# FINAL REPORT

# MOVEMENT AND HABITAT USE OF THE PLATEAU SPOT-TAILED EARLESS LIZARD (*HOLBROOKIA LACERATA*) AND THE TAMAULIPAN SPOT-TAILED EARLESS LIZARD (*HOLBROOKIA SUBCAUDALIS*): 2017-2019

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# 1.0 Introduction

Recent concerns for the conservation of Spot-tailed Earless Lizard (*Holbrookia lacerata* and *H. subcaudalis*) populations have resulted in studies to evaluate the distribution of the species and identify potential threats. Assessment of these concerns has highlighted the lack of basic life history knowledge of these two species. To evaluate the status of the species and develop conservation plans, basic information for both species has been collected to inform habitat models and species status evaluations. Biologists from the University of Texas (UT) and BIO-WEST, Inc. conducted an extensive study investigating *H. lacerata* and *H. subcaudalis* throughout their historical ranges within Texas. The combined information will provide valuable knowledge for threat evaluation and United States Fish and Wildlife Service (USFWS) Species Status Assessment (SSA) development. In order to accomplish research goals, the study was prioritized into five main tasks:

- Define *H. lacerata* and *H. subcaudalis* home range size, movement and activity patterns, and habitat use via very high frequency (VHF) radio telemetry.
- Provide movement and habitat use data on all life stages via the use of harmonic radar.
- Evaluate the performance of and conduct repeated visual encounter surveys, passive survey methods, and capture-recapture surveys to provide a method of examining species distribution, demography, and habitat associations on a landscape scale.
- Evaluate the use of arthropod community signatures to predict *H. lacerata* and *H. subcaudalis* occupancy and abundance.
- Conduct a best professional judgement threat analysis with herpetologists and other *H. lacerata* and *H. subcaudalis* researchers.

The following report provides the methodology, results, and analysis from UT / BIO-WEST (Project Team) research efforts related to the completion of these tasks during 2017, 2018, and 2019 field seasons.

## 1.1 Spot-tailed Earless Lizard

Formerly considered two subspecies of *Holbrookia lacerata,* the Plateau Spot-tailed Earless Lizard (*Holbrookia lacerata*) and the Tamaulipan Spot-tailed Earless Lizard (*Holbrookia subcaudalis*) are now considered as two separate species (Hibbitts et al. 2019, Roelke et al. 2018). These species are separated geographically by the Balcones Escarpment and exhibit clear morphological differences (Axtell 1956, 1968; Hibbitts et al. 2019). The northern species (*H. lacerata*) includes all populations north of the Balcones Escarpment in Texas, extending north to the Colorado River, east to the eastern edge of the Balcones Escarpment, and west to the Pecos River (Axtell 1956, 1968). The southern species (*H. subcaudalis*) includes all populations south of the Balcones Escarpment and exhibits larger average adult size (snout vent length 62 mm to 54 mm, respectively), higher average femoral pore counts (15.7 to 12.8, respectively), and differences in meristic characteristics such as dorsal and femoral patterns (Axtell 1956). Given the large extent of range within Texas for both species, they may be subject to threats unique to their respective eco-regions (Hibbitts et al. 2019). Based on recent records, *H. lacerata* occupies much of its historical range on the Edwards Plateau and West Texas, though few available recent records exist within its historical range along the eastern portion of the Edwards Plateau (iNaturalist 2019).

Similarly, recent publicly available records demonstrate that *H. subcaudalis* is still regularly encountered within portions of its range, particularly in the southeastern portion, with fewer recent sightings within some historically occupied areas (iNaturalist 2019). Both species can be locally abundant within highly human-impacted environments, including open fields utilized for grain agriculture and grazed pastures where there are large proportions of bare soil lacking vegetation (Roelke et al. 2018).

## 1.2 Study Area

Given that these two species are distributed across a large geographic range in Texas, the study was roughly aggregated into the following five Study Units (Figure 1) based on geographic proximity as it relates to study logistics:

**Unit 1:** Kerr, Kimble, Mason, Menard, Real counties

**Unit 2:** Edwards, Kinney, Maverick, Val Verde counties

**Unit 3:** Bee, Jim Wells, Kleberg, Nueces, Refugio, San Patricio counties

**Unit 4:** Dimmit, Duval, Jim Hogg, La Salle, Starr, Webb, Zapata counties

**Unit 5:** Tom Green, Irion, Reagan, Crockett, Schleicher, Coke, Concho, Glasscock, Runnels counties

For the purpose of organizing a variety of research tasks across the range of *H. lacerata* and *H. subcaudalis,* field efforts were organized into 24 study sites (Appendix A). Study sites were established both before the onset of field activities and during field activities (2017-2019) based on the requirements of each task. A study site is defined as an area in which there was repeated study activity (e.g., radio telemetry) and/or in which lizards were captured for study purposes (Figures 2 and 3). Not all surveyed areas (i.e., visual encounter surveys; Section 4.2) are listed as study sites.

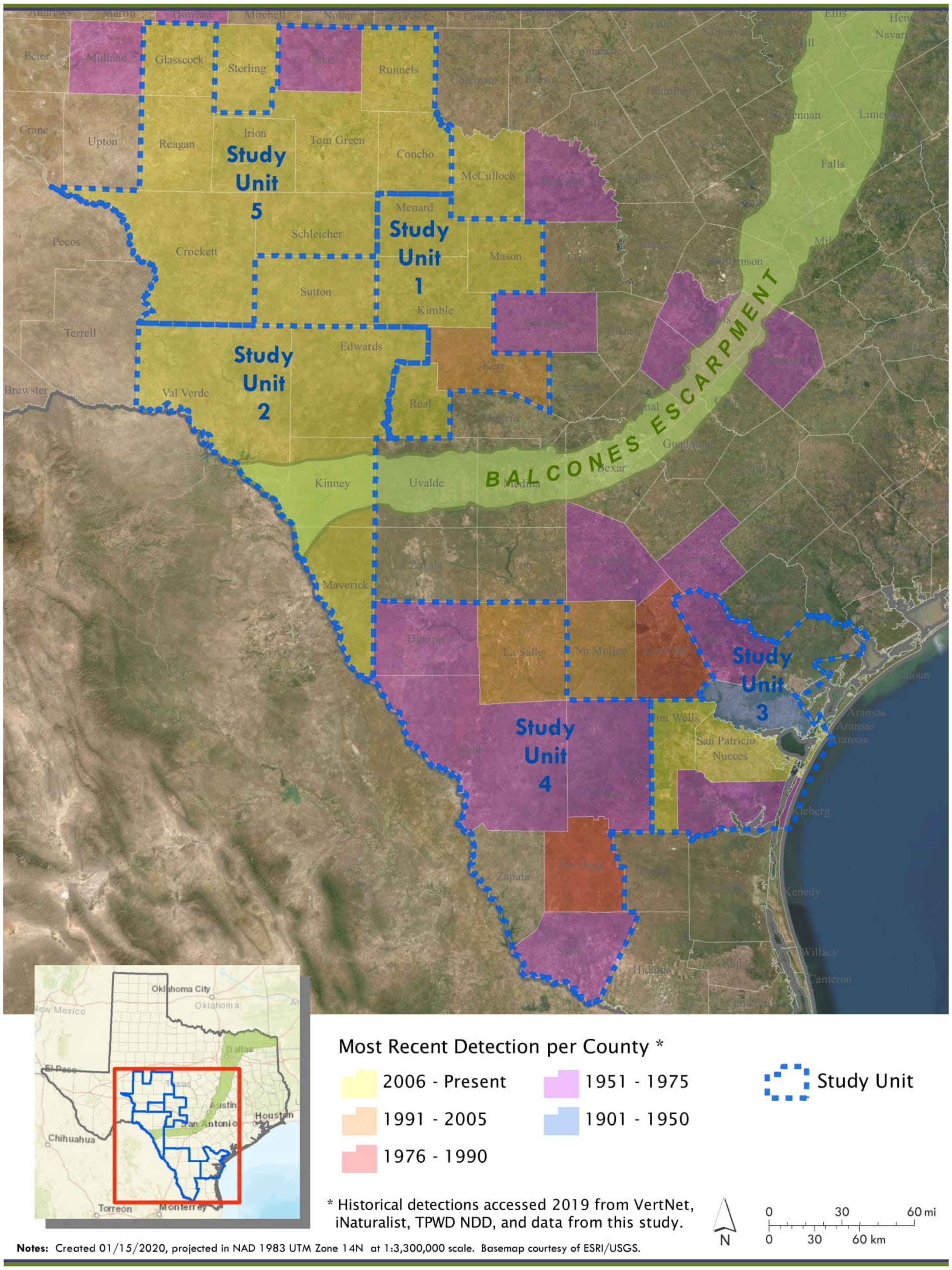


**Figure 1.** Study extent with Study Units and recency of *H. lacerata* and *H. subcaudalis* detections based on captures observed during this study and historical records.

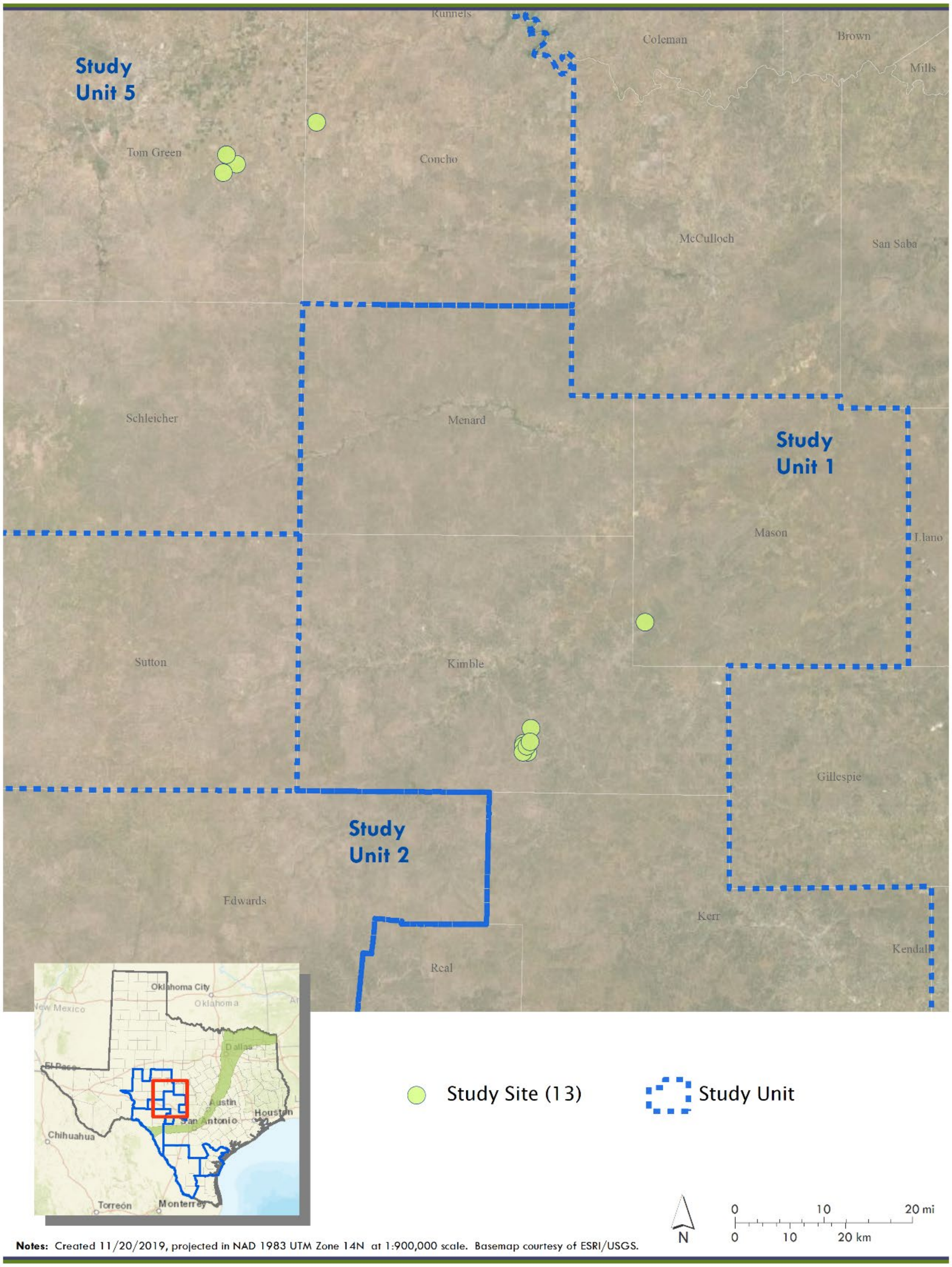
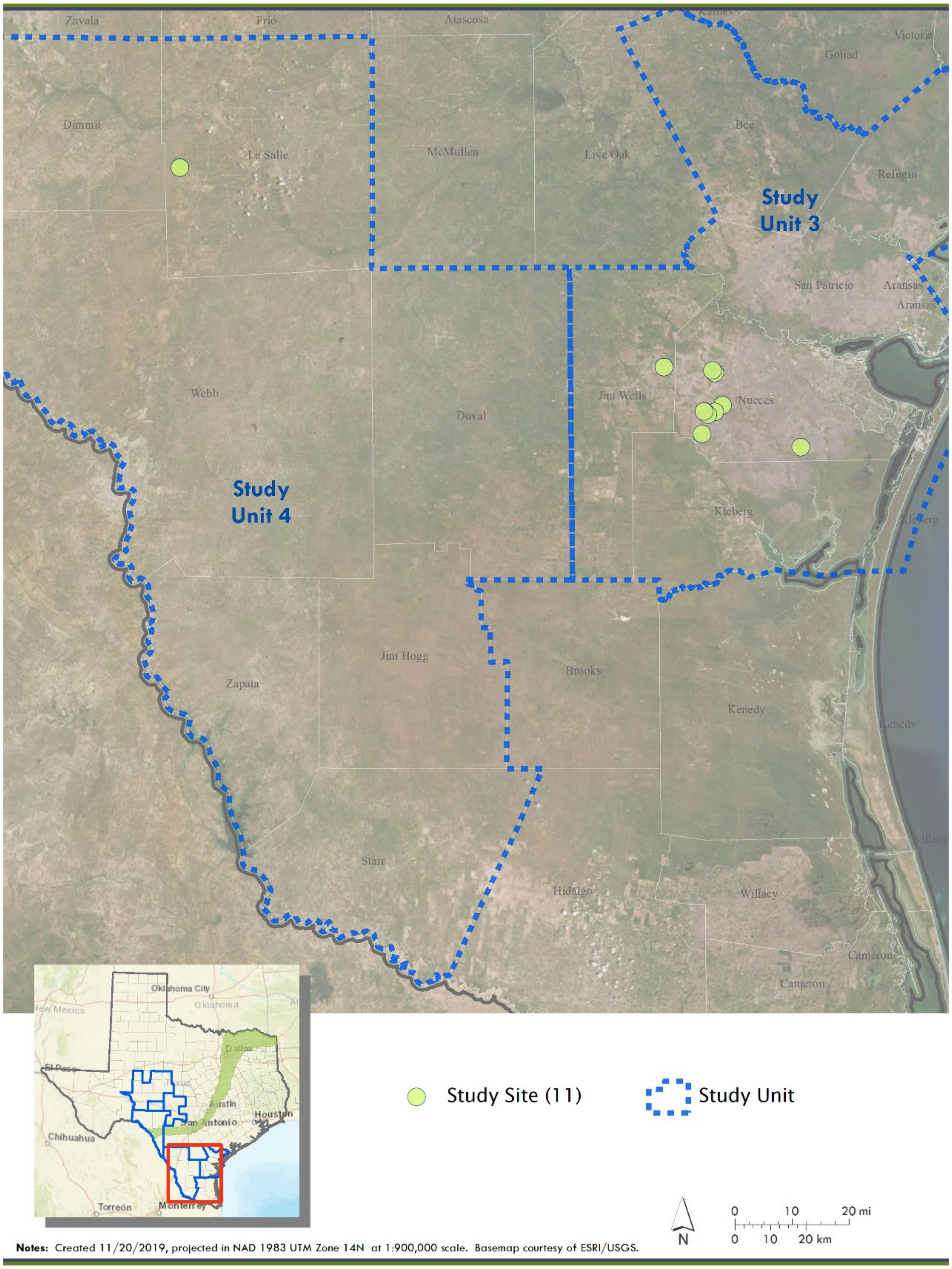


**Figure 2.** Study Units 1, 2 and 5 with distribution of study sites (n = 13) where study tasks were conducted.



**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:900,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 3.** Study Units 3 and 4 with distribution of study sites (n = 11) where study tasks were conducted.

# 2.0 Outreach and Access

As part of this study, the Project Team was engaged in activities related to the identification of, and access to appropriate field sites necessary for the completion of project tasks. These efforts began prior to the onset of field activities in Spring 2017 and continued concurrently with applied research tasks through Spring 2019. These study sites were chosen based on (1) location relative to distribution of *H. lacerata* and *H. subcaudalis*, (2) access and availability for research during field season, (3) historical observations of *H. lacerata* and *H. subcaudalis*, and (4) differing land management and use regimes. Field site access was comprised of public roadways, state parks, wildlife management areas, and state natural areas, or private property. Necessary permits were obtained from the Texas Parks and Wildlife Department (TPWD) to conduct research activities on public roadways, road rights-of way, and any state properties. The research tasks applied to these areas and the extent to which these field sites were utilized for the purposes of this study was a factor of *H. lacerata* and *H. subcaudalis* presence and task-specific requirements. Over the course of this research project (2017-2019) outreach efforts resulted in permits to access six state parks, one wildlife management area, one state natural area, and access to eight private properties (Table 1). This included access within all 5 Study Units for a total of 49,543 acres, divided between the historical ranges of both *H. lacerata* (23,751 acres) and *H. subcaudalis* (25,792 acres). Land use demographics and applied study methods varied between individual study sites.

**Table 1.** Summary of public and private areas utilized, and research activities conducted during 2017-2019 field efforts.

| Study Unit | County | Locality/Property | Access Type | Acres | Research Activities |
|---|---|---|---|---|---|
| **1** | Mason | Private Property 1 | Private Property | 1,302 | Visual encounter surveys, Arthropod surveys, |
| **1** | Kimble | Private Property 2 | Private Property | 449 | Visual encounter surveys, Arthropod surveys, Vegetation surveys, Capture-Recapture |
| **1** | Kimble | South Llano River State Park | State Park | 2,743 | Visual encounter surveys |
| **1** | Kimble | Private Property 3 | Private Property | 824 | Visual encounter surveys, Vegetation surveys, Arthropod surveys, Capture-Recapture, Radio Telemetry, Harmonic Radar, Passive surveys |
| **1** | Kimble | Private Property 4 | Private Property | 270 | Visual encounter surveys, Vegetation Surveys, Arthropod surveys, Capture-Recapture, Radio Telemetry, Harmonic Radar |
| **2** | Edwards | Devils Sinkhole SNA | State Natural Area | 1,857 | Visual encounter surveys |
| **2** | Kinney | Kickapoo Cavern State Park | State Park | 6,371 | Visual encounter surveys |
| **2** | Kinney | Private Property 5 | Private Property | 2,904 | Visual encounter surveys, Vegetation surveys |
| **3** | San Patricio | Lake Corpus Christi State Park | State Park | 388 | Visual encounter surveys |
| **3** | Nueces | Private Property 6 | Private Property | 705 | Visual surveys, Arthropod surveys, Vegetation surveys |
| **3** | Nueces | Private Property 7 | Private Property | 497 | Visual encounter surveys, Vegetation surveys, Arthropod surveys |
| **3** | San Patricio | Welder Wildlife Foundation | Private Property | 8,129 | Visual encounter surveys |
| **4** | Dimmit / La Salle | Chaparral WMA | WMA | 15,181 | Visual encounter surveys, Arthropod surveys, Vegetation surveys |
| **4** | Starr | Falcon Lake State Park | State Park | 564 | Visual encounter surveys |
| **4** | Webb | Lake Casa Blanca State Park | State Park | 328 | Visual encounter surveys |
| **5** | Tom Green | San Angelo State Park | State Park | 7,031 | Visual encounter surveys, Vegetation surveys |

## 3.0 Tracking

In order to describe *H. lacerata* and *H. subcaudalis* home range size, movement and activity patterns, and habitat use, two methods were employed for the in-field estimation of lizard locations and movement: (1) VHF radio telemetry and (2) harmonic radar.

### 3.1 Radio Telemetry

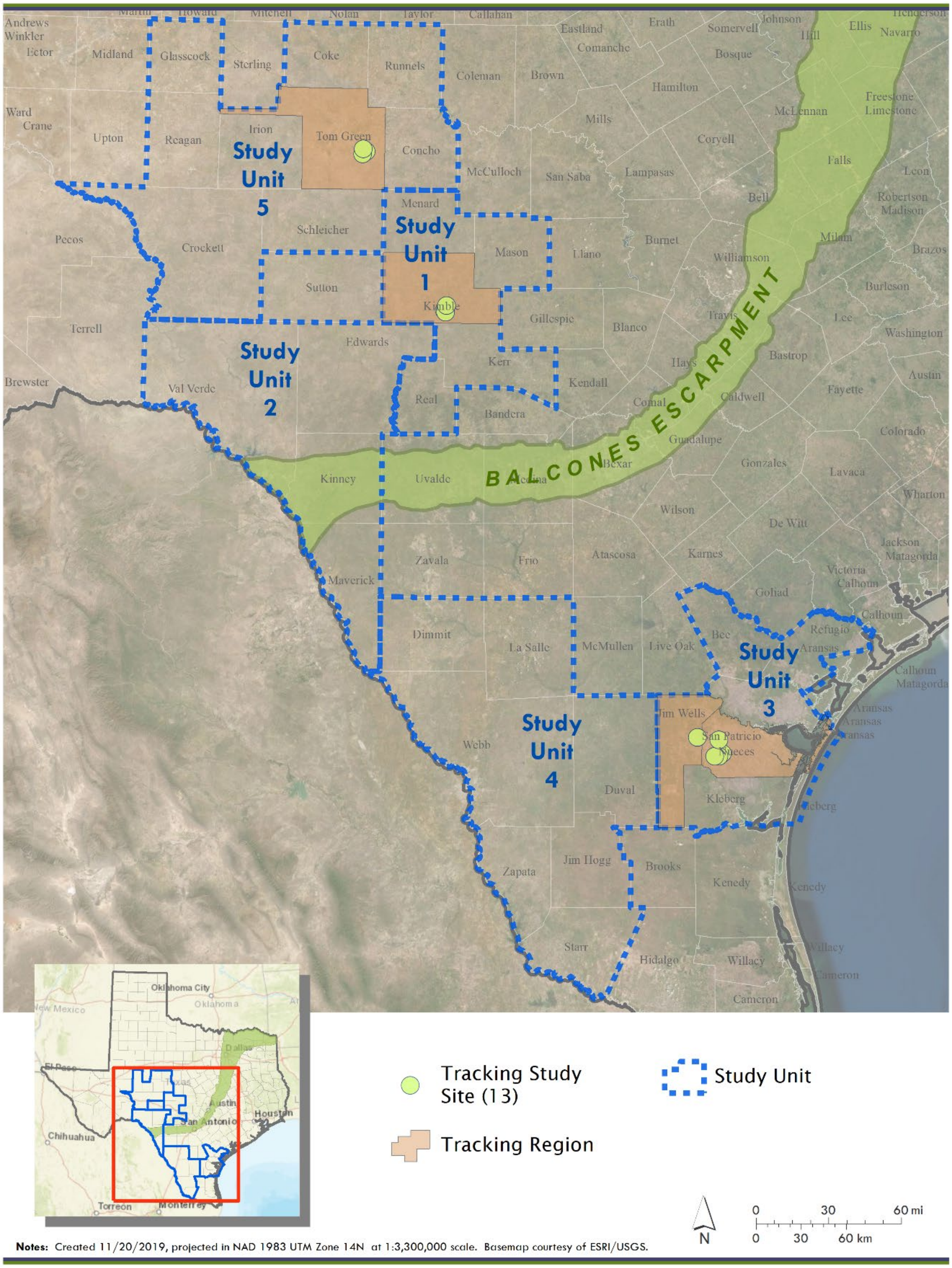
Very high frequency (VHF) radio telemetry was the first real-time technique used to track individual animals from a distance. A transmitter attached to the study animal broadcasted pulsed signals in the VHF portion of the electromagnetic spectrum (30 to 300 MHz; Figure 4). Study animals were given unique frequencies, so that individuals could be tracked during daily activity periods (Amlaner and MacDonald 1980). Radio telemetry has been proven to be an effective method for tracking the small-scale movements of organisms (Garton et al. 2001) and has been used to study aspects of behavior and spatial ecology in a variety of lizard species (Norton et al. 2019).

**Figure 4.** *Holbrookia subcaudalis* with VHF transmitter attached.

### 3.1.1 Methods

The Project Team used radio telemetry to monitor the movements of adult *H. lacerata* and *H. subcaudalis* during the active season (late Spring through early Fall) of 2017, 2018, and 2019. Telemetry study sites were chosen based on historical records, capture success, and variation in land management and use regimes (e.g., no use, grazing, prescribed fire, farming, oil and gas or other energy development). Ultimately, radio telemetry tracking was conducted at 12 study sites where *H. lacerata* and *H. subcaudalis* were present in sufficient abundance for radio telemetry, within four counties and three Study Units (Figure 5).

Once captured, snout-vent length (SVL; mm), tail length (TL; mm), and weight (g) were measured and a unique identifying toe clip was given (Perry et al. 2011). Lizards were then affixed with a 0.2-0.3 g VHF transmitter (Advanced Telemetry Systems (ATS) or Holohil Systems Ltd.) dorsally and directly posterior to the pectoral girdle using adhesive. Transmitters were attached on lizards with body weights ≥ 5.0 g, in order to keep transmitter weight within 6% of body mass. Once attached, the individual was released and then relocated at regular intervals (1–2 hours) throughout the day for up to 20 days or until failure of the transmitter or adhesive (Figure 6). The number of relocations for each individual fluctuated base on variation in tag retention time and transmitter battery life. Tracking was performed with an ATS R410 receiver and F150-3FB directional antenna (Figure 6). In order to minimize effects of disturbance by observers on lizard movement while tracking, triangulation was primarily utilized in order to remotely obtain lizard relocations. Homing (tracking study subject to its precise location) was used to locate study subjects periodically to visually verify transmitter attachment and function, as well as cryptic behaviors such as burying. Standard climate data (including temperature, relative humidity, barometric temperature, and weather observations) was recorded at the time of obtaining each lizard location.



**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:3,300,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 5.** Study extent with distribution of study sites utilized for radio telemetry study (n = 12).

**Figure 6.** VHF radio transmitter affixed to *H. lacerata* (top). Radio telemetry using ATS R410 receiver and F150-3FB directional antenna to locate a previously tagged *H. subcaudalis* (bottom).

### 3.1.2 Results

Across all field seasons conducted 2017-2019, a total of 35 adult *H. lacerata* and 30 adult *H. subcaudalis* were affixed with VHF transmitters and tracked with varying success relative to the number of relocations recorded and tracking period. Of these, 19 (54%) *H. lacerata* and 17 (57%) *H. subcaudalis* provided enough relocation points for the estimation of home ranges.

In 2017, capture efforts were focused within four study sites (A-D) in Kimble County (Study Unit 1; Figures 7-10). Within proximity to these study sites, land-use was predominately rangeland with no oil and gas activity within 1 km of estimated home ranges (Figures 7-8). These efforts produced 43 captures and only six of these captures were of sufficient weight (≥5.0 g) to be tagged with VHF transmitters. Of these, four individuals (67%) retained their transmitters for a long enough period to allow for sufficient relocations (range = 14 to 53 points) for estimation of home ranges (Table 2).

**Table 2.** Summary of radio telemetry tracking efforts in 2017. All captures respresented by *H. lacerata.*

| Unit | County | Study Site | Captures[a] | # VHF Transmitters Deployed | # Utilized for Home Range Estimations |
|---|---|---|---|---|---|
| **1** | Kimble | A | 31 | 2 | 1 |
| **1** | Kimble | B | 4 | 2 | 1 |
| **1** | Kimble | C | 2 | 1 | 0 |
| **1** | Kimble | D | 6 | 1 | 2 |
| **TOTAL** | | **4 Study Sites** | **43** | **6** | **4** |

a. Includes captured juveniles and adults above and below minimum weight for VHF transmitter attachment.

In 2018, capture efforts expanded to include Tom Green (Study Unit 5; Figures 12 --15) and Jim Wells (Study Unit 3; Figures 16-18) counties, in addition to Kimble County (Figures 7-10). Within proximity to Tom Green County study sites, land use was predominately agricultural fields with moderate oil and gas activity (Figure 12). Within a 1 km buffer around estimated home ranges, 24 well pads were identified (density = 2.2 wells/km$^2$). Within proximity to Jim Wells and Nueces counties study sites, land-use was predominately agricultural fields with high oil and gas activity, having more oil and gas installations than any other radio tracking study area (Figure 17). Within a 1 km buffer around estimated home ranges, 135 well pads were identified within Jim Wells County (density = 13.2 wells/km$^2$) and 74 well pads were identified within Nueces County (density = 6.9 wells/km$^2$). Efforts in Jim Wells County were successful within 1 study site and resulted in the capture of 13 individuals, of which 9 were affixed with VHF radio transmitters (Table 3). Of these deployed transmitters, 6 individuals (67%) were tracked long enough to provide relocation points (range = 21 to 40 points) for home range estimations. Capture efforts in Tom Green County were successful at three study sites, producing a total of nine captures and resulting in the deployment of eight VHF radio transmitters (Table 3). Of these transmitters, tag retention and battery life were sufficient to allow for enough relocations (range = 13 to 38 points) to allow for estimation of home ranges of six individuals (75%). Continued from 2017 with the addition of one study site (study site E; Figure 11), Kimble County capture efforts produced 31 lizards, resulting in the deployment of 21 VHF transmitters (Table 3). Nine (43%) of these transmitters produced sufficient lizard relocations (range = 14 to 63 points) for the estimation of home ranges.

**Table 3.** Summary of radio telemetry tracking efforts in 2018.

| Unit | County | Study Site | Species | Captures[a] | # VHF Transmitters Deployed | # Utilized for Home Range Estimations |
|---|---|---|---|---|---|---|
| 1 | Kimble | A | *H. lacerata* | 3 | 1 | 1 |
| 1 | Kimble | B | *H. lacerata* | 4 | 1 | 0 |
| 1 | Kimble | C | *H. lacerata* | 17 | 13 | 5 |
| 1 | Kimble | E | *H. lacerata* | 7 | 6 | 3 |
| 3 | Jim Wells | F | *H. subcaudalis* | 13 | 9 | 6 |
| 5 | Tom Green | G | *H. lacerata* | 3 | 3 | 3 |
| 5 | Tom Green | H | *H. lacerata* | 5 | 4 | 2 |
| 5 | Tom Green | I | *H. lacerata* | 1 | 1 | 1 |
| **TOTAL** | | **8 Study Sites** | | **53** | **38** | **21** |

a. Includes captured juveniles and adults above and below minimum weight for VHF transmitter attachment.

In order to concentrate research efforts within the range of *H. subcaudalis,* 2019 radio telemetry activities were relegated to Unit 3, with one study site in Jim Wells County and three study sites in Nueces County (Figures 16- 19). In Jim Wells, efforts involved continued research within study site F (surveyed during 2018 fieldwork) and produced 17 captures resulting in the deployment of eight VHF transmitters (Table 4). Of the eight transmitters, five (63%) produced enough relocations (range = 24 to 45 points) for the estimation of home ranges. Capture efforts in Nueces County resulted in 21 captures at three study sites. Of these 21 captures, 13 VHF radio transmitters (Table 4) were deployed with six individuals (46%) producing relocation (range = 16 to 35 points) sufficient for the estimation of home ranges.

**Table 4.** Summary of radio telemetry tracking efforts in 2019.

| Unit | County | Study Site | Species | Captures[a] | # VHF Transmitters Deployed | # Utilized for Home Range Estimations |
|---|---|---|---|---|---|---|
| **3** | Jim Wells | F | *H. subcaudalis* | 17 | 8 | 5 |
| **3** | Nueces | J | *H. subcaudalis* | 18 | 12 | 6 |
| **3** | Nueces | K | *H. subcaudalis* | 2 | 1 | 0 |
| **3** | Nueces | L | *H. subcaudalis* | 1 | 0 | 0 |
| **TOTAL** | | **5 Study Sites** | | **38** | **21** | **11** |

a. Includes captured juveniles and adults above and below minimum weight for VHF transmitter attachment.

