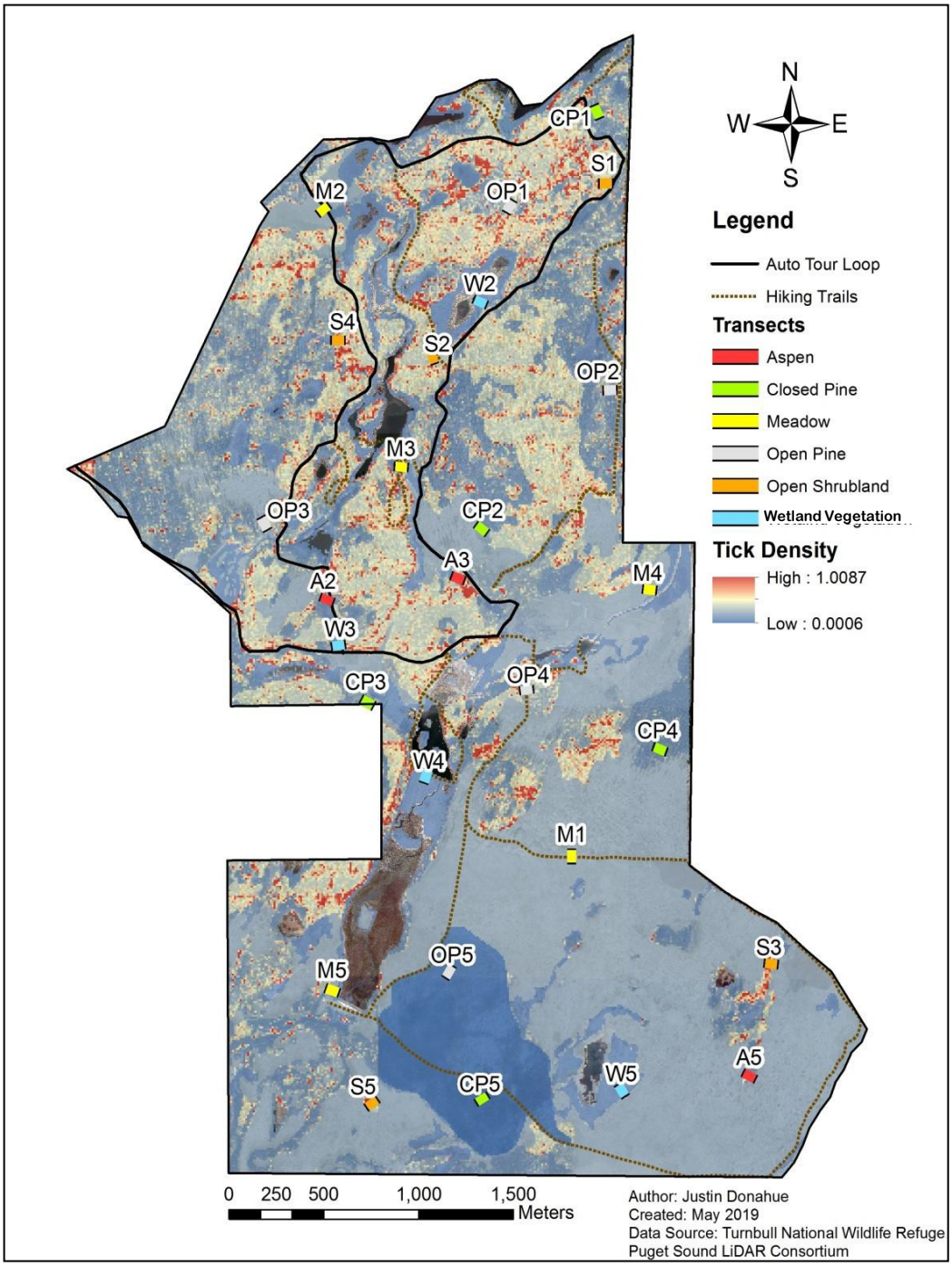

Figure 1.13: Spatial representation of questing tick density in 2018 in public use area of Turnbull National Wildlife Refuge. Tick density is predicted at a resolution of $10m^2$. Predicted density values represent number of questing ticks per $1m^2$ per a week during the tick season of 2018.

Table 2.1: PCR primer sets used in the study

Primer Set	**Gene**	**Nucleotide Sequence (5'-3')**	**Approx. Size (bp)**
Rf7.61p - Rf17.492n (Rr17)	17-kDa genus-common antigen (*rOmpB*)	GCTCTTGCAACTTCTATGTT CATTGTTCGTCAGGTTGGCG	434
Rr190.70p-Rr190.602n (Rr190)	190-kDa antigen (*rOmpA*)	ATGGCGAATATTTCTCCAAAA AGTGCAGCATTCGCTCCCCCT	532
RpCS.877p-RpCS.1258n (RpCS)	Citrate synthase (*gltA*)	GGGGGCCTGCTCACGGCGG ATTGCAAAAAGTACAGTGAACA	381

Table 2.2: Results for the 21 *Rickettsia* positive ticks using the Rr17 primer set to amplify the *rOmpB* common antigen. These matches represent the highest quality hits BLASTn identified. There are 4 ticks which were 100% identical to 3 different *Rickettsia spp.*

Tick ID	Transect	Primer Set	Read Length	BLASTn Hit Description	E-value	Ident (%)	Accession
22	S4	Rr17	410	*Rickettsia rickettsii* strain Iowa isolate Small Clone, complete genome	0.0	100.0	CP018914.1
22	S4	Rr17	410	*Rickettsia parkeri* str. Portsmouth, complete genome	0.0	100.0	CP003341.1
22	S4	Rr17	410	*Rickettsia philipii* str. 364D, complete genome	0.0	100.0	CP003308.1
23	OP3	Rr17	414	*Rickettsia rickettsii* strain Iowa isolate Small Clone, complete genome	0.0	100.0	CP018914.1
23	OP3	Rr17	414	*Rickettsia parkeri* str. Portsmouth, complete genome	0.0	100.0	CP003341.1
23	OP3	Rr17	414	*Rickettsia philipii* str. 364D, complete genome	0.0	100.0	CP003308.1
40	OP2	Rr17	432	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	99.5	CP003342.1
67	OP1	Rr17	424	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	99.8	CP003342.1
141	S3	Rr17	371	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	99.7	CP003342.1
165	CP1	Rr17	413	*Rickettsia rickettsii* strain Iowa isolate Small Clone, complete genome	0.0	100.0	CP018914.1
165	CP1	Rr17	413	*Rickettsia parkeri* str. Portsmouth, complete genome	0.0	100.0	CP003341.1
165	CP1	Rr17	413	*Rickettsia philipii* str. 364D, complete genome	0.0	100.0	CP003308.1
171	OP4	Rr17	414	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	100.0	CP003342.1
230	S4	Rr17	434	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	99.5	CP003342.1
239	S1	Rr17	436	*Rickettsia rickettsii* from Mexico 17 kDa protein gene, partial cds	0.0	99.8	DQ176856.1
243	S3	Rr17	427	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	99.8	CP003342.1

263	CP1	Rr17	434	*Rickettsia rickettsii* from Mexico 17 kDa protein gene, partial cds	0.0	100.0	DQ176856.1
325	OP1	Rr17	423	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	99.8	CP003342.1
335	S4	Rr17	425	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	99.8	CP003342.1
349	CP1	Rr17	413	*Rickettsia rickettsii* strain Iowa isolate Small Clone, complete genome	0.0	100.0	CP018914.1
349	CP1	Rr17	413	*Rickettsia parkeri* str. Portsmouth, complete genome	0.0	100.0	CP003341.1
349	CP1	Rr17	413	*Rickettsia philipii* str. 364D, complete genome	0.0	100.0	CP003308.1
381	S1	Rr17	424	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	99.8	CP003342.1
501	CP1	Rr17	284	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	9e-136	98.2	CP003342.1
506	OP1	Rr17	325	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	3e-166	99.7	CP003342.1
507	OP1	Rr17	432	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	98.8	CP003342.1
508	OP1	Rr17	324	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	3e-155	97.8	CP003342.1
510	CP1	Rr17	425	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	0.0	99.8	CP003342.1
511	CP1	Rr17	327	*Rickettsia rhipicephali* str. 3-7-female6-CWPP, complete genome	2e-167	99.7	CP003342.1

Table 2.3: Results for the 18 *Rickettsia* positive ticks using the Rr190 primer set to amplify the *rOmpA* gene. Of these, 15 ticks were collected in 2018, the other 3 were collected in 2014 (-14). These matches represent the highest quality hits BLASTn identified.

