

SURVIVAL, MOVEMENT, AND FINE-SCALE HABITAT SELECTION  
OF REINTRODUCED TEXAS HORNED LIZARDS  
(*PHRYNOSOMA CORNUTUM*).

by

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## **LIST OF ABBREVIATIONS**

<b>Abbreviation</b>	<b>Description</b>
KDE	kernel density estimate
MCP	minimum convex polygon
RIFA	Red Imported Fire Ant
THL	Texas Horned Lizard
TPWD	Texas Parks and Wildlife Department

## ABSTRACT

The historical range of the Texas Horned Lizard (*Phrynosoma cornutum*) spans most of Texas, and some surrounding states. However, the species has experienced range contraction and population declines throughout much of the range. There is no substantial evidence to specify a single cause, so it is likely the decline has been triggered by multiple factors. Possible threats include Red Imported Fire Ants (*Solenopsis invicta*), herbicides and pesticides, habitat destruction and disturbance, and collection for the pet trade. Greater conservation effort is needed for Texas Horned Lizards, requiring more detailed knowledge of their habitat requirements. The habitat description given by many researchers is vague, such as that from Burrow et al. (2001): “*a mosaic of bare ground, herbaceous vegetation, and woody vegetation in close proximity.*” I used radiotelemetry to examine the survival, movement, and fine-scale habitat selection of 23 individual horned lizards released at a reintroduction site in central Texas. The results of my study indicate an early drop in survival within the first few days post-release, followed by a relatively gradual decline in survival over the next 60 – 90 days. Only one of the 23 lizards successfully entered hibernation in the fall. Of the 23 lizards, nine were predated, especially shortly after release by snakes and raccoons. Eight lizards had an undetermined fate because their radio signals were lost. Daily movements were short (mean = 14.49 m, SD = 21.52 m), and home range sizes decreased when the initial locations post-release were removed, indicating lizards dispersed before settling into a home range. My study revealed that Texas Horned Lizards used habitat in similar proportion to what was available on the landscape, but chose locations based on thermoregulatory needs and distance to harvester ant colonies. Overall, reintroduction efforts for Texas Horned Lizards might prove successful because the lizards tend to stay close to the release site and exhibit relatively normal behavior. However, methods to increase survival, such as predator removal and initial release of more lizards, should be used to ensure greater success.

# **I. INTRODUCTION**

## **Background**

Persistently small populations or major changes in the range of a species are indications that the continued survival of the species, in local areas or globally, might be threatened. When the range of a species is reduced, populations can become isolated and numbers can dwindle, further threatening the survival of the species (Lande 1998, Keller and Waller 2002, Traill et al. 2010). Threats to species are habitat disturbance or loss, invasive species, climate change, pollution, and overexploitation. Conservation strategies include methods of protecting current populations, such as creating corridors for dispersal and migration, monitoring genetic diversity, providing supplemental food, predator removal, and protection from poachers. Additionally, conservation efforts can involve creating new populations by instituting a captive breeding program, or even through reintroduction of individuals into areas within the historic range of the species (Dodd and Seigel 1991, Fischer and Lindenmayer 2000, Seddon et al. 2007, IUCN 2013).

Reintroduction of a species has had varied success because it depends on a wide variety of factors, some of which cannot be controlled. Knowledge of life history traits of the species as well as a thorough understanding of threats to its survival are important for successful reintroductions (Dodd and Seigel 1991, IUCN 2013). Many questions need to be answered prior to a reintroduction effort, such as whether captive or wild individuals will be used, how many individuals will be released, when, where, and how they will be released, and how the reintroduction effort will be monitored for success. Thus, reintroductions of poorly understood species or with inadequate planning often fail (Germano and Bishop 2009). Genetic diversity is a factor in long term population health

and must be considered during reintroduction efforts (Miller et al. 2011). However, reintroduction of threatened species often fails for reasons other than genetic diversity (Miller et al. 2011). Successful reintroduction requires the newly released individuals to stay in the release area, survive, and reproduce, so that they form a self-sustaining population.

Habitat quality is a major factor determining reintroduction success, and habitat restoration may be needed for successful reintroduction (Dodd and Seigel 1991, Germano and Bishop 2009, Neuwald and Templeton 2013). For example, reintroduction of collared lizards to glades in the Ozark Mountains succeeded when prescribed burning restored the native habitat structure needed by the lizards (Neuwald and Templeton 2013). Also, some animals show a preference for their natal habitat. Roe et al. (2010) found that translocated snakes showed a preference for natal habitat compared to resident snakes. Thus, successful reintroduction might be increased by matching habitat for reintroduction areas to the natal habitat of the translocated individuals (Roe et al. 2010). More research is needed to determine the generality of this concept (Germano and Bishop 2009).

Certain animals, especially herpetofauna, exhibit a tendency for homing behavior, in which they attempt to return to their original location after translocation and release (Germano and Bishop 2009, Santos et al. 2009). This is a major problem for reintroduction, where the goal is for translocated individuals to stay and form a new population at the release site (Dodd and Seigel 1991, Germano and Bishop 2009, IUCN 2013). Increased movement in unfamiliar habitat may increase the risk of predation and death, further compromising the goal of establishing a new population. Thus, one method to reduce homing behavior is soft release, where animals are held in enclosures in the

new location prior to release. The holding time should be long enough to prevent excessive homing behavior upon release, but short enough to minimize stress and loss of body condition. Studies indicate that soft releases reduce the dispersal of some reptile and amphibian species (Germano and Bishop 2009), improving the success of reintroduction (Roe et al. 2010).

Individual animals for a reintroduction effort can be obtained from wild populations or from captive breeding programs. Prior experiences of individuals can influence their behavior (McPhee and Silverman 2004). While often not included in discussions of reintroduction success, behavior can be a major factor (McPhee and Silverman 2004). After translocation, abnormal behavior can place individuals at greater risk of predation, exhaustion, and death (McPhee and Silverman 2004). They may be unable to feed normally, regulate their body temperature, or perform reproductive activities such as mating and nesting (McPhee and Silverman 2004). If this occurs, then the population size may dwindle, and without successful reproduction the reintroduction effort may fail. Thus, it is important to consider the behavior of the animals and to devise reintroduction protocols to increase the likelihood of normal behavior. Captive animals often exhibit abnormal behavior compared to their wild counterparts. For example, captive-bred predators often lack proper hunting skills to feed themselves in the wild (Jule et al. 2008). Captivity can influence the behavior of animals and impact the success of reintroduction (McPhee and Silverman 2004). While captive-bred animals may seem advantageous for reintroduction because wild source populations are not impacted, the additional costs of abnormal behavior must be considered. Santos et al. (2009) found that wild animals are more successful for relocation than captive bred animals.

Furthermore, it is important to consider any additional factors that may affect reintroduction success. For example, releasing more individuals increases the chance of maintaining a larger population size, and ultimately the success of reintroduction (Germano and Bishop 2009). However, removing more individuals from the source population could be detrimental to its own health and survival (Miller et al. 2011). Also, individuals may exhibit increased dispersal when attempting to avoid competition with any resident individuals at the release site (Santos et al. 2009). Lastly, post-release monitoring is essential to determine whether reintroduction was successful or not, and to further analyze the factors influencing reintroduction of a species (Dodd and Seigel 1991, Roe et al. 2010, Miller et al. 2011, IUCN 2013).

### **Ecology of the Texas Horned Lizard**

The historical range of the Texas Horned Lizard (THL, *Phrynosoma cornutum*) spans most of Texas, all of Oklahoma, parts of Kansas and New Mexico, and most of northern and central Mexico (Sherbrooke 2003). Winton (1915) reported the “*greatest abundance is the north and south strip of Texas known as the Black and Grand prairies*” including “*Fort Worth, Dallas, Waco, Austin, and San Antonio.*” Although research indicates some populations are stable (Carpenter et al. 2014) such as those in south and far west Texas (Donaldson et al. 1994), there are many populations in decline in Texas (Johnson Linam 2008, Carpenter et al. 2014). The THL was listed as threatened in Texas on July 18, 1977 (Carpenter et al. 2014). The status of the populations in Mexico is unknown (Carpenter et al. 2014). While there has been petition for Oklahoma to list the

THL as a species of concern (Carpenter et al. 2014), THLs are doing relatively well in states outside of Texas (Henke and Fair 1998, Montgomery and Mackessy 2003).

As exhibited by its wide geographic range, the THL is capable of living in a variety of arid and semi-arid habitats. The important factors determining THL presence are the availability of harvester ants (Winton 1916, Whiting et al. 1993, Webb and Henke 2008, Eifler et al. 2012, Carpenter et al. 2014, Granberg 2014), sandy soils for burrowing (Webb and Henke 2008, Horned Lizard Watch 2010), open or bare ground to facilitate foraging (Whiting et al. 1993, Burrow et al. 2001, Montgomery and Mackessy 2003, Webb and Henke 2008, Hellgren et al. 2010, Horned Lizard Watch 2010, Wolf et al. 2013, Granberg 2014), and cover in the form of grasses and shrubs for thermoregulation and predator avoidance (Winton 1916, Whiting et al. 1993, Burrow et al. 2001, Hellgren et al. 2010, Eifler et al. 2012, Wolf et al. 2013, Granberg 2014). However, the habitat description given by many researchers is vague (Whiting et al. 1993, Henke and Fair 1998, Hellgren et al. 2010, Horned Lizard Watch 2010, Wolf et al. 2013), such as that from Burrow et al. (2001): “*a mosaic of bare ground, herbaceous vegetation, and woody vegetation in close proximity.*” Furthermore, our understanding of THL habitat use may be skewed by the ease with which they are sighted in open habitat (Burrow et al. 2001). Research using radio transmitters to track individuals can provide more accurate data and a thorough knowledge of habitat use, while removing bias toward observations in open habitat (Burrow et al. 2001).

Eifler et al. (2012) suggested that THL spend most of their time under vegetation, choosing cover based on proximity to harvester ant colonies. Moreover, THLs prefer woody cover (Hellgren et al. 2010), possibly mesquite shrubs (Eifler et al. 2012), and



herbaceous vegetation (Hellgren et al. 2010), such as forbs and grasses (Montgomery and Mackessy 2003, Webb and Henke 2008, Horned Lizard Watch 2010, Wolf et al. 2013, Granberg 2014). Prescribed fire might be an important habitat restoration tool because it increases bare ground and grasses, providing more resources for harvester ants, and thus more resources for THLs (Webb and Henke 2008, Hellgren et al. 2010). Well-drained, loose sandy soils are important for burrowing, hibernating, and nesting (Webb and Henke 2008, Horned Lizard Watch 2010). Also, road traffic is a major cause of mortality (Montgomery and Mackessy 2003), so areas with lower vehicular traffic may be more suitable for THLs (Webb and Henke 2008).

Current research suggests THLs rely on a mix of bare ground and cover for thermoregulation (Winton 1916, Burrow et al. 2001, Hellgren et al. 2010, Eifler et al. 2012, Wolf et al. 2013, Granberg 2014). As temperatures change throughout the day and season, the lizards alter their use of cool versus warm locations, as well as behavior, to maintain body temperatures (Burrow et al. 2001, Bogosian et al. 2012). While THLs have general habitat requirements for thermal suitability, they modify behavior on a fine temporal scale to maintain appropriate body temperature including movement between relatively warmer and cooler areas over the course of a day (Bogosian et al. 2012).

Seasonally, THLs enter hibernation as the weather cools by burrowing into the substrate just below the surface (Sherbrooke 2003) and emerge in the spring as temperatures warm. Timing depends upon local climate because those living in colder climates tend to enter hibernation sooner and emerge later than those living in more southern, warmer climates. In central Texas, THLs are most often sighted May through August (Fair and Henke 1999), emerging from March to early April, and going into

hibernation in October or early November (Potter and Glass 1931). Texas Horned Lizards are primarily diurnal and are most active at temperatures between 24°C and 38°C (Johnson Linam 2008, Montgomery and Mackessy 2003); however, they exhibit crepuscular activity during hot summer months when optimal temperatures occur after sunset (Lara-Resendiz 2015). They are active throughout the day during cooler months (in spring and fall), but exhibit bimodal daily activity during the warmer months (Winton 1916, Montgomery and Mackessy 2003), spending much of the afternoon under cover, presumably to prevent overheating (Montgomery and Mackessy 2003). Daily movements (net displacements) have been estimated to be 36.5-46.8 m on average (Whitford and Bryant 1979, Fair and Henke 1999). Texas Horned Lizard activity patterns also follow the activity of harvester ants, their main prey, which display a similar diurnal pattern (Burrow et al. 2001).

Although the diet of the THL mostly consists of harvester ants, some beetles and other invertebrates are occasionally eaten (Winton 1915, Pianka and Parker 1975, Donaldson et al. 1994, Sherbrooke 2003). They have been reported to eat Red Imported Fire Ants (RIFAs, *Solenopsis invicta*), but cease feeding and flee when the ants begin to swarm (Allen et al. 2004). There are 12 species of harvester ants in Texas, but the red harvester ant (*Pogonomyrmex barbatus*) is the most common. Destruction of grasslands for agriculture might negatively impact harvester ants by reducing the availability of grass seeds, their main food source (Granberg 2014). In addition, woody plant encroachment into grasslands has been correlated with reduced abundance of two harvester ant species (Whitford and Bryant 1979). Prescribed burning might be a solution by reducing woody encroachment, encouraging grassland development, and exposing

bare ground; thus, increasing availability of grass seeds and open areas to facilitate the establishment of new colonies (Webb and Henke 2008, Hellgren et al. 2010). Restoration of native grasslands might be another management strategy since McIntyre (2003) found that indigenous grasslands had the highest density of harvester ant colonies.

Texas Horned Lizards are one of the few animals that consume harvester ants (Whitford and Bryant 1979). Predation pressure from THLs can cause harvester ant colonies to become inactive, reducing their foraging activity, but not significantly reducing the number of colonies (McIntyre 2003). The density of harvester ant colonies might be a good indicator of habitat suitability for THLs (McIntyre 2003) because THLs are found in areas with more harvester ant colonies (Webb and Henke 2008). However, harvester ant distribution may not always be a limiting factor for THL populations (Whiting et al. 1993, Bogosian et al. 2012). Texas Horned Lizards feed at multiple harvester ant colonies per day (Whitford and Bryant 1979, Munger 1982, 1984a, 1984b). One study suggests that one THL requires six harvester ant colonies when there is a density of approximately three lizards per ha (Whiting et al. 1993).

