

## Survey of a Rodent and Tick Community in East-Central Texas

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**Abstract** - We conducted a survey of rodents and ticks in Brazos County in east-central Texas to learn more about native fauna that may be involved in enzootic transmission of pathogens that can cause tick-borne diseases in this region. Our objectives were to describe the species richness and seasonal activity of rodents, as well as to document their infestation with ticks over time. We captured 5 rodent species over the course of 19 months: *Sigmodon hispidus* (Hispid Cotton Rat), *Reithrodontomys fulvescens* (Fulvous Harvest Mouse), *Peromyscus leucopus* (White-footed Mouse), *Peromyscus gossypinus* (Cotton Mouse), and *Baiomys taylori* (Northern Pygmy Mouse). We observed a large increase in Hispid Cotton Rat capture success in the fall of 2013, reflecting a characteristic population boom periodically exhibited by this species. Overall tick-infestation prevalence of rodents was low (2.3%), and was comprised of juvenile ticks of 2 species—*Amblyomma maculatum* (Gulf Coast Tick) and *Ixodes scapularis* (Blacklegged Tick). The co-occurrence of tick vectors and rodent species that are known reservoirs of tick-borne pathogens underscores the importance of studies to assess tick-borne disease risk in the region.

### Introduction

Rodent species serve as reservoirs for zoonotic pathogens, many of which are transmitted by ticks (Meerburg et al. 2009). Over 70 species of rodents can be found in Texas, including 20 species that occur in east-central Texas (Schmidly 2004). Some of these species are recognized as being involved in tick-borne pathogen-transmission cycles in other areas of their distribution. For example, in the northeastern and midwestern US, *Peromyscus leucopus* (Rafinesque) (White-footed Mouse) is a reservoir for many tick-borne pathogens including those that cause ehrlichiosis (*Ehrlichia* spp.), Lyme disease (*Borrelia burgdorferi*), and babesiosis (*Babesia* spp.) (Gage et al. 1995, Hamer et al. 2010, Stafford et al. 1999). In the eastern and southeastern US, *Peromyscus gossypinus* (LeConte) (Cotton Mouse) is a reservoir for tick-borne pathogens that cause Lyme disease and human granulocytic ehrlichiosis (Magnarelli et al. 1999, Oliver 1996, Oliver et al. 2003, Rudenko et al. 2009), and *Sigmodon hispidus* Say and Ord (Hispid Cotton Rat), *Neotoma floridana* (Ord) (Eastern Woodrat), and *Orzomyomys palustris* (Harlan) (Rice Rat) are reservoir hosts for the causative agent of Lyme disease (Levin et al. 1995, Oliver 1996, Oliver et al. 2003). However, the epidemiological significance of these species in Texas is largely unknown.

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Common human-biting ticks in east-central Texas include *Amblyomma americanum* L. (Lone Star Tick), *Amblyomma maculatum* Koch (Gulf Coast Tick), *Dermacentor variabilis* (Say) (American Dog Tick), and *Ixodes scapularis* Say (Blacklegged Tick) (Merten and Durden 2000). These tick species are among the most medically important tick vectors in the US due to their ability to transmit a suite of zoonotic pathogens, including those that cause ehrlichiosis, rickettsioses, tularemia, Lyme disease, human anaplasmosis, and babesiosis in human and animal populations (Childs and Paddock 2003, Stromdahl and Hickling 2012). Given the diverse assemblage of rodent and tick species in east-central Texas, and the possibility that they could serve as reservoirs and vectors of tick-borne diseases, it is vital to learn more about the rodent and tick species in this area for better understanding disease risk in this region. This study used a mark–recapture approach to assess the rodents and ticks at a field site in Brazos County, east-central Texas.

