MAMMALIAN ASSEMBLAGE STRUCTURE AND HOST-USE PATTERNS OF THE BLACK-LEGGED TICK (*IXODES SCAPULARIS)* ACROSS ANTHROPOGENIC DISTURBANCES IN EAST TEXAS

by

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LIST OF ABBREVIATIONS

Abbreviation	Description
CDC	Centers for Disease Control
spp	species
US	United States
MM	Meso-Mammal
SM	Small Mammal
GEWMA	Gus Engeling Wildlife Management Area
BTNP	Big Thicket National Preserve
IACUC	International Animal Care and Use Committee
PCR	Polymerase Chain Reaction
Cyt*b*	Cytochrome B
BLAST	Basic Local Alignment Search Tool
Ba.ta	*Baiomys taylori*
Cr.pa	*Cryptotis parva*
Di.vi	*Didelphis virginiana*
Gl.vo	*Glaucomys volans*
Me.me	*Mephitis mephitis*
Ne.fl	*Neotoma floridana*
Oc.nu	*Ochrotomys nuttalii*
Pe.go	*Peromyscus gossypinus*
Pe.le	*Peromyscus leucopus*
Pr.lo	*Procyon lotor*
Re.fu	*Reithrodontomys fulvescens*
Si.hi	*Sigmodon hispidus*
Ur.ci	*Urocyon cinereoargenteus*

De.va ..... *Dermacentor variabilis*

Ix.sc ..... *Ixodes scapularis*

Ix.af ..... *Ixodes affinis*

Ix.co ..... *Ixodes cookei*

Ix.ki ..... *Ixodes kingi*

Ix.te ..... *Ixodes texanus*

Am.ma ..... *Amblyoma maculatum*

Am.am ..... *Amblyoma americana*

Am.in ..... *Amblyoma intornatum*

Or.tu ..... *Ornithodorous turicata*

ABSTRACT

Borrelia burgdorferi, the causative agent of Lyme disease, is responsible for infecting more than 300,000 people annually in the United States (US), with 95% of cases reported in the Northeastern US. However, human risk for contracting Lyme disease in Texas is much lower, with only 54 cases reported in 2015. Understanding the composition of mammalian reservoir host assemblages is commonly used to predict areas of greatest concern for human risk of Lyme disease. Community dynamic factors such as predation and competition greatly influence the composition of hosts present at any given time; however, anthropogenically-disturbed habitats are positively correlated to increased densities of highly competent *B. burgdorferi* reservoirs and vectors. My research objectives were to 1) assess if mammal assemblages differed across habitat disturbances; 2) determine if tick intensities were greater in disturbed habitats; 3) assess host-use patterns of tick vectors across East Texas; and 4) identify whether known vectors are associated with competent reservoirs of *B. burgdorferi*. I found that mammalian assemblages share high degrees of richness and evenness (Hurlbert's PIE = 0.77 – 0.84), although disturbed habitats have greater proportions of rare species comprising 26 – 39% of assemblages. Average individual tick intensity differed across ecoregions in sylvan habitats with 634 ticks collected from mammals at Gus Engeling Wildlife Management Area (GEWMA) and 159 ticks collected from mammals at Big Thicket National Preserve (BTNP). I suspect this difference to be the result of a 13% increase in meso-mammal

species with greater tick intensities captured at GEWMA. Furthermore, host-use patterns were observed for all *Ixodes scapularis* adults utilizing meso-mammal hosts and approximately 99% of *Dermacentor variabilis* nymphs selecting small mammal hosts. These results indicate that across evenly distributed mammalian assemblages in East Texas the prevalence of *B. burgdorferi* is expected to be low due to the rarity of competent reservoirs and association of known vectors with poor tick hosts.

1. INTRODUCTION

The etiological agent of Lyme disease belongs to the bacterial spirochete group *Borrelia burgdorferi sensu lato*. This globally distributed group is genomically and phenotypically diverse with 19 confirmed genospecies on three continents (Brisson and Dykhulzen 2004; Margos et al. 2011; Pritt et al. 2016). Adaptability gained through diversity enables this pathogen to infect multiple species of birds, mammals, and hard-bodied ticks, thus ensuring persistence and distribution in the environment. The generalist nature of this pathogen group has enabled it to become the most prevalent vector-borne disease across North America and Europe (Dennis and Hayes 2002). Furthermore, the distribution of this pathogen is expected to increase with the expansion of vector and host populations due to increased temperatures associated with climate change (Khatchikain et al. 2015; Ogden et al. 2008).

Multiple strains of the pathogen are present in North America, however the typical etiological agent of Lyme disease is *B. burgdorferi sensu stricto* (*B. burgdorferi* herein; Johnson et al. 1984). This pathogen was described in the mid 1970's as a result of clustered cases in children from the northeastern United States (US; Burgdorfer et al. 1989). However, fossil evidence suggests that *Borrelia* spirochetes existed millions of years ago (Poinar 2014; Wier et al. 2002), and museum specimens place this pathogen in North America dating back to the 19th century (Marshall et al. 1994; Persing et al. 1990). In the US, *B. burgdorferi* caused at least 37,000 confirmed cases of Lyme disease in 2015 (CDC 2016). The Centers for Disease Control reported that 95% of these cases occurred in the northeastern and upper midwestern US. Nelson et al. (2015) estimate that Lyme disease cases may exceed CDC reports by nearly 300,000 cases annually if corrected for

underreporting. Due to the high incidence rate in this region a significant amount of research is focused on uncovering the life cycle of the vector, potential hosts, and epidemiology of the pathogen (Ostfeld 2011).

In the northeastern US, *B. burgdorferi* is transmitted through the bite of infected black-legged ticks (*Ixodes scapularis*). Larval *I. scapularis* become infected through horizontal transmission when it obtains a blood meal from previously infected host reservoirs such as the white-footed deermouse (*Peromyscus leucopus*; Wilson and Reeder 2005) and shrews (*Blarina* spp. and *Sorex* spp.; Piesman 1991; LoGiudice et al. 2003). Infected larvae will overwinter then molt becoming a nymph that can infect the next host from which it feeds (Burgdorfer et al. 1989). Risk to humans is greatest during the early summer months when these small-infected nymphs (1 mm) are seeking hosts. After acquiring a blood meal, the nymph will then molt to emerge as an adult and feed once more on large mammals (i.e. deer, or humans) before reproducing. The seasonality of the *I. scapularis* lifecycle enables the avoidance of harsh abiotic factors (e.g. extreme heat or cold) and increases questing success for hosts (Troughton et al. 2007; Duik-Wasser et al. 2006). The localized LD risk to humans is dependent on pathogen prevalence within *I. scapularis* populations, but also on the availability of potential reservoir species.

Ixodes scapularis is a generalist species and will feed from reptilian or avian hosts, although mammalian hosts are typical (Anderson, J. F. 1989; Brunner and Ostfeld 2008;Durden et al. 2002; Oliver et al. 2014); therefore, the composition and abundance of mammalian hosts in an assemblage can potentially influence localized pathogen prevalence (Gilbert et al. 2001; Vuong et al. 2017). This is largely driven by the "competence" of a potential reservoir, or the variation in host physiology that alters their

capacity for initial infection with *B. burgdorferi* followed by the transfer of the pathogen to naïve vectors (Anderson, J. F. 1989; Ostfeld and Keesing 2012). For example within a mammalian assemblage, an abundance of species that are poor *I. scapularis* hosts and/or have a low competence for *B. burgdorferi* can lower the pathogen prevalence. In contrast, the pathogen prevalence may be increased with greater abundances of species that are high quality vector hosts of high reservoir competence for the pathogen (Keesing et al. 2006; LoGiudice et al. 2003). Finally, specific host behavior and physiology can lead a reduced number of infected vectors through increased predation (e.g. removal of ticks through grooming) or decreased tick molting success through acquired tick-immunity (Craig et al. 1996; Keesing et al. 2009). Therefore, variations in mammalian assemblage compositions across a landscape can increase or decrease Lyme disease risk to humans (Johnson et al. 2013; Krasnov et al. 2007). In the northeastern US mammalian assemblages vary with the degree to which habitats are fragmented or are otherwise anthropogenically-disturbed.

The prolonged impacts of human activity and habitat manipulation allows for increased zoonotic disease transmission opportunities (Bradly and Altizer 2006; Hansford et al. 2017). As habitats decrease in size or become anthropogenically-disturbed (disturbed), mammalian communities typically lose species that are low competence reservoirs for the pathogen, such as predators and niche competitors (LoGuidice et al. 2003). In the northeastern US, both disturbed and highly fragmented habitats support increased population densities of the ubiquitous white-footed deermouse, a highly competent reservoir for *B. burgdorferi* (Anderson et al. 2003; Mather et al. 1989;Wilder and Meikle 2006). Furthermore, disturbed habitats can support higher densities of *I.*

scapularis and increased proportions of infected nymphs in the environment (Allan et al. 2003). Therefore, when compared to predominantly less disturbed habitats (sylvan), there is an increased risk of pathogen transmission in disturbed habitats due to increased densities of competent reservoirs and infected vectors (Ostfeld and Keesing 2000; Suzan et al. 2015). In the northeastern US, dense human populations living near and interacting with disturbed environments during peak *I. scapularis* questing seasons further increases the incidence of infection. Disturbed habitats have increased pathogen prevalence under simplified host community structures, however alterations to mammalian assemblage composition and increasing local biodiversity can potentially lower *B. burgdorferi* prevalence.

