ECOLOGY OF A RODENT-TICK-PATHOGEN COMMUNITY IN EAST-CENTRAL TEXAS

A Thesis

by

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ABSTRACT

Rodent species worldwide are critical to the maintenance of tick-borne disease systems because they serve as hosts for ticks and reservoirs for zoonotic pathogens. To learn more about native fauna that may be involved in enzootic transmission of pathogens that can cause tick-borne diseases (TBDs), a mark-recapture study of rodents was conducted in Brazos County in east-central Texas. My objectives were to: (*i*) describe the species richness and seasonal activity of rodents; (*ii*) characterize rodent infestation with ticks over time; and (*iii*) determine the infection prevalence of rodents and ticks with selected zoonotic tick-borne pathogens.

For nineteen months, small mammals were live-trapped two nights per month and subjected to blood and ear biopsy collections. All captured mammals were checked for the presence of ticks, which were removed for diagnostic testing. Additionally, drag sampling was conducted to collect ticks from the vegetation.

Five rodent species (*Sigmodon hispidus, Reithrodontomys fulvescens*, *Peromyscus leucopus*, *P. gossypinus*, and *Baiomys taylori*) were captured over the course of the study. A large increase in *S. hispidus* capture success was seen in fall 2013, reflecting the characteristic population booms exhibited by this species. Two tick species - *Amblyomma maculatum* and *Ixodes scapularis* - were found infesting the rodents at low levels (2.33%). No ticks were found in over 14,500 meters of drag sampling the vegetation.

In an analysis of 698 ear biopsies, 3.2% of the specimens were positive for *Borrelia miyamotoi*, a spirochete that has recently been shown to cause relapsing fever in humans. One specimen (0.1%) was found to be infected with *B. lonestari*. No ticks were found to be infected with *Borrelia*. However, 4.3% of the larval *A. maculatum* pools were positive for a rickettsial endosymbiont. One larval *A. maculatum* pool and one *I. scapularis* nymph were found to be infected with *Rickettsia monacensis*, the causative agent for a Mediterranean spotted fever-like illness in Europe and North Africa. This study identifies novel TBDs in the southern United States and exposes the need for further study of TBD ecology, especially in understudied areas.

DEDICATION

I dedicate this to my family. To my parents who sacrificed everything for Sara, Luis and myself; you lifted us out of homelessness, taught us to reject the status quo, and taught me that nothing is out of reach given hard work. My parents are my heroes and I will be forever indebted to them for their support and for them pushing for me to succeed, even when things seem impossible.

To Sara (my little big sister): you were my very first friend and you are the best friend I will ever have. I'm thankful for your support through all of the milestones in my life. Even though we will go our separate ways in a few weeks, I take comfort knowing that you've found the person that truly understands and loves you. You and Joseph were meant for each other and I know you'll always be in good hands.

To Luis, you may be my younger brother, but that doesn't mean I can't look up to you. Even though we didn't always see eye-to-eye, you've shown me what true grit and determination looks like.

All of you never stopped believing in me, even at times when I didn't have confidence in myself. I couldn't have asked for a more supportive family. Thank you again for your love and support.

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Last, and most importantly, I would like to thank my family for supporting me throughout all of my endeavors and for never letting me give up, even when I felt like I wasn't getting anywhere. Your love and support has sustained me through this degree and is the fuel that pushes me to keep pushing the envelope and to reach for the stars.

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CHAPTER I

INTRODUCTION

Rodents play important roles in their ecological communities. For example, rodents can help proliferate plant species by eating and dispersing seeds or acting as pollinators. Conversely, rodents are some of the world's leading agricultural pests, causing a great deal of damage to agricultural crops worldwide. Additionally, some rodents are also capable of serving as reservoirs for zoonotic pathogens capable of causing human diseases, including those which are transmitted by ticks.

In recent years, an unprecedented number of novel tick-borne diseases (TBDs) have been detected based on the presentation and diagnostic testing of sick humans as well as through investigations of tick populations to detect etiologic agents. Such discoveries expose a critical need to better understand the ecology of TBDs in an effort to protect human and animal health. Critical aspects of TBD ecology that must be elucidated to reduce economic and public health consequences include the identification of key reservoir hosts and competent vectors as well as how the interaction of these species varies spatially and temporally.

Texas is home to many rodent and tick species, some of which serve as reservoirs and vectors for zoonotic pathogens either in Texas or in other areas of their distribution. The white-footed mouse, *Peromyscus leucopus*, is a known reservoir for many tick-borne pathogens including the agents responsible for ehrlichiosis, Lyme disease, and babesiosis. The cotton mouse, *Peromyscus gossypinus*, has also been implicated as a reservoir for tick-borne pathogens that cause Lyme disease, and human granulocytic

ehrlichiosis. The hispid cotton rat, *Sigmodon hispidus*, also serves as a reservoir host for the causative agent of Lyme disease. A laboratory experiment suggests *S. hispidus* may also be a reservoir for the Rocky Mountain spotted fever agent. The eastern woodrat, *Neotoma floridana*, and the marsh rice rat, *Oryzomys palustris*, are also thought to serve as reservoir hosts for the causative agent of Lyme disease. Whether or not these species can serve as reservoirs for tick-borne diseases in Texas is largely unknown.

Common ticks in Texas include *Amblyomma americanum*, *A. maculatum*, and *Ixodes scapularis*. *A. americanum* vectors the pathogens responsible for ehrlichiosis, Rocky Mountain spotted fever, and tularemia; *A. maculatum* vectors the pathogen responsible for spotted fever rickettsiosis; and *I. scapularis* vectors the pathogens responsible for Lyme disease, anaplasmosis, and babesiosis. In the southern United States, *I. scapularis* is rarely implicated in human-biting, and therefore the pathogens it vectors present less of a public health burden relative to the northern United States. Consequently, despite the widespread distribution of *I. scapularis* across the southern United States, less than 5% of Lyme disease cases and less than 4% of anaplasmosis cases in the United States occur across the southern United States (Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Texas, and Virginia). In contrast, *Amblyomma*-transmitted diseases are more common in the south. For example, over half of the ehrlichiosis cases and over 70% of the spotted fever rickettsiosis cases originate in this region.

Wild rodent species are important in the ecology of many TBDs because they serve as reservoir hosts for pathogens, and therefore they often are assessed as sentinels

to gauge the level of tick-borne pathogen activity within a given geographic area. As generalists, *I. scapularis, A. americanum* and *A. maculatum* all feed on members of the small mammal community including *P. leucopus*. My objective was to examine the dynamics of tick and tick-borne pathogen occurrence within a wild community of small mammals to provide information that is useful for regional assessments of human risk and public health protection in the southern United States. This study used a mark-recapture approach to assess the rodent population at a field site in Brazos County, Texas. I report the rodent species present at this field site as well as the ticks that are parasitizing them to provide background ecological information necessary in understanding tick-borne disease risk in this area. Furthermore, I also report rodent and tick infection with tick-borne pathogens.

CHAPTER II

THE STRUCTURE AND ECOLOGY OF A RODENT AND TICK COMMUNITY IN EAST-CENTRAL TEXAS

Introduction

Rodents play important roles in their ecological communities. For example, rodents can help proliferate plant species by eating and dispersing seeds (1) or acting as pollinators (2). Conversely, rodents are some of the world's leading agricultural pests, causing a great deal of damage to agricultural crops worldwide (3). Additionally, some rodents are also capable of serving as reservoirs for zoonotic pathogens capable of causing human diseases, including those which are transmitted by ticks (4).