Home range sizes might be influenced by habitat quality. Estimates of home range size in urban areas range from 0.24 ha (Wall 2014) to 1.37 ha (Granberg 2014). Fair and Henke (1999) calculated weekly home range estimates in a rural area, which ranged from 0.01 to 0.34 ha. Home range size also varies between sexes, with males having larger home ranges than females (Munger 1984a). Further research is needed to determine whether harvester ant colony density serves as a useful indicator of habitat suitability for THLs. Specifically, further research should focus on whether THL movements and home range locations are influenced by the spatial locations of harvester

ant colonies. In addition to prey availability, habitat can also be an important aspect of the spatial distribution of THLs (Whiting et al. 1993).

The life history of the THL is not thoroughly studied but is generally characterized by high adult survival, delayed maturation, and large clutch investment (Endriss et al. 2007). Estimates of survival range from 2 to 8 years (Henke and Fair 1998, Sherbrooke 2003, Horned Lizard Watch 2010). Mating likely occurs in late May or early June, and eggs are laid shortly thereafter (Ballinger 1974). Nests are dug at a slant (Cahn 1926, Ramsey 1956, Sherbrooke 2003), and a clutch contains 18 – 49 eggs with an average of 29 (Cahn 1926, Ballinger 1974). The whole process of digging and egg-laying can take many hours, often overnight (Cahn 1926, Ramsey 1956, Sherbrooke 2003, Lara-Resendiz 2015). After approximately two months of incubation, hatchlings begin to emerge in August (Winton 1915, Ramsey 1956, Ballinger 1974, Allison and Cepeda 2009). Hatchlings might forage on ant species that are smaller-bodied than harvester ants (Allison and Cepeda 2009). Maturity is reached at approximately 70 mm SVL, typically by their second hibernation (Ballinger 1974, Sherbrooke 2003).

While Winton (1915) commented that THL “*natural enemies are few and unimportant,*” predation has been found to be a leading cause of death (Munger 1986, Wolf et al. 2013). Texas Horned Lizards are not the main prey of any predator (Sherbrooke 1990, 1991, 2008), but they do form part of the diet of many mammals and birds including Greater Roadrunners (*Geococcyx californianus*), Western Coachwhips (*Masticophis flagellum*), and Western Diamondback Rattlesnakes (*Crotalus atrox*; Sherbrooke 2003, Wolf et al. 2013). Crypsis is the main predator avoidance strategy of THLs because they rely on their coloration and patterning for camouflage (Reeve 1952,

Peslak 1985, Wolf et al. 2013). Other defense strategies include defensive posturing, blood-squirting, and the hard “horns” that can harm a predator (Reeve 1952, Peslak 1985, Sherbrooke 2003). Any threats to adult survival may decimate a population quickly (Carpenter et al. 2014). Hatchling survival had the highest impact on population growth rate in one study, but causes of hatchling mortality were unclear (Wolf et al. 2014).

### **Threats to the Texas Horned Lizard**

Current research indicates the range in Texas has greatly decreased in recent decades, such that it no longer extends into eastern Texas (Henke and Fair 1998, Johnson Linam 2008). Anecdotal accounts suggest the species has experienced range contraction and regional population declines (Johnson Linam 2008). The greatest decline has occurred in east Texas with less severe decreases in parts of central Texas (Donaldson et al. 1994). Surveys also indicate the largest declines have occurred near metropolitan areas (Donaldson et al. 1994, Johnson Linam 2008). The exact cause is not known, but possible threats include RIFAs (Allen et al. 2004, Johnson Linam 2008, Webb and Henke 2008), herbicides and pesticides (Donaldson et al. 1994), habitat destruction and disturbance (Donaldson et al. 1994), and collection for the pet trade (Manaster 2002).

The decline of the THL has been largely attributed to RIFAs (Allen et al. 2004, Johnson Linam 2008). In fact, RIFAs have been implicated in the decline of other herpetofauna as well, although experimental evidence is needed to substantiate these claims (Allen et al. 2004). Red Imported Fire Ants have been spreading through Texas since the 1950s (Summerlin and Green 1977), a long enough time for the ants to become established, but not for the THL to adapt to their presence on an evolutionary or

ecological time scale. Red Imported Fire Ants are not common in the panhandle of Texas (McIntyre 2003), which could be why populations of THLs in that region are relatively stable (Henke 2003). Red Imported Fire Ants can impact THLs by directly preying on eggs, hatchlings, or hibernating individuals (Allen et al. 2004, Thawley and Langkilde 2016), or indirectly by decreasing availability of their prey, harvester ants (Hook and Porter 1990, Donaldson et al. 1994, Allen et al. 2004). Harvester ants are the main food source of THLs (Granberg 2014), so reduction or loss of this species could devastate populations. While negative correlations between RIFA presence and THL presence have been found (Johnson Linam 2008, Webb and Henke 2008), the impact of RIFAs remains unclear due to other factors typically associated with their presence, such as habitat disturbance (Donaldson et al. 1994).

Pesticides and herbicides are additional threats to the THL (Donaldson et al. 1994, Carpenter et al. 2014). These chemicals can harm lizards directly through contact, or indirectly through habitat alterations. Herbicides change the flora of an ecosystem which THLs rely on as cover from predators and for thermoregulation (Winton 1916, Burrow et al. 2001, Hellgren et al. 2010, Eifler et al. 2012, Wolf et al. 2013, Granberg 2014). Without brush cover and grasses, THLs might experience higher rates of mortality due to increased predation, and they might be unable to properly regulate their body temperature on hot summer days. While surveys indicate pesticide use is not a major factor determining THL presence in an area (Donaldson et al. 1994), pesticides kill harvester ants, the main food source for THLs. Herbicides do not directly harm harvester ants, but indirectly impact them through their food sources. Harvester ants eat the seeds of native grasses (Granberg 2014) which are harmed by broad application herbicides (Manaster

2002). Without a food source for harvester ants, populations will decline, in turn causing the decline of THLs that rely on them as a major food source.

Additional threats to the THL are habitat destruction, alteration, and fragmentation. As mentioned previously, the greatest population declines have been observed near large metropolitan areas (Donaldson et al. 1994, Johnson Linam 2008), where habitat is commonly destroyed by human development as urban and suburban areas expand. Fragments might be maintained as parks or greenspaces, but they are often isolated from other habitat areas, resulting in a fragmented set of parks surrounded by a relatively uninhabitable urban matrix (Medley et al. 1995, Wilcove et al. 1998, Collins et al. 2000). Use of residential areas by THL is limited, even if they occupied the habitat prior to construction (Wolf et al. 2013). Populations of the THL in eastern and southern Texas are likely small and fragmented (Henke 2003), and some already exhibit low genetic diversity (Wall 2014). Researchers reference the region that is “*east of a line extending from Fort Worth to Corpus Christi*” as having almost no populations of THLs remaining (Donaldson et al. 1994, Henke 2003).

Additionally, urban areas contain many meso-predators, especially domestic cats and dogs which place additional predation pressure on THLs (Donaldson et al. 1994, Carpenter et al. 2014). These predators are more common near human developments, which could be a factor in the major declines in THL populations near metropolitan areas (Carpenter et al. 2014). Some researchers suggest that reduction in predation pressure from domestic animals is needed (Donaldson et al. 1994), but others suggest that further research on predator populations, distributions, and impacts is needed (Hellgren et al. 2010, Bogosian et al. 2012).

Lastly, collection for the pet trade is another threat that might have harmed THL populations. Although it is now illegal to collect these lizards, this is a relatively recent restriction (1977; Carpenter et al. 2014). Collectors and pet traders shipped them across the country and even around the world (Manaster 2002). This export caused the deaths of countless THLs that died in shipment, or shortly thereafter from malnutrition. Even boy scouts used to collect and trade them at meets (Manaster 2002). The harvester ant diet needed by these lizards is difficult to provide in captivity, even with modern knowledge and understanding, so captive breeding programs are difficult to maintain (Sherbrooke 2003). Other species of horned lizards were also collected and traded as pets, and these actions could have caused the decline of these other species as well (Sherbrooke 2003).

While each of these factors could pose a major threat to the health of THL populations on their own, no substantial evidence has been found to indicate any one cause (Donaldson et al. 1994). Thus, it is likely that the decline has been triggered by multiple factors. Further investigation is needed to fully understand the threats these iconic lizards face, and to better understand what conservation and management methods can be developed to alleviate these threats.

## **Purpose**

The purpose of my study was to investigate the factors influencing THL movement, survival, and habitat selection following reintroduction. This study was part of a larger Texas Parks and Wildlife Department (TPWD) initiative to investigate reintroduction protocols and establish healthy, self-sustaining populations in parts of the historical range of the THL. Current THL reintroduction efforts by TPWD involve



capturing THLs from existing wild populations and translocating them to release sites in wildlife management areas. Texas Horned Lizards undergo soft release, where they are held in enclosures for a specified length of time (e.g., 1 – 3 weeks) and then allowed to disperse of their own accord. I tested the following hypotheses:

1. Most mortality will occur early, with lower mortality once lizards are settled into a home range. Then, the proportion of lizards surviving will stabilize after a period of time.
2. Surviving lizards will settle into a home range and daily movement distances will decrease with time since release.
3. The dispersal pathways and occupied home ranges will be non-random with respect to habitat features, and lizards will select areas with more harvester ant colonies and bare ground close to brush cover.
4. Lizards will select habitat to allow for thermoregulatory behavior. At high ambient temperatures, they will be in relatively cooler locations, and at low ambient temperatures they will be in relatively warmer locations.

## II. METHODS

### Study Site

The study site was Mason Mountain Wildlife Management Area (MMWMA) near Mason, Texas. Prior to TPWD acquiring the property in 1997, it was a private game ranch. The 2,146-ha ranch was designated as a Wildlife Management Area by TPWD and is dedicated to the research and management of native and exotic wildlife. Currently there are seven exotic ungulate species managed on the property. Previous research has included reintroduction and food habits of collared peccary, lizard ecology, prescribed burning, and exotic ungulate interactions with native ungulates. The property is located in the Llano Uplift ecoregion. Topography at MMWMA includes gently rolling hills with granite and limestone outcrops. There are a variety of soils ranging from clay loams to sand and gravel (Singhurst et al. 2007). Vegetation types include oak woodlands and savannas, with woody vegetation composed of different Oak species (*Quercus sp.*), Mesquite (*Prosopis glandulosa*), and Ashe Juniper (*Juniperus ashei*). The property also has large areas dominated by Prickly Pear cacti (*Opuntia sp.*), Netleaf Hackberry (*Celtis reticulata*), and Yucca (*Yucca sp.*; Singhurst et al. 2007).

### Study Design

**Initial release:** During the summer of 2015, TPWD staff released and monitored 15 THLs at MMWMA. The lizards were captured during June on private land in Irion County near San Angelo, Texas, and subsequently evaluated for health and body condition prior to transport to MMWMA. The sex, body mass, and SVL of each individual were recorded. Each lizard received a passive integrated transponder (ID100-

VB (1.25) PIT; Trovan Electronic Identification Devices Ltd., Santa Barbara, CA), and a radio transmitter (BD-2, 1.5 gram, 8-9 week battery life; Holohil Systems Ltd., Ontario, Canada) was attached to each lizard's dorsal side with eyelash glue and then secured using a collar around the neck made of fishing line covered in plastic tubing. Half of the lizards were placed in each of two holding enclosures, A and B, (3 x 3 m) located approximately 193 m apart (Figure 1). Each enclosure was placed over a harvester ant colony. Soft releases were scheduled such that one enclosure was opened after two weeks and the lizards could leave of their own accord. The other enclosure was opened after three weeks. "Opening an enclosure" entailed the opening of a small gate (approximately 10 x 5 cm) on at least one side of the enclosure.

Using radiotelemetry, lizards were located once per day for the first week, and their location, body mass, and SVL were recorded. About one week after release, the frequency of tracking was reduced to 2-3 times per week. Seven lizards died, most likely from predators, over the course of the study, and signal was lost on three. Four lizards survived until they burrowed for hibernation in late September. Mesh cages were placed over the hibernating lizards so that they could be captured and given new radio transmitters when they emerged in the spring. One lizard escaped its hibernation cage. Three lizards emerged in spring, but died in May 2016. One pair of lizards appeared to have bred because one female was gravid prior to death. None of the 15 lizards from the initial release were used in subsequent data analysis to test my hypotheses because these lizards were not tracked as frequently as lizards of the second release and no habitat data were recorded (see below). This initial release was not designed to answer the hypotheses

of this study, but instead allowed the reintroduction protocol and tracking procedure to be tested.

**Second release and tracking:** During Summer 2016, a second release of THLs occurred at MMWMA. Ten THLs were obtained from a private property in Irion County near San Angelo, Texas, and 13 were obtained from public roads near Comstock, Texas in Val Verde County. This gave a total of 23 lizards, 13 males and 10 females. Following the same protocol as above, these lizards were examined for general health, transported to MMWMA, received a PIT tag, received a radio transmitter, and were held in enclosures for soft releases. Three different enclosures were used, identified as the A, B, and C enclosures (Figure 1). Enclosure C was 171 m from enclosure B and 352 m from enclosure A. Ten lizards (6 males, 4 females) were released from enclosure A after 18 days, ten lizards (5 males, 5 females) were released from enclosure B after 12 days, and three lizards (2 males, 1 female) were released from enclosure C after 3 days.

Following the releases on 30 May, 8 June, and 12 June 2016, I located all lizards 1 - 3 times per day until 5 August 2016 using radiotelemetry. Once a lizard was located each day, I waited at least two hours before searching again. In this way, locations per lizard were dispersed throughout the day with at least two hours between sightings. This allowed detailed collection on movements and habitat use without disturbing the lizards and affecting their behavior. After 5 August 2016, tracking was reduced to once per week to monitor survival, and habitat measurements (see below) were no longer recorded.