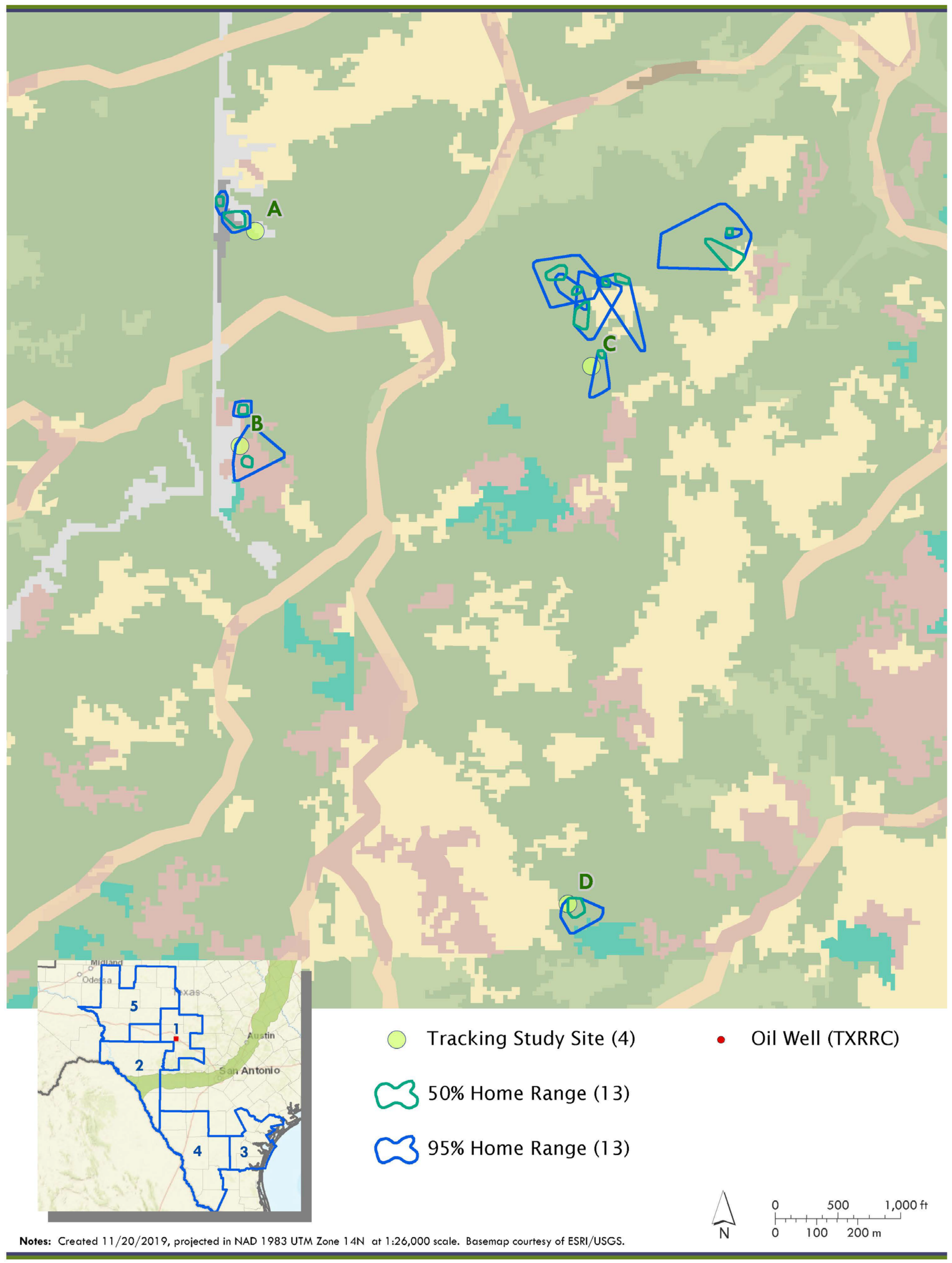
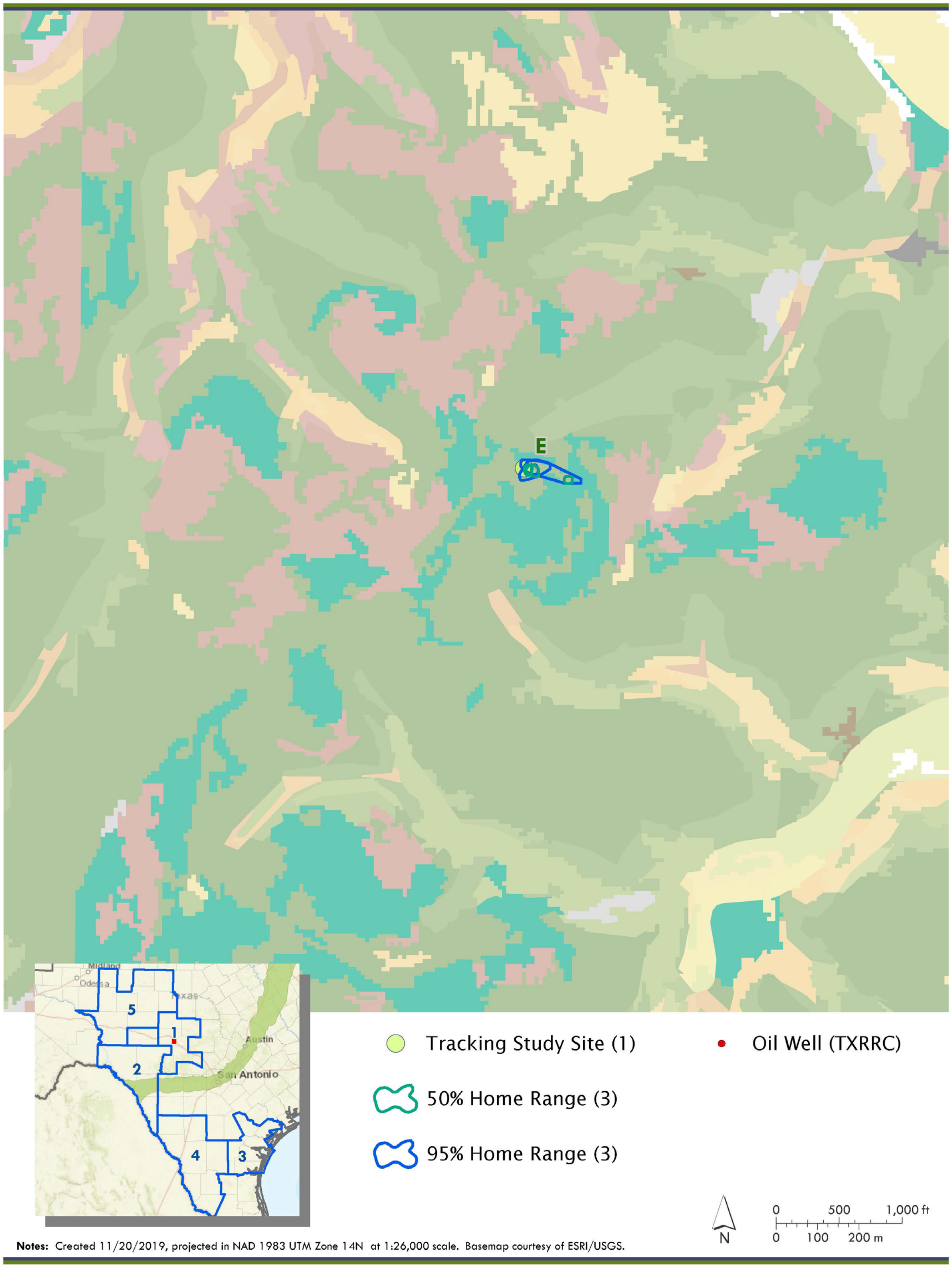


**Figure 7 .** Radio telemetry tracking study sites (n = 5) including oil and gas land-use activity in Kimble County.



**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:26,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 8.** Radio telemetry tracking study sites (n = 5) including oil and gas land-use activity in Kimble County.

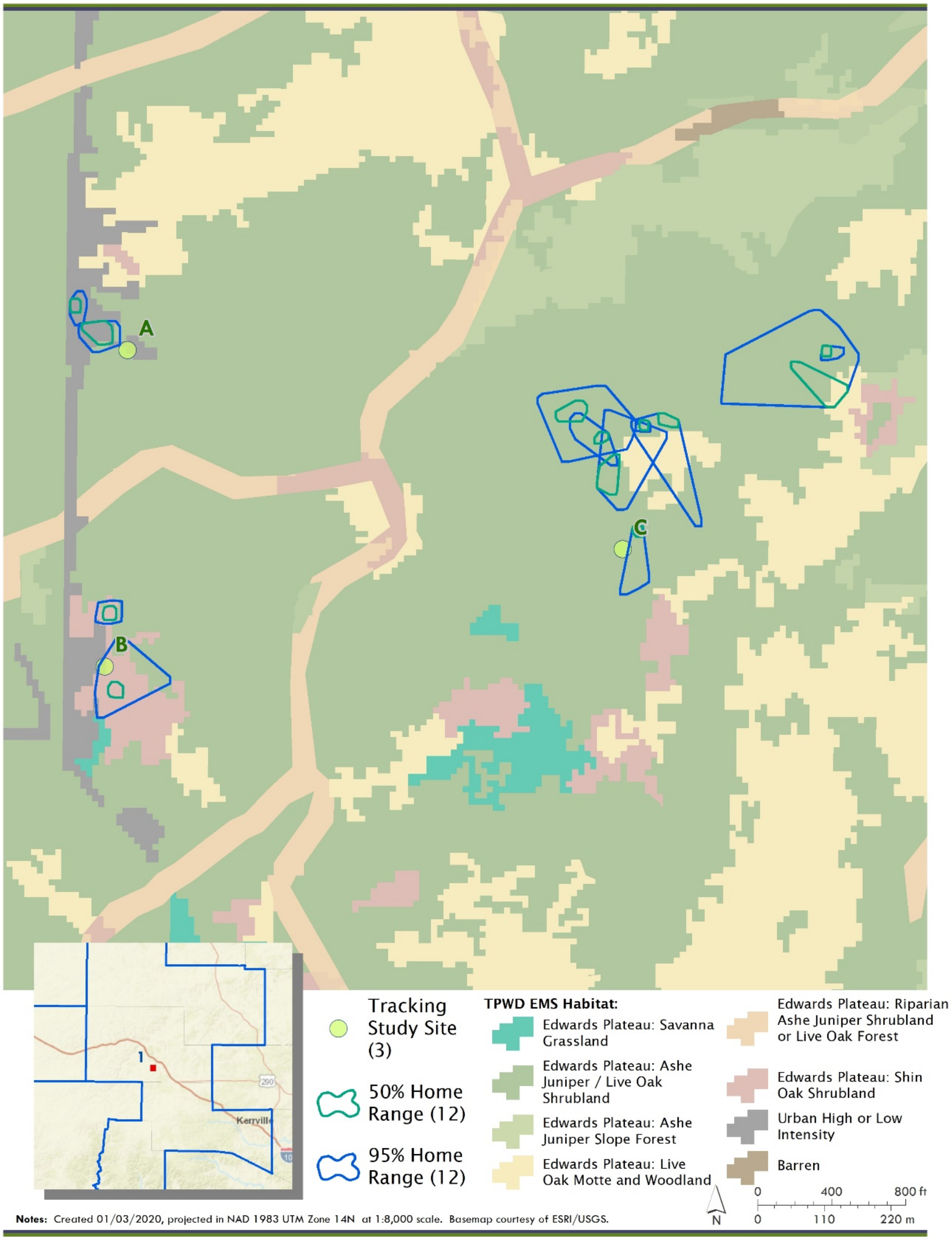
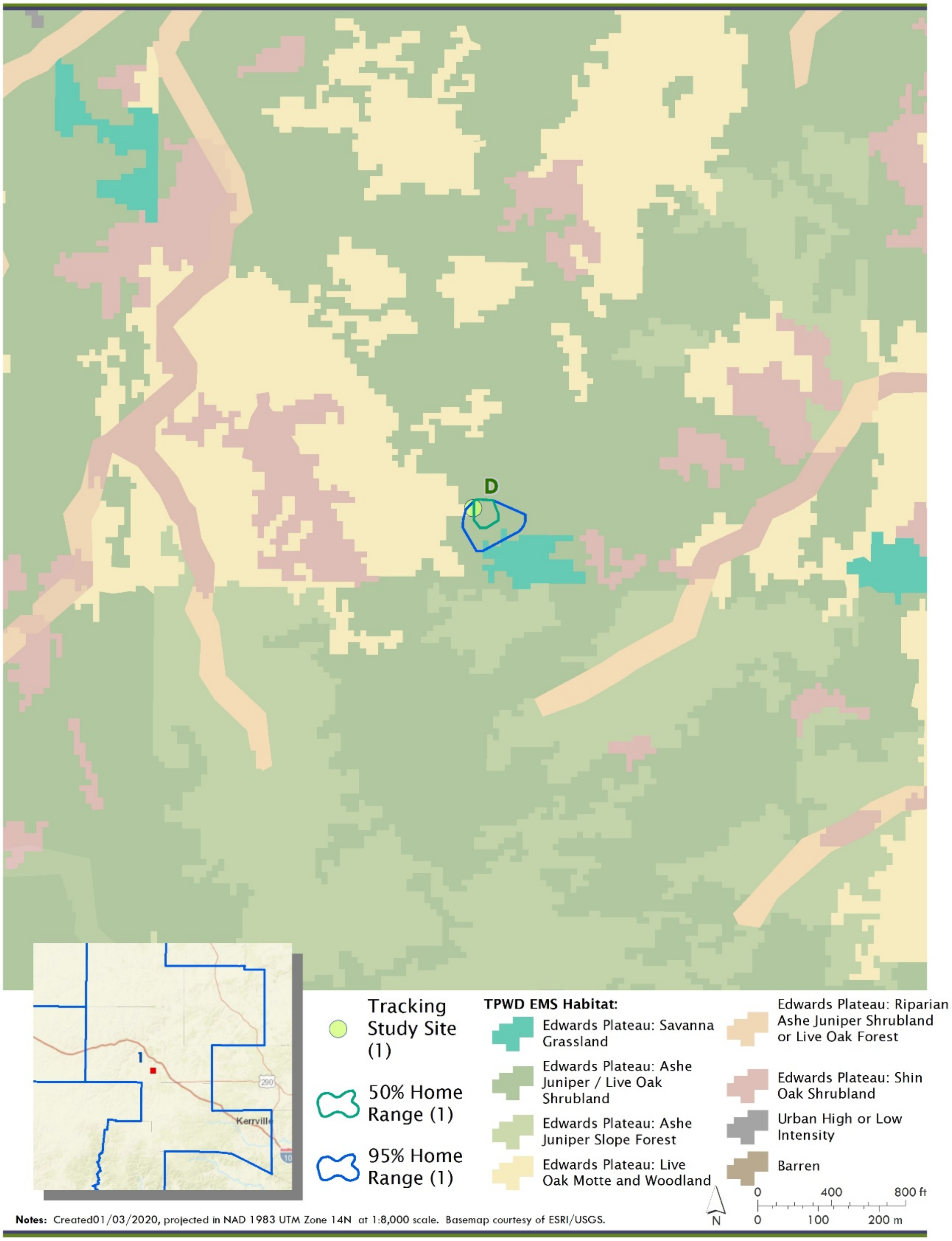


**Figure 9.** Radio telemetry tracking study sites A, B and C located within Kimble County, including associated 50% and 95% MCP home range estimations (n = 12 individuals) and land classification obtained from TPWD Ecological Mapping Systems (EMS) database.



**Notes:** Created01/03/2020, projected in NAD 1983 UTM Zone 14N at 1:8,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 10.** Radio telemetry tracking study site D located within Kimble County, including associated 50% and 95% MCP home range estimations (n = 1 individual) and land classification obtained from TPWD Ecological Mapping Systems (EMS) database.

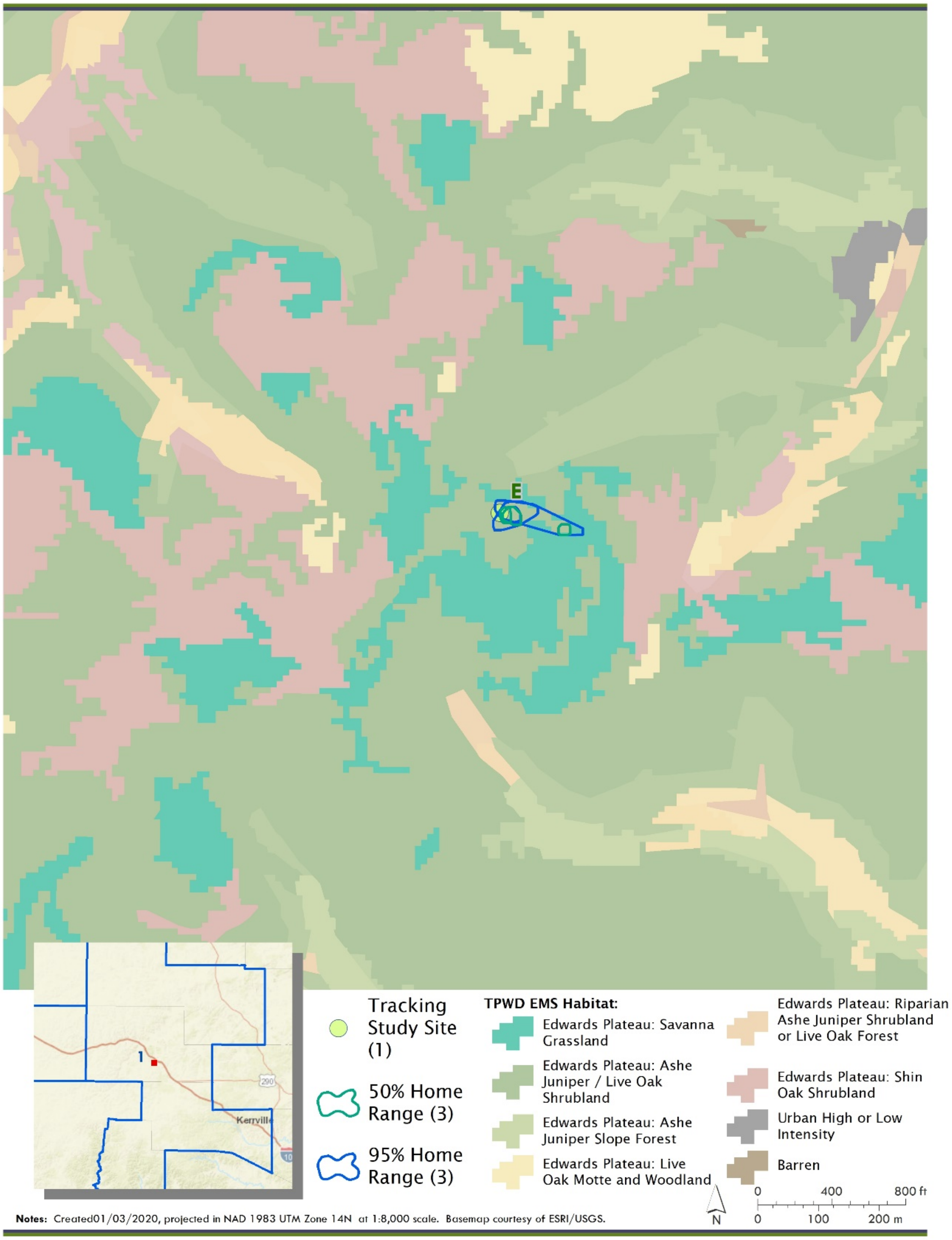
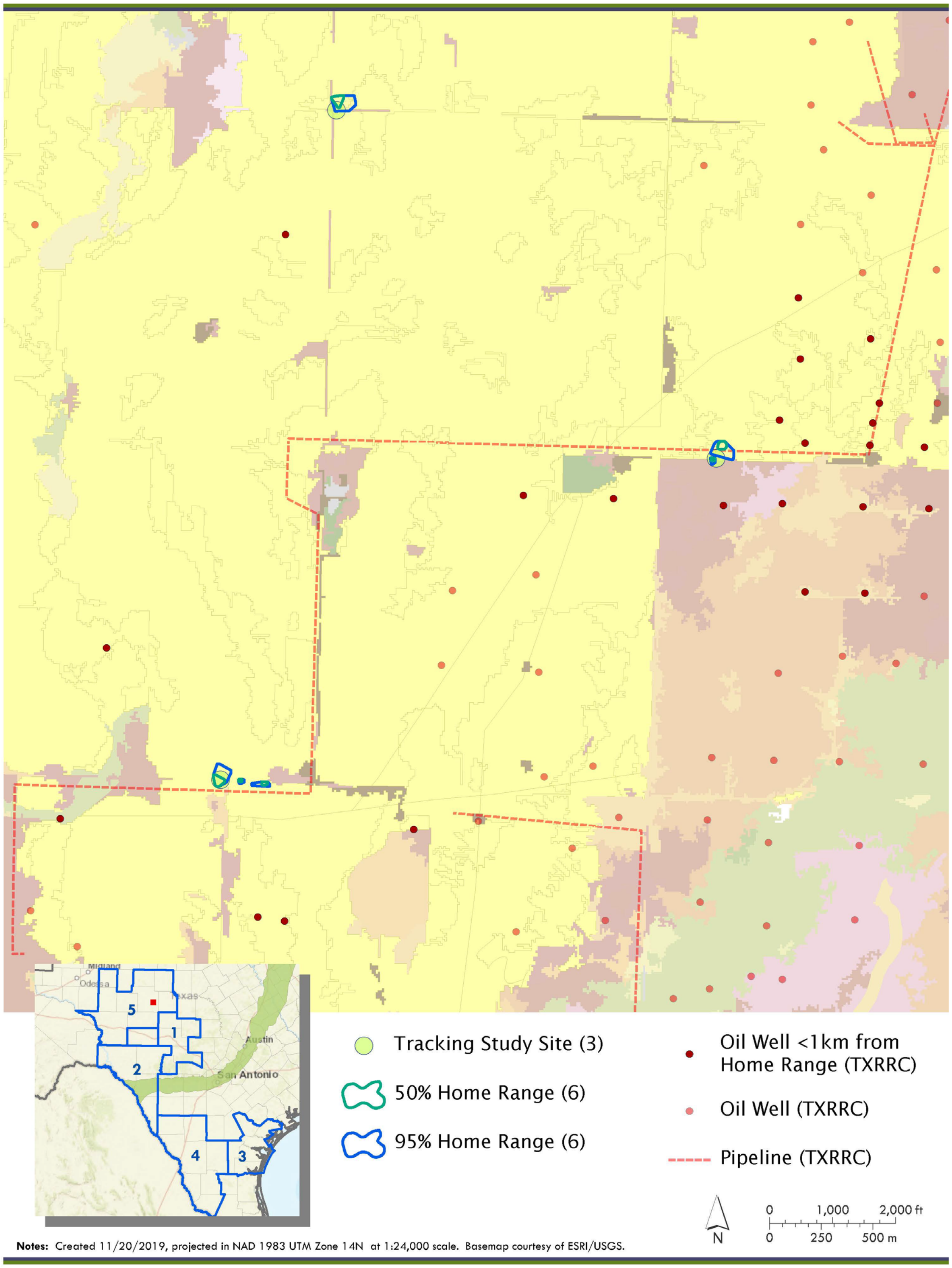
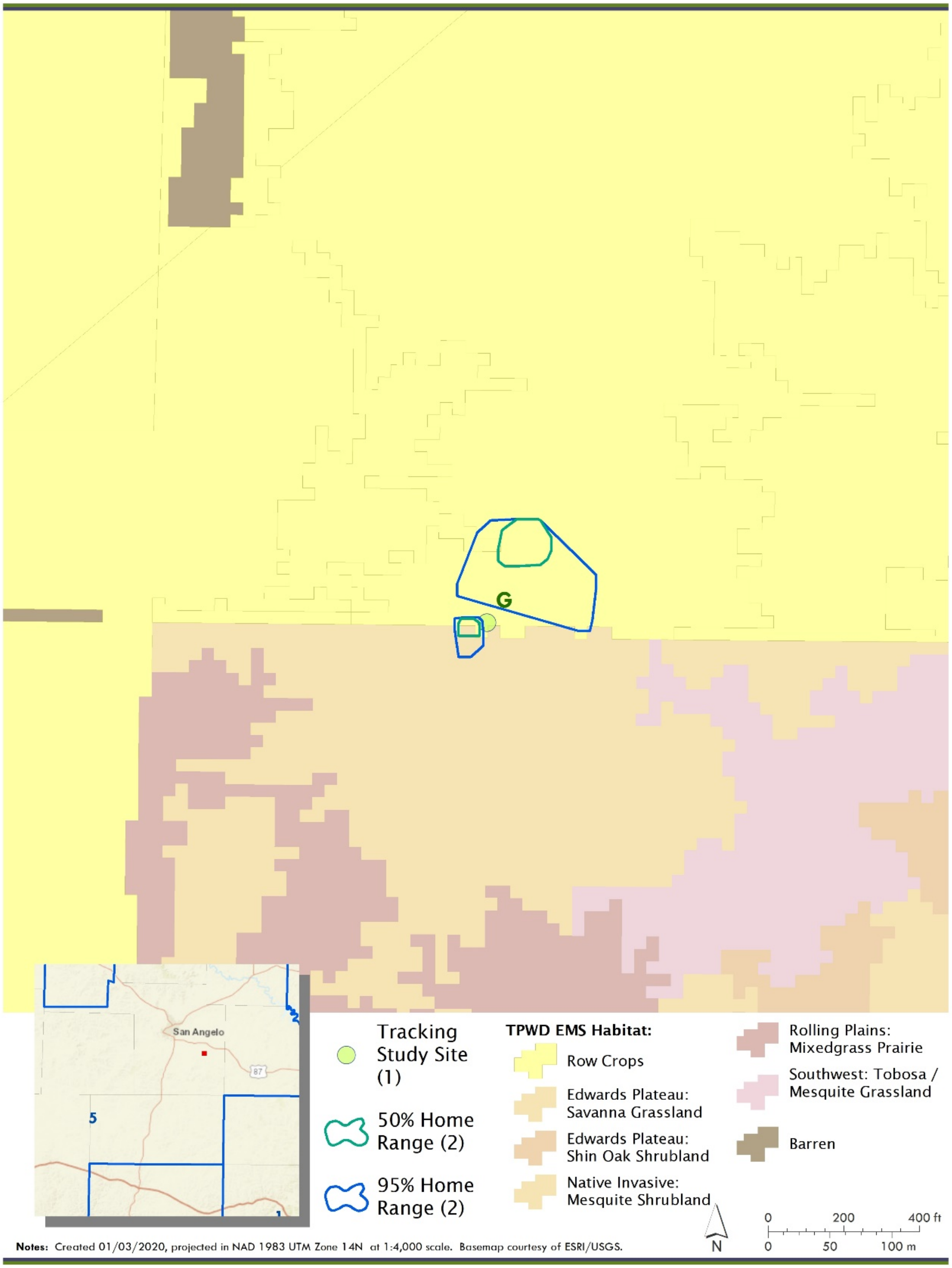


**Figure 11.** Radio telemetry tracking study site E located within Kimble County, including associated 50% and 95% MCP home range estimations (n = 3 individuals) and land classification obtained from TPWD Ecological Mapping Systems (EMS) database.



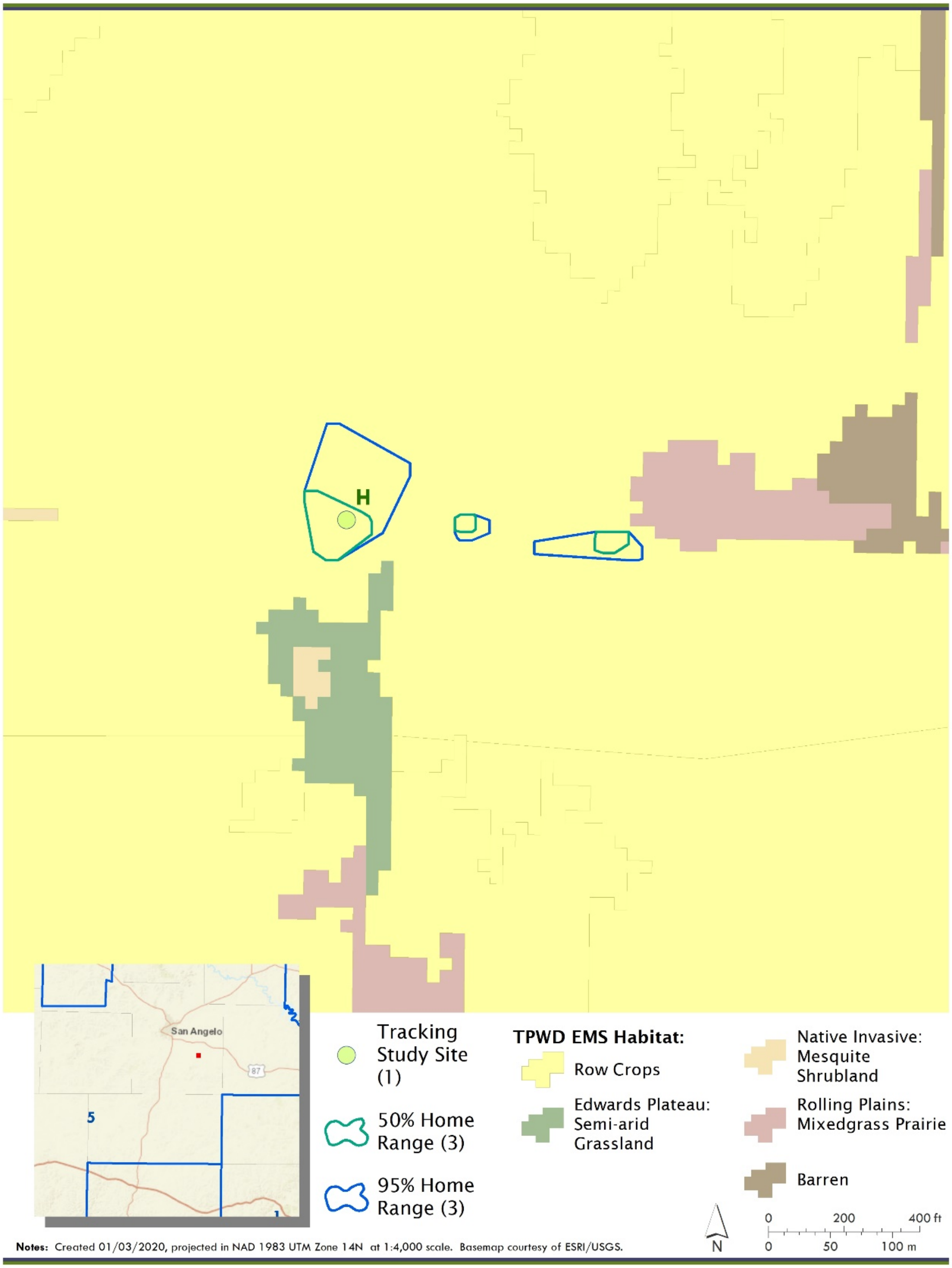
Notes: Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:24,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 12.** Radio telemetry tracking study sites (n = 3) including oil and gas land-use activity in Tom Green County.



**Notes:** Created 01/03/2020, projected in NAD 1983 UTM Zone 14N at 1:4,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 13.** Radio telemetry tracking study site G located within Tom Green County, including associated 50% and 95% MCP home range estimations (n = 2 individuals) and land classification obtained from TPWD Ecological Mapping Systems (EMS) database.



**Notes:** Created 01/03/2020, projected in NAD 1983 UTM Zone 14N at 1:4,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 14.** Radio telemetry tracking study site H located within Tom Green County, including associated 50% and 95% MCP home range estimations (n = 3 individuals) and land classification obtained from TPWD Ecological Mapping Systems (EMS) database.

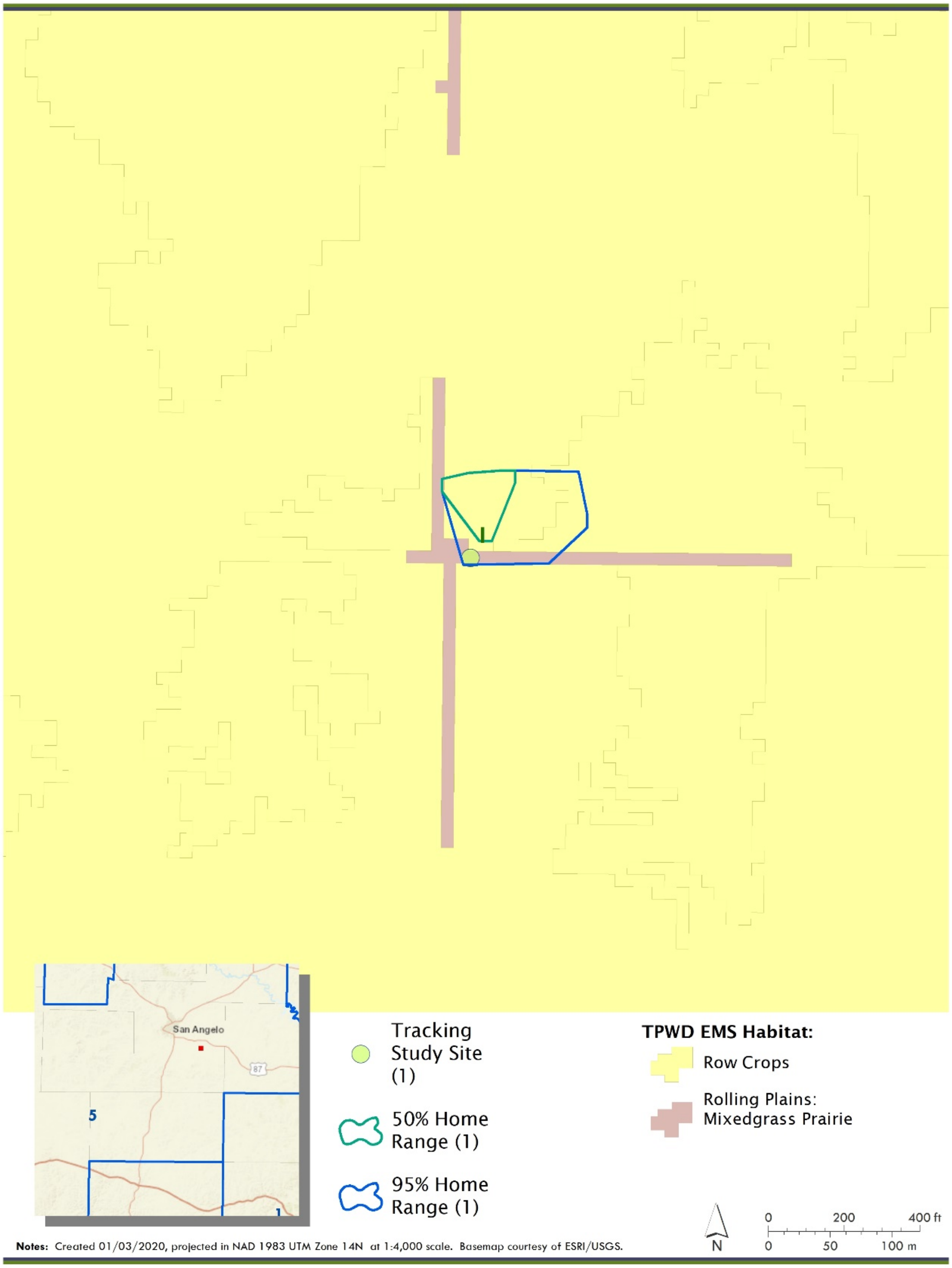


**Figure 15.** Radio telemetry tracking study site I located within Tom Green County, including associated 50% and 95% MCP home range estimations (n = 1 individual) and land classification obtained from TPWD Ecological Mapping Systems (EMS) database.