Across habitat disturbance gradients, vector and mammalian assemblages are influenced through changes in community dynamics and biodiversity. Competition and predation can modulate assemblages of vectors and hosts thereby influencing the prevalence of the pathogen (Ostfeld and LoGiudice 2003; Wood and Lafferty 2012). In the northeastern US, competition and predation pressures on the white-footed deermouse reduces the prevalence of infected *I. scapularis* nymphs in an environment (Keesing et al. 2010; LoGiudice et al. 2008). While competition among multiple tick species reduces densities of infected *I. scapularis* through partitioning of host species or reduced feeding opportunities (Apperson et al. 1993). In addition to these community interactions, variations in species richness (e.g. biodiversity) within mammalian assemblages can influence the prevalence of *B. burgdorferi*. Typically, adding species of lower competence to a community (i.e. increased biodiversity) is thought to dilute competent reservoirs, and therefore decrease the prevalence of pathogens in the environment

(Civitello et al. 2015; Suzan et al. 2009). In the Lyme disease system, dilution occurs when species added have a low competence and are also predators or niche competitors to reservoirs of greater competence (Schmidt and Ostfeld 2001). For example, an ideal dilution host is the Virginia opossum (*Didelphis virginiana)* as this species has a low competence for *B. burgdorferi*, is a poor vector host, and also a predator of both vectors and rodents (Levi et al. 2016). However, the extent to which dilution occurs varies across scales and localities since an amplification of pathogen prevalence can occur when highly competent species are added to the community (Huang et al. 2016; Norman et al. 1999; Wood and Lafferty 2012). In the northeastern US mammal assemblage, characteristics that may act to dilute or amplify the prevalence of *B. burgdorferi* are well studied; however, in areas south of this region the epidemiology of the Lyme disease system is much less understood.

Epidemiology of Lyme disease where low prevalence of *B. burgdorferi* is observed needs to be explored to better understand what factors are limiting the epidemic to the northeastern US. Outside of the epidemic, in Texas *B. burgdorferi* risk to humans appears greatly reduced; in 2015 Texas reported 0.1% of the nationally reported Lyme disease cases (CDC 2016; Walker et al. 2016). Mechanisms for this reduced prevalence in Texas are yet unknown, however, key aspects of the epidemiology are known. Habitat suitability models suggest environmental conditions are favorable to support populations of *I. scapularis* (Brownstein et al. 2003). The Texas Department of Health reported the presence of *I. scapularis* infected with *B. burgdorferi* during the 1980's (Teltow et al. 1991). Subsequent tick sampling from humans and the environment in East Texas revealed *I. scapularis* infected with *B. burgdorferi,* indicative of sustained tick

populations and pathogen persistence (Harvey and Salvato 2003; Williamson et al. 2010). These trends are not expected to vary under modeled climate change scenarios (Figure 1; Ferria-Arroyo et al. 2014). However, it remains unclear from which hosts *I. scapularis* is obtaining the pathogen and how the mammalian and tick assemblages are composed in East Texas.

Community interactions and biodiversity of mammalian and tick assemblages, as well as host-use by ticks may be driving the low prevalence of the pathogen. In East Texas two distinct ecoregions from which *B. burgdorferi* infected *I. scapularis* were collected include the Piney Woods and the Post Oak Savannah. These ecoregions differ in elevation and rainfall, resulting in unique dominant vegetation at each site. Differences in vegetation composition could provide unique microhabitats enabling *I. scapularis* foraging and survival, in addition to supporting potentially different mammalian assemblages. Compared to the northeastern US, mammalian assemblages in Texas may be influencing the epidemiology of the Lyme disease system resulting in an ineffective outbreak. I have developed a set of comparative analyses of the mammalian and tick community structure between two ecoregions in Texas to further elucidate differences in *B. burgdorferi* prevalence between Texas and the northeastern US.

The aim of this study is to compare the composition of small and medium sized mammals contributing to the assemblages from two ecoregions in Texas to identify ecological factors that contribute to differences in tick abundances and pathogen loads. Since Lyme disease risk to humans is greater in disturbed habitats, I contrasted host assemblages across disturbed and sylvan habitats from both regions to assess differences in composition and structure. I predicted that [1] disturbed habitats would consistently

have a lower mammalian diversity in both ecoregions sampled [2] disturbed habitats will evince greater numbers of *I. scapularis* on hosts ("intensity") compared to more sylvan habitats in both ecoregions, [3] host-use patterns of *I. scapularis* will be more prominent in disturbed habitats across ecoregions, and that [4] greater abundances of *I. scapularis* will be found associated with known competent reservoirs of *B. burgdorferi*.

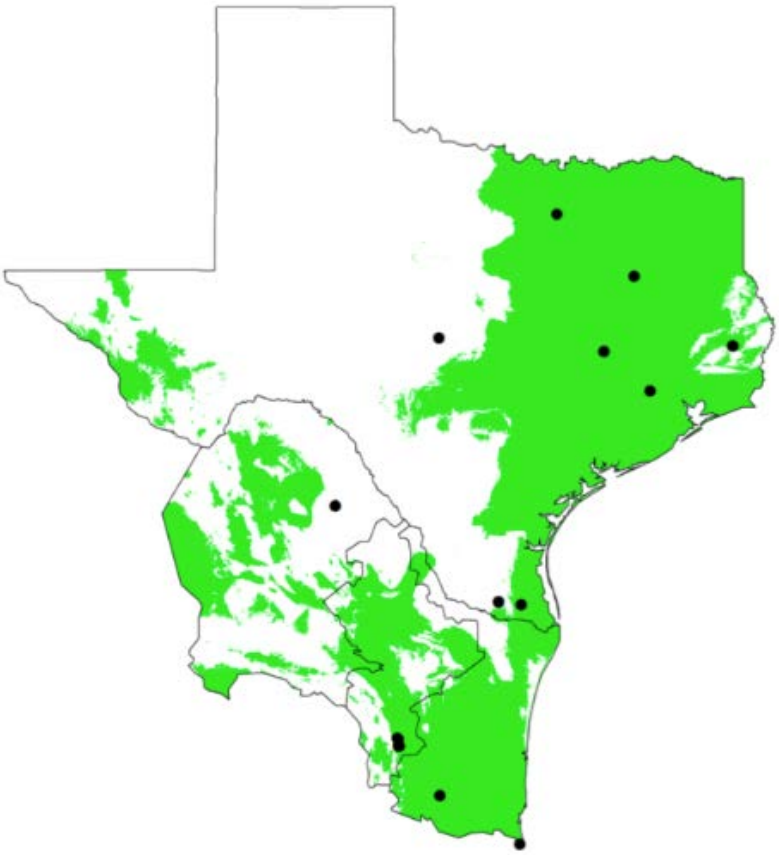

Figure 1. Suitable habitat for *Ixodes scapularis* in Texas. Points indicate sites where *I. scapularis* has been captured (taken from Feria-Arroyo et al. 2014).

2. METHODS

Site Selection

Gus Engeling Wildlife Management Area (GEWMA) was selected as the study site to represent the Post Oak Savannah ecoregion from Anderson County (UTM Zone 15 R 227000 3535000). Big Thicket National Preserve (BTNP) was selected to represent the Piney Woods ecoregion in Hardin (UTM Zone 15 R 370400 3371000), Polk (UTM Zone 15 R 338700 3393500) and Tyler Counties (UTM Zone 15 R 383000 3399200). These two ecoregions were selected based on identified suitable habitat for *I. scapularis*, and previous collection of this vector infected with *B. burgdorferi* (Feria-Arroyo et al. 2014). Satellite imagery (Google Earth V 7.1.8.3036) was used to delineate habitat into anthropogenically-disturbed and pristine habitats. Disturbed habitats were selected by proximity to buildings, major roads, agriculture, and other major human disturbance. Sylvan habitat was selected for by the absence of major human disturbance (i.e. at least 1km from agriculture, main roads, etc.), while keeping accessibility and safety in mind. Sites were then ground truthed prior to setting mammal sampling transects.

Trapping Effort

There were two distinct groups (guilds) of anticipated capture species that varied in size, diet, and home range. Four of the species will be considered meso-mammals (MM) based on their moderate body size (1Kg - 20Kg), omnivorous to carnivorous diet, and large home range (> 1Km^2). The remaining 14 species will be grouped together as small mammals (SM), because of their small body size (< 1Kg), small home range (< 1Km^2), and granivorous, insectivorous, or frugivorous diets.