### Field-Site Description

From May 2012 to November 2013, we sampled ticks and rodents monthly in the natural area around the Texas A&M Biodiversity Research and Teaching Collections, Texas A&M University, College Station, TX (30°38'47.2"N 96°17'45.9"W) along several transects (see below). Vegetation along each transect varied, generally ranging from grass and shrubs to *Quercus stellata* Wagh. (Post Oak) forest. Common vegetation encountered at this field site included Post Oak, *Schyzachyrium scoparium* (Michx.) Nash (Little Bluestem), *Sorghastrum nutans* (L.) Nash (Indiangrass), and *Ilex vomitoria* Aiton (Yaupon).

### Methods

Monthly sampling involved tick collections using standard drag-sample methodologies and rodent trapping for 2 consecutive trap nights. To trap rodents, we baited Sherman live traps (H.B. Sherman Traps, Tallahassee, FL) with sunflower seeds and set them along 4 transects (varying in length from ~0.8 km to 1.13 km), with 47–70 traps per transect (traps were ~10 m apart). To reduce trap mortalities, we avoided fire-ant mounds when setting traps, used trained personnel to conservatively apply inhalant anesthetic, placed polyfill in traps on cold nights, and set traps late in evening on hot nights and recovered captures early the following morning.

To measure overall trapping success and trapping success per species, we quantified trap effort using the effective trap-night metric (ETN) as follows: we counted each trap deployed overnight as 1 trap night. If we found a trap closed without a rodent (tripped), we counted it as half a trap night (i.e., adjusted trap night) based on the assumption that, on average, it was unavailable to capture a rodent for half a night (Nelson and Clark 1973, Sutherland 1996).

We identified to species and processed captured animals as detailed below. In some circumstances, we released animals after identification without processing due to high capture success and high temperatures, thus avoiding the stress

associated with prolonged restraint in the traps. We included all captured animals in the calculations of trap success; only those animals fully processed were included in reports of sex and tick infestation.

We weighed and visually identified captured rodents to species and sex, anesthetizing individuals using Isoflurane (Abbot Laboratories, Abbott Park, IL) when necessary to facilitate processing. We noted trap location and checked rodents for the presence of ticks, which we removed and stored in 70% ethanol. We placed an ear tag (size 1 Monel tags: 2.36-mm thick and ~0.25 g each; National Band and Tag, Newport, KY) to mark the animal in case of recapture. We recorded status and location for each individual that was recaptured. We took a 2-mm diameter ear-punch biopsy and blood sample from all new captures and, after processing was complete, released the rodents at their capture sites. We treated all animals collected during this study humanely according to the guidelines provided by the American Society of Mammalogists (Sikes et al. 2011) and the Texas A&M Animal Care and Use Committee (Permit #2012-100). We prepared all incidental mortalities as museum specimens and deposited them at the Texas A&M Biodiversity Research and Teaching Collections.

To assess phenology of off-host ticks, we sampled questing ticks using a 1-m<sup>2</sup> corduroy drag-cloth to sweep the vegetation along the trapping transects at monthly intervals (Falco and Fish 1992). Drag sampling always occurred on different days from, but within 2 weeks of, rodent trapping to avoid disrupting traps. Every 10 m, we examined drag cloths for ticks, which we removed and stored in 70% ethanol. We transported all ticks from captured rodents or obtained from drag cloths to the lab for identification to species using a dichotomous key (Sonenshine 1979).

We confirmed the species identity of ticks and rodents using the molecular methods described below. We performed total rodent and tick DNA extraction on single ear biopsies, single nymphal ticks, or pooled larval ticks (pools comprised all conspecific ticks collected from the same host individual at the same time) using commercially available kits—DNeasy<sup>®</sup> Blood and Tissue Kit (Qiagen, Valencia, CA) and EZNA<sup>®</sup> Tissue DNA Kit (Omega Bio-Tek, Norcross, GA) according to manufacturer's recommendations and using a final elution of 60 µL with elution buffer at 70 °C. We verified rodent-species identification through amplification of the cytochrome *b* gene according to the protocols of Molaei et al. (2006). We subjected 2 randomly selected specimens from all rodent species to molecular analysis to confirm species identification. In the case of *Peromyscus* specimens, where species can be difficult to distinguish based on morphologic features, we performed molecular analysis on all individuals to confirm species identity. We amplified the 12S rRNA gene according to the protocols of Beati and Keirans (2001) to confirm tick-species identification for all collected ticks. We purified polymerase chain reaction (PCR) amplicons (ExoSAP-IT<sup>®</sup>, Affymetrix, Santa Clara, CA) and sequenced them in 1 direction using ABI 3730xl DNA Sequencers (Eton Bioscience Inc, San Diego, CA and Beckman Coulter Genomics, Danvers, MA). We used Sequencher 4.9 (GeneCodes Corporation, Madison, WI) to annotate sequences and compared them to published sequences using the basic local alignment search tool

