DETECTION OF *BORRELIA BURGDORFERI SENSU LATO* INFECTION IN RODENTS FROM DISTURBED AND SYLVAN ASSEMBLAGES ACROSS TEXAS

by

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DEDICATION

The work summarized herein is dedicated to Herbert Joseph Armstrong. From Frogs to sheep to ticks, you inspired me to notice and take an interest in the world around me. I know you are smiling down from above right now as I add to the global understanding of the Lyme disease system.

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TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS v

LIST OF TABLES vii

LIST OF FIGURES ix

LIST OF ABBREVIATIONS x

ABSTRACT xii

CHAPTER

1. INTRODUCTION 1

2. MATERIALS AND METHODS 8
   - Description of field sites 8
   - Trapping effort 9
   - Animal holding and tick sampling 10
   - Laboratory methods 13
   - Statistical methods 15

3. RESULTS 17
   - Trapping effort and success 17
   - Assemblages 17
   - Ticks on rodents 18
   - Ticks on individual species 21
   - Borrelia presence 24

4. DISCUSSION 28

APPENDIX SECTION 34

LITERATURE CITED 48

LIST OF TABLES

Table **Page**

1. Reported cases of Lyme disease in Texas 5

2. Summary statistics for ticks on rodent individuals that were selected for tick sampling in Texas 18

3. Number of ticks found on individual rodent specimens in disturbed habitats for all sites sampled in Texas 19

.

4. Number of ticks found on individual rodent specimens in sylvan habitats for all sites sampled in Texas 19

5. Summary statistics for ticks on rodent individuals between habitats at each individual site sampled in Texas 20

6. Summary statistics for ticks on rodent individuals between habitats with all sites pooled for each season sampled in Texas 20

7. Results from one-way ANOVAs for the comparison of the average number of ticks per rodent individual from sites and seasons sampled during 2013-2014 in Texas 21

8. Results from one-way ANOVAs for the comparison of the average number of ticks per rodent individual among rodent species from sites and habitats sampled in Texas 22

9. Results from one-way ANOVAs for the comparison of the average number of ticks per rodent individual captured in Texas across three seasons 23

10. Summary of Poisson Likelihood Ratio Tests for the presence of *Borrelia*-positive individuals between disturbed and sylvan habitats at five Texas sites 25

11. Summary of Poisson Likelihood Ratio Tests for the presence of *Borrelia*-positive individuals between disturbed and sylvan habitats in Texas across three seasons 26

12. Summary of Poisson Likelihood Ratio Tests for the presence of Borrelia-positive individuals between disturbed and sylvan habitats and across three seasons for five sites in Texas 27

LIST OF FIGURES

Figure **Page**

1. Comparison of the number of *Borrelia*-positive individuals between disturbed and sylvan habitats for five sites sampled in Texas..... 24

2. Comparison of the number of *Borrelia*-positive individuals between disturbed and sylvan habitats for three seasons sampled in Texas..... 26

LIST OF ABBREVIATIONS

Abbreviation	Description
LD	Lyme Disease
CDC	Centers for Disease Control and Prevention
U.S.	United States
WMA	Wildlife Management Area
TPWD	Texas Parks and Wildlife Department
GE	Gus EngelingWMA
MM	Mason Mountain WMA
TR	Tejas Ranch
CH	Chaparral WMA
LP	Las Palomas WMA
PVC	Polyvinyl Chloride
IACUC	Institutional Animal Care and Use Committee
EDTA	Ethylenediaminetetraacetic Acid
DNA	Deoxyribonucleic acid
flaB	Flagella Gene
PCR	Polymerase Chain Reaction
Bb	*Borrelia burgdorferi*
TAE	Tris Base Acetic Acid EDTA Buffer

BLAST ..... Basic Local Alignment Search Tool

IBM ..... International Business Machines Corporation

SPSS ..... Statistical Package for the Social Sciences

ABSTRACT

Lyme disease (LD), caused by the bacterium *Borrelia burgdorferi*, affects tens of thousands of Americans each year. Most of the research in the United States (U.S.) is conducted in the northeastern portion of the country. Texas represents an under-studied area with low incidences of annual human infection. Studying the bacterium in an area of low incidence could answer questions about why it has a greater prevalence in other parts of its range. My study investigated tick loads on rodents and *Borrelia* prevalence in disturbed and sylvan habitats at five sites in Texas across three seasons. At four of the sites investigated, rare and relatively large bodied species that were only captured in sylvan habitats had higher tick loads than the rest of the species collected at the site. *B. burgdorferi* prevalence was found to vary seasonally, with larger numbers of infected individuals being captured in the fall. Future studies are needed to determine if the results described herein represent a consistent pattern, but this work represents a positive step toward investigating LD in the southern portion of its range.

KEYWORDS:

Ticks, Disease Ecology, Environmental Degradation, Small Mammals

1. INTRODUCTION

Lyme disease (LD) is a tick-borne emergent disease that affects around 20,000 to 30,000 Americans every year (CDC 2012). While LD is found throughout the United States (U.S.), around 50-100 cases each year occur in the state of Texas (Table 1), but the highest infection incidence occurs around the northeastern U.S. and Great Lakes regions (CDC 2012, CDC 2013). Lyme disease in the U.S. is caused by the spriochetal bacterium *Borrelia burgdorferi sensu stricto*, one of the 18 genospecies within the *Borrelia burgdorferi sensu lato* complex. Although there is evidence that *Borrelia* has existed for at least 15-20 million years (Poinar 2014), the bacterium was first detected by Steere et al. (1977) when they investigated and described a large number of mysterious child arthritis cases clustered in three eastern Connecticut communities between 1972 and 1975. It was eventually found that the bacterium causing this newly described disease occurs in many other locations worldwide and is carried from host to host by various hard-bodied tick species (Barbour and Fish 1993, Clark et al. 1998, Clark et. al 2001). Since its initial discovery, *B. burgdorferi* has been found throughout the U.S., but research mostly has been concentrated in the northeastern portion of the country (Spach et al. 1993), where the majority of LD cases are reported (CDC 2012, CDC 2013). Moreover, it has recently been discovered that the range of *B. burgdorferi* is slowly expanding out of the northeastern U.S. (Hamer et al. 2012). The expansion of *B. burgdorferi*'s range is primarily driven by the movement of reservoir species upon which the vectors feed (Hamer et al. 2012). During its lifetime, a hard-bodied tick consumes a

blood meal during each of its three growth stages – larva, nymph, and adult (Adams et al. 2003), and each blood meal may come from a unique vertebrate host. To locate these hosts, ticks undergo a behavior known as "questing" wherein they move to the edge of a piece of foliage and wait for a host to pass within reach (Sonenshine 1993, Vail and Smith 2002) . Larval and nymphal ticks tend to quest on foliage near the ground at a height that facilitates contact with mice and other small mammals while adult ticks tend to quest on taller vegetation, which facilitates contact with medium and large mammals (Adams et al. 2003, Loye and Lane 1988). After a larval or nymphal tick feeds on an animal reservoir that is infected with *B. burgdorferi*, they can become infected and transmit the pathogen to subsequent hosts. Variation in the suitability of vertebrate species as tick hosts and as reservoirs for *B. burgdorferi* results in variation of the overall prevalence of the bacterium in the environment (Schauber and Ostfeld 2002, Schmidt and Ostfeld 2001).

While many species can serve as reservoirs for *B. burgdorferi*, four mammals in particular: white-footed mice (*Peromyscus leucopus*), eastern chipmunks (*Tamias striatus*), short-tailed shrews (*Blarina brevicauda*), and masked shrews (*Sorex cinereus*), have been found to be most competent for incubation and transmission of the pathogen (Brisson et al. 2008). In studies conducted in the northeastern U.S. it has been found that variation in the presence of these four species accounts for about half of the variation in prevalence of *B. burgdorferi* in the environment (Brisson et al. 2008). Among these competent *B. burgdorferi* reservoirs, white-footed mice are most efficient at incubating and transmitting the pathogen (Keesing et al. 2006, Mather 1993, Ostfeld and Keesing 2000), as they infect 75%-95% of the larval ticks that feed on them (Ostfeld 2011).

Human infection usually occurs when a nymphal tick, that had previously fed on and become infected by a competent reservoir species, bites a person (Adams et al. 2003, Barbour and Fish 1993, Daniels et al. 1998, Spach et al. 1993).

In anthropogenically disturbed areas there is not only an increase in the number of humans present in the environment, but also generally a decrease in biodiversity, which allows more ubiquitous and generalized species to dominate local wildlife assemblages (Ostfeld et al. 2006b). White-footed mice are commonly found across a wide variety of habitat types and are often the last species remaining in highly disturbed areas (LoGiudice et al. 2008, Ostfeld and Keesing 2000, Ostfeld 2011). Conversely, areas with less anthropogenic disturbance tend to have higher species diversity and reduced relative abundances of reservoir species (Suzán et al. 2009). The addition of individuals that are less competent hosts for *B. burgdorferi* reduces the likelihood that a tick will bite an infected individual of a highly competent species and subsequently transmit the pathogen to other tick hosts (Clark et al. 2001, Keesing et al. 2006, Schauber and Ostfeld 2002, Schmidt and Ostfeld 2001). Increased biodiversity leading to a decreased pathogen transmission is known as the Dilution Effect and has been demonstrated in numerous studies (Carver et al. 2011, Ezenwa et al. 2006, Keesing et al. 2006, Ostfeld 2011, Ostfeld and Keesing 2000, Ostfeld and Keesing 2012, Magnarelli et al. 1995, Suzán et al. 2009, Wood and Lafferty 2012). Greater diversity within an environment can also result in a greater number of predators, which can further result in fewer of the most competent *B. burgdorferi* reservoirs, including white-footed mice (LoGiudice et. al. 2008). Conversely, the lack of dilution agents (predators and reservoir competitors) creates an

increased likelihood of ticks being infected with *B. burgdorferi* (Brisson et al. 2008, LoGiudice et al. 2003, Ostfeld 2011) and, consequently, increased human disease risk.

Although there has been much investigation into the ecology of the *B. burgdorferi* system in the northeastern U.S., our knowledge of the ecological relationships of this bacterium in the southern U.S. is minimal (Arroyo et al. 2014), despite the fact that LD has been known to exist as far south as Texas for almost 30 years (Rawlings 1986, Rawlings et al. 1987, Teltow et al. 1991), albeit with a relatively low prevalence (CDC 2012). However, *Ixodes scapularis* (which is the most common *B. burgdorferi* vector in the eastern U.S.) is expected to persist in Texas, and possibly expand its range, as climate change occurs (Arroyo et al. 2014), thus furthering the need to study the *B. burgdorferi* cycle within the state. Studying the ecology of this pathogen in a region of low and possibly expanding prevalence will likely aid in the scientific understanding of which factors are most influential to its high prevalence at other sites of interest.

The climate, habitats, and vertebrate species composition found in Texas are vastly different from those found in the northeastern U.S. Because these differences are so vast, current scientific knowledge of the *B. burgdorferi* system may not be applicable in the southwestern portion of its range. For example, the vertebrate species that function as primary reservoir hosts of *B. burgdorferi* in Texas have yet to be identified. Furthermore, the identity of the most influential arthropod vector responsible for transmitting the pathogen between vertebrates in this region is also unknown. DNA consistent with that of *Borrelia* spp. has been found lone star tick (*Amblyomma americanum*), cayenne tick (*A. cajennense*), American dog tick (*Dermacentor variabilis*),

and deer tick (*I. scapularis*) specimens that have been submitted to the Texas Department of Health Services (Williamson et al. 2010). Virtually no research has been done to investigate *B. burgdorferi* in Texas since the late 1980s. Despite the lack of intense investigation on the subject, human cases of LD do occur in the state (Table 1).

Table 1: Reported cases of Lyme disease in Texas† (CDC 2012).

