FINAL REPORT

IDENTIFYING BEST PRACTICES TO SURVEY FOR SPOT-TAILED EARLESS LIZARDS

Prepared for the:

Texas Comptroller of Public Accounts

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EXECUTIVE SUMMARY

We conducted a series of experiments to determine the best survey methods to assess Plateau (*Holbrookia lacerata*) and Tamaulipan (*H. subcaudalis*) spot-tailed earless lizard (STEL) populations. This was accomplished by capturing wild STEL from Tom Green and Nueces Counties, Texas, and placing them in captive scenarios where the environment and habitat could be manipulated. We first placed individual STEL in aquaria that were equipped with LED lights, UV lights, heat lamps, available prey, and a combination of LED and UV light. We determined that STEL react to the combination of LED and UV light, even when the onset of light was manipulated throughout the diel cycle. STEL emerge when UV light is approximately directly overhead, which simulates the sun at 1200 hr. Thus, STEL do not begin to emerge from their nightly burrows until ~1100 hr, and remain active during the peak intensity of UV light, which is different behavior from many ecotherms. Thus, to survey for STEL, one not only needs to know where to search, but <u>when</u> to search for them. We evaluated nine standard reptile search techniques (i.e., pitfall traps, funnel traps, rock mounds, cover boards, remote camera surveys, detection dog surveys, systematic visual searches, environmental DNA, and road cruising) to identify STEL presence and relative abundance within a 1 ha enclosure. STEL were placed inside the enclosure at known densities of 5, 10, 20, 30, and 40 lizards per ha and their presence and relative abundance was assessed daily for 3 consecutive days. Only visual searches and road cruising were successful methods; however, a density threshold was not observed nor was the number of STEL observed predictive in determining the density of STEL. Environmental DNA was investigated more thoroughly, but due to the potential 'shedding hypothesis' for reptiles, relative humidity of Texas, UV light exposure, and humic substrate, eDNA degrades too quickly for eDNA to be a practical assessment method. Road cruising was determined to be the best survey method to assess STEL, but cruising speed should not exceed 13 kph. STEL were more tolerant of an approaching vehicle than an approaching person on foot; however, Tamaulipan STEL were more wary than Plateau STEL. Tamaulipan adult STEL emerge from brumation in late-March/early April and remain active until October. Hatchlings occur from May – October, but have 2 main peaks of occurrence, one in June and another in August. Juvenile occurrence has a 1-month lag time following hatchling occurrence, but juveniles remain above ground through December, possibly to increase weight and size and for brumation and sexual reproduction, respectively. We found that STEL are associated with highly disturbed habitats, such as active crop fields, continuous mowed grasslands such as those surrounding airstrips, continuous grazed grasslands to the point of being overgrazed, and frequently burned areas. We developed a population viability analysis for STEL using the software VORTEX. We found that changes in the mortality rates of hatchlings (age class of 0–1) had the strongest effect (+) on population growth rates and extinction risk. Other important variables that also exhibited high sensitivities to change are the age of first reproduction (-), the percentage of females breeding within a population (-), female sex ratios at birth (+), and clutch size (-). Severe drought can affect STEL reproduction, which in turn lead to population decline due to low rates of juvenile recruitment. Simulations of anthropogenic impacts showed that small increases in habitat loss (e.g., 2%) can exacerbate extinction risk even for stable populations. However, habitat

restoration efforts with supplementation of captive bred individuals can yield the highest population growth as well as provide the greatest genetic diversity.

CONTRACT TASKS

Task 1: Agency will conduct a controlled experiment to determine the most efficient methods of identifying presence, relative abundance, and probability of detection of earless lizards.

A. Agency will establish a 1-hectare habitat enclosure and surrogate earless lizard species population to evaluate each collection method.

1. Agency will capture and maintain a surrogate earless lizard species population to place within the enclosure.

2. Agency will collect very-high resolution imagery to assess vegetation structure within the enclosure.

3. Agency will distribute collection methods across 100, 10 square meter grids (Figure 1).

B. Agency will test various lizard collection techniques such as pitfall traps, funnel traps, rock mounds, cover boards, remote camera surveys, detection dog surveys, systematic visual searches, and road cruising on a range of known densities of the surrogate species.

1. Agency will record the number of surrogate earless lizards encountered with each collection method and effort expended.

2. Agency will calculate relative abundance and probability of detection and determine if a density threshold is required to determine presence for each detection technique.

3. Agency will coordinate with potential end-users to incorporate practical considerations in the applications of results.

Task 2: Agency will conduct a controlled experiment to determine the efficacy of eDNA collection to detect STEL.

A. Agency will capture and maintain up to five Plateau spot-tailed earless lizards for controlled eDNA collection.

B. Agency will analyze soil samples exposed to captive STEL for various time periods to determine minimum time required to detect eDNA.

C. Agency will expose substrate to outside environment for various time periods to establish the longevity of STEL eDNA within the environment.

Task 3: Agency will employ the most effective detection techniques from Tasks 1 and 2 to conduct bimonthly (every two months) surveys for spot-tailed earless lizards at two survey sites.

A. Agency will contact stakeholders to identify properties within or near Kingsville, Laredo, Del Rio, San Angelo, and San Antonio, Texas, and select 2, 50-ha properties at each location for bimonthly assessment.

B. Agency will conduct STEL surveys using the most efficient collection methods identified in Tasks 1 and 2.

1. Agency will record GPS location, sex, weight and snout-vent length, and photograph, mark and collect fecal samples from all STEL captured.

2. Agency will collect soil samples and analyze for STEL eDNA if eDNA soil sampling is found to be an efficient method of STEL detection in Task 2. Sampling frequency will depend on eDNA longevity determined in Task 2.

3. Agency will record fortuitous encounters of all species (e.g., reptilian, avian, and mammalian) identified within each location assessment to inform STEL community and habitat associations.

C. Agency will collect very-high resolution imagery and environmental data at survey sites to evaluate the relationship between STEL occurrence and environmental properties

1. Agency will conduct drone flights to collect information on vegetation cover, spatial structure and thermal properties.

2. Agency will monitor temperature, relative humidity and dew point at known GPS locations at STEL survey sites and will model the effect of temperature at the landscape level for each property identified in Task 3 to create thermal stress maps for each study area.

D. Agency will use the results of these analyses to develop a STEL habitat suitability model.

Task 4: Agency will develop a population viability analysis to inform the long-term conservation of spot-tailed earless lizards.

A. Agency will simulate population dynamics and management strategies to evaluate function of STEL populations and efficacy of management strategies, and to identify research priorities.

B. Agency will define model parameters using data from Tasks 1-3, existing literature and stakeholder input.

TASK 1 A & B

1. EMERGENCE BEHAVIOR OF SPOT-TAILED EARLESS LIZARDS IN CAPTIVITY AND SIMULATED WILD STATE

ABSTRACT

Spot-tailed earless lizards (STEL; *Holbrookia lacerata* and *H. subcaudalis*) are an elusive, cryptic species that spend much of the diel cycle underground, which makes them difficult to locate. In conducting surveys for STEL, we observed emergence behavior during daytime hours subsequent to dispersal of morning cloud cover, increase in temperature, and a peak in ultraviolent light (UV) and light intensity levels. Following these observations, we performed laboratory experiments to test the relative influence of temperature, ambient light, UV light, and prey (prey movement) on emergence behavior of STEL. Forty-five wild-captured STEL were individually housed in 38-L aquaria, which were equipped with ceramic heat emitters, UV lights, LED lights, and video cameras. Cochran-Mantel- Haenszel test was used to answer what environmental factors elicit STEL emergence, while a linear mixed model analysis quantified the time elapsed from initiation of the environmental factor until STEL emergence. A marginal association (P = 0.0645) and no association (P = 0.76) was observed between treatment and emergence fate in Plateau STEL and Tamaulipan STEL, respectively. The combination of UV + LED light and LED light only were similar in STEL emergence (95% and 90% STEL emerged, respectively); whereas, all other pairwise comparisons were different. In a second experiment, STEL were provided UV and LED light, but the onset of light began at either 0800, 1000, or 1200. STEL emerged following light presence irrespective of time of onset. Both STEL species emerged within 2 minutes at the 75% quartile for each of the three treatments. Under natural light conditions, STEL did not emerge from their underground nightly burrow until

1048–1127 hr on any given day. After which, STEL would remain active, but individuals would bury and re-emerge throughout the day. More STEL were aboveground between 1401 – 1600 hr (4.75 ± 0.5), followed by 1201 – 1400 hr (3.75 ± 0.5), then 1601 – 1800 hr (2.78 ± 0.5), 1001 – 1200 hr (1.88 ± 0.4), 1801 – 2000 hr (0.72 ± 0.3), and lastly 0800 – 1000 hr (0.12 ± 0.02). Therefore, peak STEL detection time occurred between 1401–1600 hrs with ~48% probability of observing all possible STEL.

INTRODUCTION

Spot-tailed earless lizards (STEL; Plateau, *Holbrookia lacerata;* and Tamaulipan, *Holbrookia subcaudalis*) are diurnal lizard species that were once widely distributed across west-central and southern Texas (Hibbitts et. al., 2021). Currently, they are being considered for threatened status under the Endangered Species Act and are thus being intensively studied. Searching for these species is notoriously difficult due to their cryptic patterning and poorly understood behavior (Hibbitts et. al., 2021). As such, little is known about their diel cycle. Some publications have stated that during warmer months STEL are most active in the midmornings (0800-1000 hrs) and at dusk (1800-2000 hrs) (LaDuc et al., 2018), whereas others have reported contradictory findings such as lizard activity ceasing after the hottest part of the day (Hibbitts et al., 2021). Albeit anecdotally, we have observed STEL becoming active late in the morning (~1100 hrs), increasing through the hottest part of the day (1200-1500 hrs), and decreasing into the late afternoon (1600-2000 hrs). This activity pattern is rather different compared to closely related species such as Texas horned lizards (*Phrynosoma cornutum*) and lesser earless lizards (*H. maculate*). For example, Texas horned lizards are known to shift from unimodal activity patterns in the spring and fall, with peak activity in the middle of the afternoon, to bimodal activity in the summer with peak activity occurring during mid-morning and late afternoon

(Henke et al., 1998). Other species, such as lesser earless lizards, are reported to be most active in the late morning and decrease in the afternoon (Hager, 2001). The disparities in the published literature surrounding STEL in combination with the disjunction of their activity patterns from that of other closely related species prompts more in-depth study of their behavior.

Our objectives were: (1) to determine what environmental factors (i.e., temperature, UV light, ambient light, combination of UV and ambient light, or prey activity on surface) trigger STEL to emerge from their nightly burrow; (2) quantify the time elapsed from initiation of the environmental factor until STEL emergence, and (3) what time of the day are STEL most active, and by extension, what diel time would be most successful to survey STEL. Although we suspect that modality shifts could occur throughout STEL's active season, this study was not designed to address this.

METHODS

We hand-collected 45 spot-tailed earless lizards (20 Plateau and 25 Tamaulipan STEL) from Tom Green (31.38194 N, -100.31361 W; WGS 84) and Nueces (27.71444 N, -97.84250 W;WGS 84) counties, respectively, Texas, via road-cruising adjacent to crop fields during May – June 2021. Crop fields consisted of either milo, maize, or cotton and were over 2km away from any naturalistic habitat. The roads consisted of either caliche or bare dirt between adjacent fields. Upon capture, STEL were transported to a research lab on the TAMUK campus, where behavioral studies were conducted. For the first two trials, we set up a series of 38-L aquaria on lab tables. Individual aquaria had 10 cm deep sandy loam soil and were equipped with ceramic heat emitters, UV lights, LED lights, and a video camera. Ceramic heat emitters were used to increase temperature within aquaria without providing light. UV (A and B) lights provided ultraviolet light within the wavelength range of 290 – 320 nanometers, which is needed to regulate behaviors of feeding, mating, and activity patterns. LED lights were provided to simulate ambient daylight. House crickets (*Acheta domesticus*) were used to determine if the movement of prey on the surface would elicit STEL emergence. Dividers were placed between individual aquaria to prevent the STEL from being influenced by the adjacent enclosures' treatments. Ambient light was eliminated by mandating overhead lighting remaining off and window blinds staying shut. For the 3rd trial, which occurred outdoors under natural lighting conditions, STEL were housed in 4, 2.5-m circular diameter x 1 m tall plastic tubs with 30 cm deep of sandy loam soil spread evenly along the bottom of the tubs at The Duane Leach Research Aviary located within the Caesar Kleberg Wildlife Research Park.

Trial 1 – Emergence behavior factors

Each aquarium was equipped with a reptile 100-watt ceramic heat emitter bulb (ZooMed Laboratories, San Luis Obispo, CA 93401), ReptiSun 10.0 UVB, 13-watt, compact fluorescent lamp (ZooMed Laboratories, San Luis Obispo, CA 93401), a Repti Basking 100-watt spot LED lamp (ZooMed Laboratories, San Luis Obispo, CA 93401), and a Geeni Vivid Indoor Smart Wi-Fi Security Camera (Merkury Innovations, New York, New York 10006). Security cameras were capable of recording STEL activity and recordings were maintained until the experiments were completed. Bulbs were placed inside separate ZooMed mini-dome lamp fixtures that were fixed to the top of the aquarium and were plugged into timers to control when each bulb type would activate. Live crickets were provided daily as food after STEL emerged from their underground burrows and water was provided in shallow dishes *ad libitum*.

To understand the factors that influence STEL emergence, we manipulated heat, UV light, LED light, a combination of UV and LED light, and presence of prey (i.e., crickets). Aquaria were surrounded by black poster board so that the treatment of one enclosure would not influence adjacent enclosures. Lights to the research laboratory were not used during the experimental trials.

STEL were randomly divided amongst each of the 5 treatments, given 2 days to acclimatize to the treatment, and then on the third day behavioral data was recorded. All treatments were initiated at 0730 hr. To serve as replications, STEL were randomly rotated through each treatment until every STEL received every treatment. Time required from the initiation of a treatment until emergence onto the soil surface was recorded and categorized into six-time blocks (i.e., < 2 minutes elapsed, 3–60 minutes elapsed, 61–120 minutes elapsed, and 121 - 720 minutes elapsed, never emerged, or never buried). The cutoff of 720 minutes was used because it represented a 12-hour interval, which was considered the maximum time a STEL displayed above ground behavior in the wild. We hypothesized that an increase in temperature would be the factor most significant in triggering STEL emergence.

Trial 2 – Emergence timing

A second behavioral study was initiated to determine if the timing of emergence can be manipulated by delaying the onset of the combination of UV and LED lights. Twenty-four STEL (12 Plateau and 12 Tamaulipan STEL) were used in Trial 2. Aquaria were set-up as described in

Trial 1, except that each aquarium was set to have the combination of UV and LED lights turn on at either 0800, 1000, or 1200 hr.

Eight STEL were randomly placed into one of the three (i.e., 0800, 1000, and 1200 hr) light initiation cycles. During Trial 2, an acclimation period was not provided because we wanted to determine STEL initial response to the UV and visible light stimulus rather than a potential habituated response. STEL were randomly rotated through each treatment until every STEL received all 3 treatments. Time required from the initiation of a treatment until emergence onto the soil surface was recorded and categorized into 3 blocks (i.e., < 2 minutes from initiation, within 3-30 minutes of initiation, or within 31-60 minutes of initiation). We hypothesized that STEL emergence would be triggered during the 3-30 minute time block as some time would be required before the temperature within the aquaria would increase to trigger STEL emergence.

Trial 3 – Natural lights

To verify that STEL behavior was not altered by artificial lighting, we evenly divided 40 STEL into 4, 2.5-m circular diameter × 1 m tall plastic tubs with 30 cm deep of sandy loam soil evenly spread throughout the bottom. Tubs were maintained outdoors, protected from predators within the avian flight cage of the Duane Leach Research Aviary located within the Caesar Kleberg Wildlife Research Park, and STEL were allowed to maintain normal daily activities based on solar radiation and daylength for Kingsville, Texas (27.53222 N, -97.89036 W; WGS 84) during June–July, 2021. We recorded hourly UV light intensity during daylight hours via https://weather.com for Kingsville, Texas. Lizards were housed in groups of 10 by species, so 2 tubs contained 10 *H. lacerata* each and 2 tubs contained 10 *H. subcaudalis* each. Lizards were fed a diet of crickets and water *ad libitum* (Durtsche et al. 1997). A Geeni Vivid Smart Wi-Fi

Security Camera (Merkury Innovations, New York, New York 10006) was mounted directly above the center of each tub so lizard activity could be monitored and recorded 24-hrs/day. Time when STEL first emerged from their burrows each day was recorded. Daylight hours were divided into 6, 2-hours blocks and spanned from 0800 – 1000, 1001 – 1200, 1201 – 1400, 1401 – 1600, 1601 – 1800, and 1801 – 2000 hr. Random time within each time block and each day was selected to count the number of STEL that were aboveground within the tubs and average hourly number of STEL aboveground was recorded throughout each 24-hour period. We hypothesized that STEL emergence would begin around 1000 hr and peak around 1500 hr (mid-afternoon).