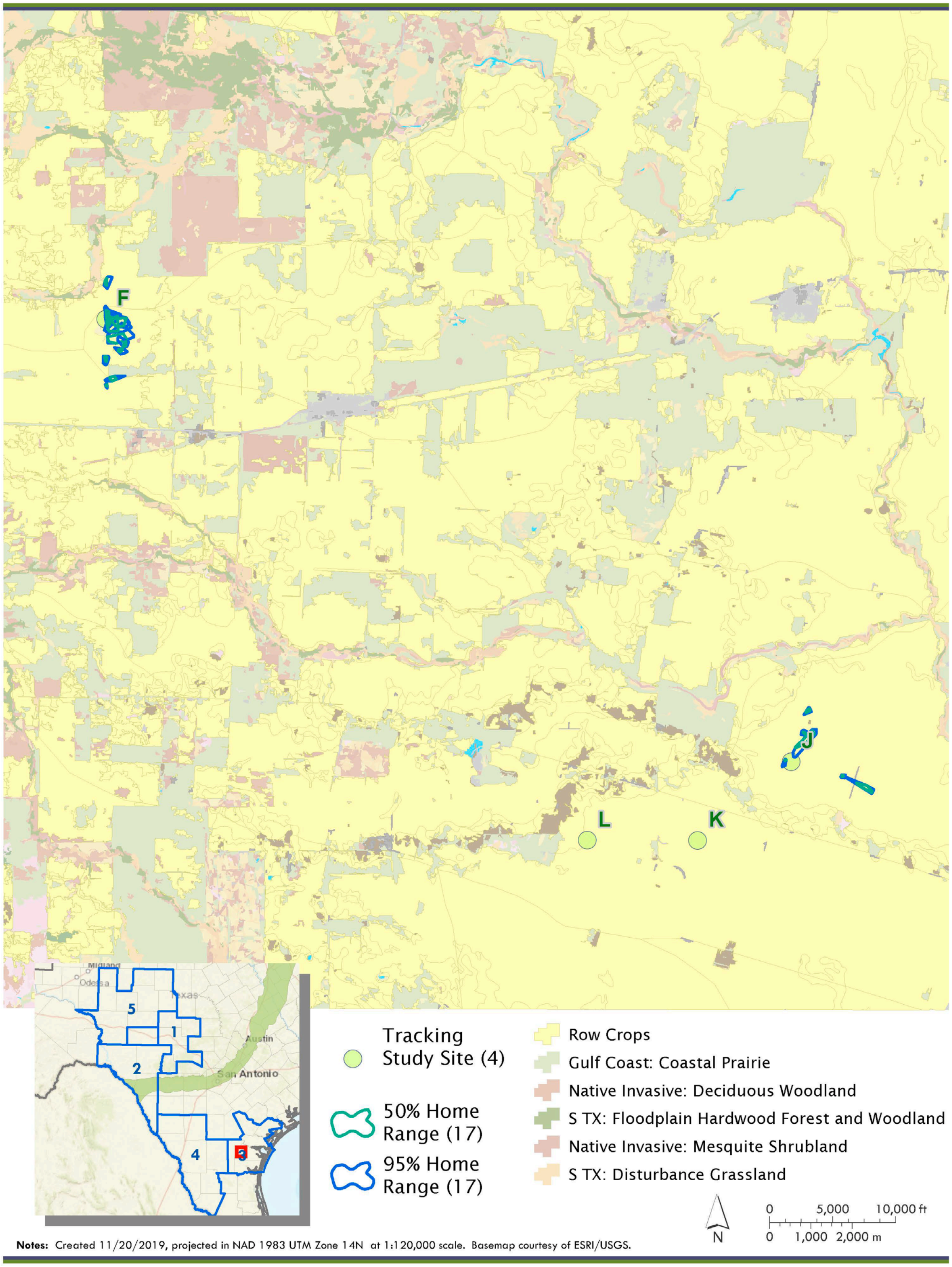
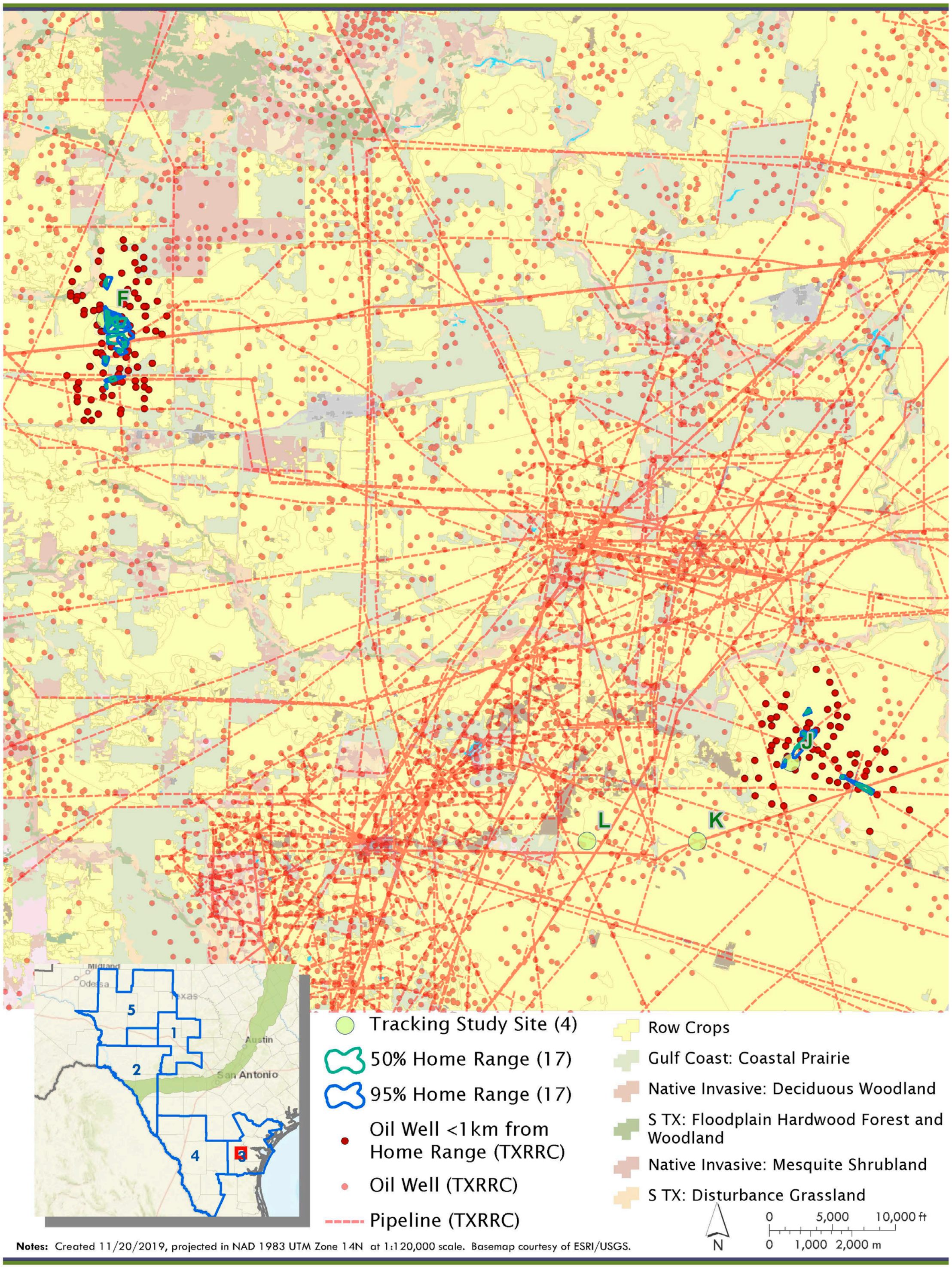
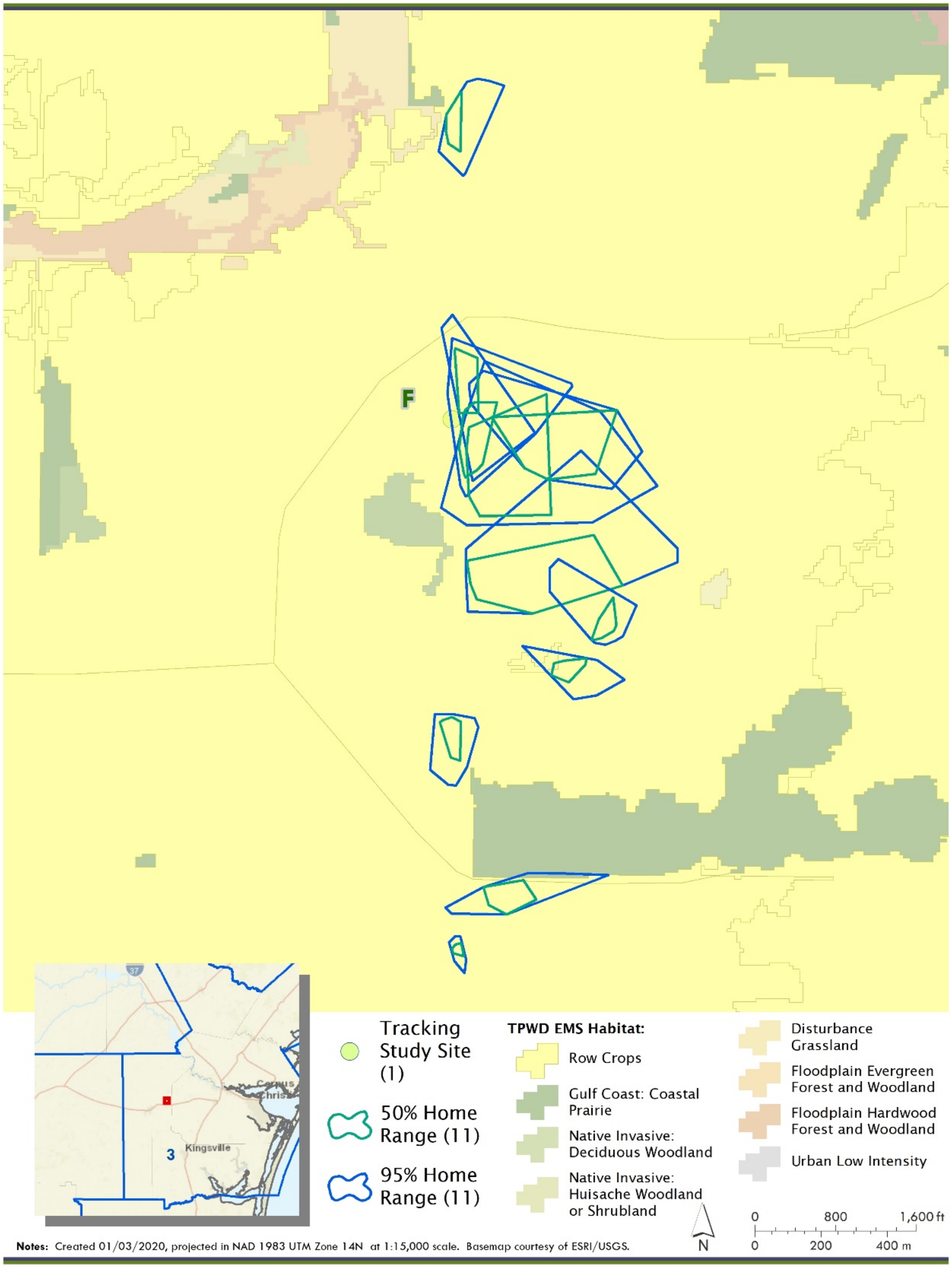


**Figure 16.** Radio telemetry tracking study sites (n = 5) in Jim Wells and Nueces Counties.



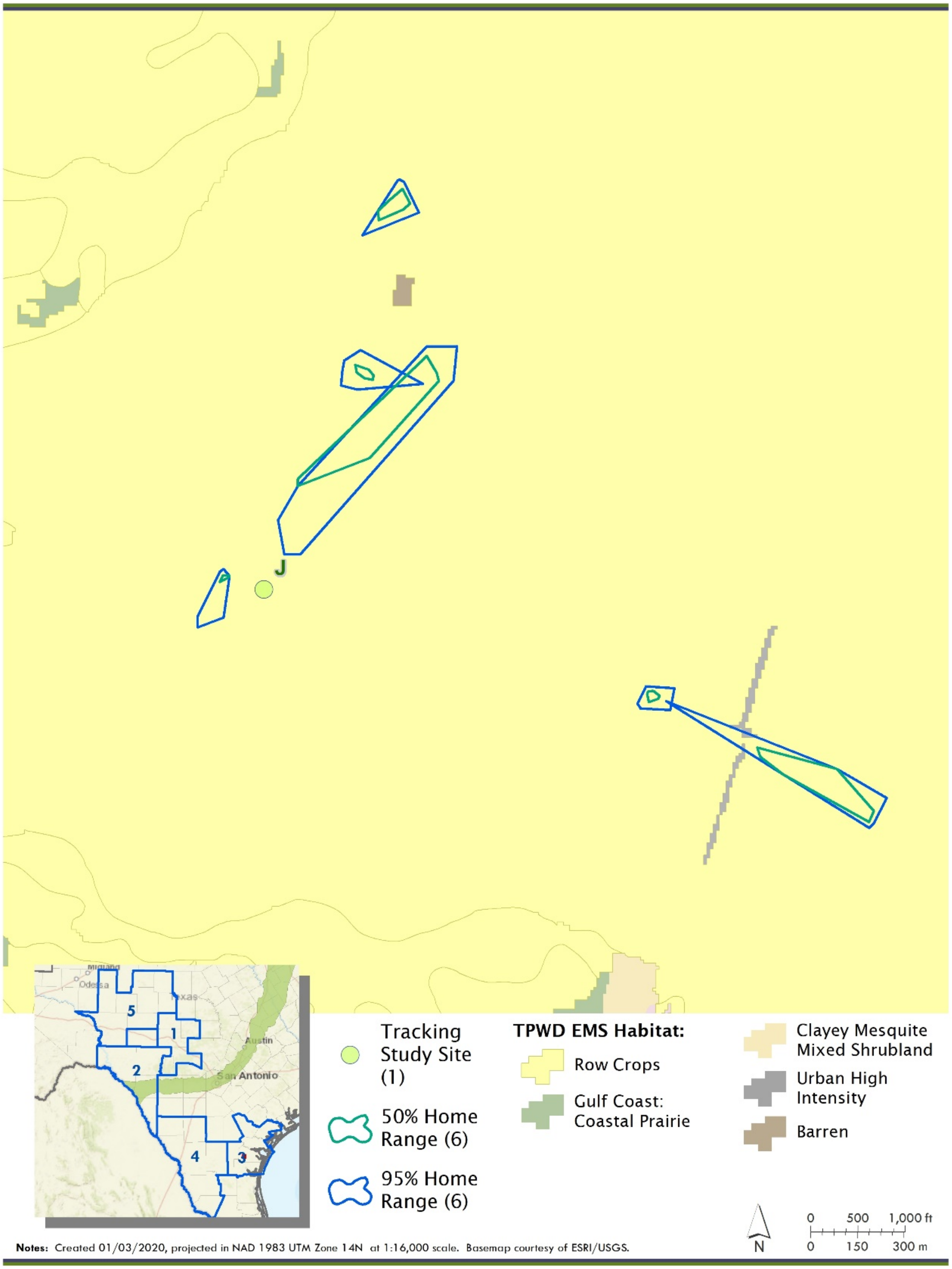
**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:120,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 17.** Radio telemetry tracking study sites (n = 5) including oil and gas land-use activity in Jim Wells and Nueces counties.



**Notes:** Created 01/03/2020, projected in NAD 1983 UTM Zone 14N at 1:15,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 18.** Radio telemetry tracking study site F located within Jim Wells County, including associated 50% and 95% MCP home range estimations (n = 11 individuals) and land classification obtained from TPWD Ecological Mapping Systems (EMS) database.



**Notes:** Created 01/03/2020, projected in NAD 1983 UTM Zone 14N at 1:16,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 19.** Radio telemetry tracking study site J located within Nueces County, including associated 50% and 95% MCP home range estimations (n = 6 individuals) and land classification obtained from TPWD Ecological Mapping Systems (EMS) database.

### 3.1.3 Discussion

Tracking success varied widely based on two main factors: (1) tag retention and (2) variation in transmitter battery life. Across all study sites and years tag retention rates varied considerably (1 to 20 tracking days). Variation in tag retention time was due to a number of factors including type of adhesive used, individual lizard shed cycle, weather, and variables affecting shearing forces (i.e., tags are shed more readily during burying behavior within rockier substrates). Of the adhesives used in this study (superglue, surgical latex, eyelash glue), eyelash glue appeared to provide the best tag adhesion. Tag retention also appeared be improved when care was taken to use a minimal application of adhesive.

Radio telemetry provided the Project Team with an opportunity to make several natural history and behavioral observations of *H. lacerata* and *H. subcaudalis* as this research was being conducted. The observations noted below provided insight into *H. lacerata* and *H. subcaudalis* habitat interactions and response to land-use.

Throughout this study, field observations of indicators of reproduction occurred for both *H. lacerata* and *H. subcaudalis.* These observations included breeding colors (i.e., forebody suffusion of orange or yellow coloration seen in females during breeding season; Hibbitts and Hibbitts 2015), courtship behavior (e.g., male-female pairing), gravidity (i.e., presence of eggs within abdomen of captured females), and hatchling presence. For *H. lacerata*, breeding colors and courtship behavior was observed April through July, and gravid females were observed as early as April (onset of field studies) and as late as July. Hatchlings were not observed until July, at which point they became locally abundant at some locations. *Holbrookia subcaudalis* showed slightly different trends, with courtship and breeding color observations occurring as early as April and continuing through July. Gravidity was observed during June and July with subsequent hatchling presence beginning in June, peaking in July, and continuing through September.

Within Unit 3, radio telemetry was conducted along a county road within Jim Wells County (study site F) during both 2018 and 2019 efforts. This site maintained a relatively large population of *H. subcaudalis* and was composed entirely of row crops along a caliche road. Crops observed were predominately cotton, with some milo and corn. This site was also notable in that an oil and gas transmission line was being actively installed through lizard habitat during 2019 tracking activities. The limit-of-disturbance (LOD) of this pipeline was approximately 40 m in width through crop fields. Increased traffic associated with construction activities did not appear to decrease lizard detection rates. In fact, one lizard was observed within the LOD on multiple occasions during radio telemetry activities. Within this study site, *H. subcaudalis* individuals were also observed utilizing the caliche lease road and pad associated with an oil and gas well-site.

On two occasions over the course of June 2018 field work within study site F, individuals of *H. subcaudalis* were observed utilizing Texas signal grass (*Urochloa texana*) as over-night cover. Heavy rains had recently inundated the surrounding fields, seeming to concentrate lizard activity near the roadsides. This observation was represented by two adult lizards affixed with VHF radio transmitters. Both individuals were observed curled up within the base of this grass in the early morning while acquiring the first relocation of the day. Generally, *H. subcaudalis* buries in soil for cover, however it is presumed flooding of fields in this area consequent of recent large rainfall events prevented these individuals from engaging in normal burying behavior.

At this same study site in July 2018, an adult male *H. subcaudalis* was observed within milo row crops before and after mechanical disking of the area. This lizard was observed actively utilizing this area and then burying over-night within a roadside area abutting the field. This area consisted predominately of Texas signal grass and bare ground. The following morning, this lizard was relocated via radio telemetry at this same location, buried within the substrate. Shortly after, this roadside area was tilled, turning over the soil and vegetation. A subsequent lizard relocation revealed that this lizard remained buried, and unharmed.

Within the row crop habitat utilized by the *H. subcaudalis* population studied during 2018 and 2019 field activities, dry conditions often created fissures within the soil. Both juvenile and adult *H. subcaudalis* were often observed employing these fissures for cover (Figure 20). Following rain events, these fissures would quickly disappear as the soil became wet, forcing lizards to find other cover.

Within Unit 1, radio telemetry was conducted within private property (study site D). Here, tracking efforts of an adult *H. lacerata* resulted in the observation of a predation event, April 2018. The VHF transmitter signal associated with this lizard was determined to be coming from inside an adult Coachwhip (*Masticophis flagellum*; Figure 21).

**Figure 20.** *Holbrookia subcaudalis* utilizing soil fissure as refugium.

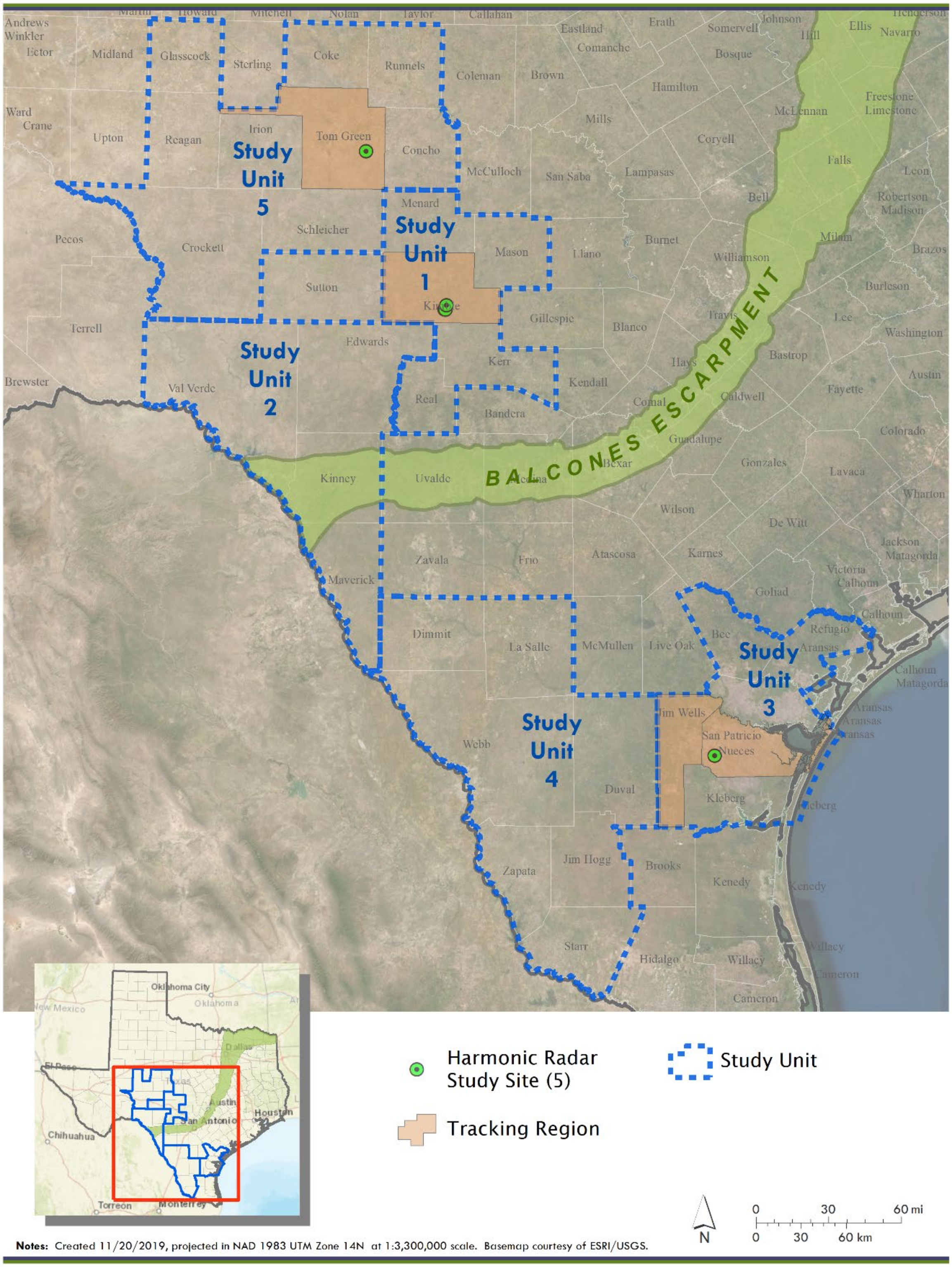
**Figure 21.** Coachwhip (*Masticophis flagellum*) captured after a predation of a radio tagged *H. lacerata* individual.

## 3.2 Harmonic Radar

This task sought to overcome some of the limitations encountered in the application of traditional radio telemetry to small organisms, specifically (1) short battery life, and (2) skewing of the data towards particular demographics (i.e., only larger adult *H. lacerata* and *H. subcaudalis* can carry VHF transmitters). The tag utilized in harmonic radar tracking consists of a copper antenna on a Polyimide (PI) carrier with a cover layer of PI material, which uses the original radar signal as an energy source, re-emitting a harmonic of the transmitted wavelength. Consequently, harmonic radar reflectors (tags) are much lighter and are passive (requiring no battery), thus even juvenile *H. lacerata* and *H. subcaudalis* can be tagged. This allows for the collection of movement and habitat use data for all life stages.

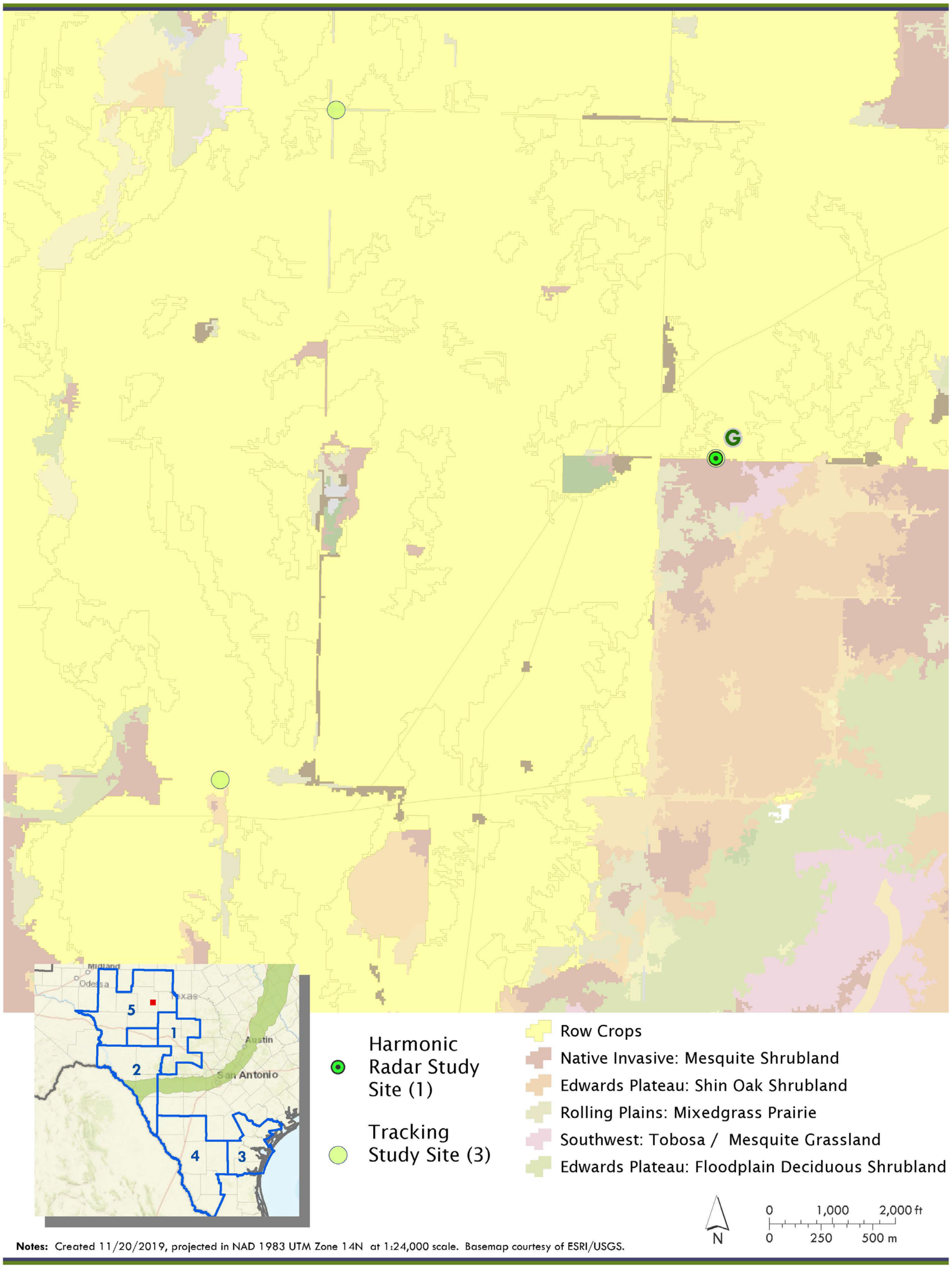
### 3.2.1 Methods

We employed harmonic radar to monitor the movements of both adult and juvenile *H. lacerata* and *H. subcaudalis* during the active season (late Spring through early Fall) of 2018 and 2019. Harmonic radar was conducted at five study sites within two Study Units, in areas where lizard captures were sufficient (Figures 22-25). Captured lizards were affixed with a 0.03 g harmonic radar reflector (RECCO® Reflector R-30CL) using a methodology analogous to that used to attach VHF transmitters (Section 3.1). Following tag placement, lizards were released and then tracked for 1-34 days during the months of April and August in 2018, and the months of July, September, and October in 2019. Tracking was performed utilizing an R9B RECCO® Detector and involved homing to obtain precise lizard relocations (Figures 26 and 27). Standard climate data (including temperature, relative humidity, barometric temperature, weather observations) was recorded at the time of obtaining each lizard relocation.



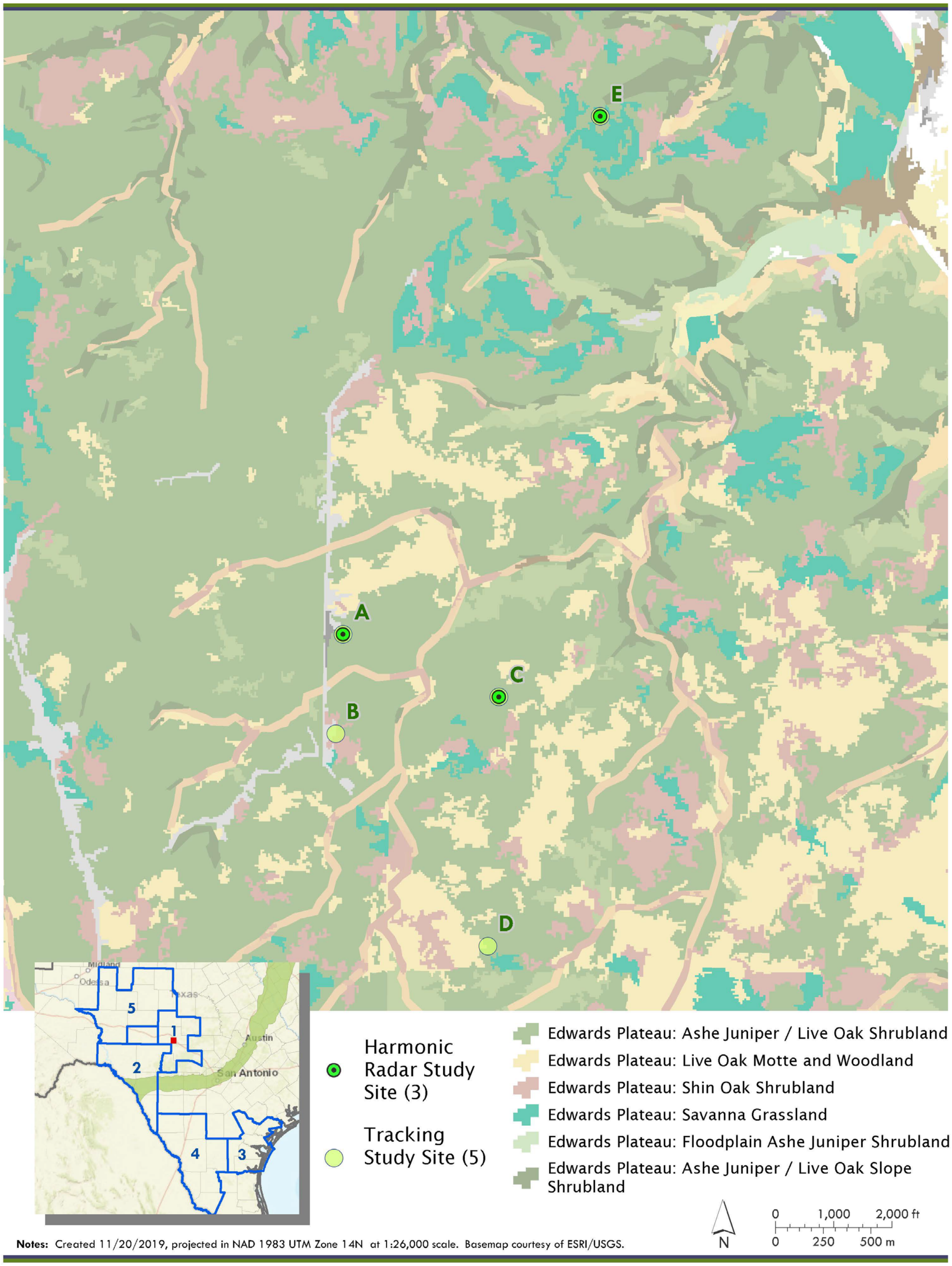
**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:3,300,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 22.** Study extent with distribution of study sites (n = 5) utilized for harmonic radar study.



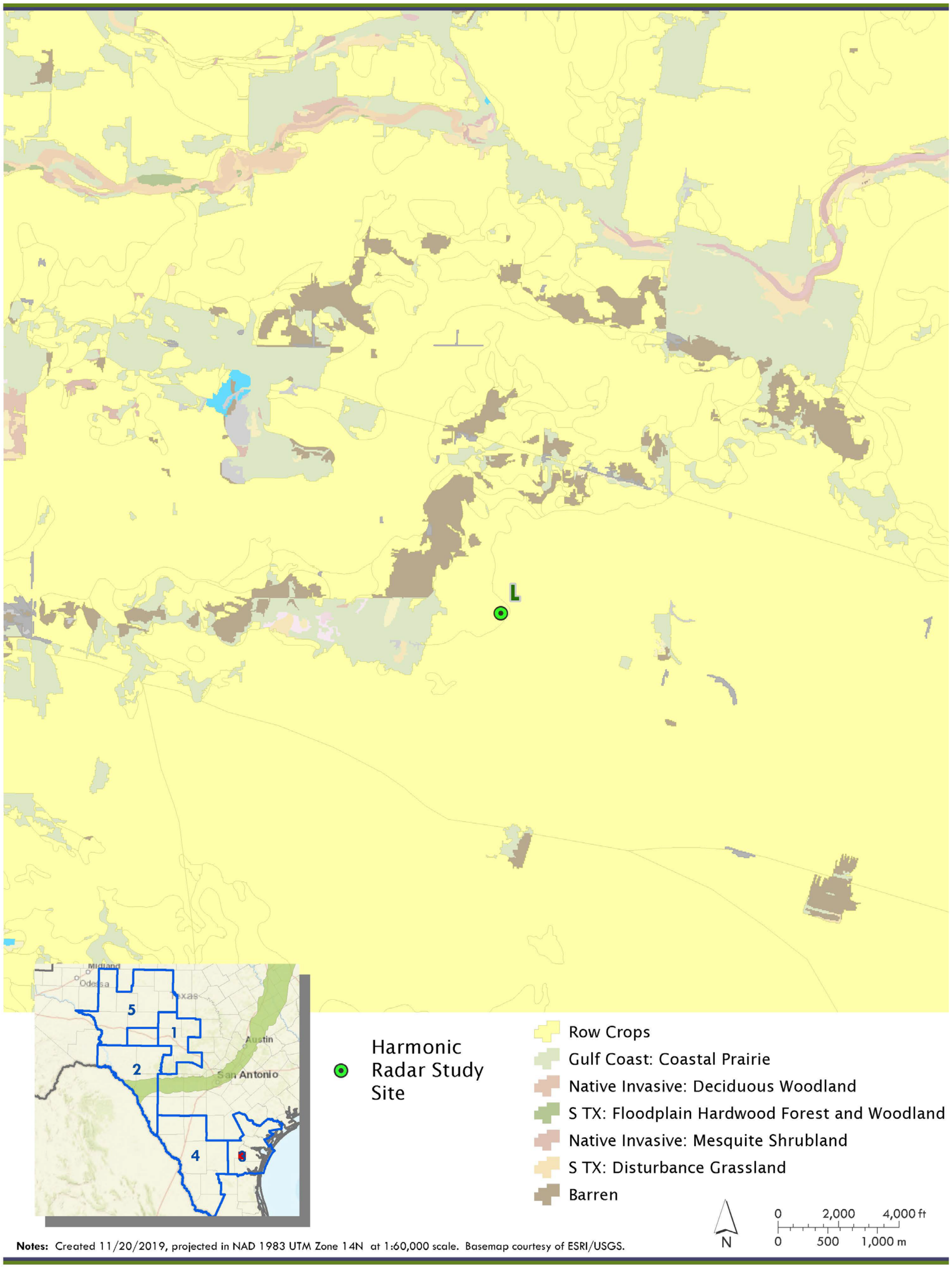
**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:24,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 23.** Tom Green County harmonic radar study site (n = 1).



**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:26,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 24.** Kimble County harmonic radar study site locations (n = 3).



**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:60,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 25.** Nueces County harmonic radar study site (n = 1).

**Figure 26.** Harmonic radar reflector affixed to *H. lacerata* <5.0 g.

**Figure 27.** Using harmonic radar to obtain a relocation point on *H. lacerata* at study site E in Kimble County.

### 3.2.2 Results

In total, 19 individuals were tagged resulting in 164 relocation points during 2018 and 2019 field activities, with the majority of lizards located within Kimble County (study sites A, C, and E; Figure 24). Two lizards were tagged outside of Kimble County, within Nueces County (study site L, Figure 25) and Tom Green County (study site G; Figure 23). Of the tracked lizards, there was varying tracking success with one providing ≥ 20 relocation points (6% of tagged lizards), and seven providing ≥ 10 relocation points (37% of tagged lizards). Tracking efforts in 2018 produced 122 relocation points from 11 individuals, and tracking efforts in 2019 produced 42 relocation points from 8 individuals (Table 5).