Upon locating a lizard, I recorded the GPS location with an ETrex 20 GPS unit (Garmin Ltd., Schaffhausen, Switzerland). I also observed the behavior (foraging, basking, other) of the lizard, recorded current weather conditions, and measured

temperature and habitat variables. Temperature data included ambient temperature, temperature of the lizard, temperature of the substrate immediately next to the lizard, and temperature of points 5 m away from the lizard in each of the four cardinal directions. I recorded surface temperatures with a NUB8380H handheld infrared thermometer (Nubee model NUB8380H, Duarte, CA) and recorded wind speed, humidity, and air temperature using a Kestrel 3000 weather meter (Kestrel, Downingtown, PA). These measurements were obtained without handling the lizards. Additionally, I recorded type of substrate and type of canopy cover at the lizard's location. If the lizard was located off the ground, a measurement of height above ground was made to the nearest 10 cm. The vegetation surrounding each lizard location was characterized using a modified line-intercept method. Vegetation type [open ground, herbaceous (grass and forbs), shrubs, cactus, trees] were recorded to the nearest 10 cm along four, 5-m line transects in each of the four cardinal directions from the lizard's location. "Open ground" was defined as areas with bare ground or herbaceous vegetation less than 5 cm in height, such that a THL could not reasonably seek shelter underneath vegetation. I collected further habitat data using the same line-intercept method from 60 points randomly located over the general area in which the lizards were released (Figure 2). This area was delineated in ArcGIS 10.4 (Environmental Systems Research Institute, Redlands, California) by computing a minimum convex polygon (MCP) around the locations of harvester ant colonies (see below).

I recorded body mass and SVL of each lizard once per week to ensure the radio transmitter weight never exceeded 10% of their body weight and to monitor overall health and body condition of the lizards. Lizards were weighed with a Pesola 0-60 g

spring scale (Pesola, Schindellegi, Switzerland), and SVL was measured to the nearest mm with a ruler. Replacement of radio transmitters was performed during weekly measurements as necessary.

Food can influence THL dispersal, survival, and habitat selection, so I systematically searched the area within 100-150 m of the release enclosures, and recorded all colonies of harvester ants. This provided a map of ant colonies within the area used by the lizards, and the study area was defined as the MCP created using these ant locations. When a lizard was located outside this area, I found and mapped the nearest harvester ant colony to the lizard's location. Once per week for six weeks, I recorded whether each of 30 randomly selected harvester ant colonies were active because predation by THLs can cause a colony to become inactive (McIntyre 2003). The activity of the nearest ant colony to each lizard was also recorded once per week for six weeks. Colonies were defined as active if at least one harvester ant was seen at the time of observation. Observation times were restricted to times of day that harvester ants were most likely to be active because temperature can affect their activity (Burrow et al. 2001).

Predators can also influence the dispersal, survival, and habitat selection of THLs, so I recorded all observations of potential predators, including Greater Roadrunners, Western Coachwhips, and Western Diamondback Rattlesnakes. The location, time, and general behavior were recorded for each observation. Survival is important to reintroduction success, so all known deaths of lizards were recorded, and the cause of death was determined to the best of my ability. When a radio signal was lost during the study, I attempted to locate the lizard from all edges of the study area for a week following loss of signal.

The Animal Use Protocol for this project was approved by the Institutional Animal Care and Use Committee of Texas State University (permit #201636162).

## **Data Analysis**

All analyses, except survival analyses, only used data collected by 5 August 2016, and were performed using 14 lizards that each had at least ten days of radio-tracking and a total of 20 or more location points. The survival analyses included all 23 lizards released in summer 2016, and all data collected until 24 October 2016.

To address the first hypothesis (survival), the following analyses were performed. Due to the right-censored nature of the data, Kaplan-Meier estimates were calculated using the *survival* package (Therneau 2015) in R (R Version 3.3.1, [www.r-project.org](http://www.r-project.org), accessed 30 August 2016). The Kaplan-Meier estimator is widely used in wildlife studies to estimate the probability of an individual in a population surviving a particular length of time (Pollock et al. 1989). Although the Kaplan-Meier estimator can be obtained from staggered-entry data, I converted all the lizard survival dates to “days post-release” prior to obtaining estimates. One estimate was performed with the assumption that all lizards that lost signal were deceased, as a lower bound, and a second estimate was performed with the assumption that all lizards that lost signal were alive, as an upper bound. Body mass was used as a measure of body condition. This was analyzed over time to detect any changes. Percent change in body mass from capture to death or end of study was also calculated. The cause of death for all lizards was examined to determine what predators impacted THLs at the study site.

To address the second hypothesis (home range and daily movement), the following analyses were performed. Daily movement for each lizard was calculated in ArcGIS 10.4, by determining the distance between consecutive days using the first location recorded each day. If points on consecutive days were not available, the distance between those days was not included. Daily movement was analyzed with respect to days since release to determine if there was a pattern, and specifically to evaluate whether daily movements decreased with time since release. This was done by comparing mean daily movement for the first seven days post-release to the mean daily movement for all subsequent days. Additionally, home range analyses were performed using the *adehabitatHR* package (Calenge 2006) in R. Minimum convex polygon and kernel density estimates (KDE) were each calculated at the 95 and 90% levels. All KDE estimates used a bivariate normal kernel function, with a grid of 1000, and an extent of 2. These home range estimates were calculated using three different sets of location data for each lizard: (1) the full set of all locations, (2) a subset where the first seven days of post-release locations were removed, and (3) a subset where the dispersal phase (prior to home range establishment) was visually estimated in ArcGIS 10.4 and those locations removed. I tested whether lizards settled into a home range by comparing the area of the home range estimates based on Datasets 2 and 3 to that of Dataset 1. This was done separately for each lizard and for each estimation method (MCP and KDE) at each probability level (95 and 90%). Estimates at the 95 and 90% levels within each estimation method, MCP and KDE, were also compared to determine the robustness of the differences in the datasets used.



To address the third hypothesis (habitat use), the following analyses were performed. Habitat use by lizards was compared to habitat availability using compositional data analysis (Aitchison 1982, Aebischer et al. 1993). This compared the habitat composition across all transects taken at lizard locations to the habitat composition across all transects taken at random locations. The distance to the nearest ant colony for each lizard location and each random point was calculated in ArcGIS 10.4. The mean distance between lizard points and ant colonies was compared to the mean distance between random points and ant colonies using Welch's *t*-test, which allows for unequal variances. Additionally, ant density was calculated using ArcGIS 10.4 across the study area and compared to the ant density in the home range of each lizard estimated using 95% MCP of Dataset 2 (locations post-7 days).

To address the fourth hypothesis (thermoregulation), I used a series of linear regressions analyzing the temperatures at and around lizard locations. Each regression was estimated separately for locations when the lizard was on the ground and for locations when the lizard was off the ground. The first pair of regressions analyzed the deviation in the ground temperature at the lizard location from the mean of the four ground temperatures, each 5 m away from the lizard location in the four cardinal directions, and used ambient temperature as a predictor. In these regressions, the ground temperature at the lizard location was the temperature that the lizard selected and the mean ground temperature at the four points 5 m away represents temperatures that the lizard likely could have selected. Thus, the deviation represents the degree to which the lizard selected relatively warmer or cooler temperatures. The second pair of regressions analyzed the lizard body temperature, and used ambient temperature as a predictor. The

third pair of regressions also analyzed lizard body temperature, but used ground temperature as a predictor. These latter regressions quantify how closely lizard body temperature correlated to environmental temperature, either ambient or ground.

For analysis of climbing behavior, a  $t$ -test was performed to compare the mean ambient temperature for lizard locations on the ground to the mean ambient temperature for lizard locations off the ground. Another  $t$ -test was performed to compare the mean ground temperature for lizard locations on the ground to the mean ground temperature for lizard locations off the ground. These tests were used to determine if lizards tended to exhibit climbing behavior at higher ambient and ground temperatures.

### III. RESULTS

#### Survival

A total of 1,349 radiotelemetry locations were collected from the 23 lizards released. However, many lizards died within the first week of release, so those were excluded from all analyses except survival. This left a total of 1,334 points collected from 14 lizards that survived more than ten days and had at least 20 locations. The mean number of locations from these lizards was 95.

Of the 23 lizards released, three died within enclosures prior to release, three died post-release before the third day of tracking, and two lost signal before the third day of tracking. The mean survival time was approximately 44 days after release, and nine lizards survived at least 54 days after release. Only one survived and successfully entered hibernation. Cause of death was determined for nine of the lizards, with five predated by Raccoons, three predated by Western Coachwhips, and one predated by a Western Diamondback Rattlesnake. Cause of death could not be determined for five lizards, and signal was lost on eight lizards. Of the lizards that lost signal, two lost signal during the more frequent summer tracking, and six lost signal after tracking frequency reduced in the fall. Table 1 shows the outcome for all 23 lizards.

The Kaplan-Meier estimator provided upper and lower bounds for survival probabilities under the assumption that all lizards that lost signal were deceased, and conversely, the assumption that all lizards that lost signal were alive. Both estimators illustrate an early drop in survival within the first few days post-release, followed by a relatively gradual decline in survival over the next 60 – 90 days (Figure 3). The two survival curves eventually stabilize at about 40 or 10% survival (depending on the

estimator) indicating the overall low survival of the released lizards through summer and into the fall (Figure 3).

Body condition of individual lizards showed little variation across time during the study. Body condition fluctuated slightly for most lizards, but none showed an overall decline (Figure 4). Additionally, there was no pattern of change in body mass between initial capture and death or end of study (Figure 4). The only lizard that showed an overall decline was the gravid female that lost body mass when she laid her eggs.

### **Daily Movement and Home Range**

The path movements of each lizard were mapped over the course of the study, from time of release until 5 August 2016 (Figure 5). Among all 14 lizards, mean path distance traveled was 1,011 m (SD = 522 m), mean net displacement distance from release (enclosure location) to the last recorded location was 100 m (SD = 65 m), mean maximum distance from release was 151 m (SD = 84 m), and mean daily movement was 15.4 m (SD = 8.1 m; Table 2). The difference in mean daily movement for the first seven days post-release (mean = 23.3 m, SD = 24.1 m) and the mean daily movement for all subsequent days (mean = 13.5, SD = 6.8 m) was not significant (paired  $t$ -test,  $t_{2\text{-tail}} = 1.70$ ,  $df = 13$ ,  $p > 0.05$ ). Nine of the 14 lizards had lower mean daily movement distances after the first seven days than during the first seven days post-release (Table 2).

Estimates of home range size varied based on whether MCP or KDE were used. The mean 95% home range size calculated using the full set of locations for each lizard was 0.86 ha (SD = 1.12 ha) using MCP and 1.88 ha (SD = 1.98 ha) using KDE (Table 3). Additionally, home range estimates depended upon the set of locations used (Table 3).

Removing locations that were assumed to be along the path of the lizard's initial dispersal substantially reduced the home range size estimates for all lizards, except L79 and L89 (Table 3). Comparing Dataset 2 (locations post-7 days) to Dataset 1 (all locations), home range size was reduced on average by 32.0% and 31.6% for MCP and KDE estimates at the 95% level, respectively, and by similar amounts at the 90% level (Table 4). Comparing Dataset 3 (locations with dispersal visually estimated) to Dataset 1 (all locations), home range size was reduced by 55.8% and 59.5% for MCP and KDE estimates at the 95% level, respectively, and by similar amounts at the 90% level (Table 4). Regardless of estimation method, MCP or KDE at 95 or 90% levels, home ranges were smaller when the initial dispersal phase was removed. Lastly, home range estimates for MCP and KDE estimates at the 95% level overlapped geographically (Figure 6, Figure 7).

### **Habitat Use**

I mapped a total of 287 harvester ant colonies surrounding the enclosures, which were used to create the MCP defining the study area (Figure 8). The study area was 26.9 ha, with an overall density of 10.7 harvester ant colonies/ha. Due to the movement of L62 outside the study area, an additional five harvester ant colonies were located, which were nearest to the locations of L62 outside the study area. This brought the total number of harvester ant colonies to 292. Although the systematic search for ants included searching for RIFAs, no RIFA mounds were found. However, RIFAs do exist in other areas of MMWMA, and there was some evidence of RIFAs within the study area.

Harvester ant activity at the random subset of 30 colonies sampled was 86% across the six weeks measured. Over the six-week monitoring period, at least 83% of harvester ant colonies were always active, except for the last week (3 August 2016) when 70% were active. Of the 30 harvester ant colonies that were monitored for activity, 18 were active for the entire six-week monitoring period, 11 were active at least 50% of the time, and one was inactive the entire period.

The mean distance to the nearest harvester ant colony was obtained for each lizard from its location points (on average 95 locations per lizard). The overall mean of these mean distances among all lizards was 8.0 m ( $n = 14$ ,  $SD = 2.3$  m), and 20.1 m for random points ( $n = 60$ ,  $SD = 17.8$  m). The difference between these was significant (Welch's  $t$ -test,  $t_{1\text{-tail}} = -5.08$ ,  $df = 66$ ,  $p < 0.0001$ ; Figure 9). The maximum mean distance from a harvester ant colony for a lizard was 13.6 m. Among all 1,334 lizard locations, only 24 (1.8%) were more than 25 m from a harvester ant colony, compared to the 13 (21.7%) random points which were more than 25 m from ant colony. Thus, lizards were always relatively close to a harvester ant colony. Additionally, the mean harvester ant density in the lizard home ranges (estimated with 95% MCP using Dataset 2) was 25.3 colonies/ha, while density in the overall study area was 10.7 colonies/ha (Figure 10).

Based on the compositional data analysis, there was no difference between mean habitat composition at all lizard locations and mean habitat composition (availability) at random locations ( $p = 0.57$ ,  $\chi^2 = 0.71$ ,  $df = 4$ ). Habitat composition at lizard locations consisted of 46% open ground, 37% herbaceous, 4% cacti, 7% shrubs, and 5% trees. Habitat composition at random locations consisted of 48% open ground, 28% herbaceous, 2% cacti, 6% shrub, and 15% trees (Figure 11). Although on average, lizards

appeared to use habitat in proportion to availability, lizards did vary in their habitat usage. For example, L62 and L94 used more open ground, whereas L65 and L77 used comparatively less (Figure 11).

### **Thermoregulation**

Ambient temperature was a decent predictor of ground temperature selection by lizards on the ground ( $R^2 = 0.19$ ,  $p < 0.0001$ , Figure 12A), and by lizards off the ground ( $R^2 = 0.12$ ,  $p < 0.0001$ , Figure 12B). At higher ambient temperatures ( $> 30^\circ\text{C}$ ), lizards were located at relatively cooler ground temperatures, and at lower ambient temperatures ( $< 30^\circ\text{C}$ ), lizards were located at relatively warmer ground temperatures. Ambient temperature was a good predictor of lizard body temperature for lizards on the ground ( $R^2 = 0.62$ ,  $p < 0.0001$ , Figure 13A), and for lizards off the ground ( $R^2 = 0.54$ ,  $p < 0.0001$ , Figure 13B). Lizard body temperature increased as ambient temperature increased. Ground temperature was a better predictor of lizard body temperature for lizards on the ground ( $R^2 = 0.91$ ,  $p < 0.0001$ , Figure 14A), and for lizards off the ground ( $R^2 = 0.84$ ,  $p < 0.0001$ , Figure 14B). Lizard body temperature increased as ground temperature increased. These relationships held regardless of whether the lizard locations were on or off the ground. Additionally, these relationships were consistent within each individual lizard (not shown), not just across all lizards.

