

BASELINE BIODIVERSITY ASSESSMENT OF SMALL MAMMALS, TICKS, AND
TICK-BORNE PATHOGENS IN SOUTH TEXAS

A Thesis

by

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ABSTRACT

Texas is a large state comprised of 10 ecological regions that provide diverse habitats to a variety of organisms. Stewardship programs throughout the state maintain healthy habitats for organisms to occupy; yet often these properties are difficult to access, which leads to unknown true organismal diversity, presence, and habitat associations, particularly in South Texas. Additionally, organisms such as small mammals are hosts for ticks and tick-borne pathogens (TBPs). Therefore, through the East Foundation's stewardship program, I created a baseline biodiversity assessment of small mammals, ticks, and tick-borne pathogens in Jim Hogg, Kenedy, Starr, and Willacy counties. I utilized several capture techniques including Sherman live traps, Macabee traps, and mist netting across three field seasons. To investigate TBPs presence, ticks and rodent ear biopsies were screened for the pathogen genera *Borrelia* and *Rickettsia* using PCR and DNA sequencing.

I captured 398 terrestrial, fossorial, and volant small mammals representing 18 species. Of these small mammals, 367 were terrestrial small mammals that were inspected for ticks. A total of 306 rodent ear biopsies were screened for TBPs, of which all were negative for *Borrelia* and *Rickettsia*. All ticks were negative for *Borrelia* and *Rickettsia*. Additionally, surveys for Texas pocket gophers revealed associations between their burrow systems and herpetofauna. A total of 125 herpetofauna were observed utilizing Texas pocket gopher mounds across East Foundation properties. This baseline assessment of small mammals, ticks, and tick-borne pathogens will serve as a reference point for future research efforts.

DEDICATION

To all those who wonder about the world around them. *“Imagination is more important than knowledge.”* – Albert Einstein

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The data analyzed for Chapter 2 were analyzed by the student with aid from Adrian A. Castellanos and Danielle K. Walkup. Dr. Toby Hibbitts and Dr. Wade Ryberg provided helpful comments on Chapter 4.

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TABLE OF CONTENTS

	Page
ABSTRACT	ii
DEDICATION	iii
ACKNOWLEDGEMENTS	iv
CONTRIBUTORS AND FUNDING SOURCES.....	vi
TABLE OF CONTENTS	vii
LIST OF FIGURES.....	ix
LIST OF TABLES	x
CHAPTER I INTRODUCTION	1
CHAPTER II BIODIVERSITY ASSESSMENT AND HABITAT ASSOCIATIONS OF SMALL MAMMALS IN SOUTH TEXAS.....	3
Introduction	3
Materials and methods.....	5
Results	8
Discussion	18
CHAPTER III ECOLOGICAL SURVEY OF SMALL MAMMALS, TICKS, AND TICK-BORNE PATHOGENS IN SOUTH TEXAS	26
Introduction	26
Materials and methods.....	30
Results	32
Discussion	36
CHAPTER IV HERPETOFAUNA ASSOCIATED WITH <i>GEOMYS PERSONATUS</i> POCKET GOPHER BURROWS ACROSS THE TEXAS SAND SHEET	46
Plains spadefoot toad (<i>Spea bombifrons</i>).....	47
Common spotted whiptail (<i>Cnemidophorus gularis</i>).....	47
Six-lined racerunner (<i>Cnemidophorus sexlineatus</i>)	48

Keeled earless lizard (<i>Holbrookia propinqua</i>)	48
Texas horned lizard (<i>Phrynosoma cornutum</i>)	48
Texas toad (<i>Anaxyrus speciosus</i>)	49
Ground skink (<i>Scincella lateralis</i>)	49
Bullsnake (<i>Pituophis catenifer</i>)	49
Texas tortoise (<i>Gopherus berlandieri</i>)	50
CHAPTER V SUMMARY	53
REFERENCES	55

LIST OF FIGURES

	Page
Figure 1. A map of South Texas with East Foundation properties: El Sauz Ranch (10,984 ha in Kenedy and Willacy counties), San Antonio Viejo Ranch (60.033 ha in Jim Hogg and Starr counties), and Santa Rosa Ranch (7,544 ha in Kenedy county).	6
Figure 2. All transects at which terrestrial, small mammals were targeted on East Foundation properties: El Sauz (Texas Sand Sheet and Gulf prairie and marshes ecoregions), San Antonio Viejo (Texas Brush Country and Texas Sand Sheet ecoregions), and Santa Rosa (Texas Sand Sheet ecoregion).....	11
Figure 3. Trapping localities of <i>Geomys personatus</i> (Texas pocket gophers) indicated by filled circles on East Foundation properties: El Sauz (Texas Sand Sheet and Gulf prairie and marshes ecoregions), San Antonio Viejo (Texas Brush Country and Texas Sand Sheet ecoregions), and Santa Rosa (Texas Sand Sheet ecoregion).....	12
Figure 4. Localities of successful mist netting events for bats species on East Foundation properties: El Sauz (Texas Sand Sheet and Gulf prairie and marshes ecoregions), San Antonio Viejo (Texas Brush Country and Texas Sand Sheet Ecoregions), and Santa Rosa (Texas Sand Sheet ecoregion).....	13

LIST OF TABLES

	Page
Table 1. Mammal species captured from East Foundation properties El Sauz, San Antonio Viejo, and Santa Rosa from June 2013 to June 2015.....	10
Table 2. Total mammal species captured (with numbers in parentheses representing retained individuals) from June 2013 to June 2015 per county.	15
Table 3. Habitat types found on East Foundation properties from June 2013 to June 2015 organized by ecoregions: Gulf prairie marshes, Texas Brush Country, and South Texas Sand Sheet.	16
Table 4. Small mammal species occupying the 15 habitat types found on East Foundation properties from June 2013 to June 2015.	17
Table 5. Terrestrial small mammals (organized by order, family, and species) retained and inspected for ticks from East Foundation properties El Sauz, San Antonio Viejo, and Santa Rosa.	34
Table 6. Small mammals (organized by order, family, and species) infested with tick species <i>Amblyomma maculatum</i> , <i>Dermacentor variabilis</i> , and possible <i>Ixodes sculptus</i> , and <i>Ixodes</i> sp.	37
Table 7. Individual rodents (all belonging to the family Cricetidae) parasitized by possible <i>Ixodes sculptus</i> and <i>Ixodes</i> species.	38
Table 8. Herpetofauna species observed to occupy <i>Geomys personatus</i> burrow systems on East Foundation properties over the course of three field seasons from 2013 to 2015.....	52

CHAPTER I

INTRODUCTION

Texas is a large state comprised of 10 ecological regions (ecoregions) and a variety of habitat types of which approximately 95% is privately owned and managed. Private land owners depend on their lands for their livelihood, which lends to managing practices that care for natural habitats very carefully. Private lands usually have healthier soils, flora, and ecosystems that provide great habitats for many organisms. Although private lands are beneficial for maintaining over all healthy environments, they may be difficult to access and result in a lowered amount of scientific studies and a general gap of knowledge concerning organismal biodiversity. South Texas has suitable habitat to support several species of small mammals. Small mammals have been previously documented in many areas of south Texas, but there are gaps in where they should occur (Schmidley and Bradley 2016). Surveys on private lands can fill in this gap of knowledge.

Small mammals are often parasitized by ectoparasites, such as ticks, that may act as vectors for diseases. Several tick-borne diseases are zoonotic, and can cause economical and health issues to humans and animals. Therefore, to understand the role of small mammals and ticks in sylvatic tick-borne disease cycles, I set out to create a baseline biodiversity assessment of small mammals, ticks, and tick-borne pathogens on East Foundation properties located within Jim Hogg, Kenedy, Starr, and Willacy Counties of south Texas. This baseline assessment may be used to monitor changes in

tick-borne pathogens and tick-borne disease prevalence over time and changes in landscape use.

Biodiversity surveys also allow researchers to discover and document important ecological interactions between species. As a result of biodiversity surveys of the East Foundation properties, the ecological role of Texas pocket gophers and their burrow systems were revealed. Texas pocket gophers are ecological engineers that create changes in habitats that many other organisms benefit from. Here, I documented the presence of herpetofauna utilizing various sections of Texas pocket gopher burrow systems. Biodiversity surveys are an old practice and are not utilized as much as they once were, but they are important for documenting species diversity and understanding ecological interactions within ecosystems.

CHAPTER II
BIODIVERSITY AND HABITAT ASSOCIATIONS OF SMALL MAMMALS IN
SOUTH TEXAS

Introduction

Texas is a large state comprised of 10 ecological regions (ecoregions) and a variety of habitat types across over 69,000,000 ha (Griffith et al. 2007). The heterogeneity of these ecoregions provides habitats for a vast variety of taxa. However, 95% of the state is privately owned (www.texaslandtrends.org). Although private lands are beneficial for maintaining over all healthy environments, they may be difficult to access and result in a lack of studies and a general gap of knowledge concerning organismal biodiversity across the state. South Texas is a large portion of the state extending south of a line from Del Rio through San Antonio to Victoria, and is particularly interesting in terms of known, and likely unknown, biodiversity. Within this large area there are three distinct ecoregions: the Gulf prairie and marshes, Texas brush country, and the south Texas Sand Sheet (Griffith et al. 2007).

The Gulf prairie and marshes ecoregion is a nearly level, slowly draining plain with streams and rivers draining to the Gulf of Mexico (Shew 1981). This ecoregion is comprised of barrier islands and oak mottes along the coastline, with tall woodlands in river bottoms and tall grass prairie remnants interspersed throughout (Griffith et al. 2007). The Texas brush country is a subtropical ecoregion once characterized by grassland interspersed with shrubs, that is now dominated by short, woody vegetation

such as honey mesquite and blackbrush (Griffith et al. 2007). The south Texas Sand Sheet is defined by a sheet of aeolian sand blown inland from the Gulf of Mexico shoreline during the Holocene period (Price 1958), which forms the namesake sand dunes found in the ecoregion (Fulbright et al. 1990). Due to the stable sand dunes, there are few streams present and most are located in the southern portion of the ecoregion, which is rich in fertile soils that are often used in cattle and wildlife hunting operations (Griffith et al. 2007). The south Texas Sand Sheet spans across eight counties and coincides with the distinct ecosystems of both the gulf prairie and marshes, and Texas brush country.

These three distinctive ecoregions provide habitats unique to Texas, yet diversity, particularly small mammal diversity, in these regions is understudied. In fact, according to VertNet (an online database of scientific collections, www.vertnet.org), only 5.16% of Texas mammal specimens housed in natural history collections (whose databases are accessible on VertNet) are from some of the larger south Texas counties within these ecoregions (e.g., Jim Hogg county: 1.55%; Kenedy county: 0.693%; Starr county: 0.227%; Willacy county; 2.69%). Notably, the East Foundation owns and manages properties that are situated across these three ecoregions in Jim Hogg, Kenedy, Starr, and Willacy counties. The East Foundation's mission is to support wildlife conservation and other public benefits of ranching and private land stewardship. The East Foundation stewardship program enables researchers to conduct fundamental biodiversity surveys to detail organismal habitat associations across ecosystems. In this study, "biodiversity" is used to detail counts of the variety of organisms, habitats, and ecosystems in a particular

area (Ricklefs and Miller 1999). The biodiversity surveys conducted on East Foundation properties are aimed to document species presence, abundance, distributions, and ecological roles within ecosystems across its lands. As part of these surveys, organisms are collected to build scientific collections for future use in education and research to expand our understanding of the natural world. Therefore, the objective of this study is to create a baseline biodiversity assessment and research collection of small mammals on East Foundation properties.