Tick ID	Transect	Primer Set	Read Length	BLASTn Hit Description	E-value	Ident (%)	Accession #
1-14	UNK	Rr190	458	*Rickettsia peacockii* isolate Dv0333 nonfunctional outer membrane protein A (ompA) gene, partial sequence	0.0	99.8	MG834531.1
2-14	UNK	Rr190	454	*Rickettsia peacockii* isolate Dv0333 nonfunctional outer membrane protein A (ompA) gene, partial sequence	0.0	100.0	MG834531.1
3-14	UNK	Rr190	458	*Rickettsia peacockii* isolate Dv0333 nonfunctional outer membrane protein A (ompA) gene, partial sequence	0.0	99.8	MG834531.1
22	S4	Rr190	506	*Rickettsia peacockii* truncated 190-kDa antigen (rOmpA) gene, complete cds	0.0	99.8	U55821.1
23	OP3	Rr190	508	*Rickettsia peacockii* truncated 190-kDa antigen (rOmpA) gene, complete cds	0.0	99.8	U55821.1
40	OP2	Rr190	452	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.3	CP013133.1
67	OP1	Rr190	463	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.4	CP013133.1
141	S3	Rr190	475	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.4	CP013133.1
165	CP1	Rr190	507	*Rickettsia peacockii* truncated 190-kDa antigen (rOmpA) gene, complete cds	0.0	100.0	U55821.1
171	OP4	Rr190	471	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.2	CP013133.1
230	S4	Rr190	452	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.3	CP013133.1
239	S1	Rr190	505	*Rickettsia peacockii* truncated 190-kDa antigen (rOmpA) gene, complete cds	0.0	100.0	U55821.1
243	S3	Rr190	472	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.2	CP013133.1
263	CP1	Rr190	510	*Rickettsia peacockii* truncated 190-kDa antigen (rOmpA) gene, complete cds	0.0	100.0	U55821.1
325	OP1	Rr190	473	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.2	CP013133.1
335	S4	Rr190	462	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.4	CP013133.1
349	CP1	Rr190	519	*Rickettsia peacockii* truncated 190-kDa antigen (rOmpA) gene, complete cds	0.0	99.8	U55821.1
381	S1	Rr190	471	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.2	CP013133.1

Table 2.4: Results for the 17 *Rickettsia* positive ticks using the RpCS primer set to amplify the *gltA* gene. Of these, 15 ticks were collected in 2018, the other 2 were collected in 2014 (-14). These matches represent the highest quality hits BLASTn identified.

Tick ID	Transect	Primer Set	Read Length	BLASTn Hit Description	E-value	Ident (%)	Accession #
2-14	UNK	RpCS	319	*Rickettsia peacockii* strain 5 citrate synthase (gltA) gene, partial cds	4e-164	100.0	KJ663738.1
3-14	UNK	RpCS	324	*Rickettsia peacockii* strain 5 citrate synthase (gltA) gene, partial cds	1e-164	99.7	KJ663738.1
22	S4	RpCS	358	*Rickettsia peacockii* strain 5 citrate synthase (gltA) gene, partial cds	0.0	100.0	KJ663738.1
23	OP3	RpCS	362	*Rickettsia peacockii* strain 5 citrate synthase (gltA) gene, partial cds	0.0	100.0	KJ663738.1
40	OP2	RpCS	324	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.7	CP003341.1
67	OP1	RpCS	357	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	100.0	CP013133.1
141	S3	RpCS	369	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	99.5	CP013133.1
165	CP1	RpCS	360	*Rickettsia peacockii* strain 5 citrate synthase (gltA) gene, partial cds	0.0	100.0	KJ663738.1
171	OP4	RpCS	315	*Rickettsia rhipicephali* strain HJ#5, complete genome	3e-161	100.00	CP013133.1
230	S4	RpCS	387	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	98.7	CP013133.1
239	S1	RpCS	361	*Rickettsia peacockii* strain 5 citrate synthase (gltA) gene, partial cds	0.0	100.0	KJ663738.1
243	S3	RpCS	324	*Rickettsia massiliae* gltA gene for citrate synthase, partial cds, note: sample:R32	1e-165	99.7	AB872797.1
263	CP1	RpCS	374	*Rickettsia peacockii* strain 5 citrate synthase (gltA) gene, partial cds	0.0	99.5	KJ663738.1
325	OP1	RpCS	324	*Rickettsia massiliae* gltA gene for citrate synthase, partial cds, note: sample:R32	1e-165	99.7	AB872797.1
335	S4	RpCS	326	*Rickettsia massiliae* gltA gene for citrate synthase, partial cds, note: sample:R32	8e-167	99.7	AB872797.1
349	CP1	RpCS	383	*Rickettsia peacockii* strain 5 citrate synthase (gltA) gene, partial cds	0.0	99.7	KJ663738.1
381	S1	RpCS	358	*Rickettsia rhipicephali* strain HJ#5, complete genome	0.0	100.0	CP013133.1

Table 2.5: Summary table for all *Rickettsia spp.* detections across the study period with each primer set. Ticks negative for *Rickettsia spp.* infections using the specified primer set are indicated as NA.

Tick ID	Rr17 Identity (*rOmpB*)	Rr190 Identity (*rOmpA*)	RpCS Identity (*gltA*)
22	*R. rickettsii, R. parkeri, R. philipii*	*R. peacockii*	*R. peacockii*
23	*R. rickettsii, R. parkeri, R. philipii*	*R. peacockii*	*R. peacockii*
40	*R. rhipicephali*	*R. rhipicephali*	*R. rhipicephali*
67	*R. rhipicephali*	*R. rhipicephali*	*R. rhipicephali*
141	*R. rhipicephali*	*R. rhipicephali*	*R. rhipicephali*
165	*R. rickettsii, R. parkeri, R. philipii*	*R. peacockii*	*R. peacockii*
171	*R. rhipicephali*	*R. rhipicephali*	*R. rhipicephali*
230	*R. rhipicephali*	*R. rhipicephali*	*R. rhipicephali*
239	*R. rickettsii*	*R. peacockii*	*R. peacockii*
243	*R. rhipicephali*	*R. rhipicephali*	*R. massiliae*
263	*R. rickettsii*	*R. peacockii*	*R. peacockii*
325	*R. rhipicephali*	*R. rhipicephali*	*R. massiliae*
335	*R. rhipicephali*	*R. rhipicephali*	*R. massiliae*
349	*R. rickettsii, R. parkeri, R. philipii*	*R. peacockii*	*R. peacockii*
381	*R. rhipicephali*	*R. rhipicephali*	*R. rhipicephali*
501	*R. rhipicephali*	NA	NA
506	*R. rhipicephali*	NA	NA
507	*R. rhipicephali*	NA	NA
508	*R. rhipicephali*	NA	NA
510	*R. rhipicephali*	NA	NA
511	*R. rhipicephali*	NA	NA
1-14	*R. rickettsii*	*R. peacockii*	NA
2-14	*R. rickettsii*	*R. peacockii*	*R. peacockii*
3-14	*R. rickettsii*	*R. peacockii*	*R. peacockii*