STATISTICAL ANALYSIS

For trial 1, we used a linear mixed model analysis with repeated measures where the fixed effects were species and treatment, random effects were lizard identification and rotation, and the dependent variable was minutes elapsed from the initiation of the treatment to STEL emergence from its nightly burrow. Species were either Plateau or Tamaulipan STEL. Treatments were heat, UV light, LED light, combination of UV and LED light, and prey availability. Multiple rotations were conducted because the same STEL were used for each treatment, which became necessary due to the low abundance of wild Plateau and Tamaulipan STEL, and STEL identification were the number of STEL/rotation/treatment. Because STEL behavior appeared altered by captivity or potential unnatural diel cycles (i.e., not all STEL would burrow and some individuals did not emerge), a post hoc test, the Cochran-Mantel-Haenszel (C-M-H) chi-square analysis, was conducted. The C-M-H test is a contingency table-based analysis that tested the differences among the treatments in frequencies of the three qualitative fates: emerged, never emerged, or never buried. Therefore, the C-M-H test was used to answer objective 1: what environmental factors elicit STEL emergence, while the linear mixed model analysis answered objective 2: quantify the time elapsed from initiation of the environmental factor until STEL emergence. However, the linear mixed model analysis only analyzed STEL that did emerge; hence, sample sizes varied among treatments. All tests were considered significant at $P \leq 0.05$. Trends in data were considered when P-values were between $0.05 < x \leq 0.10$.

For trial 2, we used the Cochran-Mantel-Haenszel (C-M-H) chi-square test as a qualitative analysis to determine differences in the frequencies of Plateau and Tamaulipan STEL that emerged within the time blocks of <2, 3 – 30, and 31 – 60 minutes after the initiation of the combination of LED and UV lights. For the quantitative analysis, we transformed the time for emergence to $\log_{10}$ of minutes to reduce the skewness. We then conducted a nonparametric permutational analysis of variance where the fixed effects were species and treatment, random effects were lizard identification and rotation, and the dependent variable was minutes elapsed from the initiation of the treatment to STEL emergence from its nightly burrow.

For trial 3, we used a linear mixed model repeated measures analysis with permutational analysis of variance due to non-normal distributions. Fixed effect was species (i.e., either Plateau or Tamaulipan STEL), random effects were STEL replication (i.e., tubs) and randomly selected time within time blocks, repeated measures included time and day effects, and the dependent variable was the number of STEL within a tub that was aboveground at a specific time. Significance was inferred at $P < 0.05$. All means are reported ±1 standard error.

RESULTS

Trial 1 – Emergence behavior factors

Concerning the C-M-H chi-square analysis to determine environmental factors that elicit STEL emergence, a marginal association was observed (P = 0.0645) in Plateau STEL between treatment and emergence fate. The combination of UV + LED light and LED light only were similar in STEL emergence (95% and 90% STEL emerged, respectively); whereas, heat, UV lights only, and prey movement resulted in 50%, 45%, and 10% of Plateau STEL emerging (Figure 1.1.1. and Figure 1.1.2). However, for Tamaulipan STEL, no association was observed (P = 0.76) between treatments and emergence fate (Figure. 1.1.1. and Figure 1.1.2.). Combination of UV and LED lights, LED lights only, heat, UV lights only, and prey movement resulted in 95%, 90%, 50%, 45%, and 20% of Tamaulipan STEL emerging (Figure 1.1.1. and Figure 1.1.2.). Heat resulted in the majority of both species of STEL never burying, while UV lights and prey movement resulted in the majority of both species of STEL never emerging from their nightly burrow (Figure 1.1.1. and Figure 1.1.2.).

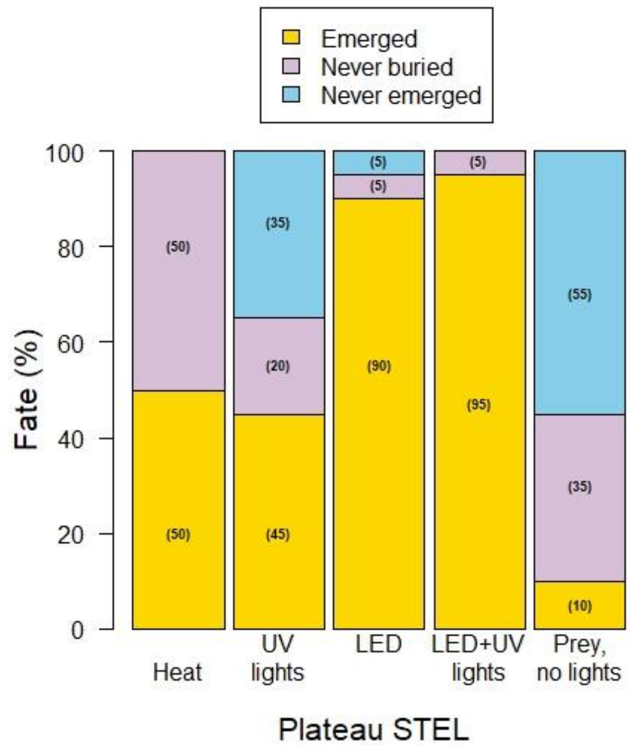


Figure 1.1.1. The outcome response of captive Plateau spot-tailed earless lizards (*Holbrookia lacerata*) to elicit emergence from underground burrows when exposed to environmental factors of heat, ultra-violet (UV) light, LED light, combination of UV and LED light, and prey.

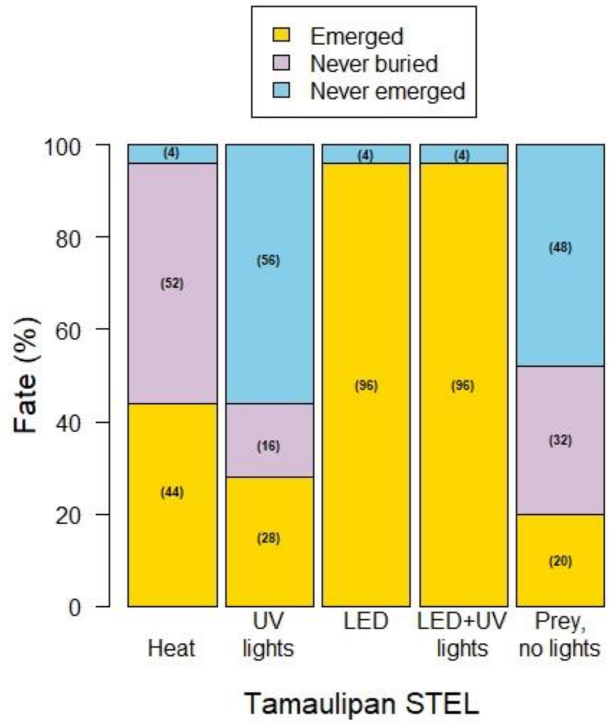


Figure 1.1.2. The outcome response of captive Tamaulipan spot-tailed earless lizards (*Holbrookia subcaudalis*) to elicit emergence from underground burrows when exposed to environmental factors of heat, ultra-violet (UV) light, LED light, combination of UV and LED light, and prey.

Table 1.1.1. Emergence behavior of spot-tailed earless lizards (20 Plateau; *Holbrookia lacerata*) and 25 Tamaulipan; *H. subcaudalis*) exposed to heat, ultra-violet light, 18-watt LED light, darkness with prey (i.e., crickets), and LED and ultra-violet light combinations. Numbers represent the number of lizards (percent) that emerged immediately (within 2 minutes), 3-60 minutes, 61-120 minutes, and 121 - 720 minutes after the onset of an emergence factor.

Time	Heat	UV light	LED light	LED + UV light	Dark + prey
< 2 min	2 (4.4)	0 (0.0)	3 (6.7)	26 (57.8)	1 (2.2)
3 – 60 min	11 (24.4)	3 (6.6)	30 (66.7)	14 (31.1)	4 (8.9)
61 – 120 min	5 (11.1)	2 (4.4)	4 (8.9)	2 (4.4)	1 (2.2)
121 – 720 min	3 (6.6)	11 (24.4)	5 (11.1)	1 (2.2)	1 (2.2)
Never bury	23 (51.1)	8 (17.8)	1 (2.2)	1 (2.2)	15 (33.3)
Never emerge	1 (2.2)	21 (46.7)	2 (4.4)	1 (2.2)	23 (51.1)

We found no significant differences between species ($F_{1,8}$ = 0.13, P = 0.73) and species × treatment interactions ($F_{4,28}$ = 0.91, P = 0.47) for the time that elapsed between the initiation of each treatment and STEL emergence. Therefore, data from both species were pooled. However, a treatment effect between treatments in the time for emergence ($F_{4,28}$ = 23.5, P < 0.0001) was observed. Pairwise comparisons between each treatment combinations were significant (P < 0.03), except for the combination of UV + LED light with LED light alone (P = 0.61 for Plateau STEL and P = 1.00 for Tamaulipan STEL). Of the STEL that emerged, UV + LED lights (12.0 ± 22.9 min), LED lights only (77.3 ± 23.3 min), and prey movement (95.0 ± 62.4 min), followed by heat (113.0 ± 32.6 min), and then UV light only (432.9 ± 37.6 min) produced the quickest emergence time by STEL from the initiation of each treatment.

Trial 2 – Emergence timing

No species effects between the frequencies of STEL that emerged within treatments were observed for Plateau STEL (P = 1.00) and Tamaulipan STEL (P = 0.12). Frequency of Plateau STEL that emerged within <2 minutes, 3 – 30 minutes, and 31 – 60 minutes was 83.3%, 16.7%, and 0%, respectively, for initiation of LED and UV lights at 0800 and 1200 hr, and 91.7%, 0%, and 8.3%, respectively, at the 1000 hr initiation of lights (Figure 1.1.3.). Frequency of Tamaulipan STEL that emerged within <2 minutes, 3 – 30 minutes, and 31 – 60 minutes was 81.0%, 9.1%, and 9.1%, respectively, for initiation of LED and UV lights at 0800 hr; 75.0%, 16.7%, and 8.3%, respectively, for the initiation of LED and UV lights at 1000 hr; and 91.7%, 8.3%, and 0%, respectively, at the 1200 hr initiation of lights (Figure 1.1.4.).

For the quantitative analysis, there were no species ($F_{1,59} = 0.68$, $P = 0.49$) or species × treatment interaction ($F_{2,59} = 2.42$, $P = 0.21$); however, a treatment effect ($F_{2,59} = 10.6$, $P = 0.02$) was noted. The 0.75 quartile was 2 minutes for each treatment, meaning that 75% of the

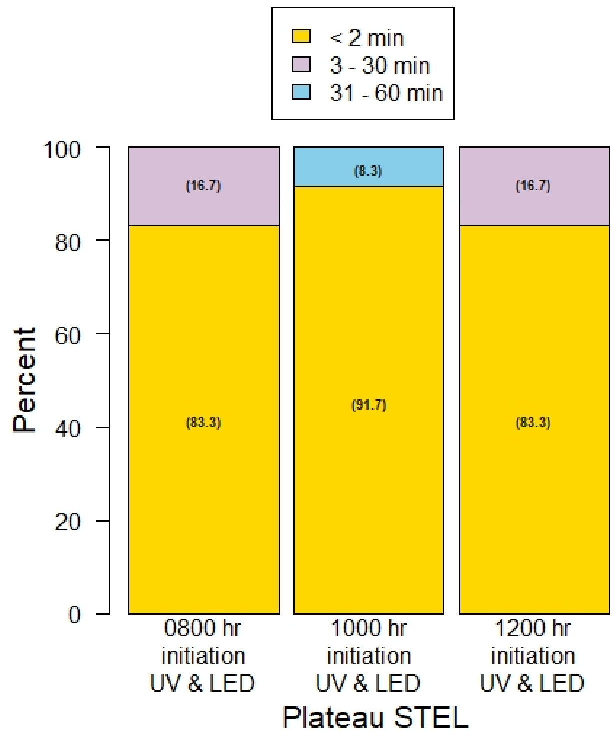


Figure 1.1.3. The outcome response of captive Plateau spot-tailed earless lizards (*Holbrookia lacerata*) to elicit emergence from underground burrows when exposed to a combination of UV and LED light at three initiation times.

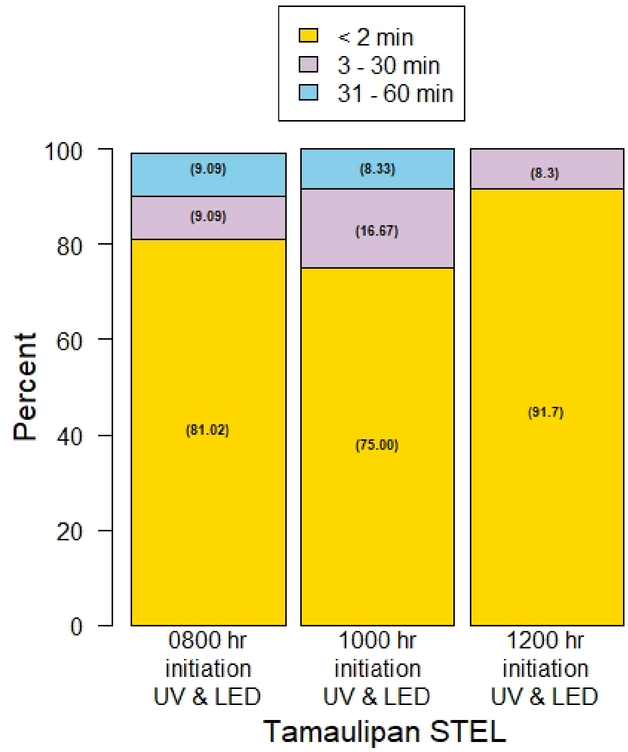


Figure 1.1.4. The outcome response of captive Tamaulipan spot-tailed earless lizards (*Holbrookia subcaudalis*) to elicit emergence from underground burrows when exposed to a combination of UV and LED light at three initiation times.

Table 1.1.2. Number of spot-tailed earless lizards (12 *Holbrookia lacerata* and 12 *Holbrookia subcaudalis*) (N=24) and how quickly they emerged following initiation of UV and LED lights. Numbers represent the number of individual lizards that emerged in <2 minutes, 3 - 30 minutes, and 31 - 60 minutes following illumination at 0800 hr, 1000 hr, and 1200 hr. Dashes represent times before the initiation of UV and LED lights.

	0800 hr			1000 hr			1200 hr		
Time (minutes)	0 – 2	3 – 30	31 – 60	0 - 2	3 – 30	31 – 60	0 - 2	3 – 30	31 - 60
0800 hr [1]	19	3	1	-	-	-	-	-	-
1000 hr	0	0	0	20	2	2	-	-	-
1200hr	0	0	0	0	0	0	21	2	1

[1]One spot-tailed earless lizard never emerged above ground within 12 hours after the initiation of UV and LED lights.

emergent times were ≤2 minutes. Even at the 0.90 quartile, emergent times were 5, 7, and 3 minutes after the initiation of LED and UV lights. Thus, STEL emerged quickly from their underground burrows once the LED and UV lights commenced, regardless of the actual time of day.

Trial 3 – Natural lights

STEL did not emerge from their underground nightly burrow until about 1100 hr (Range:1048–1127) on any given day. Post 1100 hr STEL would remain active but individuals would bury and re-emerge throughout the day (Figure 1.1.5.).

Main effects of species ($F_{1,2}$ = 0.014, P = 0.99) and species × day × time ($F_{145,300}$ = 1.12, P = 0.20) were not observed; however, differences between day ($F_{29,58}$ = 8.60, P = 0.001), time ($F_{5,300}$ = 1303.0, P = 0.001), and their interactive effects of day × time ($F_{145,300}$ = 1.51, P = 0.004) and day × species ($F_{29,58}$ = 2.54, P = 0.005) were observed. Thus, differences among times were not consistent across days, and species differed in their activity behavior throughout the 30 days of the trial. Nevertheless, because effects of day likely are related to short-term and fortuitous weather phenomena (e.g., fluctuations in cloud cover), we overlooked comparisons among days and focused on the remaining significant effect of hour. All pairwise comparisons of time intervals were different (t > 8.54, P = 0.001) from each other. More STEL were aboveground between 1401 – 1600 hr (4.75 ± 0.5), followed by 1201 – 1400 hr (3.75 ± 0.5), then 1601 – 1800 hr (2.78 ± 0.5), 1001 – 1200 hr (1.88 ± 0.4), 1801 – 2000 hr (0.72 ± 0.3), and lastly 0800 – 1000 hr (0.12 ± 0.02).