About 62% of all lizard locations had lizards located on the ground, while for the other 38% of locations, lizards exhibited climbing behavior. The proportion of lizard locations off the ground each day appears to positively correlate with the mean ambient temperature of all locations taken each day (Figure 15). The mean ambient temperature

was higher for lizard locations off the ground than locations on the ground ( $35.0^{\circ}\text{C}$  vs  $31.3^{\circ}\text{C}$ ,  $t_{1\text{-tail}} = -17.17$ ,  $df = 1332$ ,  $p < 0.001$ ), and the mean ground temperature was higher for lizard locations off the ground than locations on the ground ( $36.3^{\circ}\text{C}$  vs.  $33.6^{\circ}\text{C}$ ,  $t_{1\text{-tail}} = -8.38$ ,  $df = 1332$ ,  $p < 0.001$ ).

## **Reproduction**

There was one nest laid by L91, a female who was gravid at capture and laid her nest on June 11. The process took over 24 hours. She started digging in the evening on June 10, continued digging throughout the day on June 11, and likely laid her eggs overnight to June 12, when she filled in the nest. The nest was successful, and 25 hatchlings emerged on July 24, giving an incubation time of 43 days. All eggs recovered from the nest had hatched successfully.



#### **IV. DISCUSSION**

Successful reintroduction of any species, including the THL, requires an understanding of multiple ecological factors affecting the species. Specifically, post-monitoring programs must focus on survival, dispersal, home range establishment, habitat usage, resource availability, reproduction, and behavior. Overall, the first hypothesis (survival) was mostly supported by a large number of losses shortly after release, followed by losses spaced further apart. Many lizards died within the first few days of the study, as depicted by the steep drop in survival in both Kaplan-Meier survival estimates. This was due to heavy predation early in the study. However, the survival curves also indicated lizards that survived the first few days after release tended to survive another two months (mean = 71 days). Body condition did not change over the course of the study, so lizards that survived likely maintained natural eating habits and thermoregulatory behavior necessary for survival. Unfortunately, the overall survival of the 23 released lizards was low, with only one lizard surviving until hibernation.

Predation was a major factor causing the death of lizards early in the study, with Raccoons, Western Coachwhips, and a Western Diamondback Rattlesnake as the known predators. Active removal of raccoons appeared to reduce predation, so further study investigating predator removal around release sites is needed. Previous studies also found predation to be a leading cause of death (Munger 1986, Wolf et al. 2013). Investigation of predator population levels prior to release could provide insight to potential release success. Also, the interval between tracking times may impact the rate of lost signal on lizards, so higher frequency tracking may reduce the occurrence of lost signals. Higher

frequency tracking may improve the success of identifying the cause of death, as the investigator will find the lizard closer to its time of death, before evidence is lost.

To achieve higher survival, and thus higher reintroduction success for future releases, I suggest implementing a predator removal program prior to release, and maintaining predator removal throughout the course of the study. Additionally, I suggest releasing a large number of individuals, as survival may increase with increased population density (each individual lizard is less likely to be predated). Releasing 100-200 lizards at a time, and spacing these releases out on the landscape could provide greater reintroduction success. However, obtaining 100-200 THLs could place greater pressure on the source populations (Miller et al. 2011), so care should be taken to not overharvest and threaten the loss of any current populations. Further research on reintroduction using captive bred THLs is needed to determine whether captive bred THLs could be an alternative to wild-caught THLs for reintroduction. I suggest releases of captive-bred adults, as well as hatchlings. Hatchlings may be more susceptible to predators and RIFAs following release; however, the reduction in costs associated with raising THLs in captivity may allow a net increase in the number of individuals successfully reintroduced.

While daily distance movements did not seem to decrease with time since release, lizards did settle into home ranges, providing mixed support for the second hypothesis (home range and daily movement). The mean daily movement distance of 15.4 m was lower than that suggested in the literature of 36.5-46.8 m on average (Whitford and Bryant 1979, Fair and Henke 1999). Translocated individuals might move less than resident THLs, but this difference could be due to several other factors. For example,

variation in geographic location of the studies can induce variation in habitat quality, which could impact movements needed for adequate foraging and thermoregulation. Also, time of year in which the studies occurred, sample size, and frequency of tracking, can each impact the difference in estimated daily movements. Harvester ant activity stayed high throughout the study, suggesting that food availability was plentiful. This might explain why the reintroduced THLs, who had no native competition for harvester ants, did not disperse or move large distances. Homing behavior, which is common in herpetofauna (Germano and Bishop 2009, Santos et al. 2009), did not occur for lizards held 18 and 12 days because no lizards lost signal near release. Further evidence is provided by the short distances for maximum displacement (mean = 151 m) and start to end distances (mean = 100 m). Conversely, a three-day holding period for soft release might not be long enough, since the signal was lost on two lizards that were held only for three days. Small sample size and holding time confounded with release location and time prevent this study from drawing strong conclusions on the effect of holding time. The optimal number of holding days that reduces homing behavior and over-dispersal, but does not provide added stress to lizards, decreasing survival (Germano and Bishop 2009, Roe et al. 2010) is likely 10-18 days.

Home range size varied depending on the method and set of locations used for estimation. The approximately 30% decrease in home range size estimates, for both 95% MCP and KDE methods, using Dataset 2 (locations post-7 days) versus Dataset 1 (all locations) indicated there was a dispersal phase. This is further supported by the approximately 55-60% decrease in home range size estimates, for both 95% MCP and KDE methods, using Dataset 3 (locations with dispersal visually estimated) versus

Dataset 1 (all locations). This result was consistent between the 95 and 90% levels for determining home range, even though the 95% level gives larger, more inclusive, estimates of home range size. Thus, THLs clearly exhibited a dispersal phase after release before settling into a home range. While mean daily movement did not significantly differ between the first seven days post-release and all subsequent days, movements within the first seven days were directional (dispersal), whereas movements in subsequent days maintained a lizard's general location (home range).

The 95% MCP home range estimates using Dataset 2 (locations post-7 days) overlap for many lizards. This is not surprising due to a shared release location among lizards, as well as literature evidence that the THL is not territorial (Sherbrooke 2003, Whitford and Whitford 1973). Home range size estimates were similar to reported literature of non-translocated lizards. My results estimated mean home range size using 95% MCP and Dataset 1 (all locations) to be 0.86 ha, which is slightly smaller than the 1.37 ha home ranges reported from the same county, Mason, Texas (Granberg 2014). These estimates are greater than the 0.24 ha home ranges reported using the same methods in an urban setting, where roads likely restricted home range size (Wall 2014). However, estimates from a rural area were also smaller, ranging 0.01 to 0.34 ha, but these were calculated using weekly datasets (Fair and Henke 1999). The present study used a relatively larger sample size, with more frequent tracking, so the results are likely to provide an accurate estimation of home range size for THLs.

Home range size estimates varied greatly among individual lizards, suggesting ecologically meaningful variation among lizards in this life history characteristic. This variation could be due to variation in density of harvester ant colonies, their food source.

The mean density of harvester ant colonies in the area used by each lizard showed some variation, but was higher than the overall density in the study area. This suggests THLs may select for areas with high densities of harvester ants. However, this could be confounded with the fact that the release location was chosen for its high number of harvester ants, and only the area around the release enclosures was surveyed. It may be that this area happened to have a high density of harvester ants. Further investigation on harvester ant colony density across the geographic range of the THL is needed to better determine the role harvester ants might play as a predictor of THL presence (McIntyre 2003, Webb and Henke 2008), and aerial photography might provide a relatively easy way to quantify this (Crist and Wiens 1996, Everitt et al. 1996). THLs may not be dependent upon density of harvester ants, and distance to nearest colony may be a more important factor.

Support for the third hypothesis (habitat use) was mixed because the habitat composition of areas used by lizards did not differ from the habitat composition available in the study area, but lizards did select for locations closer to harvester ant colonies than would be randomly expected. The compositional data analysis showed that overall habitat use was random, but the habitat used by each individual varied. This could also be a matter of scale, as the study area was selected because it appeared to contain “good” THL habitat based on the vague literature description previously reported (Whiting et al. 1993, Henke and Fair 1998, Burrow et al. 2001, Hellgren et al. 2010, Horned Lizard Watch 2010, Wolf et al. 2013). Previous studies are limited by their inability to quantitatively substantiate their claims (Henke and Fair 1998), focus on only part of the year (Whiting et al. 1993), and narrow spatial scale used (Burrow et al. 2001). These descriptions have

propagated through the literature, as exhibited by Wolf et al. (2013) citing Hellgren et al. (2010), who copied the description from Burrow et al. (2001). Burrow et al. (2001) is the only other study with a large dataset (854 locations from 89 lizards); however, they only recorded habitat on the point and frame scale. This narrow focus on the point of the lizard ignores all the other habitat available to the lizard, within a few meters, but outside the area measured by Burrow et al. (2001). While the present study only analyzes 14 lizards from May to August, the large number of locations, collected throughout many months when lizards are active and on a spatial scale appropriate to THL movements, provides a comprehensive view of the habitat used by the THL.

Due to THL dependence on harvester ants as a major food source (Winton 1915, Pianka and Parker 1975, Donaldson et al. 1994, Sherbrooke 2003), it was not surprising they remained relatively close to harvester ant colonies. I suggest future reintroduction studies focus on areas with high harvester ant densities to provide a food source to released lizards. I conclude that THL used areas with habitat that provides open bare ground and herbaceous, cacti, or shrub cover in a heterogeneous spatial distribution that allows lizards to access any habitat type within just a few meters of any other. This may be largely due to the habitat selection of harvester ants, which tend to form colonies in open and disturbed habitat (Hellgren et al. 2010). The present study provides a detailed description of THL habitat use, but further study is needed in other locations throughout the historic range to better determine habitat preference of the THL.

Unfortunately, the effect of RIFAs on reintroduced THLs could not be investigated in this study due to the inability to quantify their occurrence. The presence of RIFAs in the area and on the property was confirmed by numerous sightings throughout

the summer, but the study area was devoid of mounds. It is possible that release of the phorid fly (*Pseudacteon sp.*), a predator of RIFAs, has reduced density and activity of RIFAs in the area (Gilbert et al. 2008). Future reintroduction studies should consider RIFA presence and density to determine the impact on THL reintroduction success, as evidence suggests RIFAs impact food supply (Donaldson et al. 1994, Allen et al. 2004), nest success (Allen et al. 2004), and hatchling survival (Thawley and Langkilde 2016).

Lastly, the fourth hypothesis (thermoregulation) was supported by the linear regression results. Lizards selected for relatively cooler ground temperatures at high ambient temperatures, likely as a method to prevent overheating. Additionally, lizards selected relatively warmer ground temperatures at low ambient temperatures, suggesting lizards were trying to warm themselves. Lizard temperature was influenced by ambient temperature and ground temperature, which is not surprising as these temperatures are likely correlated. Moreover, lizard climbing behavior correlated with ambient temperature and ground temperature, with lizards using climbing behavior at higher ambient and ground temperatures. This demonstrates that THLs used climbing for thermoregulation, a behavior not previously discussed in the literature. Future studies could place data loggers on individual lizards to more closely track temperatures throughout the day. These thermoregulatory responses were expected for an ectotherm, but more importantly suggest reintroduced THLs exhibit seemingly normal thermoregulatory behavior post-release, which is essential for survival.

The successful nest provides evidence that reintroduced THLs maintain natural behaviors necessary for successful reproduction. A female released during summer 2015 successfully hibernated and mated, becoming gravid during summer 2016. Unfortunately,

she was predated prior to nesting, but this provides additional evidence for successful reproduction post-reintroduction. Reproduction is an important factor in successfully establishing a population, so proper analysis of the reproductive events and the success rate of nests and hatchlings is important for future studies to consider. One method of analyzing nest success could be to monitor many females, tracking at least once per day in order to locate nests when they are laid. Then, data loggers can be placed in and around the nests to monitor environmental conditions, which can then be compared to nest success.

Overall, the main goal of reintroduction is to establish self-sustaining populations, which requires released individuals to stay in the area, survive, and reproduce successfully. For the THL, using soft release methods can help to ensure lizards stay in the area where they are released. Furthermore, there is evidence that reintroduced THLs can breed and nest successfully, so there is potential for their offspring to add to and maintain the new population. However, the main challenge to THL reintroduction success is survival. Predator removal and release of a larger number of individuals is likely to increase success. Thus, reintroductions of the THL are likely to be successful, but further study is needed to determine the best methods to increase survival.

The results of this study provide partial guidelines for reintroduction of this threatened species. Future studies should focus on better defining the methods for successful THL reintroductions, specifically focusing on survival, habitat, holding time, the role of harvester ant availability, and the impact of RIFAs. By better understanding the factors involved in the successful reintroduction of this species, we can better implement management practices to ensure the success of other reintroductions. These



guidelines can aid researchers in other reintroduction efforts, including lizards and other herpetofauna species, by providing insight to major difficulties faced during reintroduction (Ewen et al. 2014). The ultimate goal is to reintroduce the THL to parts of its historical range, reconnecting isolated populations, and ensuring the survival of a threatened and iconic species.

## V. TABLES

Table 1. Survival information for each of 23 Texas Horned Lizards released during summer 2016. THL ID# is the unique identifier for each lizard, Release Date is the date that the lizard was released from the enclosure, Outcome is the determined outcome or current status of the lizard, Last Date Observed is the date of the last observation recorded for that lizard, and Total Days is the total number of days the lizard was known to be alive since release.