(BLAST) in GenBank (Altschul et al. 1990) for identification confirmation. All remaining ear biopsies, ticks, and DNA extractions were deposited in the Texas A&M Biodiversity Research and Teaching Collections.

## Results

Over the 19-month study, we captured a total of 980 rodents, representing 797 individuals. We captured 5 species: Hispid Cotton Rat, Fulvous Harvest Mouse, White-footed Mouse, Cotton Mouse, and *Baiomys taylori* (Thomas) (Northern Pygmy Mouse). Molecular work verified all species identifications for the subset of fully processed specimens subjected to molecular confirmation of identification (Table 1); 18 *Peromyscus* were not fully processed and therefore were identified to the genus level only. We captured Hispid Cotton Rat most frequently and Northern Pygmy Mouse least frequently (Table 1). A total of 3.0% of captures were mortality events attributed to the following causes: unknown ( $n = 18$ ), predation by *Solenopsis invicta* Buren (Red Imported Fire Ant;  $n = 2$ ), cold weather ( $n = 2$ ), anesthetic overdose ( $n = 1$ ), and heat-related death ( $n = 6$ ).

Capture success was lowest in July 2012, averaging 1.0 captures per 100 ETNs (Fig. 1). Peak capture success occurred in September 2013 with an average of 44.2 total captures per 100 ETNs representing all 5 species. With the exception of September of 2013, capture success for Northern Pygmy Mouse was relatively low compared to the other species (Fig. 1). Fulvous Harvest Mouse was captured infrequently 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 success for Hispid Cotton Rat increased significantly in mid- and late 2013 (Fig. 1). Although capture success for both *Peromyscus* species was initially similar, there were some periods of time (June 2012, September–December 2012, and July–October 2013) when White-footed Mouse was caught more frequently than Cotton Mouse, and 1 period (January–June 2013) when Cotton Mouse was captured more frequently (Fig. 1).

Over the duration of the study, we recaptured 183 individuals (representing all 5 species) at least once (Table 2). These 183 recaptures included 18 individuals that

Table 1. Total number of rodent captures and recaptures listed by species throughout the duration of the 19-month study in east-central Texas, 2012–2013. \*The 18 *Peromyscus* sp. specimens were not fully processed and therefore not identified to species. Total captures = % of total captures across all species. Recaptures = % of total captures/species.

Species	Total captures	Recaptures
<i>Sigmodon hispidus</i> (Hispid Cotton Rat)	530 (54.1%)	140 (26.4%)
<i>Reithrodontomys fulvescens</i> (Fulvous Harvest Mouse)	144 (14.7%)	38 (26.4%)
<i>Peromyscus leucopus</i> (White-footed Mouse)	104 (10.6%)	54 (51.9%)
<i>Baiomys taylori</i> (Northern Pygmy Mouse)	94 (9.6%)	2 (2.1%)
<i>Peromyscus gossypinus</i> (Cotton Mouse)	90 (9.2%)	58 (64.4%)
<i>Peromyscus</i> sp.*	18 (1.8%)	
Total	980	292 (29.8%)

had clearly been captured previously but had lost their initial ear tag (as evidenced by healing circular biopsy sites in their ears and a small tear in the ear tissue where the ear tag had been placed). Counting only the times we recaptured these 183 individuals (i.e., not including their initial capture), there were a total of 292 recapture events, more than one-third (104, representing 69 individuals) of which occurred the night after a previous capture. Twenty of these individuals were only recaptured the night after their initial capture (Table 2).

