Proceedings of The National Conference
On Undergraduate Research (NCUR) 2015
Eastern Washington University, Cheney, WA
April 16-18, 2015

Spatiotemporal And Environmental Factors Affecting *Dermacentor* Tick Abundance At Turnbull National Wildlife Refuge

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Abstract

Understanding the environmental factors determining risk of exposure to disease vectors is critical for public and veterinary health. Tick populations from the Turnbull National Wildlife Refuge were collected from using drag sampling from mid-June to early August 2014 in order to determine the effect of temperature, seasonality, and habitat on tick abundance. Two species of ticks were collected, *Dermacentor andersoni* and *Dermacentor variabilis*. This study found a significant difference in overall mean abundances between transects ($p < 0.01$), as well as an approximately 5 fold decrease in mean abundance over the second half of our study ($p < 0.01$). Changes in tick abundance were significantly different across transects. The proportion of the two tick species was consistent across all transects except transect 5, which saw disproportionately more *D. variabilis* ($p < 0.05$). Tick abundance was not regulated by temperature alone, but did exhibit a seasonal dynamic, decreasing later in the summer. Tick abundance also varied by transect and even within transects themselves.

Keywords: ***Dermacentor*, Tick, Turnbull**

1. Introduction

Ticks, as vectors, transmit a wide variety of pathogens of public health or veterinary importance[4, 9]. Being able to inform people of high risk seasons or habitats can reduce transmission risk of some of these pathogens[8]. *D. andersoni* can cause tick paralysis in humans, several cases of which have been reported in Washington State[3]. *Dermacentor* ticks also act as vectors for Rocky Mountain Spotted Fever, which is one of the most dangerous tick-borne diseases in the United States[5]. The collection we did was a collaboration with the lab of Dr. Luis Matos, also at the Department of Biology at Eastern Washington University, who screened our specimens for the presence of *Rickettsia rickettsia*, the causative agent of Rocky Mountain Spotted Fever[11]. This collaboration allows us to preliminarily assess risks of exposure in the area. We also worked closely with staff at Turnbull National Wildlife Refuge (TNWR), where our study was conducted. So far only a few thorough studies have been conducted to examine ticks in Washington State, and none have been conducted at TNWR previously. The Washington State Department of Health Tick Surveillance Program identifies and screens ticks that are submitted by the public, with whom we also coordinated our program. Between the years 2011 and 2013 they were able to find non-pathogenic species of *Rickettsia* in 29 ticks from 12 counties in Washington State, including 2 from Spokane County[7]. However, this testing relies on public cooperation and submission of ticks. This screening process is not designed to study the tick behaviors, and only gives a very general sense of their distributions. It is also important to examine these factors more precisely to better serve the public health.

In addition to *Rickettsia rickettsii*, many other species of bacteria in the Rickettsia genus are transmitted by ticks, however most of these are non-pathogenic to people[10]. One study, conducted in Canada, tested 1,326 ticks and found that 84% carried non-pathogenic *Rickettsia* species[2]. These results also provided some evidence to support the hypothesis that carrying non-pathogenic *Rickettsia* species may prevent *R. rickettsii* becoming established in the tick[2]. This will be helpful in assessing risks to the public since when non-pathogenic *Rickettsia* can be detected in tick populations, we can assume a reduced risk. It is also important to understand the factors affecting their foraging behavior, typically referred to as, "questing." Ticks have been shown to be sensitive to temperature and humidity with respect to questing behavior, and that sensitivity was not specific to one age group[12]. However, it has also been demonstrated that environmental factors may differentially impact populations of *D. andersoni* that are adapted to different environments[6]. The purpose of our study was to examine the spatial and temporal distribution of ticks at TNWR, and the effects of temperature and seasonality on their abundance and distribution. We expected to find variation in tick densities in different habitat conditions, but a consistent correlation between ambient temperature and tick abundance across all transects. Along with temperature a consistent trend in tick abundance and time of season was also expected.