Sampling occurred over two trapping seasons: October 2015 to January 2016, and December 2016 to April 2017. Small mammals were captured with Sherman live traps (Model: LFATDG; Sherman Traps, Inc., Tallahassee, FL). At each location, 400 traps, placed 5-10 m apart, were set in a curvilinear transect. Traps were set 1-2 hours prior to dusk and checked the following morning within 2 hours of sunrise every day they were active. Weather conditions permitting, traps were re-baited with rolled oats and peanut butter and remained open during the diurnal hours dependent upon ambient temperatures. Similarly, meso-mammals were captured with Tomahawk live traps (sizes 20 x 7 x 7 inches and 32 x 10 x 12 inches; Tomahawk Live Trap Inc., Hazelhurst, WI) set in a curvilinear transect of 50 traps, placed 100 m apart, at each habitat. Tomahawk traps were baited with one or more of the following attractants: sardines, wet/dry cat food, boiled eggs, and marshmallows. Both sets of transects were ran for 3 nights, until holding cage availability ran out for meso-mammals (n = 5), or until permitted take limitations were reached. Total yearly trapping effort approached a total of 14690 Sherman trap-nights and 1460 Tomahawk trap-nights.

Tick and Tissue Collection

Live captured mammals were transported to Laboratory Animal Resources and Research (LARR) facilities at Texas A&M University in College Station, TX. The LARR units are climate controlled, aseptic isolation rooms set with a photoperiod to current natural conditions. Small mammals and meso-mammals were housed in separate LARR units for human and animal safety. There mammals were housed in wire-bottom cages (Model 1264C Eurostandard Type II; Tecniplast, West Chester, PA) and suspended over a tray of water for 168 hours. This allowed sufficient time for any attached ticks to

release. The mammals were then euthanized in accordance with IACUC approved methods (#2014-0227 and #2016-0243). Mammal specimens then underwent a thorough tick examination before having morphometric data collected and being identified to the species level. Mammal trap mortalities from the field were processed on site with all tissues and vouchers collected, in addition to any ticks found during examination. Tissue samples were aseptically removed and placed in liquid nitrogen until they could be transferred to long-term storage (-80°C) at Texas State University. Tissues collected included the spleen, liver, kidney, heart, lung, bladder, and articulating joint. Skull vouchers were collected from every specimen, and small mammal pelts fixed in formalin are stored at the Texas State Vertebrate Collection. In accordance with federal regulations, skull vouchers and wet mounts associated with specimens collected from BTNP will be cataloged and held at the Biodiversity Research and Teaching Collections (BRTC) at Texas A&M University.

Ticks collected from housed animals were classified by their developmental stage (adult, nymph or larvae) and identified to the species level once morphological traits develop past the first instar. Tick parasitism levels per individual, or tick intensity, was defined for the purposes of this study as the sum of all ticks collected from a mammal. Ticks will be subjected to a battery of pathogenic detection tests, including *B. burgdorferi*.

Molecular Identification of Mammalian Species

Specimen tissues were used for genetic analysis to confirm field identifications on juvenile or atypical specimens. DNA was extracted from frozen tissue samples following protocol from the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia

California) and stored at -80° C. The mitochondrial Cyt*b* gene was used due to its high degree of divergence in rodent species, allowing for identification of closely related species (Nicolas et al. 2012). Two sets of overlapping primers were amplified and sequenced for each unidentified specimen: 1) MVZ05 forward (5'- CGA AGC TTG ATA TGA AAA ACC ATC GTT G -3') and P3' reverse (5'- TCT CTC CGG TTT ACA AGA CCA AAG T -3'), and 2) LGL 765 forward (5' - GAA AAA CCA YCG TTG TWA TTC AAC T -3') and 752 reverse (5'- GCA GGA GTG TAA TTA TCG GGG TCT -3') (Alexander and Riddle 2005). These primer sets were used for mtDNA amplification via polymerase chain reaction (PCR) and as sequencing primers (methods modified from Edwards et al. 2001) in an Applied Biosystems Genetic Analyzer 3500xL (Life Technologies, Carlsbad, CA). Sequences were then be assembled in Geneous 8.1.7 (Biomatters Ltd, Auckland, New Zealand) and used in a BLAST search of the National Center of Biotechnology Information GenBank® database (NCBI 2016) to ascertain species identity of each tested sample.

Analysis

I calculated α diversity for each habitat sampled: abundance (N), richness (S), Shannon's Diversity Index (*H'*) and Hulbert's Probability of Interspecies Encounters (PIE; Hurlbert 1971; Kwak and Peterson 2007). Good's Coverage (C = 0-1) estimates were calculated for each habitat to determine how well rare species are represented. Beta (β) diversity was assessed using R version 3.4.0 with packages "Vegan" and "BiodiversityR" for the following tests (Kindt and Coe 2005; Oksanen et al. 2017; R Core Team 2017). An abundance-based measure of dissimilarity, Bray-Curtis, was calculated and visualized for mammalian assemblages across ecoregions and habitats

(Bray and Curtis 1957). Hierarchical clustering analysis was then conducted to determine which sampled sites group by dissimilarity among the mammalian assemblage composition. Differences between grouped sites were tested to determine if habitat quality or ecoregions are driving assemblage patterns. A Principal Coordinate Analysis (PCoA) was conducted to determine which species might be influencing the grouping of sites. Significance will be assessed with a permutational multivariate analysis (PERMANOVA). A Pearson Correlation was performed to assess if there were site preferences among the species analyzed.

A single factor ANOVA was conducted to assess the presence of differences in average tick intensity on individuals between habitats of all ecoregions. Tukey's post-hoc test was then applied to determine which sites contain differences. Additionally, this was performed to assess average tick intensity on species between ecoregions and habitats within ecoregions. The student's t-test was used to assess differences in the mean tick intensity from type of mammal collected (small or meso-mammal) at the regional and habitat level (East Texas, disturbed and sylvan, respectively). A Levene's Test was run for each t-test to assess equal variance of components, corrections to t-test were made accordingly.

I calculated α and β diversity for the tick assemblages to discern any patterns or preferences in host-use. For this analysis each species of host was treated as a site sampled, and the ticks present per species comprised each tick assemblage. Furthermore, tick species were separated by developmental stage (larvae, nymph, adult) for diversity analysis. Ticks not sufficiently developed for identification to the species level, or that have yet to be identified were excluded from the analysis.

Ethics Statement

The following sampling procedures were conducted under Texas Parks and Wildlife permit SPR-1112-1052. Animal capture, containment and euthanasia procedures were approved by Texas State University IACUC 201598223 and Texas A&M University IACUC 2014-0227 and 2016-0243. All work conducted at Big Thicket National Preserve was in accordance with approved protocol under the Scientific Research and Collecting Permit BITH-2015-SCI-0016.

3. RESULTS

Trap Effort and Success

As previously described, animals were trapped for three consecutive trap-nights or until available holding cages were occupied. The latter situation occurred most frequently as the maximum of five meso-mammals were typically captured within 1-2 trap nights. At each of the four sites sampled, trapping effort varied due to localized weather events and hazardous conditions. During each trapping event, trap-nights ranged from 50-147 for Tomahawk traps and 650-1281 for Sherman traps. During the first trap year, BTNP sylvan and disturbed habitats were only sampled once for meso-mammal in the fall, and GEWMA disturbed habitat was sampled once for all mammals in the fall (no replicate trap events in the late winter). All other sites and habitats were sampled twice that season (Appendix A). Furthermore, a delay in updating permits with BTNP limited when field sites were available during the second trap year and GEWMA habitats were sampled twice in succession before access to BTNP was gained. This resulted in a reduction of seasonal balance across years in the sampling design. Overall 753 mammals were collected. A total of 166 meso-mammals and 587 small mammals were captured during 1,464 Tomahawk and 14,690 Sherman trap nights respectively. The trap success rate for meso-mammal trapping was 11.3%, and 4.0% for small mammal (Appendix B). During the second year trap success decreased due an increase in trap-nights targeting meso-mammal species partitioning the environment temporally. I found that by removing crepuscular species from a site on the first trap-night increased capture rates of raccoons (*Procyon lotor*) for subsequent trap-nights.

Mammalian Assemblage Structure and Composition

Mammals captured in East Texas comprised 5 Orders, 8 Families, 14 Genera, and 15 species (Appendix C). The nine-banded armadillo (*Dasypus novemcinctus*) of the order Cingulata were excluded from these analyses, as they are not recognized as suitable hosts for *B. burgdorferi* or hard-bodied ticks. Between the sites sampled two meso-mammal and four small mammal species were ubiquitous, four small mammal species were absent from only one site, one species of meso-mammal and small mammal were unique to ecoregions, and species observed in only one habitat in one ecoregion included one meso-mammal and one small mammal species (Figure 2). This distribution resulted in a range of species richness across sites (S = 10-11), and varying abundances of total number of indivuduals within each site (N = 152-244). The composition and number of dominant species varied between sites (3-5 dominant species) based on localized average abundance measurements. However, Virginia opossums and cotton deermice remained a dominant component of each assemblage (Figure 2).

Values for Good's coverage, and rarefaction, indicate that the assemblages were well sampled (Table 1). Richness was similar across habitats and ecoregions, whereas total abundance varied between ecoregions and habitats. However, values for H' and PIE show moderate to high evenness of assemblages at each site (Table 1).

