

THE ROLE OF BIRDS AS HOSTS FOR TICKS, VECTORS OF *BORRELIA*
BURGDORFERI, IN EASTERN TEXAS

By

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LIST OF ABBREVIATIONS

Abbreviation	Description
CDC	Centers for Disease Control
US	United States
GEWMA.....	Gus Engeling Wildlife Management Area
BTNP	Big Thicket National Preserve
N.....	Abundance
S	Richness
H'	Shannon-Weiner Diversity Index
PIE.....	Hurlbert's Probability of Interspecific Encounters
BPDI	Berger-Parker Dominance Index
C.....	Good's Coverage
PCoA.....	Principle Coordinate Analysis
IP	Infestation Probability
ISI.....	Index of Species Importance

ABSTRACT

The bacterial spirochete *Borrelia burgdorferi*, the etiological agent of Lyme disease, an emerging infectious disease in the United States [US], has been detected in previous surveys in Texas. However, the northeastern and midwestern US are currently the areas with the highest abundance of cases of Lyme disease reported. Ticks of the genus *Ixodes* that are infected with *Borrelia* are solely responsible for transmission to humans, though humans are not components of the enzootic life cycle of *Borrelia*. The distribution of the black-legged tick, *Ixodes scapularis*, the primary vector of *Borrelia* to humans in the US includes most of the eastern portion of Texas. While adult *I. scapularis* ticks feed primarily on large mammals (e.g. deer), the larval and nymphal stages are generalists that will feed on numerous taxa, including small mammals, birds, and reptiles. This project, along with associated tick flagging, mammal, and reptile surveys in eastern Texas were implemented to ascertain how the *Borrelia* life cycle is structured at the local level in eastern Texas. The focus of my research was on the role that ground-dwelling and foraging birds had on the abundance and distribution of ticks in eastern Texas. The study sites chosen for this project were disturbed and more pristine sylvan habitats at Gus Engeling Wildlife Management Area [GEWMA] and Big Thicket National Preserve [BTNP]. A reference infestation rate of 5.1% was chosen to compare the observed infestation rate. In total, 211 birds were captured over two field seasons, 4 of which were found infested with *Ixodes* species ticks (1.9%). This suggests that birds are not an important vertebrate host group for maintaining tick density at the local level in eastern Texas. Several migratory bird species that have been found in other studies to be important in translocating ticks were captured at each site. A comparison of the alpha and beta diversity at and among each site revealed that the habitats at GEWMA were most similar in composition, though the disturbed habitat at BTNP

was the most diverse ($H' = 2.4840$). Ground-dwelling and foraging birds known to harbor *I. scapularis* were dominant species components of the avian assemblages at both GEWMA and BTNP. More research is needed to uncover which vertebrate host species are supporting the larval and nymphal stages of the *I. scapularis* population in eastern Texas.

I. INTRODUCTION

Lyme disease, or Lyme borreliosis, is an emerging infectious disease named for Lyme, Connecticut, where it was first identified in 1976 (Steere 1989). Lyme disease is now the most commonly reported arthropod-borne illness in the United States and Europe (Steere et al. 2004). Between 1992 and 2006, the number of cases annually reported to the CDC increased 101% (Bacon et al. 2008). And while there are roughly 30,000 reported cases of Lyme disease annually (CDC 2017C), due to misdiagnosis and underreporting the estimated annual number of Lyme disease infections in the United States is likely closer to 300,000 (Hinckley et al. 2014, Nelson et al. 2015). The emergence of Lyme disease is partly explained by the reforestation of the northeastern US and the rapid increase in deer population there, though it has likely persisted there for millennia (Barbour and Fish 1993). While the northeastern and north central US are currently the areas where Lyme disease is most prevalent (appendix A), *B. burgdorferi* has been found as far south as the Texas-Mexico transboundary region (Feria-Arroyo et al. 2014). However, comparatively few cases of Lyme disease are reported annually in Texas (CDC 2017a).

Though Lyme disease receives much notoriety from the media and healthcare industries for its high risk and rate of contraction, the disease exhibits low mortality versus other zoonotic diseases, like Hantavirus and tick-borne encephalitis which have much higher mortality rates (Bordes et al. 2015). However, if left untreated, Lyme disease can be debilitating as the disease often affects multiple body systems. The symptoms of Lyme disease can vary but typically start with a rash, erythema migrans, and can also include cardiac and neurologic symptoms, however arthritis is more

common (Borchers et al. 2015). Approximately 20% of infected humans develop no symptoms after the erythema migrans subsides (Borchers et al. 2015) and infections can even be wholly asymptomatic (Steere et al. 2003). Even after Lyme disease is treated some patients may experience chronic symptoms, however “chronic Lyme disease” is not currently a recognized disorder and is a topic of debate (Feder, Jr. et al. 2007).

The causative agents of Lyme disease are spirochetal bacteria of the genus *Borrelia* which are transmitted via parasitic feeding of ticks on vertebrate hosts, including humans (Steere 1989). However, humans are incidental hosts and not involved in the life cycle of *Borrelia*. There are at least 12 closely related species of *Borrelia* that can potentially cause Lyme disease in humans (Steere et al. 2004). Though, the 3 most likely are *B. burgdorferi*, *B. afzelii*, and *B. garinii*, with the last two being the primary cause of Lyme disease in Europe and the sole cause in Asia (Tilly et al. 2008). In the United States, *B. burgdorferi* is the primary species of concern (Steere et al. 2004). However, a new species of pathogenic *Borrelia*, *Borrelia mayonii*, was recently discovered in the upper midwestern United States (Pritt et al. 2016). Also, a potentially pathogenic strain that was recently characterized, *Borrelia lanei*, was just discovered in California (Margos et al. 2017).

The life cycle of *Borrelia* is intimately tied to the vertebrate hosts and parasitic ticks which perpetuate its presence in the environment. *Borrelia* is dependent on horizontal transmission through larval and nymphal ticks feeding on competent species (Steere et al. 2004). While several species of ticks are involved in the enzootic cycles required for the persistence of *Borrelia*, only a few species of the genus *Ixodes* are responsible for the transmission of *Borrelia* from infected vertebrate hosts to humans

(Steere et al. 2004, Tilly et al. 2008). In the United States, the most important tick vectors for transmitting *Borrelia* to humans are the blacklegged deer tick (*Ixodes scapularis*) in the northeastern and north central US and the sheep tick (*Ixodes pacificus*) in the western US (Steere et al. 2004). *I. scapularis* can also be found in the southern US from Florida to Texas (Appendix B). Additionally, *I. ricinus*, in Europe, and *I. persulcatus*, in Asia, also transmit *Borrelia* to humans. Many species of mammals, birds, and reptiles are important hosts for *Ixodes* species ticks. The life stage of the tick species is an important factor in its selection of a host, where larval and nymphal ticks often quest for small mammals, birds, and reptiles, the adult ticks more frequently parasitize larger mammals (Anderson 1989). The white-footed mouse (*Peromyscus leucopus*) is often cited as a primary reservoir host for *Borrelia* in the United States (Anderson 1989, Hamer et al. 2011, Levi et al. 2016), though eastern chipmunk (*Tamias striatus*), masked shrew (*Sorex cinereus*), and short-tailed shrew (*Blarina brevicauda*) are also competent hosts (Levi et al. 2016). Mammal assemblages, and birds to a lesser effect, can significantly dilute or amplify the density of *Borrelia* infected ticks based on the composition of those assemblages (LoGiudice et al. 2003, Levi et al. 2016).