Of the 183 recaptured individuals, 95 were Hispid Cotton Rat (51.9%), 32 Fulvous Harvest Mouse (17.5%), 28 White-footed Mouse (15.3%), 25 Cotton Mouse (13.7%), and 3 Northern Pygmy Mouse (1.6%). Of the 292 recapture events, Hispid Cotton Rat was recaptured with the highest frequency (47.9%) followed by Cotton

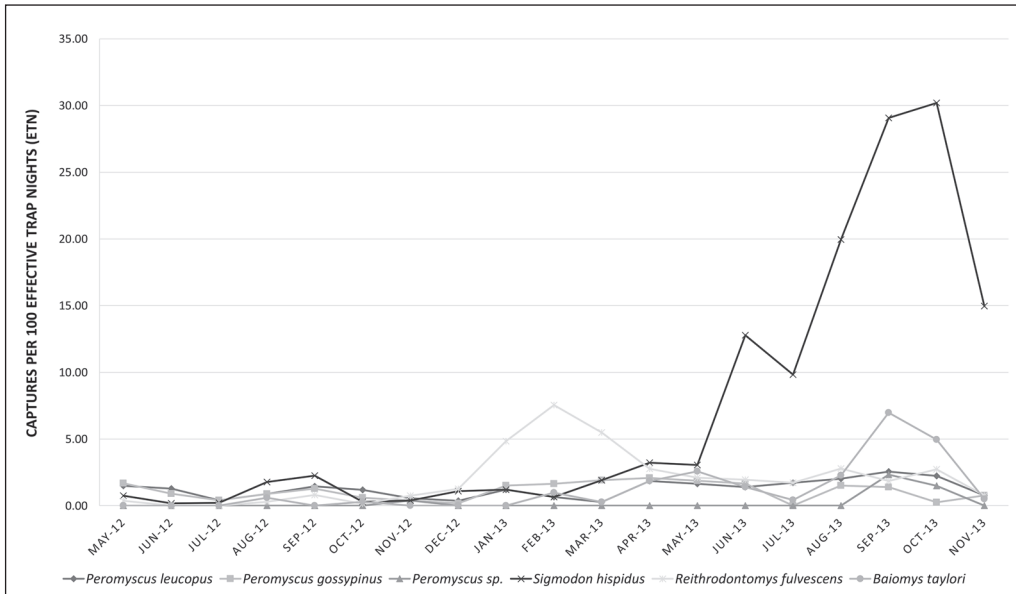


Figure 1. Captures per 100 effective trap nights (ETN) per rodent species over the 19-month study. ETN was calculated by adding all full and adjusted trap nights (see text).

Table 2. Time period in months between initial and final capture for all recaptured individuals. Values do not include recaptured individuals whose initial ear tags were lost because we could not determine initial capture date ( $n = 18$ ; see text). We recaptured a total of 20 individuals only the night after their initial capture (column 0).

Capture incidence	Time between initial and final capture (months)													Total	
	0	1	2	3	4	5	6	7	8	9	10	11	12		13
2	20	47	16	6	3	4	-	1	-	-	-	-	-	1	98
3	-	11	13	5	2	2	1	-	1	-	-	-	-	-	35
4	-	3	5	6	2	-	-	-	-	-	-	-	-	-	16
5	-	-	1	2	5	1	-	-	1	-	-	-	-	-	10
6	-	-	-	-	2	1	-	-	-	-	-	-	-	-	3
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	1	-	-	1	1	-	-	3
Total	20	61	35	19	14	8	1	2	2	-	1	1	-	1	165