Bray-Curtis dissimilarity indicates that the largest assemblage difference occurs between BTNP disturbed and GEWMA sylvan, and the smallest difference is between BTPN sylvan and GEWMA sylvan (Figure 3). The presence and abundance of raccoons (*Procyon lotor)* among sites was significant in accounting for dissimilarities (P = 0.042),

and fulvous harvest mouse (*Reithrodontomys fulvescens)* approached significance (P = 0.083). PERMANOVA showed no significance in grouping of species by habitat type or ecoregion (Df = 1, F = 1.025, P = 0.667; Df = 1, F = 2.091, P = 0.333).

Tick Intensity on Individuals

Overall, 56 meso-mammals and 467 small mammals were held in wire-bottom cages for tick collection. A total of 2093 ticks were collected from 37 meso-mammals and 299 small mammals during cage check or necropsy (Table 2).

The total number of ticks collected from animals belonging to GEWMA, BTNP, disturbed and sylvan totaled 1229, 864, 1074 and 1019 respectively. One-way ANOVA detected differences in mean number of ticks per individual within habitat type at each ecoregion and among habitats and ecoregions (Df = 3, F = 3.01, P = 0.03; Table 3). A Tukey's HSD test found GEWMA sylvan habitat to have greater average tick intensity compared to BTNP sylvan (Figure 4). Two raccoons with high tick intensities were collected from GEWMA sylvan habitat (109 and 150 ticks per animal), which may have influenced this difference in means. Summary statistics for ticks collected from individuals can be found in Appendix D.

There were no differences in average tick intensity per individual across guild types, or between habitats of guild types (Guild: t = 1.22, df = 55.75, P = 0.23; MM: t = -1.40, df = 28.971, P = 0.17; SM: t = 1.57, df = 459.72, P = 0.12; Table 4; Figure 5). Variance was assumed to be unequal only for guild-to-guild comparison, and equal for within guild comparison (Levene's test: Guild: Df = 1, F = 8.70, P < 0.01; MM: Df = 1, F = 1.64, P = 0.21; SM: Df = 1, F = 2.27, P = 0.13).

Tick Intensity on Species

Tick intensity on species was found to be different at an ecoregion scale, and at habitats within ecoregions with one-way ANOVA tests (Table 5). Tukey's HSD tests discerned which species held significantly higher tick loads from each scale examined (Figures 6 & 7). Tick intensity by species can be found in Appendix F.

At BTNP average tick intensity on gray fox (*Urocyon cinereoargenteus)* and both *Peromyscus* spp was significantly greater than other species at the ecoregional scale (Figure 6). It should be noted that only one gray fox was captured at BTNP and it had moderate tick intensity (n = 15). Within BTNP at disturbed habitats, cotton deermouse (*Peromyscus gossypinus)* had higher average tick intensity than fulvous harvest mouse, and significance was approached for the cotton mouse having a higher average tick intensity than the other two dominant species in this assemblage (P = 0.09 and 0.06, for the eastern woodrat [*Neotoma floridana*] and the short-tailed shrew [*Cryptotis parva*] respectively). At sylvan habitats in BTNP, gray fox had greater average tick intensity than all other species in the assemblage. Among the abundant species represented here, the white-footed deermouse had greater average tick intensity than Virginia opossums and short-tailed shrews, and the average tick intensity on raccoons was greater than that of short-tailed shrews.

At GEWMA the average tick intensity on cotton deermice was greater than that of eastern woodrats. The average for raccoons was greater still than cotton deermice, and all dominant species in the GEWMA assemblage (Figure 7). Within GEWMA the average tick intensity in disturbed habitats was greatest on cotton deermice when compared to white-footed deermice, eastern woodrats and fulvous harvest mice. This is the only site

that the white-footed mouse is considered a dominant component of the assemblage. At sylvan habitats in GEWMA, raccoons had the greatest tick intensity when compared to the dominant species in this assemblage.

Tick Assemblages

Overall, 2093 ticks were collected from mammalian hosts with 1343 identified to species and developmental stage (Table 6). Diversity of ticks collected from mammals comprised 4 genera, including *Ixodes* ticks comprising 60% of the species collected. However, *Ixodes* ticks were not the most abundant species as individuals belonging to *Dermacentor* represented 75% of all identified tick individuals collected (Appendix E & F).

Localized diversity metrics and Bray-Curtis dissimilarity reveal uneven distributions of ticks on and between host species sampled (Table 7). Principle Coordinate Analysis suggest that host groupings are associated with greater abundances of *Dermacentor variabilis* nymphs, *I. scapularis* larvae and adults (P = 0.024, 0.077 and 0.001 respectively). Results from the PERMANOVA indicate tick assemblages vary with host guild (Df = 1, F = 2.9, P = 0.006; Figure 8). Additionally, significance for host selection was found with *I. scapularis* adults utilizing meso-mammal hosts (phi = 0.695, P = 0.006) and *D. variabilis* nymphs utilizing small mammal hosts (phi = 0.925, P = 0.006). Correlation coefficient values suggest a strong positive association of these ticks selecting specific guild hosts.

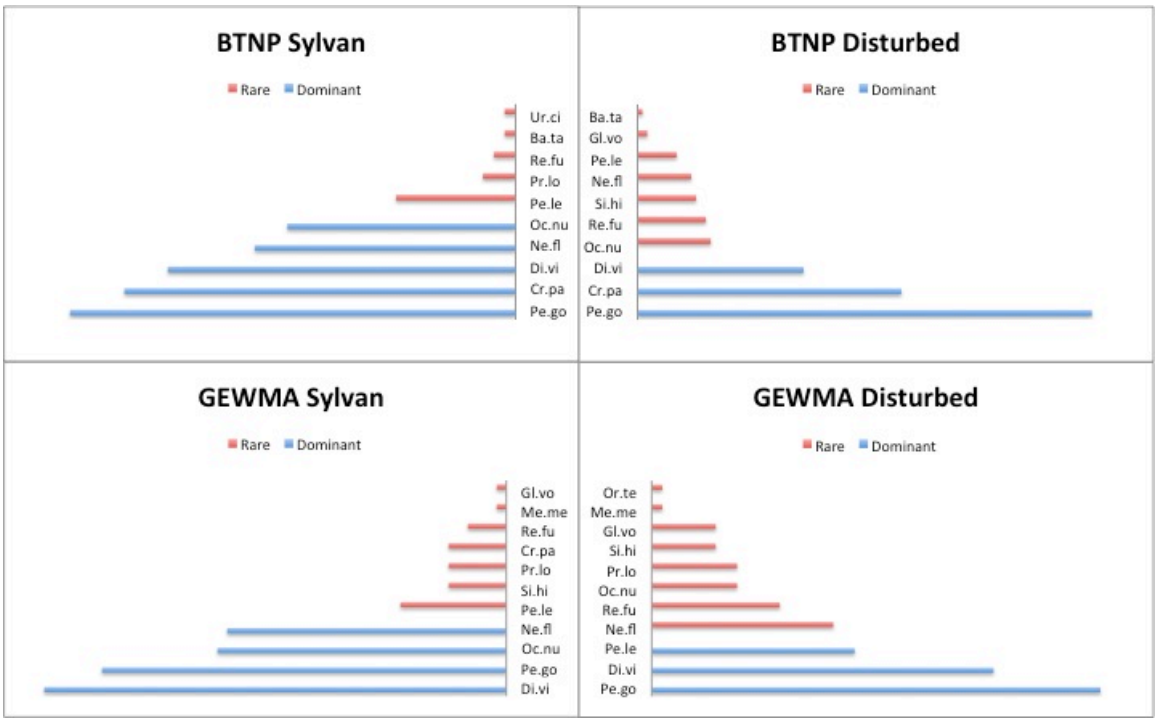

Figure 2. Rank abundance of mammal assemblages sampled. The white-footed deermouse was considered a dominant component only at GEWMA disturbed habitats, while Virginia opossums were a dominant component of all mammalian assemblages sampled.

Table 1. Mammalian assemblage metrics of alpha diversity. C – Good's Coverage, S – Richness, N – Abundance, H' – Shannon-Weiner Diversity Index, PIE – Hurlbert's Probability of Interspecies Encounters.