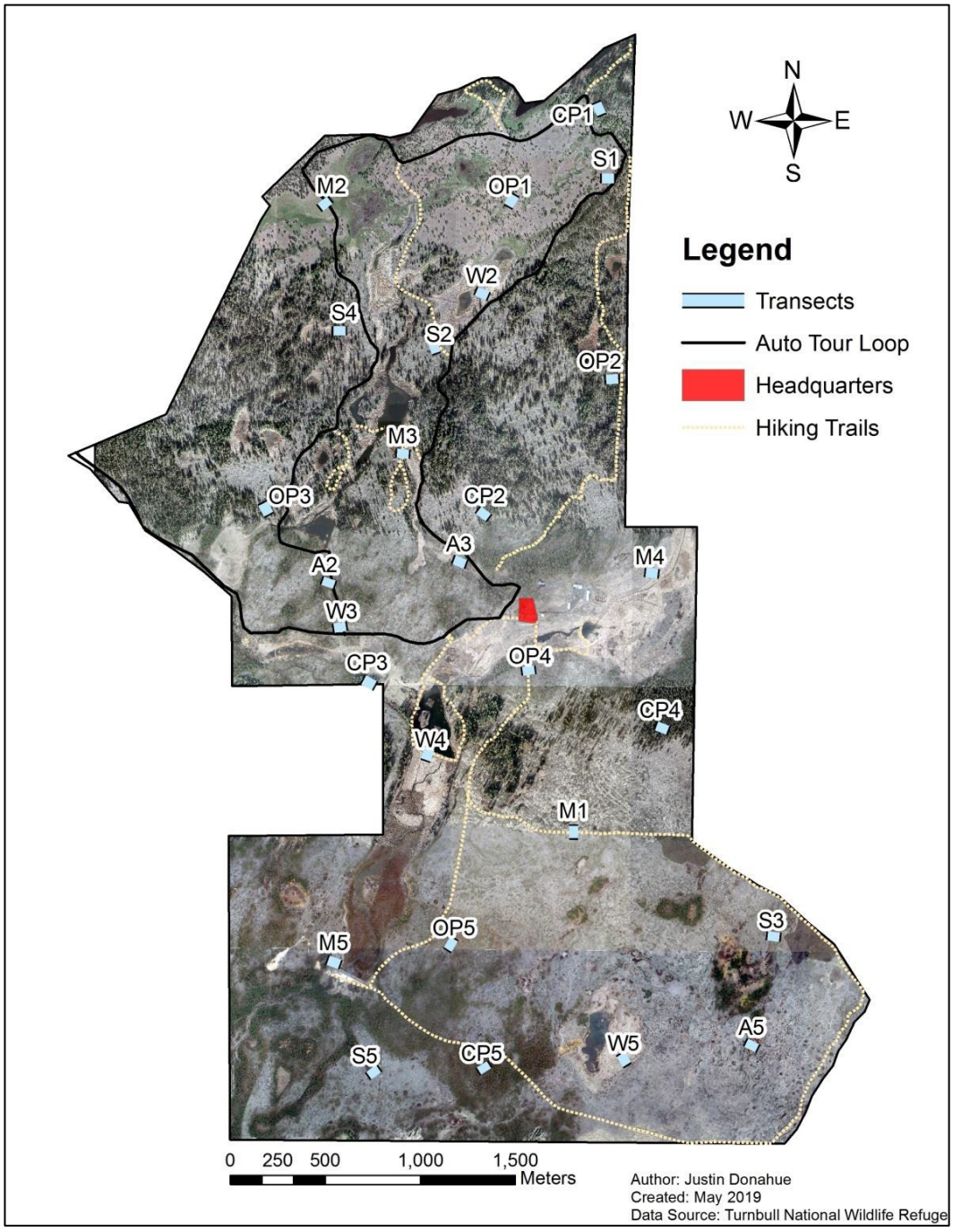

Figure 2.1: Location of 27 transects across the public use area of Turnbull National Wildlife Refuge. Ticks were collected at each of the 27 transects in the Spring of 2018. Transects are enlarged here for visibility.

Figure 2.2: Positive gel electrophoresis results for *Rickettsia spp.* using the *rOmpB* gene fragment. Wells 1-29 are as follows: 100 bp ladder, (+) control, (-) control, ticks 330-355 in sequential order. Positive *Rickettsia* detections at well 9 (tick 335) and well 23 (tick 349).

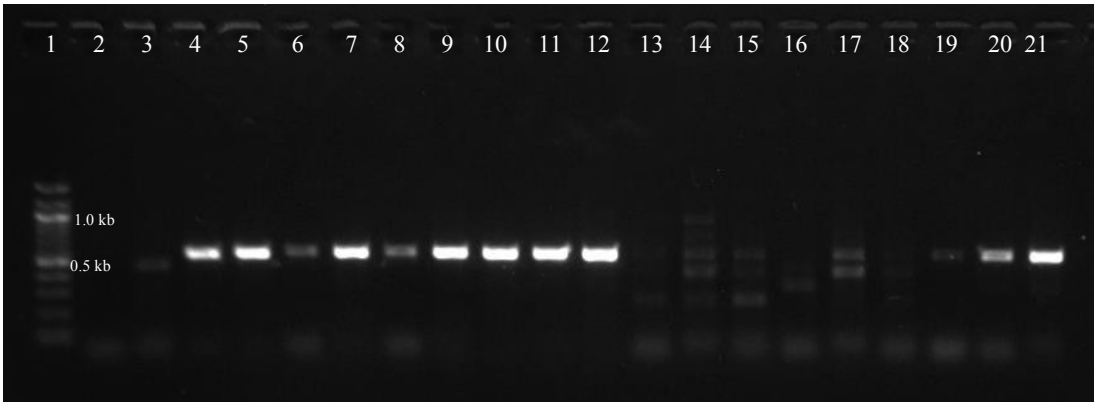

Figure 2.3: Gel electrophoresis results for *Rickettsia spp.* using the 190-kDa *rOmpA* gene fragment. Wells are as follows: 100 bp ladder, (-) control, (+) control, tick (2018); 40, 67, 141, 171, 230, 243, 325, 335, 338, 501, 506, 507, 508, 510, 511, tick (2014); 1-14, 2-14, 3-14

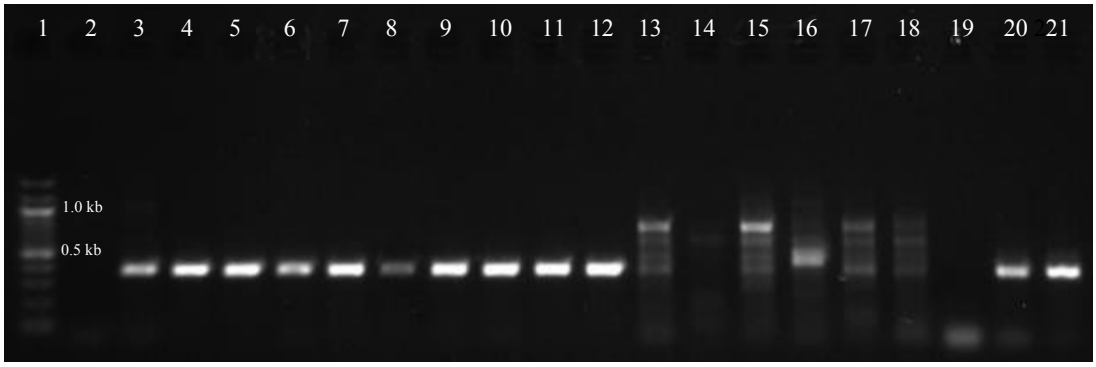

Figure 2.4: Gel electrophoresis results for *Rickettsia spp.* using the citrate synthase *gltA* gene fragment. Wells are as follows: 100 bp ladder, (-) control, (+) control, tick (2018); 40, 67, 141, 171, 230, 243, 325, 335, 338, 501, 506, 507, 508, 510, 511, tick (2014); 1-14, 2-14, 3-14

APPENDIX I: DNA Isolation

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

APPENDIX II: All DNA Sequences

QS denoted by . signifies sequencing that direction was forgone or poor quality.