**Table 5.** Summary of 2018 and 2019 harmonic radar tracking activities (n = 19 lizards).

| Year | Unit | County | Study Site | Month of Deployment | # of Relocations |
|---|---|---|---|---|---|
| 2018 | 1 | Kimble | C | April | 9 |
| 2018 | 1 | Kimble | C | April | 7 |
| 2018 | 1 | Kimble | C | April | 15 |
| 2018 | 1 | Kimble | C | August | 14 |
| 2018 | 1 | Kimble | C | August | 8 |
| 2018 | 1 | Kimble | C | August | 1 |
| 2018 | 1 | Kimble | C | April | 15 |
| 2018 | 1 | Kimble | C | August | 5 |
| 2018 | 1 | Kimble | E | April | 36 |
| 2018 | 1 | Kimble | E | April | 11 |
| 2018 | 5 | Tom Green | G | June | 1 |
| 2019 | 1 | Kimble | A | September | 1 |
| 2019 | 1 | Kimble | A | September | 1 |
| 2019 | 1 | Kimble | A | September | 10 |
| 2019 | 1 | Kimble | A | September | 6 |
| 2019 | 1 | Kimble | C | September | 7 |
| 2019 | 1 | Kimble | C | September | 12 |
| 2019 | 1 | Kimble | C | September | 4 |
| 2019 | 3 | Nueces | P | July | 1 |
| **TOTAL** | | | **5 Study Sites** | | **164** |

Across two field seasons, mean body weight for *H. lacerata* tracked using harmonic radar was 4.9 g (SE = 0.32; Table 6). Of these, nine individuals had body weights less than five grams (precluding the application of VHF radio transmitters). One *H. subcaudalis* was affixed with a harmonic radar tag within Nueces County and weighed 1.2 g.

**Table 6.** Summary of the mean body weight (g), snout vent length (mm), and tail length (mm) of *H. lacerata* utilized for harmonic radar 2018-2019 (n = 18 individuals).

| Attribute | Range | Mean | SE |
|---|---|---|---|
| Weight (g) | 2.6 – 8.5 | 4.9 | 0.32 |
| Snout to Vent Length (mm) | 37.2 – 55.7 | 49.0 | 1.13 |
| Tail Length (mm) | 27.3 – 57.0 | 45.3 | 1.72 |

### 3.2.3 Discussion

Overall, the application of harmonic radar provided this study with the ability to track the movements of nine lizards with body weights that would have otherwise precluded them from carrying tags (i.e., VHF radio transmitters). Tracking success (i.e., number of relocation points recorded per individual) was a factor of tag retention and limitations inherent to the application of harmonic radar. Similar to problems encountered during radio telemetry activities (Section 3.1), tag retention varied considerably between individuals and between study sites and was most likely due to variation in the type and application of adhesive used, weather (i.e., wet conditions), lizard shed cycle, and shearing forces related to variation in lizard burying substrate. In contrast to radio telemetry, harmonic radar presented unique challenges relating to lizard movements and signal range. The harmonic radar reflectors and associated hand-held detector had a maximum signal range of approximately 15 meters. Thus obtaining relocations of occasional large lizard movements was more time consuming than during radio tracking, and in some situations (roadsides, property boundaries) the individual could move beyond access boundaries such that it could no longer be tracked. This issue was less pronounced when tracking earlier life-stage *H. lacerata* and *H. subcaudalis* as these individuals tended to make smaller movements between relocations.

## 3.3 Home Range

The spatial distributions of animal relocations recorded in telemetry studies can be translated into estimates of home range size using statistical home range models. A widely used approach is the minimum convex polygon (MCP) method, which characterizes the animal's home range as the smallest-sized polygon encompassing the observed relocations (Moorecroft 2008). Specifically, this home range includes the area in which the animal spends 95% of its time during normal daily activities, with areas visited outside of this 95% polygon generally considered exploratory in nature (Burt 1943). The core area is the smallest area in which the individual spends 50% of its time, and generally describes the majority of activity (Van Winkle 1975; Anderson 1982). Therefore, home range studies generally apply MCP estimations using both 50% and 95% of total relocation points.

### 3.3.1 Analysis

Software package LOAS was used to calculate locations from telemetry bearings, and BIOTAS 2.0 (Ecological Software Solutions) was used to calculate home ranges and stepwise movements. The minimum convex polygon method was then used to estimate home ranges using a random selection of 50% of total relocation points per lizard (50% MCP) and 95% of total relocation points per lizard (95% MCP). This analysis includes the estimation of home ranges for individuals tracked using VHF radio telemetry and harmonic radar.

### 3.3.2 Results

Movement data on 21 *H. lacerata* (range = 3 to 17 tracking days) and 17 *H. subcaudalis* (range = 3 to 11 tracking days) were utilized for home ranges estimated using both 50% and 95% MCP, including three individuals with relocations obtained via harmonic radar. Home range estimations for *H. lacerata* using a 50% MCP produced areas ranging from 0.05 to 0.80 acres across both males and females (Table 7). Between sex, mean home range size was not significantly different for females (0.24 acres, SE = 0.05) than for males (0.24 acres, SE = 0.08) when compared using a two-sample t-test assuming unequal variances (t = 0.03, p-value = 0.49). Home range estimations for *H. subcaudalis* using a 50% MCP produced areas ranging from 0.07 to 18.93 acres across both males and females. For this species, mean home range size was significantly larger for males (6.76 acres, SE = 2.02) than for females (0.99 acres, SE = 0.23; t = -2.84, p-value < 0.05). Between species, mean home range size was significantly larger for *H. subcaudalis* (5.06 acres, SE = 1.55) than for *H. lacerata* (0.24 acres, SE = 0.05; t = 3.10 p-value <0.05).

**Table 7.** Summary of *H. lacerata* and *H. subcaudalis* home range size (acres) estimated using 50% MCP across all years (2017-2019) by species and sex.

| Species | Sex | *n*[a] | Mean # of Relocations | Mean # of Tracking Days | Mean Home Range (acres ± SE)[b] (hectares) |
|---|---|---|---|---|---|
| *H. lacerata* | Male | 10 | 24.2 | 7.1 | 0.24 ± 0.08 (0.10) |
| | Female | 11 | 35.8 | 9.3 | 0.24 ± 0.05 (0.10) |
| *H. subcaudalis* | Male | 12 | 28.3 | 6.8 | 6.76 ± 2.02 (2.74) |
| | Female | 5 | 27.6 | 7.4 | 0.99 ± 0.23 (0.40) |

a. Number includes only those individuals with enough relocation points for estimation of home range size.
b. Includes home range estimates based on both VHF radio telemetry and harmonic radar.

Home range estimations for *H. lacerata* using a 95% MCP produced areas ranging from 0.10 to 6.55 acres across both males and females (Table 8). Between sex, mean home range size was not significantly larger for males (1.51 acres, SE = 0.60) than for females (1.36 acres, SE = 0.36; t = -0.21, p-value = 0.42). Home range estimations for *H. subcaudalis* produced areas ranging from 0.82 to 51.50 acres across both males and females. For this species, mean home range size was significantly larger for males (18.60 acres, SE = 4.82) than for females (5.21 acres, SE = 1.14; t = -2.70, p-value = <0.05). Between species, mean home range size was significantly larger for *H. subcaudalis* (14.66 acres, SE = 3.70) than for *H. lacerata* (1.43 acres, SE = 0.04; t = -3.58, p-value <0.05).

**Table 8.** Summary of *H. lacerata* and *H. subcaudalis* home range size (acres) estimated using 95% MCP across all years (2017-2019) by species and sex.

| **Species** | **Sex** | ***n*[a]** | **Mean # of Relocations** | **Mean # of Tracking Days** | **Mean Home Range (acres ± SE)[b] (hectares)** |
|---|---|---|---|---|---|
| *H. lacerata* | Male | 10 | 24.2 | 7.1 | 1.51 ± 0.60 (0.61) |
| | Female | 11 | 35.8 | 9.3 | 1.36 ± 0.36 (0.55) |
| *H. subcaudalis* | Male | 12 | 28.3 | 6.8 | 18.60 ± 4.82 (7.53) |
| | Female | 5 | 27.6 | 7.4 | 5.21 ± 1.14 (2.11) |

a. Number includes only those individuals with enough relocation points for estimation of home range size.

b. Includes home range estimates based on both VHF radio telemetry and harmonic radar.

### 3.3.3 Discussion

Within this analysis there were two major disparities with regards to *H. lacerata* and *H. subcaudalis* home range sizes. Estimated areas for *H. subcaudalis* individuals were considerably larger and displayed larger differences in home range size between males and females. Large differences in body size between the two species was observed. Within all captures across this study, mean body weight of observed *H. subcaudalis* (8.8 g, SE = 0.7, n = 53) was 42.8% greater than *H. lacerata* (5.7 g, SE = 0.2, n = 51). Mean SVL for *H. subcaudalis* (55.9 mm, SE = 1.0, n = 53) was 7.6% greater than *H. lacerata* (51.8 mm, SE = 0.6, n = 51). The observed differences between the sexes in the home range sizes of *H. subcaudalis* could be explained by the need of adult males to establish larger areas so as to interact with a larger number of females during the reproductive season. Males of many lizard species have home ranges double the size of females, a characteristic important during the reproductive season (Rose 1982; Stamps 1983). This may be further evidenced by the variation in sex ratios between the northern and southern species as observed within all capture data collected across this study. Within the sampled populations of *H. lacerata*, sex ratios were 1.36:1 (F:M, n = 59). In contrast, observed sex ratios within populations of *H. subcaudalis* were 0.72:1 (F:M, n = 43). Given this variation, it may be that movements of male *H. subcaudalis* are driven to a greater extent by attempts to increase female interactions.

## 3.4 Movements

For the purposes of examining lizard movements as they relate to climatic conditions and to increase the independence of relocation points, only points recorded between 0.5 and 3.5 hours apart were used in this analysis. The collection of movement data on smaller individuals using harmonic radar was predominately collected within Kimble County (Study Unit 1; *H. lacerata*). All lizards below the minimum weight requirement for the application of VHF radio transmitters (i.e., body weights < 5.0 g, requiring the application of harmonic radar tags) were included in this analysis (smaller lizards). The resulting analysis included the movement data (n = 42 relocation points) of four individuals < 5.0 g. Body weight of these individuals ranged from 1.1 to 4.7 g, with a mean 3.0 g (SE = 0.8). Snout to vent length ranged from 31.2 to 53.4 mm with a mean of 43.6 mm (SE = 4.9).

Mean daily movement was significantly less for smaller lizards (37.8 m, SE = 9.37) than for larger lizards (59.0 m, SE = 8.03) when compared using a two-sample t-test assuming unequal variances (t = -1.71, p-value < 0.05, Figure 28). Minimum observed step-length of 0 m (distance moved between two relocation points) occurred when smaller lizards were buried in soil. Maximum step-length observed for small *H. lacerata* was 122.3 m.

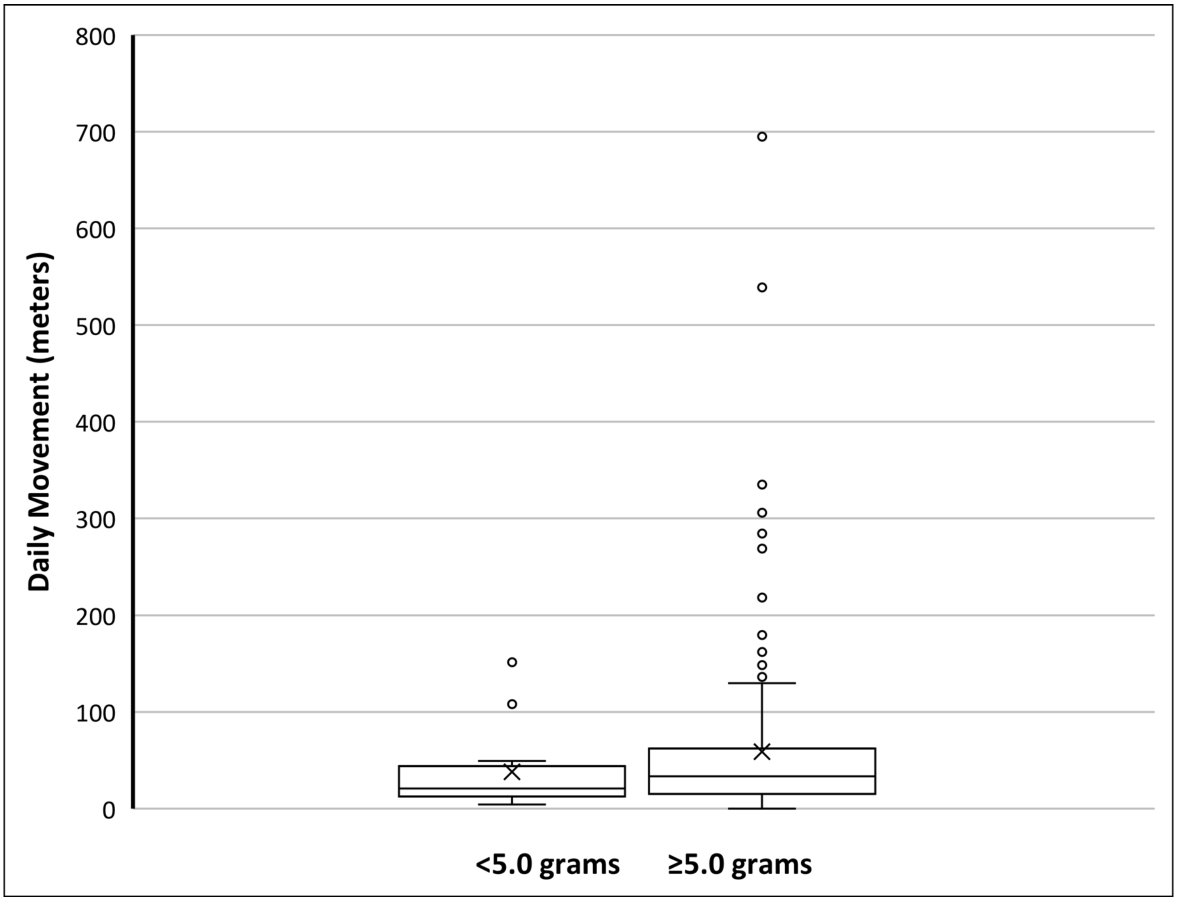


**Figure 28.** Daily movement (meters [m]) of *H. lacerata* <5.0 g (20.6 m; n = 19 days) and *H. lacerata* ≥ 5.0 g (33.3 m; n = 132 days). Box = median with 25th and 75th percentiles; with 'X' representing the mean and points representing daily movements > 1.5 x the interquartile range.

Simple linear regression was used to assess the ability of air temperature to predict smaller lizard rate of movement (m/hr). This included all data collected using harmonic radar from *H. lacerata* < 5.0 g. There was not a significant relationship (F(1,35) = 0.089, p-value = 0.77). Simple linear regression of smaller lizard rate of movement (m/hr) on relative humidity including all data collected using harmonic radar from *H. lacerata* < 5.0 g did not show a significant relationship (F(1,33) = 0.899, p-value = 0.35).

Movement data were estimated for a total of 28 adult *H. lacerata* (n = 324 relocation points) and 27 adult *H. subcaudalis* (n = 350 relocation points). Mean daily movement was significantly different for *H. lacerata* (51.4 m, SE = 6.48) than for *H. subcaudalis* (174.0 m, SE = 14.59) when compared using a two-sample t-test assuming unequal variances (t = -7.67, p-value < 0.05, Figure 29). Step-lengths ranged from 0 to 353.1 m for adult *H. lacerata* and 0 to 433.6 m for adult *H. subcaudalis.*

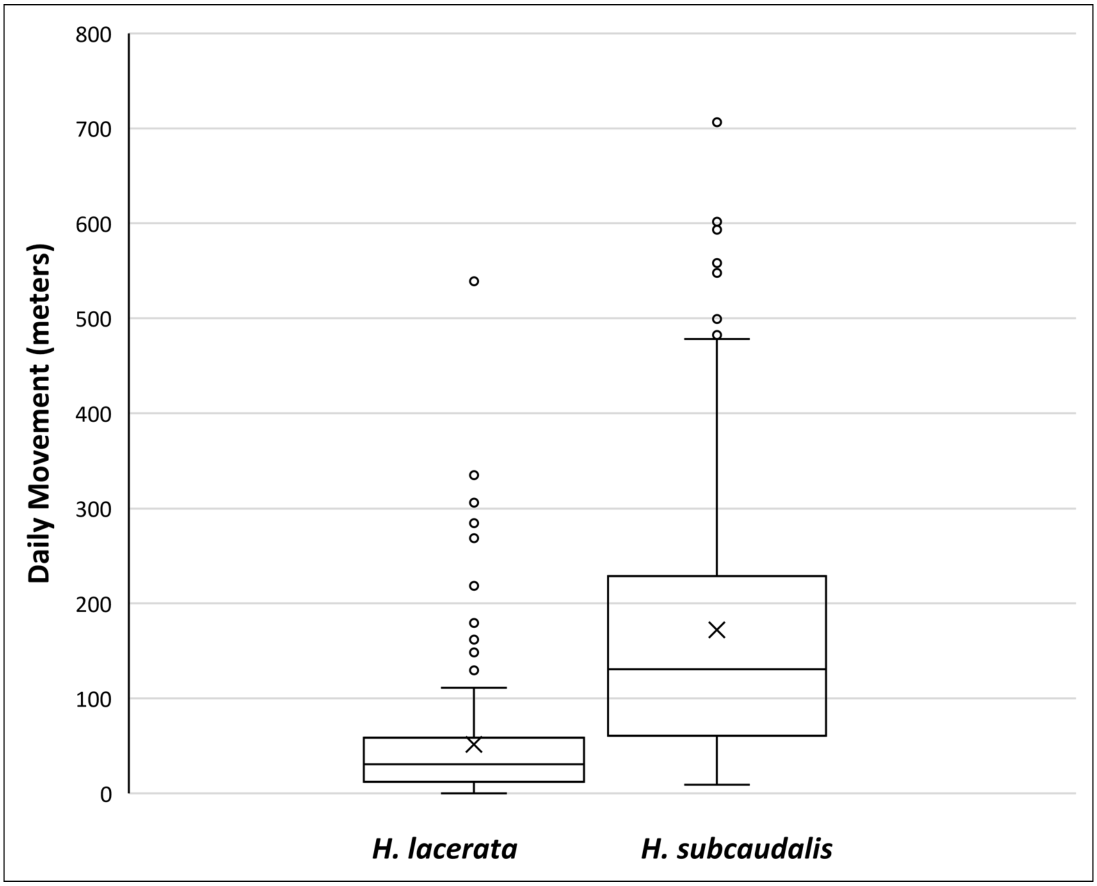


**Figure 29.** Daily movement (meters [m]) of *H. lacerata* (30.7 m; n = 130 days) and *H. subcaudalis* (130.7 m; n = 121 days). Box = median with 25th and 75th percentiles; with 'X' representing the mean and points representing daily movements > 1.5 x the interquartile range.

Within adult *H. lacerata*, mean daily movement was not significantly different between females (49.9 m, SE = 11.80) and males (57.7 m, SE = 10.39) when compared using a two-sample t-test assuming unequal variances (t = 0.49, p-value = 0.31, Figure 30).

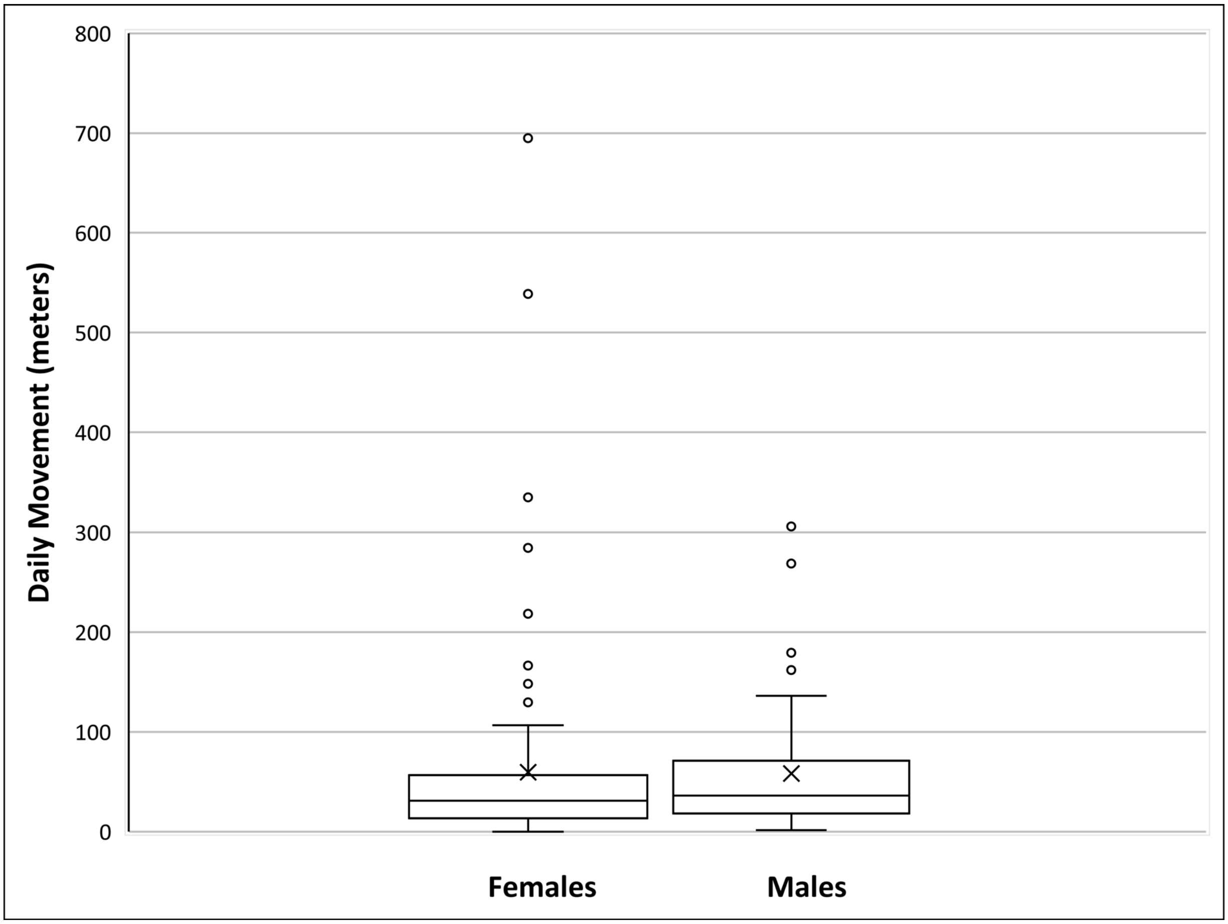


**Figure 30.** Daily movement (meters [m]) of female (30.9 m; n = 30 days) and male (34.8 m; n = 42 days) *H. lacerata*. Box = median with 25th and 75th percentiles; with 'X' representing the mean and points representing daily movements outside 1.5 x the interquartile range.

Within adult *H. subcaudalis* adults, mean daily movement was significantly different between females (120.7 m, SE = 20.67) and males (190.4 m, SE = 17.68) when compared using a two-sample t-test assuming unequal variances (t = 2.56, p-value < 0.05, Figure 31).

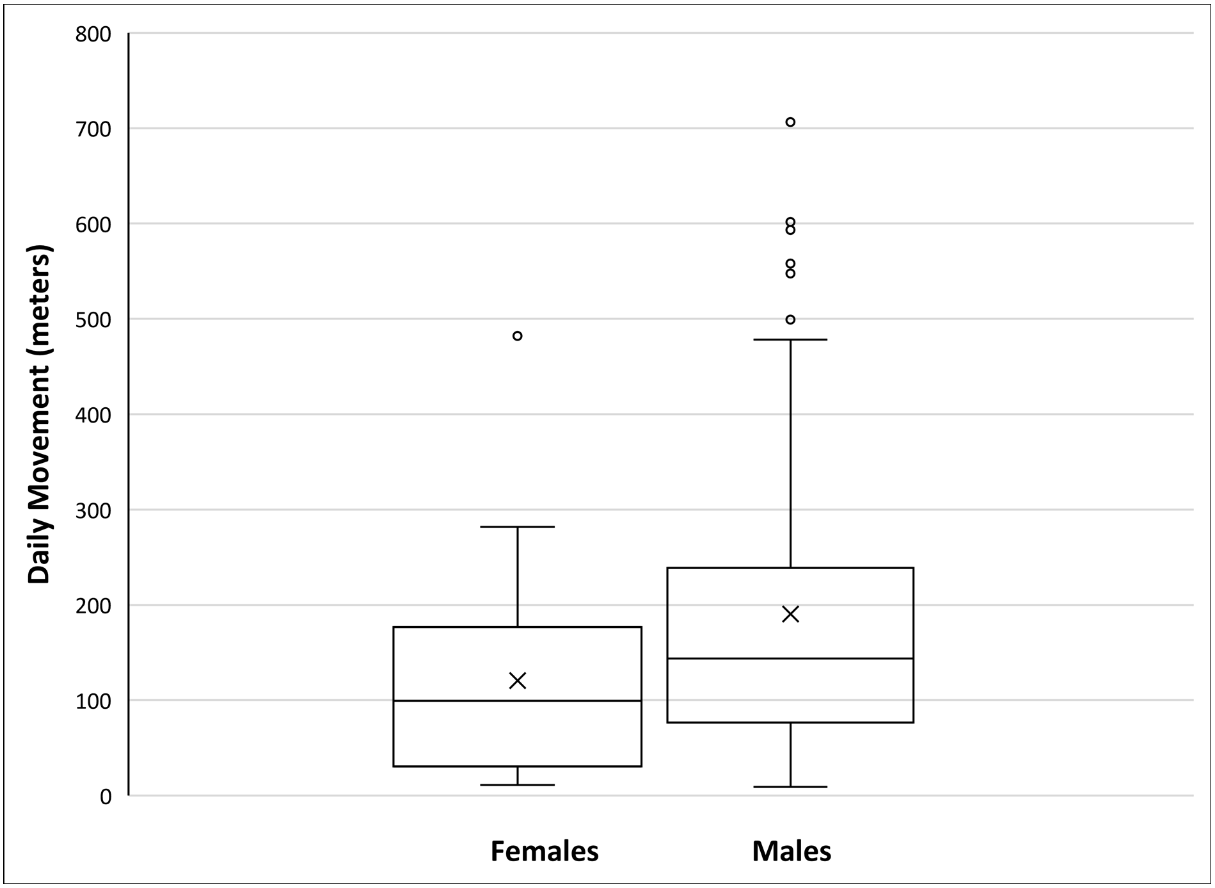


**Figure 31.** Daily movement (meters [m]) of female (99.4 m; n = 28 days) and male (143.7 m; n = 91 days) *H. subcaudalis.* Box = median with 25th and 75th percentiles; with 'X' representing the mean and points representing daily movements > 1.5 x the interquartile range.

Within *H. subcaudalis* adults, mean daily movement was not significantly different between lizards tracked in agricultural fields within harvested (166.6 m, SE = 22.9) and un-harvested (162.3 m, SE = 24.0) row crops when compared using a two-sample t-test assuming unequal variances (t = -0.13, p-value = 0.45, Figure 32).

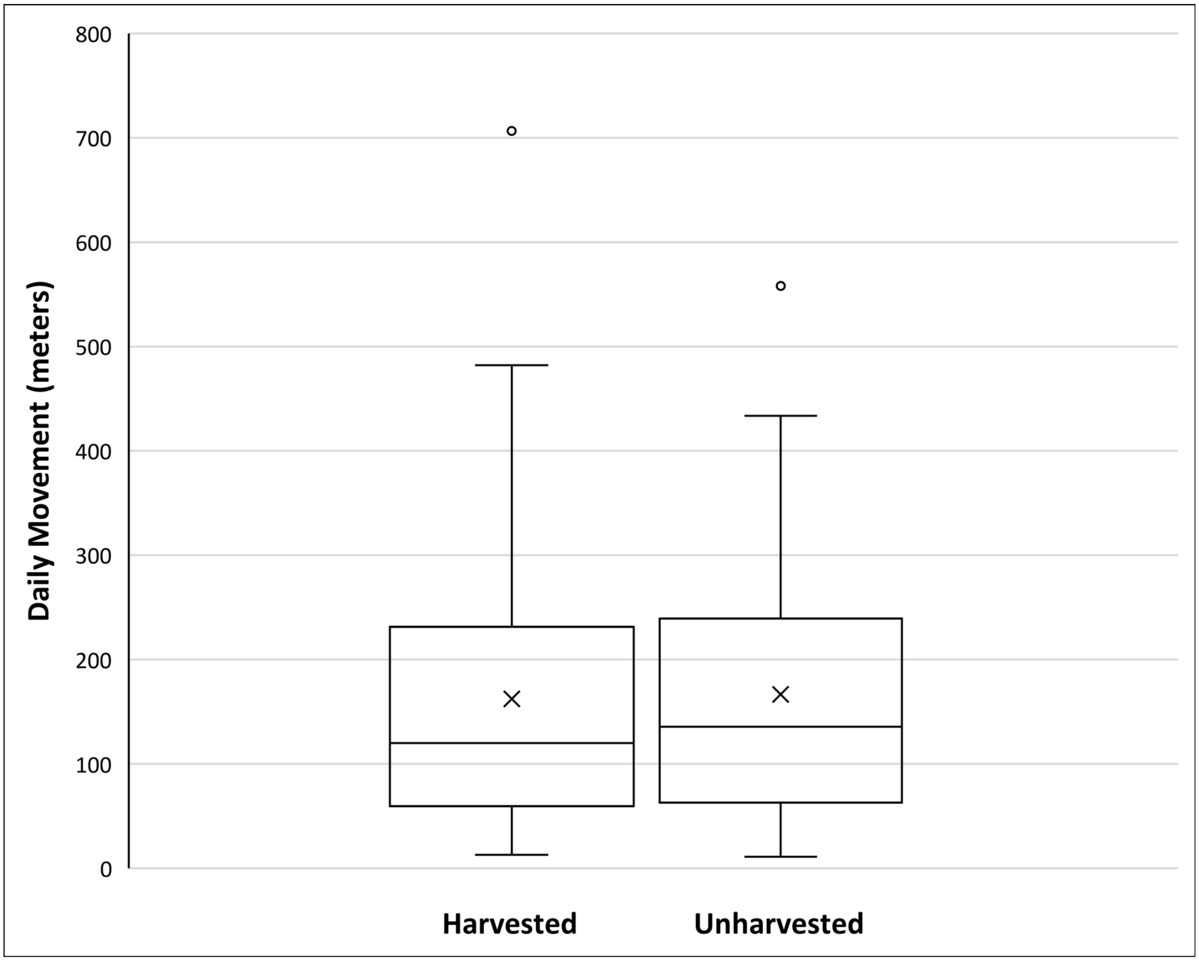


**Figure 32.** Daily movement (meters [m]) for *H. subcaudalis* within harvested (120.1 m; n = 37 days) and unharvested (135.7 m; n = 32 days) row crops. Box = median with 25th and 75th percentiles; with 'X' representing the mean and points representing daily movements > 1.5 x the interquartile range.

Simple linear regressions were used to evaluate relationships between variables associated with weather (ambient temperature, substrate temperature, and relative humidity) and rate of movement for both *H. lacerata* and *H. subcaudalis*. Daily movement was found to be significantly different between male and female *H. subcaudalis,* thus rate of movement analyses were conducted separately by sex for this species. Only those regressions showing significant relationships are depicted graphically. There was not a significant relationship of *H. lacerata* rate of movement and ambient temperature (F(1,237) = 0.041, p-value = 0.839), nor *H. lacerata* rate of movement (m/hr) and substrate temperature (F(1,55) = 0.371, p-value = 0.116). There likewise was not a significant relationship of *H. lacerata* rate of movement (m/hr) and relative humidity (F(1,173) = 0.975, p-value = 0.325).

Simple linear regression of male *H. subcaudalis* rate of movement (m/hr) on ambient temperature found no significant relationship (F(1,217) = 3.710, p-value = 0.055) at $\alpha = 0.05$. Simple linear regression of female *H. subcaudalis* rate of movement (m/hr) on ambient temperature (Figure 33) found a significant relationship (F(1,102) = 14.643, p-value $< 0.05$), with an $R^2$ of 0.126. Here, movement rate was negatively correlated with ambient temperature ($\beta = -3.14$). There was no significant regression relationship between male *H. subcaudalis* rate of movement (m/hr) and substrate temperature (F(1,220) = 0.276, p-value = 0.600). A significant regression relationship between female *H. subcaudalis* rate of movement (m/hr) and substrate temperature (Figure 34) was found (F(1,101) = 7.965, p-value $< 0.05$), with an $R^2$ of 0.073. Movement rate was negatively correlated with substrate temperature ($\beta = -0.75$). There was no significant regression relationship between male *H. subcaudalis* rate of movement (m/hr) and relative humidity (F(1,219) = 0.009, p-value = 0.924). Relative humidity was found to be positively correlated with female *H. subcaudalis* rate of movement (Figure 35, F(1,102) = 8.597, p-value $< 0.05$), with an $R^2$ of 0.078. The low $R^2$ values observed in the preceding relationships suggest that while these correlations may be significant, they explain relatively little of the variance between observed movements and the line of the regression equation.

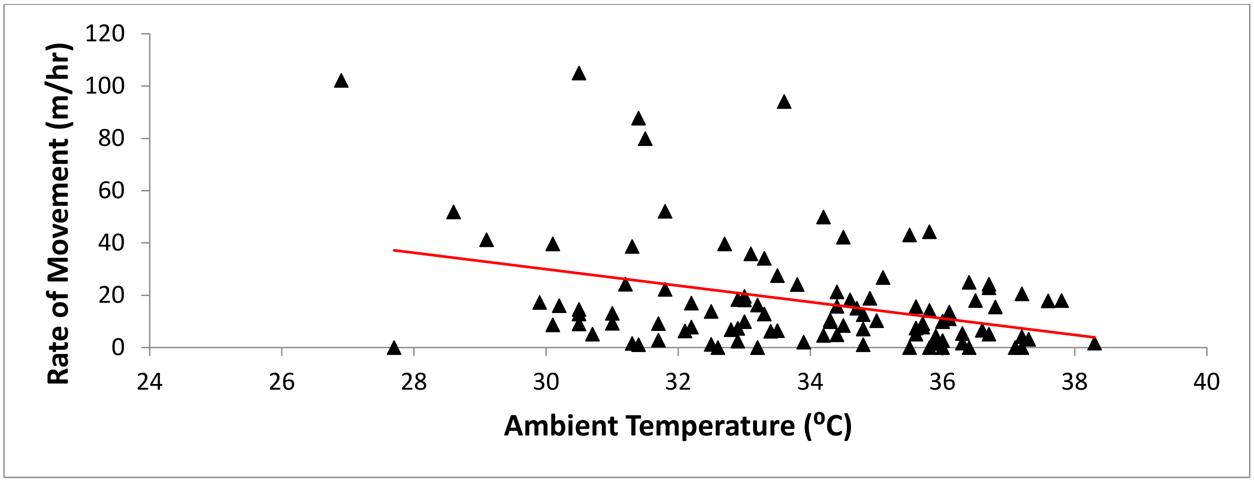


**Figure 33.** Rate of movement (m/hr) regressed on ambient temperature with linear regression line representing predicted values for individual female *H. subcaudalis* (n = 104 movements).