THL ID#	Release Date	Outcome	Last Date Observed	Total Days
L59	5/30/2016	Predation by Raccoon	5/30/2016	0
L60	5/30/2016	Predation by Raccoon	6/1/2016	2
L62	5/30/2016	Lost Signal	8/5/2016	67
L63	5/30/2016	Predation by Raccoon	6/1/2016	2
L64	5/30/2016	Predation by Coachwhip	6/18/2016	19
L65	5/30/2016	Lost Signal	9/8/2016	101
L67	5/30/2016	Lost Signal	8/30/2016	92
L68	5/30/2016	Lost Signal	8/30/2016	92
L71	5/30/2016	Predation by Raccoon	5/30/2016	0
L73	5/30/2016	Unknown	10/24/2016	147
L77	6/8/2016	Predation by Rattlesnake	6/19/2016	11
L78	6/8/2016	Predation by Coachwhip	7/2/2016	24
L79	6/8/2016	Predation by Coachwhip	7/22/2016	44
L83	6/8/2016	Unknown	6/10/2016	2
L84	6/8/2016	Lost Signal	8/22/2016	75
L86	6/8/2016	Lost Signal	8/15/2016	68
L87	6/8/2016	Predation by Raccoon	5/27/2016	0
L88	6/8/2016	Unknown	6/10/2016	2
L89	6/8/2016	Unknown	7/28/2016	50
L91	6/8/2016	Unknown	8/5/2016	58
L92	6/12/2016	Lost Signal	6/12/2016	0
L93	6/12/2016	Lost Signal	6/13/2016	1
L94	6/12/2016	Hibernating	11/7/2016	148

Table 2. Distance measurements for each of 14 Texas Horned Lizards released during summer 2016. THL ID# is the unique individual identification number for each lizard, Path Distance is the linear path distance between all lizard locations, Ending from Release is the distance between the release location and the last recorded location, Maximum from Release is the distance between the release location and the furthest recorded location from release, Mean Daily Movement is the mean distance between the first point recorded on consecutive days, and N is the total number of daily movements for each lizard.

THL ID#	Path Distance	Ending from Release	Maximum from Release	Mean Daily Movement 1st 7 Days	Mean Daily Movement Post-7 Days	N
L62	1971.8	183.4	387.3	96.8	18.0	57
L64	277.8	96.3	98.9	5.2	13.7	18
L65	846.6	52.9	63.7	15.1	7.7	62
L67	1206.2	105.4	150.4	19.4	10.7	62
L68	850.3	133.2	117.1	14.7	6.9	62
L73	815.2	211.4	213.0	9.4	7.9	62
L77	260.8	122.7	157.2	18.8	10.0	11
L78	866.5	97.3	129.7	43.7	21.1	23
L79	1428.4	196.3	243.5	19.5	25.3	41
L84	741.8	34.9	72.7	6.3	7.0	53
L86	781.6	73.9	124.3	8.8	9.5	51
L89	826.3	8.0	99.0	10.5	10.3	43
L91	1289.7	11.0	118.4	17.4	14.4	53
L94	1989.9	72.4	138.4	40.6	27.2	47
Mean	1010.9	100.0	151.0	23.3	13.5	46.1

Table 3. Home range size estimates (ha) for each of 14 Texas Horned Lizards estimated with minimum convex polygon (MCP) and kernel density estimate (KDE) methods, three different sets of lizard locations (full, post-7 days, and visual estimation of dispersal), and 95 and 90% levels.

THL ID#	Full				Post-7				Visual			
	MCP		KDE		MCP		KDE		MCP		KDE	
	95%	90%	95%	90%	95%	90%	95%	90%	95%	90%	95%	90%
L62	4.19	2.77	6.54	3.95	2.77	0.39	3.81	2.14	0.35	0.30	0.79	0.62
L64	0.18	0.06	1.34	1.04	0.05	0.05	1.04	0.78	0.01	0.01	0.02	0.02
L65	0.13	0.12	0.25	0.20	0.11	0.10	0.20	0.16	0.11	0.10	0.20	0.16
L67	0.60	0.52	0.96	0.67	0.34	0.07	0.57	0.38	0.06	0.06	0.13	0.10
L68	0.16	0.14	0.54	0.39	0.13	0.05	0.28	0.19	0.03	0.03	0.05	0.04
L73	0.80	0.78	2.64	2.13	0.71	0.61	2.14	1.72	0.63	0.60	1.87	1.51
L77	0.02	0.01	0.79	0.54	0.01	0.01	0.12	0.09	0.01	0.01	0.13	0.10
L78	1.06	0.49	2.12	1.61	0.14	0.05	0.38	0.28	0.11	0.11	0.27	0.20
L79	2.29	2.29	6.02	4.80	2.31	2.25	5.93	4.62	0.19	0.14	0.62	0.48
L84	0.10	0.10	0.23	0.18	0.09	0.09	0.19	0.15	0.10	0.10	0.20	0.17
L86	0.59	0.51	1.56	1.26	0.56	0.39	1.34	1.07	0.17	0.16	0.40	0.33
L89	0.53	0.32	1.11	0.87	0.55	0.35	1.22	0.97	0.53	0.32	1.11	0.87
L91	0.61	0.59	1.27	0.99	0.49	0.38	0.92	0.71	0.38	0.26	0.69	0.53
L94	0.77	0.22	1.04	0.75	0.19	0.17	0.70	0.53	0.23	0.22	0.78	0.58
Mean	0.86	0.64	1.88	1.39	0.60	0.35	1.35	0.98	0.21	0.17	0.52	0.41

Table 4. Percentage difference between home range estimation methods using two different datasets (Post-7 and Visual) compared to the full dataset (all locations), two methods of estimation (MCP and KDE), and two levels of confidence (95% and 90%). All comparisons were made between the listed reduced dataset and the full dataset. THL ID# is unique identifier for each Texas Horned Lizard, Post-7 indicates use of the dataset that excludes the first seven days of locations post-release, Visual indicates use of the dataset that excludes the visually estimated dispersal locations, KDE indicates use of kernel density estimate method, MCP indicates use of minimum convex polygon method, 95% indicates estimation at the 95% level, and 90% indicates estimation at the 90% level.

THL ID#	Post-7				Visual			
	MCP		KDE		MCP		KDE	
	95%	90%	95%	90%	95%	90%	95%	90%
L62	-33.9	-86.0	-41.7	-45.9	-91.7	-89.2	-88.0	-84.4
L64	-73.2	-19.6	-21.9	-25.0	-96.3	-89.6	-98.5	-98.4
L65	-19.0	-14.1	-20.9	-16.5	-19.0	-14.1	-22.3	-18.0
L67	-44.2	-86.9	-40.5	-44.3	-89.5	-88.7	-86.9	-85.3
L68	-21.1	-65.1	-48.1	-51.2	-82.8	-81.8	-90.9	-89.8
L73	-11.5	-22.4	-19.0	-19.5	-20.9	-23.4	-29.2	-29.2
L77	-50.7	-42.6	-85.2	-83.0	-16.8	-14.3	-83.4	-81.0
L78	-86.9	-89.0	-82.0	-82.6	-89.3	-78.1	-87.3	-87.5
L79	0.7	-1.6	-1.6	-3.9	-91.8	-94.0	-89.8	-89.9
L84	-11.8	-14.5	-18.3	-17.3	-3.7	-1.1	-12.3	-10.4
L86	-5.4	-23.3	-13.8	-14.6	-71.4	-69.5	-74.3	-74.0
L89	4.3	7.5	10.4	11.5	0.0	0.0	0.0	0.0
L91	-19.3	-35.2	-27.1	-28.6	-37.5	-56.3	-45.7	-46.4
L94	-75.6	-24.8	-32.6	-30.3	-70.3	-3.5	-24.4	-23.2
Mean	-32.0	-37.0	-31.6	-32.2	-55.8	-50.3	-59.5	-58.4

#### IV. FIGURES

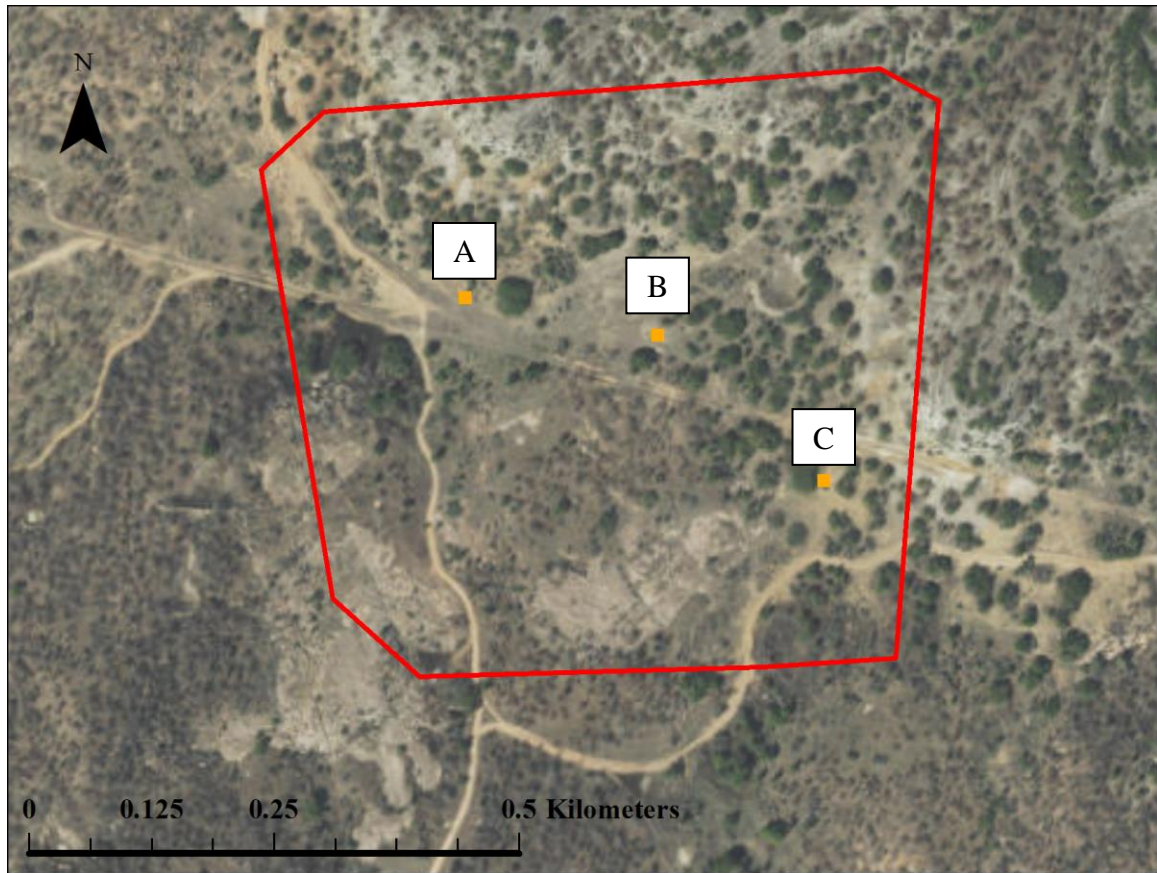


Figure 1. Map of the Texas Horned Lizard release enclosures (orange squares), with the minimum convex polygon (red polygon) delineating the study area. The enclosures were identified as A, B, and C.

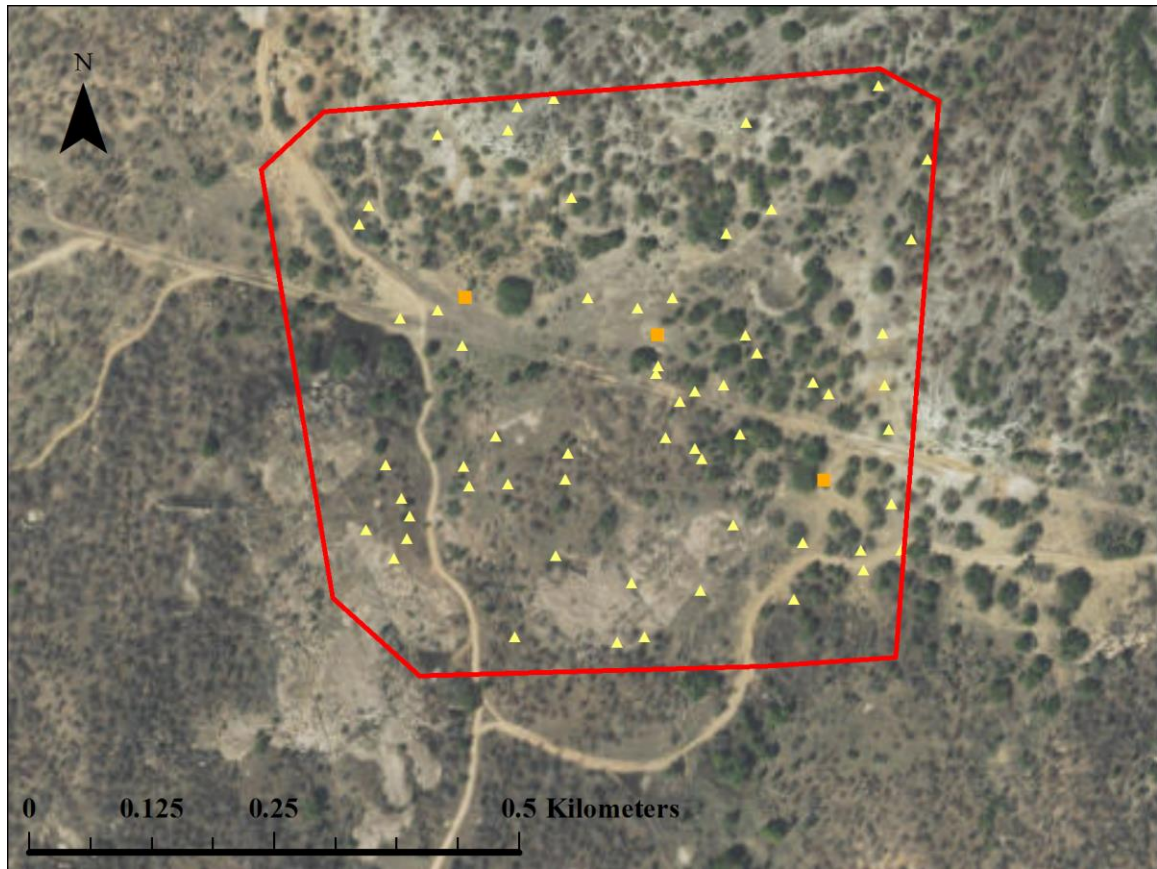


Figure 2. Map of 60 random point locations (yellow triangles) within the study area (red polygon). These points were generated using ArcGIS 10.4. The release enclosure locations for Texas Horned Lizards are also shown for reference (orange squares).