STEL were inactive during the nights and early morning hours between 2001–0800. Therefore, peak STEL detection time occurred approximately at 1500 (i.e., 1401–1600 hrs) with ~48% probability of observing all possible STEL.

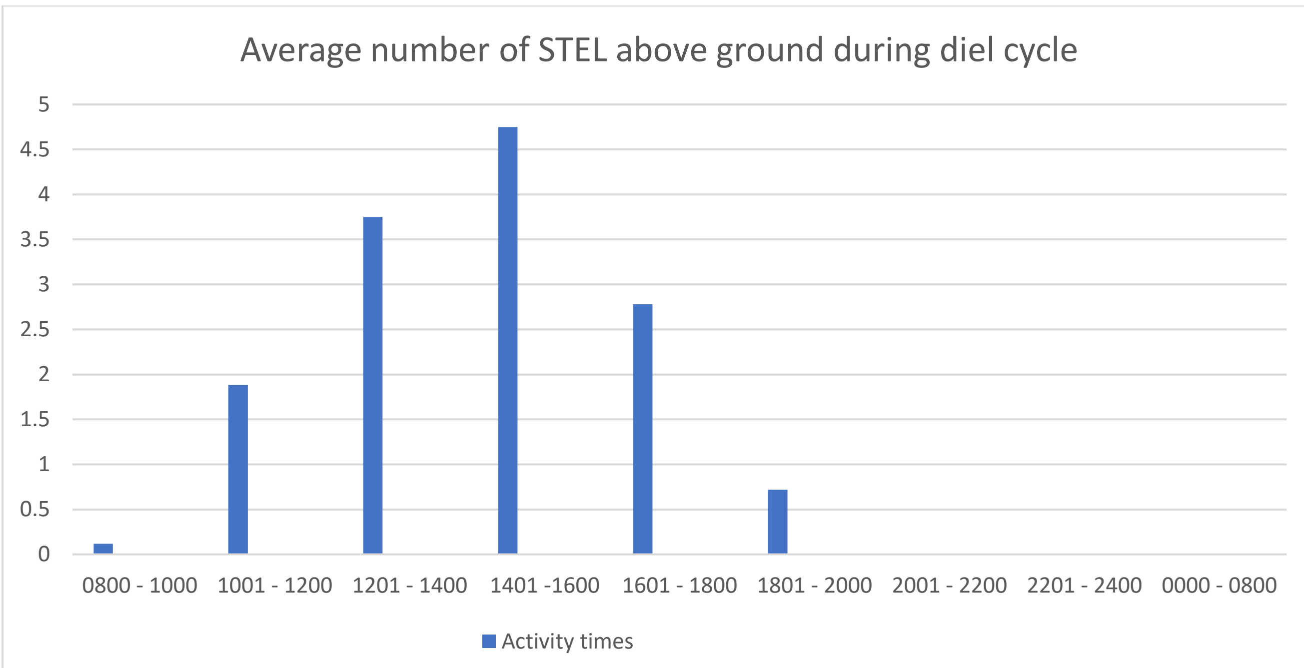


Figure. 1.1.5. Average number of spot-tailed earless lizards (*Holbrookia lacerata* and *H. subcaudalis*) observed above ground during diel cycle.

Discussion

We determined that STEL emergence is predominantly triggered by the combination of visible and UV light. There was a large discrepancy between this treatment and all other treatments including UV light and LED light independently. Our second trial demonstrated that this treatment triggers STEL emergence irrespective of time of day. Our third trial showed that STEL surface activity under natural conditions predominantly begins around 1100 hrs, is at its highest in the early to midafternoon, and tapers off into the late afternoon/early evening. This activity pattern coincides with the UV index and light intensity throughout the day, in that STEL activity peaks when UV and visible light are at their most intense. The disparity in reaction time between our laboratory trials and the trial under natural conditions, we believe, is the time for UV light to be near directly overhead. When STEL bury themselves while not under duress, such as nighttime hours, they bury their head even with, or very close to the soil surface, which is thought to enable them to detect changes in light via their parietal eye (Rangel et al., 2022). Therefore, STEL wait for UV light to be approximately directly overhead before emerging. In our laboratory experiments the UV light was mounted directly over the aquarium, which would simulate the sun at approximately 1200 hr; thus, creating the illusion of 1200 hr. Therefore, it seems that while it is the combination of visible and UV light that STEL are keying on, it is not the initiation of light, but rather the peak of intensity. In STEL native range during their active season, the increase of visible and UV light typically intensifies at 1100 hrs; whereas, in the laboratory setting there was no variability in the intensity of the lighting. Thus, in the laboratory the peak occurred upon illumination and STEL were triggered to emerge immediately. Thus, we suggest that surveys targeting STEL should start at 1000 hr and continue to ~ 1600 hr to have the opportunity to observe the majority of STEL in the area.

Ecologically, this activity pattern is rare amongst squamates. We hypothesize that this behavior is a predator avoidance mechanism. While the literature regarding depredation of *H. subcaudalis* and *H. lacerata* is sparse, the majority of published accounts are of STEL being depredated by snakes (Rangel et al, 2023). Being active during peak periods of ambient heat and UV light differentiates STEL activity patterns from that of most predatory, diurnal snake species and may aid in STEL survival. Furthermore, another documented predator of STE are Loggerhead Shrikes (*Lanius ludovicianus*; Rangel et al., 2023), which are known to hunt for prey during the first two hours of daylight (Craig, 1978). The majority (75%) of documented STEL predators hunt less during the heat of the day, which may explain STEL's delayed emergence after sunrise.

It is also of note that STEL seem to lack any significant defensive mechanisms, which may make predator avoidance even more critical. Avery et al. (1982) demonstrated that an inverse relationship existed between solar intensity and time spent basking in some lizards. Therefore, this may mean that by basking during the time of the day when light and UV radiation are at their most intense, STEL are able to spend less time on the surface exposed to predators. Further research is warranted regarding the pressures affecting STEL activity patterns.

Another potential factor that can influence daytime activity patterns of STEL is diet. However, this does not appear to be the case with STEL as gut content analysis of STEL have found arthropods known to be crepuscular or nocturnal (LaDuc et al., 2017). Our study also demonstrates that STEL are not pressured by prey movements to exhibit the activity patterns documented.

With the results of this study, researchers can more precisely target their efforts in order to locate STEL more efficiently. Searching in the bimodal fashion used for most ectotherms would exclude the time of day when STEL are most active. For STEL, knowing when to search is an important as knowing the best habitats to search

LITERATURE CITED

Avery, R.A., Bedford, J.D. and Newcombe, C.P., 1982. The role of thermoregulation in lizard biology: predatory efficiency in a temperate diurnal basker. Behavioral Ecology and Sociobiology, 11, pp.261-267.Craig, R.B., 1978. An analysis of the predatory behavior of the Loggerhead Shrike. The Auk, 95(2), pp.221-234.

Cochran, W. G., and G. M. Cox. 1957. Experimental designs. Second edition. John Wiley & Sons, New York, New York, USA.

Hager, S.B., 2001. Microhabitat use and activity patterns of Holbrookia maculata and Sceloporus undulatus at White Sands National Monument, New Mexico. *Journal of Herpetology*, *35*(2), pp.326-330.

Henke, S.E. and Fair, W.S., 1998. Management of Texas horned lizards. *Wildlife Management Bulletin of the Caesar Kleberg Wildlife Research Institute Texas A&M University-Kingsville. Management Bulletin*, (2).

Hibbitts, T.J., Walkup, D. K., LaDuc, T. J., Wolaver, B. D., Pierre, J. P., Duran, M., Neuharth, D., Frizzell, S., Adams, C. S., Johnson, T. E., Yandell, D., and Ryberg, W. A. 2021. Natural history of the spot-tailed earless lizards (*Holbrookia lacerata* and *H. subcaudalis*). Journal of Natural History 55:495-514.

LaDuc, T. J., Wolaver, B. D., Pierre, J. P., Duran, C. M., Labay, B. J., Ryeberg, W. A., Hibbitts, T. J., Roelke, C. E., Fujita, M. K., Wright, I. M., Surya, G., Shank, C. J., Holloway, P., Andrews, J. R., Ikonnikova, S. A., and McDaid, G. 2018. Collaborative research on the natural history of the enigmatic Spot-tailed Earless Lizard (*Holbrookia lacerata*) in Texas. Texas Data Repository V1: 1-259.

Neuharth, D. B., Walkup, D. K., Frizzell, S. L., Kachel, J. Z., Adams, C. S., Johnson, T. E., Hibbitts, T. J., and Ryberg, W. A. 2018. *Holbrookia lacerata* (Spot-tailed earless lizard). Burying behavior. Herpetological Review 49:536-537.

Rangel, E. D., Henke, S. E., Reyes, J., Eversole, C. B., and Ayala R. 2023. *Holbrookia lacerata* (Plateau Spot-tailed Earless Lizard) and *Holbrookia subcaudalis* (Tamaulipan Spot-tailed Earless Lizard). Predation. Herpetological Review 54:296-297

Rangel, E. D., Henke, S. E., Eversole, C. B., and Ayala R. 2023. *Holbrookia subcaudalis* (Tamaulipan Spot-tailed Earless Lizard). Predation. Herpetological Review 54:297-298

Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics. Second edition. McGraw-Hill Publishing, New York, New York, USA.

TASK 1 A & B (Continued)

2. EFFICACY OF VARIOUS SURVEY METHODS TO DETECT SPOT-TAILED EARLESS LIZARDS

ABSTRACT

Plateau (*Holbrookia lacerata*) and Tamaulipan (*Holbrookia subcaudalis*) spot-tailed earless lizard (STEL) populations have experienced declines in number and distribution. The species are currently being considered for federal threatened status. Therefore, our objectives were to determine the most effective and time-efficient methods to survey for the Plateau and Tamaulipan STEL, and to determine if a lizard density threshold is required before STEL presence is detected. We evaluated nine standard reptile search techniques (i.e., pitfall traps, funnel traps, rock mounds, cover boards, remote camera surveys, detection dog surveys, systematic visual searches, environmental DNA, and road cruising) to identify STEL presence and relative abundance within a 1 ha enclosure. The 1 ha was divided into 100, 10 x 10 m quadrants, which were delineated with flagging, and each reptile search technique was replicated 5 times and randomly placed within the enclosure without replacement. STEL were placed inside the enclosure at known densities of 5, 10, 20, 30, and 40 lizards per ha and their presence and relative abundance was assessed daily for 3 consecutive days. STEL were allowed 3-day acclimation periods between density increments. Funnel traps, rock mounds, cover boards, and remote cameras did not find STEL at any density level. Pitfall traps, visual quadrant searches, detector dogs, and eDNA detected few STEL, but only at 40 lizards/ha density. Only systematic visual searches and road cruising yielded STEL numbers at multiple densities; however, neither method could reliably predict STEL density from the STEL observed. The number of STEL observed did not increase proportionate to the number of detectable STEL, therefore, not finding

STEL does not confirm their absence in area, nor can you predict STEL density from the STEL observed. Based upon my results, the best survey techniques for STEL are visual walking searches and road cruising.

INTRODUCTION

The spot-tailed earless lizard (STEL) is an elusive, and seemingly rare, lizard that was once separated into two subspecies (*Holbrookia lacerata lacerata* and *H. l. subcaudalis*; Axtell 1968). Today the lizards are considered two distinct species, the Plateau STEL (*Holbrookia lacerata*) and the Tamaulipan STEL (*Holbrookia subcaudalis*; Hibbits et al. 2019, Maldonado et al. 2020). Initially the single species was considered to have an historical range from coastal Texas near Corpus Christi Bay, north to Austin, extended westward to Midland, Texas, and southward to include northeastern Mexico (Axtell 1968).   Today the Balcones Escarpment fault line separates the northern *H. lacerata* from the southern *H. subcaudalis* populations (Maldonado et al. 2020).

Both species have experienced sharp declines in their abundance and distribution, but the population of Tamaulipan STEL is feared to have been so severe that local extinctions have occurred (Wolaver et al. 2018). Anecdotal information suggests that Tamaulipan STEL are very rare and scattered with the only known populations in Nueces and Val Verde counties, a separation of 230 km. By comparison, populations of the Plateau STEL also are scattered but mainly occur in central Texas of Tom Green, Kimble, and Schleicher counties (iNaturalist.com; accessed 25 November 2020). Hypotheses for the decline of both species include pesticides, invasive fauna, and the invasion of exotic grasses (Duran and Axtell 2010). In addition, agricultural practices and urbanization have been offered as potential factors in the decline

(Wolaver et al. 2018); however, Axtell (1968) deemed anthropomorphic habitat modifications as advantageous to the species.

Currently, the United States Fish and Wildlife Service (USFWS) is considering 'threatened status' for STEL. In 2011, the USFWS produced a 90-day finding report that suggested federal listing status could be warranted, but critics argued that too little was known about the species and their distribution to make such a determination (Ingram 2018). Therefore, a current assessment of the STEL population was requested, but it is unknown as to which assessment method is best by which to conduct a STEL survey.

Lizard survey methods have included active assessments such as systematic searches and road cruising, passive methods such as remote cameras, pitfall traps, and funnel traps, use of artificial structures like cover boards and rock mounds to entice reptiles to for thermoregulation purposes, and newer methods such as the use of detector dogs to locate reptiles by olfaction, and environmental DNA, where reptiles leave behind traces of the DNA in soils on which they transverse. Therefore, the goal of this study was to assess various survey methods that effectively survey for the Plateau and Tamaulipan STEL. Our objectives were to: 1) evaluate the efficacy of various survey methods (i.e., systematic searches, road cruising, remote cameras, pitfall traps, funnel traps, cover boards, rock mounds, detector dogs, and environmental DNA) in determining the presence and relative abundance of STEL populations, and 2) determine if a lizard density threshold was required before STEL presence was detected.

METHODS

We removed the vegetation, plowed the soil until it was an even, pliable consistency, and constructed a 1-m tall, aluminum flashing fence that was buried 30 cm deep to enclose a square 1-ha area. The 1-ha enclosure was delineated and numbered into 100, 10 x 10 m grids, red flagging was used to mark the corners of each grid and yellow flags were placed in the center of each grid and numbered to identify the grid (Figure 1.2.1.). A driving path for an ATV was established along the outside perimeter of the fence.

We collected 40 STEL (20 Plateau and 20 Tamaulipan STEL) during May-July, 2021, from Tom Green and Nueces counties, respectively, and maintained them in captivity until STEL were randomly placed within the 1-ha enclosure described above.

At the onset of the study, STEL were placed within the 1-ha enclosure at a density of 5 (3 Plateau: 2 Tamaulipan), then 10 (5 Plateau: 5 Tamaulipan), then 20 (10 Plateau: 10 Tamaulipan), then 30 (15 Plateau: 15 Tamaulipan), and finally 40 (20 Plateau: 20 Tamaulipan) lizards for a 1-week period at each density during August - September. Lizards were provided 1 week to acclimatize to the enclosure, then daily surveys were conducted during 3 consecutive days and STEL species and number observed were recorded. Coloration was used to identify the species of STEL because Plateau STEL are a caramel tan color while Tamaulipan STEL are a slate grey color (Hibbitts et al. 2019).

To reduce the risk of predation, we maintained a predator control program throughout the study. Because we removed the vegetation and plowed the enclosure to develop suitable STEL habitat, doing so reduced the suitability of the habitat for many native predator species (Schmidly and Bradley 2016). We set Havahart traps baited with sardines around the perimeter of the enclosure and removed raccoon (*Procyon lotor*), opossum (*Didelphis virginiana*), and feral domestic cats (*Felis catus*). We would shoot skyrocket fireworks into the air randomly throughout each day to scare potential avian predators from the area. Because STEL burrow underground and remain buried throughout the night, we considered predation by owls unlikely.