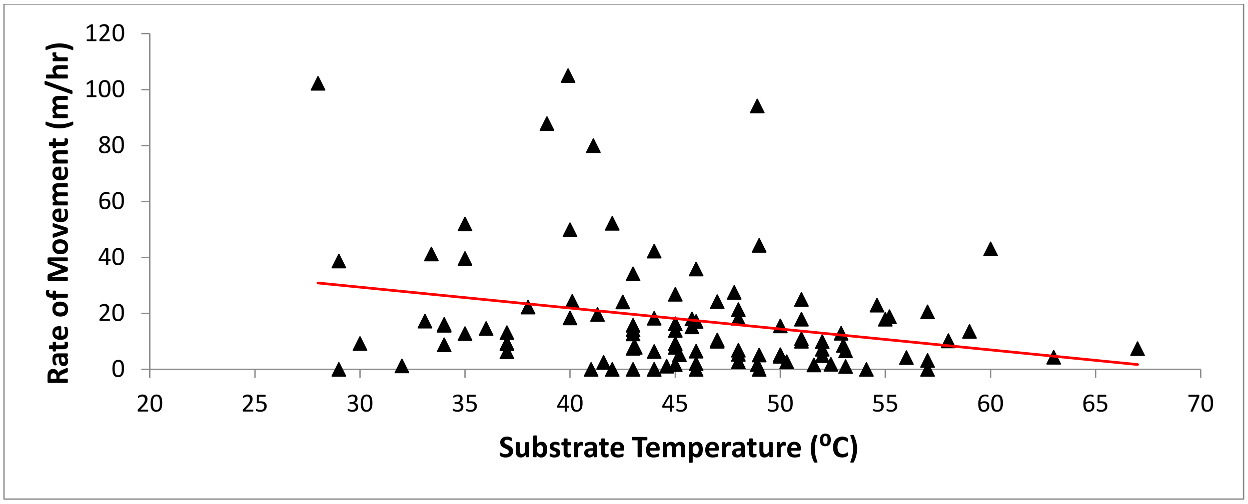


**Figure 34.** Rate of movement (m/hr) regressed on substrate temperature with linear regression line representing predicted values for individual female *H. subcaudalis* (n = 222 movements).

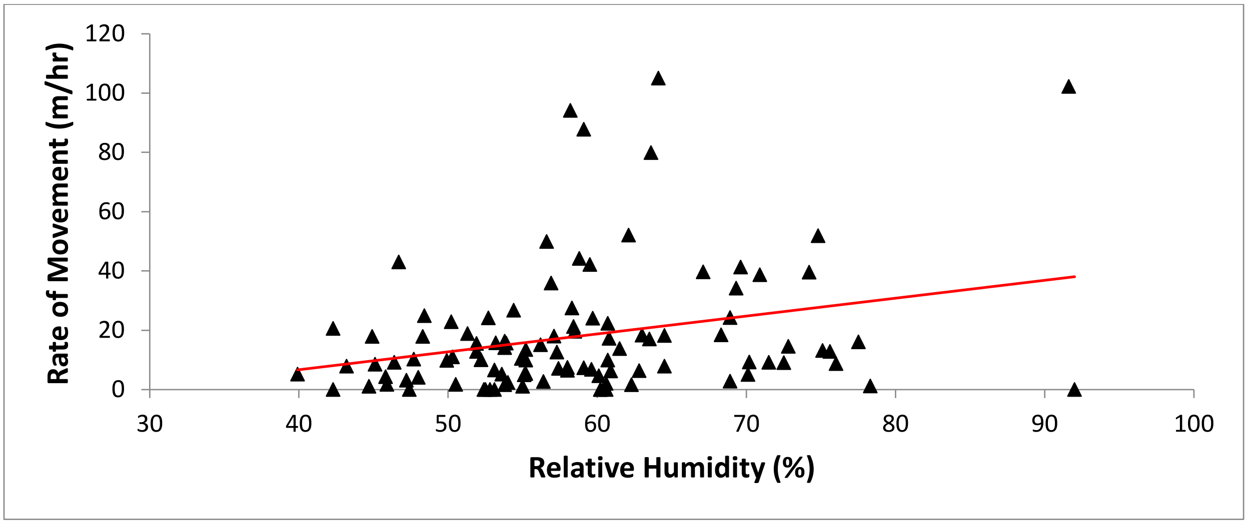


**Figure 35.** Rate of movement (m/hr) based on relative humidity with linear regression line representing predicted values for individual female *H. subcaudalis* (n = 104 movements).

Daily movements for *Holbrookia lacerata* were not significantly different between sex. This result is perhaps consistent with estimated home ranges, wherein similar sizes were observed between males and females for this species (Section 3.3). Between sex, *H. subcaudalis* displayed significant differences in mean daily movement. This characteristic may also be consistent with previous estimations of home range, as home range sizes of *H. subcaudalis* were significantly larger for males. Within the population of *H. subcaudalis* sampled for this analysis, all lizards were found within agricultural fields consisting of row crops (corn, cotton, or milo). Relative to the *H. subcaudalis* activity period, these fields were observed to follow a general trend of being at intermediate to full growth (un-harvested) states from Spring to mid-Summer and harvested states during and after late-Summer. Within this species, mean daily movements were not significantly different between harvested and un-harvested areas, suggesting that variations in habitat due to crop state does not affect movement in *H. subcaudalis.* Mean daily movement of smaller *H. lacerata* (< 5 g) was significantly smaller than larger (≥ 5 g) *H. lacerata.* It is possible this is an artifact of differences in sample size between these two groups (42 relocation points for *H. lacerata* < 5.0 g versus 369 relocation points for *H. lacerata* ≥ 5.0 g). In 2017, before harmonic radar methods were used in this study, large cohorts of juvenile *H. lacerata* were observed at some study sites. Harmonic radar was added as a task under this study in 2018, however fewer juveniles were encountered in 2018–2019 in the range of *H. lacerata*. As success in studying this size/age class of either species is driven by opportunity (availability of study subjects), fewer individuals were successfully tracked during this period. In the case of *H. subcaudalis*, in 2019 a number of hatchlings were observed, and several captured. A few attempts to utilize harmonic radar tracking on these individuals were made, however at that specific location they were able to move out of range of the harmonic radar equipment and into areas which were not accessible for tracking. Though it is more challenging than radio tracking, the collection of movement data of early life stage *H. lacerata* via harmonic radar should be continued. As study subjects present themselves it could provide valuable insight into early life stages of both species and highlight ecological differences between them.

Simple linear regressions were calculated to predict rate of movement for both *H. lacerata* and *H. subcaudalis* based on variables associated with weather. For *H. lacerata*, weather was not a good predictor of lizard rate of movement within this data, with no significant relationship of rate of lizard movement with observed air temperature, substrate temperature, or relative humidity. It is important to note, however, that these variables were not sampled throughout their entire possible range (i.e., all seasons) in this study, and relationships are likely to exist that were not captured in this study. These analyses illustrate that over the range of variable values observed, individual lizards studied were successfully able to thermoregulate in their environments such that movements rates (and thus behaviors) were consistently maintained. *Holbrookia subcaudalis* showed different trends, with significant and negative correlations between rate of movement and both ambient temperature and substrate temperature, as well as a positive correlation with relative humidity. Generally, sampling bias was avoided in these data as individuals were tracked at regular intervals regardless of weather once tags were attached. However, it was found that strong rainfall events provided conditions that improved capture success of this species, and as such these climatic patterns were targeted for study efforts when possible. This could have influenced the relationship observed with relative humidity, as study subjects were confined to non-flooded areas when tagged and subsequently dispersed into larger areas as standing water receded. Milder temperatures correlated with high humidity ($R^2 = 0.75$) after these events also contributed to this relationship. Negative relationships between movement and temperature are consistent with trends observed in the field. Activity was highest within the early morning and evening when temperatures were less severe than in the late-morning and afternoon. Within this study, relative humidity was commonly associated with increased precipitation and soil moisture. Therefore, the positive relationship between movement and relative humidity is most likely an artifact of milder temperatures (as associated with precipitation).

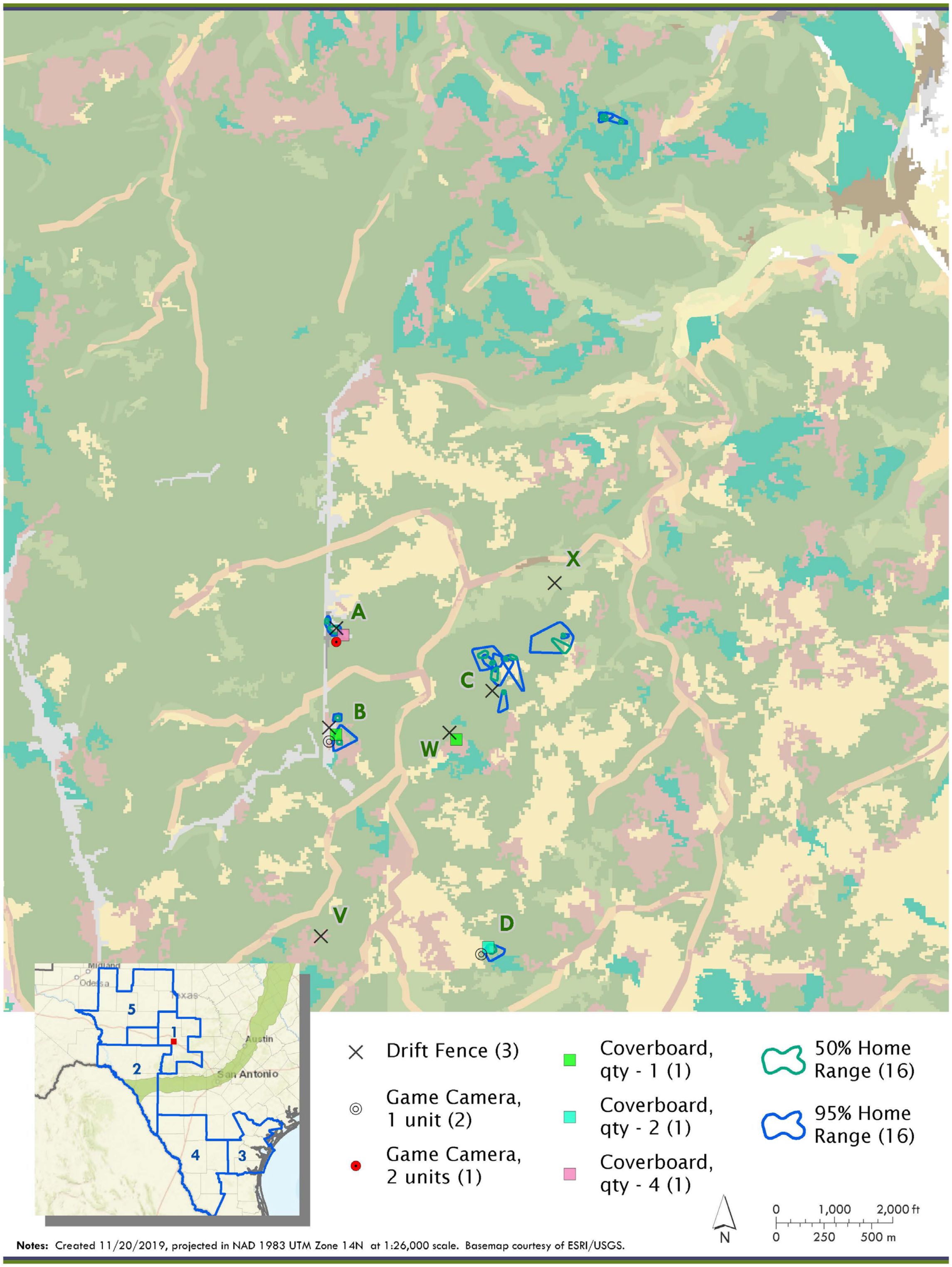
# 4.0 Passive Survey Methods and Visual Encounter Surveys

## 4.1 Passive Surveys

To assess the potential use of passive capture methods for this species, the Project Team employed three methods historically used for herpetofauna inventory. These methods included the implementation of drift fence arrays, coverboards, and game cameras within a subset of established sampling units where *H. lacerata* and *H. subcaudalis* presence had been previously observed during recent surveys.

### 4.1.1 Drift Fences

Drift fence arrays were deployed at six study sites within Kimble County (Study Unit 1) in 2017 (Figure 36). These arrays were established within either areas of known *H. lacerata* presence or areas within the species' range containing suitable habitat and proximal to occupied areas (Figure 37). All drift fence arrays were installed between July 6 and July 12, 2017. Drift fence arrays included either one central pitfall trap approximately 15 cm in diameter and 46 cm in depth (n = 4 arrays) or no central pitfall trap (n = 2). Between six and eight funnel traps were deployed at each drift fence. Drift fence arrays were actively monitored between July 6 and July 20, 2017 (Figure 38 and 39).



**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:26,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 36.** Drift fence, coverboard, and game camera placements in Kimble County.

**Figure 37.** Constructing a drift fence and pitfall trap array to sample *H. lacerata*.

**Figure 38.** Monitoring a drift fence and funnel trap array to sample *H. lacerata*.

**Figure 39.** Western diamondback rattlesnake (*Crotalus atrox*), potential predator of *H. lacerata*, captured in a funnel trap.

Drift fence monitoring efforts resulted in the capture of nine individuals from six species (Table 9). One juvenile *H. lacerata* was captured within a pitfall trap located at the drift fence installed at study site A. This study site proved to be an area locally abundant in *H. lacerata* and was subsequently utilized as a radio telemetry, harmonic radar, and capture-recapture site. Given the relatively small home range sizes and daily movements of *H. lacerata* (section 3.3) it is not surprising that capture success was low. While capture success may be a function of other species-specific characteristics (e.g., trap-shyness), decreases in lizard movement may lead to over-all decreases in lizard-fence interactions. Consequently, the implementation of drift fence arrays may have diminished efficacy for species with relatively smaller daily movements and home range sizes (Bury and Corn 1987; Corn and Bury 1990; Gibbons and Semlitsch 1982).

**Table 9.** Location and monitoring data and results from 2017 drift fence surveys in Kimble County (Unit 1).

| Drift Fence Location (Study Site) | Monitor Start | Monitor End | Monitoring Period (Days) | # Pitfalls | # Funnel Traps | Observations | Count |
|---|---|---|---|---|---|---|---|
| V | 7/10/2017 | 7/20/2017 | 10 | 1 | 8 | None | 0 |
| B | 7/10/2017 | 7/20/2017 | 10 | 1 | 8 | *Hypsiglena jani* | 1 |
| A | 7/10/2017 | 7/20/2017 | 10 | 1 | 8 | ***Holbrookia lacerata*** | **1** |
| W | 7/6/2017 | 7/20/2017 | 14 | 1 | 6 | *Plestiodon obsoletus; Rana berlandieri* | 1;3 |
| X | 7/10/2017 | 7/20/2017 | 10 | 1 | 6 | None | 0 |
| C | 7/12/2017 | 7/20/2017 | 8 | 0 | 6 | *Aspidoscelis gularis*; *Hypsiglena jani* | 1;1 |
| C | 7/12/2017 | 7/20/2017 | 8 | 0 | 6 | *Aspidoscelis gularis* | 1 |

### 4.1.2 Coverboards

Commonly used in the inventory of herpetofauna species, coverboards can be a successful method of studying more cryptic species (e.g., Harpole and Haas 1999, Pittman and Dorcas 2006, Wilgers and Horne 2006). Coverboards were installed at four study sites within Kimble (Study Unit 1) County, Texas on April 4, 2018 (Figure 36). These locations were established within areas of known *H. lacerata* presence or areas within species' range containing suitable habitat and proximal to known occupied areas. Coverboards measured 60 x 60 cm and were elevated with legs to create an approximately 4–6 cm gap between the board and ground (Figure 40). Across two field seasons (2018-2019), coverboards were checked during active searches as well as during visual encounter surveys on walking transects. No target species were observed utilizing coverboards. However, there were several instances in which non-target lizard species (e.g., Common spotted whiptail; *Aspidoscelis gularis*) were observed utilizing coverboards. Coverboards may have diminished efficacy with *H. lacerata* as burying behavior was the predominate means of avoiding high temperatures and predation, as observed during movement studies in these habitats.

### 4.1.3 Game Cameras

In order to supplement *H. lacerata* capture data and monitor the effectiveness of drift fence arrays and coverboards, game cameras were deployed in both the 2017 and 2018 seasons (Table 10). In 2017, six cameras were deployed along existing drift fence arrays in Kimble County (Unit 1) in areas known to be occupied by *H. lacerata*. These cameras were positioned approximately 1m above the ground using tripods, adjusted so that the angle of view was perpendicular to the ground, and set to record in 1-minute intervals. Between 6 July 2017 and 27 July 2017 cameras recorded a total of 46,076 photographs across 58 trap days. In 2018, two cameras were deployed at existing coverboard locations in Kimble County (Figure 36). These cameras were placed approximately 1 m off the ground, positioned so that the angle of view encompassed the coverboard, and set to record in 5-minute intervals. Between 5 March 2018 and 12 July 2018, cameras recorded a total of 29,183 photographs across 143 trap days. No *H. lacerata* were observed in the game camera photos. However, camera monitoring of coverboards did allow for the observation of non-target lizard species transiently utilizing these boards as cover (Figure 40).

**Table 10.** Summary of 2017 and 2018 game camera monitoring within Kimble County.

| Study Site | Deployment Dates | Recording period (Days) | Number of Photos Recorded | *H. lacerata* Observations |
|---|---|---|---|---|
| **B** | 7/6/2017-7/18/2017 | 13 | 10,724 | 0 |
| **B** | 7/6/2017-7/19/2017 | 14 | 10,438 | 0 |
| **A** | 7/8/2017-7/15/2017 | 8 | 5,765 | 0 |
| **A** | 7/19/2017-7/26/2017 | 8 | 6,515 | 0 |
| **W** | 7/19/2017-7/26/2017 | 8 | 6,515 | 0 |
| **D** | 7/20/2017-7/27/2017 | 7 | 6,119 | 0 |
| **W** | 4/5/2018-5/3/2018 | 30 | 8,725 | 0 |
| **D** | 4/26/2018-7/12/2018 | 113 | 20,458 | 0 |
|  | **TOTAL** | **201** | **75,259** | **0** |

**Figure 40.** Common spotted whiptail (*Aspidoscelis gularis*) utilizing a coverboard, as observed during game camera monitoring in Kimble County.

## 4.2 Visual Encounter Surveys

This task focused on repeated visual encounter surveys to evaluate a method of examining species distribution, demography and habitat associations at a broader and spatial scale. Single-season occupancy models were applied to these data and used to examine relationships between climate and habitat variables and *H. lacerata* and *H. subcaudalis* detection and occupancy.

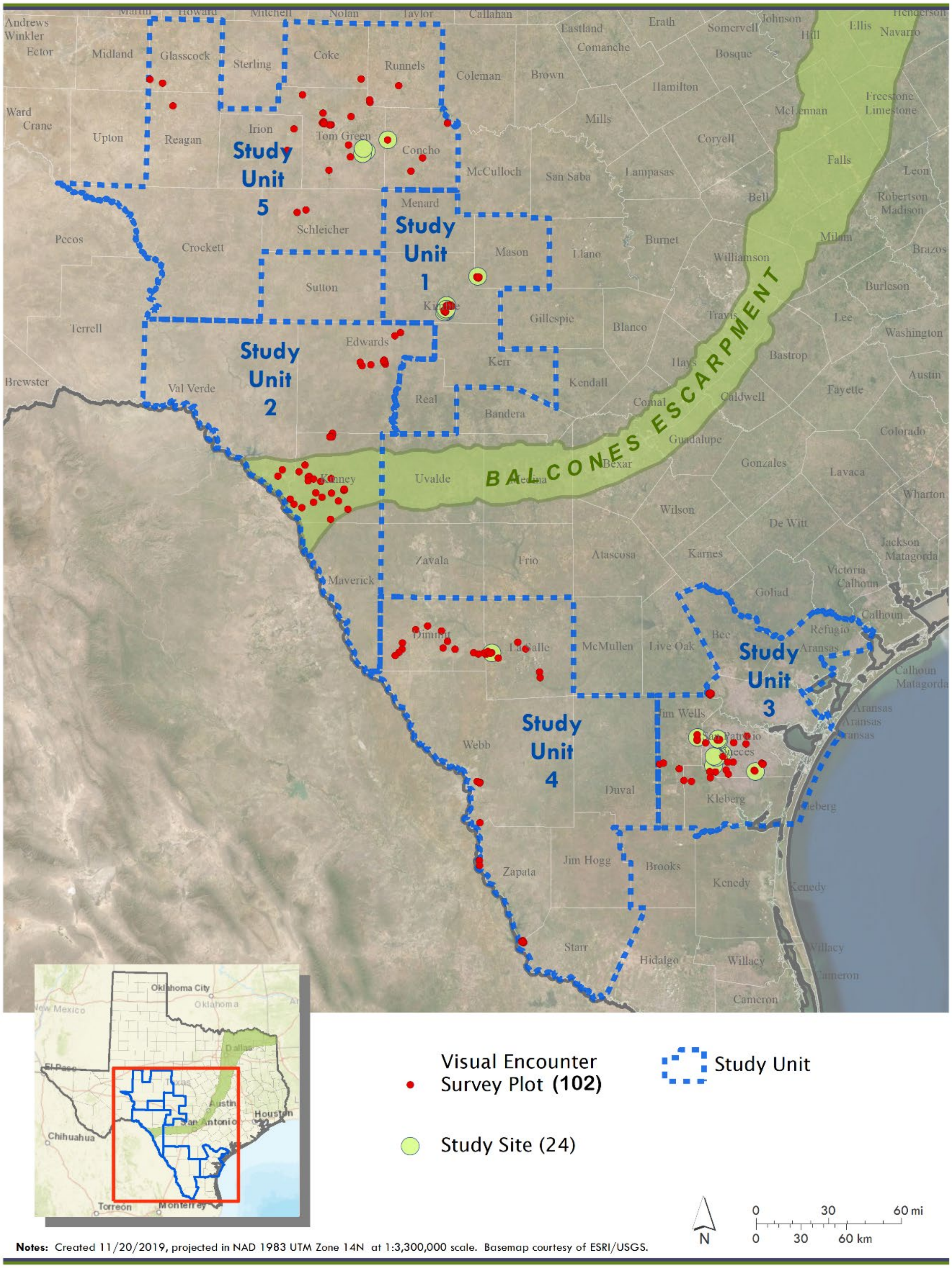
### 4.2.1 Methods

In order to establish areas to be surveyed repeatedly within seasons and across multiple seasons, formal sampling units (plots) were created. These plots consisted of 60 m x 500 m linear areas selected across the range of both species and all Study Units. Plots were further distributed based on various habitat and land-use types along roadsides, within both private and public lands, and in an effort to select areas in proximity to historical records for *H. lacerata* and *H. subcaudalis*. This led to the establishment of 102 plots prior to the onset of field efforts in 2018. Over the course of 2018 survey efforts, 13 plots were discontinued due to changes in property access, research priorities, and the onset of road construction. In 2019, an additional 11 plots were established (Figure 41). Visual encounter surveys (VES) were conducted within these plots 2–4 times within the activity season (2018) and repeated during the subsequent activity season (2019). Surveys were conducted by at least two biologists and involved methodically walking the extent of the plot actively searching for *H. lacerata* or *H. subcaudalis*. Climate data was recorded at the start and end of each survey, including air temperature (°), relative humidity (%), and barometric pressure (in Hg).

In conjunction with both this VES study and the tracking study (Section 3.0), vegetation surveys were conducted. In 2019, a total of 92 vegetation surveys were completed along plots in nine counties within all five Study Units. Across all field season, 13 vegetation surveys were completed within the home ranges of *H. lacerata* and *H. subcaudalis,* and within a 100 m buffer around the home ranges. Vegetation surveys within plots involved taking three quadrat samples at random locations within the plot area and resulted in 276 individual samples. Vegetation sampling within home ranges involved taking 5–10 quadrat samples within the home range and 10–20 samples within a 100 m buffer surrounding the home range, resulting in 135 individual samples.

A 2 m x 2 m quadrat was used to sample all sites. All vegetations within quadrats were identified to species, enumerated, and cover percentages recorded. An aerial photo was also taken of each quadrat's vegetation at approximately 3 m using a DSLR camera on a pole. Species abundance was collected by identifying each species present in the quadrat and counting the number of individuals for each species. Rhizomatous and stoloniferous plants were quantified as one individual per six inches of area surrounding an individual. Ground/plant cover percentages were categorized by plant habit types, such as forb/herb, graminoid, shrub, subshrub, tree, vine, detritus, or bare ground. Nonvascular and lichenous plants were not considered. A quadrat had the potential to contain only one plant habit type or all of plant habit types. The plant habit types were visually estimated to determine the cover percentage and all estimations were made by the same observer.

These results were compared using non-metric multi-dimensional scaling (NMDS), and no apparent differences in habitat composition within or outside of home ranges were observed, suggesting that non-random habitat selection was not occurring with respect to these habitat characteristics within the range of *H. lacerata*. Vegetation samples collected in the range of *H. subcaudalis* were homogenous across samples collected within and outside of lizard home ranges, due to this landscape being dominated primarily by row-crop monoculture. NMDS analysis of vegetation data collected within VES plots showed variation that was coincident with expected geographic variation in vegetation types across the large geographic clines sampled. Within the scale of Study Unit, analysis found no patterns related vegetation and occupancy by *H. lacerata* or *H. subcaudalis*. This result indicates that these species are most likely habitat generalists with respect to vegetation. Both species exhibit a broad range within Texas, spanning multiple eco-regions and further supporting the hypothesis that these lizards are habitat generalists.



Notes: Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:3,300,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 41.** Study extent with locations of visual encounter survey plots (2018–2019).

### 4.2.2 Results

In 2018, 254 visual encounter surveys were conducted on 102 plots within 21 counties (representing surveys within all five Study Units). Across all surveys there was a total of five *H. lacerata* and *H. subcaudalis* detections (Table 11). In 2019, surveys efforts were repeated within established sampling units and expanded to include additional areas. Focus of these efforts in 2019 was shifted to focus efforts more heavily on *H. subcaudalis,* as knowledge of the extant range of this species is more questionable than *H. lacerata*. Thus, many of the plots in the range of *H. lacerata* were removed and replaced with additional plots in the range of *H. subcaudalis*. The *H. lacerata* sites retained in 2019 were in areas with or proximal to previous captures and radio telemetry studies where individuals were marked in 2018 to provide the opportunity to refine detection estimates and collect recapture data for this species.  These efforts resulted in the collection of data from 401 visual encounter surveys conducted on 100 plots within 12 counties (representing surveys within all five Study Units), resulting in the detection of four individuals (Table 12). Across two field seasons, these efforts produced nine detections. within Study Units 1, 3, and 5. As a number of the VES plots intersected areas of other study activities, and *H. lacerata* and *H. subcaudalis* were repeatedly observed while biologists travelled among transects, for analysis purposes observations recorded within 500 m of the plots were considered evidence that the plot was in occupied habitat. This 500 m distance was determined based on our field observations of *H. lacerata* and *H. subcaudalis* movements over the course of the study.

**Table 11.** Summary of 2018 visual encounter surveys.

| **Study Unit** | **# Formal Sampling Units** | **# Survey Visits** | **# Detections within Plot** | **# Observations within 500 m of Plot[a]** | **Total # detections** |
|---|---|---|---|---|---|
| 1 | 17 | 4 | 1 | 4 | 5 |
| 2 | 24 | 3 | 0 | 0 | 0 |
| 3 | 23 | 4 | 4 | 6 | 10 |
| 4 | 19 | 3 | 0 | 0 | 0 |
| 5 | 19 | 3 | 0 | 0 | 0 |
| **TOTAL** | 102 | - | 5 | 10 | 15 |

a. Represents the number of *H. lacerata* and *H. subcaudalis* detected within a 500 m plot around the formal sampling units and outside of the visual encounter surveys, observed during field efforts related to other tasks.

**Table 12.** Summary of 2019 visual encounter surveys.

| Study Unit | # Formal Sampling Units | # Survey Visits | # Detections within Plot | # Observations within 500 m of Plot[a] | Total # detections |
|---|---|---|---|---|---|
| 1 | 14 | 5 | 1 | 4 | 5 |
| 2 | 28 | 5 | 0 | 0 | 0 |
| 3 | 25 | 5 | 1 | 4 | 5 |
| 4 | 24 | 3 | 0 | 0 | 0 |
| 5 | 9 | 3 | 2 | 3 | 5 |
| **TOTAL** | 100 | - | 4 | 11 | 15 |

a. Represents the number of *H. lacerata* and *H. subcaudalis* detected within a 500 m plot around the formal sampling units and outside of the visual encounter surveys, observed during field efforts related to other tasks.

### 4.2.3 Occupancy Modeling

As increases in land-use continue to fragment and isolate populations of imperiled species, accurate habitat assessments are becoming more important. As such, modeling of species' occupancy has become a critical conservation tool (e.g., De Wan et al. 2009; Raxworthy et al. 2003). Occupancy models can be useful in directing survey efforts (Guisan et al. 2006), providing accurate assessments of factors affecting detection or non-detection (Andelt et al. 2009), and identifying integral environmental influences for species persistence (Hamer and Mahony 2010).

Occupancy modeling is a statistical tool developed to estimate population parameters and investigate the influence of habitat variables on those parameters (Mackenzie et al. 2002). These models use repeat count data that incorporate detection probabilities and do not require identification or capture of individual animals (Mackenzie et al. 2002; Lee et al. 2011). Site occupancy (i.e., Ψ, proportion of sites occupied) was estimated using single-season occupancy models implemented in the program PRESENCE (Hines 2006) adjusted for detection probabilities (i.e., individuals may be present but go undetected; Mackenzie et al. 2006). Models were ranked according to Akaike Information criterion (Burnham 2003).

Original plans were to assess these relationships using both occupancy models and N-mixture modeling, however, sufficient *H. lacerata* and *H. subcaudalis* detections were not obtained during VES efforts for this type of analysis. Naïve occupancy, or the actual proportion of VES plots wherein lizards were detected, was 0.024 across all plots and study seasons (2018–2019). This extremely low number of detections also confounded the efficacy of study-wide assessments of detection and occupancy. Therefore, single-season occupancy models were restricted to data collected within Study Units 1 and 3. These Study Units had both higher lizard detections across surveys, and represent data collected within the ranges of both subspecies.

Because changes in counts between surveys and sites may be a product of changes in detectability and not habitat qualities, survey specific covariates with the potential to affect lizard detectability were evaluated (Mackenzie 2002). Factors which might affect lizard visibility and/or activity and therefore bias estimates of detection rates included variables associated with the weather (air temperature, substrate temperature, humidity, barometric pressure, and precipitation), and temporal variations (Julian date and time of survey). Models were created using one each of those factors potentially affecting lizard detectability (Table 13). In addition to models including survey specific covariates (n = 7 models), a predefined model with detection probability constant across surveys was used. Applying an information-theoretic approach (Burnham 2003), a multi-model selection and statistical inference was applied to establish which model(s) best explained the relationship between detection and survey specific covariates for *H. lacerata* and *H. subcaudalis*. This process was repeated independently for 2018 and 2019 VES efforts, and for Study Units 1 and 3 (n = 4 AIC selection tables).

**Table 13.** Variables used in models for determining factors affecting detection probabilities of *H. lacerata*.and *H. subcaudalis*

| **Detection Variable** | **Description** |
|---|---|
| Constant | Detection assumed constant; no detection variable applied |
| Date | Date of each survey, recorded as Julian day |
| Start time | Start time of each survey |
| Air temperature | Air temperature (°C) during each survey |
| Substrate temperature | Temperature of ground (°C) during each survey |
| Humidity | Relative humidity (%) during each survey |
| Precipitation – 1 month[a] | Cumulative rainfall (mm) preceding survey by 30 days |

a. Data aggregated from National Oceanic and Atmospheric Administration online database (NOAA 2019).

Among single-season models assessing the influence of detection probabilities on site occupancy for Study Unit 1 (*H. lacerata*) in 2018, there were three notable models (Table 14). In the first model, detection correlated negatively with the amount of precipitation preceding the survey by one month within the 4-survey period (β = -0.28, SE = 0.15). This model generated detection probability estimates ranging from 0.06 (SE = 0.08) and 0.45 (SE = 0.03) across all surveys with an occupancy estimate of 0.41 (SE = 0.19). The second model assumed detection constant across all surveys, however, relatively large standard error values across all estimations within this model suggest a poor model fit and therefore this model was not considered further. In the third model, detection probability correlated negatively with air temperature (β = -0.16, SE = 0.13). This model generated detection probability estimates ranging from 0.17 (SE = 0.18) to 0.37 (SE = 0.10) with an occupancy estimate of 0.42 (SE = 0.22).

**Table 14.** Model selection table for detection variables used within single-season occupancy models for 2018 Study Unit 1 (*H. lacerata*) visual encounter surveys. Includes relevant AIC,

ΔAIC, AIC weight (ωi), and model likelihood values. Naïve probability of occupancy across all sampling units= 0.2353 (n = 17 plots).

| Detection Model | AIC | ΔAIC | ωi | Model Likelihood | No. Parameters |
|---|---|---|---|---|---|
| Ψ(.),p(precipitation 1 month) | 34.55 | 0.00 | 0.3740 | 1.0000 | 2 |
| Ψ(.),p(.) | 34.88 | 0.33 | 0.3171 | 0.8479 | 2 |
| Ψ(.),p(air temperature) | 36.54 | 1.99 | 0.1383 | 0.3697 | 2 |
| Ψ(.),p(date of survey) | 38.20 | 3.65 | 0.0603 | 0.1612 | 2 |
| Ψ(.),p(time of survey) | 38.76 | 4.21 | 0.0456 | 0.1218 | 2 |
| Ψ(.),p(humidity) | 39.16 | 4.61 | 0.0373 | 0.0998 | 2 |
| Ψ(.),p(substrate temperature) | 39.78 | 5.23 | 0.0274 | 0.0732 | 2 |

Among single-season models assessing the influence of time specific covariates on site occupancy for Study Unit 1 (*H. lacerata*) in 2019, there were two competing models (Table 15). Detection was negatively correlated with precipitation 1 month prior to the survey (β = -0.42, SE = 0.28), generating detection probabilities ranging from 0.02 (SE = 0.04) to 0.38 (SE = 0.08) with an occupancy estimate of 0.30 (SE = 0.22). The other parsimonious model displayed a negative correlation between detection and humidity (β = -0.33, SE = 0.68), generating detection probabilities ranging from 0.04 (SE = 0.09) to 0.21 (SE = 0.17). However, relatively large standard error values for this model suggest a poor model fit and therefore this model was not considered further.