Texas is home to many rodent and tick species (5), some of which serve as reservoirs and vectors for zoonotic pathogens either in Texas or in other areas of their distribution. The white-footed mouse, *Peromyscus leucopus*, is a known reservoir for many tick-borne pathogens including the agents responsible for ehrlichiosis, Lyme disease, and babesiosis (6-8). The cotton mouse, *Peromyscus gossypinus*, has also been implicated as a reservoir for tick-borne pathogens that cause Lyme disease, and human granulocytic ehrlichiosis (9-12). The hispid cotton rat, *Sigmodon hispidus*, also serves as a reservoir host for the causative agent of Lyme disease (11). A laboratory experiment suggests *S. hispidus* may also be a reservoir for the Rocky Mountain spotted fever agent (13). The eastern woodrat, *Neotoma floridana*, and the marsh rice rat, *Oryzomys palustris*, are also thought to serve as reservoir hosts for the causative agent of Lyme

disease (10, 14). Whether or not these species can serve as reservoirs for tick-borne diseases in Texas is largely unknown.

Common ticks in Texas include *Amblyomma americanum*, *A. maculatum*, and *Ixodes scapularis* (15). *A. americanum* vectors the pathogens responsible for ehrlichiosis, Rocky Mountain spotted fever, and tularemia (16); *A. maculatum* vectors the pathogen responsible for spotted fever rickettsiosis (17); and *I. scapularis* vectors the pathogens responsible for Lyme disease, anaplasmosis, and babesiosis (8). While there are ecological and epidemiological constraints that currently limit the distributions of some of these rodent-associated tick-borne diseases, the diverse rodent and tick fauna that occurs Texas underscores the importance of learning more about rodent and tick population dynamics for understanding disease risk.

This study used a mark-recapture approach to assess the rodent population at a field site in Brazos County, Texas. I report the rodent species present at this field site as well as the ticks that are parasitizing them to provide background ecological information necessary in understanding tick-borne disease risk in this area.

Materials and Methods

For two consecutive trap nights each month from May 2012 – November 2013, small mammals were trapped at the Biodiversity Research and Teaching Collection natural area in College Station, TX (30°38'47.2"N 96°17'45.9"W). Sherman live traps (H. B. Sherman Traps, Tallahassee, FL) were baited with sunflower seeds and set along four transects, with 47 to 70 traps per transect spaced approximately 10m apart. The transects were established along vegetation types ranging from grass and shrubs to post-

oak forest. Each trap set out was counted as one trap night. If a trap was found closed without a mammal (tripped), it was counted as half a trap night (i.e., adjusted trap night) based on the assumption that it was unavailable to capture a small mammal for about half a night. The calculation for effective trap nights (the summation of all trap nights and adjusted trap nights) was used to measure overall trapping success and trapping success per species.

Captured mammals were weighed, visually identified to species and sex, noted for reproductive condition and any other anomalies, and anesthetized using Isoflurane (Abbot Laboratories, Abbott Park, IL), if necessary. Trap location was noted and mammals were checked for the presence of ticks, which were removed and stored in 70% ethanol. An ear tag (National Band and Tag, Newport, KY) was placed to mark the animal in case of recapture. A 2mm-diameter punch biopsy and blood samples were taken from each specimen for future laboratory studies investigating tick-borne pathogens. Ear biopsies were taken from both ears of new captures and from a single ear of recaptures when the time elapsed since previous capture was at least one month. All biopsies were stored in 70% ethanol. If an individual was recaptured during the same trapping period (i.e., two days in a row), the specimen was only weighed and checked for ticks. Recapture status and location was recorded for each individual. After processing was complete, the small mammals were released at their capture sites. All animals collected during this study were treated humanely according to the guidelines provided by the American Society of Mammalogists (18) and the Texas A&M Animal Care and Use Committee (permit# 2012-100).

Once in the lab, ticks were identified to species using a dichotomous key (19). Molecular laboratory work (DNA extraction, PCR amplification, and sequencing) was used to confirm species identifications on a subset of rodent and tick specimens. Due to the difficulty distinguishing some *Peromyscus* species based on morphologic features, all *Peromyscus* specimens were subjected to molecular work to determine species identification. Two randomly-selected specimens from all other species were tested to confirm visual identification. Total mammal and tick DNA extraction was performed on single ear biopsies, single nymphal ticks, or pooled larval ticks (pools comprised all conspecific ticks from the same host at the same time) using commercially available kits (DNeasy Blood and Tissue Kit, Qiagen, Valencia, CA; E.Z.N.A Tissue DNA Kit, Omega Bio-Tek, Norcross, GA) according to protocols provided in the kits and using a final elution of 60 μL with 70°C elution buffer.

Rodent identification was confirmed through amplification of the cytochrome b gene according to the protocols of Molaei et al. (20). Tick identification was confirmed through amplification of the 12S rRNA gene according to the protocols of Beati et al. (21). PCR amplicons were purified (ExoSAP-IT; Affymetrix, Santa Clara, CA) and sequenced. Sequencing for tick PCR amplicons was performed at Eton Bioscience Inc. using ABI 3730xl DNA Sequencers. Rodent PCR amplicons were sequenced at Beckman Coulter Genomics (Danvers, MA) using an ABI Prism 3730xl DNA Sequencer. Sequences were annotated using Sequencher 4.9 (GeneCodes Corporation; Madison, WI) and were compared to published sequences using the basic local

alignment search tool (BLAST) in GenBank for confirmation of visual identification (22).

Results

Over the 19 month study, there were a total of 943 small mammal captures, representing 561 individuals. Five species were captured: the hispid cotton rat (*Sigmodon hispidus*), the fulvous harvest mouse (*Reithrodontomys fulvescens*), the white-footed mouse (*Peromyscus leucopus*), the cotton mouse (*Peromyscus gossypinus*), and the northern pygmy mouse (*Baiomys taylori*). *Sigmodon hispidus* was encountered most frequently whereas *B. taylori* was encountered least frequently (Table 1). The majority of the time, there was only one individual found in each trap. However, over the course of the study there were six instances where two individuals of the same species were captured in a single trap: four instances for *R. fulvescens* (two instances each in February 2013 and March 2013), one instance for *B. taylori* (October 2013), and one instance for *S. hispidus* (October 2013). The *B. taylori* double capture occurred in a forested area while the rest occurred in grassy areas.

Although precautions were taken to reduce trap mortalities (e.g., avoidance of fire ant mounds when setting traps; conservative application of inhalant anesthetic by trained personnel only; use of polyfill in traps on cold nights; setting traps late in evening and recovery early in morning on hot nights), 3.2% of captures were mortality events attributed to the following causes: unknown ($n = 3$); predation by the red imported fire ant ($n = 15$); cold weather ($n = 2$); anesthetic overdose ($n = 1$); drowned due to rainstorm flooding of trap site ($n = 1$); and heat-related death ($n = 8$; Table 1). As

in any study working with wild animals, all necessary steps were taken to reduce mortalities.

Table 1. Total number of rodent captures, recaptures, and trap mortalities throughout the duration of the 19-month study.