Year	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012		
										Confirmed	Probable	Incidence*
Reported Cases	85	98	69	29	87	105	88	55	28	33	42	0.1

† confirmed cases presented for all years except 2012
∗ confirmed cases per 100,000 population

It has been suggested that the best way to determine human LD risk is to determine the density of questing *I. scapularis* nymphs (Diuk-Wasser et al. 2010). A standardized study of various sites east of the 100th meridian using a drag sampling method found no *I. scapularis* ticks in the state of Texas (Diuk-Wasser et al 2006). However, native Texas flora is much denser and is very structurally different from that found in the northeastern U.S. This makes it extremely difficult or impossible to implement standard drag sampling protocols at many sites in Texas.

In the southern portion of their range, immature *I. scapularis* ticks are rarely collected by researchers using standard collection methods including drag and flag sampling, (Clark et al. 1998, Mackay and Foil 2005). In the southern U.S., larvae and nymphs often quest under vegetation in areas that are inaccessible to the implements used in drag and flag sampling (Cilek and Olson 2000, Mackay and Foil 2005) and rarely move to the uppermost sections of vegetation (Arroyo et al. 2014). This combined with the fact that dense, low-lying vegetation does not allow drag cloths to make as much

contact with the leaf litter where larval and nymphal ticks generally reside means that drag and flag methods might under-sample immature ticks in the southern U.S. (Goddard and Piesman 2006). Additionally, Texas tends to be hotter, dryer and have less overall canopy cover than sites located in Northeastern forests. These conditions may put ticks at an increased risk of desiccation if they spend long periods of time in direct sunlight (Durden et al. 1996, Harlan and Foster 1990).

As a result of the factors listed above, a different system must be employed to accurately determine the presence of *B. burgdorferi* vectors in Texas. One alternate method to determine tick densities is live-capturing potential vertebrate hosts from an area and inspecting them for ticks. However, it has been shown that humans tend to under-count the number of ticks on mammalian specimens during visual inspections (Fish and Daniels 1990, Ostfeld 2011). Instead of manually inspecting every animal for ticks, holding them in cages suspended over water allows ticks to complete their feeding cycle, release on their own, and be collected (Fish and Daniels 1990, Ostfeld 2011). This method produces an accurate count of the number of ticks on any given animal and creates a standardized format for tick collection across researchers and sites. In addition to providing prevalence data, this method provides information on tick loads across different host species.

Rodents and shrews are commonly known to be the best reservoirs of *B. burgdorferi*. Nocturnal rodents will be the focus of my research because they are easily trapped and give a good estimate of the prevalence of this pathogen in a given ecosystem (LoGiudice et al. 2008). Animals used in this study will be held in cages as described above before being euthanized and tested for the presence of *Borrelia* spp. The

information obtained from this testing will be used to ascertain the presence of *B. burgdorferi* in rodent species from Texas and provide the initial guidance for future experiments to determine which species are the most significant local reservoirs in the state.

This study will seek to determine whether *B. burgdorferi* is more prevalent in disturbed or undisturbed habitats at five locations in the state of Texas. It is expected that greater human disturbance will result in reduced small mammal biodiversity, causing an increased abundance in *B. burgdorferi* reservoir species and a higher prevalence of the pathogen. If an increase in the number of reservoirs is accompanied by an increased number of hard-bodied tick vectors, it is likely that there will be a greater incidence of *Borrelia* in the environment, and therefore a greater human disease risk.

Finally, this study will also seek to determine whether *B. burgdorferi* persists at a stable level in Texas or if levels of infection fluctuate seasonally. If there is a fluctuation in the ratio of non-competent to competent hosts throughout the year, then the prevalence of the pathogen in the environment would be expected to change as well (Carver et al. 2011).

2. MATERIALS AND METHODS

DESCRIPTION OF FIELD SITES:

Nocturnal rodents were trapped at five sites throughout Texas. These sites included four Wildlife Management Areas (WMAs) under the jurisdiction of Texas Parks and Wildlife Department (TPWD) and one private ranch. Sites were selected to provide a sampling of five different habitat types: the East Central Texas Plains – Post Oak Savannah Ecoregion (Gus Engeling WMA (GE)), the Edwards Plateau Ecoregion (Mason Mountain WMA (MM)), the East Central Texas Plains – Floodplains and Low Terraces Ecoregion (Tejas Ranch (TR)), the Southern Texas Plains Ecoregion (Chaparral WMA (CH)), and the Western Gulf Coastal Plain Ecoregion (Las Palomas WMA (LP)) (Griffith et al. 2004, see Appendix I). Each site was divided into habitats that were classified as either "disturbed" or "sylvan". Disturbed habitats exhibited visible recent anthropogenic modifications while sylvan habitats exhibited the most historically natural conditions on the property. When sampling occurred (February 2013 – January 2014), all areas were still suffering the effects of a historic drought, which began in December of 2010 (U.S. Drought Monitor, 2014).

Disturbed habitats trapped at GE included the areas around a bunkhouse, a learning center, a maintenance yard, and a farm field. Sylvan habitats were post-oak savannah in various stages of natural or prescribed fire recovery. Disturbed habitats at MM included areas around bunkhouses, decommissioned ungulate holding pens, maintenance facilities, and a manmade lake. The sylvan habitat at the sites was primarily Edwards Plateau woodland with a heavy grassland component. This area has been grazed by a variety of native and nonnative ungulates for many years. To control for the

highly variable habitat created by the exposed granite domes that are characteristic of the Edwards Plateau, efforts were made to sample areas with similar geology and flora during any given sample period. Disturbed habitats at TR included areas around a farmhouse, barn, manmade lake, horse pasture, and deer feeder. The sylvan habitats primarily followed dry drainages, but also ventured into uplands of heavily forested floodplains and low terraces. A fire burned 95% of the CH site in 2008 (TPWD 2008). Disturbed habitats at this site included unburned areas surrounding the main office and parking lot, and burned areas near bunkhouses, a maintenance yard, and an outdoor storage area. Sylvan habitats were located in areas that were untouched by the fire as well as areas that were in various stages of fire recovery. Sites sampled at LP were located in the Arroyo Colorado Unit. Disturbed habitats included areas around the original homestead on the property, a maintenance/storage yard, a crop field, and roadways. Sylvan habitats were located in dense, natural western gulf coastal plains vegetation that persisted inside an oxbow created after the completion of a Corps of Engineers project to straighten the lower 41km of the Arroyo Colorado River (Lingo 2014).

TRAPPING EFFORT:

Each site was visited three times, to account for seasonal variation throughout the year. Sampling period 1, designated as the late winter trapping period, ran from February 9 to April 7, 2013. Sampling period 2, designated as the spring trapping period, ran from April 19 to June 14, 2013. Sampling period 3, designated as the fall trapping period, ran from September 8, 2013 to January 10, 2014. Additionally, there was a preliminary

trapping period conducted at MM from January 25 - 27, 2013, during which time specimens were collected and sampled, but no tick data was obtained due to logistical and methodological issues.

During each site visit, one unique sylvan and one unique disturbed transect were selected and sampled. During each visit, at least 150 Large Folding Aluminum Sherman Live Traps (H.B. Sherman Traps, Inc, Tallahassee, FL, US) were set on each transect for three nights, or until a sufficient number of animals was captured (N ≅ 30-50), for a total of between 450 and 1800 trap nights per transect (Appendix 2). Traps were placed 6 m apart in a linear, albeit not necessarily straight fashion. Care was taken to not sample identical transects during successive visits. Traps were not placed within 10 m of a prior capture without a minimum of 6 months between samples. This was done to prevent bias due to trapping within the home ranges of removed individuals. Each night all traps were opened and baited with a mixture of rolled oats, peanut butter, and imitation vanilla extract just before dusk and checked and closed just after dawn.

All animals used in this study were collected under TPWD Scientific Research Permit No. SPR-1112-1052.

ANIMAL HOLDING AND TICK SAMPLING:

Once captured, a subset of animals was held alive individually in cages with wire-mesh floors (model 1264C051, Techniplast, West Chester, PA, USA; and modified Animal Cage Assemblies with Mesh Filter Tops, Lab Products Inc., Maywood, NJ, USA). Logistical and spatial limitations constrained the maximum number of cages used during any given sampling session to 50. Due to logistical constraints, some trapping

sessions resulted in more animals being captured than could be held. In these cases, best efforts were made to select a representative sample from the captured animals. Individuals from species that were rare within the assemblages being sampled were selected first followed by a random subset of individuals from the remaining abundant species. Cages were placed above a plastic tray filled with water for 72-96 hours (Fish and Daniels 1990, Ostfeld, 2011). After this period of time most, if not all, of the ticks on each animal should have completed their feeding cycle and naturally released from their host. After falling through the mesh floor of the cage, ticks were expected to fall into the water and become trapped (Adams et al. 2003, Keesing et al. 2009, Levin et al. 2002). This method of suspending captured animals in cages over water increased the likelihood of the ticks being collected without damage to their mouthparts, which can be an important tool in identification (Adams et al. 2003). As an added precaution, petroleum jelly was smeared on the outer edges of the trays to keep ticks from crawling out (Sonenshine 1993). Food and water was suspended from the top of each cage and provided to captive animals *ad libitum*.

It was noted during the preliminary trapping session that if there was no barrier between the bottom of the cage and the water tray, fecal material as well as leftover food would fall into the water. This resulted in the water being contaminated with particulate matter, making it impossible to discern even the largest of ticks. To mitigate for this, a piece of cardboard with clear packaging tape covering all edges and imperfections was placed under the cages and above the water to keep fecal material out of the water trays. 2.5 - 6 mm non-porous spacers composed of either Polyvinyl chloride (PVC) rings or LEGO blocks (LEGO Group, Billund, Denmark) were placed between the cages and

cardboard as well as between the cardboard and the water to allow space for ticks to crawl in their search for soil and detritus. The ticks' need to find a medium in which to digest their blood-meal and molt to the next life stage resulted in a majority of the ticks crawling over the edge of the cardboard and into the water below, although some ticks were recovered from the cardboard as well as in and on portions of the cages.

After at least 72 hours, the water trays below each cage were inspected for ticks using a magnifying glass. All water was then filtered through paper or cloth filters which were inspected for ticks in the laboratory under a 1-3.5 X dissecting microscope. Additionally, a magnifying glass and dissecting needle was used to closely inspect every cage as well as the cardboard placed below each cage for any ticks. Ectoparasites collected at all stages from all animals were preserved in 95% ethanol.

To test for the presence of *B. burgdorferi*, held rodents were euthanized and processed for tissue collection. The animals were anesthetized using cotton soaked with isoflurane. After anesthetization, a cervical dislocation was performed. A toothbrush and magnifying glass were used to scan specimens for any additional ticks. Specimens were then dissected and samples from the spleen, bladder, and knee joint were collected and preserved in either liquid nitrogen or 95% ethanol. Skin and skull vouchers were prepared for each dissected specimen. Most specimens were preserved as wet museum specimens while some specimens were made into dry museum mounts. All voucher specimens and skulls are housed in the vertebrate collection at Texas State University.

All animals used in this study were collected and housed under Texas State University Institutional Animal Care and Use Committee (IACUC) protocol number 1206_0113_02.

LABORATORY METHODS:

Once collected, ectoparasites and organs were sent to the laboratory of Dr. Esteve-Gassent at Texas A&M University, Department of Veterinary Pathobiology (College Station, TX, U.S.) for *Borrelia* spp. prevalence analysis. Each sample was placed in a 1.5ml microcentrifuge tube that contained a digestion solution (200 µl Nuclei Lysis Solution©, 50 µl EDTA (0.5 M solution), 20 µl Proteinase K (20mg/ml), 5 µl RNase A Solution (4 mg/ml) (Promega Corporation, Madison, WI, U.S)) and 20 µl collagenase (hydrated to 145 U/ml) (Calbiochem©, Merck KGaA, Darmstadt, Germany) and incubated overnight at 55˚ C. Tissues were then transferred to specialized screw cap tubes containing ceramic beads and were homogenized utilizing the bead mill BeadRuptor 24 (Omni International, Inc., Kennesaw, GA, U.S.) for 4 minutes, 30 seconds at a 5.65 m/s intensity (equivalent to 210 × gravity). During this step 1.4 mm ceramic beads were used to homogenize spleens while 2.8 mm ceramic beads were used for knee samples. Samples were removed and transferred to a new microcentrifuge tube containing 250 µl of Wizard Lysis Buffer (Promega Corporation) and thoroughly mixed.