Tick ID	Gene	QS (f/r)	Read Length	Sequence (5' -3')
22	*rOmpB*	55/55	410	TATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAACACTTCTTGGCGGTG CTGGCGGCGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTGGAGTAGGTGTAGGT GCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAACAGGATAGAAGACTTG CAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGTGGTAGTAACGTAGAATGGCGTAATCCG GATAACGGCAATTACGGTTACGTAACACCTAATAAAACTTATAGAAATAGCACTGGTCAATATTGCCG TGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGGTAATGCATGCCGCCAA CCTG
22	*rOmpA*	55/56	506	TCTCCAAAATTATTTCGAAAAGCAATACAACAAGGTCTTAAAGCCGCTTTATTCACCACCTCAACCGC AGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTGTTGCTACTGATAATCATG CAGCATTTAGTGATAATATTGGCAATGGTAATTGGAATGAGATAACGGCTGAAGGGTTAATTATTATT ACTCCTGCTGACAGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGATTATACTATCACTGCAGAT GTAGCCGATCATATTATTACGGCTATAAATGTTGCGGATACTACTCCTTAGATCTAAATATTGCTCAAA ATACCGTCTTTGGTTCGATTATAACGAGAGATAACTTGTTGCCTGTTACTATTACTGCCGGCAAAAGCT TAACTTTAAATGGTAATAATGCTGTTGCTGCAAATCATGGTTTTGATGCGCCTGCCGATAATTATACAG GTTTAGGAAATATAGATTTAGGGGGA
22	*gltA*	53/54	358	CACGGCGGGGCTAATGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCTAA ATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTATATA AAAACTATGACCCGCGTGCCGTAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGCA GCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATGAATATT TTATTGAGAGAAAAT TATATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAAGCTATGGGTATACCGTCGCAAATGTTCA CTG
23	*rOmpB*	55/55	414	TTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAACACTTCTTGGCG GTGCTGGCGGCGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTGGAGTAGGTGTA GGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAACAGGATAGAAGAC TTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGTGGTAGTAACGTAGAATGGCGTAAT CCGGATAACGGCAATTACGGTTACGTAACACCTAATAAAACTTATAGAAATAGCACTGGTCAATATTG CCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGGTAATGCATGCCGC CAACCTGA

23	*rOmpA*	46/55	508	TTCTCCAAAATTATTTCGAAAAGCAATACAACAAGGTCTTAAAGCCGCTTTATTCACCACCTCAACCG CAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTGTTGCTACTGATAATCAT GCAGCATTTAGTGATAATATTGGCAATGGTAATTGGAATGAGATAACGGCTGAAGGGTTAATTATTAT TACTCCTGCTGACAGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGATTATACTATCACTGCAGA TGTAGCCGATCATATTATTACGGCTATAAATGTTGCGGATACTACTCCTTAGATCTAAATATTGCTCAA AATACCGTCTTTGGTTCGATTATAACGAGAGATAACTTGTTGCCTGTTACTATTACTGCCGGCAAAAGC TTAACTTTAAATGGTAATAATGCTGTTGCTGCAAATCATGGTTTTGATGCGCCTGCCGATAATTATACA GGTTTAGGAAATATAGATTTAGGGGGAG
23	*gltA*	52/54	362	GCTCACGGCGGGGCTAATGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCC TAAATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTAT ATAAAAACTATGACCCGCGTGCCGTAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGG GCAGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATGAAT ATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAAGCTATGGGTA TACCGTCGCA AATGTTCACTGT
40	*rOmpB*	54/55	432	GCTCTTGCAACTTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAAC ACTTCTTGGCGGTGCTGGAGGTGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTG GAGTAGGTGTAGGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAGCA GGATAGAAGACTTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGCGGTAGTAACGTA GAATGGCGTAATCCGGATAACGGCAATTACGGTTACATAACACCTAATAAAACTTATAGAAATAGCA CCGGTCAATATTGCCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGG TAATGCATGCCGCCAACCTGACGAACAA
40	*rOmpA*	55/.	452	CCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGTAGGTGTTATTTCT ACTAATAATAATGCAGCATTTAGTGACCTTGCTGTTGCCAATAATTGGAATGATATAACGGCTAAAGG GGTAGCTAATGGTACTCCTGTTGACGGTCCTCAAAATGGTACGGCATTTACTTACGGTGGTGATCATA CTATCACTGCAGATGAAGCCGGTTGTATTATTACGGCTATAAATGTTGCGGGTACTACTCCCGTAGGT CTAAATATTACTCAAAATACCGTCGTTGGTTCGATTGTGACGGGAGGTAACTTGTTGCCTGTTACTATT ACTGCCGGTAAAAGCTTAACTTTAAACGGTACTAATGCTGTTGCTGCAAATCATGGTTTTGATGCTCCT GCCGATAATTATACAGGTTTAGGAAATATAACTTTAGGGGGA
40	*gltA*	54/54	371	TGGGGGCCTGCTCACGGCGGGGCTAATGAAGTGGTAATAAATATGCTTAAAGAAATCGGTAGTTCAG AGTATATTCCTAAATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGGTTAATGGGTTTTGGT CATCGTATATATAAAAACTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAA AGGAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTT AAAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAA GCTATGGGTATACCGTCGCAA ATGTTCACTGT