Species	Total Captures	Recaptures	Trap Deaths
Sigmodon hispidus	514	190	6
Reithrodontomys fulvescens	135	60	9
Peromyscus leucopus	130	64	3
Peromyscus gossypinus	82	61	2
Baiomys taylori	82	7	10
Total	**943**	**382**	**30**

July 2012 had the lowest capture success, averaging 1.03 captures per 100 effective trap nights (Fig. 1). Peak capture success occurred in September 2013 with an average of 38.37 total captures, representing all five species, per 100 trap nights. The greatest capture success for *B. taylori* occurred in September of 2013, otherwise capture success was generally low (Fig. 1). *R. fulvescens* had a low capture rate in the summer and fall months, with increasing capture success in the winter months and the highest capture success in February 2013 (Fig. 1). Capture rates for *S. hispidus* increased significantly in mid- and late-2013 (Fig. 1). Capture success for both *Peromyscus* species began with similar captures successes. Then there were two periods of time (September 2012-January 2013 and July-October 2013) when *P. leucopus* was caught more frequently than *P. gossypinus*, including July 2013 when no *P. gossypinus* were

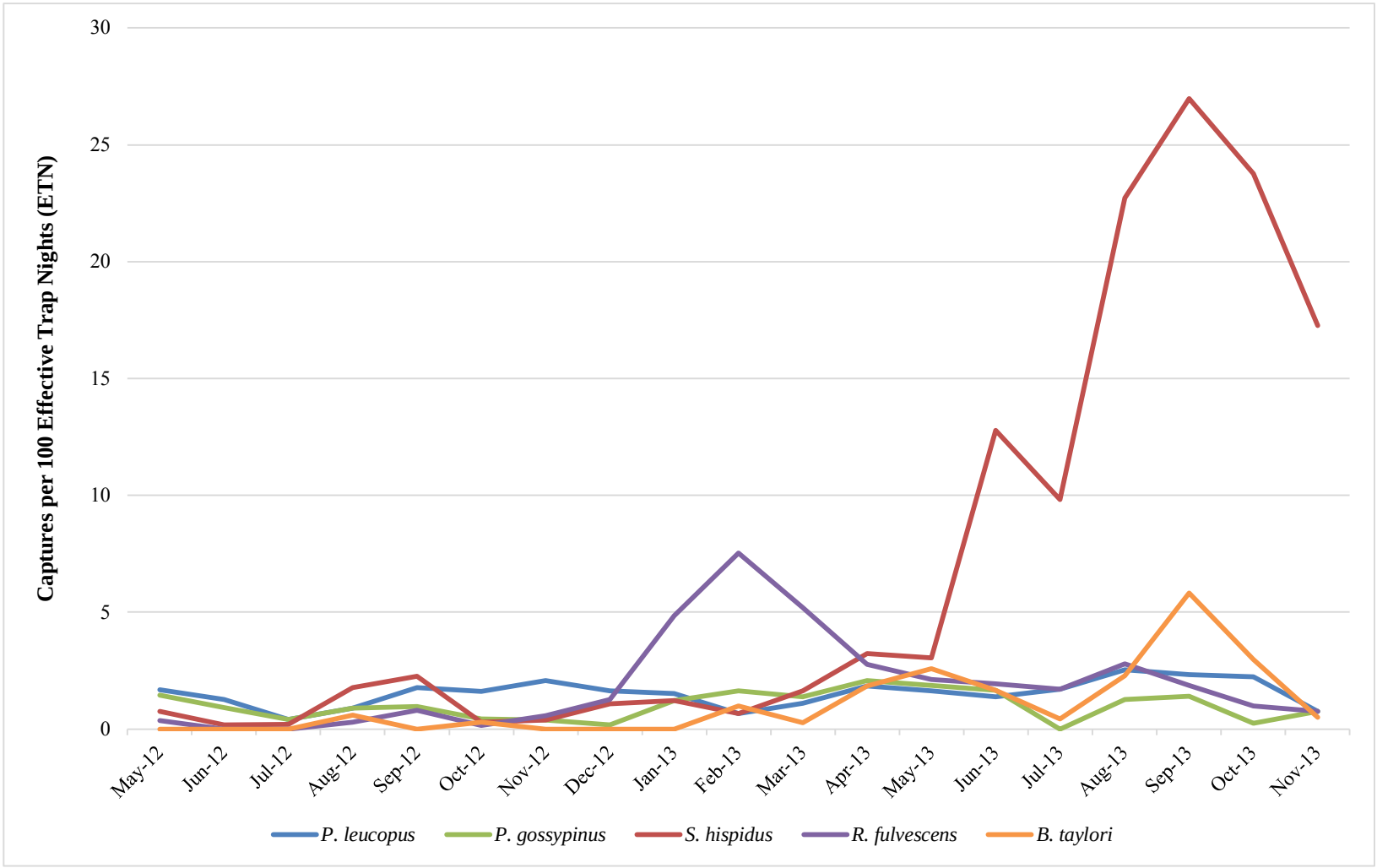

Figure 1. Effective trap nights (ETN) per rodent species over the 19 month study. ETN was calculated by adding all full and adjusted trap nights (see text)

caught (Fig. 1). In between these was a period of five months (February 2013-June 2013) when *P. gossypinus* was captured more frequently (Fig. 1).

A total of 58% of captures occurred in forested areas. Eighty-eight percent, 74%, and 62% of *P. leucopus, P. gossypinus,* and *B. taylori* captures occurred in forested areas, respectively. *S. hispidus* was consistently captured in both habitat types with 45% of captures occurring in grassy areas and 55% occurring in forested areas. *R. fulvescens* was found mostly in grassy areas with 71% of captures occurring there.

Male to female ratio was observed at 1.1:1 for all five species combined. More males were captured in May-November 2012, April-May 2013, and August-October 2013 (Fig. 2). More females were captured in January-March 2013, June-July 2013, and November 2013 (Fig. 2). Observed males outnumbered females for *P. leucopus*, *P. gossypinus*, and *R. fulvescens* with ratios of 2.1:1, 1.5:1, and 1.27:1, respectively. Observed females outnumbered males for *B. taylori* and *S. hispidus* 2.72:1 and 1.02:1, respectively.

Of the 561 captured individuals, 153 (27.2%) were recaptured during the study, including 134 individuals (representing all five species) that were captured at least twice on non-consecutive days. An additional 19 individuals were only recaptured the night after their initial capture and an additional 20 individuals were recaptured at least once, but after their original ear tag was lost (individuals were recognized as recaptures due to the presence of ear biopsy holes in their ears). Since the previous capture status of these individuals was unknown, they were not included in the overall recapture analysis above, and accordingly the recapture percentage should be interpreted as a conservative