**Table 15.** Model selection table for detection variables used within single-season occupancy models for 2019 Study Unit 1 (*H. lacerata*) visual encounter surveys. Includes relevant AIC, ΔAIC, AIC weight (ωi), and model likelihood values. Naïve probability of occupancy across all sampling units= 0.1538 (n = 13 plots).

| Detection Model | AIC | ΔAIC | ωi | Model Likelihood | No. Parameters |
|---|---|---|---|---|---|
| Ψ(.),p(precipitation 1 month) | 21.55 | 0.00 | 0.3687 | 1.000 | 2 |
| Ψ(.),p(humidity) | 23.12 | 1.57 | 0.1682 | 0.4561 | 2 |
| Ψ(.),p(time of survey) | 24.37 | 2.82 | 0.0900 | 0.2441 | 2 |
| Ψ(.),p(air temperature) | 24.42 | 2.87 | 0.0878 | 0.2381 | 2 |
| Ψ(.),p(.) | 24.61 | 3.06 | 0.0798 | 0.2165 | 2 |
| Ψ(.),p(substrate temperature) | 25.04 | 3.49 | 0.0644 | 0.1746 | 2 |
| Ψ(.),p(date of survey) | 25.19 | 3.64 | 0.0597 | 0.1620 | 2 |

Among single-season models assessing the influence of detection probability on site occupancy for Study Unit 3 (*H. subcaudalis*) in 2018, all models produced an AIC<2 and are considered equally parsimonious (Table 16). There were two notable models which presented lower standard errors and produced a good model fit. Within the first model, detection correlated negatively with time of survey (β = -0.17, SE = 0.16), generating detection probabilities ranging from 0.20 (SE = 0.21) to 0.37 (SE = 0.12) with an occupancy estimate of 0.30 (SE = 0.18). Within the second model, detection correlated positively with precipitation one month prior to the survey (β = 0.08, SE = 0.09), producing detection probabilities ranging from 0.50 (SE = 0.00) to 0.70 (SE = 0.20) with an occupancy estimate of 0.19 (SE = 0.09).

**Table 16.** Model selection table for detection variables used within single-season occupancy models for 2018 Study Unit 3 (*H. subcaudalis*) visual encounter surveys. Includes relevant AIC, ΔAIC, AIC weight (ωi), and model likelihood values. Naïve probability of occupancy across all sampling units= 0.1739 (n = 23 plots).

| Detection Model | AIC | ΔAIC | ωi | Model Likelihood | No. Parameters |
|---|---|---|---|---|---|
| Ψ(.),p(time of survey) | 34.98 | 0.00 | 0.1844 | 1.0000 | 2 |
| Ψ(.),p(precipitation 1 month) | 35.39 | 0.41 | 0.1502 | 0.8146 | 2 |
| Ψ(.),p(date of survey) | 35.83 | 0.85 | 0.1205 | 0.6538 | 2 |
| Ψ(.),p(substrate temperature) | 35.92 | 0.94 | 0.1152 | 0.6250 | 2 |
| Ψ(.),p(air temperature) | 35.99 | 1.01 | 0.1113 | 0.6035 | 2 |
| Ψ(.),p(.) | 36.00 | 1.02 | 0.1107 | 0.6005 | 2 |
| Ψ(.),p(humidity) | 36.23 | 1.25 | 0.0987 | 0.5353 | 2 |

Among single-season models assessing the influence of detection probabilities on site occupancy for Study Unit 3 (*H. subcaudalis*) in 2019, there were four parsimonious models (Table 17). Within the first model, detection correlated negatively with air temperature (β = 0.24, SE = 0.14), generating detection probabilities ranging from 0.08 (SE = 0.11) to 0.18 (SE = 0.13) with an occupancy estimate of 0.37 (SE = 0.39). Within the second model, detection correlated negatively with time of survey (β = -0.22, SE = 0.13), producing detection probabilities ranging from 0.15 (SE = 0.13) to 0.33 (SE = 0.09) with an occupancy estimate of 0.22 (SE = 0.15). Within the third model, detection correlated negatively with precipitation 1 month prior to the survey (β = -0.54, SE = 0.27), producing detection probabilities ranging from 0.01 (SE = 0.01) to 0.47 (SE = 0.02) with an occupancy estimate of 0.41 (SE = 0.27). The fourth model assumed detection constant across all surveys, however, large standard error values across all estimations within this model suggest a bad model fit and therefore this model was not considered further.

**Table 17.** Model selection table for detection variables used within single-season occupancy models for 2019 Study Unit 3 (*H. subcaudalis*) visual encounter surveys. Includes relevant AIC,

ΔAIC, AIC weight (ωi), and model likelihood values. Naïve probability of occupancy across all sampling units= 0.1200 (n = 25 plots).

| Detection Model | AIC | ΔAIC | ωi | Model Likelihood | No. Parameters |
|---|---|---|---|---|---|
| Ψ(.),p(air temperature) | 33.62 | 0.00 | 0.2768 | 1.000 | 2 |
| Ψ(.),p(time of survey) | 34.28 | 0.66 | 0.1990 | 0.7189 | 2 |
| Ψ(.),p(precipitation 1 month) | 34.41 | 0.79 | 0.1865 | 0.6737 | 2 |
| Ψ(.),p(.) | 34.41 | 0.79 | 0.1865 | 0.6737 | 2 |
| Ψ(.),p(date of survey) | 35.74 | 2.12 | 0.0959 | 0.3465 | 2 |
| Ψ(.),p(humidity) | 36.84 | 3.22 | 0.0553 | 0.1999 | 2 |

Across both years, *H. lacerata* exhibited detection rates which decreased relative to the amount of precipitation preceding the survey by one month. This relationship was not as consistent with *H. subcaudalis*, wherein precipitation correlated positively with detection in 2018 but negatively in 2019. This is likely a result of sampling error, wherein very different values of precipitation were observed prior to surveys in different years. In a previous study, it was found that the most important variable describing populations of *H. subcaudalis* south of the Balcones Escarpment was precipitation seasonality (Hibbitts et al. 2019).

Both species exhibited negative correlations between air temperature and detection. Relationships in 2018 and 2019 between detection of *H. lacerata* and *H. subcaudalis* and air temperature are corroborated by observations made during radio telemetry tracking. In these study areas during the summer months, lizards decrease activity and take refuge (thermoregulate) as heat rises during the day and are active (available for detection) increasingly less as the day goes on. The negative correlation between detection and time of survey observed within *H. subcaudalis* also reflects effects of increasing temperatures on the availability of lizards for observation.

Within sampled *H. lacerata* populations, occupancy estimates within the best models (precipitation 1 month) decreased by 27.8% between 2018 (**Ψ** = 0.41, SE = 0.19) and 2019 (**Ψ** = 0.30, SE = 0.22). Variation in occupancy estimates across the same models (precipitation 1 month) between years was also observed within sampled *H. subcaudalis* populations, with an increase of 73.3% between 2018 (**Ψ** = 0.19, SE = 0.09) and 2019 (**Ψ** = 0.41, SE = 0.27). Comparison of estimates from single season models can be misleading (note the standard error of the estimates), however, this variation is most likely caused by the low number of detections observed during the study (n = 9) as well as variations between years in the number of detections reducing the accuracy of estimations. In order to better interpret the variation across years and improve estimates of both detection and occupancy, more data should be collected within previously sampled areas and used in multiple-season models. Multi-season occupancy models are better able to parse variation within versus among sampling seasons (years) and provide a more rigorous interpretation of changes in occupancy. This analysis provides insight into variables effecting detection and demonstrates that these species can easily go undetected even when significant field efforts are made. Additionally, these results serve to inform future research as capture and survey efforts can be guided using these detection data. Given the low number of detections across a large geographic scale, and the evidence that these species are habitat generalists, persisting within a wide variety of vegetation and arthropod communities (Sections 4.2 and 6.0), identification of habitat factors affecting occupancy is critical to assessing the future of these species.

## 5.0 Capture-Recapture

Capture-recapture studies can provide accurate population estimates (i.e., abundance) in addition to other demographic estimates such as survival and longevity, provided that assumptions are met, and sampling design is robust. These data, in turn, allow for the development of population models that can be used to predict population responses to threats and alternative management scenarios (Lettink and Armstrong 2003). Capture-recapture studies can also be helpful in providing insight into the life history of a species, including growth data, and estimates such as clutch weight (i.e., observations of pre- and post-parturition). Conventional capture-recapture methods rely on uniquely marked animals, wherein individuals are uniquely identified during two or more survey events resulting in a set of encounter histories, each representing a temporal sequence of detections and non-detections (Williams et al. 2002; Royle and Young 2008).

### 5.1 Methods

In order to maximize capture numbers of both *H. lacerata* and *H. subcaudalis* to achieve the research tasks, the Project Team performed extensive surveys throughout the two species historical ranges. These surveys predominately involved active searches (e.g., visual encounter surveys or road surveys) and produced a total of 121 lizard captures within three Study Units and five Counties during the 3-year study (Table 18). For the purposes of setting up data for capture-recapture analysis, lizard captures were organized into respective study sites. Study sites affiliated with capture-recapture efforts, were defined as areas of lizard presence or activity as observed throughout the 2017, 2018 and 2019 study, and where lizards were captured. The assumption is that any study site included produced at least one capture of either *H. lacerata* or *H. subcaudalis* and was subject to repeated surveys (or capture events).

Once a lizard was captured (Figures 42 and 43), morphometric measurements were recorded, including snout-vent length (SVL; mm), tail length (TL; mm), and weight (g). Capture location (UTM) were recorded and dorsal, ventral, and lateral photographs were taken in order to assist lizard identification in the event of recapture. Climate data was recorded using a Kestrel™ 3500 weather meter and included air temperature (°C), relative humidity (%), and barometric pressure (inHg). Additionally, substrate temperature (°C) was recorded using RYOBI infrared thermometer. In order to create encounter histories for the capture-recapture study, lizards were marked using the traditional toe-clipping method which provides a unique and permanent identifying numerical marking scheme (Perry et al. 2011). All toe-clips were collected and submitted to the University of Texas Biodiversity Collections.

**Figure 42.** Capturing *H. lacerata* with a pole-noose in Kimble County.

**Figure 43.** Two captured *Holbrookia subcaudalis* (male and female), Jim Wells County.

## 5.2 Analysis

Where capture-recapture data was available (i.e., *H. lacerata* populations within Kimble County), demographic parameters were estimated. For this analysis, Kimble County study sites A-E were utilized with sites A-D combined due to geographic proximity. For both groups (study sites A-D and E) all sampling years were utilized to generate a survival (Cormack Jolly Seber) and population (robust design model) estimate. All parameters were estimated using the program MARK (White 2017).

Apparent annual survival ($\Phi$) estimates the probability of an individual being alive and available for capture from one year to the next (Cooch and White 2013). Models for annual apparent survival and recapture ($\rho$, the probability of being recaptured from one year to the next year) were used in the Cormack Jolly Seber (CJS) survival estimator, so that the parameters ($\Phi$ and $\rho$) were held either constant (.) or variable through time ($t$), producing a combination of four model iterations. The four models were compared according to AICc values (to adjust for small sample size), where the best-fitting models have the lowest AICc scores. The saturated model ($\Phi\ [t]p[t]$) was then tested for goodness-of-fit by estimating the over-dispersion parameter using median c-hat ($\hat{c}$) within MARK (Cooch and White 2013), where ≤ 3.0 indicates a good model fit. If the top-rated model was determined to be confounded, the "poor-estimated" parameters were dropped from the model, and model averaging occurred as Cooch and White (2013) suggest. For population estimates, three robust-design, full-likelihood ($p$ and $c$) models were created. These models included: recapture ($p$) and capture ($c$) probabilities set equal to each other, time varied, and time constant.

## 5.3 Results

### 5.3.1 Capture Summary

Capture efforts across all field activities and field seasons produced a total of 67 new *H. lacerata* captures (not including recaptures) within Study Unit 1 (Kimble and Concho counties) and Study Unit 5 (Tom Green County). Capture efforts were most successful in Kimble County with 58 individuals, accounting for 87% of *H. lacerata,* undoubtedly due to large areas of private land access containing suitable habitat. Capture efforts for *Holbrookia subcaudalis* were only successful in Study Unit 3 (Jim Wells and Nueces counties), accounting for all 54 new captures (Table 18).

**Table 18.** Summary of all new *H. lacerata* and *H. subcaudalis* captures (n = 121) during all field activities and all field seasons (2017-2019).

| Unit | County | Study Site | Land Use | Species | # New Captures |
|---|---|---|---|---|---|
| 1 | Kimble | A | rangeland | *H. lacerata* | 27 |
| 1 | Kimble | B | rangeland | *H. lacerata* | 5 |
| 1 | Kimble | C | rangeland | *H. lacerata* | 17 |
| 1 | Kimble | D | rangeland | *H. lacerata* | 4 |
| 1 | Kimble | E | rangeland | *H. lacerata* | 4 |
| 3 | Jim Wells | F | farming | *H. subcaudalis* | 30 |
| 5 | Tom Green | G | farming | *H. lacerata* | 3 |
| 5 | Tom Green | H | farming | *H. lacerata* | 5 |
| 5 | Tom Green | I | farming | *H. lacerata* | 1 |
| 3 | Nueces | J | farming | *H. subcaudalis* | 18 |
| 3 | Nueces | K | farming | *H. subcaudalis* | 2 |
| 3 | Nueces | L | farming | *H. subcaudalis* | 1 |
| 3 | Nueces | O | farming | *H. subcaudalis* | 1 |
| 3 | Nueces | P | farming | *H. subcaudalis* | 1 |
| 3 | Nueces | R | farming | *H. subcaudalis* | 1 |
| 5 | Concho | U | farming | *H. lacerata* | 1 |

Observed sex ratios between the two species differed with the northern species exhibiting a higher proportion of females to males within the capture data (Figure 44).

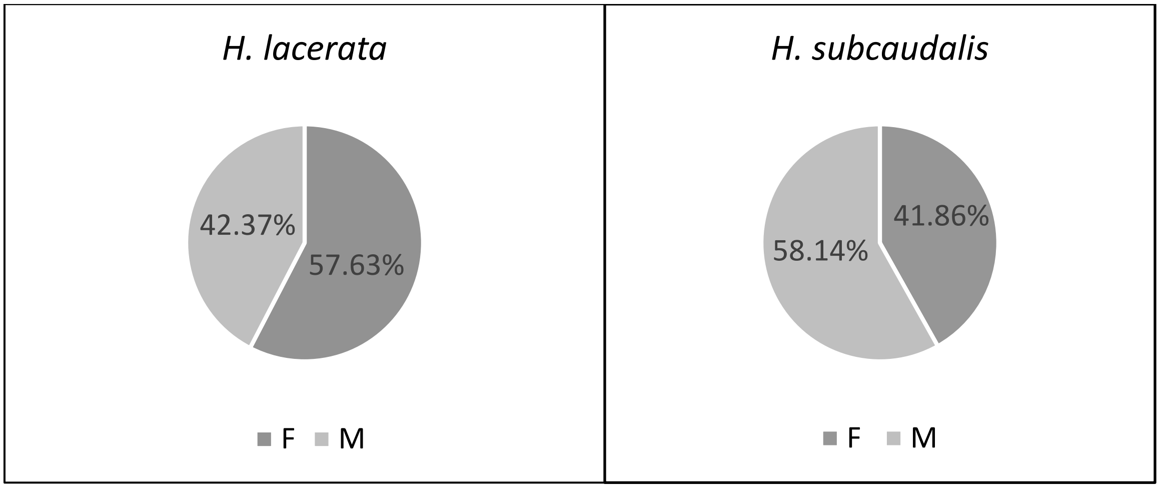


**Figure 44**. Sex ratios (female:male) observed during study for *H. lacerata* (1.36:1) and *H. subcaudalis* (0.72:1).

The following frequency histograms represent a summary of time dependent variables as they relate to captures of *H. lacerata* and *H. subcaudalis* across all field activities. In contrast to *H. lacerata,* wherein captures generally occurred within the late morning to early afternoon, captures of *H. subcaudalis* showed a more bimodal distribution with regards to time, occurring more frequently within the mid-morning and early evening (Figures 45 and 46).

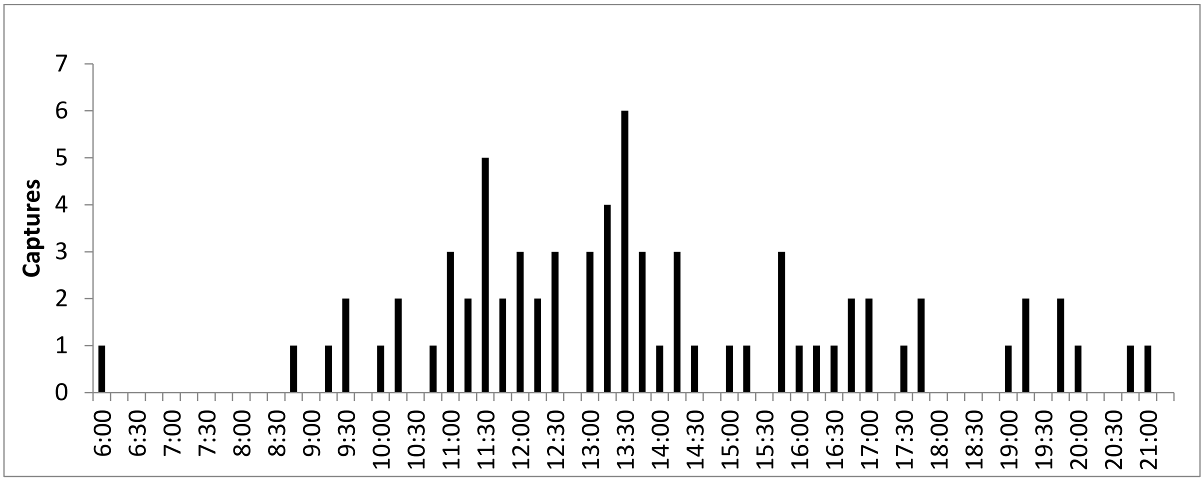


**Figure 45.** Frequency histogram showing captures of *H. lacerata* and time of day (24hr).

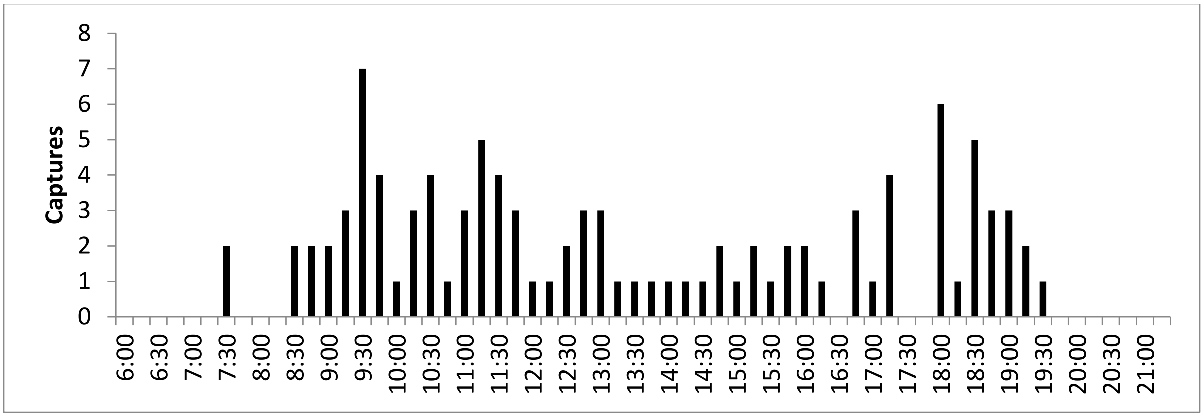


**Figure 46.** Frequency histogram showing captures of *H. subcaudalis* and time of day (24hr).

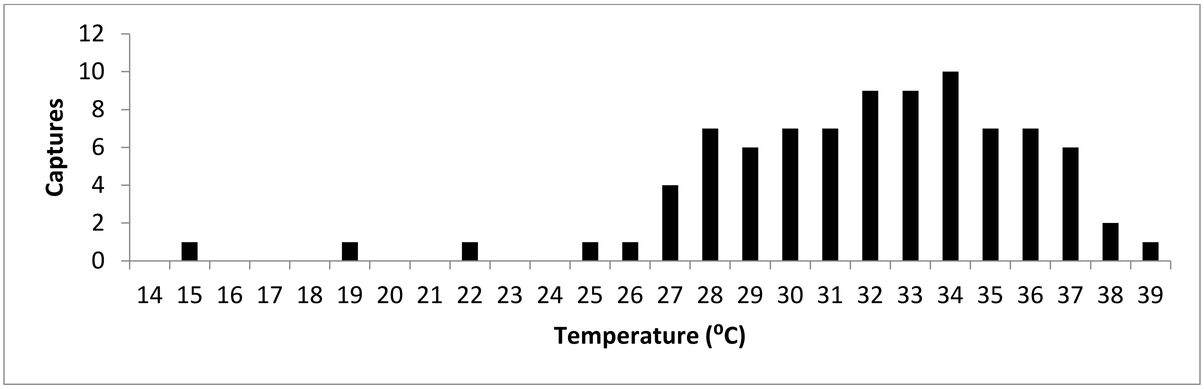


**Figure 47.** Frequency histogram showing captures of *H. lacerata* and air temperature (°C).

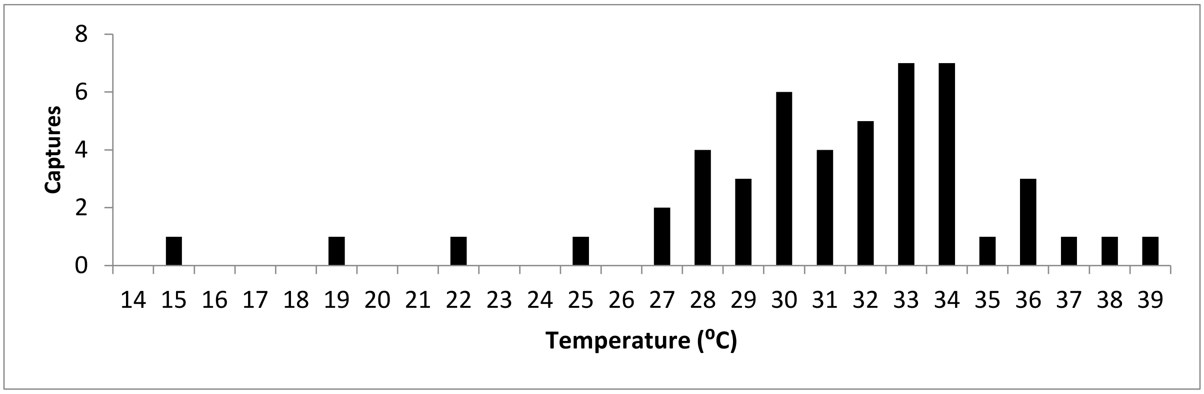


**Figure 48.** Frequency histogram showing captures of *H. subcaudalis* and air temperature (°C).

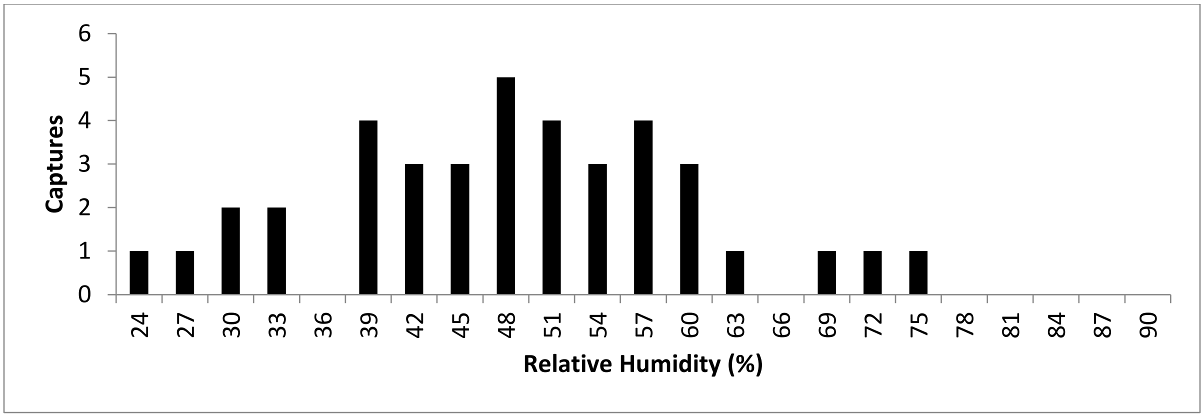


**Figure 49.** Frequency histogram showing captures of *H. lacerata* and relative humidity (%).

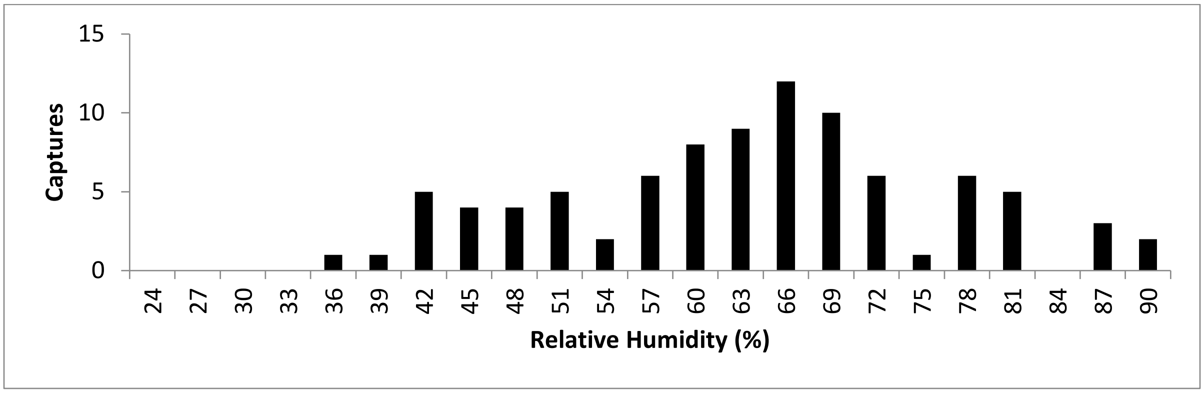


**Figure 50.** Frequency histogram showing captures of *H. subcaudalis* and relative humidity (%).

### 5.3.2 Capture-Recapture Summary

In 2017, capture-recapture sites were established within the range of *H. lacerata* at four study sites in Kimble County (study sites A-D; Figures 51-52). Across all four sites, 43 captures of *H. lacerata* occurred during this study in 2017. (Table 19). Of these, 13 represented within-season recaptures (30.2% of total captures). These data allowed for the establishment of encounter histories for 11 individuals (nine individuals recaptured once, and two individuals recaptured twice).

**Table 19.** Summary of 2017 capture-recapture efforts.

| Unit | County | Study Site | Species | # Total Captures | # New Captures | # Recaptures |
|---|---|---|---|---|---|---|
| **1** | Kimble | A | *H. lacerata* | 31 | 21 | 10 |
| **1** | Kimble | B | *H. lacerata* | 4 | 3 | 1 |
| **1** | Kimble | C | *H. lacerata* | 2 | 2 | 0 |
| **1** | Kimble | D | *H. lacerata* | 6 | 4 | 2 |
| | TOTAL | | | 43 | 30 | 13 |

In 2018, capture-recapture efforts for *H. lacerata* were repeated within Kimble County (study sites A-D with the addition of study site E) as well as expanded into Tom Green County (study sites G-I), resulting in the establishment of four new study sites (Figure 53). Additionally, one capture-recapture site was identified within the range of *H. subcaudalis* (Jim Wells County; study site F; Figure 54). Across all Kimble County study sites, there were a total of 32 captures (Table 20). Of these, nine represented within-season recaptures (28.1% of total captures), and two represented inter-season recaptures of one individual (6.3% of total captures). New encounter histories were generated for seven individuals (with six individuals recaptures once and one individual recaptured twice). Across all Tom Green County study sites, there were a total of nine captures with one within-season recapture (10.1% of total captures). Capture-recapture efforts within the range of *H. subcaudalis* were conducted at one study site and produced a total of 13 captures with no recaptures.

**Table 20.** Summary of 2018 capture-recapture efforts.

| Unit | County | Study Site | Species | # Total Captures | # New Captures | # Recaptures |
|---|---|---|---|---|---|---|
| **1** | Kimble | A | *H. lacerata* | 3 | 2 | 1 |
| **1** | Kimble | B | *H. lacerata* | 4 | 2 | 2 |
| **1** | Kimble | C | *H. lacerata* | 17 | 12 | 5 |
| **1** | Kimble | D | *H. lacerata* | 1 | 0 | 1 |
| **1** | Kimble | E | *H. lacerata* | 7 | 5 | 2 |
| **3** | Jim Wells | F | *H. subcaudalis* | 13 | 13 | 0 |
| **5** | Tom Green | G | *H. lacerata* | 3 | 3 | 0 |
| **5** | Tom Green | H | *H. lacerata* | 5 | 4 | 1 |
| **5** | Tom Green | I | *H. lacerata* | 1 | 1 | 0 |
| | TOTAL | | | 54 | 42 | 12 |

In 2019, capture-recapture efforts for *H. lacerata* resumed at three study sites within Kimble County (study sites A, C, and E; Figure 51-52) with the addition on of one study site in Concho County (study site U; Figure 53; Table 21). Within the range of *H. subcaudalis,* efforts resumed within Jim Wells County (study site F) as well as expanded to six areas within Nueces County (study sites J-L, P, and R; Figure 54). Within Kimble County, 11 total captures occurred with three within-season captures and one inter-season capture (36.4% of total captures). Within Jim Wells County, efforts produced 17 total captures and no recaptures. Within Nueces County, efforts produced 24 captures and no recaptures.

**Table 21.** Summary of 2019 capture-recapture efforts.

| Unit | County | Study Site | Species | # Total Captures | # New Captures | # Recaptures |
|---|---|---|---|---|---|---|
| **1** | Kimble | A | *H. lacerata* | 6 | 4 | 2 |
| **1** | Kimble | C | *H. lacerata* | 4 | 3 | 1 |
| **1** | Kimble | E | *H. lacerata* | 1 | 0 | 1 |
| **3** | Jim Wells | F | *H. subcaudalis* | 17 | 17 | 0 |
| **3** | Nueces | J | *H. subcaudalis* | 18 | 18 | 0 |
| **3** | Nueces | K | *H. subcaudalis* | 2 | 2 | 0 |
| **3** | Nueces | L | *H. subcaudalis* | 1 | 1 | 0 |
| **3** | Nueces | O | *H. subcaudalis* | 1 | 1 | 0 |
| **3** | Nueces | P | *H. subcaudalis* | 1 | 1 | 0 |
| **3** | Nueces | R | *H. subcaudalis* | 1 | 1 | 0 |
| **5** | Concho | U | *H. lacerata* | 1 | 1 | 0 |
|  | TOTAL |  |  | 53 | 49 | 4 |