Other tick and host systems may act as reservoirs and translocators for *Borrelia*. For instance, while *I. scapularis* and *I. pacificus* are the primary vectors for *B. burgdorferi* in the United States, *I. affinis* is also a competent vector that parasitizes birds and is thought to be an important factor in the persistence of *B. burgdorferi* in the southeastern United States (Heller et al. 2015). Another example is *I. dentatus*, which almost exclusively feeds on birds and rabbits (Hamer et al. 2012) and has tested positive for some strains of *B. burgdorferi* (Anderson et al. 1996, Oliver et al. 1996).

Birds are often overlooked as important components in the life cycles of tick-borne pathogens, even though they account for approximately 50% of animals that host ticks and tick-borne pathogens (de la Fuente et al. 2015). In a recent literature review, 11 avian tick pathogen studies in North America were examined to find an average tick infestation rate of 5.1% of birds (Loss et al. 2016). *I. scapularis* is known to parasitize at least 71 species of North American birds and almost 60% of bird species evaluated are competent reservoirs for *B. burgdorferi* (Brinkerhoff et al. 2011a). While adult *I. scapularis* feed primarily on larger mammals, the larval and nymphal ticks are host generalists and will feed on dozens of vertebrate taxa including birds (Keirans et al. 1996, LoGiudice et al. 2003). Also, ground-foraging birds have been shown to have a greater reservoir competence for *B. burgdorferi* than striped skunk (*Mephitis mephitis*), raccoon (*Procyon lotor*), and Virginia opossum (*Didelphis virginiana*) (Levi et al. 2016). Birds migrating from the tropics into Texas are possibly transporting 4 to 39 million exotic neotropical ticks into the US annually (Cohen et al. 2015). Birds caught during the spring of 2013 and 2014 at Mad Island Marsh Preserve in Matagorda county, Texas, were infested with ticks at a rate of 3.56% (n=85) of 3,844 (Cohen et al. 2015). Thus, bird migration is potentially a mechanism for long-distance dispersal of *I. scapularis* and the introduction of *Borrelia* to novel locations (Smith et al. 1996).

Anthropogenic changes in habitat due to large-scale land use modification and climate change, and small-scale biotic processes such as competition, predation, mutualism, and parasitism are important processes that drive disease distributions (Cohen et al. 2016). Climate change alone threatens to expand the range of *I. scapularis* in Canada and USA by 218% by the 2080's (Brownstein et al. 2005). The summer ranges of

many bird species that migrate to the southern US, including Texas, might then overlap more extensively with this expanded range of *I. scapularis* in Canada and northern US where *B. burgdorferi* is of high prevalence (Scott et al. 2001, Ogden et al. 2008). The concern here is that birds will increasingly be migrating from areas of high prevalence of *B. burgdorferi* to areas where it currently has low prevalence, like Texas. In fact, avian migration may be the key force driving the expansion of *Borrelia* into Canada and the southern United States and Mexico (Brinkerhoff et al. 2011b). Alarming, birds may be able to carry *Borrelia* as a latent infection for several months, only to have it reactivated and passed as a consequence of migratory restlessness (Gylfe et al. 2000, Altizer et al. 2011). And even in the apparent absence of *I. scapularis*, other bird-associated ticks may be able to maintain *Borrelia* in the environment (Hamer et al. 2011). Passerine birds may fly as much as 950 km/day during migration, and nymph ticks may remain attached to birds for 2-4 days, giving a potential long distance dispersal range of 1900-3800 kilometers, well within the range of birds flying from the northern US and Canada to Texas (Smith et al. 1996, Brinkerhoff et al. 2011a).

Borrelia burgdorferi, the etiologic agent for Lyme disease, and its primary tick vector, *Ixodes scapularis*, have previously been detected in Texas in several studies (Teltow et al. 1991, Rawlings and Teltow 1994, Feria-Arroyo et al. 2014, Szonyi et al. 2015, Adetunji et al. 2016). While most cases of Lyme disease in Texas that are reported to CDC are from central Texas counties (Appendix C), the area thought to provide the best habitat for *I. scapularis* is east Texas (Szonyi et al. 2015). However, very few tick studies have focused on avian hosts with regards to maintaining *Borrelia* populations in Texas.

The goals of this project were to determine the differences in tick infestation rates of ground-dwelling and foraging birds in eastern Texas compared to other studies, between anthropogenically disturbed and more pristine sylvan habitat types sampled, and when comparing migratory or resident species status. For this project, migratory birds are defined as those that winter in east Texas. I hypothesized that my study sites would have an infestation rate equal to that found by Loss et al. 2016, the birds sampled at disturbed habitats would have a higher infestation rate compared to those from sylvan habitats, and the migratory species captured would have a higher infestation rate compared to the resident species captured. In addition, I compared the α and β diversity of avian assemblages of the study sites to assess their similarity in composition and to determine the dominance of species that have been previously identified as important hosts for *Borrelia*-infected ticks.

II. Methods

Study Area

Gus Engeling Wildlife Management Area (GEWMA) in the Oak Woods and Prairies ecoregion and the Big Thicket National Preserve (BTNP) in the Piney Woods ecoregion in Texas (Appendix E) were selected based on their habitat suitability and previous detection of *Ixodes scapularis* infected with *Borrelia burgdorferi* (Feria-Arroyo et al. 2014). Sampling sites were chosen in coordination with mammal and reptile trapping locations for related concurrent projects. Prior to any vertebrate sampling, the sites were flagged for ticks by collaborators from Texas A&M University to locate areas

where *I. scapularis* was present. The sites were selected in both anthropogenically-disturbed and more pristine habitats.