At each lizard density, STEL were assessed by 9 collection methods previously identified (Figure 1.2.1.). I conducted pitfall trapping (Todd et al. 2007), funnel trapping

RC 1	RC 2	RC 3	RC 4	RC 5	RC 6	RC 7	RC 8	RC 9	RC 10
RC 11	PFA 12	VS 13	RM 14	15	CAM 16	17	PFA 18	CAM 19	RC 20
RC 21	DD 22	eDNA 23	FTA 24	25	26	27	eDNA 28	VS 29	RC 30
RC 31	CB 32	CAM 33	34	RM 35	36	VS 37	DD 38	FTA 39	RC 40
RC 41	RM 42	43	CB 44	45	46	CAM 47	48	CB 49	RC 50
RC 51	52	53	54	DD 55	PFA 56	57	58	59	RC 60
RC 61	PFA 62	FTA 63	CB 64	eDNA 65	66	RM 67	VS 68	FTA 69	RC 70
RC 71	DD 72	VS 73	eDNA 74	75	CAM 76	77	PFA 78	79	RC 80
RC 81	CB 82	83	FTA 84	85	RM 86	DD 87	eDNA 88	89	RC 90
RC 91	RC 92	RC 93	RC 94	RC 95	RC 96	RC 97	RC 98	RC 99	RC 100

Figure 1.2.1. Study design of the 1-ha enclosure divided into 100, 10 x 10 m grids with random placement without replacement of 9 herpetofauna collection methods (PFA = pitfall array, N=5; FTA = funnel trap arrays, N=5; RM = rock mounds, N=5; CB = cover boards, N=5; eDNA = soil samples for STEL DNA, N=5; CAM = remote cameras, N=5; DD= detector dog searches, N=10; VS = visual searches, N=10; RC = road cruising, N=4). Collection methods were conducted 3 times with lizard densities of 5-, 10-, 20-, 30-, and 40 lizards/ha.

(Gibbons and Semlitsch 1981), rock mound trapping (Willson 2016), cover board trapping (Grant et al. 1992), remote camera surveys, systematic visual encounter surveys (Pike et al. 2010), road cruising (Beane et al. 2014, Enge and Wood 2002), detector dog searches, and eDNA samples within the 1-ha enclosure. Each collection method was placed in the center of randomly selected grids within the inner 64 grids (i.e., 8 x 8) without replacement and replicated five times. The outer 36 grids were designated for road cruising, which was purposely selected so STEL could be visible from a vehicle without the vehicle driving inside the enclosure and potentially striking a STEL.

Pitfall trapping arrays (N = 5) consisted of 4, 5-liter buckets buried so the lip of the bucket was level with the ground and placed in an even-spaced triangular pattern with a central bucket. Buckets were separated by 5-m and silt fencing was partially buried upright to make a drift fence that led to each bucket. Bucket lids were removed when trapping occurred and elevated about 5 cm above the bucket with wooden sticks to provide shade inside the bucket. Lids were secured on buckets when trapping was complete for the sampling period. Buckets were checked every 8-hours when in use and captured fauna were enumerated and recorded during each 24-hour period.

Cylinder-shaped funnel traps, 1-m in length, were made of screening material that had a 30-cm opening at one end and closed at the other end. Funnel trap arrays (N = 5) consisted of a 10-m long silt fence that was partially buried upright to make a drift fence that led to a funnel trap at each end of both sides of the drift fence. Funnel traps were checked every 8-hours when in use and captured fauna were enumerated and recorded during each 24-hour period.
Funnel traps were removed when not in use.

Rock mounds (N = 5) were constructed of 14, 10 x 10 x 4 cm patio bricks piled on top of each other in a pyramid fashion. This structure allowed STEL a natural-looking hiding cover and a thermoregulation structure for basking and cooling. Rock mounds were searched by hand for STEL during surveys and STEL were enumerated and recorded. Rock mounds were reassembled after each search.

Plywood cover boards (N = 5), measuring 1.2 m x 1.2 m x 1.25 cm, were placed on top of 4 patio bricks at each corner of the plywood board so the board rested about 5 cm above the ground surface. Like the rock mounds, this structure allowed STEL hiding cover and a thermoregulation structure for basking and cooling. A capture net was placed at the back side of cover boards as they were lifted away from the searcher to entice hidden STEL to flee toward the direction of the net.

Sawhorses (N = 5), measuring 2.5 m long and 3 m tall, were equipped with 2 Reconyx remote-triggered cameras at each end that photographically recorded species that activated the sensor triggers of the camera. Cameras were placed viewing straight down, suspended from sawhorses at a height that provided a 7.7 m^2 image area. Cameras were turned on at the beginning of each survey period and recorded images continuously until the end of the survey period. Images were recorded to species.

Five grids were thoroughly searched by 3 people for 20 minutes per 10 x 10 m grid; therefore, searches were approximately 5 person-hours per survey. STEL encountered were enumerated and recorded.

Three detection dogs were trained to locate STEL odor (Stevenson et al. 2010). A live-specimen STEL was used for dog training purposes. Trained dogs, one at a time, individually searched randomly selected grids (N = 5) as directed by the trainer for 20 minutes per 10 x 10 m grid. If no STEL were found, dogs then were allowed to search the entire 1 ha enclosure and signal if it located a STEL. Number of STEL encountered were recorded.

Road cruising with an ATV was conducted by a driver and observer along the outside perimeter of the enclosure and had an unobstructed view of the outer 36 quadrants of the enclosure. The quantity of road measured ~400 m, which was driven <5 kph, observer kept watch along the path for STEL and for movement, and recorded STEL observed.

Soil samples (25 g each) from the soil surface were collected from 10 random points within a randomly selected grid and composited as a single 250-g sample. Soil composites from 5 grids were sent to an eDNA lab at the University of Victoria (British Columbia, Canada) for presence of DNA matching STEL.

In addition, the entire 1-ha enclosure was searched by 5 observers for 60 minutes for a total of 5 person-hours. Observers were spaced 5 m apart (25 m width) and walked the length of the enclosure in 4 swaths counting STEL until the entire enclosure was searched. When a STEL was encountered, observers stopped moving to determine where the STEL would relocate as a means to avoid recounting the STEL multiple times during a single survey.

Because of space and monetary constraints, a completely randomized design was not feasible (i.e., a design where treatments were independent). For example, with a single 1-ha enclosure, a STEL could not be captured within a funnel trap and pitfall trap simultaneously; therefore, such methods would not be independent. Under such a scenario, homogeneity of variances was not possible. Therefore, we analyzed only those methods that met the assumptions of independence and homogeneity of variances. Doing so did not affect the integrity of the study because so few survey methods were successful. Therefore, we used a completely randomized analysis of variance with repeated measures to determine differences in collection method yields at multiple lizard densities.

Because we placed a total of 5, 10, 20, 30, and 40 STEL into a 1-ha, outdoor enclosure, we believe the actual STEL density was known during the study. The study was conducted during a 50-day period; therefore, additional STEL due to reproduction within the enclosure was not possible because STEL egg incubation is approximately 5 weeks (Axtell 1954, 1956). Also, the enclosure walls were not conducive to STEL emigration, and although our study area was located within the historic range of Tamaulipan STEL, none have been documented at the study location (SE Henke, pers. observ.), which made immigration unlikely. Lastly, the briefness of the study and our predator control efforts reduced the likelihood of STEL loss due to depredation and STEL carcasses were not found that would indicate mortality by other causes.

To determine STEL detectability, we conducted visual systematic surveys with 3 - 5 searchers of the entire 1-ha enclosure. Searches required ~5 person-hours to search the 1-ha enclosure. We conducted searches during five time intervals (i.e., 0800 – 1000 hr, 1001 – 1200 hr, 1201 – 1400 hr, 1401 – 1600 hr, and 1600 – 1800 hr) during the same day for three consecutive days at each STEL density. The number of STEL observed during each survey was recorded and the probability of detection ± SE of detection and the 95% confidence intervals were calculated.

We used simple linear regression to estimate the relationship between (1) observable STEL density and detectable STEL density, and (2) visually-observable STEL density and detectable STEL density. The former relationship was, as expected, essentially a 1:1 relationship ($\hat{\beta} = 0.95238$); the slope of the latter was used to test the hypothesis that the number of visually-observable STEL increased with density. We compared slopes of these two regression models (Graybill, 1976) to investigate whether the density-dependent relationship between visually-observable STEL and detectable STEL density was proportional to observable STEL density. A *t* test was used to compare estimated number of STEL at two selected detectable densities.

RESULTS

Regarding the STEL housed within the tubs, STEL did not emerge from their underground nightly resting spot until about 1100 hr, then be active aboveground, but each would rebury itself and re-emerge throughout the day (Figure 1.2.2.). Aboveground activity between species was not noted ($F_{1,2} = 0.11$, $P = 0.78$), nor between days or the interactive effects ($F_{5\text{-}145,358} < 1.41$, $P > 0.08$); however, STEL activity did differ ($F_{5,358} = 608.9$, $P < 0.0001$) depending on the time of day. Although at no time of the day were all STEL aboveground at once, on average, more STEL were active aboveground between 1400 – 1600 hrs, followed by 1200 – 1400 hrs, 1600 – 1800 hrs, 1800 – 2000 hrs, and 0800 – 1000 hrs (Figure 1.2.2.). Therefore, peak STEL detection time occurred between 1200 – 1600 hrs with ~42% probability of observing all possible STEL.

Of the 9 survey methods attempted, funnel traps, rock mounds, cover boards, and remote cameras yielded no STEL at any density. Pitfall trapping captured 1 Plateau STEL, but fire ants (*Solenopsis invicta*) killed the STEL before our next check. Detector dogs and eDNA yielded 1 Tamaulipan STEL and 1 STEL (species not discernable), respectively, but only at a density of 40 STEL/ha. Remote cameras took 65,317 photographs, of which 2,763 photos (4.2%) contained white-tailed deer (*Odocoileus virginianus*), 347 (0.5%) contained insects (e.g., walking sticks (*Diapheromera femorata*), grasshoppers, (Order: Orthoptera), crickets (Order: Orthoptera), moths (Order: Lepidoptera), etc.), 222 (0.34%) contained small mammals (e.g., opossum

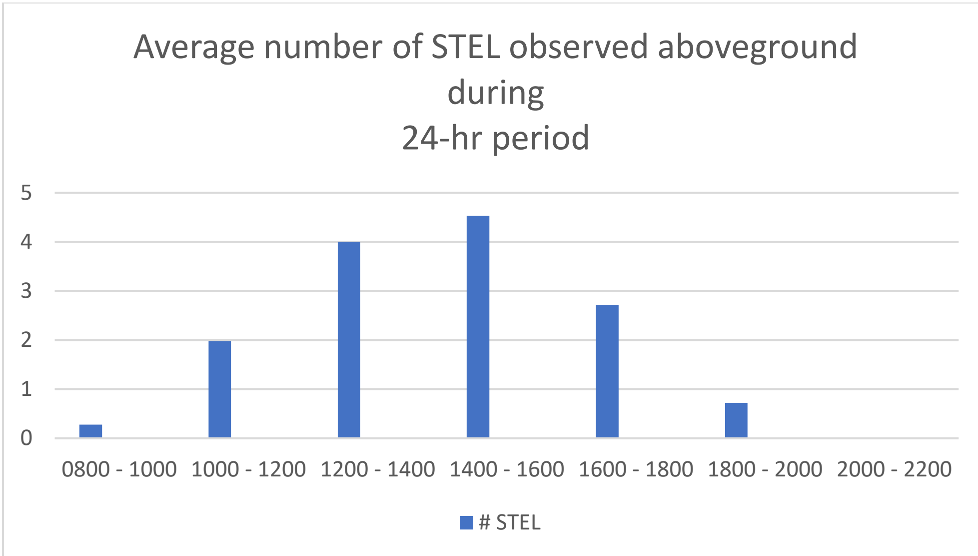


Figure 1.2.2. Average number of spot-tailed earless lizards observed aboveground within the 4, 2.5-m diameter plastic tubs out of the known 10 STEL/tub during June – July, 2021. The peak time of day to find the most STEL (~42%) occurred between 1200 – 1600 hr.

(*Didelphis virginiana*), armadillo (*Dasypus novemcinctus*), cotton rats (*Sigmodon hispidus*), and cottontail rabbits (*Sylvilagus floridanus*)), while the remaining photographs (i.e., 61,985; ~95%) were of wind-blown vegetation and resulting shadows caused by the sun's angle on the camera mount. STEL were not observed in any photograph. Only systematic visual searches of the entire enclosure and road cruising produced quantifiable results.

Comparing road cruising and systematic visual searches, differences between the methods ($F_{1,20}$ = 3.37, P = 0.08) and interactive effects with density ($F_{4,20}$ = 0.69, P = 0.61) were not noted (Figure 1.2.3.). However, a density effect ($F_{4,20}$ = 5.59, P = 0.004) was observed (Figure 1.2.4.). As the density of STEL increased, the number of STEL observed during surveys did increase once the density exceeded 20 STEL/ha (Figure 1.2.4.). However, the number of STEL observed during surveys did not increase proportionately as the density of STEL increased and the difference between observed STEL and potentially detectable STEL became greater with increasing STEL density (Figure 1.2.5.).

The detectable and observable STEL density points lie perfectly on a line with slope = 0.9524. We regressed visually observed STEL points on observable STEL density points, yielding an estimated slope $\hat{\beta} = 0.2052 \pm 0.025$ and $r^2 = 0.96, P = 0.0037$. We then selected 2 values along the abscissa for further examination. At "detectable" = 5, estimated "visually observed" is $\hat{Y}_{x=5} = 0.74954 \pm 0.1632$ (solid green dot at detectable = 5).

The corresponding "observed" point is 4.76 (solid blue dot at detectable = 5); we considered this value as known and tested the hypothesis that $\mu_{x=5} = 4.76$ with a one-sample *t* test, using $\hat{Y}_{x=5}$ as our estimate of $\mu_{x=5}$; this produced $t_{3\ df} = -24$ and P = 0.0001. Similarly, at "detectable" = 2, $\hat{Y}_{x=2} = 0.13395 \pm 0.2149$ (solid green dot at detectable = 2) with "observed" = 1.90476 (solid blue dot at detectable = 2); with $t_{3\ df} = -8.24$ and P = 0.0037.

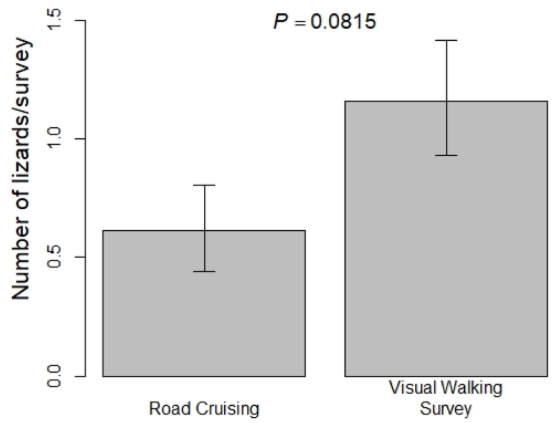


Figure 1.2.3. Best survey methods for locating spot-tailed earless lizards were visual systematic walking searches and road cruising; however, a difference between those methods was not discernable.

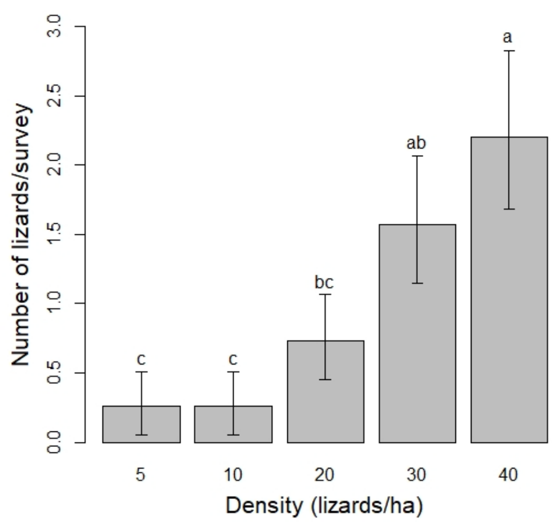


Figure 1.2.4. Although the number of spot-tailed earless lizards (STEL) observed during road cruising and walking visual searches increased with increasing lizard density ($F_{4,20}$ = 5.59, P =

0.004), the average number of STEL observed per survey only differed by 2 lizards (range = 0.25 – 2.25 STEL observed/survey).