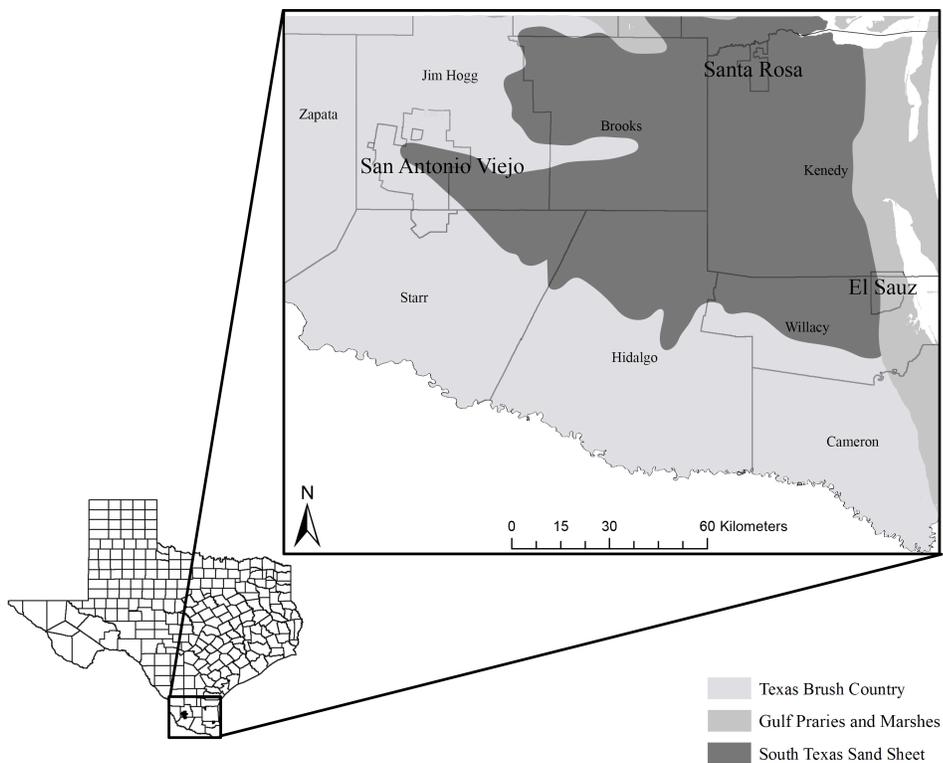
Materials and methods

Sampling - Fieldwork was conducted year-round and opportunistically from June 2013 to June 2015 on three East Foundation properties located within south Texas: El Sauz Ranch (ES), a 10,984 ha property located within southeastern Kenedy county and northwestern Willacy county (Gulf prairies and marshes and Texas Sand Sheet ecoregions); San Antonio Viejo Ranch (SAV), a 60,033 ha property located within southern Jim Hogg county and northern Starr county (Texas brush country and Texas Sand Sheet ecoregions); and Santa Rosa Ranch (SR), a 7,544 ha property within Kenedy county (Texas Sand Sheet ecoregion; Figure 1).

To capture a variety of mammalian species with differing life histories, three trapping techniques were utilized: Sherman live trapping (H.B. Sherman Traps, Tallahassee, FL) for nocturnal small mammals, Macabee trapping (Z.A. Macabee Gopher Trap Company, Los Gatos, CA) for fossorial rodents, and mist netting for bats. To determine suitable trapping localities, each property was scouted in an exploratory manner by vehicle and foot for appropriate and diverse habitats.

Field trips occurred throughout the year and were three to 20 days long. During field trips, efforts to capture small mammals occurred daily. To capture nocturnal and terrestrial small mammals, every night at dusk approximately 240 Sherman live traps were baited with sunflower seeds and set in transects of 40 to 80 traps, placed ca. 15 meters apart. Traps were checked at sunrise and small mammals were identified to species in the trap. For every trapping locality, subsets of individuals of each species were retained as voucher specimens for installation in the

Figure 1. A map of south Texas with East Foundation properties: El Sauz Ranch (10,984 ha in Kenedy and Willacy counties), San Antonio Viejo Ranch (60,033 ha in Jim Hogg and Starr counties), and Santa Rosa Ranch (7,544 ha in Kenedy county). The three ecoregions of interest (Texas Brush Country, South Texas Sand Sheet, and Gulf prairie and marshes) are indicated in gray shades.



Biodiversity Research and Teaching Collections at Texas A&M University (BRTC). To measure overall trapping success, efforts were quantified using the effective trap-nights (ETN) equation: each trap deployed overnight counted as 1 trap night. However, if the trap was found closed but empty, it was counted as 0.5 trap night, assuming that it was unavailable to rodents for half the night (Nelson and Clark 1973; Sutherland 1996).

Active fossorial rodent (pocket gopher) mounds were located by surveying each property by foot. Fresh mounds were uncovered with a shovel until tunnels were exposed. Once exposed, Macabee traps were set and checked every 15 to 20 minutes for up to 2 hours. Every pocket gopher collected was retained as a voucher specimen for the BRTC.

To capture bats, single-high and triple-high mist nets were set at dusk over water sources in a vector formation (two mist nets set across water sources in a 60 degree angle to each other) and were monitored overnight. Up to 10 specimens per bat species per locality were retained as voucher specimens for the BRTC. All animals in this study were treated humanely according to the guidelines provided by the American Society of Mammalogists (Sikes et al. 2016), the Texas A&M Animal Care and Use Committee (IACUC Animal Use Permits 2012-99 and 2015-0126), and Texas Parks and Wildlife Department Scientific Collecting Permit SPR-0409-082.

During preparation for installation into the BRTC, each collected mammal underwent a standardized protocol: collection of morphometric data (weight and body measurements), species and gender identification, reproduction status documentation, and a thorough inspection for ectoparasites. Additionally, liver and kidney tissues and

ear biopsies were collected from each specimen. Liver and kidney tissues were stored in Cryovial collection tubes in a -80°C freezer; ear biopsies and ectoparasites were stored in 70% ethanol and stored in a -20°C freezer for future studies. ArcMap, high-resolution vegetation data, and remote sensing techniques were used to identify habitat types surrounding trapping localities.

Results

Surveys resulted in a total 642 small mammal captures (398 retained) representing three orders, six families, and 18 species (Table 1). Sherman trapping resulted in 547 captures and retention of 367 small, terrestrial mammals (13 rodent species and 2 shrew species; Table 1 and Figure 2). Eleven gophers, *Geomys personatus*, were retained from approximately 130 collection attempts (Table 1 and Figure 3). Fifteen efforts to capture bats at five localities (Figure 4) resulted in 90 captures and retention of 20 bats representing two species (Table 1 and Figure 4).

In total 25,826 ETNs (ES: 8,988 ETNs; SAV: 10,846 ETNs; SR: 5,992 ETNs) occurred over 121 transects across the three properties. A transect was deemed successful if at least one capture occurred ($n = 97$). In contrast, a transect was considered unsuccessful if no captures occurred ($n = 24$). Transect success (percentage of successful transects), was highest at SAV (65.4%), and substantially lower at SR (27.3%) and ES (11.8%). For successful transects, capture success, the percentage of captured small mammals per transect, ranged from 1.25% to 72.5% per transect with an average 5.8 captures per 100 ETNs. The 72.5% capture success occurred on an ES transect of 40 ETNs.

The most abundant terrestrial small mammal species captured were rodents from the families Cricetidae and Heteromyidae (Table 1): *Peromyscus leucopus* ($n = 178$), *Onychomys leucogaster* ($n = 54$), and *Sigmodon hispidus* ($n = 64$), and *Chaetodipus hispidus* ($n = 115$) and *Perognathus merriami* ($n = 38$), respectively. The most abundant bat species captures were evening bats in the family Vespertilionidae ($n =$ approximately 90; a large number of bats, approximately 50, were captured during one netting session and I was unable to count each individual).

Collection efforts at ES yielded the highest number of captured mammals ($n = 257$), followed by SAV ($n = 247$) and SR ($n = 144$; Table 1). In terms of collection results by counties, small mammals were captured most from Kenedy County ($n = 318$; ES and SR properties), followed by Jim Hogg County ($n = 174$; SAV), Willacy County ($n = 89$; ES), and in least abundance from Starr county ($n = 61$; SAV; Table 2). Texas pocket gophers (*Geomys personatus*) were collected from all three properties ES ($n = 5$), SAV ($n = 6$), and SR ($n = 1$; Table 1) and from Jim Hogg, Kenedy, and Willacy counties, but not from Starr County, although it is within their range (Table 2).

Table 1. Mammal species captured from East Foundation properties El Sauz, San Antonio Viejo, and Santa Rosa from June 2013 to June 2015. Number of individuals captured (with numbers in parentheses representing retained individuals) is indicated per property. Mammal species are organized by order (three orders: Chiroptera, Rodentia, Soricomorpha) and family (six families: Vespertilionidae, Cricetidae, Geomyidae, Heteromyidae, Sciuridae, and Soricidae). All mammal taxonomy follows Schmidly and Bradley (2016).

Species	El Sauz	San Antonio Viejo	Santa Rosa	Total Per Species
Order Chiroptera: Vespertilionidae				
<i>Dasypterus intermedius</i> (Northern yellow bat)	0	1 (1)	0	1 (1)
<i>Nycticeius humeralis</i> (Evening bat)	79 (9)	2 (2)	8 (8)	89 (19)
Order Rodentia: Cricetidae				
<i>Baiomys taylori</i> (Northern pygmy mouse)	17 (10)	0	1 (1)	18 (11)
<i>Neotoma micropus</i> (Southern plains woodrat)	1 (1)	7 (7)	0	8 (8)
<i>Onychomys leucogaster</i> (Northern grasshopper mouse)	13 (9)	27 (24)	14 (10)	54 (43)
<i>Oryzomys texensis</i> (Texas marsh rice rat)	1(1)	0	0	1 (1)
<i>Peromyscus leucopus</i> (White-footed deermouse)	71 (53)	68 (48)	39 (17)	178 (118)
<i>Reithrodontomys fulvescens</i> (Fulvous harvest mouse)	5 (5)	1 (1)	1 (1)	7 (7)
<i>Sigmodon hispidus</i> (Hispid cotton rat)	22 (12)	18 (13)	24 (16)	64 (41)
Order Rodentia: Geomyidae				
<i>Geomys personatus</i> (Texas pocket gopher)	5 (5)	5 (5)	1 (1)	11 (11)
Order Rodentia: Heteromyidae				
<i>Chaetodipus hispidus</i> (Hispid pocket mouse)	26 (18)	48 (23)	41 (34)	115 (75)
<i>Dipodomys compactus</i> (Gulf Coast Kangaroo rat)	5 (5)	6 (6)	6 (6)	17 (17)
<i>Liomys irroratus</i> (Mexican spiny pocket mouse)	3 (3)	0	0	3 (3)
<i>Perognathus merriami</i> (Merriam's pocket mouse)	7 (4)	57 (29)	7 (5)	71 (38)
Order Rodentia: Sciuridae				
<i>Ictidomys parvidens</i> (Rio Grande ground squirrel)	0	1 (1)	0	1 (1)
<i>Xerospermophilus spilosoma</i> (Spotted ground squirrel)	0	0	1 (1)	1 (1)
Order Soricomorpha: Soricidae				
<i>Cryptotis parva</i> (Least shrew)	1 (1)	0	1 (1)	2 (2)
<i>Notiosorex crawfordi</i> (Crawford's desert shrew)	1 (1)	0	0	1 (1)
Total Per Property	257 (137)	241 (160)	144 (101)	642 (398)

Figure 2. All transects at which terrestrial, small mammals were targeted on East Foundation properties: El Sauz (Texas Sand Sheet and Gulf prairie and marshes ecoregions), San Antonio Viejo (Texas Brush Country and Texas Sand Sheet ecoregions), and Santa Rosa (Texas Sand Sheet ecoregion). Successful transects (transects in which at least one individual was caught) are indicated in green. Unsuccessful transects (transects in which trapping efforts did not yield captures) are indicated in red. Trapping occurred from June 2013 to June 2015.