During the fall and winter seasons of 2015-2017 (season 1: Oct. 2015 – Feb. 2016; season 2: Dec. 2016 – Feb. 2017), I sampled birds, targeting species that primarily forage on the ground where they are most likely to encounter questing ticks. Mist netting for birds was conducted at both GEWMA and BTNP in disturbed and more pristine sylvan habitats that were within 1 km of sites selected for small-mammal, meso-mammal, and herpetofauna trapping in related vertebrate sampling concurrent projects done by other teams. At each site, between 3 – 5 mist nets (6m and 12m x 2.6m) were deployed, depending on site logistics, along densely -forested edge habitats and the forest interior. Sampling effort (mist net m²-hours) was kept as uniform as possible between habitat types. Mist nets were opened at sunrise for 3 hours and again for 2 hours before sunset. This effort was repeated for a total of 12 days at each habitat type at GEWMA and BTNP for a total of 48 days. A FOXPRO Inferno predator caller (FOXPRO -Lewistown, PA) was used with sound files obtained from the Cornell Lab of Ornithology to attract ground-foraging birds present in east Texas. Birds caught in the nets were removed, identified to species, weighed, wing and tarsus lengths were recorded, and finally the birds were inspected for ectoparasites using illuminated head magnifier goggles and then released. Inspection of birds was focused on the nape, ear canals and coverts, rictus, and around the eyes (Schneider et al. 2015). Any ticks found on the captured birds were removed, without host injury and while attempting to remove whole, undamaged ticks, and sent to Texas A&M University for species and life stage identification.

To test the statistical significance of the infestation rates (estimated vs sampled, resident species vs migratory species, sylvan vs disturbed habitats) I used two-sample t-tests. The reference estimated infestation rate was taken from a recent literature review paper (Loss et al. 2016), where 1,981 out of 38,929 (5.1%) birds were infested.

To examine the biodiversity of ground-dwelling and foraging birds in both disturbed and sylvan habitats sampled at GEWMA and BTNP, the following α -diversity indices were calculated: abundance (N), richness (S), Shannon-Wiener diversity index (H'), Hulbert's probability of interspecies encounters (PIE), and Berger-Parker dominance index (BPDI, Berger and Parker 1970, Hurlbert 1971). The rank abundance (using N/S) of the avian assemblages at each habitat sampled was calculated to determine the dominant and rare species. Good's coverage ($C = 0-1$) estimates were calculated for each habitat to determine how well rare species are represented in the samples. To measure the diversity among the sampled habitats (β -diversity), R with "Vegan" and "BiodiversityR" packages was used to perform the following analyses: Bray-Curtis dissimilarity, hierarchical clustering, and a principle coordinate analysis (PCoA) (Bray and Curtis 1957, R Core Team 2017). The Bray-Curtis dissimilarity analysis was used to quantify the dissimilarity between the sites. The hierarchical clustering analysis and PCoA were used to further visualize this dissimilarity.

III. RESULTS

The project resulted in 211 total birds captured at the disturbed and sylvan habitats in GEWMA and BTNP (Figure 1 and Figure 2). There were a total of 24 species

caught, representing 13 families of birds (Table 1). The two field seasons included 27,955.2 mist net/m² hours of trapping effort (Table 2), resulting in a capture rate of 0.75% per mist net/m² hours. Most of the captured birds represented migratory species in Texas (Figure 3). The avian assemblage structure and composition (α -diversity) of the sampled sites was similar in all habitats except the sylvan habitat at BTNP (Table 3), which had lower H', PIE, and BPD values, indicating less diversity than the other habitats. However, the Good's coverage estimate for each habitat suggests all habitats were well sampled.

Four birds were found infested with ticks, for a total infestation rate of 1.9%. All four infested birds were captured in a disturbed habitat at GEWMA, which results in a 3.4% disturbed habitat infestation rate. Two of the birds harboring ticks were a migratory species (white-throated sparrow; *Zonotrichia albicollis*), resulting in a 1.5% migratory infestation rate. The other two infested birds represented two Texas resident species (American robin; *Turdus migratorius* and northern cardinal; *Cardinalis cardinalis*) for a resident infestation rate of 2.6%.

Five total ticks were found and removed from four birds (Table 4). Three of the ticks were *Ixodes dentatus* nymphs and one was an *Ixodes scapularis* nymph. The remaining tick was too degraded to be identified. All ticks were found during the second field season (Winter 2016-2017). A single hermit thrush (*Catharus guttatus*) was captured in a sylvan habitat site in BTNP that appeared to have an embedded tick (Figure 4). However, the possible tick was destroyed during the attempted removal and was therefore not counted.

The reference estimated infestation rate (5.1%) was significantly different than the observed infestation rate (1.9%; $t = 2.108$, $p = 0.035$). The two-sample t-test comparing the disturbed and sylvan habitats showed no statistically significant difference in the infestation rates ($t = 1.81$, $p = 0.0703$). And finally, the two-sample t-test of the migratory and resident infestation rates showed no statistically significant difference between the samples ($t = -0.588$, $p = 0.5565$).

The β -diversity metrics indicated that the disturbed and sylvan sites sampled at GEWMA were most similar based on avian assemblage composition (Figure 5). The number of hermit thrushes (HETH) captured at the BTNP sylvan habitat ($N = 17$) was the reason for that habitat to be more closely related in composition to both habitats at GEWMA than the disturbed habitat at BTNP (Figure 6). The white-throated sparrows (WTSP) were roughly 12% of total captures, and only captured at GEWMA, which significantly influenced the similarity of those avian assemblages (Figure 7).

Figure 1. Total number of birds captured during project. Totals from both disturbed and sylvan habitats in GEWMA and BTNP.

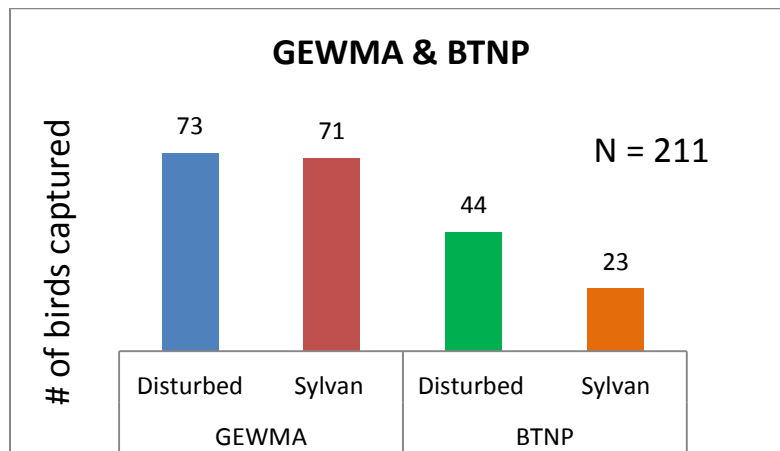


Figure 2. Rank abundance of avian assemblages sampled. Species are defined as rare (red) or dominant (blue) based on abundance/richness (N/S) at sampled habitat. The hermit thrush (HETH), a target ground-dwelling bird, was the dominant species captured at each habitat except BTNP-Disturbed. See Appendix E for index of banding codes.