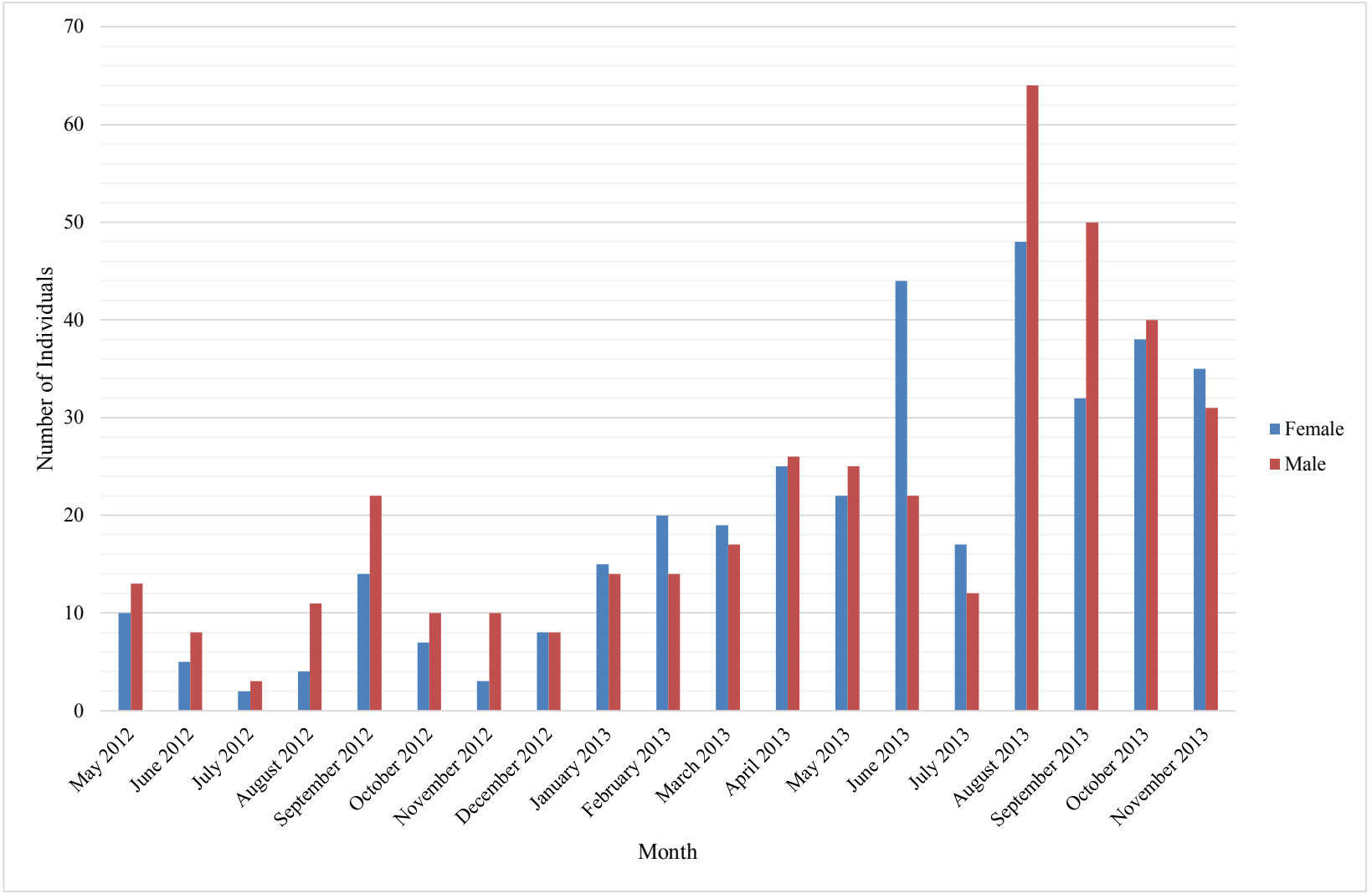

Figure 2. A monthly comparison between female and male individuals captured during the study period.

estimate. The highest recapture frequency was shown by two individuals that were recaptured on seven non-consecutive days (Table 2).

Table 2. Number of recaptured individuals and instances of recapture per species over the 19 month study. At least one individual per species was recaptured on non-consecutive days at some point during the study. A majority (72.4%) of recaptured individuals were captured twice, but two individuals were captured seven times.

Species	Number of recaptured individuals	Instances of captures (non-consecutive days)					
		2	3	4	5	6	7
Baiomys taylori	2	2	0	0	0	0	0
Peromyscus gossypinus	20	12	4	1	1	1	1
Peromyscus leucopus	23	16	5	1	1	0	0
Reithrodontomys fulvescens	21	16	2	2	0	0	1
Sigmodon hispidus	68	51	13	3	1	0	0
Total	**134**	**97**	**24**	**7**	**3**	**1**	**2**

Time between captures ranged from 1 month to 13 months between captures (Table 3). Over 40% of recaptures occurred only in the month after initial capture (Table 3). Two individuals were captured over a period of 10 months (*P. gossypinus*) and 11 months *(R. fulvescens*) between initial and final capture. One *P. gossypinus* individual was initially caught in May of 2012 and was not captured again until June of 2013, a span of 13 months.

Table 3. Time period for recaptures measured in months between trapping sessions.

Capture Incidence	Time between initial and final captures (months)													Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
2	56	21	9	5	3	1	1	0	0	0	0	0	1	**97**
3	0	10	7	3	2	1	0	1	0	0	0	0	0	**24**
4	0	0	1	4	1	1	0	0	0	0	0	0	0	**7**
5	0	0	0	2	1	0	0	0	0	0	0	0	0	**3**
6	0	0	0	0	0	0	1	0	0	0	0	0	0	**1**
7	0	0	0	0	0	0	0	0	0	1	1	0	0	**2**
Total	**56**	**31**	**17**	**14**	**7**	**3**	**2**	**1**	**0**	**1**	**1**	**0**	**1**	**134**

Although record of reproductive state was not meticulously kept, a total of 22 individuals were noted as being scrotal or pregnant. No *B. taylori* were observed to be scrotal, but five females were observed to be pregnant during the summer and fall. *P. leucopus* males were observed to be scrotal in late summer. Two *P. gossypinus* males were found to be scrotal during the summer and fall, and three females were observed to be pregnant in early summer and fall. No *R. fulvescens* males were observed to be scrotal, but four females were observed to be pregnant in spring, summer, and winter. Three *S. hispidus* males were noted to be scrotal in late summer and three females were noted as being pregnant in summer and early fall.

Nine captured *B. taylori* (11%) and one (0.2%) *S. hispidus* were found to have firm circular raised areas of skin (nodules) on their tails (Fig. 3). One of the *B. taylori* captures also had similar nodules on its feet (Fig. 3A). Three of these captures occurred

in the summer of 2013 (May and June) and six occurred in the fall of 2013 (September, October, and November).

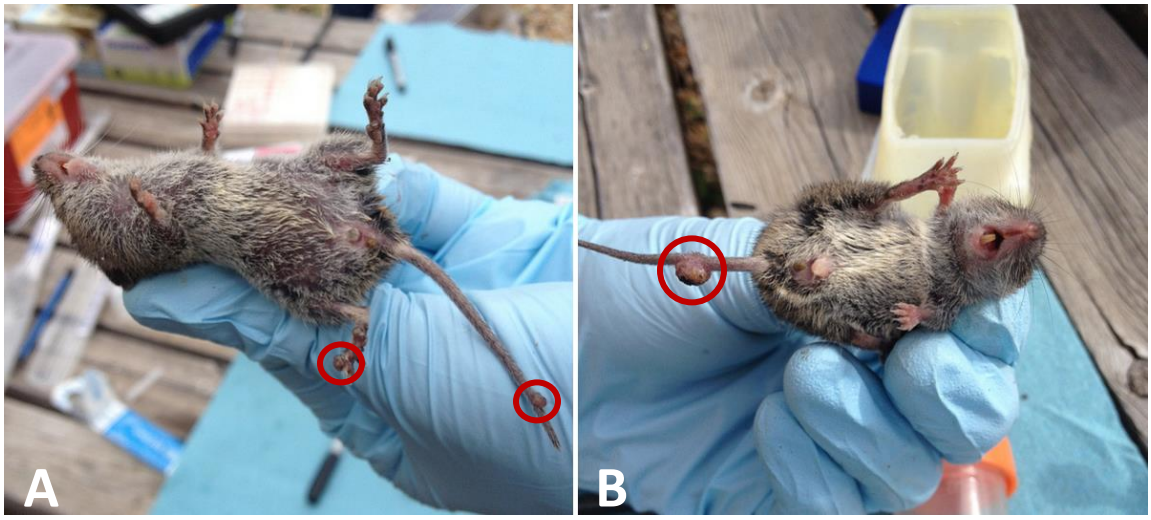

Figure 3. Two captured *B. taylori* affected with nodules. A) A captured *B. taylori* with nodules on its tail and right hind foot (left). B) Another captured *B. taylori* with a larger nodule on its tail (right). Printed with permission from Jessica Light.