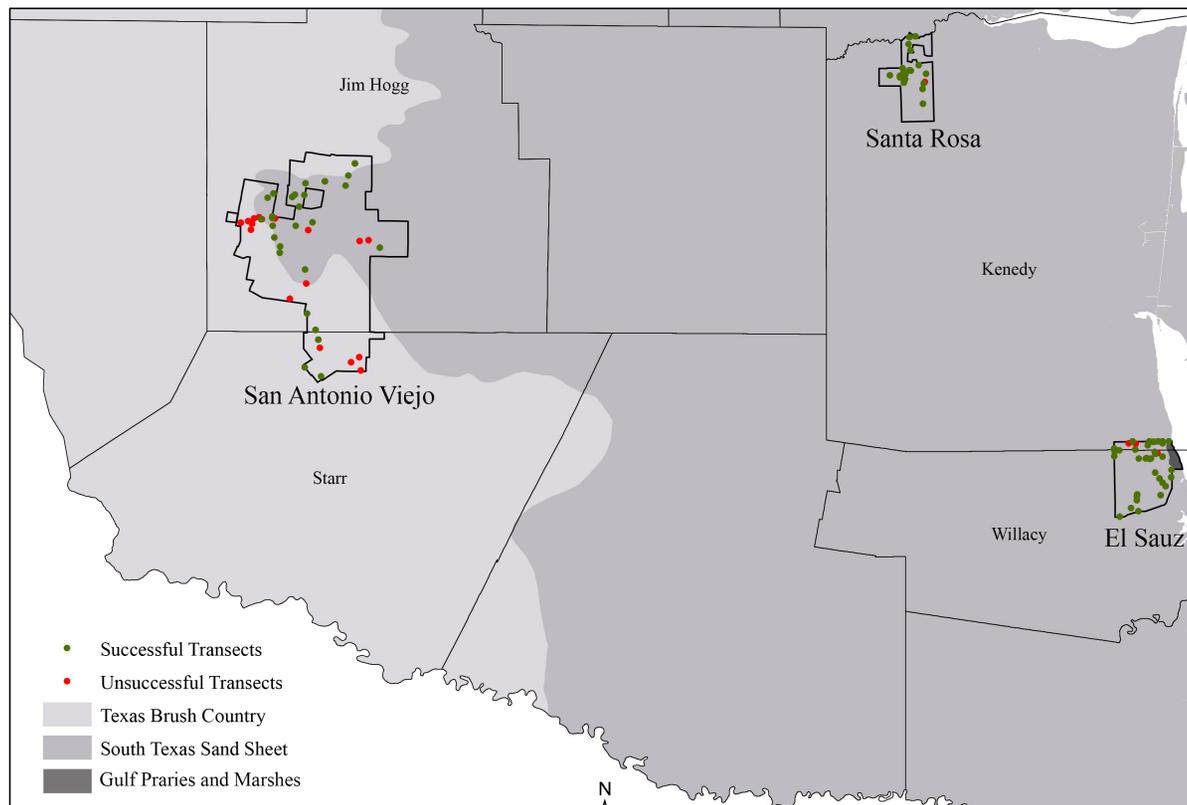


Figure 3. Trapping localities of *Geomys personatus* (Texas pocket gophers) indicated by filled circles on East Foundation properties: El Sauz (Texas Sand Sheet and Gulf prairie and marshes ecoregions), San Antonio Viejo (Texas Brush Country and Texas Sand Sheet ecoregions), and Santa Rosa (Texas Sand Sheet ecoregion). The larger sampling point at San Antonio Viejo indicates two pocket gophers that were caught from the same geographic locality. Trapping occurred from June 2013 to June 2015.

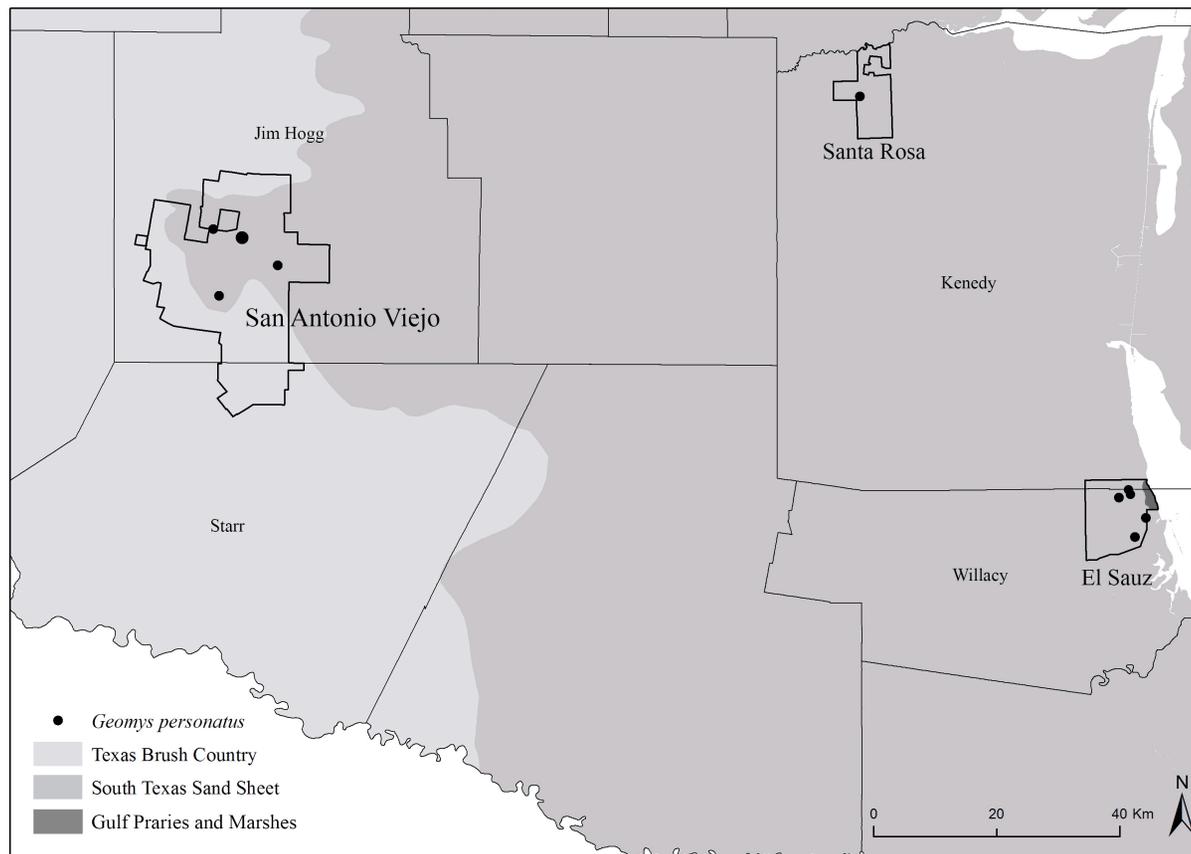
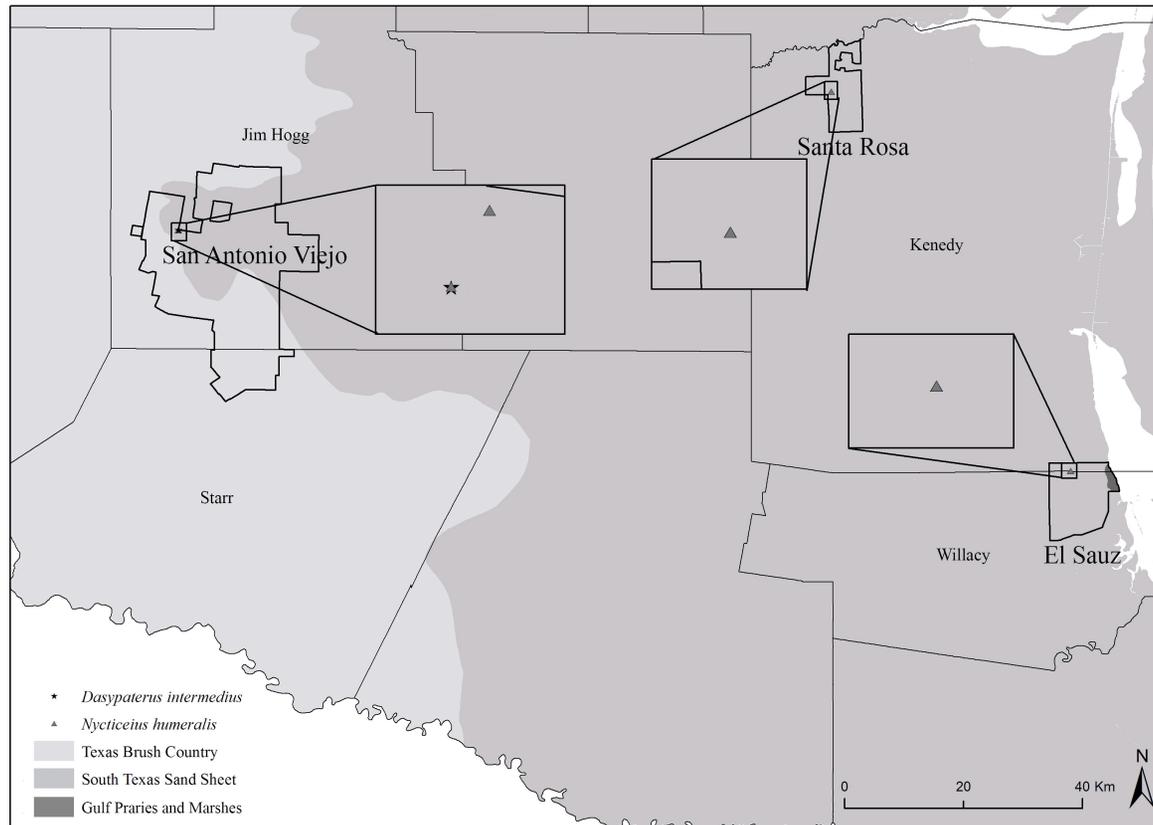


Figure 4. Localities of successful mist netting events for bat species on East Foundation properties: El Sauz (Texas Sand Sheet and Gulf prairie and marshes ecoregions), San Antonio Viejo (Texas Brush Country and Texas Sand Sheet ecoregions), and Santa Rosa (Texas Sand Sheet ecoregion). *Dasypterus intermedius* ($n = 1$) was caught from one location on San Antonio Viejo. *Nycticeius humeralis* ($n = 19$) were caught from El Sauz, San Antonio Viejo, and Santa Rosa. Netting occurred from February 2014 to June 2015.



Remote sensing techniques identified 15 unique habitat types in which mammals were captured (Table 3). One habitat type (Mesquite woodlands) occurred on all properties. Nearly half of all retained small mammals (47.7%) across all East Found properties were collected from Mesquite woodland, which also had the highest mammalian species richness ($n = 16$; Table 4). Arrowfeather grassland was present at both SAV and SR, where 6 mammal species were observed from both properties (Tables 3 and 4). Huisache woodland and live oak woodland were both present at ES and SF. Santa Rosa had the most diverse topography with 8 habitat types, each with at least 1 mammalian species recorded: Colima woodland (2 mammalian species), Gulf cordgrass grassland (4 species), Huisache woodland (6 species), Live oak woodland (8 species), Marsh hay cordgrass wetland (6 species), Mesquite woodland (6 species), Sea oxeye shrubland (1 species), and Seacoast bluestem grassland (9 species; Tables 3 and 4). Santa Antonio Viejo and Santa Rosa were comprised of 6 habitat types, each with at least 1 mammalian species recorded (Table 3). Habitats at SAV included Arrowfeather grassland (5 mammalian species), Balsam scale grassland (2 species), Blackbrush shrubland (7 species), Catclaw shrubland (2 species), Cenizo shrubland (2 species), Mesquite woodland (12 species; Tables 3 and 4). Habitats at SR included Arrowfeather grassland (2 mammalian species recorded), Bermuda grassland (6 species), Huisache woodland (3 species), Live oak woodland (3 species), Mesquite woodland (8 species), and Spiny aster wetland (4 species; Tables 3 and 4).

Table 2. Total mammal species captured (with numbers in parentheses representing retained individuals) from June 2013 to June 2015 per county. Asterisks denote new county records. All mammal taxonomy follows Schmidly and Bradley (2016) and common names are given in Table 1.