67	*rOmpB*	55/55	424	GCTCTTGCAACTTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAAC ACTTCTTGGCGGTGCTGGAGGTGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTG GAGTAGGTGTAGGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAGCA GGATAGAAGACTTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGCGGTAGTAACGTA GAATGGCGTAATCCGGATAACGGCAATTACGGTTACATAACACCTAATAAAACTTATAGAAATAGCA CCGGTCAATATTGCCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGG TAATGCATGCCGCCAACCTG
67	*rOmpA*	51/.	463	CCGCTTTATTCACCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGTA GGTGTTATTTCTACTAATAATAATGCAGCATTTAGTGACCTTGCTGTTGCCAATAATTGGAATGATATA ACGGCTAAAGGGGTAGCTAATGGTACTCCTGTTGACGGTCCTCAAAATGGTACGGCATTTACTTACGG TGGTGATCATACTATCACTGCAGATGAAGCCGGTTGTATTATTACGGCTATAAATGTTGCGGGTACTA CTCCCGTAGGTCTAAATATTACTCAAAATACCGTCGTTGGTTCGATTGTGACGGGAGGTAACTTGTTGC CTGTTACTATTACTGCCGGTAAAAGCTTAACTTTAAACGGTACTAATGCTGTTGCTGCAAATCATGGTT TTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAA CTTTAGGGGG
67	*gltA*	53/54	357	ACGGCGGGGCTAATGAAGTGGTAATAAATATGCTTAAAGAAATCGGTAGTTCAGAGTATATTCCTAA ATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGGTTAATGGGTTTTGGTCATCGTATATATA AAAACTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGCA GCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATGAATATT TTATTGAGAGAAAATTA TATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAAGCTATGGGTATACCGTCGCAA ATGTTCACTG
141	*rOmpB*	./55	371	CATTGTTCGTCAGGTTGGCGGCATGCATTACCGTATGCTTTTTGTTGTTTTCCGCCTATTACAACTGTTT GAGTGTACTCACGGCAATATTGACCGGTGCTATTTCTATAAGTTTTATTAGGTGTTATGTAACCGTAAT TGCCGTTATCCGGATTACGCCATTCTACGTTACTACCGCTAGGAGCTGTTTCTAAAGCTCTCTGTGAGG TAAGCTCTGCAAGTCTTCTATCCTGCTCATCCATACCTGCACCGATTTGTCCACCAAGAACTGCTCCAA GTAATGCACCTACACCTACTCCAACAAGCTGTCCTTTGCCCTTACCGAATTGAGAACCAAGTAATGCA CCTCCAGCACCGCCAAGAAGTGTTCC
141	*rOmpA*	43/.	475	CCGCTTTATTCACCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGTA GGTGTTATTTCTACTAATAATAATGCAGCATTTAGTGACCTTGCTGTTGCCAATAATTGGAATGATATA ACGGCTAAAGGGGTAGCTAATGGTACTCCTGTTGACGGTCCTCAAAATGGTACGGCATTTACTTACGG TGGTGATCATACTATCACTGCAGATGAAGCCGGTTGTATTATTACGGCTATAAATGTTGCGGGTACTA CTCCCGTAGGTCTAAATATTACTCAAAATACCGTCGTTGGTTCGATTGTGACGGGAGGTAACTTGTTGC CTGTTACTATTACTGCCGGTAAAAGCTTAACTTTAAACGGTACTAATGCTGTTGCTGCAAATCATGGTT TTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAACTTTAGGGGGAGCGAATGCTGC
141	*gltA*	52/54	369	ACGGCGGGGCTAATGAAGTGGTAATAAATATGCTTAAAGAAATCGGTAGTTCAGAGTATATTCCTAA ATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGGTTAATGGGTTTTGGTCATCGTATATATA AAAACTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGCA

				GCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATGAATATT TTATTGAGAGAAAATTAT ATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAAGCTATGGGTATACCGTCGCAAATGTTCACTG TACTTTTTTGCA
165	*rOmpB*	55/55	413	TTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAACACTTCTTGGCG GTGCTGGCGGCGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTGGAGTAGGTGTA GGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAACAGGATAGAAGAC TTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGTGGTAGTAACGTAGAATGGCGTAAT CCGGATAACGGCAATTACGGTTACGTAACACCTAATAAAACTTATAGAAATAGCACTGGTCAATATTG CCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGGTAATGCATGCCGC CAACCTG
165	*rOmpB*	55/55	413	TTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAACACTTCTTGGCG GTGCTGGCGGCGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTGGAGTAGGTGTA GGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAACAGGATAGAAGAC TTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGTGGTAGTAACGTAGAATGGCGTAAT CCGGATAACGGCAATTACGGTTACGTAACACCTAATAAAACTTATAGAAATAGCACTGGTCAATATTG CCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGGTAATGCATGCCGC CAACCTG
165	*rOmpA*	55/56	507	TCTCCAAAATTATTTCGAAAAGCAATACAACAAGGTCTTAAAGCCGCTTTATTCACCACCTCAACCGC AGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTGTTGCTACTGATAATCATG CAGCATTTAGTGATAATATTGGCAATGGTAATTGGAATGAGATAACGGCTGAAGGGTTAATTATTATT ACTCCTGCTGACAGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGATTATACTATCACTGCAGAT GTAGCCGATCATATTATTACGGCTATAAATGTTGCGGATACTACTCCTTAGATCTAAATATTGCTCAAA ATACCGTCTTTGGTTCGATTATAACGAGAGGTAACTTGTTGCCTGTTACTATTACTGCCGGCAAAAGCT TAACTTTAAATGGTAATAATGCTGTTGCTGCAAATCATGGTTTTGATGCGCCTGCCGATAATTATACAG GTTTAGGAAATATAGATTT AGGGGGAG
165	*gltA*	53/53	360	CACGGCGGGGCTAATGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCTAA ATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTATATA AAAACTATGACCCGCGTGCCGTAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGCA GCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATGAATATT TTATTGAGAGAAAAT TATATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAAGCTATGGGTATACCGTCGC AAATGTTCACTGTA
171	*rOmpB*	55/55	414	CTTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAACACTTCTTGGC GGTGCTGGAGGTGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTGGAGTAGGTGT AGGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAGCAGGATAGAAGA

				CTTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGCGGTAGTAACGTAGAATGGCGTAA TCCGGATAACGGCAATTACGGTTACATAACACCTAATAAAACTTATAGAAATAGCACCGGTCAATATT GCCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGGTAATGCATGCCG CCAACCTG
171	*rOmpA*	55/.	471	GTCTTAAGCCGCTTTATTCACCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTG TTGCTGTAGGTGTTATTTCTACTAATAATAATGCAGCATTTAGTGACCTTGCTGTTGCCAATAATTGGA ATGATATAACGGCTAAAGGGGTAGCTAATGGTACTCCTGTTGACGGTCCTCAAAATGGTACGGCATTT ACTTACGGTGGTGATCATACTATCACTGCAGATGAAGCCGGTTGTATTATTACGGCTATAAATGTTGC GGGTACTACTCCCGTAGGTCTAAATATTACTCAAAATACCGTCGTTGGTTCGATTGTGACGGGAGGTA ACTTGTTGCCTGTTACTATTACTGCCGGTAAAAGCTTAACTTTAAACGGTACTAATGCTGTTGCTGCAA ATCATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAACTTTAGGGGG
171	*gltA*	53/.	315	TCGGTAGTTCAGAGTATATTCCTAAATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGGTTA ATGGGTTTTGGTCATCGTATATATAAAAACTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAA AGAAGTATTAAAGGAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAA GCTATCGCTCTTA AAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAAG CTATGGGTATACCGTCGCAAATGTTCACTG
230	*rOmpB*	40/55	434	GCTCTTGCAACTTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAAC ACTTCTTGGCGGTGCTGGAGGTGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTG GAGTAGGTGTAGGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAGCA GGATAGAAGACTTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGCGGTAGTAACGTA GAATGGCGTAATCCGGATAACGGCAATTACGGTTACATAACACCTAATAAAACTTATAGAAATAGCA CCGGTCAATATTGCCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGG TAATGCATGCCGCCAACCTGACGAACAATG
230	*rOmpA*	54/.	452	CCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGTAGGTGTTATTTCT ACTAATAATAATGCAGCATTTAGTGACCTTGCTGTTGCCAATAATTGGAATGATATAACGGCTAAAGG GGTAGCTAATGGTACTCCTGTTGACGGTCCTCAAAATGGTACGGCATTTACTTACGGTGGTGATCATA CTATCACTGCAGATGAAGCCGGTTGTATTATTACGGCTATAAATGTTGCGGGTACTACTCCCGTAGGT CTAAATATTACTCAAAATACCGTCGTTGGTTCGATTGTGACGGGAGGTAACTTGTTGCCTGTTACTATT ACTGCCGGTAAAAGCTTAACTTTAAACGGTACTAATGCTGTTGCTGCAAATCATGGTTTTGATGCTCCT GCCGATAATTATACAGGTTTAGGAAATATAACTTTAGGGGGA
230	*gltA*	53/54	379	TGGGGGCCTGCTCACGGCGGGGCTAATGAAGTGGTAATAAATATGCTTAAAGAAATCGGTAGTTCAG AGTATATTCCTAAATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGGTTAATGGGTTTTGGT CATCGTATATATAAAAACTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAA AGGAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTT AAAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAA GCTATGGGTATACCGTCGCAA