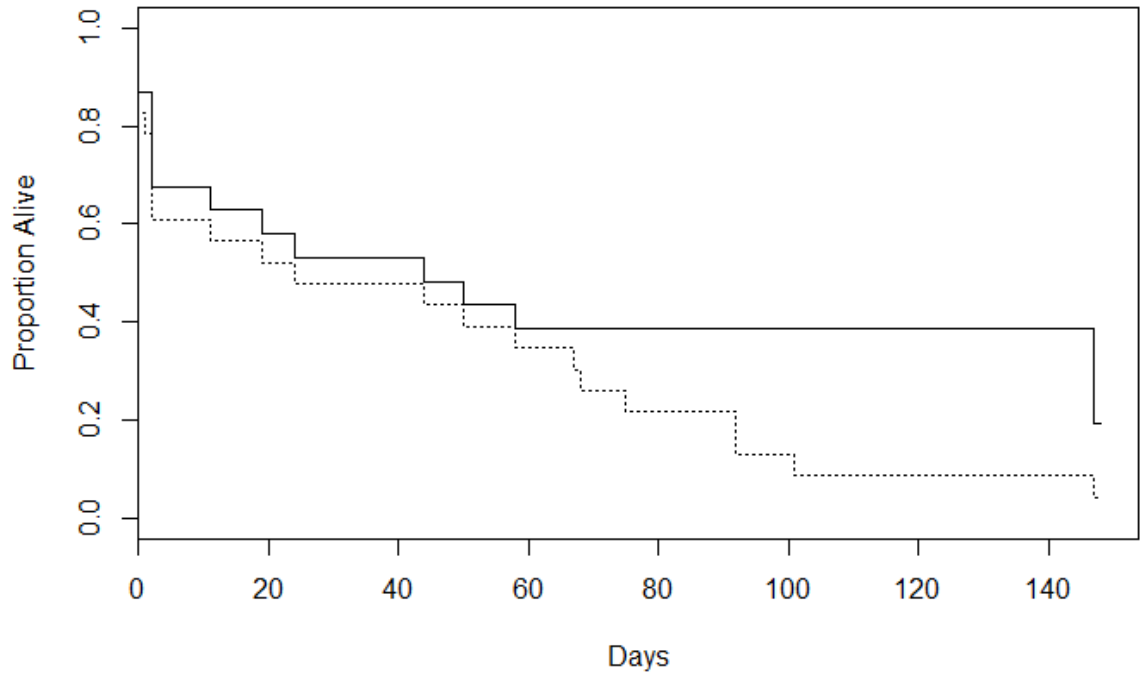


Figure 3. Kaplan-Meier survival curves calculated for 23 Texas Horned Lizards released in summer 2016. The solid line represents the upper bound for survival estimates, where lizards that lost signal were assumed to be alive. The dashed line represents the lower bound for survival estimates, where lizards that lost signal were assumed to be dead.



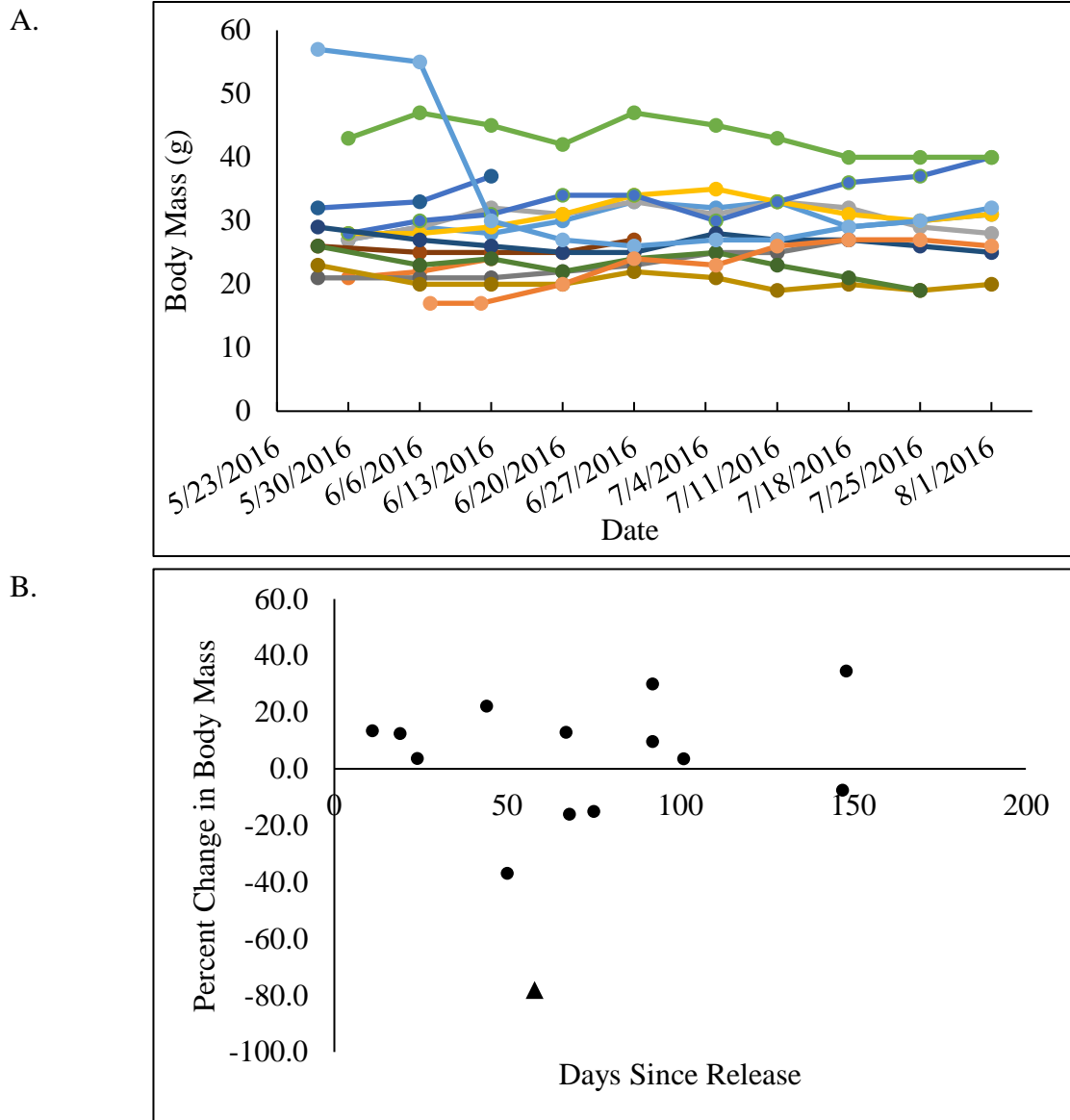


Figure 4. Body mass (g) of 14 Texas Horned Lizards during radio-tracking in summer 2016 shown across time (A) and as percent change in body mass (B). Each colored line (A) represents a single lizard. Percent change in body mass was calculated as the percent deviation between the initial mass and the last recorded mass of each lizard. The triangle symbol for percent change in body mass represents the gravid female that lost mass when she laid her eggs.

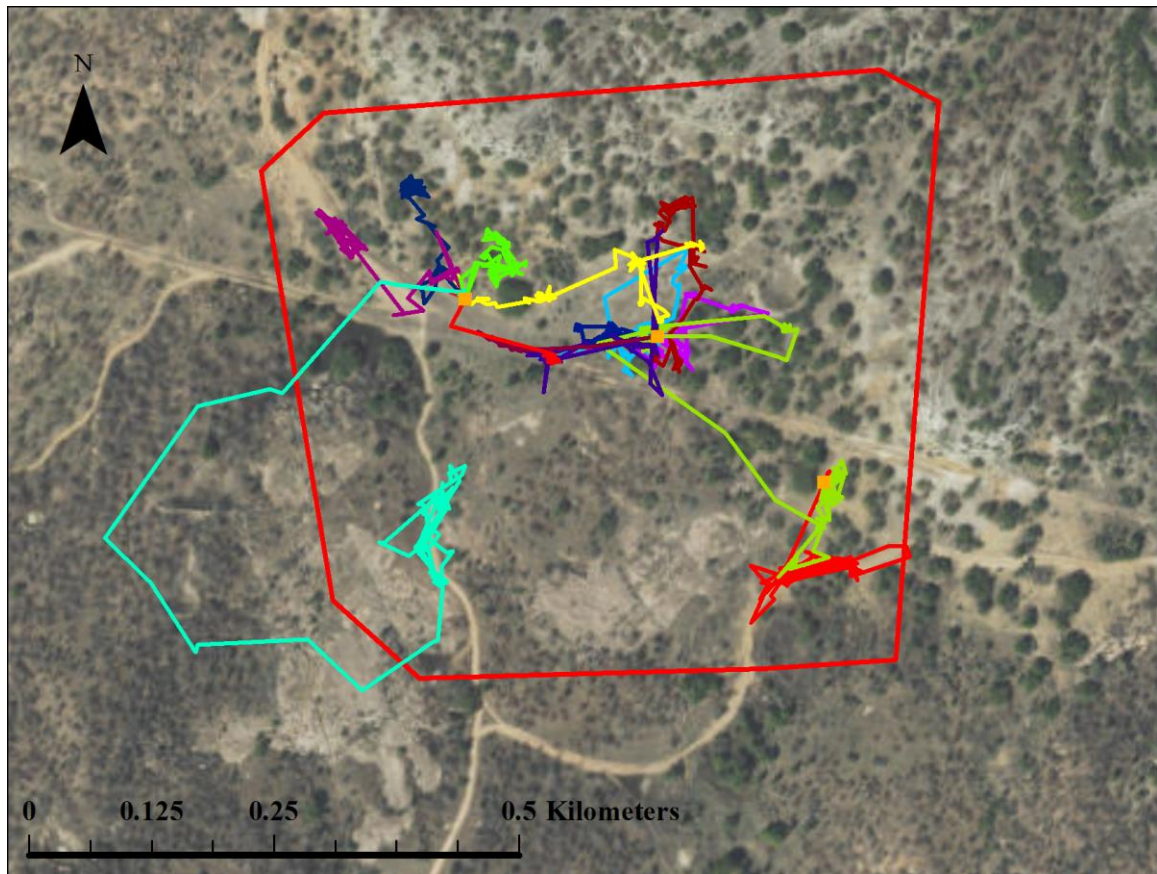


Figure 5. Map of individual Texas Horned Lizard movements (colored lines) following release from enclosures (orange boxes). The study area (red polygon) is provided for reference. One lizard moved outside the study area for eight locations.

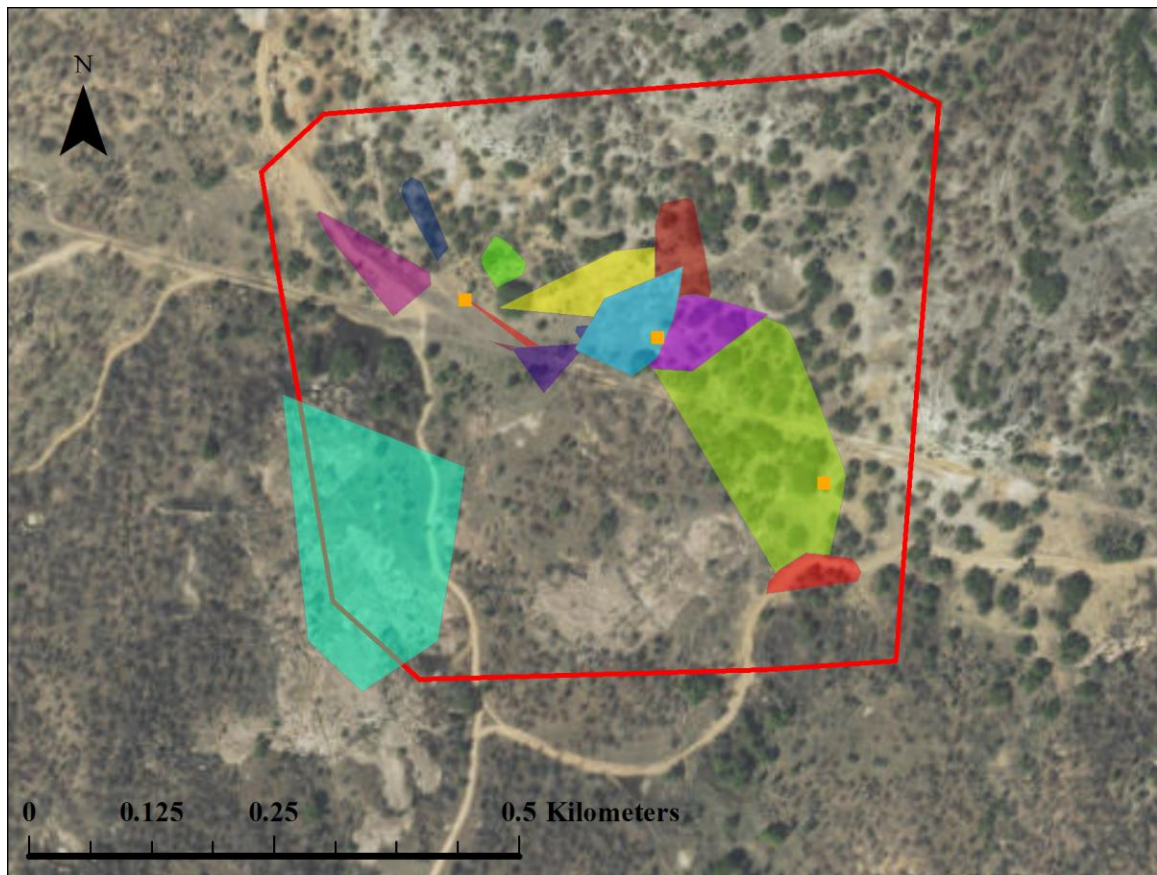


Figure 6. Map of home range estimates for each of 14 Texas Horned Lizards (colored polygons) estimated with 95% MCP using Dataset 2 (locations post-7 days). The study area (red polygon) is provided for reference.