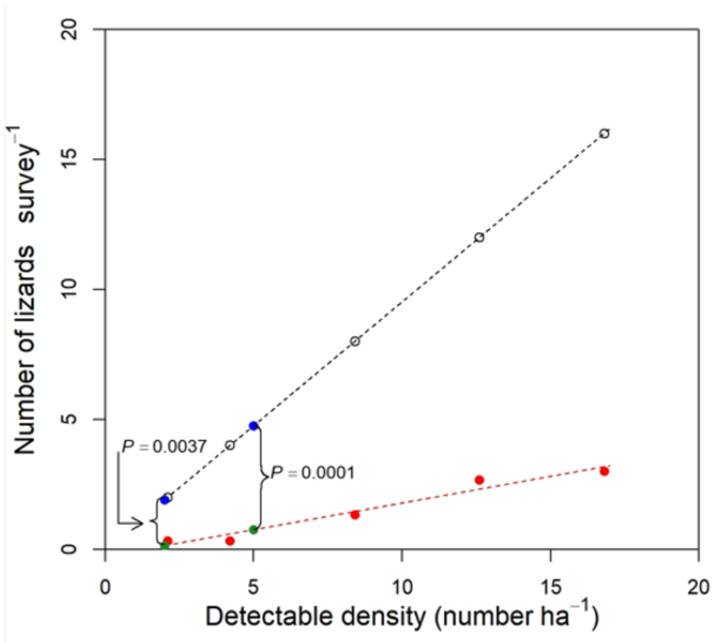


Figure 1.2.5. Comparing the detectable and observable number of STEL at various lizard densities (open black dots) with the number of STEL actually observed (red dots), a significant difference in slope exists. This makes it impossible to use the number of STEL observed to extrapolate population size. If one observes a STEL, it confirms their presence but tells nothing of abundance. If one does not observe STEL, it does not confirm their absence.

Overall, 55 STEL were observed during the 75 systematic visual searches, with 0, 9, 37, 9, and 0 STEL observations occurring during the 0800 – 1000 hr, 1001 – 1200 hr, 1201 – 1400 hr, 1401 – 1600 hr, and 1601 – 1800 hr surveys, respectively (Table 1.2.1). Detection of STEL was unlikely at any STEL density during the time intervals of 0800-1000 hr and 1601 – 1800 hr. Detection probabilities ranged from 1.7% to 13.3% but did not increase proportionally as STEL density increased (Table 1.2.1).

DISCUSSION

We determined that the best methods to survey STEL are road cruising and walking searches. Road cruising has the advantages of traversing a greater distance than walking within the same allotment of time, and one can get closer to STEL with vehicles than approach them on foot (Rangel et al. 2022a). However not all locations are accessible by vehicle so walking searches would be appropriate in such cases.

Many survey methods and techniques were unsuccessful. Perhaps STEL within our study did not traverse the enclosure as much as expected. Hibbitts et al. (2021) stated that STEL had a much larger home range (e.g., 2 – 7 ha) than other lizard species of similar life history characteristics. However, if STEL did not move from quadrant to quadrant, then perhaps they did not encounter the various structures and drift fences, which would account for their lack of presence by many of the attempted techniques.

Such low capture rates by pitfall and funnel trapping did not warrant the effort expended. Time required to dig pitfall buckets and array drift fences into the ground, especially in areas of harder clay soils, was time-consuming. Additionally, the use of trapping methods in areas where fire ants are present is not advisable because STEL escape from ants becomes limited, and as I found, had mortal consequences.

Table 1.2.1. Average number of spot-tailed earless lizards (STEL; *Holbrookia lacerata* and *H. subcaudalis*), equally placed within a 1-ha enclosure at 5 different densities, that were observed and the related percent detectable (odds; 1 out of x) during 3 systematic searches of the enclosure, which occurred within 5 times during the same day in August – September, 2021, in southern Texas. Because STEL were not observed during time intervals 0800 – 1000 hr and 1601 – 1800 hr, the probability of detection at those times were unlikely at any STEL density and therefore, not listed below.

	Survey times (N = 3 surveys conducted at each time and density)											
	1001-1200 hr				1201-1400 hr				1401-1600 hr			
Density	STEL #[1]	Prob ± SE[2]	Low CI[3]	Up C[i4]	STEL #	Prob ± SE	Low CI	Up CI	STEL #	Prob ± SE	Low CI	Up CI
5	0	Unlikely[5]	--[6]	--	2	0.133 ± 0.09	0.0336	0.4054	0	Unlikely	--	--
10	1	0.033 ± 0.03	0.0047	0.2020	2	0.067 ± 0.04	0.0167	0.2307	0	Unlikely	--	--
20	1	0.017 ± 0.02	0.0023	0.1090	6	0.100 ± 0.04	0.0456	0.2053	1	0.017 ± 0.02	0.0023	0.1090
30	3	0.033 ± 0.0.02	0.0108	0.0983	12	0.133 ± 0.04	0.0773	0.2203	2	0.022 ± 0.02	0.0056	0.0845
40	4	0.033 ± 0.02	0.0126	0.0854	15	0.125 ± 0.03	0.0768	0.1970	6	0.050 ± 0.02	0.0226	0.1068

[1]Total number of STEL observed during 3 surveys at each time interval and each STEL density.

[2]Probability of detecting a STEL ± SE during visual systematic surveys at each time interval and each STEL density.

[3]Lower 95% confidence interval.

[4]Upper 95% confidence interval.

[5]If STEL were not observed during any of the 3 visual systematic surveys, the probability of STEL detection was unlikely rather than zero.

[6]If STEL were not observed during surveys, then confidence intervals were undefined.

Much of the currently known distribution of STEL occurs in association with crop fields (Kahl et al., 2022); therefore, we built our enclosure to have characteristics consistent of crop fields (i.e., plowed, pliable soil for ease of burying, volunteer forbs and grasses to serve as cover and to entice insects as a food source). However, by doing so, perhaps the need for artificial structures was eliminated. STEL have been noted to bury themselves throughout a diel cycle (Rangel et al. 2022b), which we provided ample habitat in which to bury. Although STEL also have been observed to use rocks for basking and crevices for hiding and/or thermoregulation, those observations were in areas of hard-compacted soils (Hibbitts et al. 2019).

It was surprising that STEL were not caught by remote cameras. Approximately 1% of our enclosure was monitored by cameras and >65,000 photographs were taken, even of quite small subjects such as crickets and grasshoppers clearly visible. Because STEL have a documented home range of about 2.2 ha and 7.7 ha (Minimum Convex Polygon estimator) for Plateau and Tamaulipan STEL, respectively, with little overlap between conspecifics (Hibbitts et al. 2021), and with our final density of 40 STEL within our 1 ha enclosure potentially requiring up to 44-154X the size of enclosure area, it does seem reasonable that at least one STEL would have passed within the camera range.

Although detector dogs have experienced much success with other reptile species such as eastern box turtles (*Terrapene carolina carolina*; Kapfer et al. 212), forest geckos (*Hoplodactylus granulatus*; Browne et al. 2015), and brown tree snakes (*Boiga irregularis*; Engeman et al. 2002), detector dogs in our study were unable to locate STEL. Though the three dogs each appeared highly motivated and kept their noses to the ground, none ever signaled that a STEL was located. We acknowledge that the issue may have been with the dogs that were trained; however, we equally surmise that perhaps STEL do not produce a scent, or possibly they produce a substance that can hide their scent from potential predators. However, this is only speculative and needs further study.

Environmental DNA is an emerging tool that has promise as a survey technique for STEL. However, at present, we equate the method to ‘finding a needle in the haystack.’ One must collect a soil sample from upon which a STEL rested. Close to an actual resting spot will still produce a negative result. For example, eDNA was successful in locating Burmese python (*Python bivittatus*) refugia, but only with telemetry-monitored pythons. The method was not successful in locating snake refugia without the knowledge of known sites. We believe the method could potentially be successful if an attractant could be developed to entice STEL to a specific site. Such has been the case in attracting sharp-tailed snakes (*Contia tenuis*) to use asphalt shingles for thermoregulation and then swabbing the artificial cover object for eDNA detection (Matthias et al. 2021).

It is worthy to note that even with the knowledge of the actual density of STEL, one will, at best, observe less than half because of their burying behavior. Unfortunately, even knowing this, STEL density was not predictable. Even an assessment of relative abundance between areas could be problematic. At low densities, the number of observable STEL did not differ. Even as densities became greater, observable STEL numbers did not increase substantially, or in proportion to their increasing density. Therefore, observing STEL in an area only reliably allows one to note their presence. As with many rare and elusive species, not finding them in an area does not necessarily equate to their absence from that area, but only that they were not observed on a specific date and time.

Literature Cited

Axtell, R. W. 1954. The systematic relationships of certain lizards in two species groups of the genus Holbrookia. M.S. Thesis. University of Texas, Austin, Texas, USA.

Axtell, R. W. 1956. A solution to the long-neglected Holbrookia lacerata problem and the description of two new species of Holbrookia. Bulletin of the Chicago Academy of Sciences 10:163-179.Beane, J. C., S. P. Graham, T. J. Thorp, A. M. Mills, P. M. Dixon, and J. W. Gibbons. 2014. Natural history of the southern of the southern hognose snake (*Heterodon simus*) in North Carolina, USA. Copeia 2014:168-175.

Browne, C. M., K. J. Stafford, and R. A. Fordham. 2015. The detection and identification of tuatera and gecko scents by dogs. Journal of Veterinary Behavior 10:496-503.

Duran, M., and R. W. Axtell. 2010. A rangewide inventory and habitat model for the spot-tailed earless lizard, *Holbrookia lacerata*. Report submitted to Texas Parks and Wildlife Department, Austin, Texas.

Durtsche, R. D., P. J. Gier, M. Fuller, W. I. Lutterschmidt, R. Bradley, C. K. Meier, and S. C. Hardy. 1997. Ontogenetic variation in the autoecology of the Greater Earless Lizard *Cophosaurus texanus*. Ecography 20:336-346.

Enge, K. M., and K. N. Wood. 2002. A pedestrian road survey of an upland snake community in Florida. Southeastern Naturalist 1:365-380.

Engeman, R. M., D. S. Vice, D. York, and K. S. Gruver. 2002. Sustained evaluation of the effectiveness of detector dogs for locating brown tree snakes in cargo outbound from Guam. International Biodeterioration and Biodegradation 49:101-106.

Gibbons, J. W., and R. D. Semlitsch. 1981. Terrestrial drift fences with pitfall traps: an effective technique for quantitative sampling of animal populations. Brimleyana 7:1-16.

Grant, B. W., A. D. Tucker, J. E. Lovich, and R. Shine. 1992. The use of coverboards in estimating patterns of reptile and amphibian biodiversity. Pages 378-403 *in* D. R. McCullough and R. H. Barrett, editors. Wildlife 2001. Elsevier Science, London, England, United Kingdom.

Hibbitts, T. J., W. A. Ryberg, J. A. Harvey, G. Voelker, A. M. Lawing, and C. S. Adams. 2019. Phylogenetic structure of *Holbrookia lacerata* (Cope 1880) (Squamata: Phrynosomatidae): one species or two? Zootaxa 4619:1.6.

Hibbitts, T. J., D. K. Walkup, T. J. LaDuc, B. D. Wolaver, J. P. Pierre, M. Duran, D. Neuharth, S. Frizzell, C. S. Adams, T. E. Johnson, D. Yandell, and W. A Ryberg. 2021. Natural history of the spot-tailed earless lizards (*Holbrookia lacerata* and *H. subcaudalis*). Journal of Natural History 55:495-514.

Ingram, M. 2018. The Endangered Species Act in Texas: a survey and history. Texas Public Policy Foundation, Austin, Texas.

Kahl, S. S., C. Portillo-Quintero, R. Cox, N. McIntyre, and G. Perry. 2022. Landscape assessment of west and south Texas grasslands to inform conservation of spot-tailed earless lizards in Texas. Final Report to Comptroller's Office of Public Accounts of Texas. Austin, Texas. 50pp.

Kapfer, J. M., D. J. Munoz, and T. Tomasek. 2012. Use of wildlife detector dogs to study eastern box turtle (*Terrapene carolina carolina*) populations. Herpetological Conservation and Biology 7:169-175.

Maldonado, J. A., T. J. Firneno, Jr., C. E. Roelke, N. D. Rains, J. Mwgiri, and T. K. Fujita. 2020. Transcriptome sequencing reveals signatures of positive selection in the spot-tailed earless lizard. Plos ONE: https://doi.org/10.1371/journal.pone.0234504.

Matthias, L., M. J. Allison, C. Y. Maslovat, N. Hobbs, and C. C. Helbing. 2021. Improving ecological surveys for the detection of cryptic, fossorial snakes using eDNA on and under artificial cover objects. Ecological Indicators 131. https://doi.org/10.1016/j.ecolind.2021.108187.

Pike, D. A., B. M. Croak, J. K. Ibb, and R. Shine 2010. Subtle – but easily reversible – anthropogenic disturbance seriously degrades habitat quality for rock-dwelling reptiles. Animal Conservation 13:411-418.

Rangel, E. D., S. E. Henke, C. A. Moeller, L. Willard, C. B. Eversole, and R. A. Ayala. 2022a. Approach tolerances and escape distances of Plateau (*Holbrookia lacerata*) and Tamaulipan (*H. subcaudalis*) spot-tailed earless lizards. Herpetology Notes 15:267-270.

Rangel, E. D., S. E. Henke, C. A. Moeller, L. Willard, C. B. Eversole, and R. A. Ayala. 2022b. *Holbrookia lacerata* (Plateau spot-tailed earless lizards) and *H. subcaudalis* (Tamaulipan spot-tailed earless lizard) burying behavior. Herpetological Review 53:497-498.

Stevenson, D. J., K. R. Ravenscroft, R. T. Zappalorti, M. D. Ravenscroft, S. W. Weigley, and C. L. Jenkins. 2010. Using a wildlife detector dog for locating eastern indigo snakes (*Drymarchon couperi*). Herpetological Review 41:437-442.

Todd, B. D., C. T. Winne, J. D. Wilson, and J. W. Gibbons. 2007. Getting the drift: examining the effects of timing, trap type, and taxon on herpetofaunal drift fence surveys. American Midland Naturalist 158:292-305.

Willson, J. D. 2016. Surface-dwelling reptiles. Pages 125-138 *in* C. K. Dodd, Jr., editor. Reptile Ecology and Conservation, Oxford University Press, Oxford, England, United Kingdom.

TASK 2 A, B, & C

ASSESSMENT OF ENVIRONMENTAL DNA (eDNA) TO DETERMINE THE PRESENCE OF SPOT-TAILED EARLESS LIZARDS

ABSTRACT

Spot-tailed earless lizards (STEL; *Holbrookia lacerata* and *H. subcaudalis*) are cryptic, elusive lizards that have been identified for potential federal threatened status listing. Environmental DNA (eDNA) has been offered as having potential to assess areas for the presence of STEL because eDNA is less sensitive than traditional surveys for detecting reptiles. Therefore, we assessed the potential of using eDNA as a viable survey technique to assess for the presence of STEL. Although we could not develop assays specific to the species level that accurately assessed the presence of *H. lacerata* and *H. subcaudalis*, we could reliably predict the presence of the genus *Holbrookia* with 67% accuracy. A 33% false negative rate was due to humic substances in the soil, which inhibited PCR amplification. We found that STEL needed to be in contact with soil substrate for at least 24 hours to reliably detect eDNA, but that humid conditions common to Texas during the summer degraded eDNA quickly. In addition, locating specific sites where STEL had rested in the soil for adequate time was equated to 'finding a needle in a haystack.' Lures or pheromones to entice STEL to specific areas for eDNA sampling are suggested, but such lures have not been identified. Therefore, eDNA is not recommended as a survey technique to assess STEL presence at this time.

INTRODUCTION

The spot-tailed earless lizard (*Holbrookia lacerata*) is an elusive, and seemingly rare lizard that was once separated into two subspecies (*H. l. lacerata* and *H. l. subcaudalis*; Axtell

1968). Today the lizards are considered two distinct species, the Plateau spot-tailed earless lizard (*Holbrookia lacerata*) and the Tamaulipan spot-tailed earless lizard (*Holbrookia subcaudalis*; Hibbits et al. 2019, Maldonado et al. 2020).

Initially the single species was considered to have an historical range from coastal Texas between Baffin Bay and Corpus Christi Bay, north to Austin, extended westward to Midland, Texas, and included northeastern Mexico (Axtell 1968). Today the Balcones Escarpment fault line separates the northern *H. lacerata* from the southern *H. subcaudalis* populations (Maldonado et al. 2020).

Both species have experienced sharp declines in their abundance and distribution, but the population of Tamaulipan spot-tailed earless lizards is feared to have been so severe that local extinctions have occurred (Wolaver et al. 2018). Fragmented populations can lead to isolated pockets of remaining populations and result in homozygosity, which in turn, can result in loss of genetic diversity and lead to inbreeding depression (Maldonado et al. 2020). Anecdotal information suggests that Tamaulipan spot-tailed earless lizards are very rare and scattered; whereas, the Plateau spot-tailed earless lizards may occur in small concentrations throughout its historic range (iNaturalist.com; accessed 25 November 2020). Hypotheses for the decline of both species include pesticides, invasive fauna, and the invasion of brush and exotic grasses (Duran and Axtell 2010). In addition, agricultural practices and urbanization have been offered as potential factors in the decline (Wolaver et al. 2018); however, Axtell (1968) deemed anthropomorphic habitat modifications as advantageous to the species.