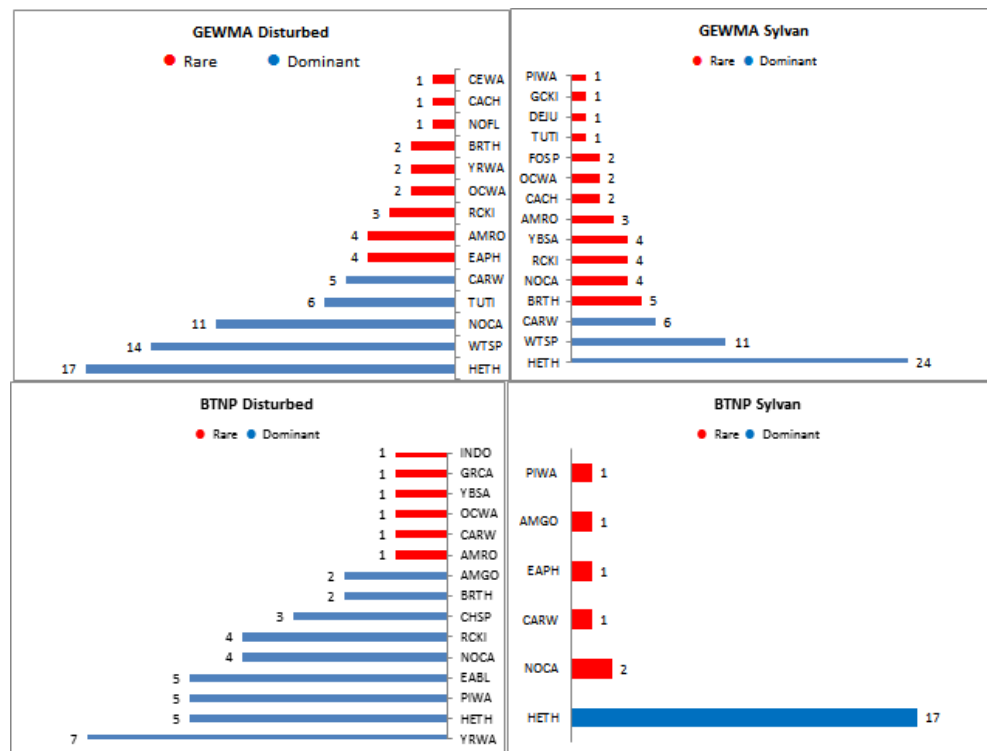


Table 1. Number of bird species captured listed by avian family. There were a total of 24 unique species caught during the project.

	# Birds Captured (species)			
	BTNP	BTNP	GEWMA	GEWMA
Avian Family	Disturbed	Sylvan	Disturbed	Sylvan
Picidae	1	0	1	1
Tyrannidae	0	1	1	0
Troglodytidae	1	1	1	1
Mimidae	2	0	1	1
Cardinalidae	1	1	1	1
Turdidae	2	1	2	2
Paridae	0	0	2	2
Regulidae	2	0	1	2
Parulidae	3	1	2	2
Emberizidae	1	0	1	3
Fringillidae	1	1	0	0
Columbidae	1	0	0	0
Bombycillidae	0	0	1	0
Total	15	6	14	15

Table 2. Sampling effort in both disturbed and sylvan habitats.

Season 1	BTNP Disturbed	BTNP Sylvan	GEWMA Disturbed	GEWMA Sylvan	Total
Mist Net m ² hours	3,744	3,510	3,276	2,979.6	13,509.6
Season 2	BTNP Disturbed	BTNP Sylvan	GEWMA Disturbed	GEWMA Sylvan	Total
Mist Net m ² hours	3,744	3,744	3,681.6	3,276	14,445.6
Sum					27,955.2

Figure 3. Number of migratory and Texas resident birds captured. Over-wintering migratory (N_m) and Texas winter resident (N_r) birds captured at GEWMA and BTNP over both field seasons.

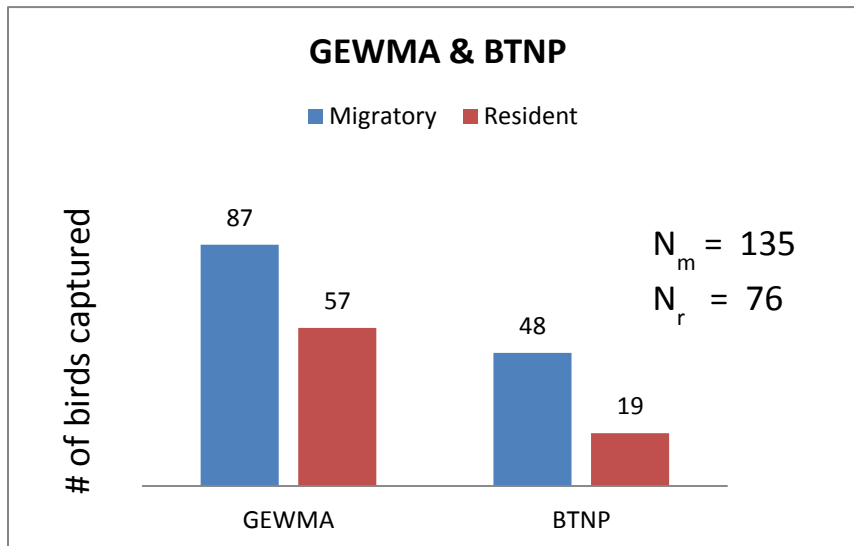


Figure 4. Hermit thrush (*Catharus guttatus*) captured with possible embedded tick. BTNP sylvan site. Possible tick shown in red circle.



Table 3. Avian assemblage metrics of α -diversity. N – Abundance, S – Richness, H' – Shannon-Weiner Diversity Index, PIE – Hurlbert's Probability of Interspecific Encounters, C – Good's Coverage, BPD I – Berger-Parker Dominance Index.

Site	Habitat	N	S	H'	PIE	C	BPD I
GEWMA	Disturbed	73	14	2.2517	0.8763	0.9286	4.2941
	Sylvan	71	15	2.2128	0.8471	0.9333	2.9583
BTNP	Disturbed	43	15	2.4840	0.9247	0.9333	6.1429
	Sylvan	23	6	0.9811	0.4585	0.8333	1.3529

Table 4. Ticks collected from captured birds. Recovered ticks were identified to species and life stage.