A total of 98 ticks were taken off small mammals over the course of the study. An example of a tick on a rodent host is shown in Figure 4. In total, I found a 2.3% (22 of 943) tick infestation of small mammals. Ticks comprised larvae and nymphs of two species: *Ixodes scapularis* and *Amblyomma maculatum.* Tick burden on infested individuals ranged from 1-40 larvae and 1-10 nymphs (Fig. 5). Forty *A. maculatum* larvae were found on a single *S. hispidus* in September 2013. *Ixodes scapularis* nymphs were found exclusively on *S. hispidus* in September 2012, and July and August 2013. *Amblyomma maculatum* larvae were found on one *B. taylori* individual in September

2013, one *P. leucopus* in May 2013, and four *S. hispidus* in September 2012, two in July 2013, and four in September 2013. *Amblyomma maculatum* nymphs were found on one *P. leucopus* individual in August 2013, and three *S. hispidus* in September 2012 and five in August 2013 (Fig. 5). One *S. hispidus* individual was co-infested with *I. scapularis* and an *A. maculatum* larva and another was infested with larval and nymphal *A. maculatum*. No ticks were found on *R. fulvescens* and *P. gossypinus*. Approximately 55% of the ticks (n = 54) were found on rodents from a grassy habitat with no forest canopy (including an individual that had 40 *A. maculatum* larvae). The remaining 44 ticks, including all 3 *I. scapularis*, were found in forested areas. No apparent sex bias was seen in tick infestation as 11 females and 11 males were found to be infested with ticks.

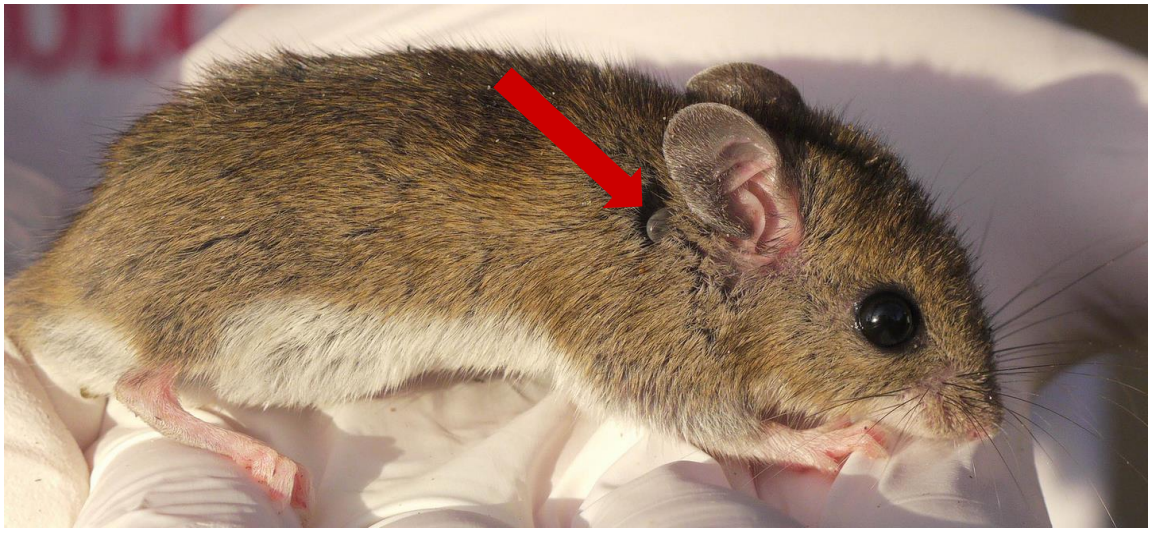

Figure 4. A captured *Peromyscus leucopus* specimen with an attached engorged *A. maculatum* nymph. Printed with permission from Sarah Hamer.

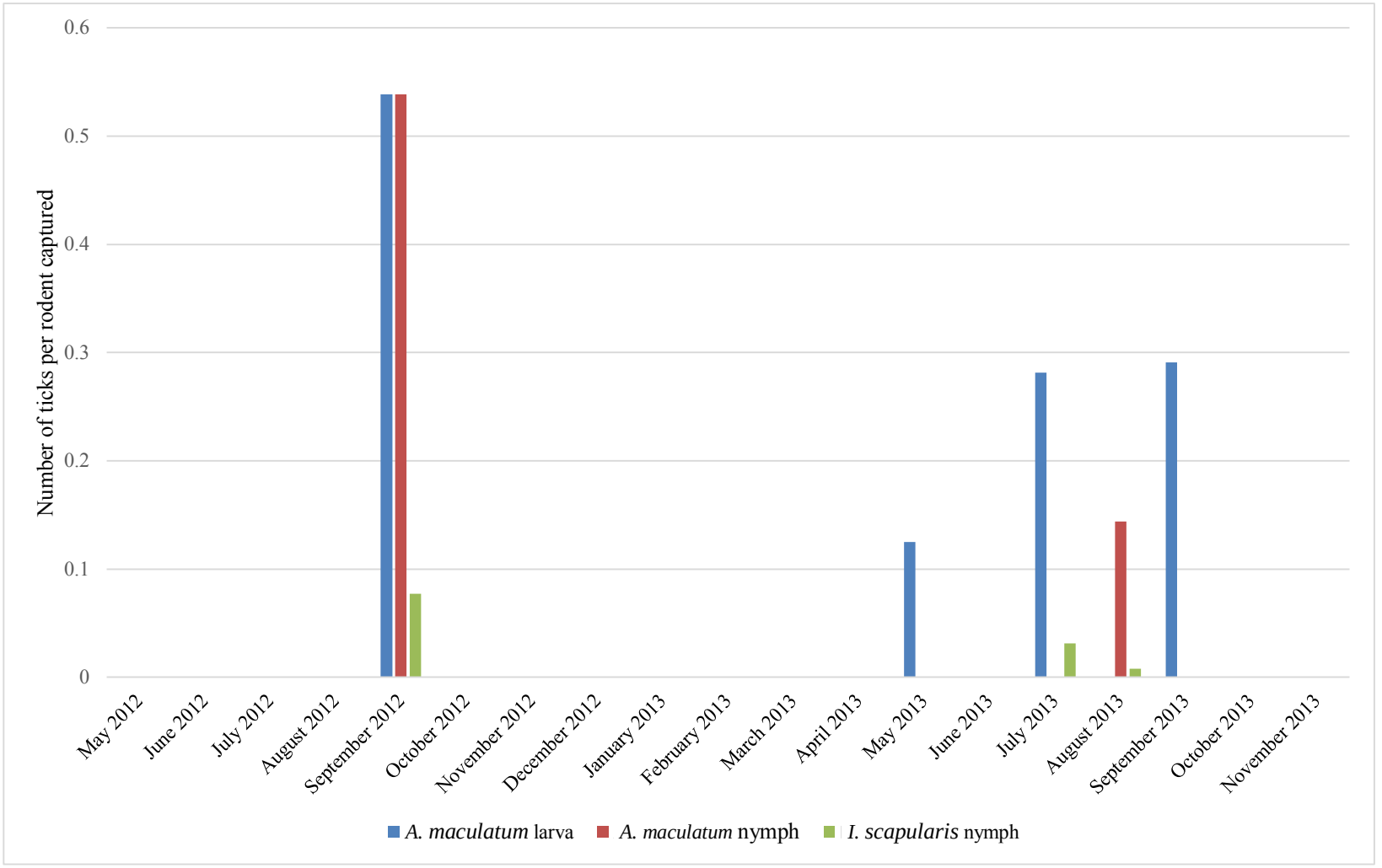

Figure 5. Phenology of ticks removed from rodents measured in number of ticks collected per rodent. Forty-eight *A. maculatum* larvae were collected in September 2013, 40 of which came from a single *S. hispidus*.