Once samples were prepared and placed in lysis buffer solution, they were purified using a Wizard SV Minicolumn with a vacuum adaptor (Vac-Man Laboratory Vacuum Manifold A7231, Promega Corporation). After samples were purified, the minicolumn was washed with 800 µl of Column Wash Solution (diluted with 95% ethanol) four separate times then dried. After drying, the minicolumn filter was moved to a new 1.5 ml centrifuge tube. 200 µl of nuclease free water was added to the dried filter and it was incubated for 3 minutes at room temperature. The tube was then centrifuged at

13.000 x gravity for 1 minute to create an elution that contained DNA from both the specimen and any internal bacteria. This elution was stored at -20˚ C for later use.

After DNA purification, each sample was diluted using a 1:5 mix of distilled water and DNA. The diluted DNA was then placed in tube containing 12.5 µl Accustart Supermix (Quanta Biosciences, Gaithersburg, MD, U.S.), 8.5 µl distilled water, and 2 µl of Primer Mix (a 1:1:3 mix of Forward Primer (Integrated DNA Technologies, Inc., Coralville, IA, U.S.), Reverse Primer (Integrated DNA Technologies), and distilled water). The PCR mix prepared was used to detect the flagella gene (*flaB*) as previously described by Jaulhac et al. (2000). A Polymerase Chain Reaction (PCR) was performed on each sample using an Eppendorf PCR machine (Mastercycler Pro-Thermal cycle 950040015, Eppendorf, Hauppauge, NY, U.S.). Bb MSK5 cultured in the lab was used as a positive control for *B. burgdorferi sensu stricto* and distilled water was used as a negative control. After running the PCR program for flagella gene developed by Jaulhac et al. (2000), gel electrophoresis was run using 1% agrose gel (1% agrose dissolved in TAE buffer and added to 20µl Ethidium Bromide) to separate the amplicons. Orange G dye was used to mix the PCR product before loading the 5 µl PCR product on the gel, and a 1000 base pair ladder was used for reference of positive band size. Gels were run at 90 V for 40 minutes and read under Ultraviolet light. Positive samples were sent to Eton Biosciences (San Diego, CA, U.S.) and sequences were provided to the Esteve-Gassent lab, where they were analyzed through BLAST® (National Center for Biotechnology Information database www.blast.ncbi.nlm.nih.gov) using MacVector 12.6 software (MacVector Inc., Cary, NC, U.S.) to facilitate identification. For my study,

individuals were considered to be positive for *B. burgdorferi* if the sample was identified as any strain of *Borrelia* spp.

STATISTICAL METHODS:

Independent measures t-tests were run to determine differences in tick loads between disturbed and sylvan sites as well as differences across seasons. Additionally, Levene's tests were utilized to determine whether equal variances were assumed for each t-test. One-way ANOVAs were used to determine differences in the average number of ticks across species at each site and habitat as well as differences across seasons.

Due to the large number of *Borrelia*-negative individuals, Poisson log-linear analysis was utilized to investigate differences in *Borrelia* presence across habitats and seasons. Both Poisson likelihood ratio tests and Pearson chi-square tests were used to determine differences between datasets that pooled individuals across multiple sites. When applicable, individuals collected during the preliminary sampling period were grouped with the late winter specimens for analysis of *B. burgdorferi* presence.

Due to issues with sample collection (space limitations, trap mortalities, holding mortalities, and other logistical issues), some differences occurred in the numbers of individuals included in each test. All rodents captured (N = 592) were utilized to tally assemblage data for each site. Most individuals (N = 561) were also sampled for *Borrelia* presence (Many mortalities that occurred during trapping or while animals were being held in cages were unsuitable for sampling by the time that they were discovered. Additionally, due to logistical reasons, a very small number of collected samples were

excluded.). A representative subset of captured animals were placed in cages and utilized for tick sampling (N = 463).

All statistics were completed in IBM SPSS Statistics (IBM, North Castle, NY, U.S.) software.

3. RESULTS

TRAPPING EFFORT AND SUCCESS:

During each season, 150-525 trap-nights were conducted in each habitat type at every site (Appendix II). Trapping was conducted until the number of captures between disturbed and sylvan habitats filled most of the holding cages available. During the first visit to each site only 23 cages were available; 50 cages were available during all subsequent visits. A total of 592 individuals were captured during 10,950 trap-nights. Of these, there were 591 rodents (mice and rats) and 1 least shrew (*Cryptotis parva*). This resulted in an overall trapping success of 5.41%.

Overall, 393 animals were captured in disturbed habitats during 5475 trap-nights (trapping success 7.18%). A total of 199 animals were captured over 5475 trap-nights in sylvan habitats (trapping success 3.63%). Trapping effort was 600, 3025, 3425, and 3900 trap-nights for the pre-trapping, late winter, spring, and fall trapping periods respectively. This resulted in 22, 156, 203, and 211 captures and 3.67%, 5.16%, 5.93%, and 5.41% trapping success (pre-trapping, late winter, spring, and fall respectively).

ASSEMBLAGES:

Captured rodents represented 10 genera (Appendix III). Due to difficulties with identification to species, some *Peromyscus* individuals (primarily juveniles and subadults) were classified as *Peromyscus* spp. Between 6 and 9 species (not including *Peromyscus* spp.) were captured in each habitat at each site (Appendix IV). Each habitat had between 2 and 5 dominant species as defined by Berger and Parker (1970).

TICKS ON RODENTS:

A total of 463 rodents were held in cages over water to ascertain tick loads (Table 2). A single southern plains woodrat (*Neotoma micropus*) captured during the spring sampling period in the sylvan habitat at LP had an unusually large tick load (counting was ended at 741 ticks – it is estimated that there were over 1,000 ticks present on the individual) and was excluded from all analyses due to the fact that it was an obviously extreme and unusual case (80 ticks or fewer were found on all other individuals). Ticks were found in the water below cages, on the cardboard trays between the cages and the water, on the petroleum jelly that lined each water tray, and on the animals after they had been euthanized. Ticks were only found on 98 specimens.

Table 2: Summary statistics for ticks on rodent individuals that were selected for tick sampling in Texas.

Summary Statistic	Value
Total Number of individuals Sampled for Ticks	462
Average Number of Ticks Per individual rodent	0.88
Standard Deviation	4.62
Minimum Number of Ticks found on an individual	0
Maximum Number of Ticks found on an individual	80

Overall, 323 ticks were found on animals in disturbed habitats and 83 ticks were found on animals in sylvan habitats (Table 3 and 4). In tests comparing mean numbers of ticks between habitat types with all sites pooled, equal variances were assumed (Levene's test, $F = 5.464$, $p = .020$). Overall, with all sites pooled, there was no difference in the average number of ticks per animal between disturbed and sylvan habitats as a whole ($t = 1.471$, $df = 460$, $p = 0.142$).

Table 3: Number of ticks found on individual rodent specimens in disturbed habitats for all sites sampled in Texas.

Site	Number of Ticks
Gus Engeling WMA (GE)	45
Mason Mountain WMA (MM)	26
Tejas Ranch (TR)	31
Chaparral WMA (CH)	114
Las Palomas WMA (LP)	107
TOTAL	**323**

Table 4: Number of ticks found on individual rodent specimens in sylvan habitats for all sites sampled in Texas.

Site	Number of Ticks
Gus Engeling WMA (GE)	6
Mason Mountain WMA (MM)	1
Tejas Ranch (TR)	29
Chaparral WMA (CH)	27
Las Palomas WMA (LP)	20
TOTAL	**83**

The mean number of ticks per rodent individual between the disturbed and sylvan habitats was the same for all sites (GE, $t = 1.547$, $df = 46.164$, $p = 0.129$; MM, $t = 1.209$, $df = 98.000$, $p = 0.229$; TR, $t = 0.066$, $df = 60.690$, $p = 0.947$; CH, $t = 0.416$, $df = 111.000$, $p = 0.678$; LP, $t = 1.250$, $df = 105.041$, $p = 0.214$). Equal variances were assumed only for MM (Levene's Test, $F = 5.420$, $p = 0.022$) but were not assumed for any other sites (Levene's Test: GE, $F = 3.259$, $p = 0.076$; TR, $F = 0.522$, $p = 0.472$; CH, $F = 0.426$, $p = 0.515$; LP, $F = 1.173$, $p = 0.281$). It should be noted that at all sites there was a high variation (standard deviation) for the mean number of ticks found on each individual rodent (Table 5, Appendix V).

Table 5: Summary statistics for ticks on rodent individuals between habitats at each individual site sampled in Texas.

Site	Habitat	Number of Rodents Tested	Number of Rodents with 0 Ticks	Minimum Number of Ticks	Maximum Number of Ticks	Mean Number of Ticks	Standard Deviation
Gus Engeling WMA (GE)	Disturbed	42	29	0	21	1.07	3.37
	Sylvan	25	21	0	3	0.24	0.66
Mason Mountain WMA (MM)	Disturbed	47	43	0	22	0.55	3.22
	Sylvan	53	52	0	1	0.02	0.14
Tejas Ranch (TR)	Disturbed	37	30	0	12	0.84	2.50
	Sylvan	36	24	0	6	0.81	1.56
Chaparral WMA (CH)	Disturbed	84	71	0	80	1.36	8.92
	Sylvan	30	22	0	15	0.90	2.81
Las Palomas WMA (LP)	Disturbed	77	48	0	36	1.39	4.55
	Sylvan	31	25	0	7	0.65	1.62

Overall, with all sites combined, there were no differences in the average number of ticks present on mice between habitats across seasons (late winter, $t = 1.325$, $df = 111.161$, $p = 0.188$; spring, $t = 1.145$, $df = 141.567$, $p = 0.254$; fall, $t = 1.050$, $df = 117.965$, $p = 0.296$). Levene's test was not significant for any season overall (late winter, $F = 2.869$, $p = 0.093$; spring, $F = 2.318$, $p = 0.130$; fall, $F = 2.000$, $p = 0.159$), so equal variances were not assumed. As was the case in each site individually, there was a high variation (standard deviation) in tick loads between habitats across seasons (Table 6).

Table 6: Summary statistics for ticks on rodent individuals between habitats with all sites pooled for each season sampled in Texas.

Season	Habitat	Number of Rodents Tested	Number of Rodents with 0 Ticks	Minimum Number of Ticks	Maximum Number of Ticks	Mean Number of Ticks	Standard Deviation
Late Winter	Disturbed	76	54	0	21	1.04	2.94
	Sylvan	49	39	0	6	0.53	1.29
Spring	Disturbed	116	92	0	80	1.45	7.98
	Sylvan	62	51	0	15	12.30	93.31
Fall	Disturbed	95	75	0	36	0.80	3.84
	Sylvan	64	54	0	7	0.36	1.16

When sites were individually analyzed for seasonal differences, there was a heterogeneous response in average tick loads across habitats. Three sites (MM, CH, LP) did not show any differences in the mean number of ticks per rodent across seasons (Table 7). Differences across seasons at GE approached significance, suggesting that there may have been a higher number of ticks per animal in the late winter (Table 7). TR exhibited a difference in the mean number of ticks across seasons, indicating that there were more ticks on individual animals in the spring than any other season (Table 7).

Table 7: Results from one-way ANOVAs for the comparison of the average number of ticks per rodent individual from sites and seasons sampled during 2013-2014 in Texas.