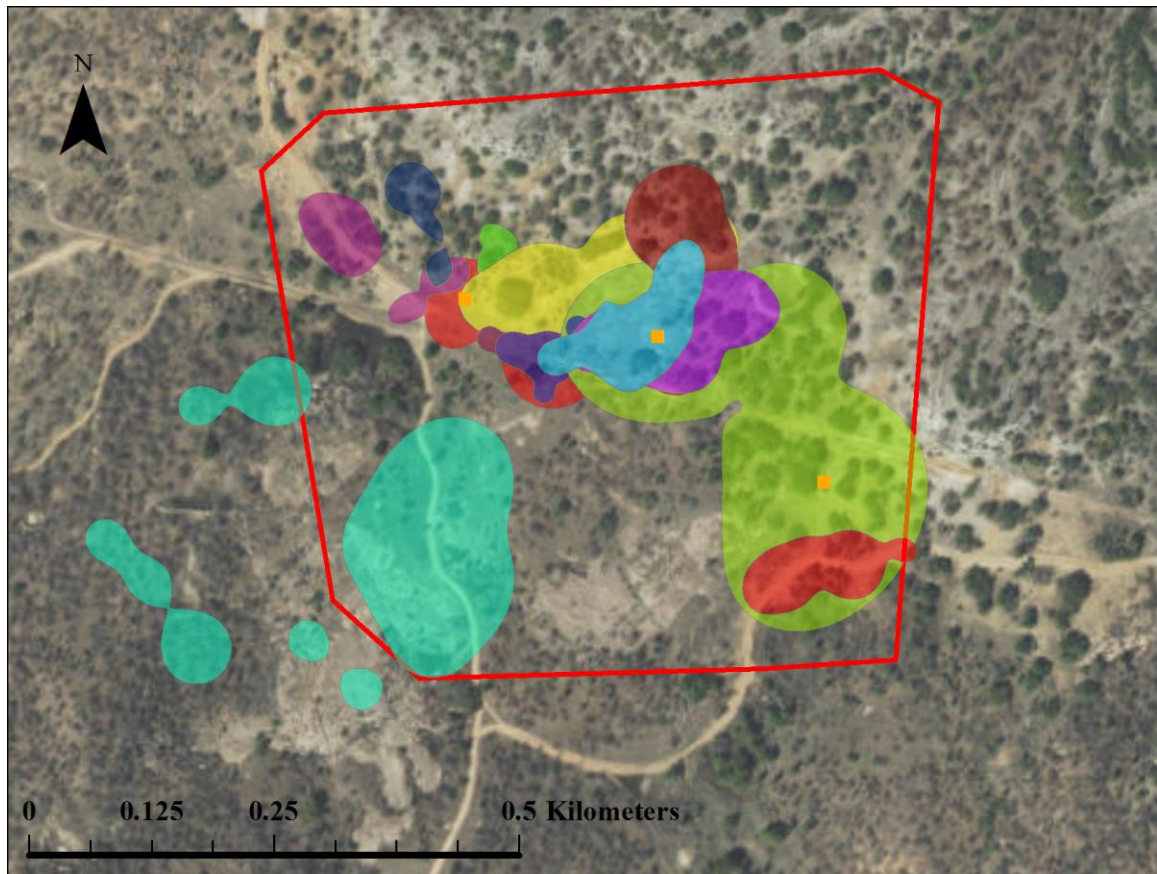


Figure 7. Map of home range estimates for each of 14 Texas Horned Lizards (colored polygons) estimated with 95% KDE using Dataset 2 (locations post-7 days). The study area (red polygon) is provided for reference.

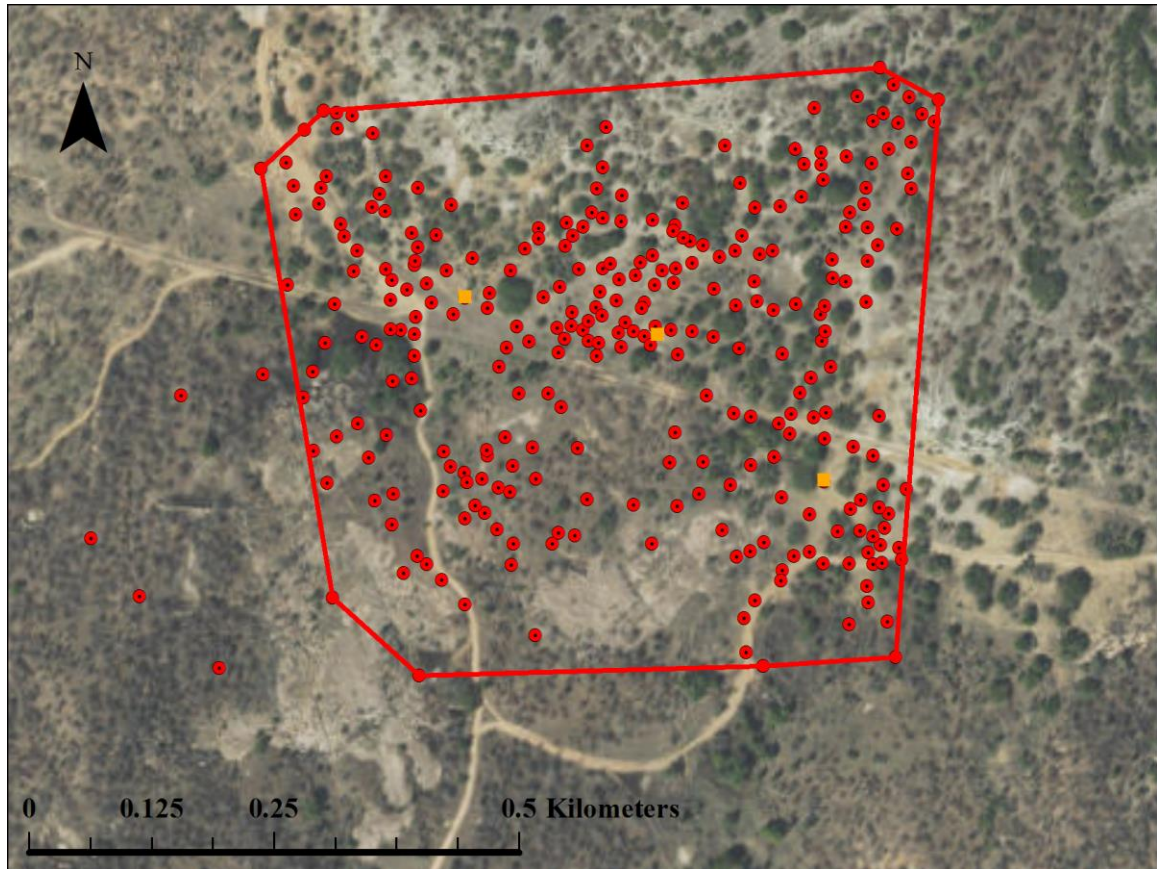


Figure 8. Map of harvester ant colonies (red circles) with minimum convex polygon (red line) delineating the study area around the release enclosures (orange squares). Harvester ant colonies outside the MCP were located when a Texas Horned Lizard moved outside the study area. There were a total of 287 colonies within the MCP, and five outside the MCP.

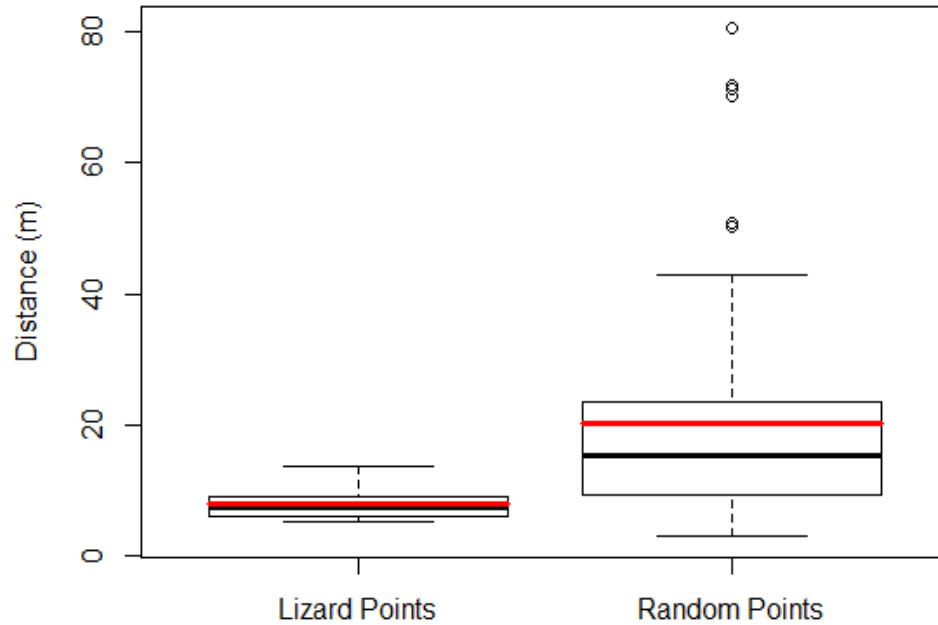


Figure 9. Boxplots depicting distribution of distance to the nearest ant colony averaged for each of 14 Texas Horned Lizard's locations, and for each of 60 random points. Red bars are the means, bold black bars are the medians (50<sup>th</sup> percentiles), boxes represent 25<sup>th</sup> and 75<sup>th</sup> percentiles, whiskers show maximum and minimum values within 95%, and dots are outliers. The difference was significant (Welch's *t*-test,  $t_{1\text{-tail}} = -5.08$ ,  $df = 66$ ,  $p < 0.0001$ ).

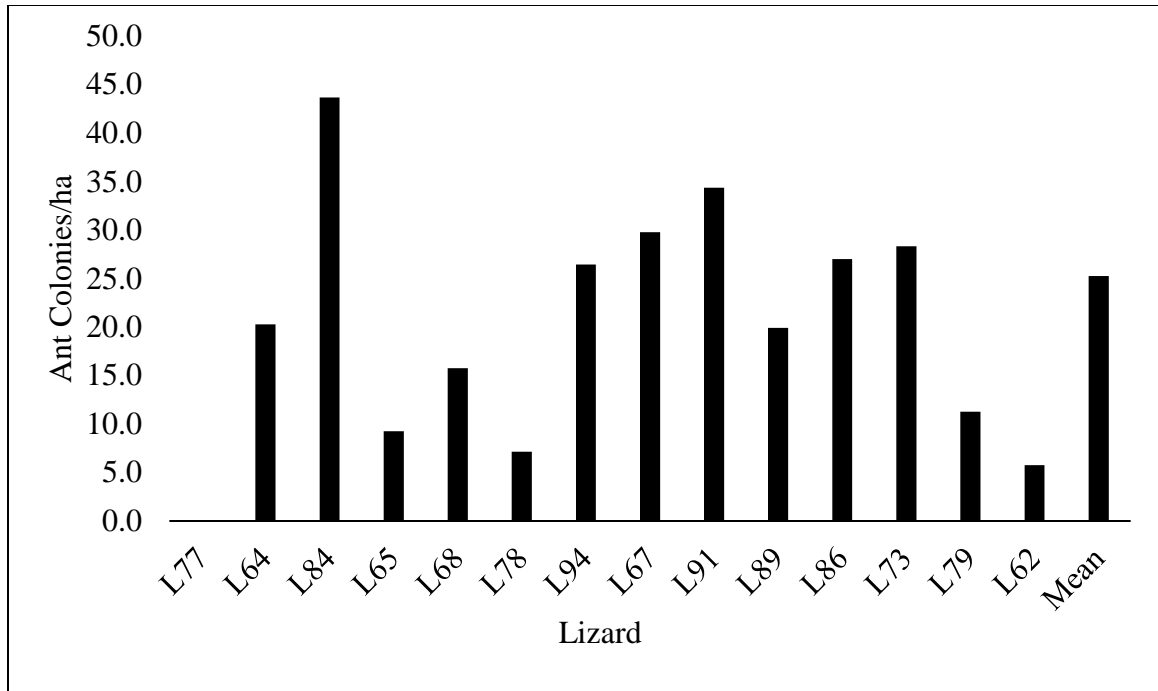


Figure 10. Density of harvester ant colonies (colonies/ha) within estimated home range for each of 14 Texas Horned Lizards. Lizards are arranged left to right in increasing home range size using 95% MCP with Dataset 2 (locations post-7 days). The estimated home range for L77 did not contain any harvester ant colonies. Mean harvester ant density across all lizards is also shown.

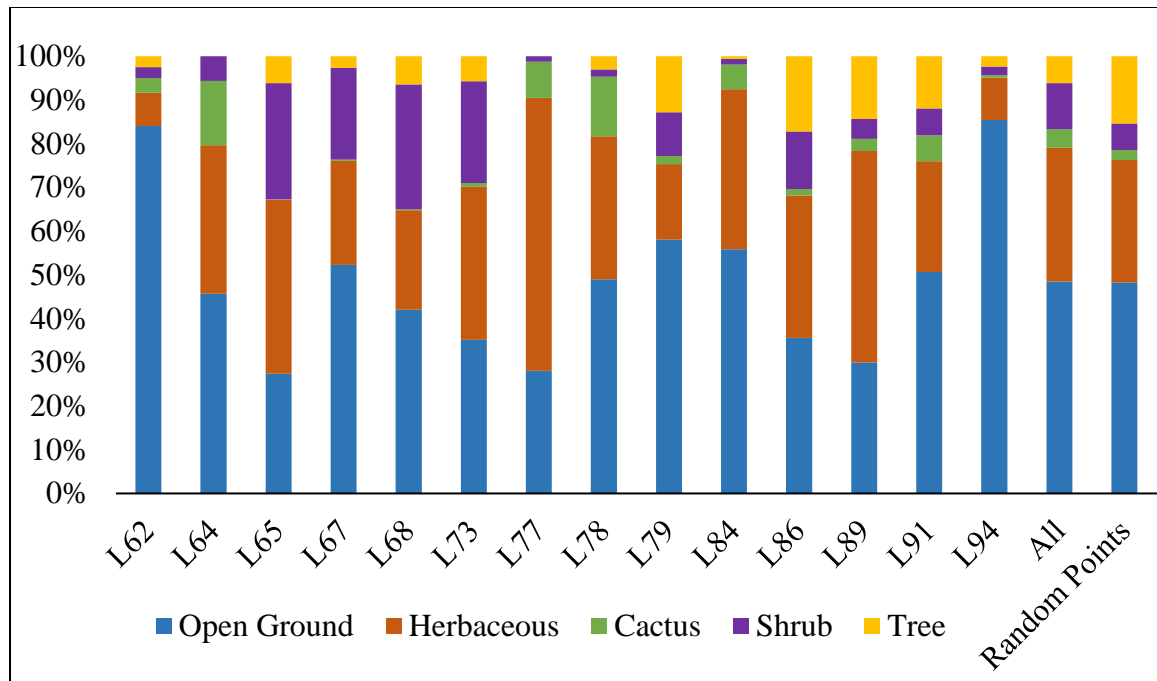


Figure 11. Average habitat composition for each Texas Horned Lizard, across all lizards, and across 60 random points. Compositional data analysis showed average habitat use by lizards was not different from random availability ( $p > 0.05$ ). Some individual lizards may be using habitat different than random.