Mammal Species	Jim Hogg	Kenedy	Starr	Willacy	TOTAL
Order Chiroptera: Vespertilionidae					
<i>Dasypterus intermedius</i>	1 (1)*	0	0	0	1 (1)
<i>Nycticeius humeralis</i>	2 (2)*	87 (17)	0	0	89 (19)
Order Rodentia: Cricetidae					
<i>Baiomys taylori</i>	0	2 (2)	0	16 (9)	18 (11)
<i>Neotoma texensis</i>	3 (3)	1 (1)	4 (4)	0	8 (8)
<i>Onychomys leucogaster</i>	20 (17)	22 (16)	7 (7)	5 (3)	54 (43)
<i>Oryzomys texensis</i>	0	1 (1)	0	0	1 (1)
<i>Peromyscus leucopus</i>	59 (39)	74 (53)	9 (9)	36 (17)	178 (118)
<i>Reithrodontomys fulvescens</i>	1 (1)	4 (4)	0	2 (2)	7 (7)
<i>Sigmodon hispidus</i>	1 (1)	38 (22)	12 (12)	13 (6)	64 (41)
Order Rodentia: Geomyidae					
<i>Geomys personatus</i>	5 (5)	2 (2)	0	4 (4)	11 (11)
Order Rodentia: Heteromyidae					
<i>Chaetodipus hispidus</i>	28 (14)	59 (44)	20 (9)	8 (8)	115 (75)
<i>Dipodomys compactus</i>	5 (5)	10 (10)	0	2 (2)	17 (17)
<i>Liomys irroratus</i>	0	3 (3)	0	0	3 (3)
<i>Perognathus merriami</i>	48 (24)	11 (6)	9 (5)	3 (3)*	71 (38)
Order Rodentia: Sciuridae					
<i>Ictidomys parvidens</i>	1 (1)	0	0	0	1 (1)
<i>Xerospermophilus spilosoma</i>	0	1 (1)	0	0	1 (1)
Order Soricomorpha: Soricidae					
<i>Cryptotis parva</i>	0	2 (2)*	0	0	2 (2)
<i>Notiosorex crawfordi</i>	0	1 (1)*	0	0	1 (1)
TOTAL	174 (113)	318 (185)	61 (46)	89 (54)	642 (398)

Table 3. Habitat types found on East Foundation properties from June 2013 to June 2015 organized by ecoregions: Gulf prairie and marshes, Texas brush country, and south Texas Sand Sheet. Habitat types are categorized based on dominant vegetation type, for a total of 15 habitats. East Foundation properties are as follows: El Sauz (ES), San Antonio Viejo (SAV), and Santa Rosa (SR). Number of small mammal species found on each habitat type (per ecoregion and East Foundation property) is indicated in parentheses.

Habitat Types	ECOREGIONS OF SOUTH TEXAS		
	Gulf Coast Prairie and Marshes	Texas Brush Country	Texas Sand Sheet
Arrowfeather grassland			SAV (5) SR (2)
Balsam scale grassland			SAV (2)
Bermuda grassland			SR (6)
Blackbrush shrubland		SAV (3)	SAV (4)
Catclaw shrubland			SAV (2)
Cenizo shrubland		SAV (2)	
Colima woodland			ES (2)
Gulf cordgrass Grassland	ES (4)		
Huisache woodland			ES (6) SR (3)
Live oak woodland			ES (8) SR (3)
Marshhay cordgrass wetland	ES (1)		ES (5)
Mesquite woodland		SAV (5)	ES (6) SAV (7) SR (8)
Sea oxeye shrubland			ES (1)
Seacoast bluestem Grassland			ES (9)
Spiny aster wetland			SR (4)

Table 4. Small mammal species occupying the 15 habitat types found on East Foundation properties from June 2013 to June 2015. Presence of a species within a habitat is given by an “X” and absence by “—“. Habitat abbreviations are as follows: Arrowfeather grassland (AFG), Balsam scale grassland (BSG), Bermuda grassland (BG), Blackbrush shrubland (BBS), Catclaw shrubland (CCS), Cenizo shrubland (CS), Colima woodland (CW), Gulf cordgrass grassland (GCG), Huisache grassland (HG), Live oak woodland (LOW), Marsh hay cordgrass wetland (MHW), Mesquite woodland (MW), Seacoast bluestem grassland (SBG), Sea oxeye shrubland (SOS), and Spiny aster woodland (SAW). All mammal taxonomy follows Schmidly and Bradley (2016) and common names are given in Table 1.

Species	AFG	BSG	BG	BBS	CCS	CS	CW	GCG	HG	LOW	MHW	MW	SBG	SOS	SAW
<i>Dasypterus intermedius</i>	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
<i>Nycticeius humeralis</i>	-	-	X	-	-	-	-	-	-	X	-	X	-	-	-
<i>Baiomys taylori</i>	-	-	X	-	-	-	-	X	-	-	X	X	-	-	-
<i>Neotoma micropus</i>	X	-	-	X	-	-	-	-	X	-	-	X	-	-	-
<i>Onychomys leucogaster</i>	X	-	-	-	-	X	-	-	X	X	X	X	X	-	-
<i>Oryzomys texensis</i>	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
<i>Peromyscus leucopus</i>	-	X	X	X	-	X	X	X	X	X	X	X	X	-	-
<i>Reithrodontomys fulvescens</i>	-	-	-	-	X	-	-	-	X	X	-	X	X	X	-
<i>Sigmodon hispidus</i>	-	-	-	X	-	-	-	X	X	X	-	X	-	-	X
<i>Geomys personatus</i>	X	-	X	-	-	-	-	X	-	X	-	X	X	-	-
<i>Chaetodipus hispidus</i>	X	-	X	X	X	-	X	-	X	X	X	X	X	-	X
<i>Dipodomys compactus</i>	X	-	-	X	-	-	-	-	-	X	X	X	X	-	X
<i>Liomys irroratus</i>	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
<i>Perognathus merriami</i>	X	X	X	X	-	-	-	-	-	-	X	X	X	-	-
<i>Ictidomys parvidens</i>	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
<i>Xerospermophilus spilosoma</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
<i>Cryptotis parva</i>	-	-	-	-	-	-	-	-	-	-	-	X	X	-	-
<i>Notiosorex crawfordi</i>	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-
Total Number of Species Per Habitat	6	2	6	6	2	2	2	4	6	8	6	16	9	1	4

Discussion

Biodiversity surveys are necessary to assess organismal diversity across spatial and temporal scales. Preliminary surveys are particularly useful for monitoring changes in organismal diversity in response to landscape use. This baseline biodiversity survey of East Foundation properties gives important insight on the accuracy of published data on small mammal diversity (e.g., *The Mammals of Texas*; Schmidly and Bradley 2016) across Jim Hogg, Kenedy, Starr, and Willacy counties. I cross-referenced my capture data with Schmidly and Bradley (2016), and examined VertNet records as well. As a result of this comparison, I discovered new county records for five mammalian species: *Dasypterus intermedius* (northern yellow bat; Jim Hogg county), *Nycticeius humeralis* (evening bat; Jim Hogg county), *Perognathus merriami* (Merriam's pocket mouse; Willacy county), *Cryptotis parva* (least shrew; Kenedy county), and *Notiosorex crawfordi* (Crawford's desert shrew; Kenedy county; Table 2). These new county records are not out of geographic ranges (Schmidly and Bradley 2016), but they are the first known county records for these species within this region of Texas. These findings highlight the importance of continuing biodiversity surveys to confirm species presence, abundance, and distributions over geographic regions.

Although, I captured a total of 18 mammalian species (2 bat species, 14 rodent species, and 2 shrew species), all within their proposed geographic range, I was not successful in capturing as many small mammal and bat species as expected (Schmidly and Bradley 2016). In order to determine each species' geographic ranges, museum specimens were reviewed for their capture localities and habitat data to create

distribution maps for each species (Schmidly and Bradley 2016). According to Schmidly and Bradley (2016), there are 13 bat species, 17 rodent species, and 3 soricomorph species distributed over the areas where the East Foundation properties occur. Species not captured include 3 rodent species (*Oryzomys couesi*, *Peromyscus maniculatus*, and *Dipodomys ordii*). The fact that I did not encounter all possible small mammal species may be due to the type of trapping techniques used, the exploratory study design, the size of the study sites, or that fact that some species simply are not present on East Foundation properties.

I used standard model Sherman live traps (LFA-TDG, 7.5 x 9 x 23 cm) and captured 15 of 19 terrestrial, non-fossorial small mammal species that are thought to occur in this region. Larger Sherman live trap models (XLK, 7.7 x 9.5 x 30.5 cm; and XLF15, 10 x 11.5 x 38 cm; www.shermantraps.com) could have been useful to capture small mammals with larger body sizes and tails. For example, the 8 *Neotoma micropus* captured as part of this study occupied the entirety of standard Sherman live trap models. Pit fall traps could also have increased capture numbers for mammals that weigh less than 10 grams (Pankakoski 1979), such as shrews. Often these small mammals are too light to offset the trigger in Sherman live traps (although I note that I did catch 3 individuals of the 2 shrew species that occur on East Foundation properties in Sherman traps; Table 1).

Another topic to consider when using terrestrial live traps is bait choice. For this research I used black sunflower seeds as bait in Sherman live traps. I briefly compared trapping success between black sunflower seeds and rolled oats along several transects at

SAV and found no difference in trapping success. I chose to use black sunflower seeds as bait throughout the course of the study for three reasons: (1) large bags of black sunflower seeds are inexpensive; (2) black sunflower seeds are successful in attracting small mammals, particularly rodents, to traps; and (3) my study sites harbor red imported fire ant populations, which may prey on small mammals that are captured in the trap after being attracted by alternative bait choices (such as rolled oats, peanut butter, a combination of rolled oats and peanut butter, and freeze-dried insects; Beer 1964; Patric 1970; Buchalczyk and Olszewski 1971). Use of additional, variable baits could attract a larger variety of terrestrial small mammal species. For example, using freeze-dried insects as bait could potentially increase insectivorous small mammal captures (the three shrew individuals I caught were more than likely attracted to traps by the harvester ants that often invaded traps; A. Galán, pers. obs.).

The three rodent species that could have been captured based on my trapping technique and bait choice but were not (*Oryzomys couesi*, Couesi's marsh rat; *Peromyscus maniculatus*, deer mouse; and *Dipodomys ordii*, Ord's kangaroo rat), may have not been captured for several reasons. *Oryzomys couesi* is a marsh habitat specialist that are known from county records in Kenedy (27 specimens from 1985 to 1987) and Willacy (1 specimen from 1985), but that may be out competed by *Oryzomys texensis*. Although I trapped in marsh habitats on ES, and captured 1 *O. texensis*, future efforts to trap more extensively will help determine if *O. couesi* is present on East Foundation properties. *Peromyscus maniculatus* occupies a variety of habitats statewide, but they are uncommon in the eastern, southern, and coastal portions of the state (Schmidly and

Bradley 2016), and in fact only 1 specimen is known from Kenedy County. Therefore, *Peromyscus maniculatus* may not be present on East Foundation properties. *Dipodomys ordii* is a sand dune specialist known from Jim Hogg County (79 specimens from 1939 to 1976), Kenedy County (6 specimens from 1949 to 2004), and Willacy County (2 specimens from 1969 to 1982). *Dipodomys ordii* may be out competed by *D. compactus*, which occupies the same habitat and was captured from study sites. Future work to extensively trap targeted habitats for these rodents may clarify whether they are present on East Foundation properties.

Fossorial mammals (pocket gophers and moles) were targeted using harpoon-style (Macabee) traps. However, live-traps have been shown to successfully capture fossorial rodents as well (Baker and Williams 1972; Connior 2009). Although *G. personatus* have previously been captured in Starr County, I was not able to collect *G. personatus* from this area despite my efforts. I located and attempted to open 8 *G. personatus* mounds across Starr County. However, due to the rocky topography of this region of SAV, I was unable to open tunnels completely. For future efforts, use of Macabee and live traps concurrently, as well as a small pickaxe for opening gopher mound tunnels in rocky habitats more easily, may increase trapping success.