Site	Habitat	C	S	N	H'	PIE
BTNP	Disturbed	0.9959	10	244	1.7733	0.7736
	Sylvan	0.9884	10	172	1.8717	0.8258
GEWMA	Disturbed	0.9868	11	153	2.0200	0.8362
	Sylvan	0.9891	11	184	1.9179	0.8211

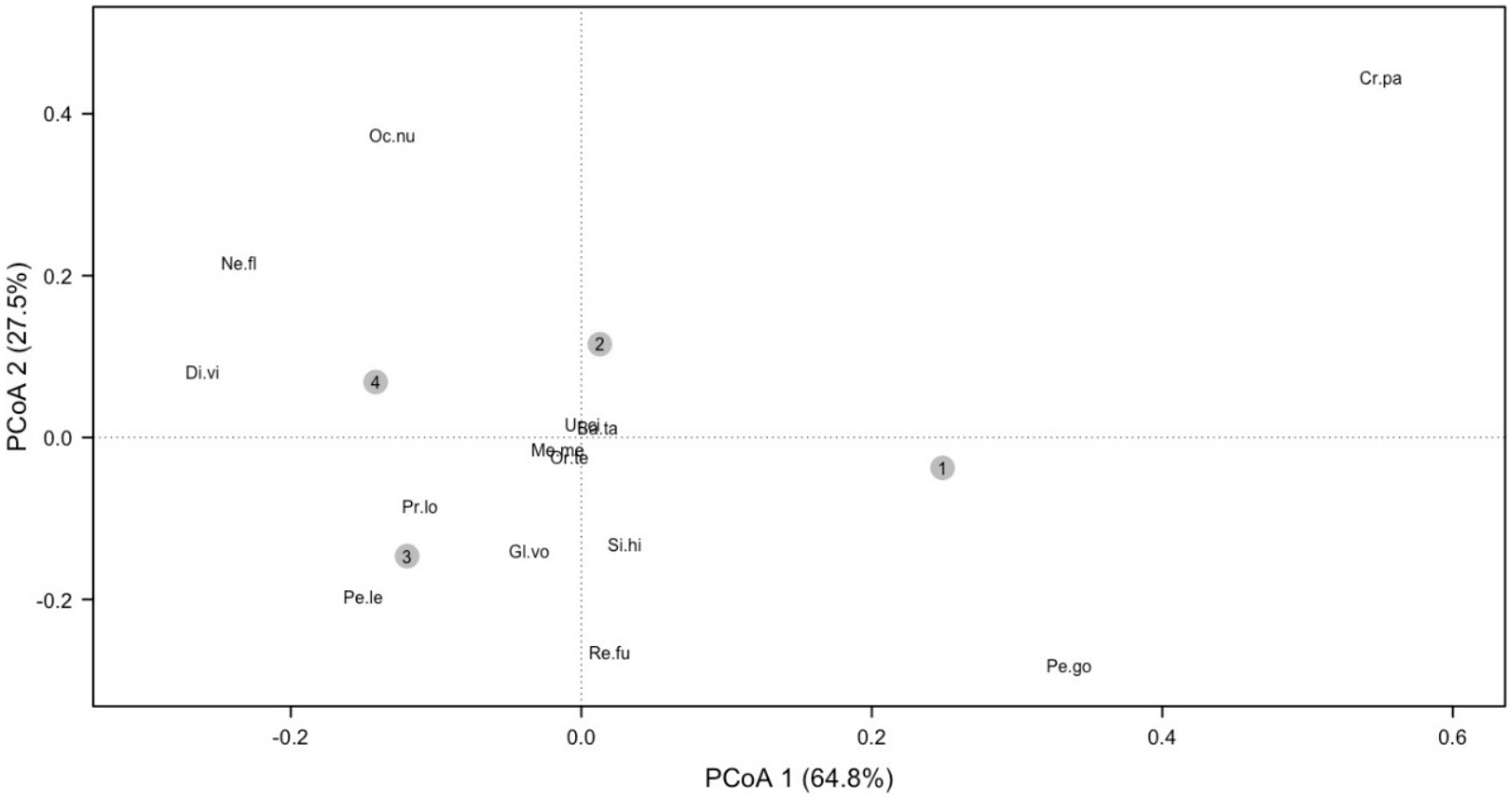

Figure 3. Principal Coordinate Analysis of East Texas mammalian assemblages. Results from Permutaional ANOVA suggest mammal species sampled have a homogenous distribution across East Texas. 1 - BTNP disturbed, 2 - BTNP sylvan, 3 - GEWMA disturbed, 4 - GEWMA sylvan.

Table 2. Summary statistics for ticks collected from mammals.

	Total Host Individuals	Host Individuals with ≥1 Tick	Total Ticks Recovered	Average Number of Ticks per Individual Host	Standard Deviation	Median	Maximum
All	527	336	2093	4.06	9.67	1	150
MM	55	37	419	7.69	24.34	1	150
SM	467	299	1674	3.64	5.8	1	31

Table 3. Individual average tick intensity by area sampled. Results from one-way ANOVA comparing individual average tick intensity by ecoregion and habitat indicate differences across East Texas.

Site	Habitat	Df	F	P
GEWMA	Sylvan			
	Disturbed	3	3.009	0.03
BTNP	Sylvan			
	Disturbed			

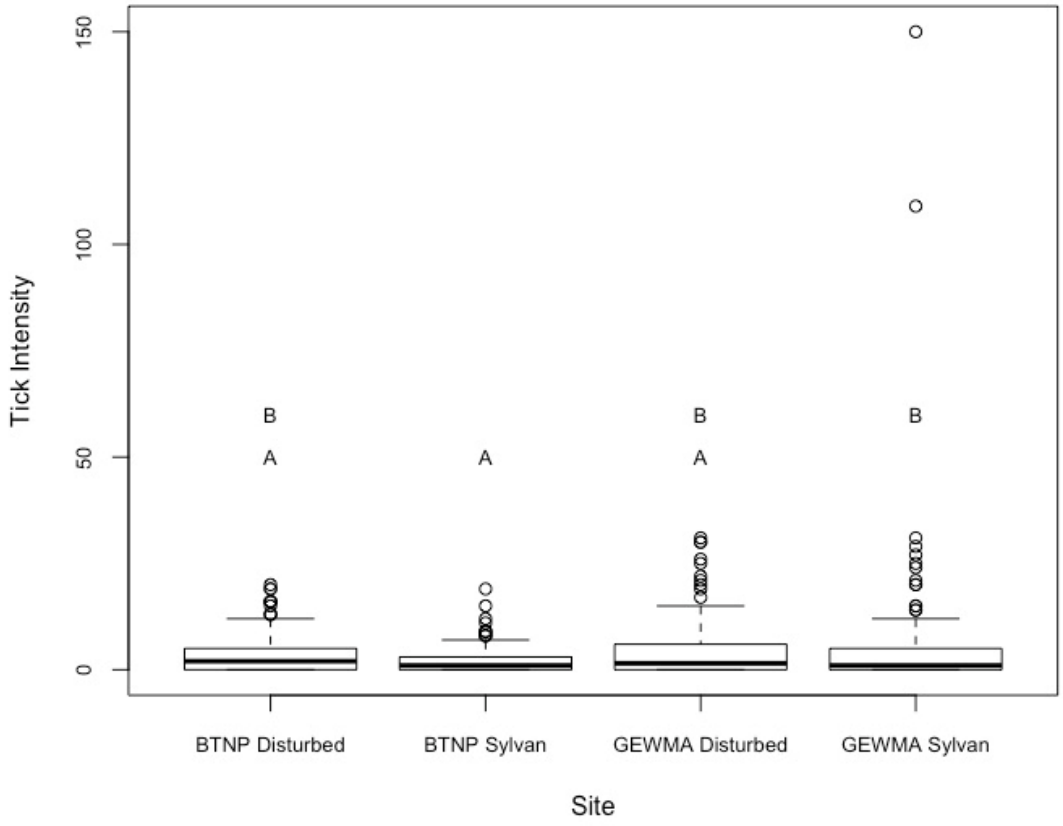

Figure 4. Average tick intensity on mammals collected. Significant difference in average tick intensity on mammals was found between GEWMA sylvan and BTNP sylvan sites.

Table 4. Tick intensity on guild members. Summary statistics for student's t-tests comparing average tick intensity between guilds, as well as within guild at disturbed and sylvan habitats.

Guild	Habitat	t	df	P
ALL		1.22	55.75	0.23
MM	Disturbed Sylvan	-1.4	28.97	0.17
SM	Disturbed Sylvan	1.57	459.72	0.12