2. Materials and Methods

2.1 Study Area

Ticks were sampled from 6 different locations at Turnbull National Wildlife Refuge (TNWR). The sites were chosen based on the presence of wildlife trails, observations of wildlife, scat, fur, signs of animal bedding, and reported tick activity from the staff at TNWR. At each site we measured out one 100m transect, divided into 10m increments. Transect 1 (N 47° 26.376', W 117° 32.700' to N 47° 26.399', W 117° 32.650') was located in a meadow just west of a large wetland, outside the public use area of the refuge. Transect 2 (N 47° 25.953', W 117° 32.709' to N 47° 25.997', W 117° 32.742') was inside the public use area, just off of the autotour route. This transect went through a small *Populus tremuloides* (American aspen) stand and was flanked by small ephemeral water bodies on both sides. Transect 3 (N 47° 25.410', W 117° 32.928' to N 47° 25.463', W 117° 32.919') was also off of the autotour route, and went across a small area of scabland and basalt mounds. This transect was located between two small ponds, passed through a small *Pinus ponderosa* (Ponderosa pine) stand, and an intersection of wildlife trails. Transect 4 (N 47° 27.233', W 117° 34.435' to N 47° 27.228', W 117° 34.438') was located at the Turnbull Lab for Ecological Studies (TLES). Transect 4 ran alongside a large pond, mostly through *Phalaris arundinacea* (reed canarygrass). Transect 5 (N 47° 27.311', W 117° 34.448' to N 47° 27.345', W 117° 34.391') was also located at TLES, and covered a large patch of *Symphoricarpos albus* (common snowberry), as well as an intersection of two wildlife trails. Transect 6 (N 47° 24.890', W 117° 33.203' to N 47° 24.836', W 117° 33.187') was located outside the public access area of the refuge in an area of closed-canopy *P. ponderosa* stands. *S. albus* was also a main component of Transect 6.

2.2 Sampling

Tick collection took place in the summer of 2014 from June 19th until August 2nd for transects 1, 2, 3, 4, and 5. Transect 6 was added on June 24th. The ticks were collected between the hours of 6:00am and 10:00am. Each transect was collected on once a day, for 3 days a week. The ambient temperature was recorded from the start of each transect about 10cm above the ground. Using a 1m wide by 1.5m long white cloth attached to a dowel, weighted down by a chain, tick drags were conducted. At each of the 10m increments the collector checked themselves and the cloth, front and back, for ticks. Any ticks found were stored in vials containing a 75% ethanol solution. The distance to first encountering a tick within a transect was also recorded. The stored ticks were transferred to the lab at Eastern Washington University in order to be processed.

2.3 Processing

In the lab ticks were identified to species, and their sex and life stage determined. Tick sex was determined by the scutum. The scutum covers the entire dorsal side on both *Dermacentor andersoni* and *Dermacentor variabilis* males, while the scutum only partially covers the dorsal side of the females of both species. Adults were recognizable due to the presence of the genital orifice, nymphs were much smaller and lacked a genital orifice. The species could be distinguished by the size and abundance of the goblet cells on the spiracular plate[13]. In *D. variabilis* there were many small cells, consistent in size throughout the spiracular plate. In *D. andersoni* the goblet cells closest to the spiracle were large, and few in number. The cells on the edge of the spiracular plate were similar in size as those in *D. variabilis.*

All specimens were frozen for DNA extraction later by Dr. Luis Matos's lab in order to be tested for the presence of *R. rickettsii.*

2.4 Statistical analysis

This study compared mean tick density between all transects individually as well as across time. For time, we used two different time scales, two week periods (of which there were three), and the first and second half of our study. We fitted statistical models that included transects and one of these time scales, as well as the interaction between transects and either of these temporal classifications using ANOVAs in the statistical software R version 3.1.2. Since ANOVAs assume a normal distribution of tick abundance, which may not have been the case in the field, we also conducted Poisson regressions on the same comparisons, which are more appropriate for count variables. All of the models were then compared based on sample-size corrected Akaike information criterion (AICc) values.