Additionally, although there was evidence of moles (*Scalopus aquaticus*) on East Foundation properties, I did not capture mole specimens during my field seasons. Moles occupy large areas and often abandon and reoccupy burrow systems several months apart (Harvey 1976). Therefore, it is difficult to determine if a burrow is active unless activity is obvious. According to VertNet, 1 mole specimen was caught from Willacy

County in 1947 and another from Kenedy County in 1974. Future efforts may include marking and monitoring burrows for signs of activity over longer periods of time to ensure capture.

For capturing bats, I used single-high and triple-high mist nets and captured 2 species *Dasypterus intermedius* and *Nycticeius humeralis* (Table 1). An additional 11 species of bats have geographic ranges overlapping with my study sites (e.g., Brazilian free-tailed bats, ghost-faced bats, Mexican long-nosed bats, and vesperilionid bats such as pallid bats, silver-haired bats, and several *Myotis* species). However, only 3 of these species have previously been collected from the areas in which East Foundation properties occur: the Brazilian free-tailed bat (*Tadarida brasileinsis*), southern yellow bat (*Dasypterus ega*), and Yuma myotis (*Myotis yumanensis*). In my attempts to net bats, mist nets were set in a vector in one of two combinations: two single-high nets in a vector or a single-high net and a triple-high net set in a vector. Mist nets were set in a vector formation over water sources to target bats during foraging events. With this formation, when bats flew in to capture an invertebrate or drink water, they could potentially fly into one net or use echolocation to avoid the net. In attempting to avoid the net by righting their flight, bats accidentally fly into the adjacent mist net. Despite this advantageous net arrangement, 10 of my 15 attempts to capture bats via mist netting resulted in zero captures. I did find that the triple-high nets are better suited for capturing individuals flying at higher altitude. For instance, the sole *Dasypterus intermedius* captured was collected in the top-most tier of a triple-high net. Previous to this capture, *Nycticeius humeralis* was the only species captured in either single or triple-high nets,

and when it was caught in triple-high nets it was caught no higher than the second tier. Mist nets are useful for capturing and collecting bats but they sample a small area relative to that used by free-flying bats and may introduce sampling bias (Kunz and Kurta 1988). Using triple-high nets allows sampling more of the open area that free-flying bats use. Harp nets may also be considered; however, this type of net works best at cave openings or known roost sites rather than flyways or over water sources. Additionally, I suggest using ultrasonic detecting systems to survey areas for bats prior to setting up mist nets, which could confirm if an area would be more likely to result in captures, especially across large sampling areas with several potential roosting sites (Dixon et al. 2014).

In general, my surveys were exploratory and often limited by time and technician availability. Exploratory, convenient sampling is appropriate for this baseline assessment study as it aims to document species presence in large areas when time and technicians are a limiting factor (Morrison et al. 2008). A way to help alleviate time constraints could have been to choose transects and trapping localities based on a grid system generated with suitable habitat characteristics in mind. This would have been especially helpful given the large sizes of the East Foundation properties. Additionally, capture success could have been higher if I had used a methodical approach in which I utilized additional types of trapping techniques (i.e., pit fall traps) and baits choices (i.e., freeze-dried insects), although black sunflower seeds did yield high capture rates along some transects (see above). Notably, capture success may have been reduced due to small mammals responding to the scent of the traps (Brouard et al. 2015). Small

mammals use olfactory cues to learn about their environment including detecting the presence of conspecifics and competing species (Shillito 1963). If the scent of another individual is present in a trap, there could be an increase in trap avoidance. Many mammal species also may be wary of traps introduced to their environments, again due to differences in olfactory cues, also resulting in avoidance of traps (Brouard et al. 2015). This trap avoidance behavior may be eliminated by thoroughly cleaning traps out every day and allowing mammals to acclimate to baited traps within their habitats, possibly resulting in higher capture rates (Shillito 1963). However, it is important to note that the region in which the East Foundation properties occur recently came out of a three-year drought period, which allowed for an abundance of plants to produce food sources for terrestrial small mammals. This may have also played a role in lower capture success.

In this study, I identified 15 unique habitats within the 3 distinct ecoregions found in this area of south Texas (Tables 3 and 4). Within each of these habitats were a minimum of 1 and a maximum of 16 small mammal species (Table 4). All small mammal habitat associations were expected based on organismal biology (Schmidly and Bradley 2016). However, new county records were discovered for 5 species in Jim Hogg, Kenedy, and Willacy counties (Table 2). This highlights the importance of surveying biodiversity within these unique and expansive ecoregions to uncover the extent of organismal diversity within the area.

The secondary objective for conducting these biodiversity surveys was to build a scientific collection for the East Foundation and to supplement the BRTC collection at

Texas A&M University. Scientific collections provide innumerable benefits to education and scientific research. These collections house millions of specimens, historical images, documents, and other materials that are invaluable sources of primary data for researchers working in a variety of fields (Mares 2009). Researchers can use scientific collections to answer questions relating to evolution, ecology, epidemiology, ecotoxicology, pathology, public health, ecological niche modeling, conservation and management and more (Baker 1994; Illoldi-Rangel 2004). Perhaps the most important and applicable use for scientific collections is to conduct research across temporal scales (Avila-Arcos 2012) and on species occurring in geographic areas that are difficult to access (Ponder 2001). Notably, specimens provide a reference point that documents past biodiversity and provides baseline materials necessary to forecast species distributions and extinction risk (Newbold 2010). This baseline biodiversity assessment provides the foundation for which changes in small mammal presence, distribution, and assemblages in south Texas may be monitored over time with respect to changes in landscape use and climate.

CHAPTER III
ECOLOGICAL SURVEY OF SMALL MAMMALS, TICKS, AND TICK-BORNE
PATHOGENS OF SOUTH TEXAS

Introduction

In wild populations, small mammals are often parasitized by ectoparasites such as ticks. Ticks may transmit pathogens to small mammals. Additionally, several of tick-borne pathogens (TBPs) cause zoonotic tick-borne diseases (TBDs); that is, they are spread between animals and humans. Vector-borne zoonotic diseases (zoonoses) are naturally maintained in animal diseases systems by interactions between vectors, usually hemaphagous arthropods, and reservoir hosts in a process known as sylvatic disease cycles. Zoonoses become problematic when an infected vector, in this case a pathogen carrying tick, bites an incidental host such as humans. Humans are incidental dead-end hosts for most TBPs, host species in which pathogens have little or no transmission to subsequent hosts due to tick feeding behavior. The accumulation of TBPs often leads to the manifestation of the disease (i.e., TBD) in these incidental hosts. TBD outbreaks become a serious possibility and concern when incidental hosts are infected. Humans working closely with wildlife are especially susceptible to TBPs due to the increased chance to encounter ticks. All in all, TBPs and TBDs are a major concern for human and veterinary health, particularly at the human-wildlife-domestic animal interface (Oliver 1989; Walker et al. 2014).

Most TBPs are transmitted by generalist tick species that feed upon a multitude of hosts other than cattle (Walker et al. 2014). In sylvatic disease cycles, wildlife act as reservoir hosts for TBPs and may act as sentinel species for monitoring TBP levels within an ecosystem (Kim et al. 2006; Hamer et al. 2010; Williamson et al. 2010). The most notable wildlife reservoir host species are rodents and depending on the geographic region, several different rodent species can act as reservoir hosts for TBPs. As reservoir hosts, rodents provide pathogens a suitable environment to reproduce without manifesting into diseases. This allows TBPs to reach infectious levels within the rodent reservoir hosts. In these sylvatic cycles, rodent reservoir hosts are infected with TBPs via a ticks' feeding event, which occur over several days at a time (Burgdorfer 1957). Larval and nymphal ticks acquire TBPs by ingesting blood meals from infected rodents. After ingesting an infected blood meal, larval and nymphal ticks molt into their next instar and transmit TBPs to their next life stage, in a process called transstadial transmission (Nicholson et al. 2009; Williamson et al. 2010). Additionally, some TBPs may also be transmitted transovarially from infected adult female ticks to offspring, such as *Borrelia miyamotoi* (Rollend 2013), which can then pass TBPs to reservoir hosts. Thus, ticks can acquire TBPs several ways, with adults passing pathogens on to larger hosts, including incidental hosts such as humans.

In the United States, the most common human-afflicting TBDs are caused by *Rickettsia* and *Borrelia* pathogen species (Pepin et al. 2012). *Borrelia* species are gram-negative spirochetes with flagella for mobility (Parola and Raoult 2001) and are the causative agents of Lyme borreliosis (caused by *B. burgdorferi*) and relapsing fevers (*B.*

miyamotoi, which has been known to infect tick populations but only recently has been identified as the causative agent of human disease; Barbour et al. 2009; Platonov et al. 2011; Krause et al. 2013). *Rickettsia* species are obligate intracellular bacteria and are causative agents for spotted fever rickettsiosis in their incidental dead-end hosts (Raoult and Roux 1997). Historically, TBD research has focused in areas of the United States where disease cases were reported: the northeast, midwest, and western United States (Diuk-Wasser et al. 2012; Hamer et al. 2012; Pepkin et al. 2012). Little research has focused on the southern United States, although this area harbors stable populations of multiple tick and reservoir host species that are important in TBD ecology. For example, ixodid (hard bodied) tick species such as the Gulf Coast tick (*Amblyomma maculatum*), lone star tick (*A. americanum*), Cayenne tick (*A. cajennense*), blacklegged tick (*Ixodes scapularis*), rabbit tick (*Haemaphysalis leporispalustris*), and American dog tick (*Dermacentor variabilis*) are common in the southern United States and can transmit a variety of TBPs (Barbour et al. 2009; Diuk-Wasser et al. 2012). *Borrelia* pathogenic species are vectored primarily by *Ixodes* tick species and Lyme borreliosis is primarily transmitted to humans by *Ixodes scapularis* (Barbour et al. 2009; Williamson et al. 2010; Pepkin et al. 2012). *Amblyomma*, *Dermacentor*, and *Haemaphysalis* tick species vector *Rickettsia* pathogen species. Furthermore, *A. americanum* is the most implicated tick species in TBPs transmission to humans in the southern United States (Barbour et al. 2009; Stromdahl et al. 2012; Adams et al. 2013).

Sylvatic TBDs research has not been extensively studied in Texas, where all of these tick species and a large number of potential reservoir hosts occur. Sixteen studies

concerning TBDs in Texas have covered several topics such as opportunistically screening ticks found on humans for pathogens (Mitchell et al. 2016); investigating the ecology of rodent and tick communities (Rodriguez et al. 2015) and the role of interactions between ticks and small mammals and red imported fire ants (Castellanos et al. 2016); investigating strains of TBPs in livestock (Guerrero et al. 2007); reporting case studies of animal and human patients presenting with clinical signs of disease (Whitney et al. 2007; Allison et al. 2011; Piccione et al. 2016); and using biotic and abiotic data to generate predictive models of tick species distribution and TBDs dispersal across the state (Sanders et al. 2008; Pound 2010; Wang et al. 2015). My study is one of few to specifically examine sylvatic TBP cycles in rodent communities in south Texas.

South Texas is a potential hot spot for TBDs. There are approximately 20 rodent species (Schmidly and Bradley 2016) that may act as potential hosts for tick species (Armed Forces Pest Management Board 2012). Although rodents are known to play an integral role in maintaining and transmitting pathogens (Kurtenbach et al. 1994; Shih and Chao 1998; Singla et al. 2008; Meerburg et al. 2009), the relative potential for serving as competent reservoir hosts differs among rodent species (Mather et al. 1989; Brown and Lane 1996; Burkot et al. 1999; Humair et al. 1999; Sinski et al. 2006). It is generally unknown which, if any, south Texas rodents and ticks may be important in TBP sylvatic cycles. The objectives of this study were to (1) determine which tick and potential reservoir rodent species are present in south Texas, and (2) to screen native fauna to create a baseline assessment of TBP prevalence to aid in determining risk assessment to human and veterinary health in the region.