Discussion

I describe the seasonal dynamics of a rodent and tick community in east-central Texas, and these ecological data are important considering the potential role of these species in the enzootic maintenance of pathogens that may cause tick-borne diseases. Overall, five rodent species were encountered at this field site in Brazos County, of which *P. leucopus*, *P. gossypinus*, and *S. hispidus* have previously been implicated as reservoirs for tick-borne pathogens in other regions of the country. Their presence, along with the presence of other rodent species and the tick vectors *I. scapularis* and *A. maculatum*, allows the possibility for the maintenance of TBD pathogens at this location.

While all five captured rodent species have previously been described in Brazos county (5, 23), a large study conducted over a 6 year period (1977-1983) in Brazos County neglected to capture *P. gossypinus* (24). Based on the results of that study, my findings of *P. gossypinus* at this field site may represent a range expansion or increase in population density of this species over the past 30 years. Alternatively, given that the morphologic differentiation between *P. leucopus* and *P. gossypinus* is subtle (*P. leucopus* has a hind foot length of 17-25mm and a skull length of 24-29mm while *P. gossypinus* typically has a hind foot length greater than 22mm and a skull length greater than 27mm), it is possible that previous studies overlooked *P. gossypinus.* In my study, all *Peromyscus* individuals were subjected to molecular methods for identification of species thus I am confident of my species identifications.

Capture success for all species was generally low in 2012 with an average capture success of 3.53 captures per 100 ETN. Capture success increased to 18.74

captures per 100 ETN in 2013. *S. hispidus* was associated with the greatest increase, when capture success increased from an average of 1.29 captures per 100 ETN in May 2012-May 2013 to 18.89 captures per 100 ETN in June–November 2013. Fluctuations in *S. hispidus* populations have been reported as early as the late 1920s. An observational study found an increase in the local population of *S. hispidus* in 1928 after previous observations in 1927 found low numbers of *S. hispidus* in the same area (25). Another study found a statewide rise and decline in the *S. hispidus* population of Texas (26). Grant et al. (24) also noted a fluctuation in the *S. hispidus* at their field site in Brazos County. Their analyses indicated that this fluctuation was not significantly correlated with mean temperature or precipitation. However, a significant correlation between the fluctuation and the number of days above 100°F (37.8°C) was found. Haines (26) reports that similar fluctuations were seen in Georgia and Tennessee. These previous studies suggest that population fluctuations in *S. hispidus* are to be expected.

R. fulvescens and *B. taylori* also showed seasonal variations in their captures. A large peak was seen in the winter and a smaller peak in the summer during my study. This is in line with previous reports of a bimodal population density pattern (27). In my study, a large peak was seen in early fall and a smaller peak in late spring with low capture success in the summer months. This is again consistent with previous reports of population peaks in early fall and winter (28). Neither *Peromyscus* species exhibited a seasonal variation in capture success.

While most captures involved a single individual in a trap, double captures were seen in three of the five species. Three of the four *R. fulvescens* double-capture

instances involved members of the opposite sex. The third instance involved two males in a trap. The *B. taylori* double-capture involved two males, and the *S. hispidus* double capture involved two females. In a previous study, *R. fulvescens* and *B. taylori* were involved in multiple capture events (MCE) (29). It was concluded that while *R. fulvescens* mating pairs may participate in short-term co-travelling, *B. taylori* MCEs were not apparently related to reproduction. My data appear to support these previous findings. *S. hispidus* has also been involved in MCEs in a previous study (30). That study observed more same sex MCEs than opposite sex MCEs, with male-male captures being most common. Even though it did not occur here, *P. leucopus* has also been involved in MCEs, mostly intersex pairs, indicating possible mating pairs travelling together (31).

Habitat associations can be deduced based on capture data from this study. Over half of all captures occurred in forested areas. For example, *P. leucopus* and *P. gossypinus* were overwhelmingly caught in forested areas. *P. leucopus* is known to prefer areas with a canopy (32) and *P. gossypinus* is known to prefer bottomland hardwood forests (33). *B. taylori* is known to be most commonly found in grassy areas, but can also be found in forested areas (28) where I captured over 60% of the specimens of this species. *S. hispidus* was found approximately equally in forested and grassy areas and has been previously described to be most commonly caught in grassy areas (34). Only *R. fulvescens* showed a preference for grassy areas with approximately 70% of captures taking place in such areas. This is consistent with previous findings indicating *R. fulvescens* prefers grassy fields (35).

My observations for reproductive state are mostly consistent with previous knowledge of each species as all are known to breed year-round (27, 28, 32-34). I found pregnant *B. taylori* in the summer and fall and it is known that *B. taylori* mates year-round with peaks in late fall and early spring (28). Both *P. leucopus* and *P. gossypinus* are also known to mate year-round in Texas (32, 33) and my findings of reproductive activity during the summer and fall are in line with these findings. *S. hispidus* breed throughout the year in Texas with peaks in fall and spring (34); I found evidence of reproductive activity during the fall reproductive peak. Although *R. fulvescens* has reproductive peaks in late spring and early fall (27), my observations did not include reproductive activity during this time.

The nodules affecting *B. taylori* and *S. hispidus* were only observed in the summer and fall of 2013. Because samples were not taken, the cause or origin of these nodules remains undetermined, although their appearance is suggestive of viral papillomas or polyps. There is a growing concern for *Leishmania* in the state, for which rodents are known to be reservoirs and skin lesions would be expected. However, an etiology of *Leishmania* for the observed rodent lesions seems unlikely, as such lesions are typically ulcerative and not proliferative.

This study found two species of ticks on mammals at this field site, both of which have been previously recorded in Texas (15, 36). Previous studies have found that coastal *A. maculatum* populations have a different phenology than that of inland populations. Coastal populations showed a peak of larval and nymphal feeding in January and February (37) while inland populations show peak larval and nymphal

feeding in the summer (38). While it appears the population in this field site is following the phenology of inland populations, I cannot make any definitive conclusions on *A. maculatum* phenology at this time because of the low and inconsistent collection of ticks over the course of the study.

In this study, I was unable to conclude that a population of *I. scapularis* was established at this field site as only 3 nymphs were collected from small mammals. I am also unable to make any definitive conclusions of *I. scapularis* phenology. However, two of the *I. scapularis* nymphs were collected in the summer months and the third was collected at the beginning of fall. This is consistent with previous studies that have found *I. scapularis* nymphs and larvae active during the summer months (39, 40). Further studies should incorporate additional methods of tick captures in order to make better conclusions on tick populations and phenology.

While an established population of *I. scapularis* could not be confirmed, its presence along with that of *A. maculatum* and rodent reservoir hosts presents a possible threat of TBDs to the human population of Brazos County. Further studies should be conducted in order to monitor this community of rodents and ticks into the future.

CHAPTER III

TICK-BORNE PATHOGENS IN A RODENT COMMUNITY IN EAST-CENTRAL TEXAS

Introduction

In recent years, an unprecedented number of novel tick-borne diseases (TBDs) have been detected based on the presentation and diagnostic testing of sick humans as well as through investigations of tick populations to detect etiologic agents (41-45). Such discoveries expose a critical need to better understand the ecology of TBDs in an effort to protect human and animal health. Critical aspects of TBD ecology that must be elucidated to reduce economic and public health consequences include the identification of key reservoir hosts and competent vectors as well as how the interaction of these species varies spatially and temporally.

The southern United States harbors several species of ticks that can transmit zoonotic pathogens to native fauna and humans. The most common human-biting tick in the southern United States is *Amblyomma americanum* (the lone star tick; 46), which serves as a vector for the agents of ehrlichiosis, Rocky Mountain spotted fever, and tularemia. *Amblyomma maculatum* (the Gulf Coast tick) and *Ixodes scapularis* (the blacklegged tick) also are widely distributed (15) across the south. The former transmits agents of spotted fever rickettsiosis, and the latter transmits the agents of Lyme disease, anaplasmosis, and babesiosis.