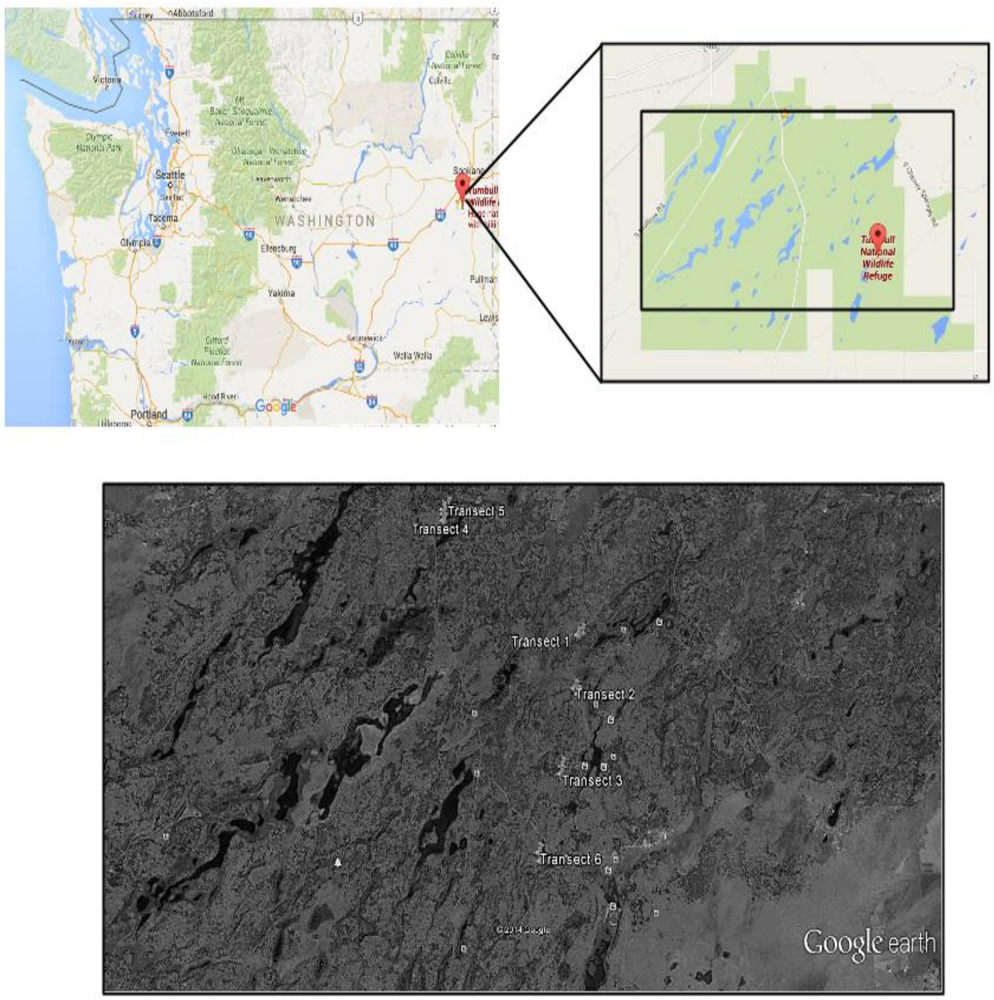

Figure 1: Aerial map of study site and transects. Inset shows the location of TNWR within the State of Washington. Box shows relative location of aerial map.

3. Results

During the course of the study 66 ticks were collected from two species, *Dermacentor andersoni* (Rocky Mountain wood tick) and *Dermacentor variabilis* (American dog tick). Of the two species *D. variabilis* was disproportionately more common on transects 1 and 5 using a Chi squared test to compare the proportions across each transect, assuming an equal proportion for all transects (Figure 2, $p < 0.001$).

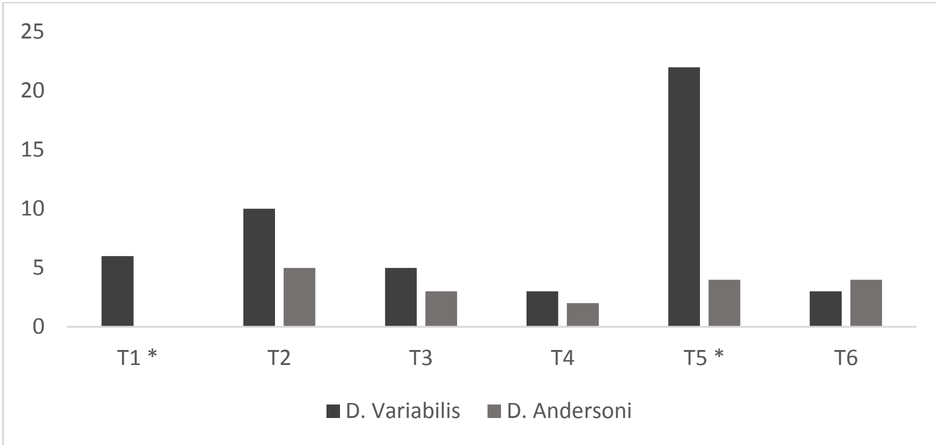

Figure 2: Total number of ticks, by species, collected on each transect.
Asterisks are used to denote significant differences.