Materials and methods

Sampling - Study sites for this research were on three south Texas properties managed by the East Foundation in Jim Hogg, Kenedy, Starr, and Willacy counties (El Sauz (ES), San Antonio Viejo (SAV), and Santa Rosa (SR); Chapter II, Figure 1). On each of these properties, biodiversity surveys of small mammals were conducted opportunistically from June 2013 to December 2015 (Chapter II). Small mammals for this study are the same as the ones trapped and collected in Chapter II (see above), where field trips occurred throughout the year and were 3 to 20 days long. During field trips, efforts to capture small mammals occurred nightly at dusk. Approximately 240 Sherman live traps were baited with sunflower seeds and set in transects of 40 to 80 traps, placed ca. 15 meters apart. Traps were checked at sunrise and small mammals were identified to species in the trap. For every trapping locality, subsets of specimens of each species were retained as voucher specimens for installation in the Biodiversity Research and Teaching Collections at Texas A&M University (BRTC). Small mammals were captured and humanely euthanized individually, according to guidelines of the American Society of Mammalogists (Sikes et al. 2016) and Texas A&M University Animal Care and Use permits (permits # 2012-99 and # 2015-0126) while in the field. Each euthanized small mammal was placed individually into a Ziploc bag with all pertinent locality data. All of the ticks for this study were collected directly from small mammals that were euthanized in the field, during museum preparation that occurred within a week of field collection.

During preparation for installation into the BRTC, each collected mammal underwent a standardized protocol listed in Chapter II. Ticks were removed using

forceps, identified morphologically using dichotomous keys (Sonenshine 1979; Kierans and Durden 1998; Sonenshine and Roe 2014), and stored in 70% ethanol in a -20°C freezer. Additionally, two 2-mm-diameter ear punch biopsies were collected from most rodent mammal specimens, and stored in 70% ethanol at -20°C for pathogen screening in the laboratory.

Laboratory methods - In the laboratory, total DNA was extracted from rodent ear biopsies and collected ticks using the E.Z.N.A. Tissue DNA extraction kit (Omega Bio-tek, Inc. Norcross, GA, USA) according to manufacturer's recommendations except with a final elution of 60 µL of elution buffer at 70°C (Bunikis et al. 2004, Williamson et al. 2010). Tick morphological identifications were confirmed via purified polymerase chain reaction (PCR) of a fragment (360 base pairs [bp]) of the mitochondrial 12S rDNA gene by utilizing two conserved primers (TIB and T2A) following protocols developed by Beati and Keirans (2001).

Ear biopsies and ticks were subjected to *Borrelia* and *Rickettsia* pathogen screening. *Borrelia* species were detected using a nested PCR for the 16S - 23S rRNA intergenic spacer region (IGS; primers: IGS-F, IGS-R, IGS-Fn, and IGS-Rn) following protocols developed by Bunikis et al. (2004) for a final product of approximately 900 bp for Lyme group *Borrelia* and 500 bp for relapsing fever *Borrelia*. *Rickettsia* species were detected using a traditional PCR protocol targeting 617 bp of the citrate synthase (gltA) gene using primers (RrCS 372 and RrCS 989) and protocols developed by Williamson et al. (2010). This PCR was used only to test for positive amplification of *Rickettsia* across ear biopsy and tick samples. If any samples were positive for

Rickettsia, a second PCR targeting the outer membrane protein A (ompA; 612 bp) gene was performed for *Rickettsia* identification to species (Zhang et al. 2006). Purified water served as negative controls for both pathogen assays. Included in each PCR respectively, were *Rickettsia* and *Borrelia* positive controls that were obtained from field-collected ticks previously determined to be positive for each pathogen (*A. maculatum* collected from Attwater Prairie Chicken National Wildlife Refuge in Texas; and *I. scapularis* collected from the midwestern United States, respectively). Tick identification and pathogen PCR amplicons were visualized by gel electrophoresis and positive PCR reactions were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA). Amplicons were sequenced in both directions using PCR primers at Yale University's DNA Analysis Facility (New Haven, CT). Sequences were annotated using Sequencher 4.10.1 (GeneCodes Corporation; Madison, WI) and were compared to published sequences using the basic local alignment search tool (BLAST) in GenBank for species identification.

Results

Across the three East Foundation properties, a total of 367 small mammals were retained, representing 13 rodent species and 2 soricomorph (insectivore) species (Table 5). Seven of the 15 species were collected from all three properties: *Chaetodipus hispidus* (hispid pocket mouse), *Dipodomys compactus* (Gulf Coast kangaroo rat), *Onychomys leucogaster* (northern grasshopper mouse), *Perognathus merriami* (Merriam's pocket mouse), *Peromyscus leucopus* (white-footed mouse), *Reithrodontomys fulvescens* (fulvous harvest mouse), and *Sigmodon hispidus* (hispid

cotton rat). Of the remaining ten species, *Baiomys taylori* (northern pygmy mouse) and *Cryptotis parva* (least shrew) were collected only from ES and Santa Rosa SR ranches. *Neotoma micropus* (southern plains woodrat) was solely collected from SAV and ES ranches. *Liomys irroratus* (Mexican spiny pocket mouse), *Notiosorex crawfordi* (Crawford's desert shrew), and *Oryzomys texensis* (Texas marsh rice rat) were collected only from ES (Table 5). The two squirrel species, *Ictidomys parvidens* (Rio Grande ground squirrel) and *Xerospermophilus spilosoma* (spotted ground squirrel), were each caught only at SAV and SR, respectively.

All small mammals were inspected for ticks; 47 individual small mammals were parasitized by ticks for an overall tick prevalence of 12.8 % (Table 6). The 47 individuals infested with ticks represent seven rodent species: *Baiomys taylori*, *Chaetodipus hispidus*, *Onychomys leucogaster*, *Perognathus merriami*, *Peromyscus leucopus*, *Reithrodontomys fulvescens*, and *Sigmodon hispidus* (Table 6). *Peromyscus leucopus* had the highest rate of infestation (27.1%), followed by *Onychomys leucogaster* (18.6%), and *Reithrodontomys fulvescens* (14.3%). Notably, only 7 *Reithrodontomys fulvescens* individuals were retained, of which 1 was parasitized (Table 6). *Baiomys taylori* and *Sigmodon hispidus* had moderate rates of tick infestations, 9.1% and 7.3% respectively. *Chaetodipus hispidus* had low overall tick infestations of 2.7% (Table 6). Ticks were not found on *Dipodomys compactus*, *Cryptotis parva*, *Liomys irroratus*, *Neotoma micropus*, *Notiosorex crawfordi*, *Oryzomys texensis*, *Ictidomys parvidens*, or *Xerospermophilus spilosoma*.

Table 5. Terrestrial small mammals (organized by order, family, and species) retained and inspected for ticks from East Foundation properties El Sauz, San Antonio Viejo, and Santa Rosa. Mammal taxonomy follows Schmidly and Bradley (2016). Mammal common names are given in Table 1.

Mammal Species	El Sauz	San Antonio Viejo	Santa Rosa	Species Total
Order Rodentia: Cricetidae				
<i>Baiomys taylori</i>	10	0	1	11
<i>Neotoma micropus</i>	1	7	0	8
<i>Onychomys leucogaster</i>	9	24	10	43
<i>Oryzomys texensis</i>	1	0	0	1
<i>Peromyscus leucopus</i>	53	48	17	118
<i>Reithrodontomys fulvescens</i>	5	1	1	7
<i>Sigmodon hispidus</i>	12	13	16	41
Order Rodentia: Heteromyidae				
<i>Chaetodipus hispidus</i>	18	23	34	75
<i>Dipodomys compactus</i>	5	6	6	17
<i>Liomys irroratus</i>	3	0	0	3
<i>Perognathus merriami</i>	4	29	5	38
Order Rodentia: Sciuridae				
<i>Ictidomys parvidens</i>	0	1	0	1
<i>Xerospermophilus spilosoma</i>	0	0	1	1
Order Soricomorpha: Soricidae				
<i>Cryptotis parva</i>	1	0	1	2
<i>Notiosorex crawfordi</i>	1	0	0	1
Total Per Property	123	152	92	367

In total, 146 ticks (all larvae and nymphs) were collected from rodents across all three properties (Table 6). Before molecular identification confirmation, ticks were preliminarily identified morphologically using a microscope and dichotomous keys yielding *Amblyomma maculatum* ($n = 59$; 41 larvae, 18 nymphs), *Dermacentor variabilis* ($n = 28$; 19 larvae, 9 nymphs), and 59 ticks (44 larvae, 15 nymphs) that were difficult to identify confidently morphologically. To validate morphological identification, the 146 ticks were divided into 64 molecular samples: 42 individual nymph samples and 22 larval pools. Larval pools consisted of 1 to 7 larval ticks, all from the same host individual, homogenized together to make a DNA sample. Molecular analyses validated some and resolved other morphological identifications (Table 6): *Amblyomma maculatum* ($n = 9$; 6 larvae and 3 nymphs), *Dermacentor variabilis* ($n = 120$; 81 larvae and 39 nymphs), a species with close homology (96% identity and 98% query coverage) to *Ixodes sculptus* ($n = 13$; all larvae), and a possible *Ixodes* species ($n = 4$; all larvae). These possible *Ixodes* larvae could not be confidently assigned a species identification due to multiple BLAST hits (including a soft tick, *Carios capensis*, and *I. sculptus*). *Dermacentor variabilis* was the most prevalent tick species, parasitizing 5 of 15 small mammals species, and was encountered most frequently at ES (Table 6). *Amblyomma maculatum* was encountered only at ES and was found to parasitize *Cheatodipus hispidus*, *Onychomys leucogaster*, and *Peromyscus leucopus* (Table 6). The possible *Ixodes sculptus* ticks were found on *Onychomys leucogaster* and *Peromyscus leucopus* at SAV (Table 7). The possible *Ixodes* ticks were collected only from

Peromyscus leucopus ($n = 2$) at SAV (Table 3). Ticks were collected in equal numbers from ES ($n = 67$) and SAV ($n = 67$), with 12 ticks collected from SR (Table 7).

Of the 367 collected small mammals, 306 were examined for *Borrelia* and *Rickettsia* species. Species not examined included 2 squirrel and 3 shrew specimens, and 51 other specimens. Shrews were not examined due to lack of external ears from which to biopsy and the other specimens (including squirrels) were not examined because ear biopsies were not initially collected during preparation for installation into the BRTC. All 306 biopsies were negative for both *Borrelia* and *Rickettsia* species. All tick DNA samples ($n = 64$) were also screened for *Borrelia* and *Rickettsia* species and yielded negative results for both genera.

Discussion

This study is one of few to use empirical data to investigate TBD sylvatic cycles and TBP prevalence in rodent communities in south Texas. Here, I created a baseline biodiversity assessment of potential reservoir hosts, ticks, and tick-borne pathogens. This will allow researchers to monitor changes in pathogen transmission cycles over time, land use, and small mammal assemblages across the area.

Table 6. Small mammals (organized by order, family, and species) infested with tick species *Amblyomma maculatum*, *Dermacentor variabilis*, and possible *Ixodes sculptus*, and *Ixodes* sp. Tick life stage (larvae, nymph) is also indicated. The number of infested small mammals is represented as a whole fraction (with percent of infestation in parentheses). Superscripts correspond to East Foundation properties where on-host ticks were found: (a) El Sauz, (b) San Antonio Viejo, and (c) Santa Rosa. Ticks were not found on *Dipodomys compactus*, *Cryptotis parva*, *Liomys irroratus*, *Neotoma micropus*, *Notiosorex crawfordi*, *Oryzomys texensis*, *Ictidomys mexicanus*, or *Xerospermophilus spilosoma*. Mammal common names are given in Table 1.