				ATGTTCACTGTATTTTTTT
239	*rOmpB*	55/55	436	GCTCTTGCCAACTTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAA CACTTCTTGGCGGTGCTGGCGGCGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTT GGAGTAGGTGTAGGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAAC AGGATAGAAGACTTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGTGGTAGTAACGT AGAATGGCGTAATCCGGATAACGGCAATTACGGTTACGTAACACCTAATAAAACTTATAGAAATAGC ACTGGTCAATATTGCCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACG GTAATGCATGCCGCCAACCTGACGAACAATGA
239	*rOmpA*	55/55	505	TCTCCAAAATTATTTCGAAAAGCAATACAACAAGGTCTTAAAGCCGCTTTATTCACCACCTCAACCGC AGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTGTTGCTACTGATAATCATG CAGCATTTAGTGATAATATTGGCAATGGTAATTGGAATGAGATAACGGCTGAAGGGTTAATTATTATT ACTCCTGCTGACAGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGATTATACTATCACTGCAGAT GTAGCCGATCATATTATTACGGCTATAAATGTTGCGGATACTACTCCTTAGATCTAAATATTGCTCAAA ATACCGTCTTTGGTTCGATTATAACGAGAGGTAACTTGTTGCCTGTTACTATTACTGCCGGCAAAAGCT TAACTTTAAATGGTAATAATGCTGTTGCTGCAAATCATGGTTTTGATGCGCCTGCCGATAATTATACAG GTTTAGGAAATATAGATTTAGGGGG
239	*gltA*	53/54	361	CCACGGCGGGGCTAATGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCTA AATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTATAT AAAAACTATGACCCGCGTGCCGTAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGC AGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATGAATAT TTTATTGAGAGAAA ATTATATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAAGCTATGGGTATACCGTCGCAAATGTT CACTGTA
243	*rOmpB*	56/55	427	GCTCTTGCAACTTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAAC ACTTCTTGGCGGTGCTGGAGGTGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTG GAGTAGGTGTAGGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAGCA GGATAGAAGACTTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGCGGTAGTAACGTA GAATGGCGTAATCCGGATAACGGCAATTACGGTTACATAACACCTAATAAAACTTATAGAAATAGCA CCGGTCAATATTGCCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGG TAATGCATGCCGCCAACCTGACC
243	*rOmpA*	55/.	472	AGGTCTTAAGCCGCTTTATTCACCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGG TGTTGCTGTAGGTGTTATTTCTACTAATAATAATGCAGCATTTAGTGACCTTGCTGTTGCCAATAATTG GAATGATATAACGGCTAAAGGGGTAGCTAATGGTACTCCTGTTGACGGTCCTCAAAATGGTACGGCAT TTACTTACGGTGGTGATCATACTATCACTGCAGATGAAGCCGGTTGTATTATTACGGCTATAAATGTTG CGGGTACTACTCCCGTAGGTCTAAATATTACTCAAAATACCGTCGTTGGTTCGATTGTGACGGGAGGT AACTTGTTGCCTGTTACTATTACTGCCGGTAAAAGCTTAACTTTAAACGGTACTAATGCTGTTGCTGCA AATCATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAACTTTAGGGG

243	*gltA*	54/.	324	GCTTAAGAATCGGTAGTTCAGAGTATATTCCTAAATATATAGCTAAAGCTAAGGATAAAAATGATC CATTTAGGTTAATGGGTTTTGGTCATCGTATATATAAAAACTATGACCCGCGTGCCGCAGTACTTAAA GAAACGTGCAAAGAAGTATTAAAGGAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAA TAGAACTTGAAGCTATCGCTCTTAAAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGAT TTTTATTCGGGTATTATCTATAAAGCTATGGGTATACCGTCGCAAATGTTCACTG
263	*rOmpB*	52/54	434	GCTCTTGCAACTTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAAC ACTTCTTGGCGGTGCTGGCGGCGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTG GAGTAGGTGTAGGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAACA GGATAGAAGACTTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGTGGTAGTAACGTA GAATGGCGTAATCCGGATAACGGCAATTACGGTTACGTAACACCTAATAAAACTTATAGAAATAGCA CTGGTCAATATTGCCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGG TAATGCATGCCGCCAACCTGACGAACAATG
263	*rOmpA*	54/54	510	ATTCTCCAAAATTATTTCGAAAAGCAATACAACAAGGTCTTAAAGCCGCTTTATTCACCACCTCAACC GCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTGTTGCTACTGATAATCA TGCAGCATTTAGTGATAATATTGGCAATGGTAATTGGAATGAGATAACGGCTGAAGGGTTAATTATTA TTACTCCTGCTGACAGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGATTATACTATCACTGCAG ATGTAGCCGATCATATTATTACGGCTATAAATGTTGCGGATACTACTCCTTAGATCTAAATATTGCTCA AAATACCGTCTTTGGTTCGATTATAACGAGAGGTAACTTGTTGCCTGTTACTATTACTGCCGGCAAAA GCTTAACTTTAAATGGTAATAATGCTGTTGCTGCAAATCATGGTTTTGATGCGCCTGCCGATAATTATA CAGGTTTAGGAAATATAGAT TTAGGGGGAGC
263	*gltA*	39/53	374	CACGGCGGGGCTAATGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGTATATTCCTAA ATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCATCGTGTATATA AAAACTATGACCCGCGTGCCGTAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGCA GCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATGAATATT TTATTGAGAGAATAATT ATATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAAGCTATGGGTATACCGTCGCAAATGTTCAC TGTATTTTTTTGCAATT
325	*rOmpB*	55/54	423	TTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAACACTTCTTGGCG GTGCTGGAGGTGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTGGAGTAGGTGTA GGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAGCAGGATAGAAGAC TTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGCGGTAGTAACGTAGAATGGCGTAAT CCGGATAACGGCAATTACGGTTACATAACACCTAATAAAACTTATAGAAATAGCACCGGTCAATATTG CCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGGTAATGCATGCCGC CAACCTGACGAACAATG
325	*rOmpA*	36/.	473	AGGTCTTAAGCCGCTTTATTCACCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGG TGTTGCTGTAGGTGTTATTTCTACTAATAATAATGCAGCATTTAGTGACCTTGCTGTTGCCAATAATTG