Mouse (19.9%), White-footed Mouse (18.5%), Fulvous Harvest Mouse (13.0%), and Northern Pygmy Mouse (0.7%) (Table 1). As a proportion of the total captures of each species across the study, recaptures were most common in Cotton Mouse, followed by White-footed Mouse, Hispid Cotton Rat, Fulvous Harvest Mouse, and Northern Pygmy Mouse (Table 1). Many of the recaptured individuals were captured a total of 2 times (59.4%), but 3 individuals (2 Cotton Mouse and 1 Fulvous Harvest Mouse) were captured 8 times, which was the highest recapture frequency over the course of the 19-month study (Table 2). Time between captures of an individual ranged from 1 to 13 months (Table 2). The individuals with the longest time between initial and final captures included 1 Cotton Mouse (10 months), 1 Fulvous Harvest Mouse (11 months), and 1 Cotton Mouse (13 months) (Table 2).

We fully processed 779 of the total 980 total captures, and of these specimens, we were able to determine sex in 767 individuals for a male–female ratio of 1.1:1 for all 5 species combined. We captured more males in May–November 2012, April–May 2013, and August–October 2013; more females were captured in January–March 2013, June–July 2013, and November 2013. Observed Cotton Mouse, White-footed Mouse, and Fulvous Harvest Mouse males outnumbered females with ratios of 1.5:1, 2.1:1, and 1.3:1, respectively, and observed Northern Pygmy Mouse females outnumbered males 2.9:1.

We removed a total of 98 ticks from 22 rodents, representing Northern Pygmy Mouse, White-footed Mouse, and Hispid Cotton Rat, over the course of the study, resulting a 2.2% (22 of 980) tick-infestation rate among captures. We collected larvae and nymphs of 2 species: Blacklegged Tick (nymphs only, representing 3.1% of all ticks) and Gulf Coast Tick (larvae and nymphs, representing 71.4% and 25.5% of all ticks, respectively). Molecular work verified all tick-species identifications. Tick burden on infested individuals ranged from 1 to 40 larvae and 1 to 10 nymphs, with the majority of rodents infested with ticks in the late summer months (July–September). Specifically, we found the 3 Blacklegged Tick nymphs in July, August, and September. The Gulf Coast Tick larvae were present in May, July, and September, and the nymphs were present in August and September. We counted 40 Gulf Coast Tick larvae on a single Hispid Cotton Mouse. Blacklegged Tick nymphs were found exclusively on Hispid Cotton Rat. We located Gulf Coast Tick larvae on 1 Northern Pygmy Mouse, 1 White-footed Mouse, and 10 Hispid Cotton Rat individuals over the course of the study. We observed Gulf Coast Tick nymphs on 1 White-footed Mouse and 8 Hispid Cotton Rat individuals. One Hispid Cotton Rat was co-infested with a Blacklegged Tick nymph and a Gulf Coast Tick larva, and another was infested with larval and nymphal Gulf Coast Ticks; no ticks were found on Fulvous Harvest Mouse or Cotton Mouse. Of the 22 infested rodents, 11 were female and 11 were male; therefore, we observed no apparent sex bias in tick infestation, given the male:female ratio of captured rodents was 1.1:1. Although we collected no ticks on drag cloths in over 14,500 m<sup>2</sup> of drag sampling across the 19-month study, we documented 2 ticks—an adult Gulf Coast Tick and an adult American Dog Tick—crawling on technicians during drag sampling and setting of live traps.

## Discussion

We described the rodent and tick community at a focal field site in east-central Texas; these data are important in considering the potential role of these species in the enzootic maintenance of pathogens that may cause tick-borne diseases. Overall, we encountered 5 rodent species at this field site in Brazos County, 3 of which (White-footed Mouse, Cotton Mouse, and Hispid Cotton Rat) have been previously implicated as reservoirs for tick-borne pathogens in other regions of the country. To date, the epidemiological significance of these species in Texas is largely unknown. Therefore, future research will test samples collected as part of this study for pathogen presence and identity to better understand the role these species may play in the tick-borne disease maintenance in east-central Texas.