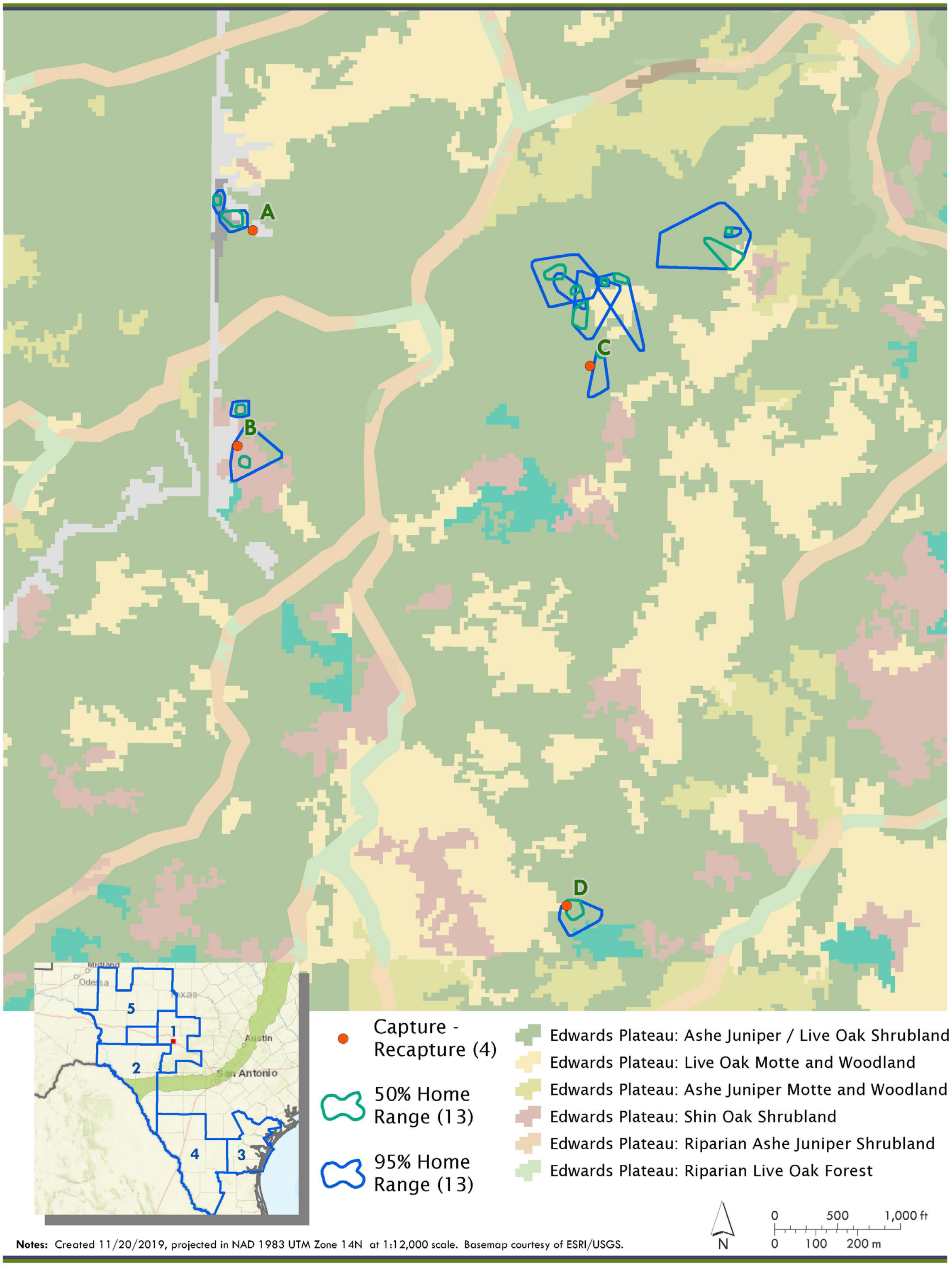


**Figure 51.** Kimble County capture-recapture study sites (n = 5).

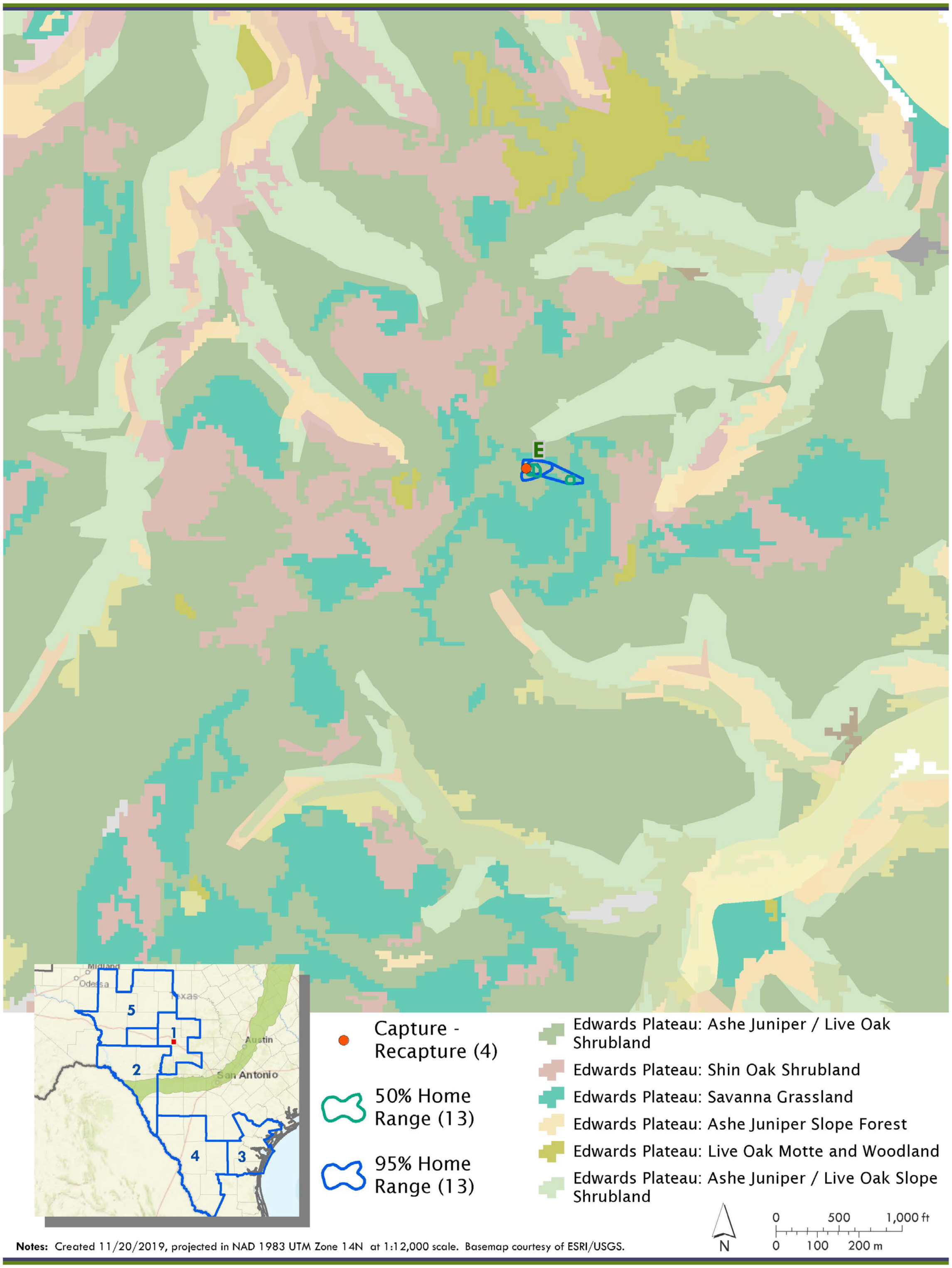
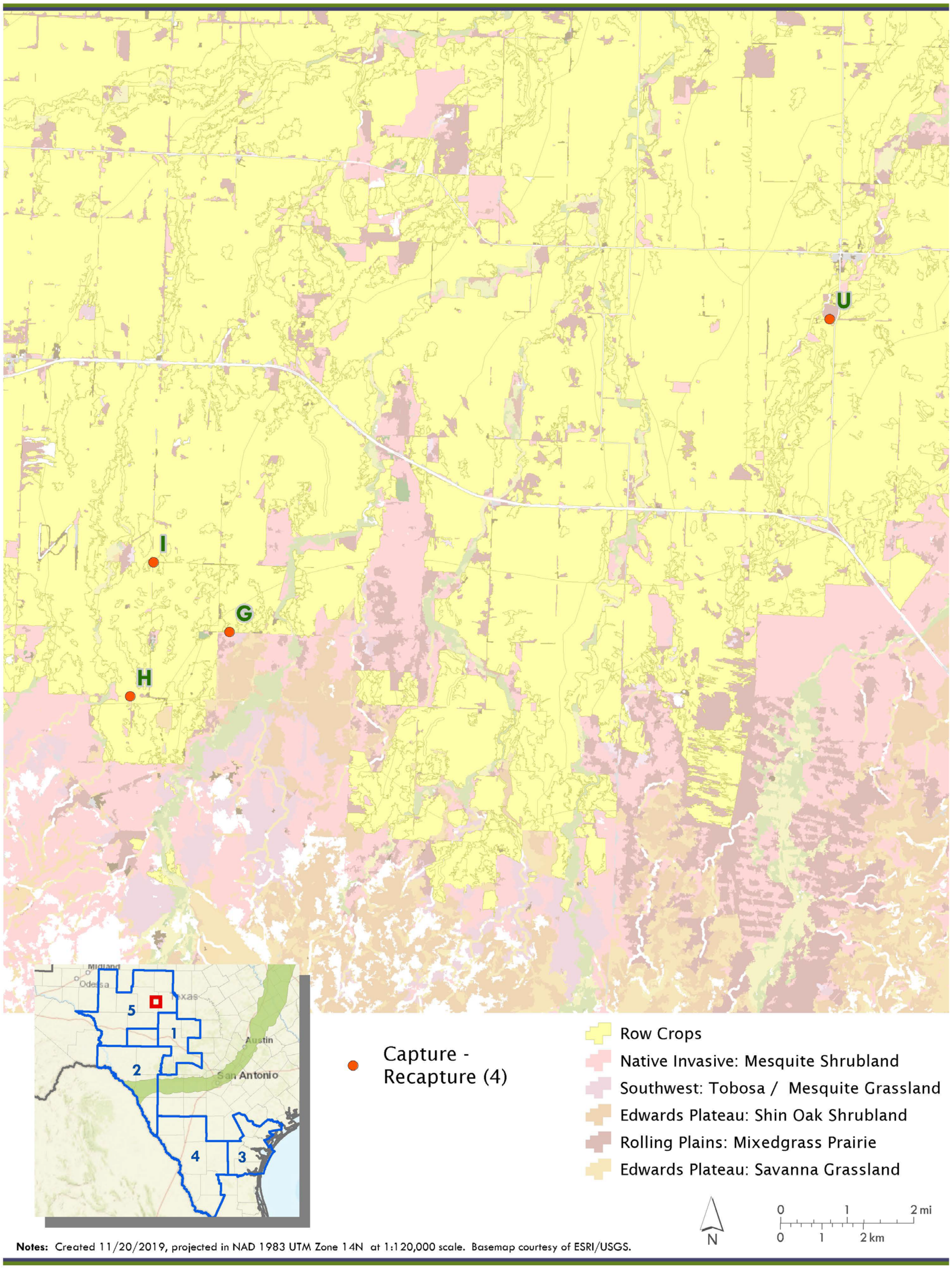
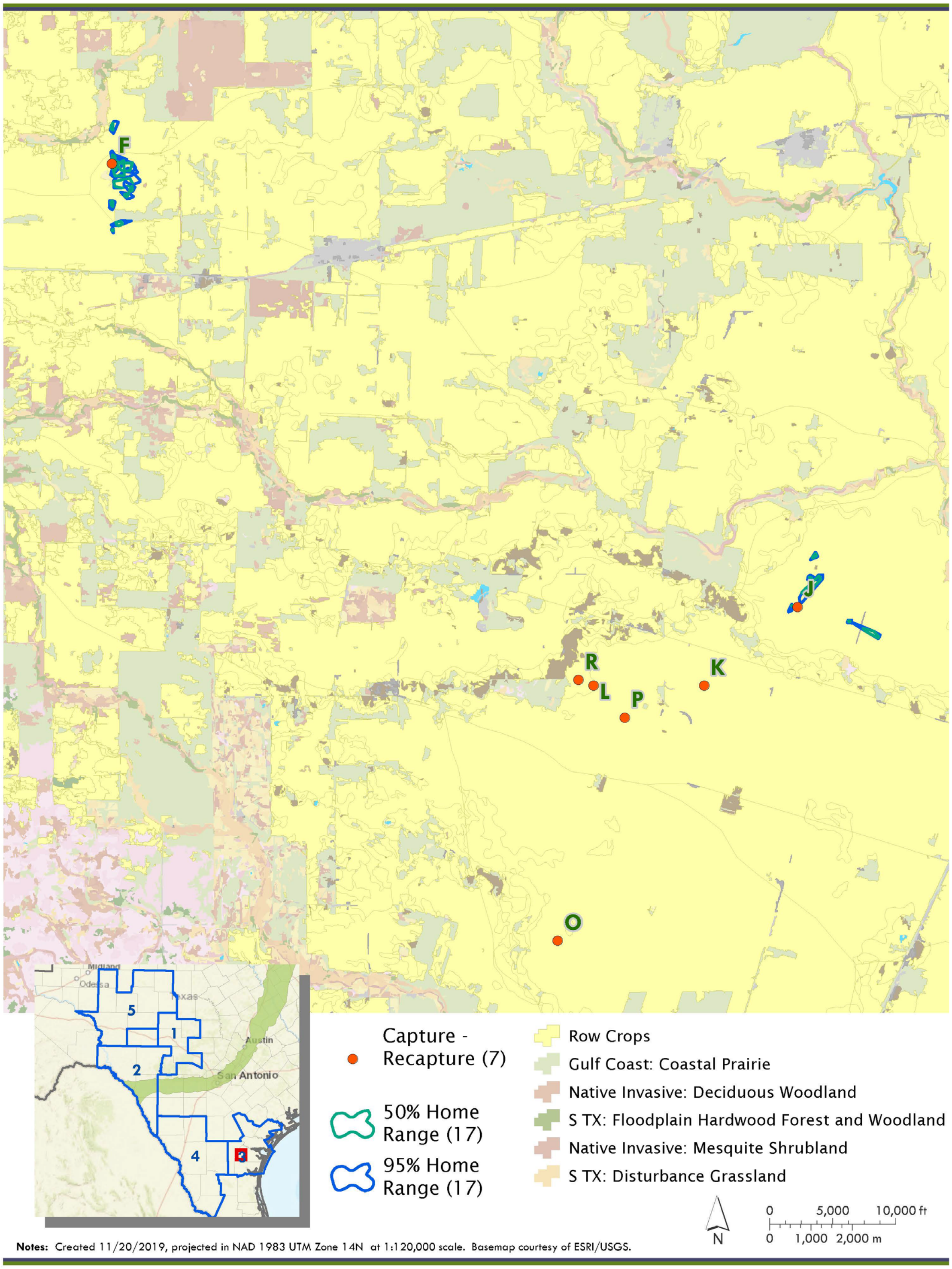


**Figure 52.** Kimble County capture-recapture study sites (n = 5).



**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:120,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 53.** Capture-recapture study sites in Concho and Tom Green counties (n = 4).



**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:120,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 54.** Capture-recapture study sites in Jim Wells and Nueces counties (n = 7).

### 5.3.4 Growth Measurements

Recapture numbers were very low within all study sites except for within those associated with Kimble County. Capture efforts at study sites A through E resulted in a total 28 recaptures across 3 field seasons (2017-2019). These recaptures allowed for the calculation of *H. lacerata* growth rates. Interestingly, one adult female *H. lacerata* was captured both before and after parturition (within a 7-day span). This individual was observed as gravid on initial capture and having deposited eggs during the subsequent capture, showing a reduction in body weight from 9.1 to 5.3 g (41.8% decrease) and indicating that clutch weight was approximately 3.8 g. This individual was excluded from the assessment of growth rates (Table 22).

**Table 22.** Growth measurements estimated from capture-recapture data collected from *H. lacerata* within Kimble County (mean days between captures = 64.7).

| Attribute | # Individuals | Mean Growth Per Week ± SE |
|---|---|---|
| Body Weight | 19 | -0.14 g ± 0.21 |
| Snout to Vent Length | 17 | 0.49 mm ± 0.42 |
| Tail Length | 17 | 0.14 mm ± 0.63 |

### 5.3.5 Analysis

For *H. lacerata* across all years (2017–2019) within combined study sites A-D apparent annual survival estimates ranged from 55.4 to 70.5%, with a small and consistent increase from year one to year three. Study site E produced similar results with an apparent annual survival estimate of 54.8% (Table 23).

**Table 23.** Model averaging results for *H. lacerata* apparent annual survival (ϕ) from 2017 to 2019 in Kimble County (study sites A-D).

| Year | Study Site/s | Survival Estimate (ϕ) | Standard Error | Lower and Upper 95% Confidence Intervals |
|---|---|---|---|---|
| 2017 - 2018 | A-D | 0.5536 | 0.1831 | 0.1936 – 0.8650 |
| 2018 - 2019 | A-D | 0.6797 | 0.1529 | 0.3488 – 0.8937 |
| 2019 - 2020 | A-D | 0.7054 | 0.0201 | 0.6629 – 0.7446 |
| 2018 - 2019 | E | 0.5479 | 0.1830 | 0.1947 – 0.8587 |

A total of 18 capture events during three primary occasions (2017–2019) were used to estimate population sizes at study sites A-D, and 12 capture events during two primary occasions (2018–2019) were used to estimate study site E in a robust design population model (Table 24). Population estimates for study sites A-D appear to show a decrease across years, however, this is most likely a result of changes in effort through time as radio telemetry efforts were shifted increasingly to Study Unit 3 to assess populations of *H. subcaudalis*. Capture-recapture data was insufficient for the reliable estimation of population size within study site E. Research was not conducted at study site E until 2018.

**Table 24.** *Holbrookia lacerata* robust design population model results for populations sampled in Kimble County (2017–2019).

| Year | Study Site/s | Population Estimate | Standard Error | Lower and Upper 95% Confidence Intervals |
|---|---|---|---|---|
| 2017 - 2018 | A-D | 33.65 | 1.50 | 33.05 – 42.32 |
| 2018 - 2019 | A-D | 19.00 | 2.08 | 18.08 – 30.62 |
| 2019 - 2020 | A-D | 10.50 | 9.54 | 7.20 – 68.24 |

### 5.3.6 Discussion

Attempts to recapture marked individuals were more successful for *H. lacerata,* with recaptures constituting 32.6% of total captures across all Kimble County sites and field seasons. This was also the only location were data on inter-season recaptures was collected. Obtaining recaptures within populations of *H. subcaudalis* proved to be more difficult. While capture rates were relatively high (n = 54 captures across seven study sites), no recaptures were obtained. This species has observed home range sizes significantly larger than *H. lacerata* (Section 3.3), reducing the probability of re-encountering an individual. Furthermore, study populations of *H. subcaudalis* were not identified until 2018. Continued capture events within established sites over a longer time-period is needed to increase the frequency of recaptures in these data and refine estimates of population parameters for both *H. lacerata* and *H. subcaudalis*.

Within Kimble County study sites, apparent annual survival estimates fall within the bounds of survivorship as estimated in studies of other lizards in the family Phrynosomatidae, wherein estimates range from as low as 13% to as high as 90% for adults (e.g., Ortega-Leon et al. 2007; Tinkle et al. 1986). Population estimates (abundance) appear to be relatively consistent with *H. lacerata* numbers as observed in the field during studies, especially in regards to year 2017-2018 in study sites A-D.

Recapture efforts and the subsequent development of encounter histories were the result of field work conducted in conjunction with other study activities (e.g., VES, tracking) rather than formal recapture sampling. Moreover, 2018 and 2019 research priorities were shifted to place more focus on *H. subcaudalis,* reducing radio telemetry and other study efforts within Kimble County during these years and this change in effort is likely the cause for the observed decrease in population estimates at Kimble county sites. Given these considerations, however, capture-recapture numbers were sufficient for the establishment of baseline data for continued capture-recapture studies and the estimation of some demographic parameters of this population. Further study and standardization of future work effort will most likely produce more consistent abundance estimates across years.

## 6.0 Arthropod Community Pilot Study

The goal of this pilot study was to evaluate the utility of insect community signatures to predict occupancy and abundance for *H. lacerata* and *H. subcaudalis*. This involved employing an alternative method to traditional "habitat" delimitation to identify ecological differences that correlate with occupancy and abundance. This task also sought to assess, on a fine scale, effects of disturbances or land use on ecosystem health that may not be easily or directly observable in these lizard species.

Vertebrate habitat and ecosystems or regions are most commonly described by abiotic or vegetative criteria, however these descriptions do not always possess enough resolution to explain differences observed in the distribution and abundance of these taxa on a landscape scale. Often overlooked in terrestrial vertebrate studies, except as a food source, terrestrial invertebrate communities are extraordinarily diverse, speciose, and exhibit a very broad range of niche allocation and specialization. The short generation time of invertebrate taxa also means that they are likely to show a response to ecosystem changes more rapidly than vertebrates or vegetation. The use of invertebrate data to describe vertebrate habitat and ecosystems provides much higher definition than traditional methods (Olson et al. 2001).

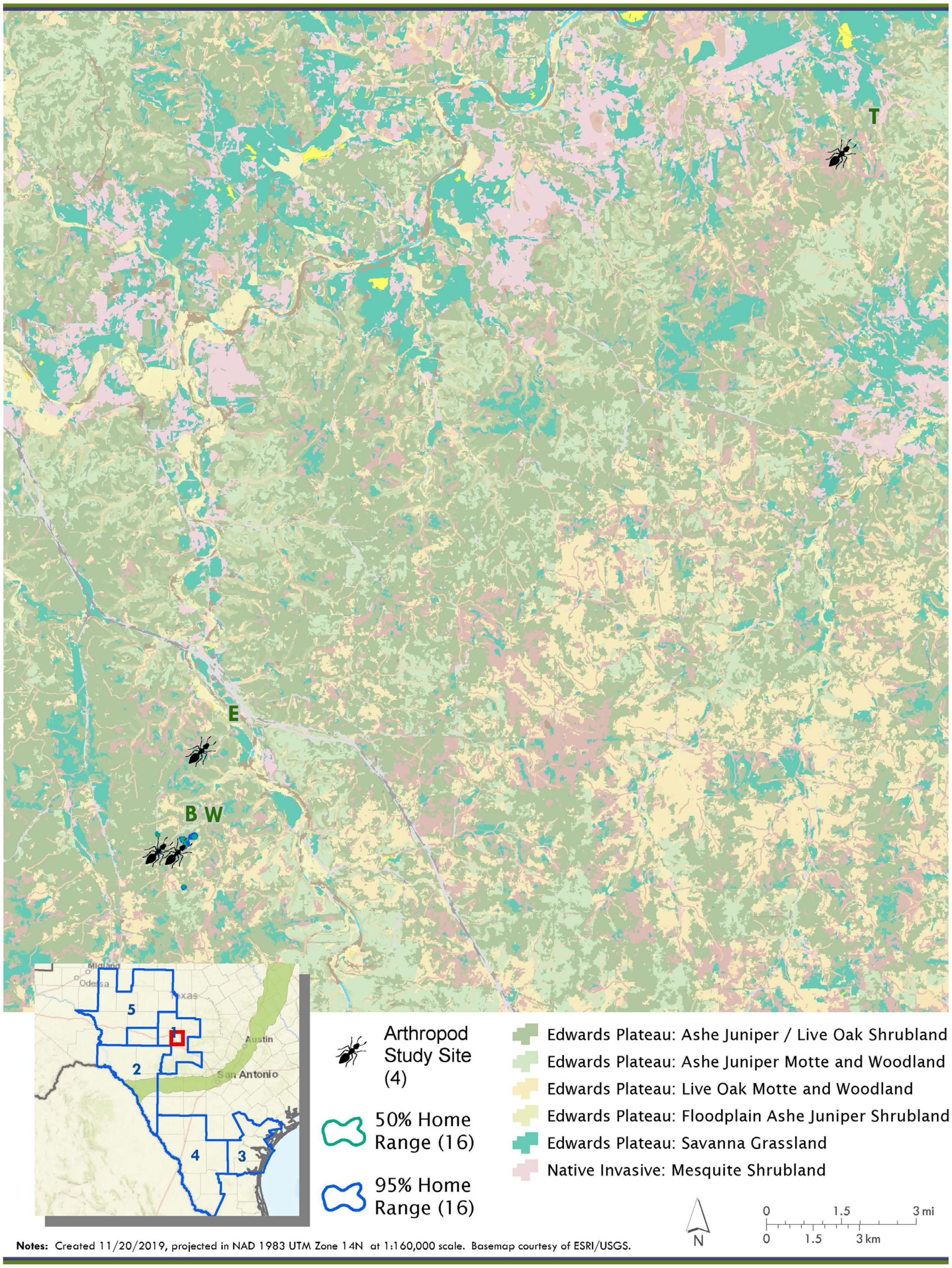
### 6.1 Methods

Arthropods were sampled at 10 study sites (sites). This included five sites within the range of *H. lacerata* located in Study Units 1 (Figure 55) and 5 (Figure 56), and five sites within the range of *H. subcaudalis* located in Study Units 3 (Figure 57) and 4 (Figure 58). Site selection was informed by known presence or absence of *H. lacerata* or *H. subcaudalis* based on study activities or historical records, and to span geographic variation and land use within the range of each species to the greatest extent possible.

At each site, arthropods were sampled by pitfall trapping and sweep netting. Pitfall traps were established by using a hand auger to form a hole 12 cm deep by 9 cm in diameter to accommodate a 500 mL plastic sample cup. The cup was placed in the ground so that the upper lip was flush with or lower than the ground surface and was filled with 150 mL of propylene glycol. A coverboard was made of two 50 cm pieces of plywood and was attached to a roof to discourage non-target wildlife and protect from precipitation (Figure 59). Five pitfall traps were established 10 m apart along three transects that were spaced 50 m apart for a total of 15 traps per site. Pitfall traps were deployed for four days (9 July 2018–12 July 2018) and then retrieved. During retrieval of pitfall traps, 250 mL of 95% isopropyl alcohol was added to each sample cup. Samples were transported to the laboratory where they were washed through a 250 µm mesh sieve (US standard 60), then combined into a single sample cup with 75% isopropyl alcohol.

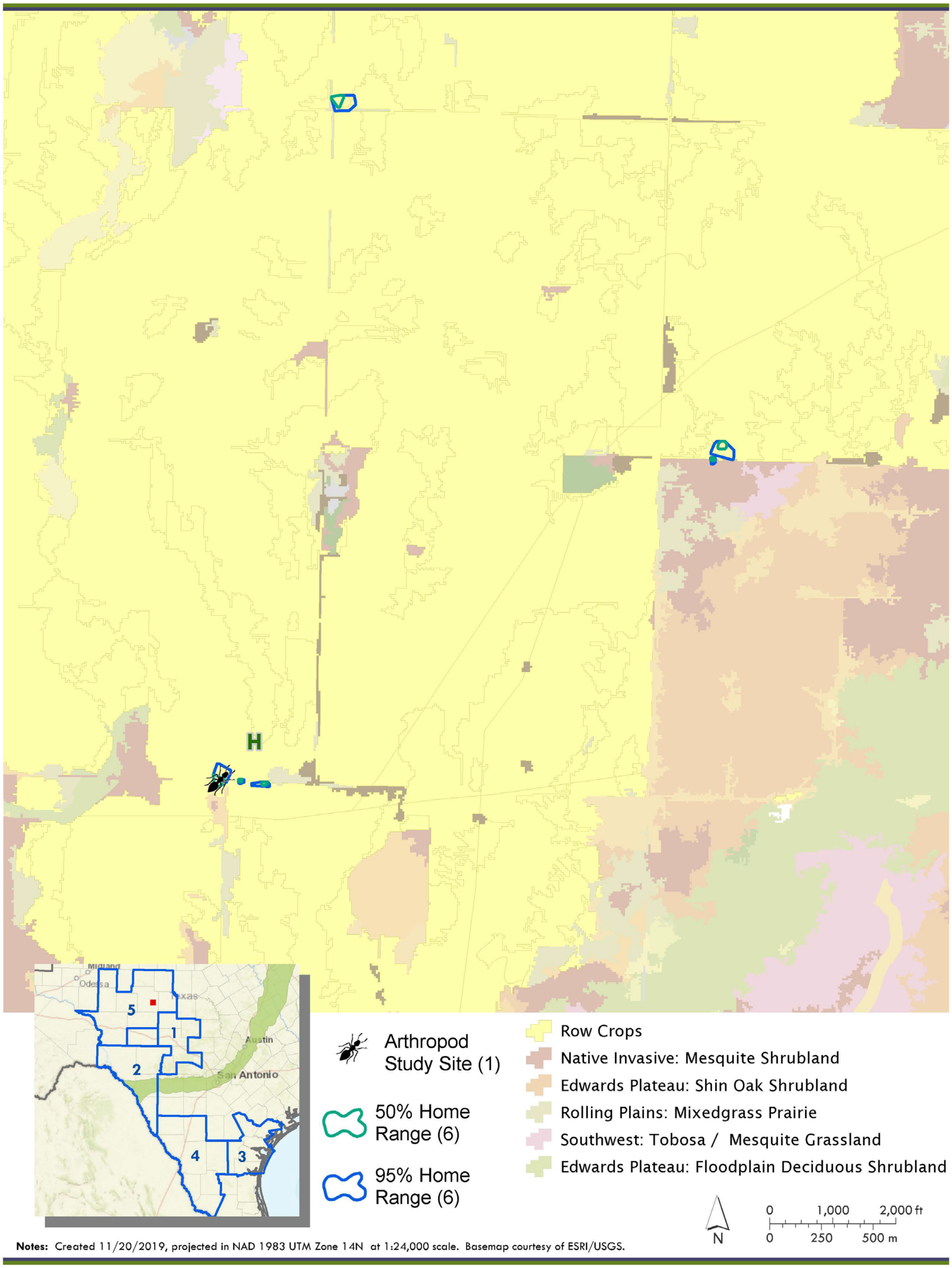
At each site, sweep-net sampling was conducted by sweeping standing vegetation along each of the 50 m pitfall transects just before the traps were set. The sampling method was conducted with a heavy-duty insect net with a 38 cm diameter opening. The net was swept vigorously through standing vegetation until the length of the transect was completed. Contents were periodically transferred to a 9.5 L freezer bag during the sweeping of the transect. Sample bags were placed in a cooler with dry ice and later transported to the laboratory where they were stored in a freezer.

Collected samples were sorted into major taxonomic groups, then identified using available keys (Brust et al. 2018, Capinera et al. 2004, Fisher and Cover 2007, Triplehorn and Johnson 2005, Ubick et al. 2017). Identification efforts focused on food items previously described as important to the diet of *H. lacerata* and *H. subcaudalis* (Wright and LaDuc 2018). This predominately included Orthoptera (grasshoppers, crickets) and Araneae (spiders) as well as more diverse groups of insects, Coleoptera (beetles), Hemiptera (true bugs), Formicidae (ants), and Mutilidae (velvet ants). Formicids and mutilids were only identified from the pitfall samples because they were not present in sweep samples from every site. The dataset was inspected for ambiguous taxa; damaged or immature individuals left at family level were removed if several other taxa within the family were identified to genus or better. Other specimens that were retained at the family level were kept in the data set in circumstances where they were unique from the other taxa identified (e.g. Chrysomelidae, *Eleodes*). In circumstances where only one lower level was identified, the higher-level taxon was rolled down when there was confidence that all individuals were the same, or the lower level taxon was rolled up when it was evident that multiple taxa occurred.



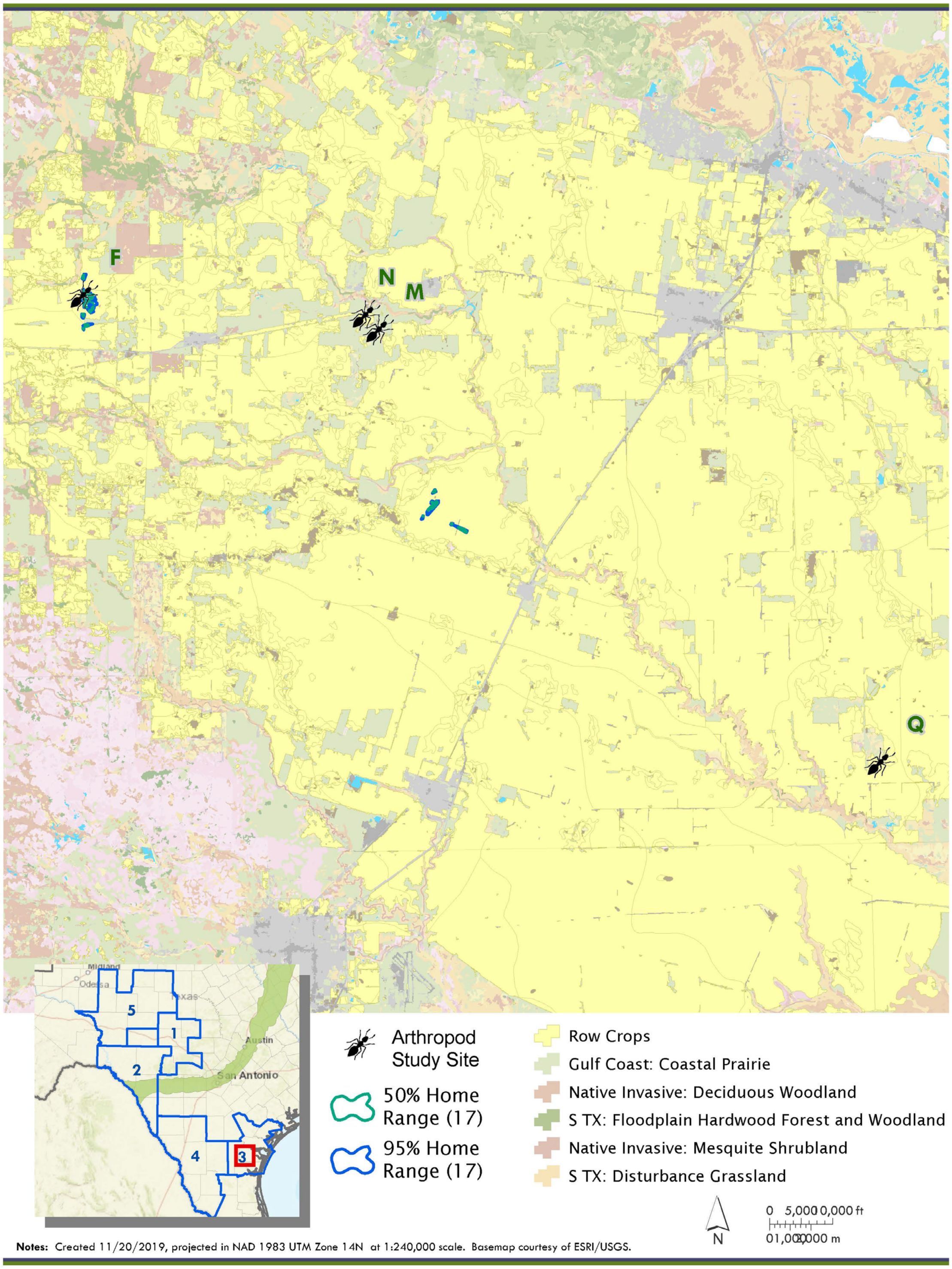
**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:160,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 55.** Arthropod study sites in Kimble and Mason counties (Unit 1).



**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:24,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 56.** Arthropod study site in Tom Green County (Unit 5).



**Notes:** Created 11/20/2019, projected in NAD 1983 UTM Zone 14N at 1:240,000 scale. Basemap courtesy of ESRI/USGS.

**Figure 57**. Arthropod study sites in Jim Wells and Nueces counties (Unit 3).

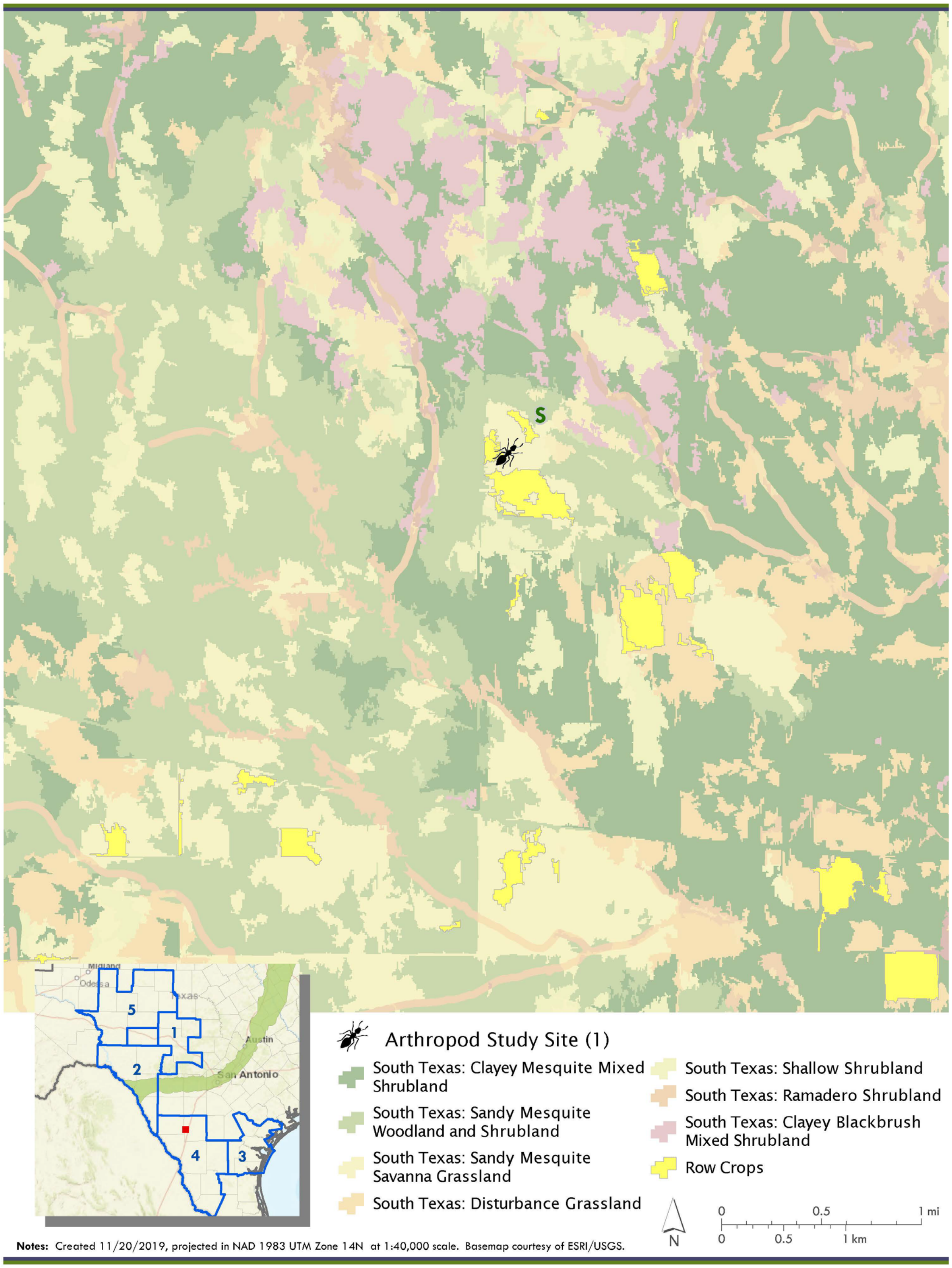


**Figure 58.** Arthropod study site in La Salle County (Unit 4).

**Figure 59.** Example of a pitfall trap. When set, the fence was placed directly over the cup.

### 6.1.2 Analysis

Arthropod community structures were analyzed from two perspectives: (1) presence or absence of *H. lacerata* or *H. subcaudalis* at sites and (2) in which species' range the site occurred. Species range was considered a geographic proxy for ecoregion/expected geographic effect on arthropod composition. All arthropod taxa from both sampling methods were used to calculate taxonomic richness, Simpson's Diversity Index, Fisher's Alpha, and Pielou's J (evenness) for each site and a two-tailed t-test was used to compare presence or absence sites and between the ranges of *H. lacerata* and *H. subcaudalis* for each of these metrics, separately. Non-metric multidimensional scaling (NMDS) was used to show site variables ordinated within arthropod community structure. Rare and ubiquitous arthropod taxa were removed from the data set to control for noise by excluding arthropod taxa that only occurred at 1–2 sites and 9–10 sites, respectively. Both sampling methods were pooled and considered separately to determine if one sampling method was a better representation of the arthropod community structure in relation to the presence of *H. lacerata* and *H. subcaudalis*. Analysis of similarities (ANOSIM) was used to show if there was a difference in community structures of detection sites with non-detection sites, and among species designation sites. Similarity Percentage (SIMPER) analysis was used to identify species associated with select site classes.