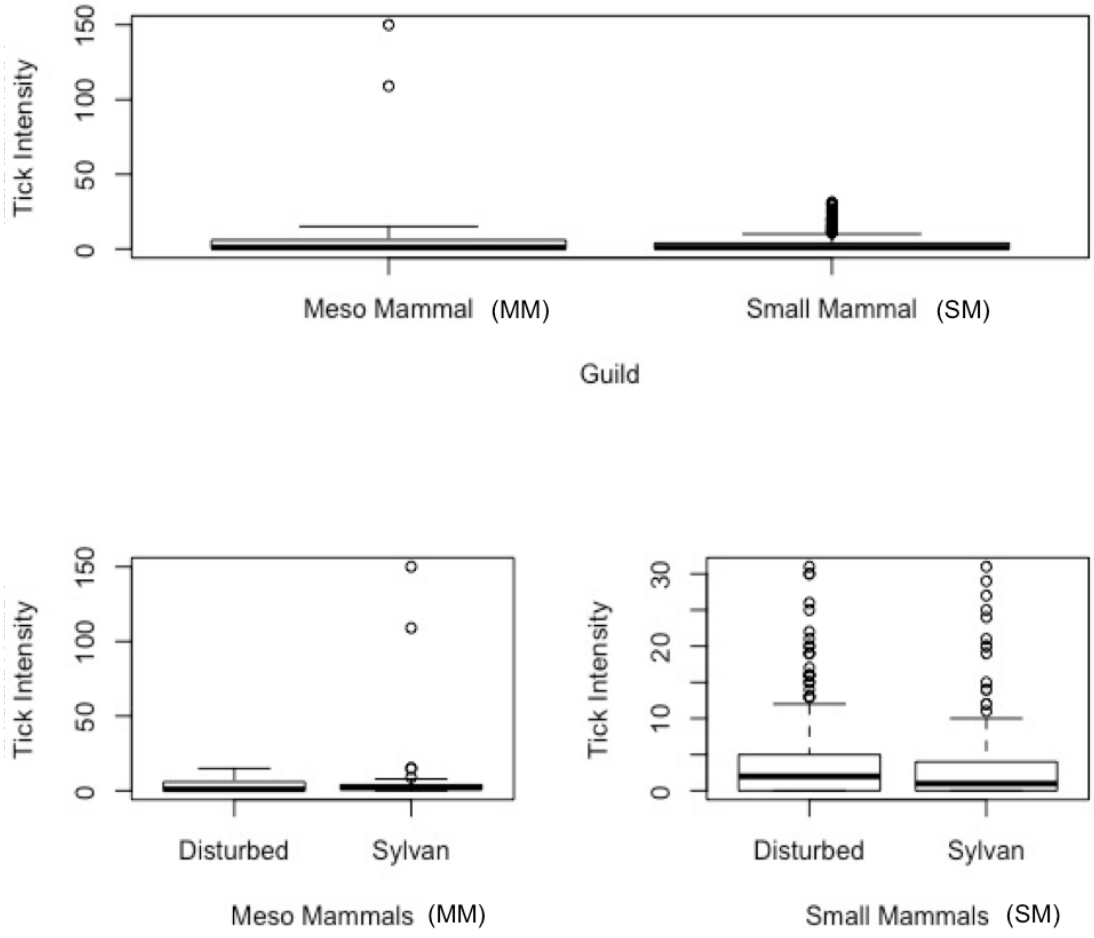

Figure 5. Average tick intensity between guild members. No significant differences in individual tick intensities were found between guild members, or within guild members captured in different habitats. This suggests that body size of host and habitat type were not deterministic of average tick intensity.

Table 5. Average tick intensity on species by area sampled. Results from one-way ANOVAs suggest average tick intensities vary by species across ecoregions and habitat disturbances.

Ecoregion	Habitat	Df	F	P
BTNP	All	11	7.41	< 0.01
	Disturbed	9	3.68	< 0.01
	Sylvan	9	8.45	< 0.01
GEWMA	All	9	5.88	< 0.01
	Disturbed	9	4.164	< 0.01
	Sylvan	7	8.87	< 0.01

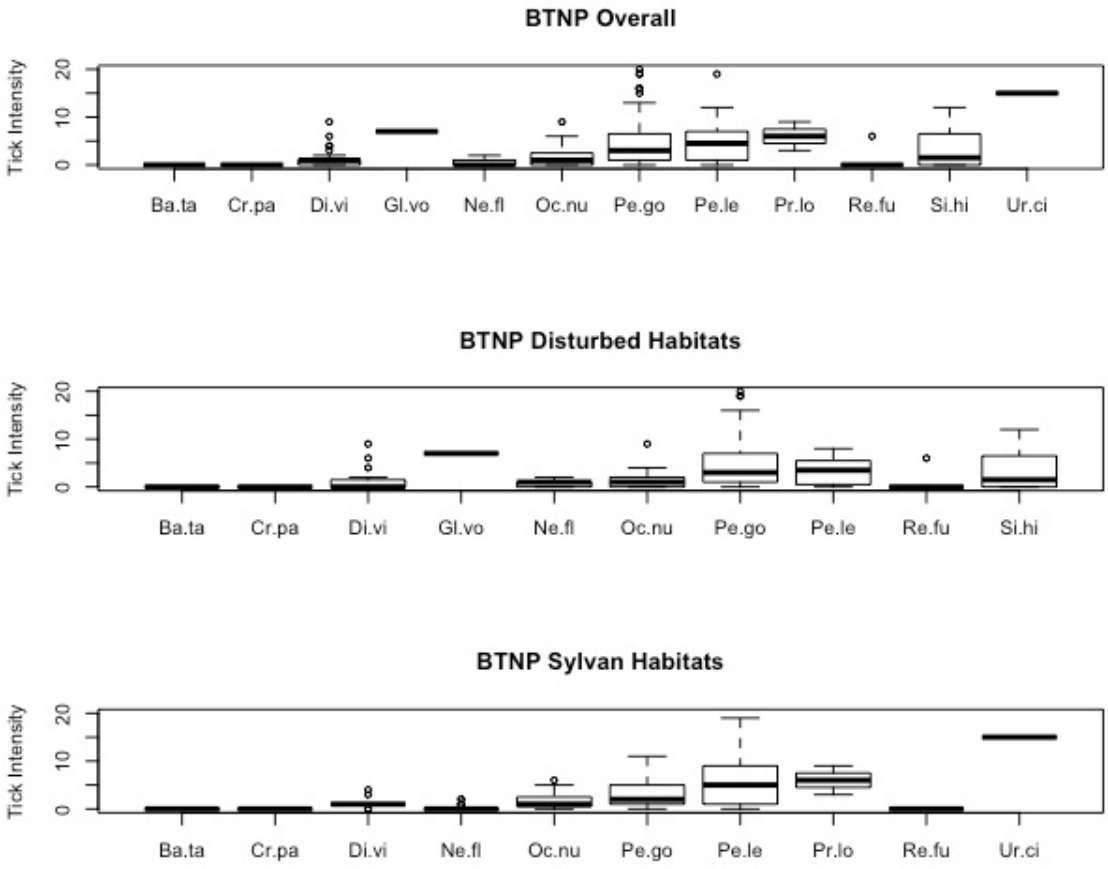

Figure 6. Average tick intensity on mammal host species at Big Thicket National Preserve. Overall select small and meso-mammal species had greater tick intensities than other species present. In disturbed habitats both *Peromyscus* species had greater tick intensities compared to other species collected, and in sylvan habitats gray fox (*Urocyon cinereoargenteus*) had greater tick intensities.

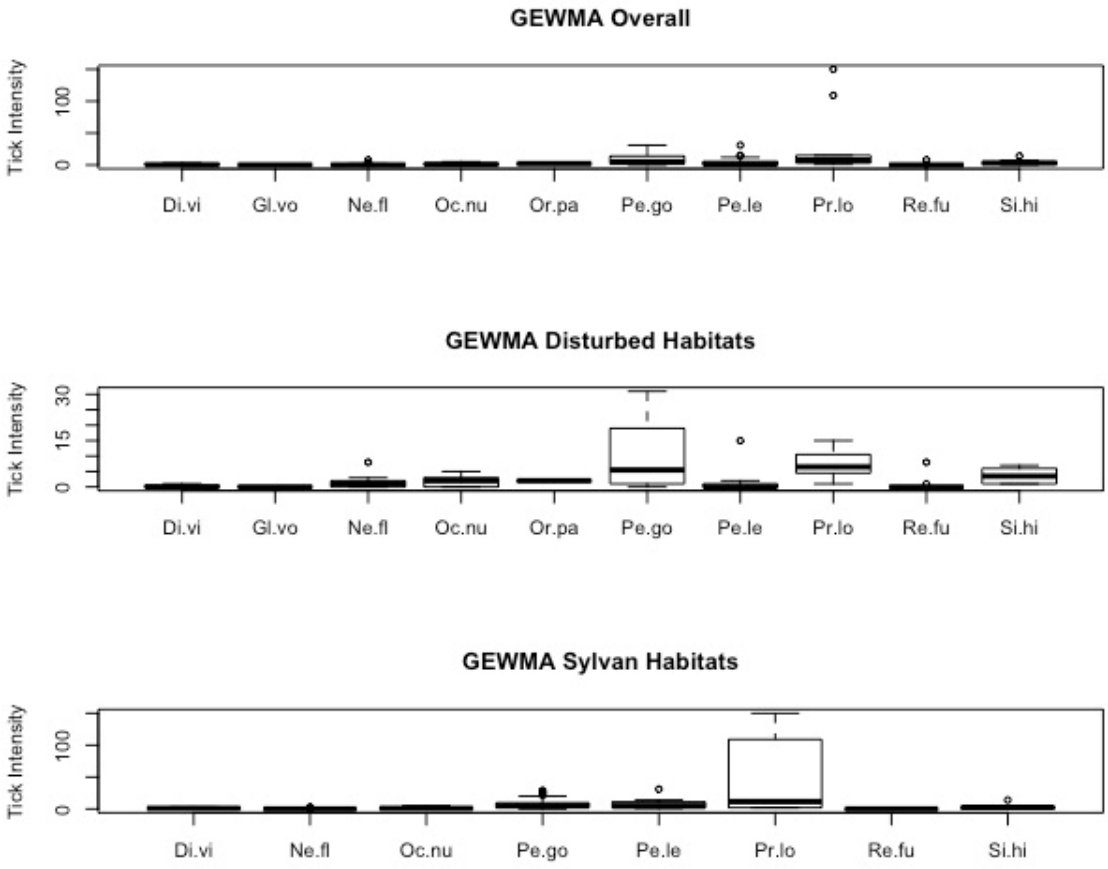

Figure 7. Average tick intensity on mammal host species at Gus Engeling Wildlife Management Area. Overall select small and meso-mammal species had greater tick intensities than other species present. In disturbed habitats cotton deermice (*Peromyscus gossypinus*) had greater tick intensities than other species sampled, and in sylvan habitats raccoons (*Procyon lotor*) had greater tick intensities.