In the southern United States, *I. scapularis* is rarely implicated in human-biting (46), and therefore the pathogens it vectors present less of a public health burden relative to the northern United States. Consequently, despite the widespread distribution of *I. scapularis* across the southern United States, less than 5% of Lyme disease cases and less than 4% of anaplasmosis cases in the United States occur across the southern United States (Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Texas, and Virginia; 47). In contrast, *Amblyomma*-transmitted diseases are more common in the south. For example, over half of the ehrlichiosis cases and over 70% of the spotted fever rickettsiosis cases originate in this region (47).

Wild rodent species are important in the ecology of many TBDs because they serve as reservoir hosts for pathogens, and therefore they often are assessed as sentinels to gauge the level of tick-borne pathogen activity within a given geographic area (8, 48). As generalists, *I. scapularis, A. americanum* and *A. maculatum* all feed on members of the small mammal community (49) including the white-footed mouse, *Peromyscus leucopus*, which is a known reservoir for many tick-borne pathogens (6-8). My objective was to examine the dynamics of tick and tick-borne pathogen occurrence within a wild community of small mammals to provide information that is useful for regional assessments of human risk and public health protection in the southern United States.

Materials and Methods

For two consecutive trap nights each month from May 2012 – November 2013, small mammals were live-trapped at the Biodiversity Research and Teaching Collection

natural area in College Station, TX (30°38'47.2"N 96°17'45.9"W). Captured mammals were visually identified to species and sex, weighed, and ear tagged (National Band and Tag, Newport, KY). Mammals were inspected thoroughly for ticks, which were removed for analysis and stored in 70% ethanol. From each captured a mammal, a blood sample and a 2mm-diameter ear biopsy was taken to quantify pathogen presence and stored in 70% ethanol. All animals collected during this study were treated humanely according to the guidelines provided by the American Society of Mammalogists (18) and the Texas A&M Animal Care and Use Committee (Permit# 2012-100).

To assess phenology of off-host ticks, questing ticks were sampled using a $1m^2$ corduroy drag cloth to sweep the vegetation along the trapping transects at monthly intervals (50).

In the laboratory, ticks were identified to species and life stage using a dichotomous key (19). Total tick and mammal DNA extraction was performed using commercially available kits (DNeasy Blood and Tissue Kit; Qiagen, Valencia, CA, and E.Z.N.A Tissue DNA Kit; Omega Bio-Tek, Norcross, GA) according to manufacturer's instructions, but with a final elution of 60 µL with 70°C elution buffer. Prior to extraction, ticks were macerated with a sterile scalpel. The DNA from adult and nymphal ticks was extracted individually, whereas larval ticks of the same species and host were pooled for extraction. For mammals, one ear biopsy per capture was selected for extraction.

Three separate PCRs for the detection of *Borrelia, Rickettsia*, and *Ehrlichia* genus pathogens were performed using the following previously described assays: a

nested PCRs for the 16S-23S rRNA intergenic spacer region (IGS) of *Borrelia* species (51) using DNA of *B. burgdorferi* and *B. miyamotoi* from field-collected Midwestern *I. scapularis* as positive controls; a conventional PCR for the citrate synthase gene of *Rickettsia* species (617 bp; 52) using DNA of *R. amblyommii* from field-collected *A. americanum* as a positive control; and a conventional PCR for the 16S rRNA gene of *Ehrlichia* species using DNA from *E. muris* and *E. chaffeensis* from field-collected ticks as positive controls (431 bp; 53). PCR products were visualized using gel electrophoresis. All rodent ear biopsies were tested for *Borrelia*, and subset was tested for *Rickettsia*. A subset of rodent blood samples was tested for *Ehrlichia*. All ticks were tested for *Borrelia*, *Rickettsia*, and *Ehrlichia*. PCR amplicons were purified (ExoSAP-IT; Affymetrix, Santa Clara, CA) and sequenced in both directions using the same primers used in the respective PCRs; for *Borrelia* the inner primers for the IGS nested reaction were used for sequencing. Sequencing was performed at Eton Bioscience Inc. using ABI 3730xl DNA Sequencers and sequences were annotated using Sequencher 4.9 (GeneCodes Corporation; Madison, WI). Annotated sequences were compared to published sequences using the basic local alignment search tool (BLAST) in GenBank for species identification (22).

Results

Over the 19 months of study, there were a total of 943 small mammal captures, representing 621 individuals. Five species were captured during the course of the study: the hispid cotton rat (*Sigmodon hispidus*; n = 514 captures and 384 individuals), the fulvous harvest mouse (*Reithrodontomys fulvescens*; n = 135 and 75 individuals), the

white-footed mouse (*Peromyscus leucopus*; *n* = 130 and 66 individuals), the northern pygmy mouse (*Baiomys taylori*; *n* = 82 and 75 individuals), and the cotton mouse (*Peromyscus gossypinus*; *n* = 82 and 21 individuals.

In an analysis of 698 rodent ear biopsies representing 491 individuals, we detected infection with a *Borrelia* pathogen in 23 biopsies from 22 different individuals. DNA sequencing revealed that 22 specimens (3.2%) were infected with *Borrelia miyamotoi* and a single specimen (0.1%) was infected with *B. lonestari* (Table 4). No mammals were infected with *B. burgdorferi*. *Borrelia miyamotoi* was detected in four rodent species: *S. hispidus* (*n* = 9), *P. gossypinus* (*n* = 6), *R. fulvescens* (*n* = 4), and *P. leucopus* (*n* = 3). One *P. gossypinus* individual was shown to be infected with *B. miyamotoi* on two occasions (September 2012 and March 2013). Three recaptured individuals were determined to be infected at their last capture only (i.e., became infected during the study).

A subset (*n* = 163) of rodent ear biopsies was tested for the presence of *Rickettsia,* and results were uniformly negative. A subset of rodent blood samples (*n* = 24) was tested for the presence of *Ehrlichia* without detection.

The tick infestation prevalence of small mammals was 2.3% (Table 5). A total of 98 ticks was collected from small mammals (*B. taylori, P. leucopus,* and *S. hispidus*; Table II). All ticks collected from rodent hosts were larvae and nymphs of two species: *A. maculatum* and *I. scapularis* (Table 5). The largest burden of ticks on hosts was 40 *A. maculatum* larvae collected from one *S. hispidus* individual. Two ticks (adult *A.*

Table 4. Infection prevalence in rodents. Numbers of ear biopsies and blood samples from rodents are indicated, as is number of individuals (and percentage prevalence) infected with each pathogen. Primers used were specific to *Borrelia, Rickettsia*, and *Ehrlichia* genus (see text). No samples were positive for *B. burgdorferi*.

Species	*Borrelia*			*Rickettsia*		*Ehrlichia*	
	Biopsies Tested	*B. miyamotoi*	*B. lonestari*	Biopsies Tested	Positive	Blood Samples Tested	Positive
Baiomys taylori	62	0	0	4	0	0	0
Peromyscus gossypinus	72	6 (8.3%)	0	36	0	7	0
Peromyscus leucopus	88	3 (3.4%)	0	42	0	6	0
Reithrodontomys fulvescens	123	4 (2.9%)	0	44	0	4	0
Sigmodon hispidus	353	9 (2.3%)	1 (0.3%)	37	0	7	0
Total	**698**	**22 (3.2%)**	**1 (0.1%)**	**163**	**0**	**24**	**0**

Table 5. Ticks collected off rodents. Number of rodent hosts checked and infested with ticks is indicated (with percentage prevalence in parentheses), as is identification and life stage of identified ticks.