Bird/Tick ID#	Tick Species	Tick Stage	Location	Habitat	Bird Species	Bird ID#	Date Collected
B1T1	<i>Ixodes dentatus</i>	Nymph	Gus Engeling WMA	Disturbed	<i>Turdus migratorius</i>	GED48	12/12/2016
B2T1	<i>Ixodes scapularis</i>	Nymph	Gus Engeling WMA	Disturbed	<i>Zonotrichia albicollis</i>	GED60	12/13/2016
B3T1	<i>Ixodes dentatus</i>	Nymph	Gus Engeling WMA	Disturbed	<i>Zonotrichia albicollis</i>	GED68	1/3/2017
B3T2	Unknown	Nymph	Gus Engeling WMA	Disturbed	<i>Zonotrichia albicollis</i>	GED68	1/3/2017
B4T1	<i>Ixodes dentatus</i>	Nymph	Gus Engeling WMA	Disturbed	<i>Cardinalis cardinalis</i>	GED65	1/3/2017

Figure 5. Bray-Curtis dissimilarity heat map. Darker colors signify less distance (more similarity) among sampled sites. The habitats sampled at GEWMA are most similar in composition of avian assemblage. 1 – GEWMA disturbed, 2 – GEWMA sylvan, 3 – BTNP disturbed, 4 – BTNP sylvan.

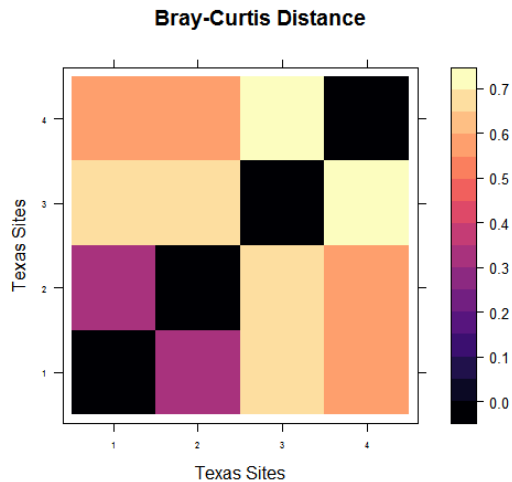


Figure 6. Dendrogram of hierarchical cluster analysis. Lighter colors indicate greater abundance of captured birds at each site. GEWMA sites are more similar. 1 – GEWMA disturbed, 2 – GEMWMA sylvan, 3 – BTNP disturbed, 4 – BTNP sylvan.

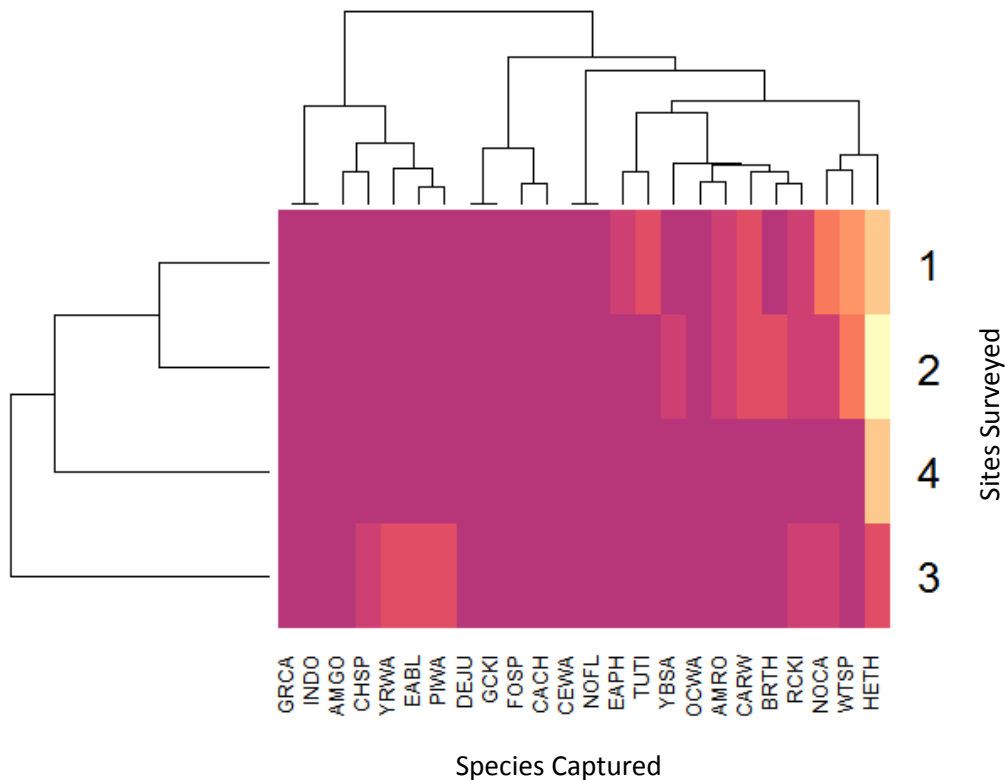
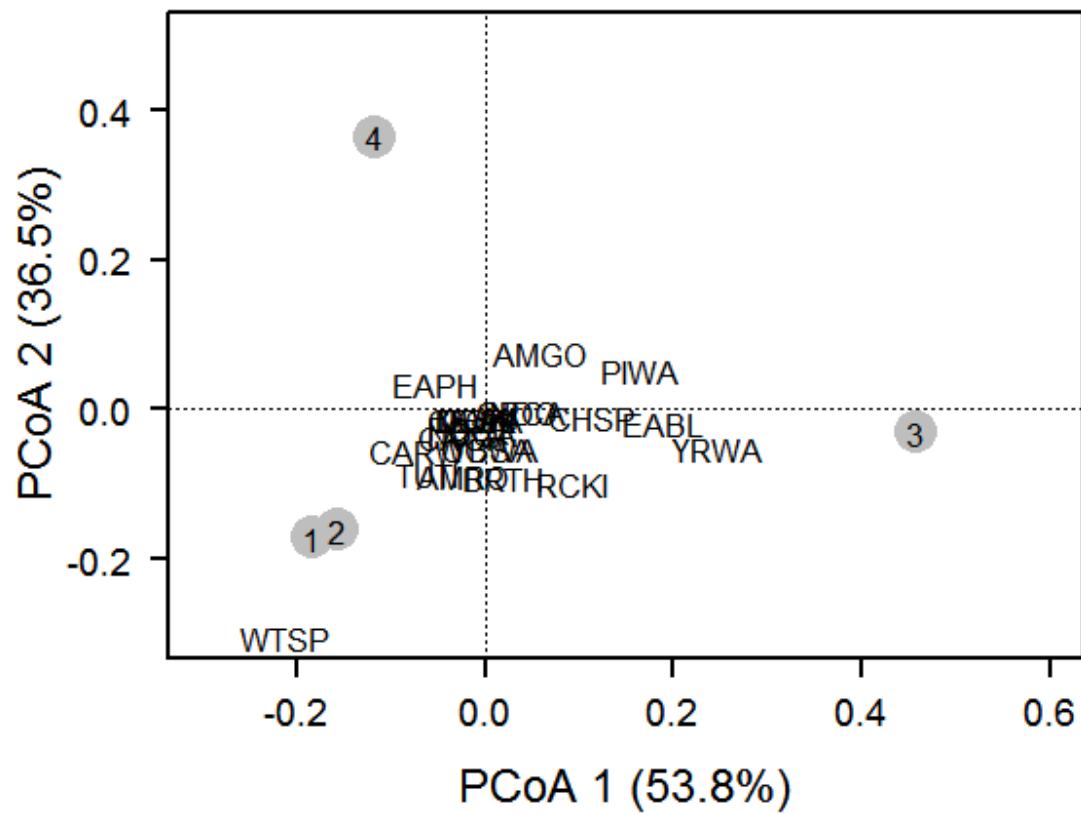