Site	Season	Total Number of Rodent Individuals	Number of Rodents WIth No Ticks	Maximum Number of Ticks	Number of Rodents With Ticks	ANOVA		
						P	F	DF
Gus Engeling WMA (GE)	**All**	**67**	**50**	**21**	**17**	0.080	2.634	2
	Late Winter	23	15	21	8			
	Spring	35	26	2	9			
	Fall	9	9	0	0			
Mason Mountain WMA (MM)	**All**	**100**	**95**	**22**	**5**	0.349	1.065	2
	Late Winter	39	37	2	2			
	Spring	33	30	22	3			
	Fall	28	28	0	0			
Tejas Ranch (TR)	**All**	**73**	**54**	**12**	**19**	0.005	5.742	2
	Late Winter	19	9	12	10			
	Spring	26	24	1	2			
	Fall	28	21	9	7			
Chaparral WMA (CH)	**All**	**114**	**93**	**80**	**21**	0.310	1.182	2
	Late Winter	22	19	1	3			
	Spring	50	41	80	9			
	Fall	42	33	3	9			
Las Palomas WMA (LP)	**All**	**108**	**73**	**36**	**35**	0.915	0.089	2
	Late Winter	22	13	4	9			
	Spring	34	22	17	12			
	Fall	52	38	36	14			

TICKS ON INDIVIDUAL SPECIES:

Ticks gathered from individual rodents at each site were analyzed at the species level using a one-way ANOVA. The average number of ticks per individual was

analyzed at both the habitat level and at the site level. In general, differences in average numbers of ticks per individual across species were heterogeneous, but one common pattern was that species from rodent assemblages from sylvan habitats at all sites had no difference in the average number of ticks per individual (Table 8, Appendix VI). Results for each site were as follows: At GE, Marsh rice rats (*Oryzomys palustris*) had more ticks per animal than any other species sampled in both the disturbed habitat and the site overall (Table 8, Appendix VI). It should be noted that only two *O. palustris* were captured throughout the entirety of the sampling effort, both at GE. There were more ticks per animal on white-throated woodrats (*Neotoma leucodon*) than any other species

Table 8: Results from one-way ANOVAs for the comparison of the average number of ticks per rodent individual among rodent species from sites and habitats sampled in Texas.

Site	Habitat	Total Number of Rodent Individuals	Number of Rodent Species	ANOVA		
				P	F	DF
Gus Engeling WMA (GE)	All	67	8	< .001	10.106	7
	Disturbed	42	7	< .001	7.338	6
	Sylvan	25	6	0.953	0.213	5
Mason Mountain WMA (MM)	All	100	8	< .001	6.171	7
	Disturbed	47	6	< .001	7.569	5
	Sylvan	53	7	0.935	0.298	6
Tejas Ranch (TR)	All	73	8	0.893	0.409	7
	Disturbed	37	8	0.990	0.164	7
	Sylvan	36	6	0.856	0.383	5
Chaparral WMA (CH)	All	114	10	0.011	2.550	9
	Disturbed	84	9	0.043	2.136	8
	Sylvan	30	8	0.225	1.480	7
Las Palomas WMA (LP)	All	108	9	< .001	3.900	8
	Disturbed	77	9	0.014	2.640	8
	Sylvan	31	6	0.121	1.956	5

sampled at MM in the disturbed habitat and the site overall (Table 8, Appendix VI). At TR, the average number of ticks per individual among rodent species was the same independent of whether the analysis was done at the habitat or site level (Table 8). At CH, the average number of ticks per rodent individual was highest on southern plains woodrats (*Neotoma micropus*) in both the disturbed habitat and the site overall (Table 8, Appendix VI). Finally, at LP, hispid cotton rats (*Sigmodon hispidus*) had more ticks per individual than any other species sampled in both the disturbed habitat and the site overall (Table 8, Appendix VI).

In addition to the spatial and habitat level fluctuation in the average number of ticks per species, temporal variation was also present. There were differences in the average number of ticks per individual among rodent species during the late winter and spring sampling periods but tick loads were the same among species for the fall (Table 9, Appendix VII). More ticks per animal were present on *O. palustris* than on any other species sampled in the late winter period (Table 9, Appendix VII) and *N. micropus* and *N. leucodon* had a higher number of ticks per animal than any other species sampled in the spring period (Table 9, Appendix VII).

Table 9: Results from one-way ANOVAs for the comparison of the average number of ticks per rodent individual captured in Texas across three seasons.

Season	Total Number of Rodent Individuals	Number of Rodent Species	ANOVA		
			P	F	DF
Late Winter	125	16	< .001	4.673246	15
Spring	178	14	< .001	22.439	13
Fall	159	12	.732	.706	11

BORRELIA PRESENCE:

A total of 561 individual rodents (94.76% of all individuals captured) were tested for presence of *Borrelia* spp. Up to three tissues (spleen, knee, and bladder) were tested from each animal. Of these, 9.45% returned a positive result for at least one of the tissues examined. Eleven different strains of *Borrelia* were found in the animals tested (*B. americana* strain SCW-30f, *B. burgdorferi* isolate 5N17L2-IC, *B. burgdorferi* isolate J8P5N-IR, *B. burgdorferi* Ka-21, *B. burgdorferi* NCHS6, *B. burgdorferi* strain CAhS-1, *B. burgdorferi* strain OHHS-1, *B. burgdorferi* strain Ka-21, *B. burgdorferi* strain Ka-21.10, *B. japonica*, and *Borrelia* spp. THL-14). Molecular analysis of these strains is the focus of a collaborating project and not included in this document. For the purposes of all analyses contained herein, all eleven strains were grouped together as *B. burgdorferi*.

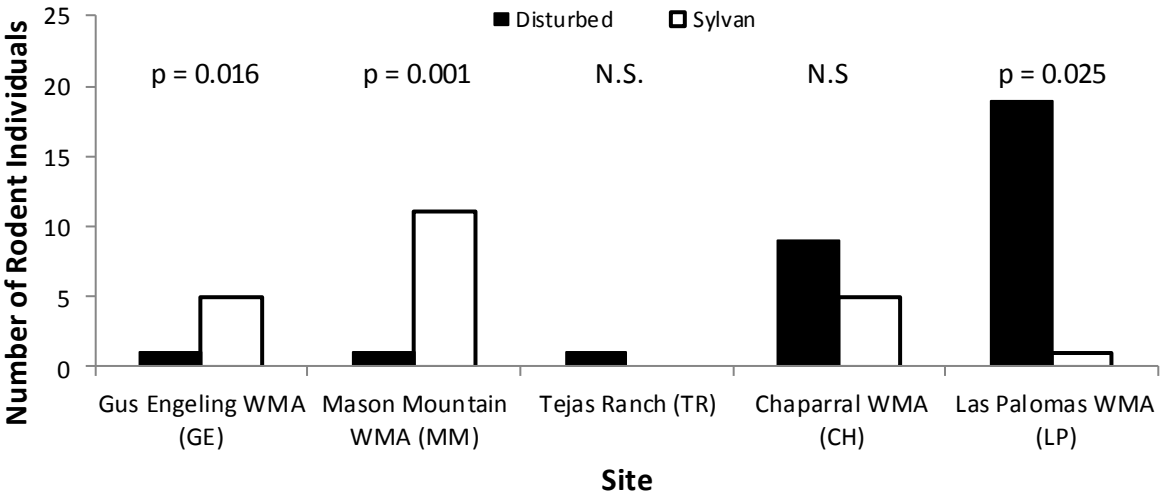

Figure 1: Comparison of the number of *Borrelia* positive individuals between disturbed and sylvan habitats for five sites sampled in Texas. A statistical comparison was done via a Poisson Likelihood Ratio test. Full results are in table 10.

When all data was pooled, there was no difference in the number of *B. burgdorferi*-positive rodents found in sylvan and disturbed habitats (Poisson Likelihood Ratio, t = 1.785, p = 0.182, df = 1; Pearson Chi-Square, t = 1.843, p = 0.175, df = 1). However, more individuals were captured in disturbed habitats (66.39% of all captures), which resulted in a greater overall number of *B. burgdorferi*-positive individuals in disturbed habitats than in sylvan habitats (N=31 and 22 respectively, Figure 1).

In the two northernmost trapping locations (GE and MM) a greater number of individual rodents were infected with *B. burgdorferi* in sylvan habitats (Figure 1, Table 10). It is worth noting that this held true despite the fact that nearly twice as many animals were captured in the disturbed habitat at GE (Appendix IV). At the southernmost site (LP), where > 75% of the individuals captured came from the disturbed habitat (Appendix IV), there was a higher number of individual rodents infected with *B. burgdorferi* in disturbed habitats (Figure 1, Table 10). Neither CH or TR exhibited differences in the number of individual animals infected with *B. burgdorferi* between disturbed and sylvan habitats (Table 10). As was the case with GE, > 75% of the individuals captured at CH came from the disturbed habitat (Appendix IV).

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Table 10: Summary of Poisson Likelihood Ratio Tests for the presence of *Borrelia*-positive individuals between disturbed and sylvan habitats at five Texas sites.

Site	Value	df	P
Gus Engeling WMA (GE)	5.839	1	.016
Mason Mountain WMA (MM)	10.696	1	.001
Tejas Ranch (TR)	1.238	1	.266
Chaparral WMA (CH)	1.370	1	.242
Las Palomas WMA (LP)	5.044	1	.025

After determining differences in the number of *B. burgdorferi*-infected individuals at each site, possible variations between seasons were analyzed. When data from all sites was pooled, there were differences seen across seasons (Poisson Likelihood Ratio, $t = 82.516$, $p < 0.001$, $df = 1$; Person Chi-Square, $t = 81.318$, $p < 0.001$, $df = 2$). More infected rodents were found during the fall trapping period than in the late winter or spring. However, there were no significant differences between disturbed and sylvan habitats for any of the three trapping periods (although the fall trapping period approached significance) (Table 11, Figure 2).

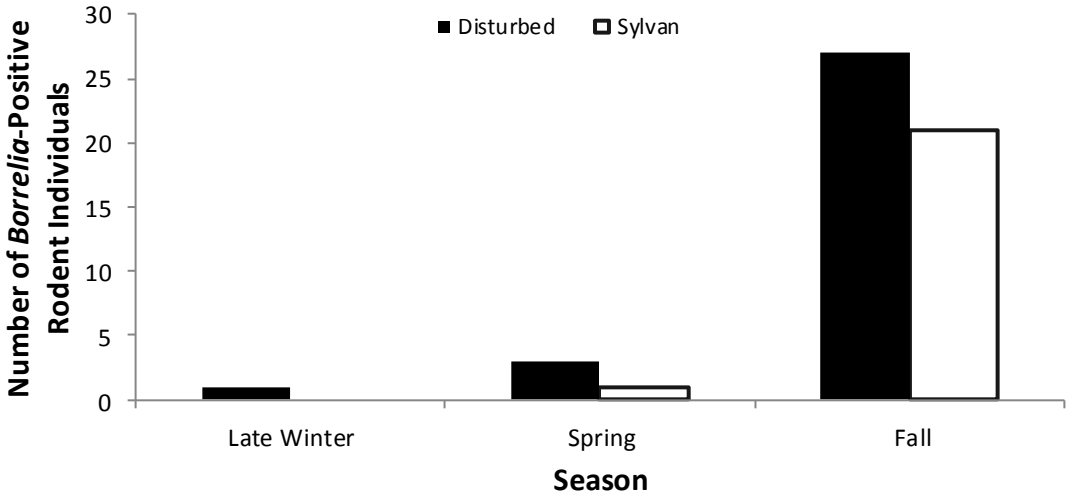


Figure 2: Comparison of the number of *Borrelia*-positive individuals between disturbed and sylvan habitats for three seasons sampled in Texas. A statistical comparison was done via a Poisson Likelihood Ratio test. Full results are in table 11.

Table 11: Summary of Poisson Likelihood Ratio Tests for the presence of *Borrelia*-positive individuals between disturbed and sylvan habitats in Texas across three seasons.