Species	Checked for ticks	Infested with ticks	*A. maculatum*		*I. scapularis*	
			Larva	Nymph	Larva	Nymph
Baiomys taylori	82	1 (1.2%)	1	0	0	0
Peromyscus gossypinus	82	0	0	0	0	0
Peromyscus leucopus	130	2 (1.5%)	6	4	0	0
Reithrodontomys fulvescens	135	0	0	0	0	0
Sigmodon hispidus	514	19 (3.7%)	63	21	0	3
Total	943	22 (2.3%)	70	25	0	3

maculatum and adult *D. variabilis)* were found crawling on technicians. No ticks were collected on drag cloths in over 14,500 m^2 of drag sampling across the 19-month study. All 100 ticks were screened *Borrelia*, *Ehrlichia*, and *Rickettsia* pathogens. No ticks were found to be infected with *Borrelia* or *Ehrlichia* (Table 6). Three of 15 *A. maculatum* pools representing 70 larvae (4.3%) and one of 25 *A. maculatum* nymphs (4%) tested positive for a rickettsial endosymbiont most similar to Genbank GU131156. One *I. scapularis* nymph and one *A. maculatum* larval pool of 6 larvae were infected with a pathogen that matched with 100% identity to *Rickettsia monacensis* sequences found on GenBank (Table III). All rodent and tick pathogen sequences were submitted to GenBank (GenBank accession numbers XXXX-XXXX for *Borrelia miyamotoi and B. lonestari*, XXXX-XXXX for *Rickettsia monacensis*, and XXXX-XXX for *Rickettsia* endosymbionts).

Discussion

This study underscores the importance of field-based wildlife studies to learn about the ecology of emerging human pathogens especially in the southern United States where there is a lot of confusion about the ecology and etiology of TBDs (46). I report *B. miyamotoi* infection in a community of wild rodents (2.8% overall infection prevalence) in east-central Texas. *Borrelia miyamotoi* was recently recognized as a human pathogen after human cases were confirmed in Russia and the United States (42, 45). Although *Ixodes* ticks are recognized as the main vector of *B. miyamotoi* (54-57), *I. scapularis* was rarely encountered at my field site, with only 3 of 943 rodents infested with *I. scapularis* across 19 months, and no ticks collected during drag sampling of over

Table 6. Infection prevalence in ticks. Numbers of ticks sampled are indicated, as is number of individuals (and percentage prevalence) infected with each pathogen. Primers used were specific to *Borrelia, Rickettsia*, and *Ehrlichia* genus (see text). No samples were positive for *B. burgdorferi*.

Species	Samples Tested			*B. miyamotoi*	*B. lonestari*	*R. monacensis*		Rickettsial endosymbiont		*Ehrlichia*
	Larva	Nymph	Adult			Larva	Nymph	Larva	Nymph	
Amblyomma maculatum	70	25	1	0	0	1 (1.4%)*	0	3 (4.3%)**	1	0
Ixodes scapularis	0	3	0	0	0	0	1 (33%)	0	0	0
Dermacentor variabilis	0	0	1	0	0	0	0	0	0	0

*Larval pool of 6 ticks. Thus, minimum infection prevalence is reported.

**Includes 3 larval pools totaling 11 ticks. Thus, minimum infection prevalence is reported.

14,500 m^2 of vegetation. Accordingly, *I. scapularis* at this field site does not meet the CDC criteria for establishment (58), and the rare nymphal specimens we encountered may result from bird drop-offs of larvae earlier in the season or other importation events. Other tick species may therefore be involved in maintaining *B. miyamotoi* in this small mammal community. Future studies should consider using tick traps and exploration of ticks on other wildlife species in addition to drag sampling in order to maximize tick collections to explore this possibility.

B. miyamotoi typically co-occurs in vector and wildlife populations with *B. burgdorferi*, but its infection prevalence is normally an order of magnitude lower than *B. burgdorferi*. For example, a study in Connecticut where *I. scapularis* is established found 12.4% and 6.5% of captured mice were infected with *B. burgdorferi* and *B. miyamotoi*, respectively (59). In Lyme disease endemic areas, previous studies have found that *I. scapularis* and *I. pacificus* typically have a *B. miyamotoi* infection prevalence of 1-2% in adult ticks (8, 55, 60). Another study in New York found 64% of *I. scapularis* ticks were infected with *B. burgdorferi* while only 2% were infected with *B. miyamotoi* (61). In this Texas study, however, *B. miyamotoi* infection in rodents (2.8%) was present without *B. burgdorferi*. Notably, the current infection prevalence reported in this study is based only on samples confirmed with a sequence of the IGS region, and without this stringent criterion the rodent infection prevalence could be even higher.

Wildlife reservoirs for *B. miyamotoi* in the United States are largely unknown. We document *B. miyamotoi* infection in four small mammal species (*S. hispidus, P.*

gossypinus, P. leucopus, and *R. fulvescens*), although future studies are needed to test reservoir competence. *P. gossypinus*, *R. fulvescens* and *S. hispidus* have not previously been reported to be infected, whereas *P. leucopus* has previously been shown to be infected with *B. miyamotoi* (8). *B. miyamotoi* infection has also been seen in the bank vole (*Myodes glareolus*), old world field mice (Apodemus spp.; 62), and wild birds (63, 64). My findings of infected rodents indicate that *B. miyamotoi* may be maintained in multiple wildlife reservoir species in the apparent absence of both *I. scapularis* and *B. burgdorferi*.

Two percent of the ticks (Table 6) found in this study were infected with *Rickettsia monacensis* - a spotted fever pathogen associated with *Ixodes ricinus* ticks in Europe and North Africa (65, 66) that has been shown to cause a Mediterranean spotted fever-like illness in humans (67). This agent has not previously been reported in North America. While human risk cannot be determined based on this study alone, my findings of *R. monacensis* in native wildlife-associated ticks that are capable of biting humans underscores the importance of this ecological study for identifying pathogens that may emerge in human populations in the future.

TBDs are likely to become greater health concerns as vectors and pathogens continue experiencing range expansions exposing increasingly larger populations to disease (68, 69). All in all, my study identifies novel TBDs in the southern United States (*B. miyamotoi* and *R. monacensis*), while at the same time highlights that much more work needs to be done to understand the ecology of TBDs, especially in understudied areas.

CHAPTER IV

CONCLUSION

I describe the seasonal dynamics of a rodent and tick community in east-central Texas, and these ecological data are important considering the potential role of these species in the enzootic maintenance of pathogens that may cause tick-borne diseases. Overall, five rodent species were encountered at this field site in Brazos County, of which *P. leucopus*, *P. gossypinus*, and *S. hispidus* have previously been implicated as reservoirs for tick-borne pathogens in other regions of the country. Their presence allows the possibility for the maintenance of TBD pathogens at this location.

While an established population of *I. scapularis* could not be confirmed, its presence along with that of *A. maculatum* and rodent reservoir hosts presents a possible threat of TBDs to the human population of Brazos County. Further studies should be conducted in order to monitor this community of rodents and ticks into the future.

This study also underscores the importance of field-based wildlife studies to learn about the ecology of emerging human pathogens especially in the southern United States where there is a lot of confusion about the ecology and etiology of TBDs. I report *B. miyamotoi* infection in a community of wild rodents (2.8% overall infection prevalence) in east-central Texas. *Borrelia miyamotoi* was recently recognized as a human pathogen after human cases were confirmed in Russia and the United States. *B. miyamotoi* typically co-occurs in vector and wildlife populations with *B. burgdorferi*, but its infection prevalence is normally an order of magnitude lower than *B. burgdorferi*. Furthermore, *Ixodes* ticks are recognized as the main vector of *B. miyamotoi*. My

findings of infected rodents indicate that *B. miyamotoi* may be maintained in multiple wildlife reservoir species in the apparent absence of both *I. scapularis* and *B. burgdorferi*.

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