Host Species	Infested with Ticks	<i>A. maculatum</i>		<i>D. variabilis</i>		<i>I. sculptus</i>		<i>Ixodes</i> sp.	
		Larva	Nymph	Larva	Nymph	Larva	Nymph	Larva	Nymph
Order Rodentia: Cricetidae									
<i>Baiomys taylori</i>	1/11 (9.1%)	0	0	2 ^a	0	0	0	0	0
<i>Onychomys leucogaster</i>	8/43 (18.6%)	1 ^a	0	1 ^a , 12 ^b	1 ^b	6 ^b	0	0	0
<i>Peromyscus leucopus</i>	32/118 (27.1%)	5 ^a	0	26 ^a , 24 ^b , 6 ^c	8 ^a , 13 ^b , 6 ^c	7 ^b	0	4 ^b	0
<i>Reithrodontomys fulvescens</i>	1/7 (14.3%)	0	0	4 ^a	0	0	0	0	0
<i>Sigmodon hispidus</i>	3/41 (7.3%)	0	0	6 ^a	11 ^a	0	0	0	0
Order Rodentia: Heteromyidae									
<i>Chaetodipus hispidus</i>	2/75 (2.7%)	0	3 ^a	0	0	0	0	0	0
TOTAL	47/367 (12.8%)	6 (6 ^a)	3 (3 ^a)	81 (39 ^a , 36 ^b , 6 ^c)	39 (19 ^a , 14 ^b , 6 ^c)	13 (13 ^b)	0	4 (4 ^b)	0

Table 7. Individual rodents (all belonging to the family Cricetidae) parasitized by possible *Ixodes sculptus* and *Ixodes* species. Unique identifier numbers for each rodent host is given in parentheses after the species name. East Foundation properties (ranch) and counties where on-host ticks were found also are given: El Sauz (ES), San Antonio Viejo (SAV), and Santa Rosa (SR). Number of ticks (nymphs, larvae) is presented and mammal common names are given in Table 1.

Host Species	Ranch	County	<i>I. sculptus</i>	<i>Ixodes</i> sp.
<i>Peromyscus leucopus</i> (APG 4)	SAV	Jim Hogg	0	1 (0, 1)
<i>Onychomys leucogaster</i> (APG 112)	SAV	Starr	4 (0, 4)	0
<i>Onychomys leucogaster</i> (HAF 7)	SAV	Starr	2 (0, 2)	0
<i>Peromyscus leucopus</i> (APG 120)	SAV	Starr	7 (0, 7)	0
<i>Peromyscus leucopus</i> (APG 5)	SAV	Starr	0	3 (0, 3)
		Total	13 (0, 13)	4 (0, 4)

Small mammal surveys yielded mammal species I expected to encounter in south Texas (Table 5), with the exception of three rodent species: *Oryzomys couesi* (Coues' rice rat), *Peromyscus maniculatus* (North American deermouse), and *Dipodomys ordii* (Ord's kangaroo rat; see Chapter II discussion). Additional, more extensive sampling may be necessary to detect these species on East Foundation properties. Furthermore, study sites were sampled in an opportunistic, exploratory manner with little consideration for the individual behavioral tendencies of small mammals with concern to trapping methods (Kanda and Hatzel 2014). Allowing mammals to acclimate to baited traps within their habitats could lead to higher capture rates. However, because the study sites are large, ranging from 7,544 ha to 660,033 ha, I focused to survey as extensively as possible within the given time frame and the limitation of working independently.

Previous research has noted 3 of the 13 retained rodent species, *Peromyscus leucopus*, *Oryzomys texensis* (previously *Oryzomys palustris*) and *Sigmodon hispidus*,

act as reservoir hosts for *Borrelia* and *Rickettsia* pathogens throughout the northeastern and midwestern United States (*Peromyscus leucopus*) and east and southeastern United States (*Sigmodon hispidus* and *Oryzomys texensis*; Gage et al. 1995; Levin et al. 1995; Oliver 1996; Magnarelli et al. 1999; Stafford et al. 1999; Oliver et al. 2003; Rudenko et al. 2009; Hamer et al. 2010). Although I found these, and all other rodent species, to be free of *Borrelia* and *Rickettsia* infections in three separate wild rodent communities (El Sauz, San Antonio Viejo, and Santa Rosa East Foundation properties) over three years, my baseline data can be used to monitor any fluctuations in *Borrelia* and *Rickettsia* transmission cycles across south Texas. Future work includes collecting and screening ear biopsies from museum specimens from which ear biopsies were not initially collected. Additionally, screening ear biopsies for *Borrelia* is an appropriate means to determine *Borrelia* prevalence because the spirochete is slow moving and is localized to the point of tick bite. However, *Rickettsia* species enter the blood stream quickly, therefore blood screens for *Rickettsia* would be more appropriate.

Notably, although I only screened for *Borrelia* and *Rickettsia* species, *Peromyscus leucopus* and *Sigmodon hispidus* are implicated in the transmission of other tick-borne zoonoses such as anaplasmosis, ehrlichiosis, and typhus (Jensenius et al. 2009; Rar et al. 2011). Therefore, future studies could include screening rodents for other tick-borne zoonoses to fully understand which TBDs, if any, are occurring within the region.

I report two ixodid tick species within my study sites: *Amblyomma maculatum*, and *Dermacentor variabilis* (Table 6). I also report two possible *Ixodes* species: *Ixodes*

sculptus ($n = 13$), and *Ixodes* sp. ($n = 4$) ticks (Table 6). Notably, my tick morphological and molecular identifications were conflictive for several tick specimens. I initially identified 59 *Amblyomma maculatum* (41 larvae, 18 nymphs), 28 *Dermacentor variabilis* (19 larvae, 9 nymphs), and 59 unidentifiable ticks (44 larvae, 15 nymphs) morphologically. Molecular screening revealed only 9 *Amblyomma maculatum* (6 larvae and 3 nymphs), 120 *Dermacentor variabilis* (81 larvae and 39 nymphs), 13 possible *Ixodes sculptus* larvae, and 4 possible *Ixodes* larvae (see results above). These discrepancies between morphological and molecular identifications were due to difficulty identifying key morphological features on engorged ticks. The erroneous morphological tick species identifications that were resolved through molecular identification highlight the importance of genetic analyses, especially when attempting to identify larval and nymphal tick specimens without sufficient experience identifying engorged ticks.

The possible *Ixodes* ticks collected as part of this research are interesting. All were collected on SAV, the property with the most suitable habitat for this tick genus (deciduous, dry forests, woodlands and few grasslands; Guerra et al. 2002). The sequence data from 2 larval pools (APG 4 pool $n = 1$ larva, and APG 5 pool $n = 3$ larvae; Table 7) were most similar to an argasid (soft tick), *Carios capensis* (83% identity with 95% coverage; GenBank AB075953.1), which is commonly associated with seabirds (Reeves et al. 2006). However, morphologically these ticks looked like ixodid ticks, not argasids. At this point, the identification of these ticks is unknown and further work (amplification and sequencing of additional molecular markers) is required to resolve the

identity of these ticks. Unfortunately, all larval tick specimens were destroyed during the DNA extraction process. Thus, an additional possibility is to target future collection work at the same localities to see if additional ticks could be collected.

The other 3 tick DNA samples (HAF 7 pool $n = 2$ larvae, APG 112 pool $n = 4$ larvae, and APG 120 pool $n = 7$ larvae; Table 7) resulted with BLAST sequences most similar (96% identity and 98% query coverage) to *Ixodes sculptus*, a tick species that is normally found in the midwestern United States (Salkeld et al. 2006). Although it is far from its known range, research has found *I. sculptus* ticks on swift foxes from the Texas Panhandle from 2000 – 2001 (Pence et al. 2004). This tick species is most commonly associated with ground burrowing rodents, such as ground squirrels, and other rodent species known to use their burrows (e.g., *Onychomys* species; Salkeld et al. 2006). Notably, ground squirrels and grasshopper mice (genus *Onychomys*) are ubiquitous throughout Texas (Schmidly and Bradley 2016). Ticks disperse by attaching to mammalian, avian, and herpetofauna hosts that can travel large distances (Bloemer and Zimmerman 1988). Furthermore, despite the findings of the Pence et al. (2004) study, it appears that no one is regularly searching for *I. sculptus* in south Texas; therefore it may be that this tick species may already occur in Texas. All in all, the occurrence of *I. sculptus* on swift foxes in the Texas panhandle coupled with available burrowing rodent hosts, the natural movement patterns of meso- and large mammals (Bloemer and Zimmerman 1988; McQuiston et al. 2011), and the lack of *I. sculptus* surveillance indicates a possible southward geographic range of *I. sculptus*. I intend to screen additional molecular markers to verify the identification of these larval pools. If my

identification of *I. sculptus* holds following additional molecular work, my finding of 12 individual *I. sculptus* ticks fits the criterion set forth by the Centers for Disease Control and Prevention for demonstrating an established population of this species within the sampling locality and collection period (six individual ticks or a minimum of two different life stages; Dennis et al. 1998).

Very little is known about the vector competence of *I. sculptus* for TBDs although it has been reported to carry the causative agents of Tularemia, and Colorado tick fever (Thorpe et al. 1965; CDC 1976). *Amblyomma maculatum* transmits *Rickettsia parkeri*, the causative agent for Rocky Mountain spotted fever and tick paralysis (Espinoza-Gomez et al. 2011; Parola et al. 2013). *Dermacentor variabilis* vectors *Rickettsia rickettsii*, another causative agent of Rocky Mountain spotted fever (Piesman and Gage 1996). Although previous studies in Texas found *Amblyomma maculatum* to harbor rickettsial endosymbionts and a low prevalence of *R. parkeri* (Castellanos et al. 2016), and *Dermacentor variabilis* to be infected with a low prevalence of SF rickettsial pathogens (Williamson et al. 2010), the tick samples in this study yielded negative results for both TBPs of interest, *Borrelia* and *Rickettsia*.

Rates of tick parasitism were relatively high for some rodent species within this study, but several rodent species were captured in low numbers (e.g., *Baiomys taylori*, *Neotoma micropus*, *Oryzomys texensis*, *Reithrodontomys fulvescens*, and *Liomys irroratus*; Table 1). Therefore, their associations with ticks and pathogens in this region are still largely unknown. Low pathogen detection, low capture success of some small mammal species, and low tick parasitism occurrences, complicates elucidating if these

small mammal species act as reservoir hosts. Additionally, in this study I focused to screen non-sciurid rodents for TBPs due to the abundance and diversity of these rodents captured throughout the study. However, studies show that shrews and squirrels are competent reservoir hosts for TBPs such as Lyme borreliosis (Anderson 1984; Parola and Raoult 2001). All members of small mammal communities should continue to be monitored and surveyed for TBPs as their role as sentinel species in TBD ecology is imperative to understanding the dynamics within these disease systems.

Past research in Texas has primarily used environmental data to create models to predict TBD distribution (Guerrero et al. 2007; Sanders et al. 2008; Pound 2010), but did not take into account movement and behavior of potential TBD reservoir hosts including small, medium, and large mammals (Bloemer and Zimmerman 1988). Therefore, behavior of potential reservoir hosts should also be considered as an important factor in the ecology of TBD cycles.

The goal of this study was to gather empirical field-collected data to create a baseline assessment of potential TBPs small mammal reservoir hosts, ticks, and tick-borne pathogens across various habitats found throughout south Texas. All rodents and ticks were negative for both *Borrelia* and *Rickettsia* species. The absence of *Borrelia*, more specifically *B. burgdorferi*, in small mammals and ticks is most likely due to the absence of *Ixodes scapularis* ticks on the study sites. Other pathogenic *Borrelia* species that cause relapsing fevers are primarily vectored by argasid ticks, such as those belonging to the genus *Ornithodoros* (Parola and Raoult 2001; Meri et al. 2006), which I did not encounter during the course of the study.