Diversity indices were calculated with the *vegan* package version 2.4-4 (https://CRAN.R-project.org/package=vegan) for R statistical software version 3.4.1 (R Core Team 2017) and t-test were performed with base R. NMDS, ANOSIM, and SIMPER analyses were performed in Primer (Clarke and Gorley 2006).

## 6.2 Results

A total of 138 unique taxa were identified from 5,456 individuals sampled within the orders Araneae, Orthoptera, Hemiptera, Coleoptera and the family Formicidae. A total of 4,222 individuals were identified to 84 unique taxa from pitfall traps while 1,234 individuals were identified to 80 unique taxa from sweep samples. Only 24 taxa occurred in both pitfall and sweep samples; however, Formicidae were only identified from pitfall samples and these represented 18 unique taxa among 2,707 individuals.

Analysis of the diversity indices are given in Table 25. In general, there were no statistical differences between detected or designated sites with any of the metrics at α = 0.05.

**Table 25.** Means and standard deviation of diversity indices where *H. lacerata* or *H. subcaudalis* were detected vs. not detected and among the species-designated areas. Results of t-tests are also given.

| | **Detected (both species)** | **Not detected** | **t-value** | **p-value** |
|---|---|---|---|---|
| **Simpson diversity** | 0.867 ± 0.052 | 0.843 ± 0.091 | 0.513 | 0.626 |
| **Fisher's alpha** | 11.601 ± 2.234 | 9.544 ± 1.026 | 1.871 | 0.114 |
| **Taxa richness** | 43.2 ± 10.5 | 36.8 ± 3.0 | 1.312 | 0.250 |
| **Pielou's J** | 0.707 ± 0.078 | 0.696 ± 0.077 | 0.208 | 0.841 |
| | ***H. subcaudalis*** | ***H. lacerata*** | **t-value** | **p-value** |
| **Simpson diversity** | 0.871 ± 0.040 | 0.840 ± 0.095 | -0.147 | 0.539 |
| **Fisher's alpha** | 9.379 ± 1.136 | 11.766 ± 1.960 | 2.356 | 0.054 |
| **Taxa richness** | 38.4 ± 3.8 | 41.6 ± 11.1 | 0.609 | 0.570 |
| **Pielou's J** | 0.696 ± 0.078 | 0.707 ± 0.077 | 0.225 | 0.828 |

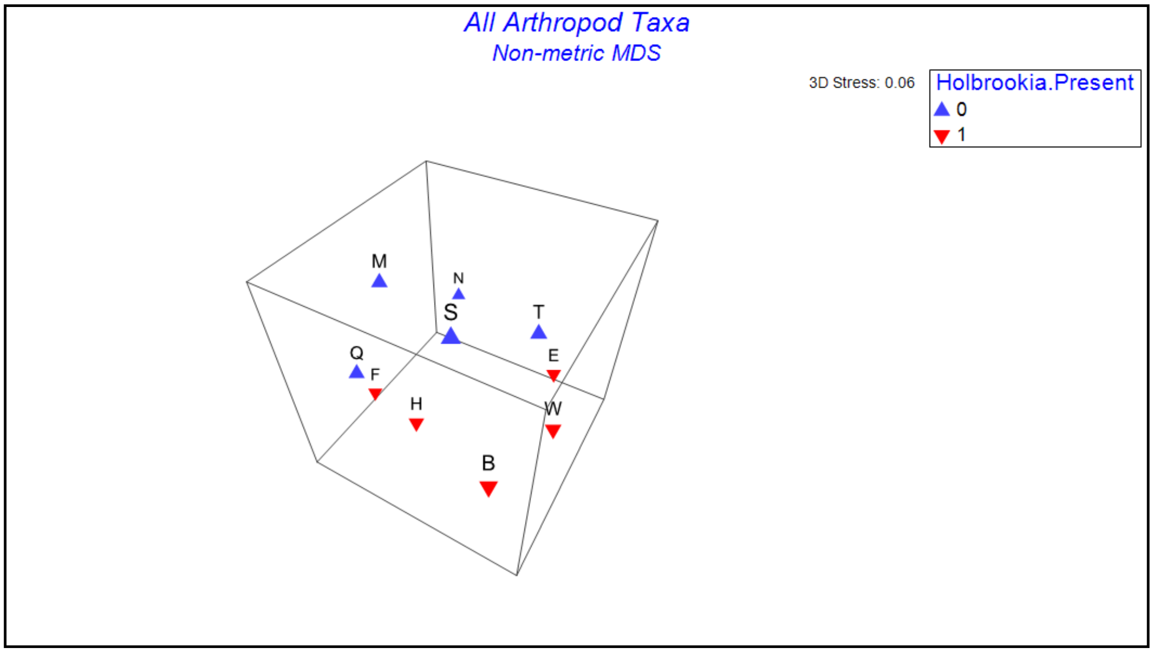


**Figure 60.** Non-metric multidimensional scaling of study sites ordinated over arthropod community space. Sites where *H. lacerata* or *H. subcaudalis* were detected are given in red.

Preliminary NMDS analyses indicated that three dimensions were necessary to maintain low stress. Visualization of the full arthropod data set NMDS (minus rare and ubiquitous taxa as described above) illustrated some possible structure separating sites where *H. lacerata or H. subcaudalis* were detected from sites where *H. lacerata or H. subcaudalis* were not detected (stress = 0.06, Figure 60). However, ANOSIM did not support that these site classes were different ($R^2$ = 0.112; p-value = 0.238). Ordination of the arthropod data set consisting of only pitfall samples indicated that there were unique structures representing sites where *H. lacerata or H. subcaudalis* were present vs absent (stress = 0.04, Figure 62). ANOSIM supported that these site classes were different at $\alpha = 0.10$ ($R^2$ = 0.23; p-value = 0.095).

The clearest structure apparent in the NMDS plot of the entire arthropod data set was that of geographic variation (species range) (stress = 0.06, Figure 61). ANOSIM supported that these site classes were different ($R^2$ = 0.56; p-value = 0.016), and this meets expectations that arthropod community structure variation would exist at this geographic scale. Similarly, ordination of the arthropod data set consisting of only pitfall samples, indicated that there existed strong separation correlated with geographic separation of species ranges (stress = 0.04, Figure 63). ANOSIM again supported that these site classes were different ($R^2$ = 0.54; p-value = 0.024).

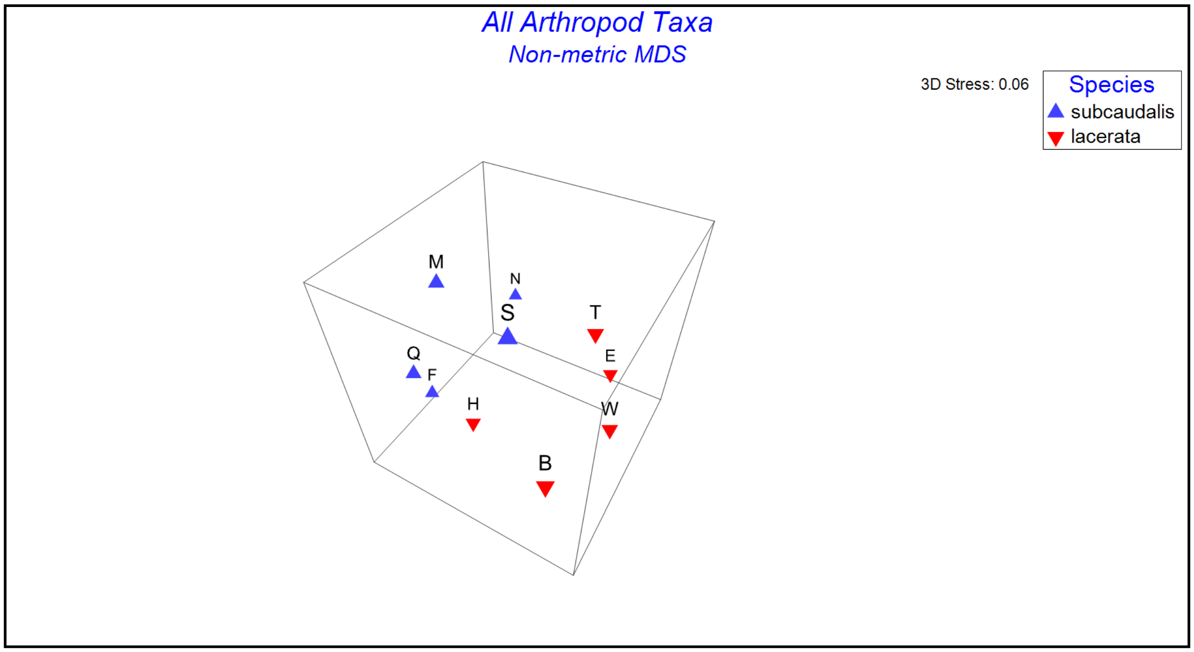


**Figure 61.** Non-metric multidimensional scaling of study sites ordinated over arthropod community space. Sites are colored by lizard species designation.

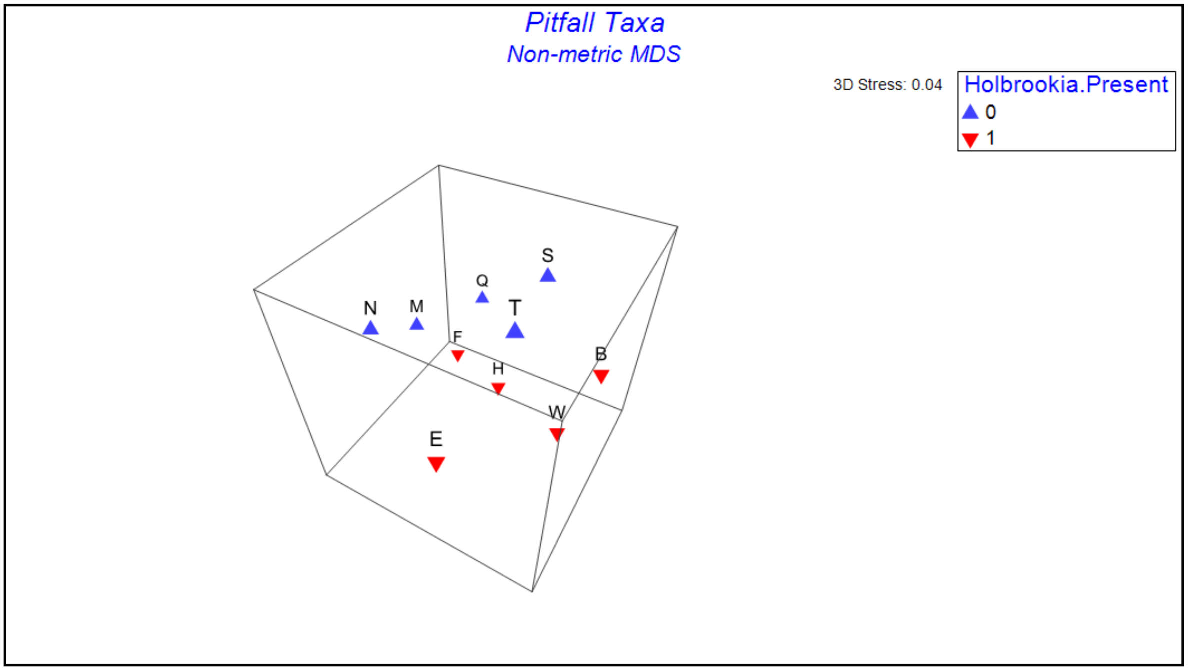


**Figure 62.** Non-metric multidimensional scaling of study sites ordinated over arthropod community space with pitfall samples only. Sites where *H. lacerata* or *H. subcaudalis* were detected are given in red.

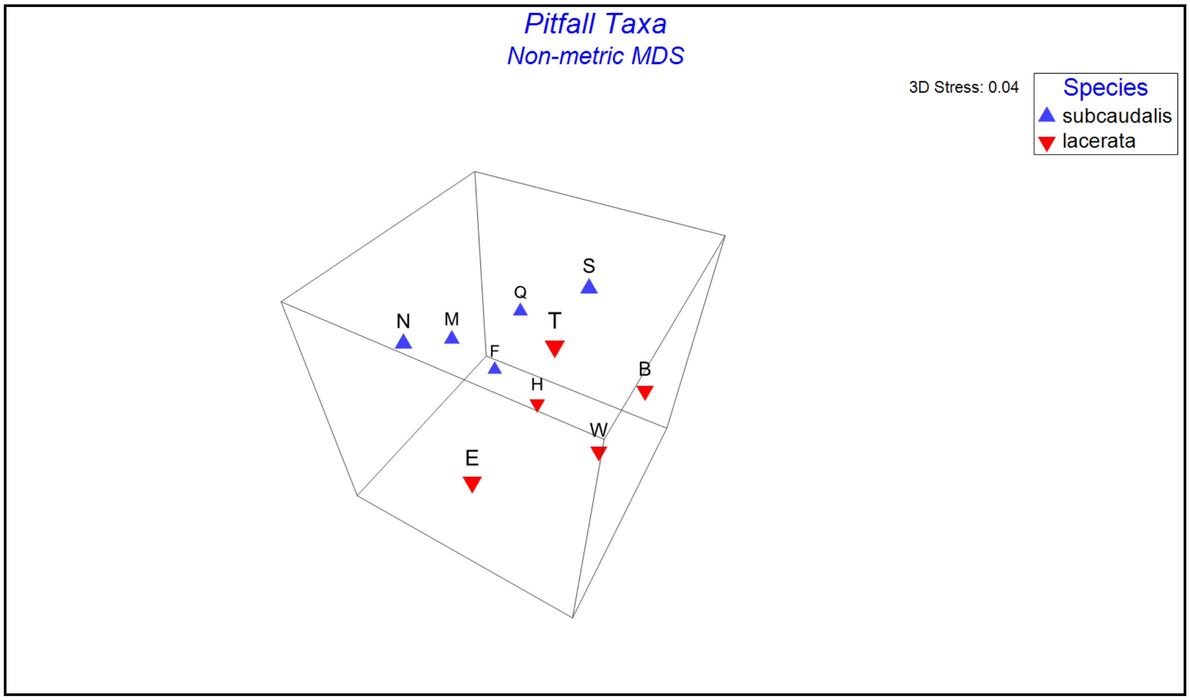


**Figure 63.** Non-metric multidimensional scaling of study sites ordinated over arthropod community space with pitfall samples only. Sites are color coded by lizard species designation.

NMDS analysis of the arthropod data set consisting of only sweep samples, did not indicate that there were unique structures representing sites by species range (stress = 0.06, Figure 65). This is clearly visible in the overlap of sites within different ranges in the NMDS plot. Ordination of the sweep samples data likewise did not produce any visible patterns in the arthropod data when plotted by presence or absence of *H. lacerata or H. subcaudalis* (stress = 0.06, Figure 64).

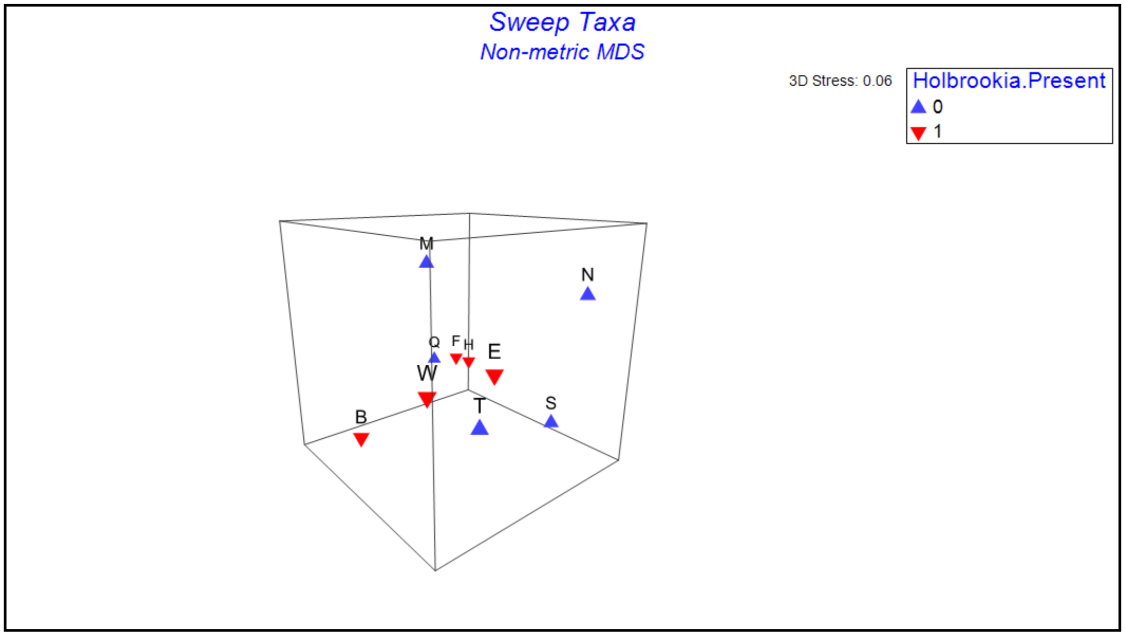


**Figure 64.** Non-metric multidimensional scaling of study sites ordinated over arthropod community space with sweep samples only. Sites where *H. lacerata* or *H. subcaudalis* were detected are given in red.

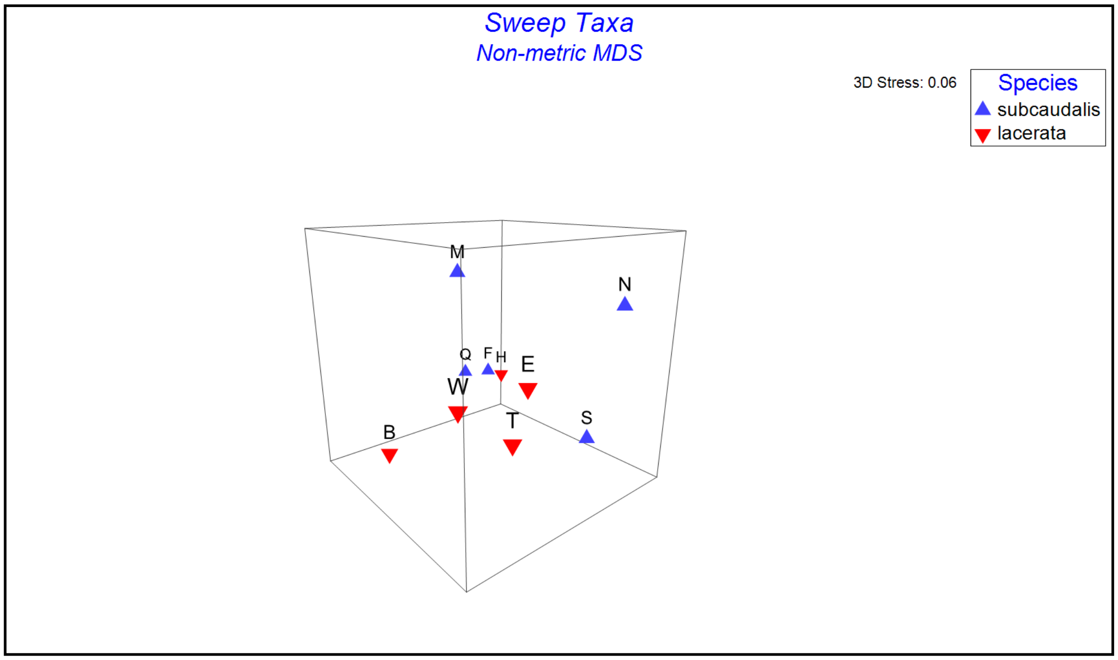


**Figure 65.** Non-metric multidimensional scaling of study sites ordinated over arthropod community space with sweep samples only. Sites are color coded by lizard species designation.

## 6.3 Discussion

The ordination results suggest that the community structures of pitfall traps and sweep nets are different from each other and that pitfall traps are more useful for capturing an aspect of the arthropod community structure most likely related to habitat suitability for *H. lacerata* and *H. subcaudalis*. This makes sense as many of the arthropods sampled in the pitfall traps are mostly associated with the ground and are more reflective of the conditions experienced by *H. lacerata* and *H. subcaudalis* compared to arthropods that are typically found on vegetation.

This was a pilot study with a relatively small number of sites (replicates) widely distributed geographically and over ecological clines, this was initially by design to capture maximal arthropod diversity during the pilot study. However, experiment-wise error resulted in unequal distribution of occupied sites between the two species' ranges (1 of 5 *H. subcaudalis* sites had detections, and 4 of 5 *H. lacerata* sites had detections). This also created an effect where a small number of sites within each range are very strongly dissimilar from the others, confounding the visibility of other patterns in the data.

SIMPER analyses of the most significant ANOSIM data sets (pitfall samples based on species designation) were examined to find which taxa were associated with geographic ecotype. The ant genera *Pogonomyrmex, Monomorium,* and *Pheidole*, the spider genus *Psilochorus*, the grasshopper *Encoptolophus costalis*, and true bugs from the family Rhyparochromidae, were more associated with sites designated within the range of *H. lacerata*. Spiders in the genera *Nesticus* and *Schizocosa*, the darkling beetles (Tenebrionidae excluding the genus *Eleodes*), the ground beetles (Carabidae excluding the genera *Brachinus*, *Calosoma*, and *Cicindela*), the click beetles (Elateridae), the ant-like flower beetles (Anthicidae), and velvet ants (Mutilidae) were more associated with the range of *H. subcaudalis*.

Some of the more important taxa related to the habitat of *H. lacerata* and *H. subcaudalis* were spiders and ants. Both of these groups have many ground dwelling species that are important components of the habitat. Ground spiders are active or hide and wait predators that require varying conditions for their retreats; as an example, *Nesticus* require conditions that are similar to subterranean habitats (Ubick et al. 2017). Ants as well may require specific habitat conditions and have been shown to be indicators of soil function (De Bruyn 1999). Potentially confounding results, spiders and ants were mainly identified to genus level, whereas beetle and true bugs were generally retained at the family level. It is likely that finer resolution of all taxa to species level may help ascertain more specific habitat requirements.

Results of the arthropod surveys indicate that while the community structures reflect ecological components of habitat suitability for *H. lacerata* and *H. subcaudalis*, strong ecological differences between the ranges of these species correlates most strongly with the differences in arthropod communities. Furthermore, it was determined that the pitfall samples alone were better at identifying these community structures. This suggests that *H. lacerata* or *H. subcaudalis* more frequently interact with the same type of habitats as the ground-dwelling arthropods. The arthropod community is a factor related to habitat quality for *H. lacerata* or *H. subcaudalis*. Arthropod community structure is a result of the general ecology of an area, including the physico-chemical and biological components that make up a habitat. Although *H. lacerata* and *H. subcaudalis* are dietary generalists (Wright and LaDuc 2018), the quality and availability of arthropods as food items could be factor with regard to the current-day distributions of these lizards.

# 7.0 Summary and Conclusion

Project Team biologists have been investigating the Plateau spot-tailed earless lizard (*Holbrookia lacerata*) and Tamaulipan spot-tailed earless lizard (*Holbrookia subcaudalis*) throughout their historical range within Texas for several years, building on previous work by Dr. Travis LaDuc (UT) and colleagues. This research, sponsored by the Texas Comptroller of Public Accounts, sought to inform the listing determination process and the development of a "best professional judgment" threats analysis by species experts. The study employed VHF radio telemetry to define species home range size, movement and activity patterns, and habitat use, and sought to use harmonic radar reflector tags to provide analogous data on earlier life stages that cannot carry VHF radio tags. Additionally, the Project Team evaluated the performance of repeated visual encounter surveys, passive survey methods, and capture-recapture surveys to provide a method of examining species distribution, demography, occupancy and detection. A pilot study of arthropod community signatures and their relationship to *H. lacerata* and *H. subcaudalis* occupancy and abundance was also completed.

Extensive surveys were conducted throughout the historical range of both species. These surveys predominately involved active searches (e.g., walking transects or road rights-of way) and produced a total of 153 captures within three Study Units (Tables 18–21; Figure 1) and 5 counties during the 3-year study (2017–2019). Study subjects of *H. lacerata* and *H. subcaudalis* of sufficient size and abundance for VHF radio telemetry studies were located within four counties during three field seasons. In total, 52 individuals were affixed with VHF radio transmitters, producing data sufficient for movement and home range estimations of 19 *H. lacerata* (585 total relocation points) and 17 *H. subcaudalis* (478 total relocation points). These data were supplemented by 2018 and 2019 harmonic radar tracking efforts in which a total of 19 individuals were affixed with harmonic radar reflectors within three counties (Kimble, Tom Green, and Nueces counties), producing data sufficient for *H. lacerata* movement analysis of 13 individuals and home range estimations for three individuals. The utilization of harmonic radar allowed for tracking lizards as small as 1.2 g (body weight) and 29.3 mm (SVL). This method allowed lizards to be relocated for a longer period of time, as the tags require no battery, with tag retention up to 34 days. During the application of this method in this study, the limitations and advantages of this equipment were realized.

The two species studied appeared to differ strongly in their movements and home range size, and these behavioral differences support their elevation to species status. These differences also suggest there may be underlying ecological differences in the habitat available to each species. Mean home range size estimated using both 50% and 95% Minimum Convex Polygon (MCP) methods was significantly smaller for study subjects of *H. lacerata* than *H. subcaudalis.* Observed daily lizard movements showed similar patterns across both species, with mean daily movements significantly greater for *H. subcaudalis* individuals. There was no significant difference in mean home range sizes between male and female individuals of *H. lacerata*, however, *H. subcaudalis* males exhibited a significantly larger home range size than females. Harmonic radar tracking provided initial comparison data for *H. lacerata* individuals smaller than 5 g, and the mean daily movements of these individuals were significantly less than larger individuals studied. Additional data from applying this tracking method to smaller individuals is needed to determine how they disperse from their hatching locations to surrounding habitat, survival rates, and how they transition into and interact with surrounding populations.

Vegetation and ground cover quadrat sampling (n = 135 samples) was also conducted within and outside (100 m buffer) a selection of *H, lacerata* and *H. subcaudalis* home ranges. No apparent differences in habitat composition within or outside of home ranges were observed, suggesting that non-random habitat selection was not occurring with respect to habitat as defined by vegetation.

Passive capture methods (drift fence arrays, coverboards, and game cameras) were evaluated as methods for sampling *H. lacerata.* Methods were employed within areas of known *H. lacerata* presence in Kimble County as part of 2017–2019 field activities. Seven drift fence arrays with accompanying pitfall and funnel traps were established in 2017 and monitored for up to 14 days. These efforts produced one *H. lacerata* capture, suggesting that this method is not effective due to the shorter movements and smaller home ranges observed in this species. Based on movement and home range data collected during this study for *H. subcaudalis,* however, it is likely that this method may be viable to increase capture success of this species. In 2018, eight coverboards were deployed within Kimble County, and checked throughout the field season in 2018 and 2019. No *H. lacerata* were observed utilizing these structures as cover. This species was regularly observed burying in soil or hiding in vegetative cover during tracking studies and seems to prefer this behavior to seeking refuge under rocks or other analogues to coverboards preferred by other sympatric lizard species such as the greater earless lizard (*Cophosaurus texanus*). In the same areas, eight game cameras were deployed along-side drift arrays and coverboards in order to monitor the effectiveness of these methods. No *H. lacerata* were observed, however, camera monitoring did allow for the observation of non-target lizard species interacting with drift fence arrays and coverboards.

Visual encounter surveys (VES) of 60 m x 500 m linear plots were conducted repeatedly across multiple seasons. These plots were established in 2018 within various habitat and land-use types along roadsides, and within both private and public lands throughout the historical ranges of *H. lacerata* and *H. subcaudalis.* In 2018, 254 visual encounter surveys were conducted on 102 sample units within 21 counties (representing surveys within all five Study Units). In 2019, survey efforts were repeated within a selection established plots in the range of *H. lacerata* and expanded to include additional areas in the range of *H. subcaudalis* resulting in 401 visual encounter surveys conducted on 100 sampling units within 12 counties. Across two field seasons, these efforts produced four *H. lacerata* and five *H. subcaudalis* detections. In conjunction with this task, vegetation and ground cover quadrat sampling (n = 276 samples) was conducted within a selection of formal sampling units. Provisional multivariate analysis of this vegetation data does not support any strong relationship between occupancy by *H. lacerata* or *H. subcaudalis* but does illustrate the strong geographic stratification of the vegetative communities across the ranges of these two species. Vegetation surveys conducted with both VES and VHF tracking efforts suggest that fine-scale vegetative characteristics are unlikely to successfully define occupancy of habitat for *H. lacerata* on a range-wide scale due to their large geographic range and occurrence across numerous vegetative associations. Where populations of *H. lacerata* and *H. subcaudalis* occur in areas dominated by agricultural crops, this prevents stratification of habitat suitability via fine-scale vegetation data as there is little or no variation present in vegetative communities. At a larger scale, vegetative cover data is best used to determine areas that may be categorically unsuitable (e.g. forested areas) or potentially suitable (e.g. rangeland). Occupancy modeling estimated that approximately 30–40 % of potential *H. lacerata* habitat surveyed was occupied during study seasons, and that approximately 20–40 % of *H. subcaudalis* potential habitat surveyed was occupied over the two study seasons. Detection probabilities from all but one of the models evaluated were < 0.5, validating the difficulty in substantiating presence of these species when they are present. This analysis also determined that *H. lacerata* and *H. subcaudalis* detection probability are influenced by the amount of precipitation preceding a survey and temperature at the time the survey is conducted.

Capture-recapture efforts were most successful within the range of *H. lacerata*, with 28 recaptures (including three inter-season recaptures) across 83 total captures. Within these populations apparent annual survival ranged from 55 to 70%, with population estimates up to 33 individuals across four study sites, but additional data is required for more accurate estimations and to ascertain the significance of trends. No recaptures of the 54 individual *H. subcaudalis* captures were obtained, however study populations of this species were not identified until 2018 whereas *H. lacerata* study populations were identified and marking began in 2017. *Holbrookia subcaudalis* was also found to be more challenging to capture than *H. lacerata*, and future efforts may be aided with the use of passive collection techniques. In summary, capture-recapture efforts are well underway for study populations of *H. lacerata*, and initial marking effort has been made for study populations of *H. subcaudalis,* increasing chances of successful future recapture studies.

Arthropods were collected via pitfall trapping and sweep-netting at five study sites within the range of *H. lacerata* and five study sites within the range of *H. subcaudalis.* This resulted in the identification 138 unique taxa from 5,456 individuals. Analysis of these data suggest that pitfall traps are more useful for capturing an aspect of the arthropod community structure most likely related to habitat suitability for *H. lacerata* and *H. subcaudalis*. Analyses did not demonstrate that community distinctions in pitfall-trap samples were associated with the presence of *Holbrookia sp.* at study sites, largely showing grouping of samples concordant with expected geographic variation. These results are likely a result of small sample size, and wide geographic distribution of samples in this pilot study. It is possible that the arthropod community is a factor related to quality of habitat for *H. lacerata* and *H. subcaudalis*, however this appears difficult to parse from geographic variation in arthropod communities.

Over the course of this study, numerous populations of both *H. lacerata* and *H. subcaudalis* were located in different parts of their respective ranges. These populations were found to be persistent from year to year, to be locally abundant, engaging in reproductive behaviors and producing offspring. Populations of *H. lacerata* were studied in areas of intensive agriculture (croplands) and rangeland habitats. Populations of *H. subcaudalis* were studied in vast expanses of intensive agriculture in the eastern portion of their range with significant oil and gas activity, including large pipeline construction that occurred in the study area. Populations of both species appear to be robust to agricultural activities. Populations of *H. subcaudalis* in proximity to well pads and pipeline construction appeared to be robust to these activities to the extent observed in this study. It is important to note in this regard that pipeline construction observed during this study was occurring during the “active season” for this species, where they were most likely able to avoid heavy construction activity and thus direct mortality. Construction appeared to be complete by the time that hatchling lizards were observed proximal to the construction area, likewise avoiding direct impacts to this vulnerable life stage.

As limited life history data for these two species was available when this study began, there are still many questions to be answered to assist in the development of management strategies. To succeed in this, future research should include efforts that focus on a smaller scale than the landscape level focus of this study to garner more accurate and refined views at the population level for *H. lacerata* and *H. subcaudalis.* Furthermore, studies should investigate activity patterns outside of the season in which the species is generally studied to determine habitat use and requirements during the remainder of the year to inform how to best protect these species from direct impacts during those periods. The present study demonstrated that it is reasonable to pursue capture-recapture studies of this species to inform managers of temporal trends in variation of population demographic attributes, fill in gaps in life history knowledge, and monitor the viability and status of these species in the future. This research should continue to capitalize on the previous marking effort. Estimates of detection illustrate that this species can be easily missed by standard survey methods which can have implications in the assessment of current and future occupancy of habitat by these two species. Continued refinement of detection estimates will be useful in determining the reliability of absence data in assessing the extant range and changes in the status of both species.

In conclusion, this study has greatly contributed to the best available science involving *H. lacerata* and *H. subcaudalis*. Future studies are needed to refine our knowledge of populations of both lizard species to contribute directly to the USFWS SSA process and the overall knowledge base for long-term management and protection of these species.

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# Appendices

**Appendix A.** Summary of all study sites used across all study tasks

| Study Unit | County | Study Site | Study Activities Conducted |
|---|---|---|---|
| 1 | Kimble | A | telemetry, harmonic radar, drift fence, cover boards, game cameras, capture-recapture |
| 1 | Kimble | B | telemetry, drift fence, cover boards, game cameras, capture-recapture, arthropod |
| 1 | Kimble | C | telemetry, harmonic radar, drift fence, capture-recapture |
| 1 | Kimble | D | telemetry, cover boards, game cameras, capture-recapture |
| 1 | Kimble | E | telemetry, harmonic radar, capture-recapture, arthropod |
| 1 | Mason | T | arthropod |
| 1 | Kimble | V | drift fence |
| 1 | Kimble | W | drift fence, cover boards, game cameras, arthropod |
| 1 | Kimble | X | drift fence |
| 3 | Jim Wells | F | telemetry, capture-recapture, arthropod |
| 3 | Nueces | J | telemetry, capture-recapture |
| 3 | Nueces | K | telemetry, capture-recapture |
| 3 | Nueces | L | telemetry, harmonic radar, capture-recapture |
| 3 | Nueces | M | telemetry, arthropod |
| 3 | Nueces | N | arthropod |
| 3 | Nueces | O | capture-recapture |
| 3 | Nueces | P | capture-recapture |
| 3 | Nueces | Q | arthropod |
| 3 | Nueces | R | capture-recapture |
| 4 | La Salle | S | arthropod |
| 5 | Tom Green | G | telemetry, harmonic radar, capture-recapture |
| 5 | Tom Green | H | telemetry, capture-recapture, arthropod |
| 5 | Tom Green | I | telemetry, capture-recapture |
| 5 | Concho | U | capture-recapture |