There was a significant difference in the abundance of ticks per transect, with transect 5 having a significantly higher abundance than all transects except transect 2 (Figure 3).

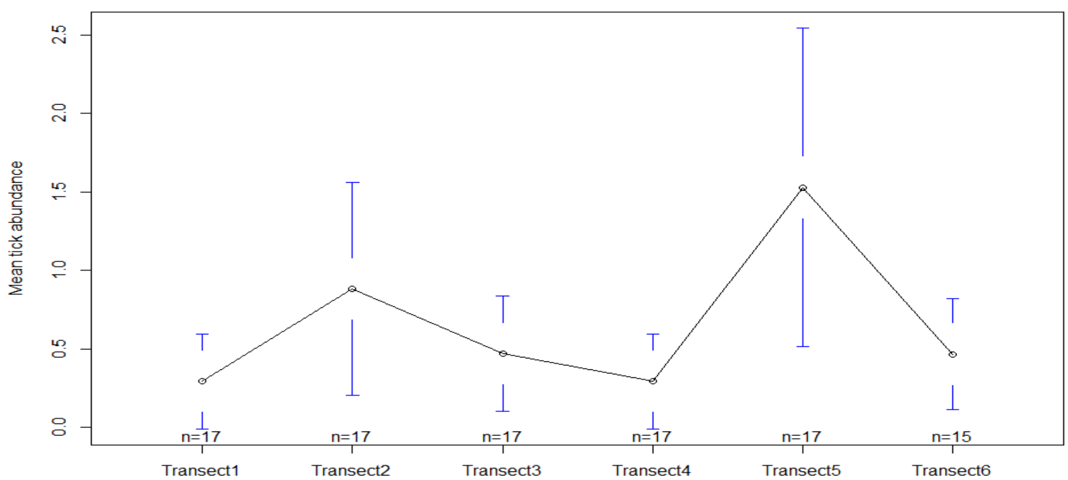

Figure 3: Mean tick abundance, represented as number of ticks per drag, by transect.
Bars represent 95% confidence intervals

The differences between transects also varied by time, and were much larger in the first half than in the second half of the study (Figure 4).

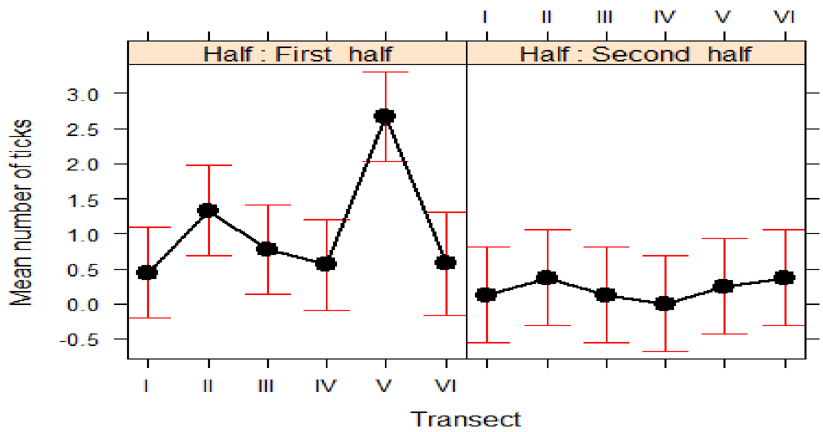

Figure 4. Mean tick density at the five transects during the two study periods and their 95% confidence intervals, predicted by the two-way ANOVA model.

The results from the two-way ANOVA showed a significant interaction between time periods using two week periods and across transects (Table 1). This model assumes a normal distribution of tick abundance at each transect.

Table 1: Two-way ANOVA comparing the effects of time across transects.

	df	SS	MS	F	P
Transect	5	19.41	3.883	4.629	0.000904
Two Week	2	13.00	6.502	7.751	0.000825
Interaction	10	33.24	3.324	3.963	0.000197
Residuals	82	68.78	0.839		

Using a Poisson regression explained 40.6% of the variation in or data, and gave us a better AICc score (Table 2). The Poisson regression gave us similar results to the ANOVA model; abundance was higher on transect 5 compared to 1 and 4, but not 2, 3 and 6, as well as a significant decrease in tick abundance in the second half of the study for transect 5 (Figure 5).