The lack of pathogen prevalence may be due to high biodiversity and abundance of small mammals found on East Foundation properties. The East Foundation's stewardship style of land management maintains habitat in near pristine conditions, which promotes high host diversity and abundance, and thus potentially keeping TBPs and TBDs out of the ecosystem. Small mammal hosts vary in their competency to act as TBP reservoirs; therefore, a higher diversity of potential reservoir hosts directly influences TBP prevalence and transmission based on this observed difference in competency (LoGiudice et al. 2003). Abundance of small mammal hosts is also important because more hosts (of varying degree of reservoir hosts competency) limits the tick burden (number of ticks found on a host) on each host that is parasitized. For example, a higher population density of chipmunks lowered the tick-burden of white-footed deermice and vice versa in forests of Dutchess County, New York (Schmidt et al. 1999). This means that ticks are parasitizing highly competent reservoir hosts at a lower rate, thus lowering the prevalence of TBPs in ticks. Combined, reservoir competency and abundance of small mammals promote the "dilution effect". Simply, the dilution effect implies that higher host diversity for questing ticks lowers the prevalence of TBPs because ticks may predate on hosts with low reservoir competency. Another factor to consider for low pathogen prevalence is climate and habitat heterogeneity. Ticks are susceptible to desiccation and south Texas experiences high temperatures that may alter tick behavior and rate of development in ticks.

In conclusion, south Texas is an area of unique and high habitat heterogeneity and connectivity that is geographically situated among a potential hot spot for infectious

diseases. TBDs are increasingly becoming a greater concern for human and veterinary public health. Surveying for ticks, TBPs, and potential reservoir hosts in the south Texas border region is vital for One Health paradigm in the area. Future directions should include screening meso- and large mammals for tick-borne pathogens; establishing long-term monitoring systems to account for host species population cycles, investigating microhabitats occupied by tick species, and determining alternative methods for controlling tick populations.

CHAPTER IV

HERPETOFAUNA ASSOCIATED WITH *GEOMYS PERSONATUS* POCKET

GOPHER BURROWS ACROSS THE TEXAS SAND SHEET

The Texas pocket gopher (*Geomys personatus*; Rodentia: Geomyidae) is a fossorial rodent endemic to the native coastal prairies and deep, sandy soils found throughout northeastern Tamaulipas in Mexico and much of south Texas (Williams 1982; Schmidly and Bradley 2016). The mounds of Texas pocket gopher burrow systems are prominent features of the natural habitats of the Texas Sand Sheet. *Geomys personatus* burrow systems are comprised of several chambers and tunnels extending up to 30 m in length, and may be as much as 3 m deep (Williams 1982). Pocket gophers alter the structure of their communities via direct and indirect mechanisms such as creating habitats for organisms and altering habitat compositions often leading to greater flora biodiversity (Cameron 2000).

Many organisms other than pocket gophers use the extensive burrow systems for foraging and shelter (Cameron 2000; Hafner et al. 2003). During efforts to capture *G. personatus*, I noticed the presence of herpetofauna within pocket gopher burrow systems. The observations occurred on East Foundation's properties distributed across the Texas Sand Sheet: El Sauz (ES), San Antonio Viejo Ranch (SAV), and Santa Rosa Ranch (SR) (Chapter II, Fig. 1). Over the course of three field seasons from 2013 to 2015, I observed nine herpetofauna species (Table 8) occupying *G. personatus* burrow systems. I captured the herpetofauna by hand and used a dichotomous key to verify

species (Dixon 2013). In total, I observed 125 individual amphibians and reptiles within and surrounding pocket gopher burrows across the East Foundation properties (Table 8). Descriptions of the observations are below.

Plains spadefoot toad (*Spea bombifrons*)

Plains spadefoot toads are found in loose soils of grasslands, sand hills, semi-desert and desert shrub throughout most of south Texas, including Jim Hogg, Kenedy, Starr, and Willacy counties (Dixon 2013). During the summer, their burrows are shallow but in winter their burrows may be up to 4.6 m deep (Tipton et al. 2012). I observed 26 individuals of this species within the loose soil of pocket gopher mounds no more than 1/3 m below the surface on SAV and ES during summer months of 2013 and 2014 (Table 8). Although this species can be found on all East Foundation properties, most of the fieldwork at SR took place during winter months, which may explain the lack of observations of spadefoot toads on SR.

Common spotted whiptail (*Cnemidophorus gularis*)

Common spotted whiptails are found throughout most of Texas, except for the Texas panhandle, and pockets of east and west Texas (Dixon 2013). They are often encountered in leaf litter during their active periods but can be found in subterranean burrows and shade during periods of high temperatures (Hibbitts and Hibbitts 2015). I observed 21 individuals of this species at SAV (Table 8) within gopher mound tunnels, while I was setting traps. Although this species was only found within pocket gopher mounds on SAV during late summer field seasons, I did encounter this species on ES

and SR while searching for *G. personatus* mounds to excavate during spring field seasons.

Six-lined racerunner (*Cnemidophorus sexlineatus*)

This species occurs throughout most of east and south Texas, including Starr and Willacy counties (Dixon 2013). Racerunners are found in open grasslands with open and sandy soils. I observed 17 individuals of this species in shallow (15-23 cm) tunnels within pocket gopher mounds on SAV (Starr County) and ES (Willacy County; Table 8) during late spring field seasons. One observation was of two juvenile lizards in the same burrow system.

Keeled earless lizard (*Holbrookia propinqua*)

Keeled earless lizards are found in sandy soils of dunes throughout south Texas (Hibbitts and Hibbitts 2015). I observed 17 individuals of this species occupying pocket gopher mounds in the same manner as racerunners across SAV, ES, and SR (Table 8) in summer field seasons.

Texas horned lizard (*Phrynosoma cornutum*)

Texas horned lizards are found throughout most of Texas and occur in arid to semi-arid grasslands, savannahs, and thorn scrub forests (Hibbitts and Hibbitts 2015). This species is known to seek refuge in animal burrows under mesquite trees, near harvester ant mounds (*Pogonomyrmex barbatus*) (Eifler et al. 2012). I observed 17 individuals of this species foraging on invertebrates while atop pocket gopher mounds across SAV, ES, and SR, but primarily on SAV ($n = 14$; Table 8). All encounters across the properties occurred during mid-summer field seasons. Although occurrences at ES

and SR were low, the large number of encounters at SAV indicates that the association of this lizard with pocket gopher burrows may not be random.

Texas toad (Anaxyrus speciosus)

Texas toads inhabit sandy soils of prairies and grasslands in areas with high humidity or high water-retention capabilities (Tipton et al. 2012) to include Jim Hogg, Kenedy, Starr, and Willacy counties. I observed 13 individuals 5-8 cm below ground level within the humid tunnels of pocket gopher burrow systems. Most encounters ($n = 7$) occurred in mounds close to water sources (cattle tanks) on ES and SR in the late spring and summer; while other encounters occurred days after rains during late spring field seasons (Table 8).

Ground skink (Scincella lateralis)

Ground skinks inhabit forest leaf litter across the eastern two-thirds of Texas, including Kenedy, Starr, and Willacy Counties (Brooks 1967; Dixon 2013). I observed eight individuals of this species within loose surface soils of abandoned pocket gopher mounds found in an oak forest in SR (Table 8) in spring 2014. Although this species' range occurs across all East Foundation properties, SR is the only property surveyed extensively for *G. personatus* within oak forest areas. To verify if ground skinks also are associated with pocket gopher mounds in SAVR and ES, additional surveys in appropriate habitat are necessary.

Bullsnake (Pituophis catenifer)

Bullsnakes have an extensive range throughout Texas and are present on all East Foundation properties. This species inhabits a wide variety of habitats and is often

ubiquitous within its home range (Kapfer et al. 2008). I observed four individuals of this species on SAV (Table 8) during summer 2014. Two snakes were observed within tunnels while setting traps, another snake was found in a shallow chamber immediately above a pocket gopher tunnel, and the last observation was taken as a bullsnake entered a previously opened pocket gopher mound. Although these are a small number of observations, bullsnakes are known to prey upon pocket gophers and inhabit habitats with abundant small mammals such as pocket gophers (Schmidt and Davis 1941; Dixon and Werler 2005). Therefore, these observations likely reflect real, rather than random, associations.

Texas tortoise (*Gopherus berlandieri*)

The Texas tortoise inhabits thornscrub forests throughout southern Texas (Hibbitts and Hibbitts 2016). I observed two individuals of this species on SR (Table 8) during summer 2014. One tortoise was observed excavating an abandoned pocket gopher mound and another entering a previously opened pocket gopher burrow system. Although this is a low number of observations, this species is known to occupy the burrows of mammals such as armadillos, badgers, and pocket gophers rather than excavating their own extensive burrows (Kazmaier et al. 2001). Therefore this association is likely real rather than random.

Fossorial rodents such as pocket gophers act as ecological engineers by creating burrow systems which are extensive, alter plant community structures, create deep soils, and increase soil moisture levels (Cox et al. 1995). The burrow systems of pocket gophers create optimal habitat for not just the rodents, but for a suite of associated taxa

(Cameron 2000; Hafner et al. 2003). These observations are the first to extensively document amphibian and reptile use of pocket gopher burrows within this part of Texas and showcase the important functions Texas pocket gopher burrow systems play for herpetofauna, and likely for other organisms as well, across the Texas Sand Sheet.

Table 8. Herpetofauna species observed to occupy *Geomys personatus* burrow systems on East Foundation properties over the course of three field seasons from 2013 to 2015. Properties include (a) San Antonio Viejo Ranch, (b) El Sauz Ranch, and (c) Santa Rosa Ranch (Fig. 1). Observations are ordered from most to least where an “observation” refers to every time an individual was observed during efforts to capture *G. personatus*.

Herpetofauna Species	Total Observations	Observation Per Property
Plains spadefoot toad (<i>Spea bombifrons</i>)	26	10 ^a , 16 ^b
Common spotted whiptail (<i>Cnemidophorus gularis</i>)	21	21 ^a
Six-lined racerunner (<i>Cnemidophorus sexlineatus</i>)	17	12 ^a , 5 ^b
Keeled earless lizard (<i>Holbrookia propinqua</i>)	17	11 ^a , 3 ^b , 3 ^c
Texas horned lizard (<i>Phrynosoma cornutum</i>)	17	14 ^a , 1 ^b , 2 ^c
Texas toad (<i>Anaxyrus speciosus</i>)	13	8 ^b , 5 ^c
Ground skink (<i>Scincella lateralis</i>)	8	8 ^c
Bullsnake (<i>Pituophis catenifer</i>)	4	4 ^a
Texas tortoise (<i>Gopherus berlandieri</i>)	2	2 ^c
Total Observations		125

CHAPTER V

SUMMARY

Although biodiversity surveys are not as common as before, they continue to be useful for a plethora of question-based research. Through investigating organismal diversity on East Foundation properties, I found that reference material for noting mammalian biodiversity (e.g., Mammals of Texas and VertNet) are constantly kept up to date in part to scientific collections that are built from such biodiversity surveys.

Scientific collections are invaluable resources for conducting interdisciplinary research.

I was able to investigate the ecology of tick-borne pathogens within three rodent communities across three distinctive ecoregions: Gulf prairie and marshes, Texas Brush Country, and the south Texas Sand Sheet. My findings show that both small mammals and ticks from these ecoregions were void of tick-borne pathogens. However, this study highlights the importance of empirical field-based gathered data from wildlife studies to understand the mechanisms of sylvatic tick-borne pathogen cycles through out Texas.

Additionally, the possible geographic range expansion of *Ixodes sculptus* showcases the need to increase efforts to survey for novel ticks and tick-borne pathogens in Texas.

Furthermore, microhabitats for tick populations is a subject that should be explored with future endeavors to study tick-borne diseases in Texas. Because Texas has diverse ecoregions, microhabitats may vary widely in their ability to support ticks. Even if ecoregions are diverse, microhabitats may be stable and thus several tick species could expand their geographic range, such as *Ixodes sculptus*.

Through these surveys, I found that more than ground-welling and nesting rodent made use of Texas pocket gopher burrows, but so do herpetofauna. I was also able to note behavioral ecology of herpetofauna that was previously not documented extensively. This baseline assessment of small mammals in south Texas served to answer question from several disciplines and will continue to be used as such.

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