Season	Value	df	P
Late Winter	0.920	1	.338
Spring	0.087	1	.769
Fall	3.587	1	.058

When investigated with a site-specific focus, more *B. burgdorferi*-positive animals were found in the fall than the other two trapping periods at 4 of the 5 sites (GE, MM, CH, and LP) (Table 12). The only site that did not show significant differences across seasons was TR, where the only *Borrelia*-positive animal discovered on site was captured in the fall (Table 12).

Table 12: Summary of Poisson Likelihood Ratio Tests for the presence of Borrelia-positive individuals between disturbed and sylvan habitats and across three seasons for five sites in Texas.

Site	Value	df	P
Gus Engeling WMA (GE)	32.307	2	< 0.001
Mason Mountain WMA (MM)	28.046	2	< 0.001
Tejas Ranch (TR)	1.840	2	0.399
Chaparral WMA (CH)	21.167	2	< 0.001
Las Palomas WMA (LP)	19.026	2	< 0.001

4. DISCUSSION

Contrary to expectations, there were no differences in the mean number of ticks across disturbed or sylvan habitats. This may have been due to the high variation in tick loads within each habitat and site. Additionally, with the exception of TR, there was no change in the average number of ticks per individual across seasons within individual sites or when all sites were pooled together. Despite the large variances in the data, all animals can be classified into three categories: no ticks, very few ticks (N = 5 – 25), or many ticks (N = 35+).

Analysis of the number of ticks per individual revealed no differences between species in sylvan habitats. Conversely, with the exception of TR, there was a single species in the disturbed habitat of each site that had a higher tick load than any other species. Interestingly, the species with the highest tick load changed across sites (GE, *Oryzomys palustris*; MM, *Neotoma leucodon*; CH, *N. micropus*; LP, *Sigmodon hispidus*). In all cases, this was a relatively large bodied species that was in low abundance and classified as "rare" (Berger and Parker 1970) within each individual rodent assemblage (Appendix IV). None of the species that had high tick loads in a given disturbed habitat were captured in the sylvan habitats of same site.

In spite of the fact that *Peromyscus* species were numerically dominant at most assemblages, no species of *Peromyscus* were among those with the highest tick loads. This is important because species within the genus *Peromyscus* are highly competent reservoirs that are commonly regarded as an integral part of the *B. burgdorferi*

transmission cycle (Brisson et al. 2008, Keesing et al. 2006, Mather 1993, Ostfeld and Keesing 2000).

The amount of parasite biomass that an individual host can support tends to be correlated with the host's body mass (Poulin 2007). Many large animals may not persist in as great of numbers in anthropogenically disturbed as they do in undisturbed areas. *O. palustris, N. leucodon*, *N. micropus,* and *S. hispidus* all have a larger body mass than any species of *Peromyscus* captured for my study. These rodents may be providing a host to ticks that would otherwise be feeding on deer and other non-competent hosts (Ostfeld 2011) including various mammalian, reptile, and avian species (Barbour and Fish 1993) that do not exist in Texas. My results suggest that large-bodied rodent species that have high average tick loads may act as dilution agents that lower the infection risk for other species by hosting a larger share of the ticks present in the environment (Keesing et al. 2006, Ostfeld and Keesing 2012). Instead of infecting a new, highly competent host, an infected vector that feeds on a low or incompetent host creates an abortive transmission event and thereby reduces the overall presence of the pathogen in the system (Keesing et al. 2006, Ostfeld 2011). In order to ascertain the extent to which these species act as dilution agents, their role as competent reservoirs for *B. burgdorferi* must first be assessed.

Of the individuals that represented the species with the highest average tick load at each site, only one returned a positive result for *B. burgdorferi*. That individual was a *S. hispidus* from LP. *B. burgdorferi* was not found in *O. palustris*, *N. leucodon*, or *N. micropus* at any site; however the pathogen was discovered in *S. hispidus* at all sites

except TR. Discovering the reason for the low infection rate of *S. hispidus* at LP, where it had a higher tick load than any other species, is deserving of further research.

Medium and large mammals act as important reservoirs for the maintenance of *Ixodes scapularis* ticks (Lane et al. 1991). In my study, hard ticks including *I. scapularis* as well as *Amblyomma* spp., *A. americanum*, *A. auricularium, A. darwini, A. parvum, Bothriocroton concolor*, *Dermacentor andersoni, D. variabilis*, *I. affinis*, *I. banksi*, *Rhipicephalus* spp., and *R. turanicus* as well as the soft tick *Carios capensis* were discovered on collected rodents. These additional tick species may have slightly different host preferences than *I. scapularis*. Although the range of *O. palustris* extends into New Jersey, *N. leucodon*, *N. micropus* and *S. hispidus* do not occur in the northeastern portion of the U.S. (Wilson and Reeder 2005). The presence of additional tick species as well as additional hosts leaves open the possibility that these larger rodents may be playing a role in tick maintenance in Texas. More work must be done in order to discover the role of larger rodents in Texas and how they compare to other known tick hosts that are poor competency reservoirs for *B. burgdorferi*. This will be the focus of future projects that will build upon the data presented here.

Human disturbance exhibited a heterogeneous response on *B. burgdorferi* presence. However, the number of individuals that were infected with the pathogen showed temporal differences. At all sites except TR, a higher number of individual animals were found to be infected with *B. burgdorferi* in the fall than in either the late winter or the spring. Although it was not statistically significant, the only positive animal at TR was captured during the fall season, which holds true to the pattern exhibited by the rest of the sites.

There are multiple scenarios that may explain the temporal differences in *Borrelia* spp. prevalence. The strains of *Borrelia* spp. that persist in Texas may have a detrimental effect on rodent species. Effects could include changes in metabolism, changes in gene expression, and behavioral differences, all of which can result in decreased fitness and survivorship (Schwanz et al. 2012, Vendeville et al. 2005). Lower fitness or survivorship could make an animal less likely to survive winter. This would result in a lower number of infected rodents at the start of the year. If this is the case for Texas, then it may be that *B. burgdorferi* is sustained through the winter months within the ticks that transmit the bacterium (DeBoer et al. 1993). Alternately, rodents may not be the most important *Borrelia* spp. reservoir in Texas. Other common reservoirs for *Borrelia* spp., including various species of chipmunk, shrews, robins, lagomorphs, raccoons and other medium sized mammals (Anderson et al. 1983, Brisson et al. 2008, Ostfeld 2011, Logiudice et al. 2003, Tallekint and Jaenson 1994), that were outside of the scope of this project's trapping effort also live throughout the state and may be of greater importance in Texas than in other areas. Additionally, *Borrelia* spp. prevalence in Texas may be driven by the reproductive phenology of hard-bodied ticks (Ogden et al. 2008), which may result in increases and reductions of the pathogen throughout the year. All of these avenues are deserving and needing of further research.

Another possible scenario for an increased prevalence of *B. burgdorferi* at the end of 2013 may simply have been that conditions were favorable for an increase in both the spirochete and its vectors. Every week of April, 2012 had a lower drought index across the state (nearly 50% of the state was classified as not in drought conditions) than any time two years previous or since (U.S. Drought Monitor, 2014). This may have created

more abundant food sources for rodents, which could have expanded the population size in the following year (Jones et al. 1998, Ostfeld et al. 1996, Windberg 1998). The inflated number of hosts in the system may have provided ticks hatching that summer an increased likelihood of biting and surviving on an infected host and passing on *B. burgdorferi* the following year (Jones et al. 1998, Ostfeld et al. 1996). This lends some support to Schauber et al. (2005) assertion that food abundance may play a key role in LD prevalence in the following year. Additionally, this increased number of infected hosts in 2013 may result in an increase in infected nymphal ticks in 2014 (Ostfeld et al. 2006a).

While low competency reservoirs diluting bacterial prevalence within rodent assemblages may be a factor in keeping the overall incidence of LD in Texas low, it was also found that the prevalence of *B. burgdorferi* in the state exhibited a temporal pattern. A multi-year sample is necessary to ascertain whether or not this is a true pattern or simply a one-time phenomenon.

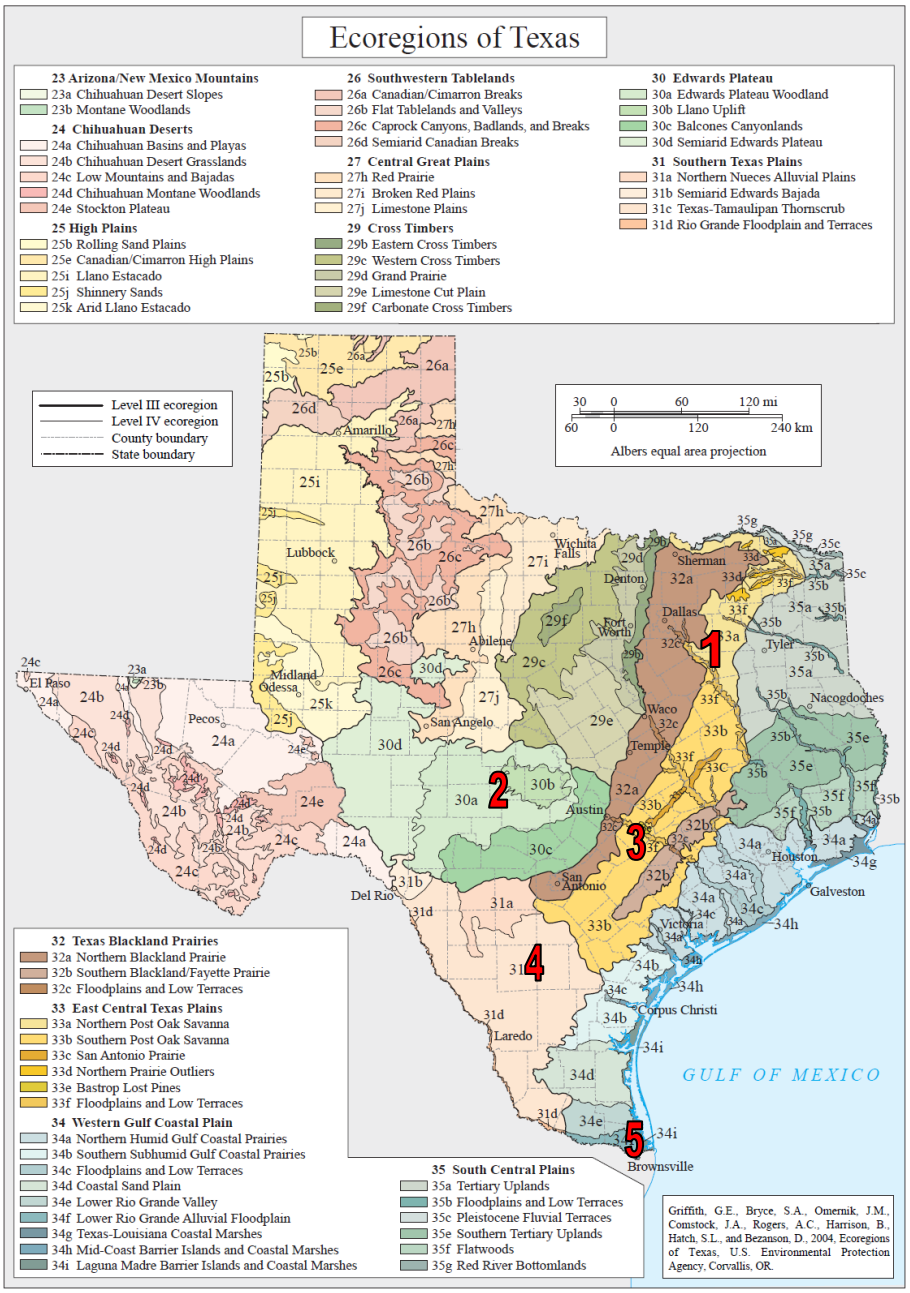
The fact that there is likely an increased incidence of *B. burgdorferi* in rodents in the fall could mean that many Texans are at risk. During the summer and fall, many people head outdoors for hunting or camping before rain and winter weather set in. If rodents are suddenly testing positive for *B. burgdorferi* in the fall, it may be that the highest number of infected ticks is occurring shortly beforehand, when people are outdoors. Other temporal data, including prevalence of *B. burgdorferi* in humans, ticks, pets, and wild animals must be gathered and patterns between these datasets should be investigated. If there is a central time period during which a large number of nymphal

and adult ticks are infecting new hosts, then that period is also likely to pose a high risk of infection in humans.