				GAATGATATAACGGCTAAAGGGGTAGCTAATGGTACTCCTGTTGACGGTCCTCAAAATGGTACGGCAT TTACTTACGGTGGTGATCATACTATCACTGCAGATGAAGCCGGTTGTATTATTACGGCTATAAATGTTG CGGGTACTACTCCCGTAGGTCTAAATATTACTCAAAATACCGTCGTTGGTTCGATTGTGACGGGAGGT AACTTGTTGCCTGTTACTATTACTGCCGGTAAAAGCTTAACTTTAAACGGTACTAATGCTGTTGCTGCA AATCATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAACTTTAGGGGG
325	*gltA*	53/.	324	GCTTAAGAATCGGTAGTTCAGAGTATATTCCTAAATATATAGCTAAAGCTAAGGATAAAAATGATCCA TTTAGGTTAATGGGTTTTGGTCATCGTATATATAAAAACTATGACCCGCGTGCCGCAGTACTTAAAGA AACGTGCAAAGAAGTATTAAAGGAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAATA GAACTTGAAGCTATCGCTCTTAAAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTT TATTCGGGTATTATCTATAAAGCTATGGGTATACCGTCGCAAATGTTCACTG
335	*rOmpB*	55/55	425	GCTCTTGCAACTTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAAC ACTTCTTGGCGGTGCTGGAGGTGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTG GAGTAGGTGTAGGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAGCA GGATAGAAGACTTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGCGGTAGTAACGTA GAATGGCGTAATCCGGATAACGGCAATTACGGTTACATAACACCTAATAAAACTTATAGAAATAGCA CCGGTCAATATTGCCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGG TAATGCATGCCGCCAACCTGA
335	*rOmpA*	53/.	462	CGCTTTATTCACCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGTAG GTGTTATTTCTACTAATAATAATGCAGCATTTAGTGACCTTGCTGTTGCCAATAATTGGAATGATATAA CGGCTAAAGGGGTAGCTAATGGTACTCCTGTTGACGGTCCTCAAAATGGTACGGCATTTACTTACGGT GGTGATCA TACTATCACTGCAGATGAAGCCGGTTGTATTATTACGGCTATAAATGTTGCGGGTACTACTCCCGTAG GTCTAAATATTACTCAAAATACCGTCGTTGGTTCGATTGTGACGGGAGGTAACTTGTTGCCTGTTACTA TTACTGCCGGTAAAAGCTTAACTTTAAACGGTACTAATGCTGTTGCTGCAAATCATGGTTTTGATGCTC CTGCCGATAATTATACAGGTTTAGGAAATATAACTTTAGGGGG
335	*gltA*	54/.	326	ATGCTTAAGAATCGGTAGTTCAGAGTATATTCCTAAATATATAGCTAAAGCTAAGGATAAAAATGATC CATTTAGGTTAATGGGTTTTGGTCATCGTATATATAAAAACTATGACCCGCGTGCCGCAGTACTTAAA GAAACGTGCAAAGAAGTATTAAAGGAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAA TAGAACTTGAAGCTATCGCTCTTAAAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGAT TTTTATTCGGGTATTATCTATAAAGCTATGGGTATACCGTCGCAAATGTTCACTG
349	*rOmpB*	55/55	413	TTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAACACTTCTTGGCG GTGCTGGCGGCGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTGGAGTAGGTGTA GGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAACAGGATAGAAGAC TTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGTGGTAGTAACGTAGAATGGCGTAAT CCGGATAACGGCAATTACGGTTACGTAACACCTAATAAAACTTATAGAAATAGCACTGGTCAATATTG CCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGGTAATGCATGCCGC CAACCTG

349	*rOmpA*	55/52	519	TATGGCGAAATATTTCTCCAAAATTATTTCGAAAAGCAATACAACAAGGTCTTAAAGCCGCTTTATTC ACCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTGTTGC TACTGATAATCATGCAGCATTTAGTGATAATATTGGCAATGGTAATTGGAATGAGATAACGGCTGAAG GGTTAATTATTATTACTCCTGCTGACAGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGATTATA CTATCACTGCAGATGTAGCCGATCATATTATTACGGCTATAAATGTTGCGGATACTACTCCTTAGATCT AAATATTGCTCAAAATA CCGTCTTTGGTTCGATTATAACGAGAGGTAACTTGTTGCCTGTTACTATTACTGCCGGCAAAAGCTTAA CTTTAAATGGTAATAATGCTGTTGCTGCAAATCATGGTTTTGATGCGCCTGCCGATAATTATACAGGTT TAGGAAATATAGATTTAGGGGG
349	*gltA*	51/54	383	GGGCCTTGCTCACGGCGGGGCTAATGAAGCGGTAATAAATATGCTTAAAGAAATCGGTAGTTCTGAGT ATATTCCTAAATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGATTAATGGGTTTTGGTCAT CGTGTATATAAAAACTATGACCCGCGTGCCGTAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGG AACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAA GATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAAGCT ATGGGTATACCGTCGC AAATGTTCACTGTACTTTTTTGCAATA
381	*rOmpB*	55/55	424	TTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAACACTTCTTGGCG GTGCTGGAGGTGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTGGAGTAGGTGTA GGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAGCAGGATAGAAGAC TTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGCGGTAGTAACGTAGAATGGCGTAAT CCGGATAACGGCAATTACGGTTACATAACACCTAATAAAACTTATAGAAATAGCACCGGTCAATATTG CCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGGTAATGCATGCCGC CAACCTGACGAACAATGA
381	*rOmpA*	38/.	471	GTCTTAAGCCGCTTTATTCACCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGG TGTTGCTGTAGGTGTTATTTCTACTAATAATAATGCAGCATTTAGTGACCTTGCTGTTGCCAATAATTG GAATGATATAACGGCTAAAGGGGTAGCTAATGGTACTCCTGTTGACGGTCCTCAAAATGGTACGGCAT TTACTTACGGTGGTGATCATACTATCACTGCAGATGAAGCCGGTTGTATTATTACGGCTATAAATGTTG CGGGTACTACTCCCGTAGGTCTAAATATTACTCAAAATACCGTCGTTGGTTCGATTGTGACGGGAGGT AACTTGTTGCCTGTTACTATTACTGCCGGTAAAAGCTTAACTTTAAACGGTACTAATGCTGTTGCTGCA AATCATGGTTTTGATGCTCCTGCCGATAATTATACAGGTTTAGGAAATATAACTTTAGGGGG
381	*gltA*	54/54	358	CACGGCGGGGCTAATGAAGTGGTAATAAATATGCTTAAAGAAATCGGTAGTTCAGAGTATATTCCTAA ATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGGTTAATGGGTTTTGGTCATCGTATATATA AAAACTATGACCCGCGTGCCGCAGTACTTAAAGAAACGTGCAAAGAAGTATTAAAGGAACTCGGGCA GCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGAAGCTATCGCTCTTAAAGATGAATATT TTATTGAGAGAAAATTA TATCCAAATGTTGATTTTTATTCGGGTATTATCTATAAAGCTATGGGTATACCGTCGCAAATGTTCACT G