Figure 7. Principle coordinate analysis of sampled habitats and species composition. Figure displays the differences among sampled habitats (PCoA 1) and species composition (PCoA 2) at GEWMA and BTNP. 1 – GEMWA disturbed, 2 – GEWMA sylvan, 3 – BTNP disturbed, 4 – BTNP sylvan.



IV. DISCUSSION

The low total observed infestation rate (1.9%) suggests that, at the local level, in east Texas, birds are not a vertebrate host group important to maintain tick populations. However, the total observed infestation rate, while lower than the estimated infestation rate, was similar to the rate found in other studies (Ogden et al. 2008, Hamer et al. 2012). Also, the fact that all of the tick specimens that were removed and identified were *Ixodes* species which are known to carry *B. burdorferi* is a relevant observation.

The t-test results from the habitat type and migration status comparisons were not statistically significant, but the comparisons are still relevant to understanding the persistence of *Borrelia* in Texas. A single *I. scapularis* was removed from a white-throated sparrow [WTSP], which is a migratory species that winters in Texas and breeds in New England through Canada. The WTSP has been identified as a primary avian host for *I. scapularis* in several long-term, large sample size studies (Ogden et al. 2008, Brinkerhoff et al. 2011a, Hamer et al. 2011, Hamer et al. 2012). Some migratory bird species that winter in Texas may be influential in translocating *Borrelia*-infected ticks from TX to the northern US and into Canada during spring migration (Scott et al. 2001, Ogden et al. 2008). However, the highest abundance of larval and nymphal *I. scapularis* ticks in the northern and midwestern US occurs during the late summer and fall respectively (Brinkerhoff et al. 2011). This temporal peak of sub-adult tick abundance occurs as fall migration begins, when long distance migrants like the WTSP and HETH leave for wintering grounds in Texas. Birds in eastern Texas may not be an important vertebrate group for maintaining tick populations, though they still might be responsible

for the persistence *Borrelia* and its introduction into novel areas throughout Texas (Szonyi et al. 2015).

Two indices for estimating the importance of particular bird species as tick hosts are: infestation probability [IP], measured by population size, range overlap with sampling area, and captured bird sample size; and the index of species importance [ISI], which is calculated by multiplying species' estimated continental population size by mean tick density for carrying ticks (Loss et al. 2016). Though IP was originally calculated for eastern and western North America, eight species captured at GEWMA and BTNP overlap with this index (Loss et al. 2016). Six species that were captured at GEWMA and BTNP are listed on the ISI (Loss et al. 2016). In fact, the infested northern cardinal and American robin that were captured at GEWMA represent the top two species in terms of ISI. In addition, 71.6% (n=151) of the 211 captured birds were ground dwelling/foraging target species and most of the other bird species captured, while not targets, are known to harbor ticks (Cornell Lab of Ornithology 2015).

By comparing the diversity of the avian assemblages sampled at GEWMA and BTNP, we see both important similarities and differences in terms of composition and abundance of avian hosts known to harbor *Ixodes* species ticks. The disturbed and sylvan habitats at GEWMA were the most similar in β -diversity metrics, due to their larger species overlap. All the dominant species at GEWMA have higher IP or ISI values (Loss et al. 2016). The abundance of WTSP at GEWMA is another reason the habitats were so similar. The disturbed habitat in BTNP had the highest H', PIE, and BPD values indicating it was the most diverse avian habitat sampled. The sylvan habitat at BTNP had the lowest values for richness indices and also the lowest Good's coverage value,

suggesting it was not sampled thoroughly enough. All the sampled habitats had dominant species that were known for carrying *Ixodes* species ticks (Hamer et al. 2011, Schneider et al. 2015). Avian assemblage diversity is likely not as important for sustaining *Borrelia* as mammal diversity, where removal of a few key species has a notable amplification on tick density (Levi et al. 2016).

The number of unsuccessful (zero captures) morning or evening sampling events did not influence the observed infestation rate, but likely reduced the number of infested birds captured. Unsuccessful events were especially pronounced at BTNP where 43.8% (n=21) of sampling events were fruitless versus 10.4% (n=5) at GEWMA. The capture rate at GEWMA, 1.1% per mist net/m² hours, was more than double the capture rate at BTNP, 0.45% per mist net/m² hours. However, one sampling event in the disturbed habitat at BTNP produced the highest single event capture total of the project (n=19). Another challenging obstacle to overcome was the skill required to detect ticks on birds, especially while in the field. Engorged imbedded ticks might be slightly more easily detected, but the larvae and nymphs are very small (~1 mm) and easily missed. While control of duplicate captures was attempted by taking vital measurements of the birds, the measurements were not individually exclusive, with much overlap within species. Ideally the captured birds would have been banded but that required experience and permitting that was not feasible with the time-table for this project.

If the observed infestation rate at GEWMA and BTNP was higher, the number of birds captured in this project would have been adequate to assess tick density and other metrics as done in similar projects (Durden et al. 2001, Schneider et al. 2015). Due to the lower infestation rate observed, some methods may need to be modified to improve the

likelihood of capturing enough infested birds to continue this line of research at these sites. Increasing sampling effort: the number of mist nets deployed, additional sampling days, and sampling at more sites could improve capture abundance. However, including blood sampling to test for seroprevalence of *Borrelia* might elucidate more about its life cycle in eastern Texas than just increasing capture abundance. Because ticks take a blood meal from their hosts and can drop off in only a few days, there may have been several birds that were captured that had previously been parasitized by *Borrelia* infected ticks. Sampling for currently infested birds is only a snapshot of the bird-tick-*Borrelia* cycle and may not reveal enough on its own. The additional cost and time to acquire permitting and IACUC approval for blood sampling and bird banding made these unfeasible once this project was under way. However, including these methods is highly recommended for the continuation of this research.