In order to properly assess the LD threat in Texas, the main *B. burgdorferi* reservoir, vector, and dilution species must be identified, the life cycle of these species must be understood, and the temporal pattern of infection across multiple biotic levels must be investigated. The work described herein should be used as a basis for future investigation into the *B. burgdorferi* system in Texas and throughout the southwestern portion of the bacterium's range.

APPENDIX SECTION

Appendix I:



Map of sample sites. Sites ordered from North to South are Gus Engeling WMA (GE) (1), Mason Mountain WMA (MM) (2), Tejas Ranch (TR) (3), Chaparral WMA (CH) (4), and Las Palomas WMA (LP) (5).

Appendix II: Overview of trapping effort and success for each site, habitat, and season sampled. Sites arranged in the order that they were sampled.

Location	Habitat	Trapping Period	Number of Captures	Number of Traps Set	Trapping Success
Mason Mountain WMA (MM)	Disturbed	Preliminary	11	300	3.67%
	Sylvan		11	300	3.67%
Gus Engeling WMA (GE)	Disturbed	Late Winter	15	450	3.33%
	Sylvan		13	450	2.89%
Tejas Ranch (TR)	Disturbed	Late Winter	12	450	2.67%
	Sylvan		9	450	2.00%
Las Palomas WMA (LP)	Disturbed	Late Winter	30	150	20.00%
	Sylvan		10	150	6.67%
Chaparral WMA (CH)	Disturbed	Late Winter	18	175	10.29%
	Sylvan		7	150	4.67%
Mason Mountain WMA (MM)	Disturbed	Late Winter	29	300	9.67%
	Sylvan		13	300	4.33%
Tejas Ranch (TR)	Disturbed	Spring	10	500	2.00%
	Sylvan		16	525	3.05%
Chaparral WMA (CH)	Disturbed	Spring	56	150	37.33%
	Sylvan		9	150	6.00%
Gus Engeling WMA (GE)	Disturbed	Spring	35	450	7.78%
	Sylvan		8	450	1.78%
Mason Mountain WMA (MM)	Disturbed	Spring	15	300	5.00%
	Sylvan		18	300	6.00%
Las Palomas WMA (LP)	Disturbed	Spring	23	300	7.67%
	Sylvan		13	300	4.33%
Mason Mountain WMA (MM)	Disturbed	Fall	5	450	1.11%
	Sylvan		22	450	4.89%
Gus Engeling WMA (GE)	Disturbed	Fall	2	450	0.44%
	Sylvan		7	450	1.56%
Chaparral WMA (CH)	Disturbed	Fall	28	450	6.22%
	Sylvan		14	450	3.11%
Las Palomas WMA (LP)	Disturbed	Fall	84	150	56.00%
	Sylvan		18	150	12.00%
Tejas Ranch (TR)	Disturbed	Fall	20	450	4.44%
	Sylvan		11	450	2.44%
TOTAL			**592**	**10950**	**5.41%**

Appendix III: Checklist of rodent species captured across all sites

Family / Subfamily / Genus	Species	Species Code	Adult Body Mass (g)
Heteromyidae			
Heteromyinae			
	Liomys irroratus	Lioirr	50-60
Perognathinae			
	Chaetodipus hispidus	Chahis	30-47
	Perognathus merriami	Permer	7-9
Cricetidae			
Neotominae			
	Baiomys taylori	Baitay	7-10
	Neotoma floridana	Neoflo	200-350
	Neotoma leucodon	Neoleu	136-294
	Neotoma micropus	Neomic	204-243
	Ochrotomys nuttalli	Ochnut	15-25
	Onychomys leucogaster	Onyleu	27-46
	Oryzomys palustris	Orypal	40-68
	Peromyscus attwateri	Peratt	25-35
	Peromyscus leucopus	Perleu	15-25
	Peromyscus maniculatus	Perman	25-32
	Peromyscus pectoralis	Perpec	24-39
	Peromyscus spp.	Perspp	--
	Reithrodontomys fulvescens	Reiful	14-30
Sigmodontinae			
	Sigmodon hispidus	Sighis	80-150
Muridae			
Murinae			
	Mus musculus	Musmus	17-25

Taxonomic designations from Wilson and Reeder (2005).
Species code derived from first 3 letters of genus and first 3 letters of species.
Adult body mass from Schmidly (2004).

Appendix IV: Species rank distributoin per habitat and site and relative abundance of *Borrelia burgdorferi* per season.

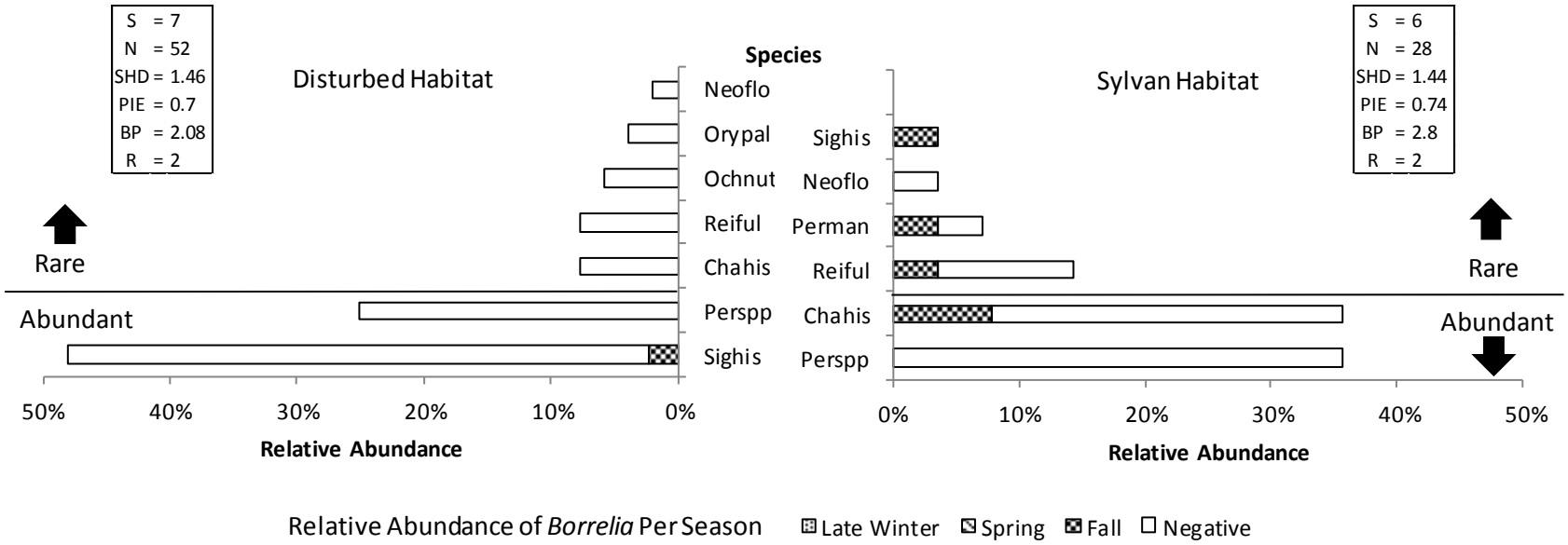


Figure 1: Relative abundance of *Borrelia* per season at Gus Engeling WMA. Species rank distributions were determined by weighting proportional abundance for disturbed and sylvan habitats from pooled data from all sampling periods. The relative abundance of *B. burgdorferi* per season sampled is indicated by the following fill patterns: stippled areas represent late winter, striped areas represent spring, and checkered areas represent fall. Un-filled boxes represent the relative abundance of a given species that was found to be negative for *B. burgdorferi*. A line on each graph denotes rare (species with a relative frequency < the inverse of species richness) and abundant (species with a relative frequency > the inverse of species richness) species. See Appendix 3 for species codes. Abbreviations are as follows: S = species richness (number of species captured), N = sample size (number of individuals captured), SHD = Shannon-diversity index (Pielou 1975), PIE = Hurlbert's PIE (Probability of Interspecies Encounter) index (Hurlbert 1971) (Using EcoSim700 (Entsminger 2012)), BP = Berger-Parker dominance index (Berger and Parker 1970), R = number of rare species.

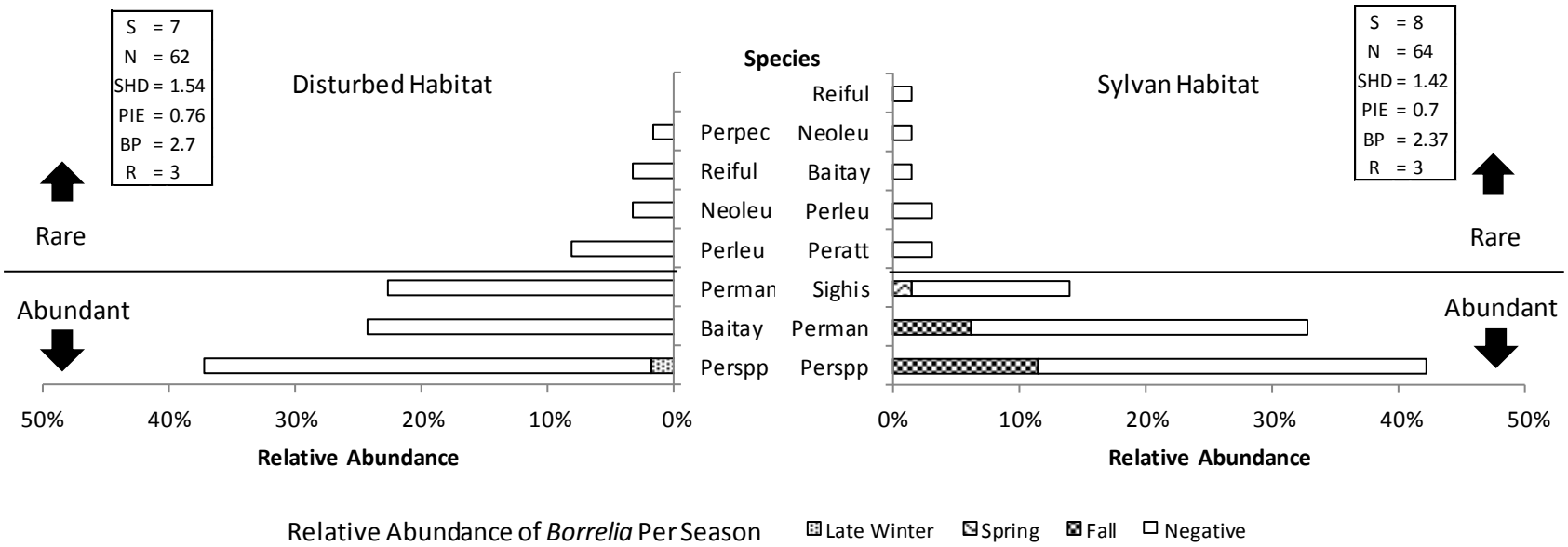


Figure 2: Relative abundance of *Borrelia* per season at Mason Mountain WMA. Species rank distributions were determined by weighting proportional abundance for disturbed and sylvan habitats from pooled data from all sampling periods. The relative abundance of *B. burgdorferi* per season sampled is indicated by the following fill patterns: stippled areas represent late winter, striped areas represent spring, and checkered areas represent fall. Un-filled boxes represent the relative abundance of a given species that was found to be negative for *B. burgdorferi*. A line on each graph denotes rare (species with a relative frequency < the inverse of species richness) and abundant (species with a relative frequency > the inverse of species richness) species. See Appendix 3 for species codes. Abbreviations are as follows: S = species richness (number of species captured), N = sample size (number of individuals captured), SHD = Shannon-diversity index (Pielou 1975), PIE = Hurlbert's PIE (Probability of Interspecies Encounter) index (Hurlbert 1971) (Using EcoSim700 (Entsminger 2012)), BP = Berger-Parker dominance index (Berger and Parker 1970), R = number of rare species.