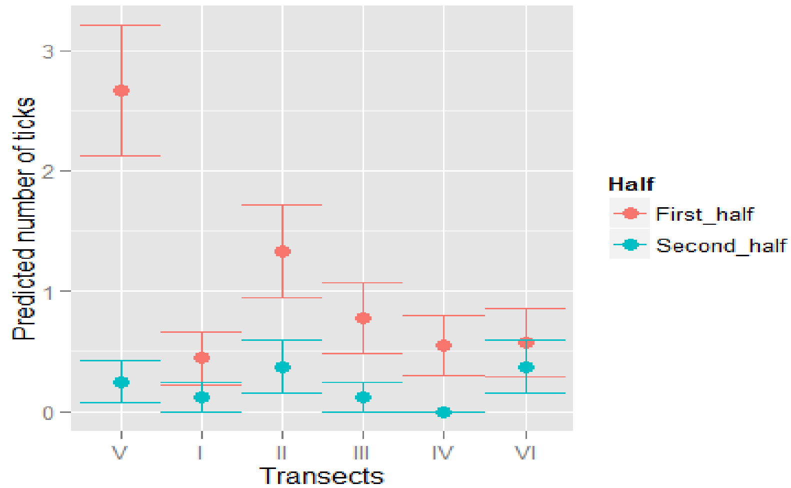

Figure 5: Mean tick density and its standard error in the first and second period of the study in the 6 transects dragged, as predicted by the Poisson GLM model.

The Poisson model predicted the differences between transects to disappear by the second half of our study. The results of the AICc analysis determined that the Poisson regressions were the best fit models for all of our analyses (Table 2). This model does not assume a normal distribution of tick abundance at each transect.

Table 2: AICc comparison between statistical models.

Model	**AICc**
ANOVA Transects only	313.0052
ANOVA Transects + Which Half of the Study	304.5420
ANOVA Transects + Which Two Week Period of the Study	311.4254
ANOVA Transects +Which Half of the Study + Their Interaction	293.8668
Poisson Transect Only	229.6500
Poisson Transect + Which Half of the Study	224.4100
Poisson Transect + Which Two Week Period of the Study	215.8600
Poisson Transect + Which Half of the Study + Their Interaction	210.4200

4. Conclusion

The study showed what seems to be a seasonal dynamic, causing tick numbers to drop in the second half of our study, but the mechanism causing this change needs to be further investigated. Relative humidity has been shown to have an impact on the questing behavior of ticks[6, 12]. While temperature and relative humidity are related to one another there wasn't a significant impact on tick density due to temperature alone (results not shown). In future studies we will need to collect relative humidity data to corroborate the findings of previous studies. Our study has also demonstrated a high degree of spatial heterogeneity in tick abundance, with large differences between our sites in terms of tick abundance. For our study it appeared that transect five experienced the most favorable environmental conditions for tick abundance and questing behaviors. Transect five had several wildlife trails and a lot of snowberry, which may help explain the higher abundances it experienced. The conditions necessary for questing have been shown to be mostly environmental conditions in *Ixodes* ticks[12]. However, substances on the legs of deer and ears of dogs have been shown to attract *D. variabilis* ticks among others to places associated with host presence, potentially explaining this association[1].

There seems to be multiple factors driving the abundance of *Dermacentor* ticks at Turnbull National Wildlife Refuge. Although there were significant changes in tick abundance over time on some of our transects, others had relatively low numbers of ticks for the entire study. This may be attributed to the differences in plant communities, animal species present, or microclimatic factors. We did not collect data on any of these factors, which may be driving tick abundances more than temperature. Turnbull National Wildlife Refuge also implements prescribed burning as part of their timber management practices which can have an impact on tick densities[9]. Future studies of tick demographics should investigate if this is occurring at Turnbull. It is important for researchers to collaborate with the public, and public agencies to maintain continued tick surveillance and reduce exposure risks to tick-borne pathogens.

5. Acknowledgements

The author wishes to express appreciation for the help of Mike Rule, the Refuge Biologist at Turnbull National Wildlife Refuge, and Elizabeth Dykstra, the Public Health Entomologist at the Washington State Department of Health, as well as for the contributions of Dr. Luis Matos, Professor at Eastern Washington University, Department of Biology.

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