The Plateau spot-tailed earless lizard was petitioned by WildEarth Guardians for federal protection in January 2010 (Ingram 2018). The USFWS in 2011 produced a '90-day finding' report that suggested that listing the spot-tailed earless lizard as threatened may be warranted, placing much emphasis on the distributional overlap between spot-tailed earless lizards and red-imported fire ants (*Solenopsis invicta*). Critics of the USFWS findings argue that too little of actual data of the species' abundances and distributions occur, and without such data, a federal listing is unwarranted.

Use of environmental DNA (eDNA) has been offered as having potential to assess areas for the presence of spot-tailed earless lizards because eDNA is less sensitive than traditional surveys for detecting reptiles (Fediajevaite et al. 2021). Environmental DNA is organismal cellular material, such as excrement, skin cells, mucous, etc., that can be found and monitored in the environment (Rees et al. 2014). Application of eDNA has been used to determine the presence of rare species, cryptic species, and for early detection of invasive species (Matthias et al. 2021). Although much research involving the use of eDNA has been in aquatic habitats (Beng and Corlett 2020, Ruppert et al. 2019), studies also successfully detected snake eDNA in soil samples from the field (Katz et al. 2021, Matthias et al. 2021).

Therefore, our goal was to determine if eDNA could be a viable survey technique to assess the presence of spot-tailed earless lizards within their historic range. Specific objectives were to 1) determine if DNA from *Holbrookia lacerata* and *H. subcaudalis* could be isolated for use in eDNA analyses, 2) quantify the time required with soil contact to determine the presence of eDNA, 3) determine the longevity of eDNA detectability with exposure to UV light, humidity, and the combination of UV light and humidity, and 4) determine if a threshold of STEL density is required to detect the presence of eDNA.

METHODS

We hand-collected 24 spot-tailed earless lizards (12 Plateau and 12 Tamaulipan STEL) from Tom Green (31.38194 N, -100.31361 W; WGS 84) and Nueces (27.71444 N, -97.84250 W; WGS 84) counties, respectively, via road-cruising adjacent to crop fields during May, 2021. STEL were transported to the Texas A&M University-Kingsville campus and placed in individual 25 × 15 ×15 cm plastic containers with screen mesh lids that contained 500-g of sandy loam soil. Each container was equipped with a reptile 100-watt ceramic heat emitter bulb (ZooMed Laboratories, San Luis Obispo, CA 93401), ReptiSun 10.0 UVB, 13-watt, compact fluorescent lamp (ZooMed Laboratories, San Luis Obispo, CA 93401), and a Repti Basking 100-watt spot LED lamp (ZooMed Laboratories, San Luis Obispo, CA 93401). Bulbs were placed inside separate ZooMed mini-dome lamp fixtures that were fixed to the top of the container and were plugged into timers to control to simulate natural daylength. Live crickets were provided daily as food after STEL emerged from their underground burrows and water was provided in shallow dishes *ad libitum*.

For objective 1, we collected tissue, blood, and swabs from STEL ((*H. lacerata*), 6M:6F; (*H. subcaudalis*), 4M:8F), related species (i.e., Texas horned lizards (*Phrynosoma cornutum*), 2M:3F), and abundant reptile species that were associated within the STEL community (i.e., Texas spiny lizard (*Sceloporus olivaceus*), 2M; six-lined racerunner (*Aspidoscelis sexlineata*), 3M:5F) as voucher specimens for DNA verification. Tissue samples from each lizard included one toe clipping and the tip of the tail, placed in separate sterile vials with 2 mL of RNA*later* solution (Thermo Fisher Scientific, Waltham, MA 02451). Blood from the toe and tail clippings were blotted with Whatman 903™ Protein Saver Cards (Cytiva, Buckinghamshire, UK). Separate cloacal, mouth, and body swabs with Puritan PurFlock Ultra Sterile swabs (Puritan

Medical Products Company, Guilford, MA 04443) were collected from each lizard and placed in individual separate sterile vials. All samples were marked for species, sample type, and individualized number. Mitogenome sequence in GenBank for *H. lacerata* as developed by Hibbitts et al. (2019) were used to conduct eDNA assays.

To obtain positive eDNA samples from STEL, STEL were maintained in the containers described above for 30 days. Each day the STEL would be removed from its container while a researcher, wearing latex gloves, would thoroughly mix the soil in the container to avoid potential eDNA being isolated in a portion of the container. After the 30-day period of exposure to STEL, soil from each container was placed in individual freezer bags, and stored frozen until analysis.

As a blind test, we randomly selected 9 soil bags for which *H. lacerata* had contact, 9 bags for which *H. subcaudalis* had contact, and collected 9 bags with 500-g of sandy soil from Kleberg County, Texas, which was outside the historic range of STEL as control samples. Bags were randomly ordered and labeled 1- 27. Blind test was devised to verify that eDNA could be accurately detected from soil samples. Voucher specimens and their associated samples, and soil samples were sent to the Helbing Lab, University of Victoria, British Columbia, Canada, for eDNA analyses.

For objective 2, STEL were maintained in containers as previously described, except STEL contact on the soil substrate was for 10 minutes, 1 hour, 12 hours, 24 hours, and 48 hours. Five replications for each time period were collected. Once the time period for STEL contact on the soil expired, STEL were removed, and soil was placed in individually identified freezer bags and stored frozen until analyses. This test was devised to determine the minimum quantity of contact time with soil for STEL eDNA to become detectable.

For objective 3, STEL were maintained in containers as previously described and allowed to be in contact with the soil for 24 hours. After which, the containers, sans STEL and lids, were placed in an environmental chamber that exposed the containers to 12 hours of UV light/day for 1, 2, 5, 10, 20, and 30 days. Five replicate containers at each of the 6 UV light exposure levels were collected. After which, soil was placed in individually identified freezer bags and stored frozen until analyses. The above-described experiment was repeated twice. The first time, instead of UV light, the environmental chamber was maintained at 70% relative humidity for 24 hours/day for 1, 2, 5, 10, 20, and 30 days. The second time, a combination of 70% relative humidity and 12 hours of UV light/day for 1, 2, 5, 10, 20, and 30 days was conducted. These tests were devised to determine the longevity for possible eDNA detection under typical south-central Texas summer conditions (i.e., 12 hour/day exposure to UV light, ~70% RH, 30 – 40 C; https://www.weather.gov).

For objective 4, see Methods section of Task 1 A&B, Chapter 2, *Efficacy of various survey methods to detect spot-tailed earless lizards*. In brief, a 1-ha outdoor enclosure that was subdivided into 10 × 10 m quadrants was built, in which 5, then 10, then 20, then 30 and lastly 40 STEL were placed within the enclosure. At each STEL density, including prior to the placement of STEL into the enclosure at 0 density as a control, 5 randomly selected quadrants were selected. The top 5mm of soil from the selected quadrant was collected with a hand-held, cordless Dust Buster vacuum (Black + Decker, Baltimore, MD 21202) that had a plastic bag liner inside the collection chamber to avoid cross-contamination between samples. A 500-g sample of soil was collected from each quadrant, placed in a freezer bag marked for STEL density and quadrant number, and stored frozen until analyses.

All samples were shipped frozen to the Helbing Lab of the University of Victoria (Victoria, British Columbia, Canada V8P 5C2) for eDNA analyses. Environmental DNA analyses were conducted according to the methods of Veldhoen et al. (2016) and Langlois et al. (2021). In brief, samples were randomized to avoid operator or plate bias by assigning a DNA Processing Number (DPN) to each sample prior to eDNA extraction and analysis and samples were processed in DPN order. Immediately after DNA extraction, samples were cleaned using a PCR inhibitor removal protocol as a preliminary measure to remove any potential qPCR inhibitory compounds present, such as tannins and organic acids, which can inhibit the function of the enzymes used in qPCR. All samples then underwent the Integrit-DNA quality control process to determine DNA viability. The Integrit-DNA step tests for chloroplast DNA in the sample. Environmental samples have high concentrations of chloroplast DNA present, and so it acts as a natural and reliable positive control. If chloroplast DNA is not detected to an appropriate level in the sample, then it is unlikely that target DNA, which is present at much lower concentrations than chloroplast DNA, will be detected and false negatives would be expected. If a sample failed the Integrit-DNA test, the sample was cleaned using the PCR inhibitor removal protocol a second time. The samples were then re-tested with the integrit-DNA assay to determine if an improvement in sample quality occurred, before running samples on the target assay. If a sample did not pass the quality control, its data was viewed with caution in taxon-specific results, which commonly results in false negatives or an underestimation of eDNA copy number. As an additional quality control measure, each qPCR plate was run with both positive and negative controls to ensure reliable data was produced. For example, if all qPCR plate positive controls and all no template plate negative controls were positive and negative, respectively, then the plates were of good quality. All target DNA samples were tested in 8 replicates on a qPCR plate; therefore, frequencies indicate how many individual wells detected a positive result for the target species. Samples were considered ‘positive’ if 2 or more individual wells positively reacted for DNA of the target species, ‘suspected’ if only 1 individual well reacted positively, and ‘not detected’ if the frequency was 0/8 for all replicates.

Chi-square analyses were conducted to determine equal proportionality amongst various datasets (e.g., if false negative samples during the blind test were similar between the two species of STEL). Significance was inferred at $P < 0.05$.

RESULTS

Our plan to develop assays specifically to detect eDNA of both *H. lacerata* and *H. subcaudalis* failed. The available full mitogenome in GenBank appeared to be from a different population than the samples we collected, and thus, failed to amplify DNA. Using just the partial mitogenome, the DNA from both *H. lacerata* and *H. subcaudalis* was amplified; therefore, we conducted assays for eDNA at the genus *Holbrookia* level. Assay was able to differentiate between *Holbrookia* spp. DNA from DNA of associated community-level reptile species.

Within the blind test of known *Holbrookia* spp. soil samples and controls, 9 soil samples were correctly identified as containing *Holbrookia* eDNA with 7 of those samples having all 8 individual plate wells as positive and 2 samples having 4 of their 8 individual plate wells as positive (Table 2.1.1). All 9 control soil samples were correctly identified as negative for *Holbrookia* spp. eDNA (Table 2.1.1). However, 9 soil samples were incorrectly identified as negative for *Holbrookia* spp. eDNA, when in fact, they should have contained *Holbrookia* spp. eDNA (Table 2.1.1). Of the 9 false negative results, 4 and 5 samples were from *H. subcaudalis* and *H. lacerata*, respectively, which did not differ ($\chi^2 = 0.22$, $P = 0.64$) proportionately between the two species. False positive results were not determined (Table 2.1.1). Therefore, our assay to assess eDNA for *Holbrookia* spp. had a 67% accuracy.

Table 2.1.1. Accuracy of the genetic assay to determine environmental DNA from soil samples that had contact and no contact with spot-tailed earless lizards (*Holbrookia lacerata* and *H. subcaudalis*).

		Actual results		
		Positive	Negative (Control)	
Assay results for eDNA	Positive:	9 (True positives)	0	(False positives)
	Negative:	9 (False negatives)	9	(True controls)

For objective 2, prevalence of positive soil samples for *Holbrookia* spp. eDNA was 20% (N = 5) for soil samples that had STEL contact for 10 minutes, 1 hr, 6 hr, and 12 hr, but increased to 100% prevalence once STEL was in contact with soil for 24+ hr (Table 2.1.2). In addition, the mean number of eDNA positive plate wells/sample (N = 8) was <2 wells for up to 12 hr of soil contact, but increased to >5 wells for 24 and 46 hr of soil contact (Table 2.1.2). The proportion of positive plates wells increased significantly (χ^2 = 67.7, P < 0.0001) with 24 and 48 hrs of STEL contact with the soil, which accounted for 67% of the χ^2-value.

Table 2.1.2. Determination of longevity that *Holbrookia* spp. needed to be in contact with soil substrate (i.e., 10 minutes, 1 hr, 6 hr, 12 hr, 24 hr, or 48 hr) to deposit detectable DNA in soil.

Time interval	Prevalence (N =5)[1]	Frequency (N = 8 wells)	
		$\bar{x}$ ± SE[2]	%[3]
10 minutes	25%[4]	1.2 ± 2.5	15.6
1 hr	20%	0.6 ± 0.9	5.0
6 hr	20%	0.8 ± 1.3	7.5
12 hr	20%	1.6 ± 3.6	20.0
24 hr	100%	6.4 ± 2.5	80.0
48 hr	100%	5.4 ± 2.4	67.5

[1]Prevalence = (Number of positive *Holbrookia* spp. eDNA soil samples)/5 samples/time interval.

[2]Mean number (± SE) of positive *Holbrookia* spp. eDNA wells/8 plate wells/sample.

[3]Percent of positive *Holbrookia* spp. eDNA wells/40 wells/time interval.

[4]One sample failed quality control; therefore, values are based on 4 samples and 32 well plates.

For objective 3, eDNA degraded quickly in the presence of UL light and humidity (Table 2.1.3). In the presence of UL light only, 17% (i.e., 5 of 30) of soil samples displayed detectable eDNA for *Holbrookia* spp. up to 5 days exposure. However, positive eDNA for *Holbrookia* spp. was not detectable within any sample after 5 days exposure to UV light (Table 2.1.3). Exposure to humidity and the combination of humidity and UV light was more erratic (Table 2.1.3), with only 10% (3 of 30) of soil samples displaying detectable eDNA for *Holbrookia* spp. Therefore, we experienced an 83 – 90% chance of receiving a false negative result for detectable *Holbrookia* spp. eDNA when soil is exposed to 70% humidity and UV light.

Table 2.1.3. Effect of UV light and humidity on the longevity (i.e., 1-, 2- 5-, 10-, 20-, and 30-days) of detectable *Holbrookia* spp. eDNA within soil samples after *Holbrookia* spp. had 24-hr of soil contact.

		Frequency			Frequency			Frequency	
Time interval[1]	Prevalence[2]	$\bar{x} \pm SE$[3]	%[4]	Prevalence[2]	$\bar{x} \pm SE$[3]	%[4]	Prevalence[2]	$\bar{x} \pm SE$[3]	%[4]
1	20%	0.6 ± 0.6	7.5	0%	0.0 ± 0.0	0.0	0%	0.0 ± 0.0	0.0
2	40%	1.4 ± 1.0	17.5	0%	0.0 ± 0.0	0.0	20%	1.6 ± 1.6	20.0
5	40%	1.6 ± 1.0	20.0	0%	0.0 ± 0.0	0.0	0%	0.0 ± 0.0	0.0
10	0%	0.0 ± 0.0	0.0	20%	1.2 ± 1.2	15.0	40%	1.2 ± 0.8	15.0
20	0%	0.0 ± 0.0	0.0	40%	1.4 ± 1.0	17.5	0%	0.0 ± 0.0	0.0
30	0%	0.0 ± 0.0	0.0	0%	0.0 ± 0.0	0.0	0%	0.0 ± 0.0	0.0

[1]Days

[2]Prevalence = (Number of positive *Holbrookia* spp. eDNA soil samples)/5 samples/time interval.

[3]Mean number (± SE) of positive *Holbrookia* spp. eDNA wells/8 plate wells/sample.

[4]Percent of positive *Holbrookia* spp. eDNA wells/40 wells/time interval.

For objective 4, only 1 of 30 samples (3.3%) yielded detectable eDNA for *Holbrookia* spp. Four of the 8 plate wells yielded detectable eDNA for *Holbrookia* spp. from a soil sample when STEL density was 40 STEL/ha, which constituted a prevalence of 20%, mean frequency of 0.8 ± 0.8, and a percent frequency of 10%.

DISCUSSION

Environmental DNA does not appear to be a viable survey method for STEL at this time. The methodology is still in its infancy, so perhaps as advancements in technology and methodology occur, the use of eDNA for terrestrial lizards can be reassessed.

We acknowledge we did have problems in the development of assays that would be species specific (i.e., *H. lacerata and H. subcaudalis*); however, we believe our approach in the development of a genus-level assay (i.e., *Holbrookia* spp.) was still useful because the *Holbrookia* spp. in Texas do not appear to have overlapping distributions (Hibbitts et al. 2021). Plateau STEL and Tamaulipan STEL are separated by the Balcones Escarpment (Hibbitts et al. 2021), and other *Holbrookia* spp. are found in different habitat types (e.g., keeled earless lizard (*H. propinqua*) are associated with sand dunes (Axtell 1983) and lesser earless lizard (*H. maculata*) are more common in deserts (Hager 2001). Thus, a genus-level assay for our target species did not create complications for interpretation.