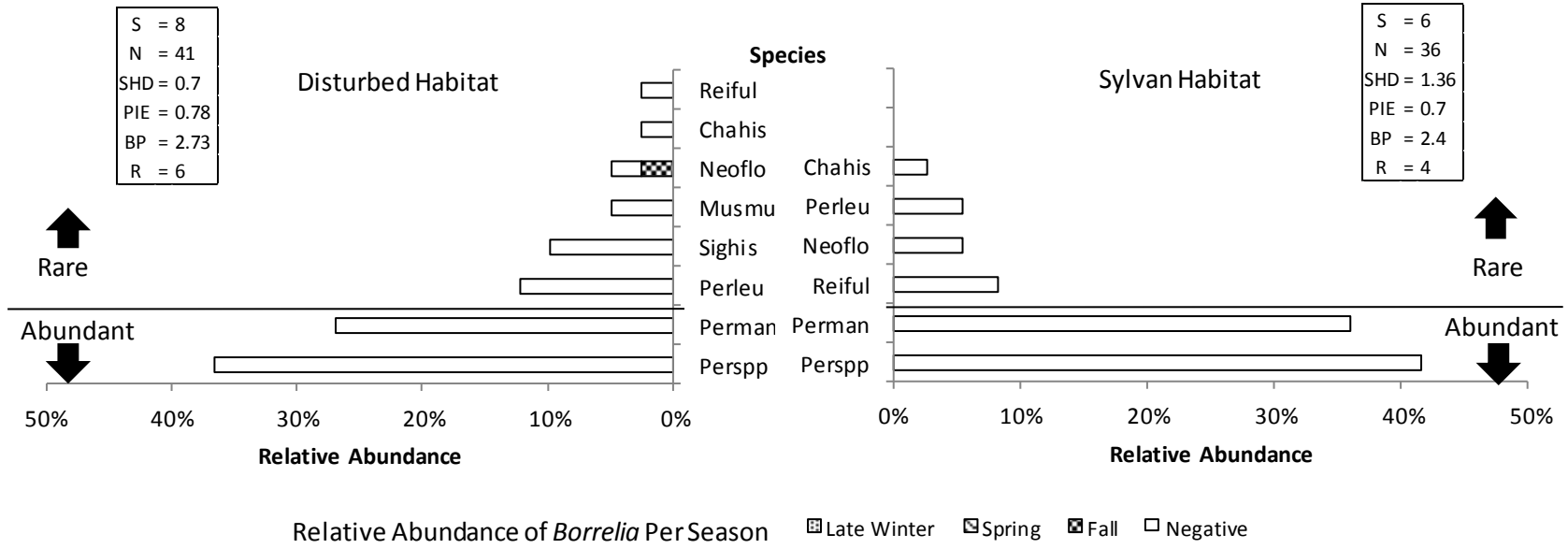


Figure 3: Relative abundance of *Borrelia* per season at Tejas Ranch. Species rank distributions were determined by weighting proportional abundance for disturbed and sylvan habitats from pooled data from all sampling periods. The relative abundance of *B. burgdorferi* per season sampled is indicated by the following fill patterns: stippled areas represent late winter, striped areas represent spring, and checkered areas represent fall. Un-filled boxes represent the relative abundance of a given species that was found to be negative for *B. burgdorferi*. A line on each graph denotes rare (species with a relative frequency < the inverse of species richness) and abundant (species with a relative frequency > the inverse of species richness) species. See Appendix 3 for species codes. Abbreviations are as follows: S = species richness (number of species captured), N = sample size (number of individuals captured), SHD = Shannon-diversity index (Pielou 1975), PIE = Hurlbert's PIE (Probability of Interspecies Encounter) index (Hurlbert 1971) (Using EcoSim700 (Entsminger 2012)), BP = Berger-Parker dominance index (Berger and Parker 1970), R = number of rare species.

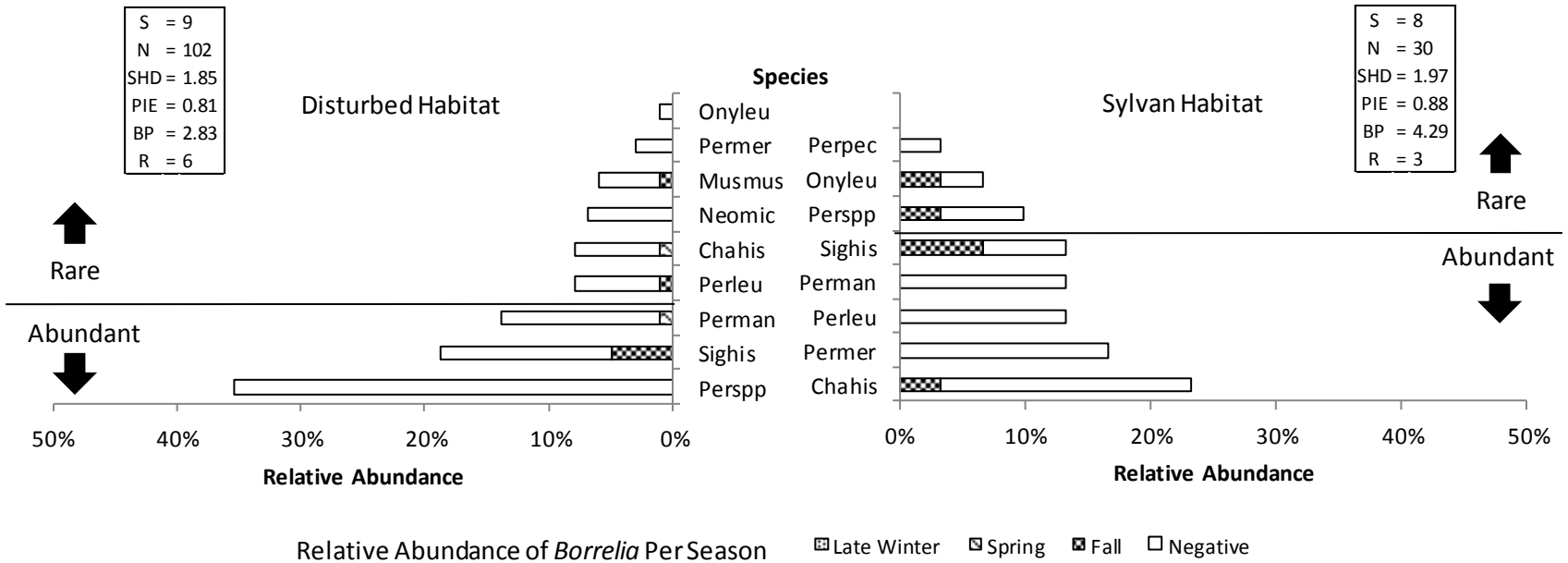


Figure 4: Relative abundance of *Borrelia* per season at Chaparral WMA. Species rank distributions were determined by weighting proportional abundance for disturbed and sylvan habitats from pooled data from all sampling periods. The relative abundance of *B. burgdorferi* per season sampled is indicated by the following fill patterns: stippled areas represent late winter, striped areas represent spring, and checkered areas represent fall. Un-filled boxes represent the relative abundance of a given species that was found to be negative for *B. burgdorferi*. A line on each graph denotes rare (species with a relative frequency < the inverse of species richness) and abundant (species with a relative frequency > the inverse of species richness) species. See Appendix 3 for species codes. Abbreviations are as follows: S = species richness (number of species captured), N = sample size (number of individuals captured), SHD = Shannon-diversity index (Pielou 1975), PIE = Hurlbert's PIE (Probability of Interspecies Encounter) index (Hurlbert 1971) (Using EcoSim700 (Entsminger 2012)), BP = Berger-Parker dominance index (Berger and Parker 1970), R = number of rare species.

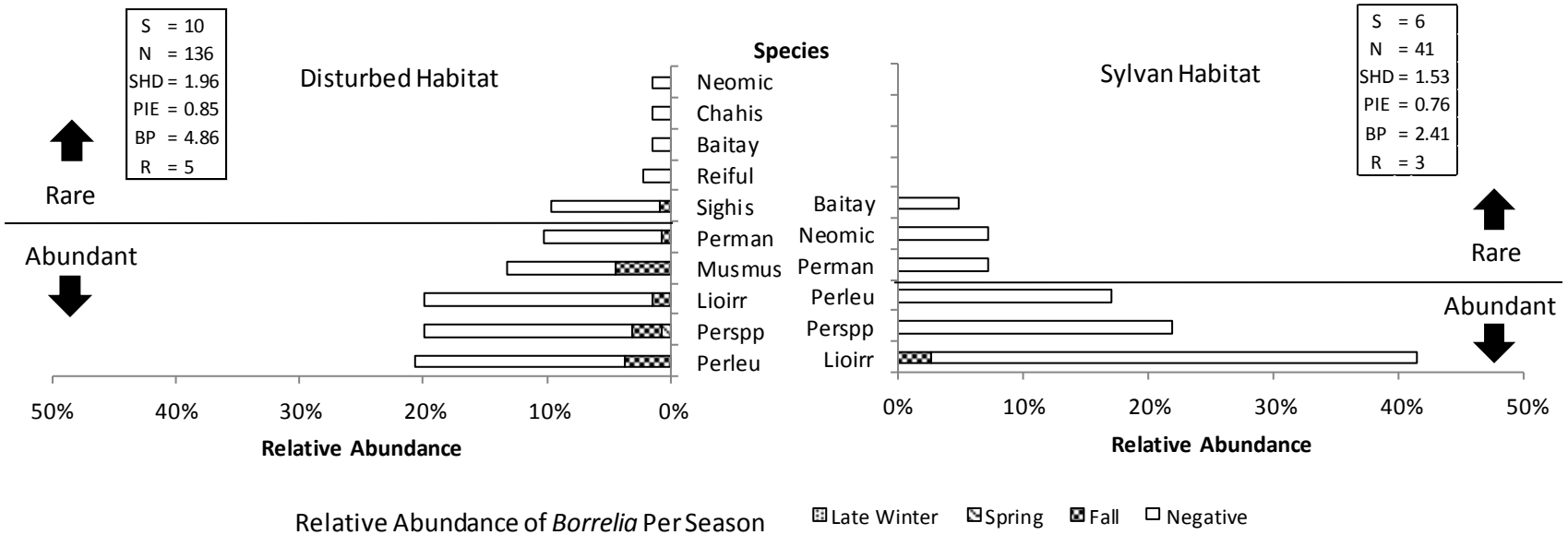
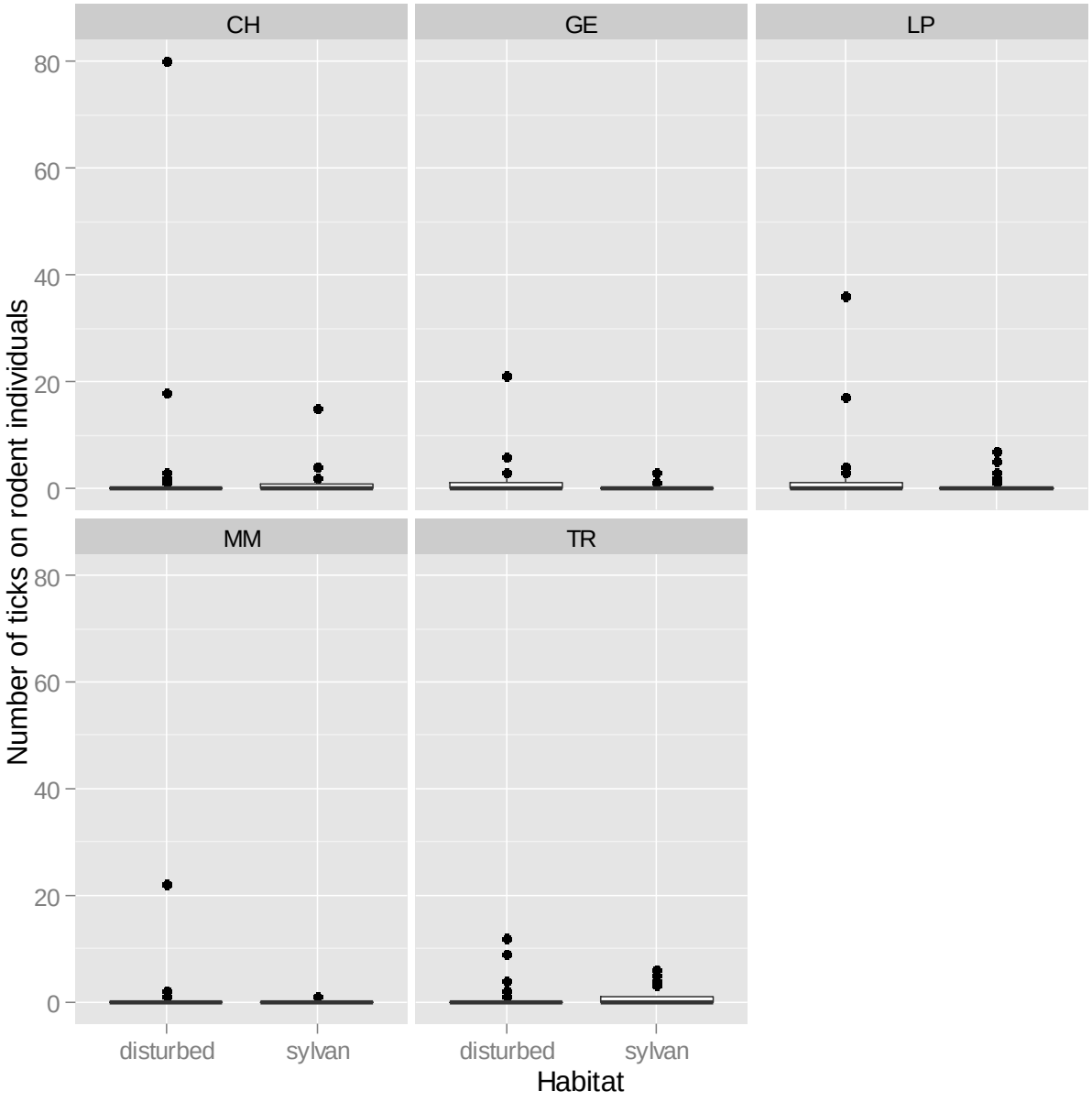