Table 6. Abundance of tick species collected from mammalian hosts in East Texas.

Tick Genus	Host Species										
	Di.vi	**Gl.vo**	**Ne.fl**	**Oc.nu**	**Or.pa**	**Pe.go**	**Pe.le**	**Pr.lo**	**Re.fu**	**Si.hi**	**Ur.ci**
Amblyoma	-	-	1	1	-	12	1	112	6	10	5
Dermacentor	2	6	27	90	1	727	97	4	3	56	1
Ixodes	11	-	-	-	-	-	-	164	-	-	5
Ornithodorous	-	-	-	-	-	-	-	1	-	-	-
Unknown	36	-	17	37	1	511	46	74	2	22	4

Table 7. Tick assemblage metrics of alpha diversity. C – Good's Coverage, S – Richness, N – Abundance, H' – Shannon-Weiner Diversity Index, PIE – Hurlbert's Probability of Interspecies Encounters.

Host	C	S	N	H'	PIE
Di.vi	1.0000	2	49	0.4101	0.2449
Gl.vo	1.0000	1	7	0.0000	0.0000
Ne.fl	1.0000	2	44	0.6711	0.4781
Oc.nu	0.9890	2	128	0.0605	0.0217
Or.pa	0.0000	2	2	0.6931	0.5000
Pe.go	0.9974	4	1320	0.0961	0.0339
Pe.le	0.9898	2	144	0.0569	0.0202
Pr.lo	0.9866	9	355	0.9078	0.5224
Re.fu	1.0000	2	11	0.6365	0.4444
Si.hi	1.0000	2	88	0.4176	0.2509
Ur.ci	0.9167	3	14	0.9184	0.5694

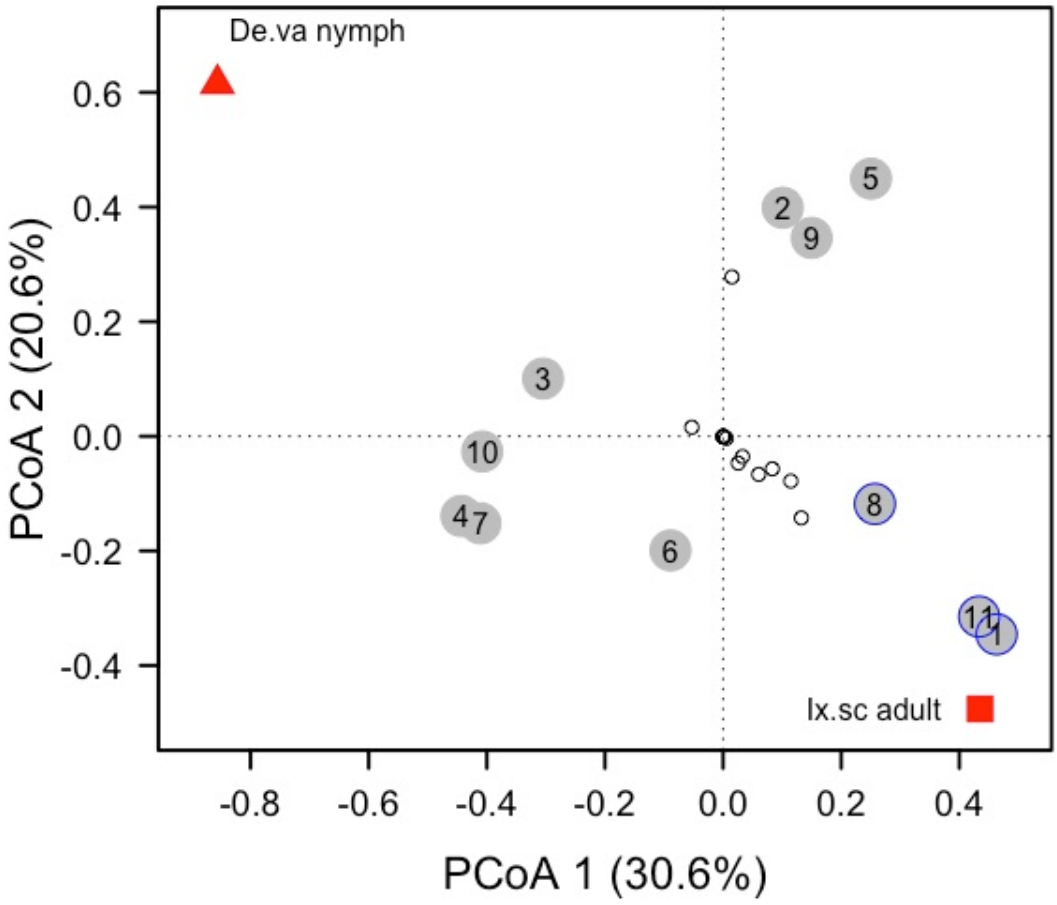

Figure 8. Principle Coordinate Analysis of East Texas tick assemblages. Tick assemblages were highly varied across all mammal host species; however, small mammal hosts (gray-filled circles) were preferred by *Dermacentor variabilis* nymphs (red triangle) and meso-mammal hosts (blue outlined circles) were preferred by *Ixodes scapularis* adults (red square). Open circles are tick species that showed no significant preference of host.

4. DISCUSSION

Contrasting expected results, anthropogenically-disturbed habitats did not consistently have lower mammalian assemblage diversity compared to sylvan habitats. However, there was turnover of rare species within ecoregions at disturbed and sylvan habitats, and across ecoregions. This suggests that anthropogenic variations in habitat quality may be more suitable for species present, in addition to limiting successful establishment of certain new species from the greater East Texas mammalian assemblage. Despite these differences, cotton deermice and Virginia opossums were a dominant component of each assemblage sampled. This is significant as *Peromyscus* species are high quality tick hosts and some are highly competent for *B. burgdorferi*, while opossums have a strong dilution effect on pathogen prevalence.

Differences in mammalian assemblages may then be influencing the abundances of ticks in the environment. At BTNP high abundance of *Peromyscus* species and short-tailed shrews were observed, while in GEWMA high abundances and greater diversities of meso-mammals were observed. Analysis of individual tick intensity across anthropogenically-disturbed habitats revealed greater numbers on individuals collected from GEWMA sylvan sites compared to BTNP sylvan. This may be due to the relative body size, and home range area, of meso-mammal compared to small mammal hosts. However, no difference in individual tick intensity was observed between mammal hosts when data was pooled. This suggests that larger body size, or area of home range, did not extrapolate to greater tick intensity. Similarly, when compared across disturbed or sylvan habitats there were no differences in individual tick intensity for meso-mammal or small mammal hosts. Overall, these results suggest that mammal exposure to ticks across

disturbance gradients in East Texas is similar, but sites have greater individual tick intensity based on abundance of mammals.

Examining tick intensity by species revealed differences at each ecoregion, and within habitats of those ecoregions. *Peromyscus* species were significant hosts for tick meals in disturbed habitats, and in sylvan habitats meso-mammal species consistently had greater tick intensities. Therefore, GEWMA sylvan habitats produced higher individual and species level tick intensity as a result of greater meso-mammal richness and abundance. However, the composition of the tick assemblage doesn't show grouping of tick species in GEWMA sylvan habitats, or within any ecoregion and habitat combination. This could be a result of the even distribution within mammalian assemblages across all sites sampled.

Across this matrix of potential mammalian hosts, differences in tick assemblages suggested a preference in host guild type among the most abundant tick genera collected (*Dermacentor* and *Ixodes*). Host preference was observed for the nymph *D. variabilis* on small mammal hosts, whereas the adult stage of *I. scapularis* preferentially selected meso-mammal hosts. Furthermore, all *Ixodes* species were collected from meso-mammal hosts. It is of note that numerous genospecies of *B. burgdorferi* can be vectored by Ixodid ticks, and the roles of these less dominant *Ixodes* species are yet unknown. Unfortunately sampling efforts were not comparable across seasons; therefore the phenology for stages of *Dermacentor* or *Ixodes* ticks, and their host-use patterns in East Texas remains unanswered. Until a more complete understanding of *Ixodes* species host-use patterns is developed for this region it may be difficult to assess the acquisition of the pathogen by these competent vectors.

Low prevalence of *B. burgdorferi* in Texas can potentially be explained by ecological factors such as competition or predation among reservoir hosts and vectors. Ubiquitous local small mammals may be outcompeting species of higher competency, such as the white-footed mouse. Furthermore, observed densities of captured omnivorous predators (e.g. Virginia opossums) were high (3 per hectare), which could result in added pressure on underperforming mammalian species and tick populations. These factors may be decreasing the availability of reservoirs with high competence from pathogen transmission events. Among vectors guild-level partitioning of hosts, and competition, could further reduce the number of reservoirs becoming infected with *B. burgdorferi*. Meso-mammals are typically poor tick hosts in addition to having a known low competency for the pathogen (Craig et al. 1996; Keesing et al. 2009), yet *Ixodes* ticks were found only on these animals. The restriction of potential host for *Ixodes* species ticks may be a result of inter-specific competition among hard-bodied ticks, as 75% of the identified tick assemblage belonged to one species, *D. variabilis*. The tick assemblage data suggests that *D. variabilis* is present on a wide range of SM hosts, with *Peromyscus* species selected for 83% of the time. Exclusion of the vector tick from potentially gaining or transmitting *B. burgdorferi* from highly competent rodent species may be an important limiting factor in the Lyme disease system in Texas, but this may not be the only obstacle for the transmission of this pathogen.