We did experience issues with false negative results in our blind test because some samples did not pass the Integrit-DNA quality control protocol, which indicated sample degradation and/or high concentrations of inhibitors such as tannins and humic substances. Such samples are likely to result in false negatives or an underestimation of positive frequencies because they inhibit PCR amplification (Thomsen and Willerslev 2015).

Environmental DNA studies that use water as the sampling medium are well-established and have been more successful (Beng and Corlett 2020, Ruppert et al. 2019), than eDNA studies that use soil as the sampling substrate (Kucherenko et al (2018). Katz et al. (2021) and Matthias et al. (2021) were able to detect the presence of snake eDNA in soil samples, but Ratsch et al. (2020) was unsuccessful in detecting Kirkland's snake (*Clonophis kirklandii*) eDNA from soil samples.

Another possible limitation with eDNA, at least between Reptilia and other classes, may be the morphological differences in the integument system, which results in different rates of DNA shedding. Adams et al. (2019) developed the 'Shedding Hypothesis', which states that an animal with a keratinized outer layer, such as reptiles, might shed less DNA than species with semipermeable skin; thus, reducing detectability of reptiles in the environment.

Our results highlight the need for STEL to be in contact with soil for at least 24 hours to find detectable eDNA. We believe this requirement to be problematic because behaviorally during a diel period, STEL do not remain in a single location (Rangel 2023). Instead, we have observed STEL emerging and burying into soil substrate multiple times throughout the day, but not necessarily in the same location. Site fidelity in burial locations has not been demonstrated by STEL. The nightly burrow location would be the longest diel location (~15 hrs) in which STEL would be in contact with the soil, but the likelihood of finding the exact location to sample can be equated to finding 'a needle in a haystack.' This was demonstrated by our locating only 1 site within a 1 ha area that contained detectable eDNA from *Holbrookia* spp. One objective of our study was to determine if a STEL density threshold was needed before detectable eDNA would be discovered. Even though we found eDNA at the highest density of STEL, we cannot reliably state that such a density was needed before detectable eDNA can be found. It is equally possible that such a discovery was by random chance.

We attempted to improve our chances of finding exact STEL locations by vacuuming large areas, a concept originated by Valentin et al. (2020, 2021) with dampen paint rollers; however, doing so may also increase chances of collecting inhibitory compounds that quicken the degradation of eDNA. Therefore, a tradeoff between problems and benefits may occur.

To enhance the likelihood of locating eDNA of *Holbrookia* spp., we hypothesized that if a lure was developed to entice STEL to an area and maintain them in the area for an adequate period, then one would only need to sample the soil around the lure for eDNA. Such a concept has been successful for sharp-tailed snakes (*Contia tenuis*; Matthias et al. 2021) and for invasive carp (*Cyprinus carpio*; Ghosal et al. 2022). We attempted various lures, such as lights and cover boards, to entice crickets, a prey preference (unpubl. data) of STEL, to an area. We also used visual cues of a female STEL in mating colors by painting plastic lizards with the appropriate RGB color code on its sides. Unfortunately, nothing to date was successful to lure STEL to a specified location. However, we believe if the right lure or pheromone can be developed, it will enhance the success of using eDNA as a survey method.

Until a successful lure for STEL is developed, and/or eDNA technology advances such that reptile eDNA can be easily detected from soil, eDNA is not recommended as a survey technique for STEL. Traditional survey techniques for STEL (i.e., road surveys and visual systematic searches) are superior to eDNA as a survey method. In addition, traditional surveys can yield more information such as relative abundance and density; whereas, eDNA at present yields presence/absence data.

LITERATURE CITED

Axtell, R. W. 1968. *Holbrookia lacerata*. Catalogue of American Amphibians and Reptiles 56:1-2.

Axtell, R. W. 1983. *Holbrookia propinqua*. Catalogue of American Amphibians and Reptiles. Society for the Study of Amphibians and Reptiles. 341:1-2 pp.

Beng, K. C., and R. T. Corlett. 2020. Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges, and prospects. Biodiversity and Conservation 29:2089 – 2121.

Duran, M., and R. W. Axtell. 2010. A rangewide inventory and habitat model for the spot-tailed earless lizard, *Holbrookia lacerata*. Report submitted to Texas Parks and Wildlife Department, Austin, Texas.

Fediajevaite, J., V. Priestley, R. Arnold, and V. Savolainen. 2021. Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. Ecology and Evolution 11:4803 – 4815.

Ghosal, R., A. A. Coulter, and P. W. Sorensen. 2022. Proof-of-concept studies demonstrate that food and pheromone stimuli can be used to attract invasive carp so their presence can be readily measured using environmental DNA. Fishes 7:176. https://doi.org/10.3390/fishes7040176.

Hager, S. B. 2001. Microhabitat use and activity pattern of *Holbrookia maculata* and *Sceloporus undulatus* at White Sands National Monument, New Mexico. Journal of Herpetology 35:326-330.

Hibbitts, T. J., W. A. Ryberg, J. A. Harvey, G. Voelker, A. M. Lawing, and C. S. Adams. 2019. Phylogenetic structure of *Holbrookia lacerata* (Cope 1880) (Squamata: Phrynosomatidae): one species or two? Zootaxa 4619:1.6.

Katz, A. D., L. R. Harper, E. C. Sternhagen, S. E. Pearce, C. A. Melder, J. H. Sperry, and M. A. Davis. 2021. Environmental DNA is effective in detecting the federally threatened Louisiana pinesnake (*Pituophis ruthveni*). Environmental DNA 3:409 – 425.

Langlois, V.S., M. J. Allison, L. C. Bergman, T. A. To, and C. C. Helbing. 2021. The need for robust qPCR-based eDNA detection assays in environmental monitoring and species inventories. *Environmental DNA* 3:519-527. doi:10/gmfj7s

Maldonado, J. A., T. J. Firneno, Jr., C. E. Roelke, N. D. Rains, J. Mwgiri, and T. K. Fujita. 2020. Transcriptome sequencing reveals signatures of positive selection in the spot-tailed earless lizard. Plos ONE: https://doi.org/10.1371/journal.pone.0234504.

Matthias, L., M. J. Allison, C. Y. Maslovat, J. Hobbs, C. C. Helbing. 2021. Improving ecological surveys for the detection of cryptic, fossorial snakes using eDNA on and under artificial cover objects. Ecological Indicators 131:108187. https://doi.org/10.101g/j.ecolind.2021.108187.

Rees, H. C., B. C. Maddison, D. J. Middleditch, J. R. M. Patmore, K. C. Gough, and E. Crispo. 2014. REVIEW: The detection of aquatic animal species using environmental DNA – A review of eDNA as a survey tool in ecology. Journal of Applied Ecology 51:1450 – 1459.

Ruppert, K. M., R. J. Kline, and M. S. Rahman. 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Global Ecology and Conservation 17:e00547. https://doi.org/10.10116/j.geocco.2019.e00547.

Veldhoen, N, J. Hobbs, G. Ikonomou, M. Hii, M. Lesperance, and C. C. Helbing. 2016. Implementation of novel design features for qPCR-based eDNA assessment. *PLOS ONE* 11:e0164907. doi:10/f9rpnh

Wolaver, B. D., J. P. Pierre, S. A. Ikonnikova, J. R. Andrews, G. McDaid, W. A. Ryberg, C. M. Duran, and T. J. Hibbitts. 2018. An improved approach for forecasting ecological impacts from future drilling in unconventional shale oil and gas plays. Environmental Management 61:805-818.

TASK 3 A & B

APPROACH TOLERANCE AND ESCAPE DISTANCES OF PLATEAU AND TAMAULIPAN SPOT-TAILED EARLESS LIZARDS

ABSTRACT

The Tamaulipan and Plateau spot-tailed earless lizards (STEL; *Holbrookia subcaudalis* and *H. lacerata*, respectively) are species of conservation concern and are currently being considered for federal threatened status in the United States. It is imperative to determine escape behavior in response to survey methods because altered behavior could affect detectability of the species, and thus, lead to incorrect conclusions about abundance and population status. We conducted driving and walking transects to determine lizard tolerance (i.e., flight initiation distance) to approaching vehicles and humans and the distance they fled (i.e., escape distance) when approached too close. Both Tamaulipan and Plateau STEL were more tolerant of approaching vehicles than humans. However, Tamaulipan STEL had larger flight initiation distances and had longer escape distances when disturbed by humans and vehicles than Plateau STEL. Thus, driving may offer the best method to positively identify STEL during surveys.

INTRODUCTION

The Plateau spot-tailed earless lizard (*Holbrookia lacerata*; also known as the northern spot-tailed earless lizard; STEL) and Tamaulipan spot-tailed earless lizard (*H. subcaudalis*; also known as southern STEL) are phrynosomatid lizards that are known to inhabit grasslands, agricultural fields, oak (*Quercus* sp.)-juniper (*Juniperus* sp.) woodlands, mesquite (*Prosopis* sp.) brushlands, and anthropogenically disturbed areas (Hibbitts et al., 2021). Prior to 2019, the two lizards were considered subspecies (*H. l. lacerata* and *H. l. subcaudalis*, respectively), but recently they have been recognized as two distinct species (Hibbitts et al., 2019). The Plateau

STEL are endemic to central Texas while Tamaulipan STEL are found within the Tamaulipan Biotic Province of southern Texas and adjacent northern Mexico, with the Balcones escarpment geographically separating the two species (Hibbitts et al., 2019).

Both species, Plateau and Tamaulipan STEL, have experienced population declines in recent decades making them species of conservation concern (Duran and Axtell, 2010). So much in fact that WildEarth Guardians (2010) petitioned to have Plateau STEL considered for federal threatened or endangered listing under the Endangered Species Act in the United States (United States Fish and Wildlife Service, 2011). Tamaulipan STEL already are considered a threatened species by Secretariat of Environment and Natural Resources (SEMARNAT) in Mexico (Lazcano et al., 2019). However, their status in the United States is currently being assessed.

For any species petitioned for listing, it is imperative to obtain baseline information of population abundance and distribution to determine if population declines and range reductions are occurring in order to make an informed decision concerning federal listing. Hibbitts et al. (2021) noted that both Plateau and Tamaulipan STEL fled upon approach to avoid detection during walking surveys, but they did not quantify the distance at which the individual lizard of either species first fled from an approaching threat (i.e., flight initiation distance). Because STEL have a cryptic color pattern (Axtell, 1956) and spend a portion of their day buried (Neuharth et al., 2018), these factors, coupled with their wariness, can create difficulties in obtaining such baseline information. Therefore, our objectives were to determine: (1) the approachability of Plateau and Tamaulipan STEL to walking and driving surveys (hereafter, flight initiation distance), and (2) the distance they fled when approached too close (hereafter, escape distance). If both, or either species of STEL, are wary of approaching humans (e.g., on foot or in a vehicle), as to render them undetectable, it could lead to incorrect conclusions concerning their abundance or distribution, which could affect their status for being listed as threatened or endangered.

METHODS

Two sites were selected based on recent records of STEL occurrence. The site selected for Plateau STEL was caliche and dirt roads within oak-juniper rangeland used for cattle grazing in Kimble County (30.47694 N, -99.78457 W; WGS 84), Texas, USA. Additionally, the site selected for Tamaulipan STEL was dirt roads surrounded by cotton (*Gossypium* sp.) fields in Nueces County (27.71565 N, -97.86863 W), Texas, USA.

Driving and walking surveys were conducted with 2 observers at both sites during August 2021, and the survey method was randomly selected for each surveyed road at each site. Roads were only surveyed once to avoid potential habituation to survey methods by STEL. Survey roads were either driven at 8 kph or walked at 2-3 kph down the center of the road. During driving surveys, observer 1 would sit on the hood at the front of the truck while observer 2 drove the truck. During walking surveys, both observers would walk side-by-side. When a STEL was observed, observer 1 would be driven or walked slowly directly toward the STEL until the STEL would flee. Observer 2 would mark the location with a Presco steel wire stake flag (Forestry Suppliers, Jackson, MS 39284) as to where the observers were when they first spotted the STEL fleeing (i.e., location 1). Upon the STEL fleeing, observer 1 would continue to the location where the STEL was first observed and mark the location with a Presco steel wire stake flag (i.e., location 2), while observer 2 would observe and mark the location where the STEL stopped its initial flee attempt (location 3). Distances between locations 1 and 2 were considered the flight initiation distances or approach tolerance of STEL, and the distances between locations 2 and 3 were the escape distances. Distances between the 3 marked locations were measured with a 50 m tape measure and recorded for each STEL.

We used a general linear model analysis of variance (SAS Institute, Inc., 1994) to test the main effects of species and approach method (i.e., approach by vehicle or walking human), and their interaction, on the flight initiation distances and escape distances of Tamaulipan and Plateau STEL. If significant interactions were detected, single variates of the interaction were analyzed separately within each grouping of the other main effect. Homogeneity of variances among treatments was evaluated with the Bartlett's test (Steel and Torrie, 1980). Distributions of residual errors were tested and verified for normality via the Shapiro-Wilk test. All means are reported ± 1 standard error.

RESULTS

We located 12 Tamaulipan and 13 Plateau STEL for which we were able to obtain distance measurements. We observed species by treatment interactions ($F_{1,46}$ = 55.4; P < 0.0001 and $F_{1,46}$ = 13.3; P < 0.0007) for both flight initiation and escape distances, respectively. Similar behavior patterns were observed by the two species with respect to the method of approach (i.e., vehicle and human) in their flight initiation and escape distances. There was no difference ($F_{1,23}$ = 0.08; P = 0.78) between species when each was approached by a vehicle, allowing the truck to get within 2 m before fleeing (Table 3.1.1.). However, Tamaulipan STEL were warier ($F_{1,23}$ = 87.9; P < 0.0001) than Plateau STEL when approached by humans. Human observers were able to approach Plateau STEL nearly twice as close as Tamaulipan STEL (Table 3.1.1.). Both species, Tamaulipan and Plateau STEL, were more tolerant ($F_{1,22}$ = 159.0; P < 0.0001 and $F_{1,24}$ = 10.2; P < 0.004, respectively) of a vehicle approaching them than a human (Table 3.1.1.). In addition, both species were similar ($F_{1,23}$ = 0.08; P = 0.78) in their mean escape distance when approached by a vehicle, but Tamaulipan STEL fled ($F_{1,23}$ = 14.0; P = 0.001) a farther distance than Plateau STEL when approached by humans (Table 5.1.). Also, both species, Tamaulipan and Plateau STEL had a greater escape distance ($F_{1,22}$ = 25.5; P <0.0001 and $F_{1,24}$ = 34.0; P < 0.0001, respectively) when approached by humans compared to a vehicle (Table 3.1.1).

DISCUSSION

Both species of STEL were warier of approaching humans than of approaching vehicles, which can hamper surveys if walking transects are used. Hibbitts et al. (2021) noted similar behavior by both species of STEL. Because both STEL species co-occur with other species of sprinting lizards, such as six-lined racerunners (*Aspidoscelis sexlineatus*), obtaining close proximity for positive identification could be difficult when conducting walking transects, especially if vegetation partially obstructs the view. Therefore, driving surveys for STEL appears to be a superior method because 1) observers can get closer to STEL before they flee, and 2) the higher vantage points from a vehicle, especially standing in the bed of a truck, provides a comprehensive and wide view of the area.

Table 3.1.1. Average minimum distance (m) tolerated (i.e., flight initiation distance) and escape distance by Tamaulipan (*Holbrookia subcaudalis*) and Plateau (*H. lacerata*) spot-tailed earless lizards to an approaching vehicle or walking human.

	Tamaulipan spot-tailed earless lizard (N = 12)				Plateau spot-tailed earless lizard (N = 13)			
	Vehicle (N=6)		Human (N=6)		Vehicle (N=7)		Human (N=6)	
	$\bar{x} \pm$ SE	Range	$\bar{x} \pm$ SE	Range	$\bar{x} \pm$ SE	Range	$\bar{x} \pm$ SE	Range
Flight Initiation distance (m)	2.1 ± 0.2 Aa[1,2]	1.0 – 3.0	5.2 ± 0.2 Ab	4.2 – 6.1	2.0 ± 0.1Aa	1.4 – 2.9	2.7 ± 0.2Bb	1.8 – 4.0
Escape distance (m)	2.3 ± 0.1 Aa	1.6 – 3.0	12.2 ± 2.0 Ab	7.3 – 32.1	2.1 ± 0.1 Aa	1.6 – 2.8	4.9 ± 0.5 Bb	2.2 – 7.9

[1]Means with the same capital letter are not significant (P > 0.05) between species within the same approach method.