Figure 5: Relative abundance of *Borrelia* per season at Las Palomas WMA. Species rank distributions were determined by weighting proportional abundance for disturbed and sylvan habitats from pooled data from all sampling periods. The relative abundance of *B. burgdorferi* per season sampled is indicated by the following fill patterns: stippled areas represent late winter, striped areas represent spring, and checkered areas represent fall. Un-filled boxes represent the relative abundance of a given species that was found to be negative for *B. burgdorferi*. A line on each graph denotes rare (species with a relative frequency < the inverse of species richness) and abundant (species with a relative frequency > the inverse of species richness) species. See Appendix 3 for species codes. Abbreviations are as follows: S = species richness (number of species captured), N = sample size (number of individuals captured), SHD = Shannon-diversity index (Pielou 1975), PIE = Hurlbert's PIE (Probability of Interspecies Encounter) index (Hurlbert 1971) (Using EcoSim700 (Entsminger 2012)), BP = Berger-Parker dominance index (Berger and Parker 1970), R = number of rare species.

Appendix V:



Summary of numbers of ticks on individual animals across disturbed and sylvan habitats at five sites in Texas. Mean number of ticks ranged from 0.02 – 1.36 and standard deviations ranged from 0.14 – 8.92.

Appendix VI: Descriptive statistics of captures in disturbed and sylvan habitats at each site. For descriptions of species codes, see Appendix III.

Gus Engeling WMA (GE) Disturbed Habitat

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Sighis	17	0.35	0.61	0	2	6
Perspp	11	1.27	1.95	0	6	14
Chahis	4	0.00	0.00	0	0	0
Reiful	4	0.00	0.00	0	0	0
Ochnut	3	0.33	0.58	0	1	1
Orypal	2	12.00	12.73	3	21	24
Neoflo	1	0.00	N/A	0	0	0
Total	**42**	**1.07**	**3.37**	**0**	**21**	**45**

Gus Engeling WMA (GE)Sylvan Habitat

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Chahis	9	0.33	1.00	0	3	3
Perspp	8	0.25	0.46	0	1	2
Reiful	4	0.00	0.00	0	0	0
Perman	2	0.50	0.71	0	1	1
Neoflo	1	0.00	N/A	0	0	0
Sighis	1	0.00	N/A	0	0	0
Total	**25**	**0.24**	**0.66**	**0**	**3**	**6**

Mason Mountain WMA (MM) Disturbed Habitat

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Perspp	15	0.20	0.56	0	2	3
Perman	13	0.08	0.28	0	1	1
Baitay	12	0.00	0.00	0	0	0
Perleu	4	0.00	0.00	0	0	0
Neoleu	2	11.00	15.56	0	22	22
Perpec	1	0.00	N/A	0	0	0
Total	**47**	**0.55**	**3.22**	**0**	**22**	**26**

Mason Mountain WMA (MM) Sylvan Habitat

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Perman	23	0.00	0.00	0	0	0
Perspp	18	0.06	0.24	0	1	1
Sighis	5	0.00	0.00	0	0	0
Perleu	3	0.00	0.00	0	0	0
Peratt	2	0.00	0.00	0	0	0
Baitay	1	0.00	N/A	0	0	0
Neoleu	1	0.00	N/A	0	0	0
Total	**53**	**0.02**	**0.14**	**0**	**1**	**1**

Tejas Ranch (TR) Disturbed Habitat

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Perspp	15	1.07	3.20	0	12	16
Perman	9	1.33	2.96	0	9	12
Sighis	4	0.50	1.00	0	2	2
Perleu	3	0.00	0.00	0	0	0
Musmus	2	0.00	0.00	0	0	0
Neoflo	2	0.50	0.71	0	1	1
Chahis	1	0.00	N/A	0	0	0
Reiful	1	0.00	N/A	0	0	0
Total	**37**	**0.84**	**2.50**	**0**	**12**	**31**

Tejas Ranch (TR) Sylvan Habitat

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Perman	14	1.00	1.71	0	5	14
Perspp	14	1.00	1.80	0	6	14
Reiful	3	0.00	0.00	0	0	0
Neoflo	2	0.50	0.71	0	1	1
Perleu	2	0.00	0.00	0	0	0
Chahis	1	0.00	N/A	0	0	0
Total	**36**	**0.81**	**1.56**	**0**	**6**	**29**

Chaparral WMA (CH) Disturbed Habitat

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Perspp	27	0.15	0.36	0	1	4
Sighis	16	0.63	1.09	0	3	10
Perman	10	0.10	0.32	0	1	1
Chahis	8	0.00	0.00	0	0	0
Neomic	7	14.00	29.87	0	80	98
Musmus	6	0.17	0.41	0	1	1
Perleu	6	0.00	0.00	0	0	0
Permer	3	0.00	0.00	0	0	0
Onyleu	1	0.00	N/A	0	0	0
Total	**84**	**1.36**	**8.92**	**0**	**80**	**114**

Chaparral WMA (CH) Sylvan Habitat

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Chahis	7	0.00	0.00	0	0	0
Permer	5	0.20	0.45	0	1	1
Perleu	4	1.25	1.89	0	4	5
Perman	4	4.75	6.90	0	15	19
Sighis	4	0.00	0.00	0	0	0
Perspp	3	0.33	0.58	0	1	1
Onyleu	2	0.50	0.71	0	1	1
Perpec	1	0.00	N/A	0	0	0
Total	**30**	**0.90**	**2.81**	**0**	**15**	**27**

Las Palomas WMA (LP) Disturbed Habitat

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Perspp	20	1.10	1.33	0	4	22
Lioirr	16	0.13	0.34	0	1	2
Perleu	13	0.77	1.24	0	4	10
Sighis	9	7.33	11.91	0	36	66
Musmus	8	0.13	0.35	0	1	1
Perman	6	1.00	1.67	0	4	6
Baitay	2	0.00	0.00	0	0	0
Reiful	2	0.00	0.00	0	0	0
Neomic	1	0.00	N/A	0	0	0
Total	**77**	**1.39**	**4.55**	**0**	**36**	**107**

Las Palomas WMA (LP) Sylvan Habitat

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Lioirr	12	0.00	0.00	0	0	0
Perspp	7	1.14	2.04	0	5	8
Perleu	6	0.33	0.82	0	2	2
Perman	3	2.67	3.79	0	7	8
Baitay	2	0.00	0.00	0	0	0
Neomic	1	2.00	N/A	2	2	2
Total	**31**	**0.65**	**1.62**	**0**	**7**	**20**

Appendix VII: Descriptive satistics of captures across seasons. For descriptions of species codes, see Appendix III.

Late Winter

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Perspp	46	1.13	2.29	0	12	52
Perman	18	0.83	1.42	0	4	15
Baitay	13	0.00	0.00	0	0	0
Perleu	11	0.09	0.30	0	1	1
Reiful	9	0.00	0.00	0	0	0
Permer	8	0.13	0.35	0	1	1
Sighis	5	1.40	1.34	0	3	7
Neoflo	3	0.00	0.00	0	0	0
Chahis	2	1.50	2.12	0	3	3
Neomic	2	1.00	1.41	0	2	2
Orypal	2	12.00	12.73	3	21	24
Peratt	2	0.00	0.00	0	0	0
Lioirr	1	0.00	N/A	0	0	0
Neoleu	1	0.00	N/A	0	0	0
Onyleu	1	0.00	N/A	0	0	0
Perpec	1	0.00	N/A	0	0	0
Total	**125**	**0.84**	**2.43**	**0**	**21**	**105**

Spring

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Perspp	54	0.37	0.92	0	5	20
Perman	32	0.66	2.67	0	15	21
Sighis	26	0.96	3.33	0	17	25
Lioirr	18	0.11	0.32	0	1	2
Chahis	15	0.00	0.00	0	0	0
Perleu	15	0.87	1.46	0	4	13
Reiful	4	0.00	0.00	0	0	0
Musmus	3	0.00	0.00	0	0	0
Ochnut	3	0.33	0.58	0	1	1
Baitay	2	0.00	0.00	0	0	0
Neoleu	2	11.00	15.56	0	22	22
Neomic	2	49.00	43.84	18	80	98
Onyleu	1	0.00	N/A	0	0	0
Perpec	1	0.00	N/A	0	0	0
Total	**178**	**1.13**	**6.56**	**0**	**80**	**202**

Fall

Rodent Species	Number of Rodents Captured	Mean Number of Ticks	Standard Deviation	Minimum Number of Ticks	Maximum Number of Ticks	Total Number of ticks
Perspp	38	0.34	0.85	0	3	13
Perman	34	0.76	2.08	0	9	26
Sighis	25	2.08	7.15	0	36	52
Perleu	15	0.20	0.56	0	2	3
Chahis	13	0.00	0.00	0	0	0
Musmus	13	0.15	0.38	0	1	2
Lioirr	9	0.00	0.00	0	0	0
Neomic	5	0.00	0.00	0	0	0
Neoflo	3	0.67	0.58	0	1	2
Baitay	2	0.00	0.00	0	0	0
Onyleu	1	1.00	N/A	1	1	1
Reiful	1	0.00	N/A	0	0	0
Total	**159**	**0.62**	**3.06**	**0**	**36**	**99**

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