In conclusion, this project was successful in capturing ground foraging/dwelling birds in East Texas at two different field sites. Two *Ixodes* tick species were detected that have been identified as vectors for *Borrelia burgdorferi*, the spirochetal bacterium that causes Lyme disease (Battaly et al. 1987, Barbour et al. 1993, Steere et al. 2004). Both GEWMA and BTNP had diverse avian assemblages that included several dominant ground-dwelling and foraging species known to harbor ticks. The sylvan habitat at BTNP needs additional sampling to assess if it is equally diverse. In addition to a larger sample size of birds, the inclusion of blood sampling for seroprevalence of *Borrelia* is suggested to enhance any further research into the bird-tick-*Borrelia* cycle in eastern Texas. The life cycle of *I. scapularis* at GEWMA and BTNP is still not fully known. The flagging done at GEWMA and BTNP captured more questing adult *I. scapularis* ticks and less larvae

and nymphs. This project and the associated mammal and reptile *Borrelia* projects did not find an adequate number of sub-adult stage *I. scapularis* ticks using these vertebrate hosts to explain their adult abundance in these habitats. These tick projects done at GEWMA and BTNP leave more questions to be answered about the life cycle of *I. scapularis* in eastern Texas.

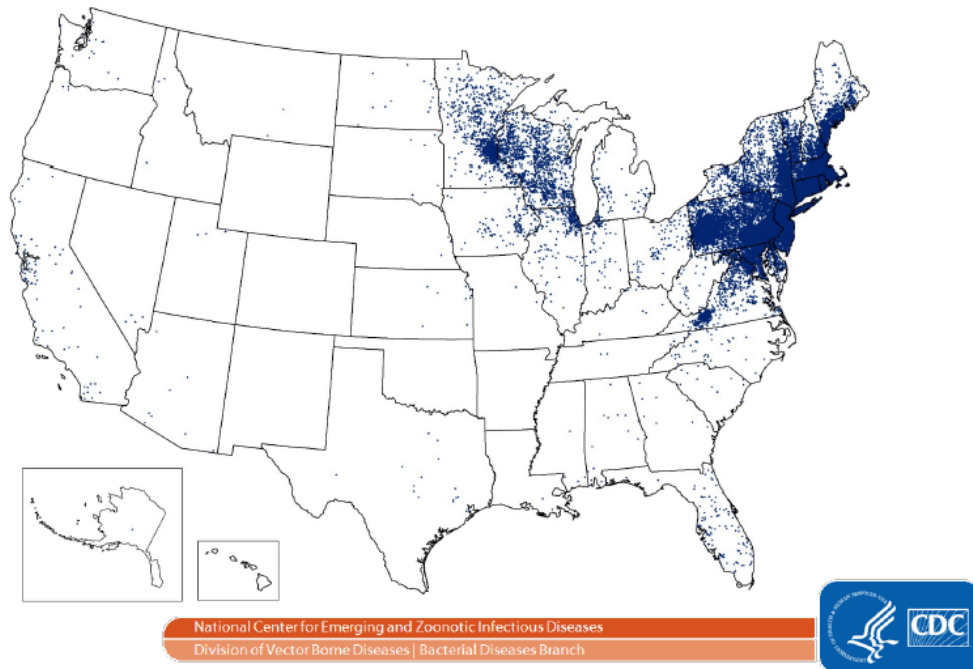
APPENDIX SECTION

Appendix A. Cases of Lyme disease reported during 2015 in the United States showing “hot spots” in NE and North Midwest. Texas has a comparatively low number of cases reported. (CDC 2017a).

https://www.cdc.gov/lyme/resources/reportedcasesoflymedisease_2015.pdf

Reported Cases of Lyme Disease—United States, 2015

Each dot represents one case of Lyme disease and is placed randomly in the patient’s county of residence. The presence of a dot in a state does not necessarily mean that Lyme disease was acquired in that state. People travel between states, and the place of residence is sometimes different from the place where the patient became infected.



Appendix B. Approximate distribution of *Ixodes scapularis* in the United States. (CDC 2017b). https://www.cdc.gov/ticks/geographic_distribution.html