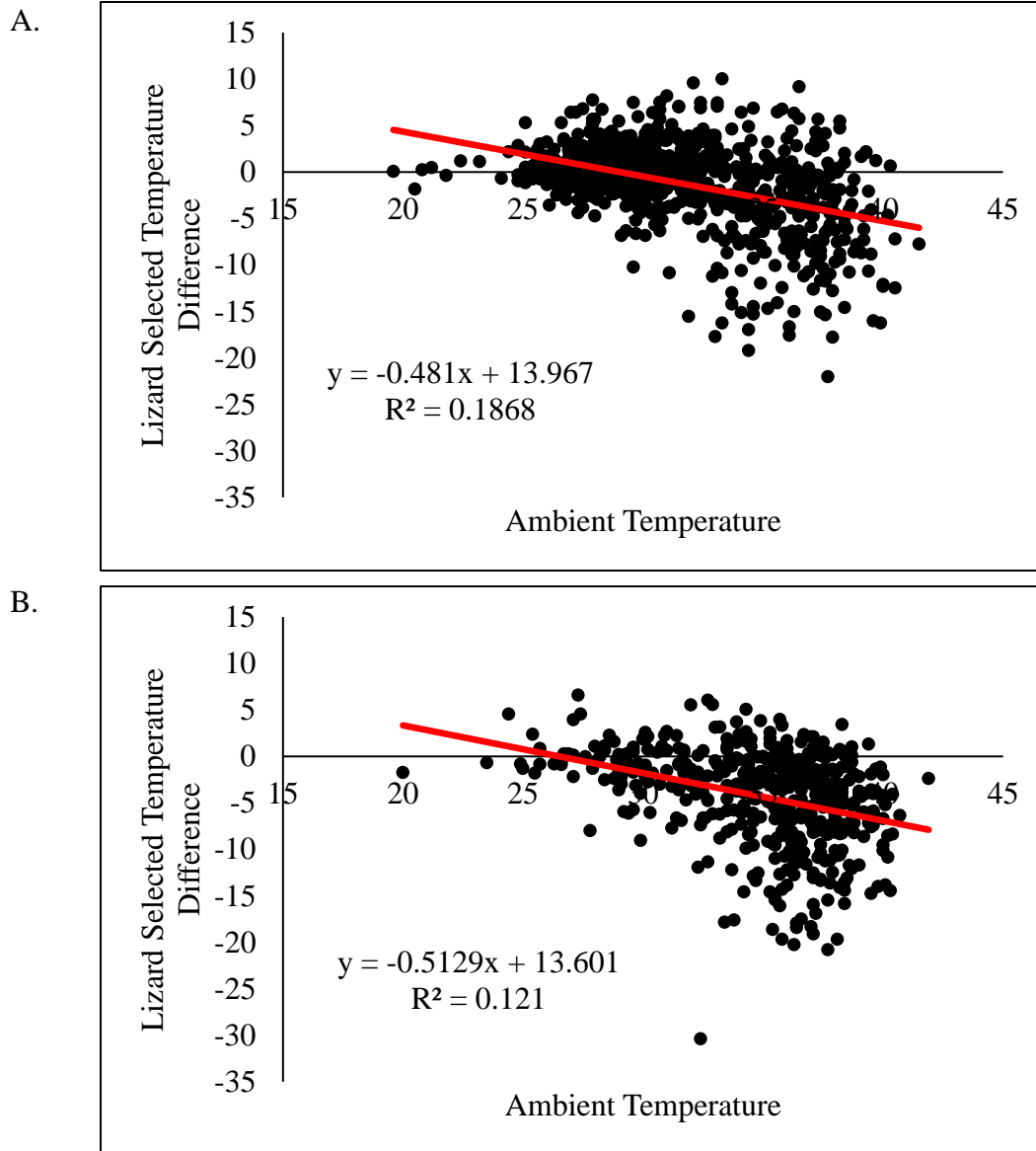
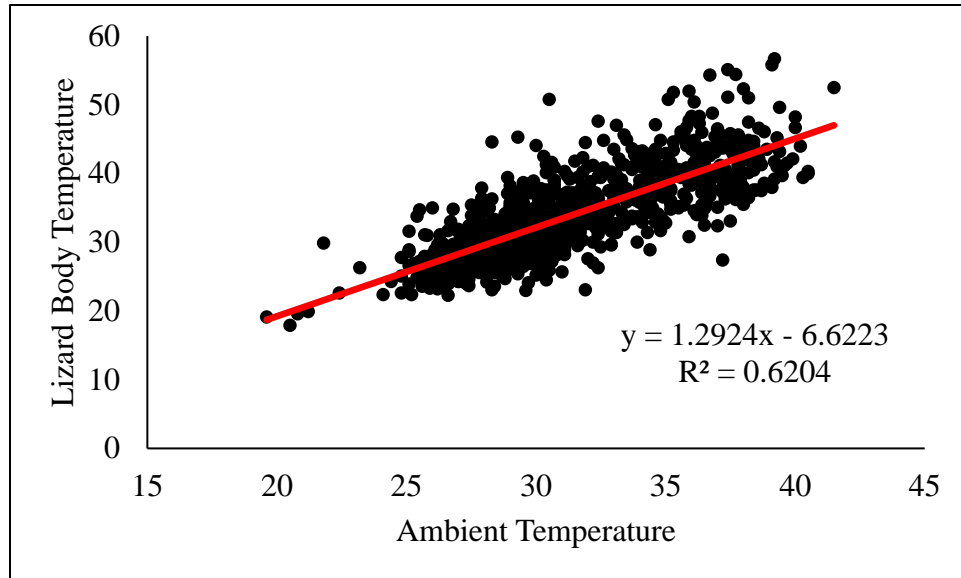


Figure 12. Ground temperature selection based on ambient temperature for Texas Horned Lizards on the ground (A) and off the ground (B). Lizard selected temperature difference is the deviation between the ground temperature at the lizard location and the mean ground temperature at four points, each 5 m away from the lizard location in the four cardinal directions.

A.



B.

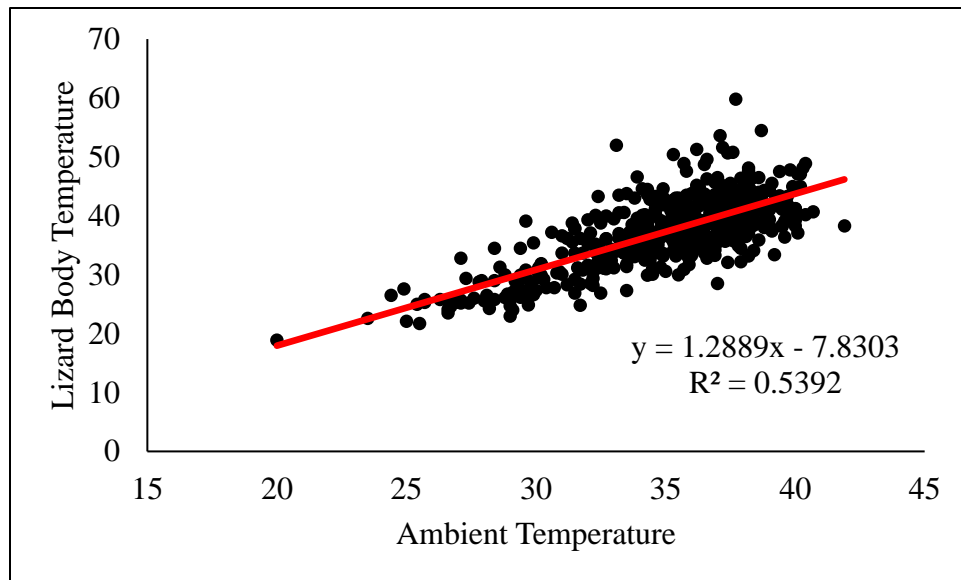


Figure 13. Lizard body temperature based on ambient temperature for Texas Horned Lizards located on the ground (A) and off the ground (B).

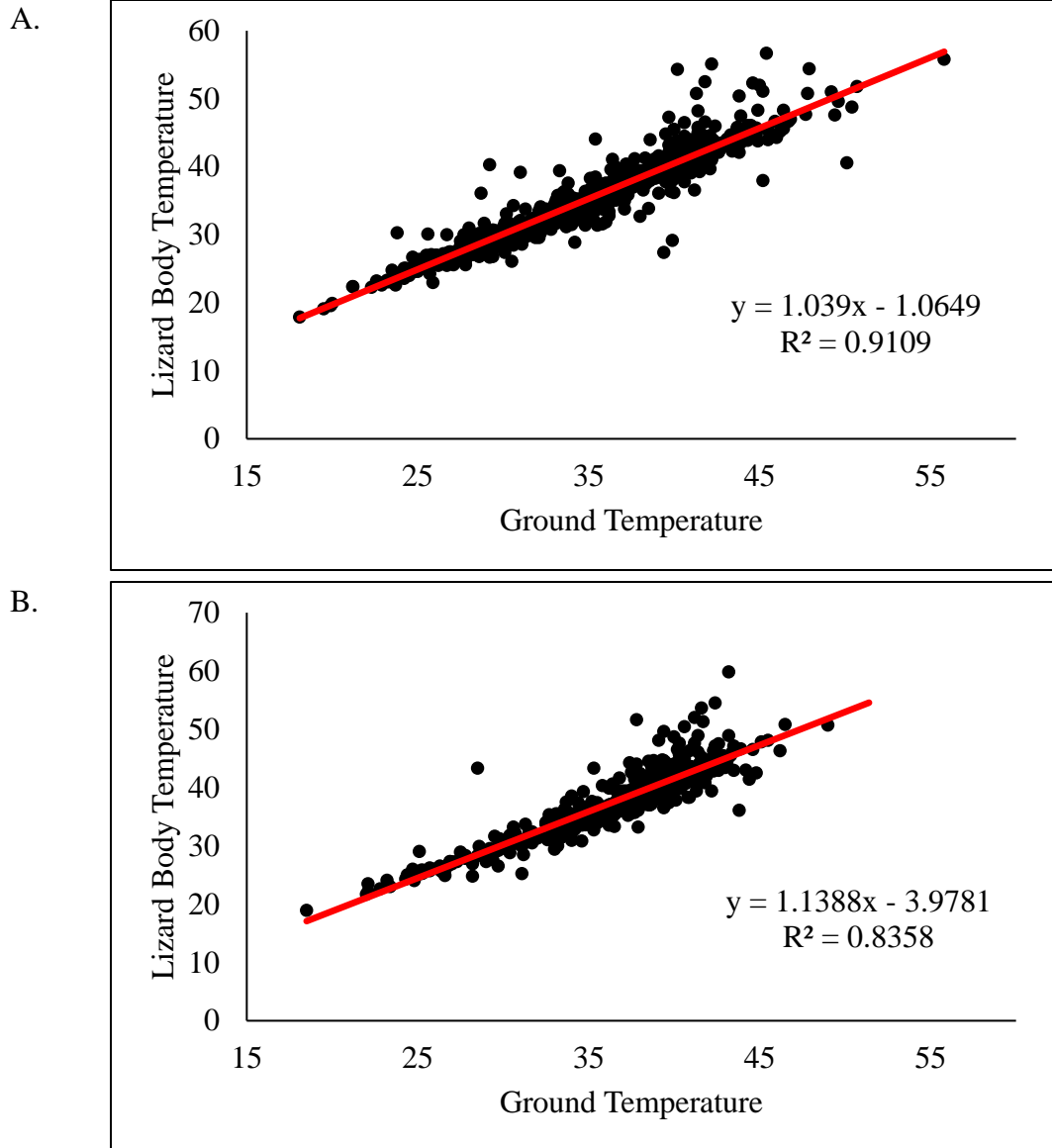


Figure 14. Lizard body temperature based on ground temperature for Texas Horned Lizards located on the ground (A) and off the ground (B).

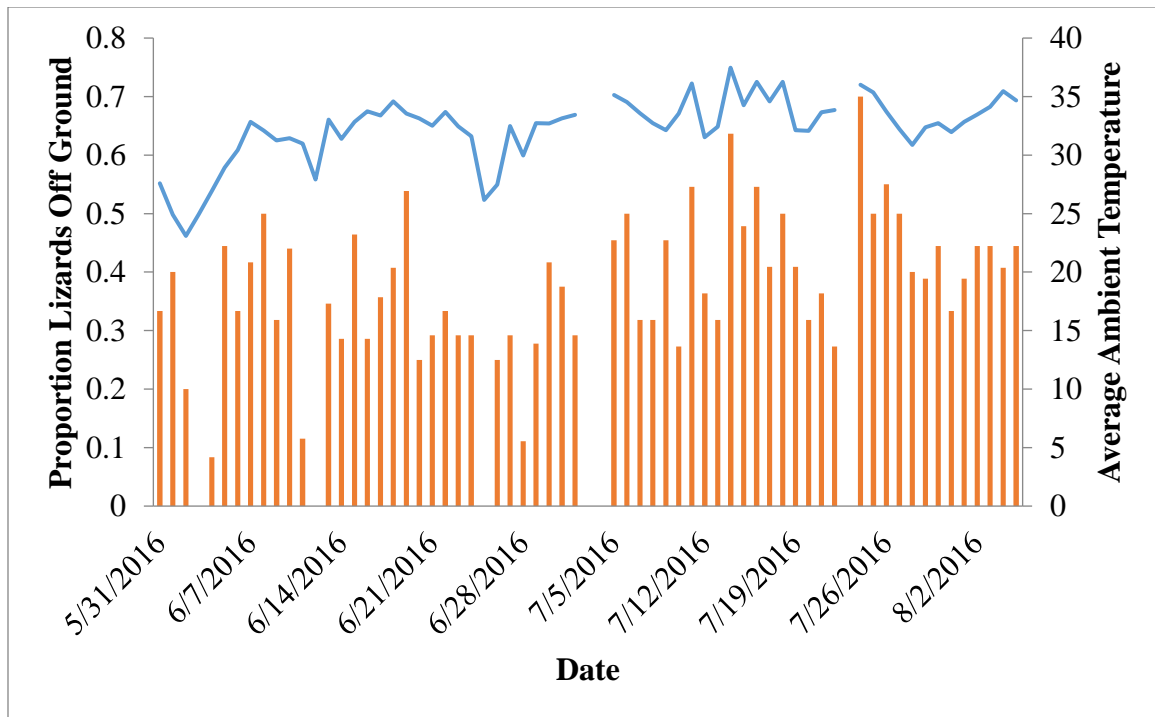


Figure 15. Proportion of locations that were off the ground each day for 14 Texas Horned Lizards in summer 2016, along with mean ambient temperature. Each orange bar represents the proportion of lizard locations that were off the ground that day. The blue line above represents the mean ambient temperature of all recorded locations that day.

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