Capture success for all species was lower in 2012 than 2013, with averages of 3.2 and 19.8 captures per 100 ETNs, respectively (Fig. 1). During this time, Hispid Cotton Rat capture success increased from an average of 1.3 captures per 100 ETNs in May 2012–May 2013 to 19.5 captures per 100 ETNs in June–November 2013. Fluctuations in Hispid Cotton Rat populations are not uncommon, and have been reported as early as the late 1920s. In fact, numerous studies suggest that population fluctuations in Hispid Cotton Rat are to be expected (Grant et al. 1985, Haines 1963, Strecker 1929). Fulvous Cotton Mouse and Northern Pygmy Mouse also showed seasonal variations in capture rates, with overall low capture success in the summer months, likely due to abundant food resources in the area such that these rodent species were not as attracted to the baits in the traps. During our study, we observed a large peak for Fulvous Cotton Mouse captures in the winter and a smaller peak in the summer, which is congruent with previous reports of a bimodal population-density pattern (Spencer and Cameron 1982). In Northern Pygmy Mouse, we documented a large peak in early fall and a smaller peak in late spring, which is again consistent with previous reports of population peaks in early fall and winter (Eshelman and Cameron 1987). Neither *Peromyscus* species exhibited noticeable seasonal variation in capture success. Notably, activity patterns may also be linked to reproductive status because both *Peromyscus* species are known to breed year-round in the southern parts of their ranges (data available upon request; Cameron and Spencer 1981, Eshelman and Cameron 1987, Lackey et al. 1985, Spencer and Cameron 1982, Wolfe and Linzey 1977).

We found 2 species of ticks (Gulf Coast Tick and Blacklegged Tick) on rodents, both of which have been previously recorded in Texas (Bishopp and Trembley 1945, Merten and Durden 2000). Tick infestation of rodents was 2.2% and comprised mainly of Gulf Coast Ticks, nearly half of which were found on a single Hispid Cotton Rat. Our results make statistical inferences regarding tick populations and phenology difficult. However, we are able to make some general observations for each tick species. With nymphal and larval activity highest in the summer, it appears that at our collecting locality Gulf Coast Tick may be following a phenology similar to those of inland (vs coastal) populations. Previous studies have found that coastal Gulf Coast Ticks showed a peak of larval and nymphal feeding in January and February (Teel et al. 1998), whereas inland populations showed peak larval and

nymphal feeding in the summer (Barker et al. 2004). The numbers of Blacklegged Ticks in this study were especially low, with only 3 nymphs collected from rodents. The Centers for Disease Control and Prevention criterion for demonstrating an established population of Blacklegged Tick is that a minimum of 6 individual ticks or a minimum of 2 different life stages must be present in a given collection period (Dennis et al. 1998). Our sampling methodologies may not have been sufficient to detect the minimum number of Blacklegged Ticks to recognize the species as established. The limited number of collected Blacklegged Ticks in our study does not afford any conclusions about seasonal phenology, although we note that our 3 specimens were collected in the summer and early fall, consistent with previous studies that have found nymphs and larvae of this species active during the summer months (Falco et al. 1999, Kollars et al. 1999).

Previous studies have shown that both Blacklegged Ticks and Gulf Coast Ticks infest larger mammals and birds (Piesman and Spielman 1979, Teel et al. 2010). Further studies should incorporate additional tick-capture methods to make broader conclusions on tick populations and phenology at this collection site. In addition, it should be noted that this field site contained abundant Red Imported Fire Ants, which are known to prey on ticks (Burns and Melancon 1977, Harris and Burns 1972). Therefore, the presence of Red Imported Fire Ants, in addition to other possible mammal and bird tick hosts, may have contributed to the low apparent tick prevalence in our study.

Although tick abundance was low and we only assessed rodents, the presence of Blacklegged Ticks and Gulf Coast Ticks, as well as multiple rodent species that are known reservoirs for various tick-borne pathogens, suggests that future studies should be conducted to monitor the risk of tick-borne disease to the wildlife, livestock, and human populations in east-central Texas.

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