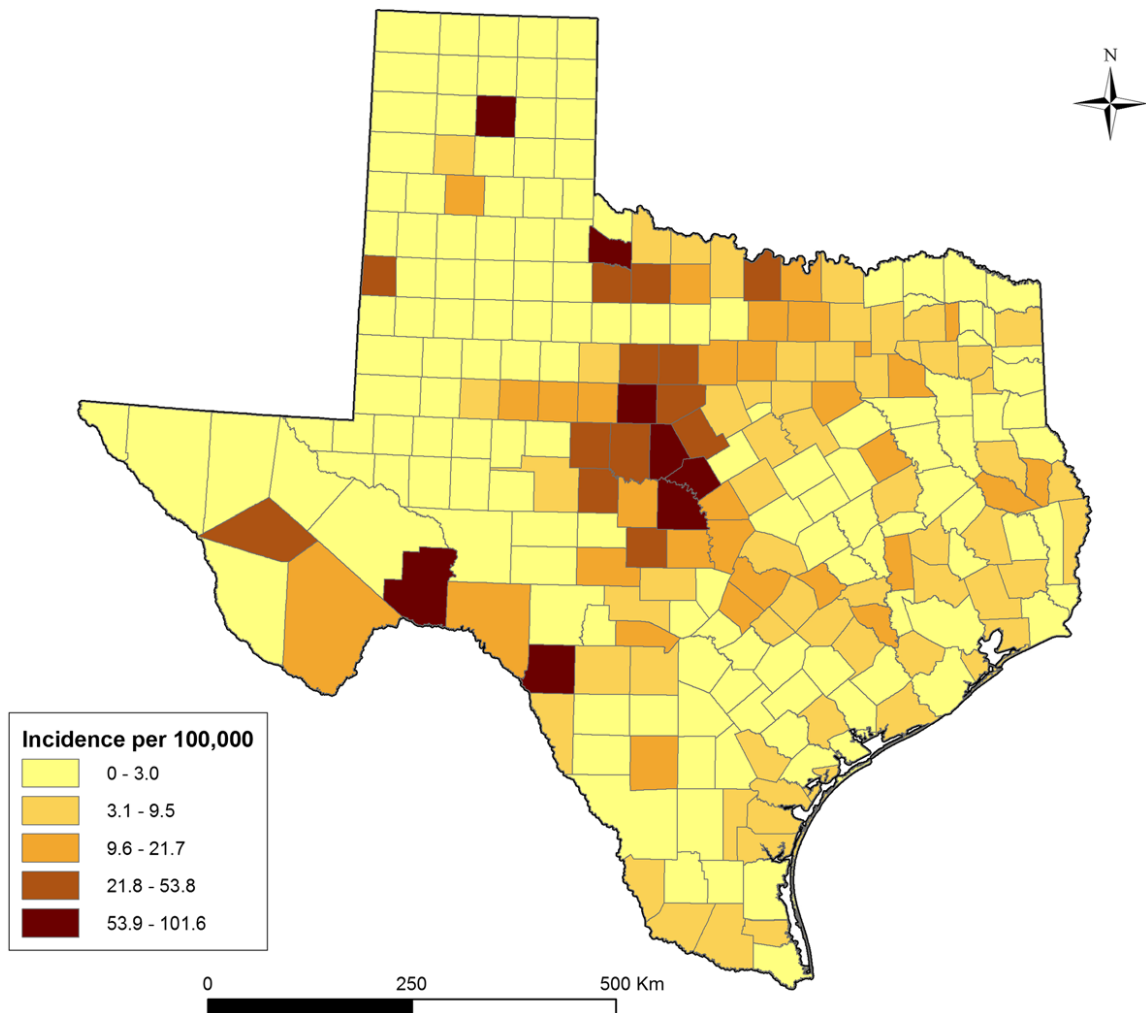


ABOUT THIS MAP: This map is not meant to represent risks for a specific tick-borne disease, because disease transmission is influenced by multiple factors beyond mere tick presence. This map has been designed to answer the question "What ticks should I be concerned about at a regional scale?" Please consult a local public health authority or USDA Agricultural Extension Office to determine more specific information at the state, county, or municipal level. Background data for this map is from the US National Atlas.

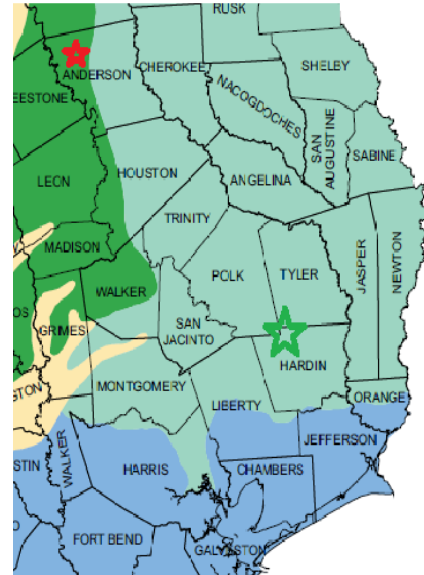
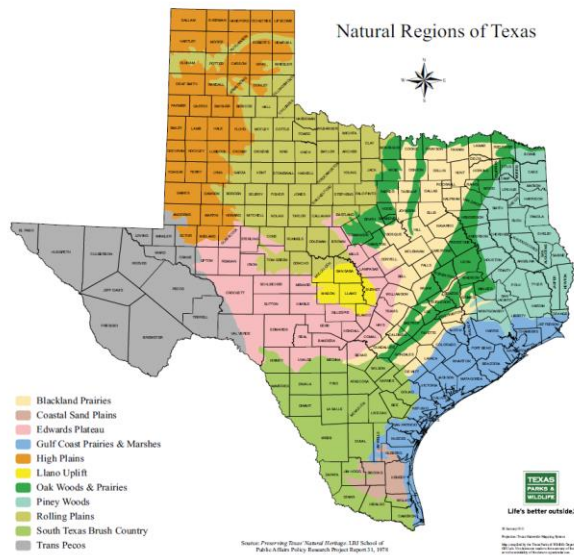
National Center for Emerging and Zoonotic Infectious Diseases
Division of Vector-Borne Diseases



Appendix C. Choropleth map showing crude cumulative Lyme disease incidence from 2000 to 2011 in Texas. The eastern counties, thought to be the most suitable habitat for *Ixodes scapularis*, had lower LD incidence during the period. Misdiagnosis, reporting county of residence versus location of original infection, and clusters of human-biting infected ticks were possible explanations for the unexpected results (Szonyi et al. 2015).



Appendix D. Ecoregions of Texas. Big Thicket National Preserve is comprised of multiple units (green star) located in the piney woods ecoregion. Gus Engeling WMA is found in nw Anderson county (red star) in the oak woods & prairies ecoregion. (TPWD 2017).



Appendix E. USGS Bird Banding Laboratory banding codes for species captured.

Capture List		
Banding Code	Common Name	Species Name
AMRO	American Robin	<i>Turdus migratorius</i>
AMGO	American Goldfinch	<i>Spinus tristis</i>
BRTH	Brown Thrasher	<i>Toxostoma rufum</i>
CACH	Carolina Chickadee	<i>Poecile carolinensis</i>
CARW	Carolina Wren	<i>Thryothorus ludovicianus</i>
CEWA	Cedar Waxwing	<i>Bombycilla cedrorum</i>
CHSP	Chipping Sparrow	<i>Spizella passerina</i>
DEJU	Dark-eyed Junco	<i>Junco hyemalis</i>
EABL	Eastern Bluebird	<i>Sialia sialis</i>
EAPH	Eastern Phoebe	<i>Sayornis phoebe</i>
FOSP	Fox Sparrow	<i>Passerella iliaca</i>
GCKI	Golden-crowned Kinglet	<i>Regulus satrapa</i>
GRCA	Gray Catbird	<i>Dumetella carolinensis</i>
HETH	Hermit Thrush	<i>Catharus guttatus</i>
INDO	Inca Dove	<i>Columbina inca</i>
NOCA	Northern Cardinal	<i>Cardinalis cardinalis</i>
NOFL	Northern Flicker	<i>Colaptes auratus</i>
OCWA	Orange-crowned Warbler	<i>Oreothlypis celata</i>
PIWA	Pine Warbler	<i>Setophaga pinus</i>
RCKI	Ruby-crowned Kinglet	<i>Regulus calendula</i>
TUTI	Tufted Titmouse	<i>Baeolophus bicolor</i>
WTSP	White-throated Sparrow	<i>Zonotrichia albicollis</i>
YBSA	Yellow-bellied Sapsucker	<i>Sphyrapicus varius</i>
YRWA	Yellow-rumped Warbler	<i>Setophaga coronata</i>

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