Some potential obstacles to what may be limiting this pathogen in Texas are potential hosts, vector competition, and host competency. The findings of this study are not a complete assessment of potential hosts for *I. scapularis* ticks, or reservoirs for *B. burgdorferi*. Other ground dwelling mammals known to harbor ticks were observed

during the course of sampling (eastern cottontail [*Sylvilagus floridanus*] and eastern gray squirrel [*Sciurus carolinensis*]), however the targeted methods used did not attract these species for capture. Furthermore many ground-dwelling birds known to aide in the dispersal of *I. scapularis* and *B. burgdorferi*, and reptiles, were observed at all sites in East Texas, and should be included in future studies (Hamer et al. 2012; Heylen et al. 2012; Apperson et al. 1993). The relative competence of local species (mammal, avian, and reptile) needs to be examined. This is key to understanding if potential hosts may be acting to dilute competent reservoirs, or become rescue hosts when typical reservoir abundance declines. Finally, assessing the local phenology of tick species in Texas by extending the sampling season could help elucidate host-use patterns for species and potential competition between species. Through addressing these issues in future studies a more complete idea of what may be limiting the human risk to Lyme disease in Texas will be revealed. This information could then be applied to the Lyme disease system in the northeastern US to help mitigate risk where greater densities of people are affected.

Host-use patterns of hard-bodied ticks used in conjunction with the mammalian assemblage data collected herein provides a useful tool for targeted monitoring of potential reservoirs for *B. burgdorferi* in Texas. However, on a larger scale this study provides the tools necessary to create a multi-pathogen predictive model that encompasses numerous vectors of known zoonotic agents within the ecological crossroads of Texas. A predictive model such as this is needed now more than ever, as the effects of climate change and anthropogenic-disturbance increase human exposure to potential emerging infectious diseases.

APPENDIX SECTION

Appendix A. Trapping effort was conducted from October 2015 – January 2016, and December 2016 – April 2017. Due to weather and logistics some trapping events were aborted during year 1, and in year 2 most fall sampling was shifted to the late winter period. See main text for complete explanation.

Year	Site	Habitat	Targeted Trapping	Sampling Events		Sampling Event Totals
				Fall	Winter	
1	BTNP					6
		Disturbed	MM	1	-	1
			SM	1	1	2
		Sylvan	MM	1	-	1
			SM	1	1	2
	GEWMA					6
		Disturbed	MM	1	-	1
			SM	1	-	1
		Sylvan	MM	1	1	2
			SM	1	1	2
2	BTNP					8
		Disturbed	MM	-	2	2
			SM	-	2	2
		Sylvan	MM	-	2	2
			SM	-	2	2
	GEWMA					8
		Disturbed	MM	-	2	2
			SM	-	2	2
		Sylvan	MM	1	1	2
			SM	1	1	2

Appendix B. Trapping effort and capture success across sylvan and disturbed habitats in the Piney Woods and Post Oak Savannah ecoregions.

Year	Site	Habitat	Trap Nights		Captures		Trap Success	
			MM	SM	MM	SM	MM	SM
1	BTNP	Disturbed	96	2050	14	124	14.58%	6.05%
		Sylvan	50	1319	10	82	20.00%	6.22%
	GEWMA	Disturbed	88	1281	16	19	18.18%	1.48%
		Sylvan	237	1827	33	56	13.92%	3.07%
	Total		471	6477	73	281	15.50%	4.34%
2	BTNP	Disturbed	262	2200	20	86	7.63%	3.91%
		Sylvan	219	1671	26	54	11.87%	3.23%
	GEWMA	Disturbed	275	2106	25	93	9.10%	4.42%
		Sylvan	237	2236	22	73	9.28%	3.27%
	Total		993	8213	93	306	9.37%	3.73%
	Grand Total		1464	14690	166	587	11.33%	3.99%

Appendix C. Mammalian species captured across East Texas, including capture abundances and subset held for tick collection.

Order Family Genus Species	Species Code	Guild	Number Captured	Held for Tick
Carnivora				
Canidae				
Urocyon cinereoargenteus	Ur.ci	MM	1	1
Mehpitidae				
Mephitis mephitis **	Me.me	MM	2	-
Procyonidae				
Procyon lotor	Pr.lo	MM	17	17
Cingulata				
Dasypodidae				
Dasypus novemcinctus *	Da.no	-	1	-
Didelphimorphia				
Didelphidae				
Didelphis virginiana	Di.vi	MM	146	38
Eulipotyphla				
Soricidae				
Cryptotis parva	Cr.pa	SM	96	14
Rodentia				
Cricetidae				
Baiomys taylori	Ba.ta	SM	2	2
Neotoma floridana	Ne.fl	SM	82	81
Ochrotomys nuttalii	Oc.nu	SM	74	70
Oryzomys paulustris	Or.pa	SM	1	1
Peromyscus gossypinus	Pe.go	SM	218	204
Peromyscus leucopus	Pe.le	SM	50	42
Reithrodontomys fulvescens	Re.fu	SM	32	31
Sigmodon hispidus	Si.hi	SM	24	24
Sciuridae				
Glaucomys volans	Gl.vo	SM	9	2

*species is not a typical tick host or host for the *Borrelia burgdorferi* pathogen

**species not held for tick collection

Appendix D. Summary statistics for ticks collected from all mammal species included in tick intensity evaluations.

Tick Hosts Examined		Ticks Collected				
Species	**(n)**	**Average**	**Sd**	**Median**	**Range**	**Total**
Pe.go	204	6.02	7.10	3	0-31	1250
Po.lo	17	21.35	41.56	7	0-150	355
Pe.le	42	4.60	6.38	2	0-31	144
Oc.nu	70	1.87	2.96	1	0-21	128
Si.hi	24	3.63	3.99	2.5	0-14	88
Ne.fl	81	0.63	1.19	0	0-8	45
Di.vi	38	1.24	1.91	1	0-9	49
Ur.ci	1	15.00	N/A	15	0-15	15
Gl.vo	2	3.50	4.95	3.5	0-7	6
Or.pa	1	2.00	N/A	2	0-2	2
Cr.pa	14	0.00	0.00	0	0	0

Appendix E. Tick species collected from mammals, identified, and utilized in study. 750 ticks were excluded from analysis as they were not yet identified to species or developmental stage.

Order				
	Family			
		Genus **Species**	**Species code**	**Identified**
Ixodida				
	Ixodidae			
		Amblyoma americanum	Am.am	6
		Amblyoma inornatum	Am.in	1
		Amblyoma maculatum	Am.ma	140
		Dermacentor variabilis	De.va	1014
		Ixodes affinis	Ix.af	1
		Ixodes cookei	Ix.co	1
		Ixodes kingii	Ix.ki	2
		Ixodes scapularis	Ix.sc	20
		Ixodes texicanus	Ix.te	157
	Argasidae			
		Ornithodorous turicata	Or.tu	1

Appendix F. Ticks collected from mammals in East Texas, totals provided for tick species and overall tick abundance on host species.

Tick		Host Species										
Specie	**Stage**	**Di.v**	**Gl.v**	**Ne.f**	**Oc.n**	**Or.p**	**Pe.g**	**Pe.l**	**Pr.l**	**Re.f**	**Si.h**	**Ur.c**
Am.am	Nymp	-	-	-	-	-	-	-	-	-	-	1
	Adult	-	-	-	-	-	-	-	-	1	-	4
Am.in	Nymp	-	-	-	-	-	1	-	-	-	-	-
Am.ma	Larvae	-	-	-	-	-	-	-	109	-	-	-
	Nymp	-	-	1	1	-	10	1	1	6	10	-
	Adult	-	-	-	-	-	1	-	-	-	-	-
De.va	Larvae	2	-	-	-	-	2	14	2	1	-	-
	Nymp	-	5	26	80	1	700	81	1	2	48	-
	Adult	-	1	1	10	-	25	2	1	-	8	1
Ix.af	Nymp	-	-	-	-	-	-	-	1	-	-	-
Ix.co	Nymp	-	-	-	-	-	-	-	1	-	-	-
Ix.ki	Nymp	-	-	-	-	-	-	-	2	-	-	-
Ix.sc	Larvae	2	-	-	-	-	-	-	1	-	-	-
	Adult	9	-	-	-	-	-	-	3	-	-	5
Ix.te	Nymp	-	-	-	-	-	-	-	150	-	-	-
	Adult	-	-	-	-	-	-	-	7	-	-	-
Or.tu	Nymp	-	-	-	-	-	1	-	1	-	-	-

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