501	*rOmpB*	24/.	284	CTGGCGGTATGAATAAACAAGGTACAGGATCACTTCTTGGCGGTGCTGGAGGTGCATTACTTGGTTCT CAATTCGGTAAGGGCAAAGGACAGCTTGTTGGAGTAGGTGTAGGTGCATTACTTGGAGCAGTTCTTGG TGGACAAATCGGTGCAGGTATGGATGAGCAGGATAGAAGACTTGCAGAGCTTACCTCACAGAGAGCT TTAGAAACAGCTCCTAGCCGTAGTTAGTTAGAATGGCGTAATCCGGATAACGGCAATTACGGTTACAT AACACCTAATAAA
506	*rOmpB*	./54	325	ATTGTTCGTCAGGTTGGCGGCATGCATTACCGTATGCTTTTTGTTGTTTTCCGCCTATTACAACTGTTTG AGTGTACTCACGGCAATATTGACCGGTGCTATTTCTATAAGTTTTATTAGGTGTTATGTAACCGTAATT GCCGTTATCCGGATTACGCCATTCTACGTTACTACCGCTAGGAGCTGTTTCTAAAGCTCTCTGTGAGGT AAGCTCTGCAAGTCTTCTATCCTGCTCATCCATACCTGCACCGATTTGTCCACCAAGAACTGCTCCAAG TAATGCACCTACACCTACTCCAACAAGCTGTCCTTTGCCCTTACCGAA
507	*rOmpB*	36/52	432	CTTGCAACTTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAACACT TCTTGGCGGTGCTGGAGGTGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTGGAG TAGGTGTAGGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAGCAGGAT AGAAGACTTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGCGGTAGTTATTTAGAATG GCGTAATCCGGATAACGGCAATTACGGTTACATAACACCTAATAAAACTTATAGAAATAGCACCGGTC AATATTGCCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGGTAATGC ATGCCGCCAACCTGACGAAC AATGA
508	*rOmpB*	./21	324	TCATTGTTCGTCAGGTGGGCGGCATGCATTACCGTATGCTTTTTGTTGTTTTCCGCCTATTACAACTGTT TGAGTGTACTCACGGCAATATTGACCGGGGCTATTTTTATAAGTTTTATTAGGGGTTATGTAACCGTAA TTGCCGTTATCCGGATTACGCCATTTTACGTTACTACCGCTAGGAGCTGTTTTTAAAGCTCTCTGTGAG GTAAGCTCTGCAAGTCTTCTATCCTGCTCATCCATACCTGCACCGATTTGTCCACCAAGAACTGCTCCA AGTAATGCACCTACACCTACTCCAACAAGCTGTCCTTTGCCCTTACC
510	*rOmpB*	35/43	425	GCTCTTGCAACTTCTATGTTACAAGCCTGTAACGGTCCGGGCGGTATGAATAAACAAGGTACAGGAAC ACTTCTTGGCGGTGCTGGAGGTGCATTACTTGGTTCTCAATTCGGTAAGGGCAAAGGACAGCTTGTTG GAGTAGGTGTAGGTGCATTACTTGGAGCAGTTCTTGGTGGACAAATCGGTGCAGGTATGGATGAGCA GGATAGAAGACTTGCAGAGCTTACCTCACAGAGAGCTTTAGAAACAGCTCCTAGCGGTAGTAACGTA GAATGGCGTAATCCGGATAACGGCAATTACGGTTACATAACACCTAATAAAACTTATAGAAATAGCA CCGGTCAATATTGCCGTGAGTACACTCAAACAGTTGTAATAGGCGGAAAACAACAAAAAGCATACGG TAATGCATGCCGCCAACCTGA
511	*rOmpB*	./49	327	TGTTCGTCAGGTTGGCGGCATGCATTACCGTATGCTTTTTGTTGTTTTCCGCCTATTACAACTGTTTGAG TGTACTCACGGCAATATTGACCGGTGCTATTTCTATAAGTTTTATTAGGTGTTATGTAACCGTAATTGC CGTTATCCGGATTACGCCATTCTACGTTACTACCGCTAGGAGCTGTTTCTAAAGCTCTCTGTGAGGTAA GCTCTGCAAGTCTTCTATCCTGCTCATCCATACCTGCACCGATTTGTCCACCAAGAACTGCTCCAAGTA ATGCACCTACACCTACTCCAACAAGCTGTCCTTTGCCCTTACCGAATTGA
1-14	*rOmpA*	53/.	485	TATTCACCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTT GTTGCTACTGATAATCATGCAGCATTTAGTGATAATATTGGCAATGGTAATTGGAATGAGATAACGGC

				TGAAGGGTTAATTATTATTACTCCTGCTGACAGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGA TTATACTATCACTGCAGATGTAGCCGATCATATTATTACGGCTATAAATGTTGCGGATACTACTCCTTA GATCTAAATATTGCTCAAAATACCGTCTTTGGTTCGATTATAACGAGAGATAACTTGTTGCCTGTTACT ATTACTGCCGGCAAAAGCTTAACTTTAAATGGTAATAATGCTGTTGCTGCAAATCATGGTTTTGATGC GCCTGCCGATAATTATACAGGTTTAGGAAATATAGATTTAGGGGGAG
2-14	*rOmpA*	55/.	454	CCACCTCAACCGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGTTGTTGCT ACTGATAATCATGCAGCATTTAGTGATAATATTGGCAATGGTAATTGGAATGAGATAACGGCTGAAGG GTTAATTATTATTACTCCTGCTGACAGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTGATTATAC TATCACTGCAGATGTAGCCGATCATATTATTACGGCTATAAATGTTGCGGATACTACTCCTTAGATCTA AATATTGCTCAAAATACCGTCTTTGGTTCGATTATAACGAGAGGTAACTTGTTGCCTGTTACTATTACT GCCGGCAAAAGCTTAA CTTTAAATGGTAATAATGCTGTTGCTGCAAATCATGGTTTTGATGCGCCTGCCGATAATTATACAGGTT TAGGAAATATAGATTTAGGGGGAGCG
2-14	*gltA*	52/.	319	ATCGGTAGTTCTGAGTATATTCCTAAATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTAGATT AATGGGTTTTGGTCATCGTGTATATAAAAACTATGACCCGCGTGCCGTAGTACTTAAAGAAACGTGCA AAGAAGTATTAAAGGAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTTGA AGCTATC GCTCTTAAAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCGGGTATTATC TATAAAGCTATGGGTATACCGTCGCAAATGTTCACTGTAT
3-14	*rOmpA*	54/.	458	TATTCACCACCTCAACCAGCAGCGATAATGCTGAGTAGTAGCGGGGCACTCGGTGTTGCTGCAGGTGT TGTTGCTACTGATAATCATGCAGCATTTAGTGATAATATTGGCAATGGTAATTGGAATGAGATAACGG CTGAAGGGTTAATTATTATTACTCCTGCTGACAGTCCTCAAAACAATTGGGCATTTACTTACGGTGGTG ATTATACTATCACTGCAGATGTAGCCGATCATATTATTACGGCTATAAATGTTGCGGATACTACTCCTT AGATCTAAATATTGCTCAAAATACCGTCTTTGGTTCGATTATAACGAGAGGTAACTTGTTGCCTGTTAC TATTACTGCCGGCAAAA GCTTAACTTTAAATGGTAATAATGCTGTTGCTGCAAATCATGGTTTTGATGCGCCTGCCGATAATTATA CAGGTTTAGGAAATATAGATTTAGGGGGA
3-14	*gltA*	54/.	324	AAGAATCGGTAGTTCTGAGTATATTCCTAAATATATAGCTAAAGCTAAGGATAAAAATGATCCATTTA GATTAATGGGTTTTGGTCATCGTGTATATAAAAACTATGACCCGCGTGCCGTAGTACTTAAAGAAACG TG CAAAGAAGTATTAAAGGAACTCGGGCAGCTAGACAACAATCCGCTCTTACAAATAGCAATAGAACTT GAAGCTATCGCTCTTAAAGATGAATATTTTATTGAGAGAAAATTATATCCAAATGTTGATTTTTATTCG GGTATTATCTATAAAGCTATGGGTATACCGTCGCAAATGTTCACTGTATT

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Presentations:

J. L. Donahue. 2019. Detection of *Rickettsia spp.* in Ticks at Turnbull National Wildlife Refuge, WA. Eastern Washington University Research and Creative Works Symposium, Cheney, Washington

J. L. Donahue. 2018. Factors Influencing *Dermacentor* Tick Density at Turnbull National Wildlife Refuge, WA. Eastern Washington University Research Symposium, Cheney, Washington

J. L. Donahue. 2018. Evaluating Factors Influencing *Dermacentor* Tick Density in Eastern Washington. Entomological Society of America Annual Conference, Vancouver, British Columbia, Canada

J. L. Donahue and K. Duckett. 2016. Heavy Metal Analysis of the Coeur d'Alene River System. Geological Society of America, Moscow, Idaho