[2]Means with the same lowercase letter are not significant (P > 0.05) between approach methods within the same species.

Tamaulipan STEL appear warier than Plateau STEL, and although not measured, seemed much faster than Plateau STEL. We noted that Plateau STEL co-occur with Plains Lubber grasshoppers (PLG; *Brachystola magna*), which, in Texas, only occur in the western portion of the state, adults are flightless, and they have similar color, color pattern, and size (i.e., brown-green color with conspicuous black dots along its back; 43-55 mm in length; Burleson, 1974) as Plateau STEL. In addition, PLGs prefer cropland field margins and use roadside plants for cover (Burleson, 1974) much like Plateau STEL. We hypothesized that because PLGs can occur in densities up to 10 grasshoppers/m^2 and they secrete a non-toxic, but foul smelling and distasteful foam when harassed (Burleson, 1974), that Plateau STEL may use mimicry of the PLGs as a potential anti-predation technique. Thus, perhaps Plateau STEL did not evolve to require greater wariness and speed as Tamaulipan STEL. However, such a hypothesis is speculative and requires future testing.

Although not quantified within our study, it is noteworthy that on several occasions the observers first heard movement in the vegetation located on the edge of the road, which caused them to stop and scan the area for lizards. Upon successfully observing a STEL within the vegetation, the methods of the study continued. Because STEL use their surroundings as camouflage in which to hide, observers should attempt to use both vision and hearing when assessing STEL abundance during driving surveys.

LITERATURE CITED

Axtell, R.W. (1956): A solution to the long neglected *Holbrookia lacerata* problem, and the description of two new subspecies of *Holbrookia*. Bulletin of the Chicago Academy of Science **10** (11): 161-181.

Burleson, W.H. (1974): A two-year life cycle in *Brachystola magna* (Orthoptera Acrididae) with notes on rearing and food preference. Annals of Entomological Society of America **67**(3): 526-528.

Duran, C.M., Axtell R.W. (2010): A rangewide inventory and habitat model for the spot-tailed earless lizard (*Holbrookia lacerata*). Unpublished report, Texas Parks and Wildlife Department, Austin, Texas, USA. 37p.

Hibbitts, T.J., Ryberg W.A., Harvey J.A., Voelker G., Lawing A.M., Adams C.S., Neuharth D.B., Dittmer D.E., Duran C.M., Wolaver B.D., Pierre J.P., Labay B.J., LaDuc T.J. (2019): Phylogenetic structure of *Holbrookia lacerata* (Cope 1880) (Squamata: Phrynosomatidae): one species or two? Zootaxa **4619**(1): 139-15

Hibbitts, T.J., Walkup D.K., LaDuc T.J., Wolaver B.D., Pierre J.P., Duran M., Neuharth D., Frizzell S., Adams C.S., Johnson T.E., Yandell D., Ryberg W.A. (2021): Natural history of the spot-tailed earless lizards (*Holbrookia lacerata* and *H. subcaudalis*). Journal of Natural History **55**(7-8): 495-514.

Lazcano, D., Nevarez-de Los Reyes M., Garcia-Padilla E., Johnson J.D., Mata-Silva V., DeSantis D.L., Wilson L.D. (2019): The herpetofauna of Coahuila, Mexico: Composition, distribution, and conservation status. Amphibian Reptile Conservation **13**(2): 31-94(e189).

Neuharth, D.B., Walkup D.K., Frizzell S.L., Kachel J.Z., Adams C.S., Johnson T.E., Hibbitts T.J., Ryberg W.A. (2018): *Holbrookia lacerata* (Spot-tailed earless lizard). Burying behavior. Herpetological Review **49**(3): 536-537.

SAS Institute, Inc. (1994). SAS/STAT user's guide, Version 9.4, SAS Institute Inc., Cary, North Carolina, USA. 10,923pp.

United States Fish and Wildlife Service. (2011): Endangered and threatened wildlife and plants; 90-day finding on a petition to list the spot-tailed earless lizard as endangered or threatened. Federal Register **76**: 30082-30087.

WildEarth Guardians. (2010): Petition to list the spot-tailed earless lizard (*Holbrookia lacerata*) under the U. S. Endangered Species Act. Petition submitted to the U. S. Secretary of Interior acting through the U. S. Fish and Wildlife Service. Washington, D.C., USA. 22p.

AGE DIFFERENCES IN MONTHLY ACTIVITY AND BEHAVIOR OF TAMAULIPAN SPOT-TAILED EARLESS LIZARDS (*HOLBROOKIA SUBCAUDALIS*)

ABSTRACT: Tamaulipan spot-tailed earless lizards (*Holbrookia subcaudalis*; STEL) are an elusive species whose populations appear to be declining in number and distribution. So much so that the species is being considered for federal threatened status by the U.S. Fish and Wildlife Service. Therefore, it is imperative to understand the activity patterns displayed by various age STEL to know when to assess their populations. We conducted monthly surveys during 2022 along a 38.6-km road within crop fields where known populations of Tamaulipan STEL occurred and we standardized abundance to the number of minutes and miles per STEL observed. Overall, Tamaulipan STEL populations emerge in March and can be found through December, at which point their brumation period occurs in January and February. Activity of STEL peak during summer months of June – August. A month x age interaction occurred for both the number of minutes and miles per STEL observed; however, the same basic activity pattern occurred for each measurement. Adult STEL could be found from March – November with their greatest abundance observed during June – September. Hatchling STEL occurred as early as late May and were found each month through October; however, two peaks of occurrence were noted in June and August. Juvenile STEL were observed from June – December, but their abundance increased in a 1-month lag time behind hatchling peak abundances, which suggest STEL are capable of fast growth and can transition from hatchling to juvenile size within an approximate 30-day period. Juvenile STEL enter brumation later than adults, perhaps as a strategy to extend

the growth period before entering brumation, which may increase their survivability and decrease time to sexual maturity.

INTRODUCTION

Tamaulipan spot-tailed earless lizards (*Holbrookia subcaudalis*; here after referred to as STEL) are elusive Phrynosomatidae species whose abundance and distribution has dramatically declined. Historically, Tamaulipan STEL occurred south of the Balcones escarpment from Corpus Christi to San Antonio to Del Rio, Texas, USA, with the exception of some southern coastal counties (Axtell 1968). Today only a few remnant populations appear to exist in Nueces, Jim Wells, McMullen, and Val Verde counties of southern Texas (Hibbitts et al. 2021, Rangel 2023). Due to perceived population declines, Tamaulipan STEL are currently undergoing a status review for federal threatened listing (WildEarth Guardians 2010, USFWS 2011).

Tamaulipan STEL average 61 mm snout-vent length and are a gray-green color with two distinct dorsolateral sequences of dark body blotches (Axtell 1968). Tamaulipan STEL prefer highly disturbed habitats near roadsides, crop fields, and air fields (Hibbitts et al. 2021). However, because of their small size, cryptic coloration within their preferred habitat, and few and scattered populations, Tamaulipan STEL can be difficult to detect.

Rangel et al. (2022) found that road cruising was an efficient method to observe and capture STEL. Biologists typically conduct road cruising for reptiles immediately after sunrise and sunset because reptiles seek to bask in the early morning sun and garner the warmth from roads, respectively (Dodd et al. 1989, Rosen and Lowe 1994, McDiarmid 2012).

Henke and Montemayor (1998) hypothesized that Texas horned lizards (THL; *Phrynosoma cornutum*) would be most active during the warmest months of summer, but instead found their peak activity occurred in May, which perhaps was a result of mate-seeking activity. In addition, THL were active aboveground during March–October (Henke and Montemayor 1998). Because Tamaulipan STEL are a member of the Phrynosomatidae family, we hypothesized that they would behave similar to THL. Therefore, our objectives were to determine: 1) age-specific monthly activity of Tamaulipan STEL, and 2) the phenology when Tamaulipan STEL are active aboveground.

METHODS

Study Area

We conducted monthly surveys along a 38.6-km non-paved road that traversed through crop fields (cotton, *Gossypium* sp., and maize, *Zea mays*) in Nueces County (27.7156^{o}N, -97.8686^{o}W; 5 m elevation), Texas, USA. We selected this site because the area had a known viable population of Tamaulipan STEL (Rangel et al. 2022).

Surveys

Road cruising was conducted 1 day per week for 3 weeks each month from January–December 2022. Surveys began after 1200 hr on sunny days with <10% cloud cover and < 20 kph wind speeds. Such weather parameters were selected because a companion study determined that such parameters yielded the greatest number of STEL per km and/or per minute ratio (Rangel et. al. 2022). Road cruising was selected as the survey method because it has been documented as a superior method to locate STEL (Rangel et al. 2022), and STEL exhibit a greater approach tolerance to road cruising (Rangel et al. 2022). Survey route was driven at 8 kph and consisted of 2 – 3 observers, the driver and additional observers standing in bed of the truck for a higher vantage point (Rangel et al. 2022). The beginning and ending point of the

38.6-km driving route was marked with Presco steel wire stake flags (Forestry Suppliers, Jackson, MS 39284) for consistency between surveys. The number of STEL observed during the survey was recorded. When possible, STEL were captured, measured for snout-vent length (SVL), and released at capture site. Tamaulipan STEL that had SVL ≤30 mm, 31 – 53 mm, and ≥54 mm were considered hatchlings, juveniles, and adults, respectively. For STEL that escaped capture (i.e., N = 52, 13%), their SVL was estimated by experienced observers, and thus, placed in the appropriate age category. To test the accuracy of the observers identifying age class, observers would report perceived age class upon encounter, then proceed to capture the STEL, measure the SVL, and determine the appropriate age class based upon the measurements listed above. Estimated age class upon first encounter was compared to actual age class based on SVL measurement to determine accuracy of observers. The time required to drive the route from beginning to end for each survey was recorded, sans the minutes required during captures. As a means to standardize STEL abundance so it can be used across multiple habitats and roads, we calculated the number of km driven per STEL observed for each age class and the number of minutes driven per STEL observed for each age class.

Data Analysis

We conducted two analyses of STEL abundance, one using the combined data and another that considered age as a factor of observed abundance. For the combined analysis, we used a linear mixed model with month as a fixed effect (SAS 2012). Because of non-normal distribution of data, we used a back-transformed mean, which estimates the median, and used the upper and lower 1 standard error values above and below, respectively, the back-transformed mean for pairwise comparisons when month was significant (SAS 2012). We used a linear mixed model analysis with fixed effects of age and month and their interaction to determine if age affects monthly observations of abundance (SAS 2012). Because of non-normal distribution of data (i.e., several months with 0 abundance for a certain age class), we used permutation-based analyses on log(Y+1) data to estimate *P*-values (SAS 2012). Means and standard errors were computed on the observed scale. Chi-square analysis was conducted to determine if capture success between age classes was proportional with their observed occurrence and if monthly differences of capture success by age class was proportional with their monthly observed occurrence.

RESULTS

Tamaulipan STEL were first observed 25 March 2022 and were observed each month until 23 December 2022. Tamaulipan STEL of any age class were not observed during January and February surveys. During 2022, we observed 92 hatchling, 110 juvenile, and 190 adult STEL. We had an overall capture success of 87%; 12, 14, and 26 hatchling, juvenile, and adult STEL, respectively, escaped capture. Observers had a 96% (326/340) accuracy in correctly estimating STEL age class upon first observation. Observers incorrectly identified 3 of 80 STEL (3.8%) as hatchlings when they were actually juvenile-sized, 7 of 96 STEL (7.3%) as juveniles when 1 (1%) was actually hatchling-sized and 6 (6.2%) were actually adult-sized STEL, and 4 of 164 (2.4%) as adults when they were actually juvenile-sized STEL. Capture success was not different ($\chi^2 = 0.06$, $P = 0.97$) between hatchling, juvenile, and adult STEL, nor were monthly differences in capture success ($\chi^2 < 3.98$, $P > 0.97$) noted within an age category.

Monthly differences were noted in the overall STEL abundance for the number of km driven per STEL observed ($F_{9,18} = 89.1$, $P < 0.0001$) and for the number of minutes driven per STEL observed ($F_{9,18} = 102.5$, $P < 0.0001$). Overall, more STEL were observed during August, followed by June, July, and September, then October, May, April, November and March, and lastly December, January, and February (Table 3.2.1, Figs 3.2.1 and 3.2.2).

Age × month interactions were noted for the number of minutes driven per STEL observed ($F_{22,66}$ = 24.5, P = 0.001) and for the number of km driven per STEL observed ($F_{22,66}$ = 16.5, P = 0.001) across age classes.

Hatchlings were first observed on 17 May 2022 and were last observed on 27 October 2022. Number of minutes driven per hatchling observed ($F_{11,22}$ = 391.7, P = 0.001) and number of km driven per hatchling observed ($F_{11,22}$ = 112.7, P = 0.001) differed by month (Table 3.2.2, Figure 3.2.3). The greatest number of hatchlings were observed during August, followed by

Table 3.2.1. Average quantity of minutes or miles traveled during three monthly surveys to locate a Tamaulipan spot-tailed earless lizard (*Holbrookia subcaudalis*; STEL) on the same 38.6 km non-paved road in Nueces County, Texas, during 2022.

	Minutes/Number of STEL observed			Miles/Number of STEL observed		
Month[1]	Estimated median[2]	Lower SE[3]	Upper SE[3]	Estimated median[2]	Lower SE[3]	Upper SE[3]
March	53.8 b[4]	45.2	63.9	5.8 ab	4.5	7.4
April	32.1 c	27.5	37.4	3.3 c	2.9	3.7
May	19.3 d	17.8	20.9	2.0 d	2.0	2.0
June	10.9 g	9.8	12.2	1.1 f	1.1	1.2
July	10.8 g	10.2	11.3	1.1 f	1.1	1.2
August	10.3 g	9.7	10.9	1.0 g	0.9	1.0
September	14.1 f	13.4	14.9	1.3 f	1.2	1.3
October	16.3 e	15.5	17.2	1.6 e	1.6	1.7
November	44.1 bc	39.4	49.3	4.9 b	4.3	5.5
December	123.8 a	94.2	162.7	13.2 a	9.5	18.3

[1]January and February were not included because STEL were not observed during these months due to brumation period.

[2]Due to number of surveys/month with no observations of STEL, back-transformed mean (log(Y+1), which estimates a median, was used for analysis.

[3]Lower and upper 1 standard error values below and above the back-transformed mean.

[4]Same lower case letter for each month are not different (P > 0.05) between months.

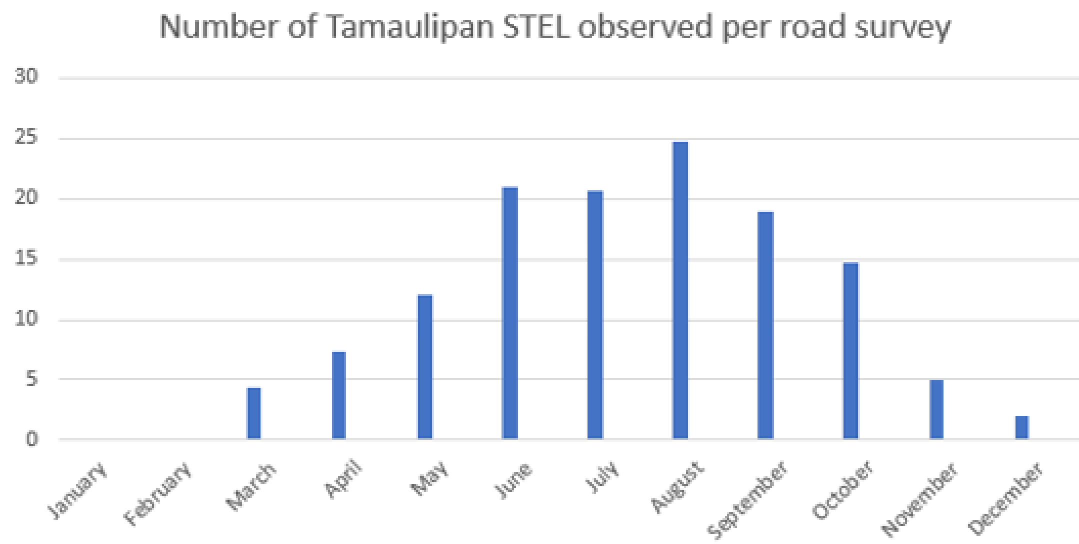


Figure 3.2.1. Average number of Tamaulipan spot-tailed earless lizards (Holbrookia subcaudalis; STEL) observed during three monthly, 36-km road surveys through crop fields with known populations of STEL in Nueces County, Texas, during 2022.

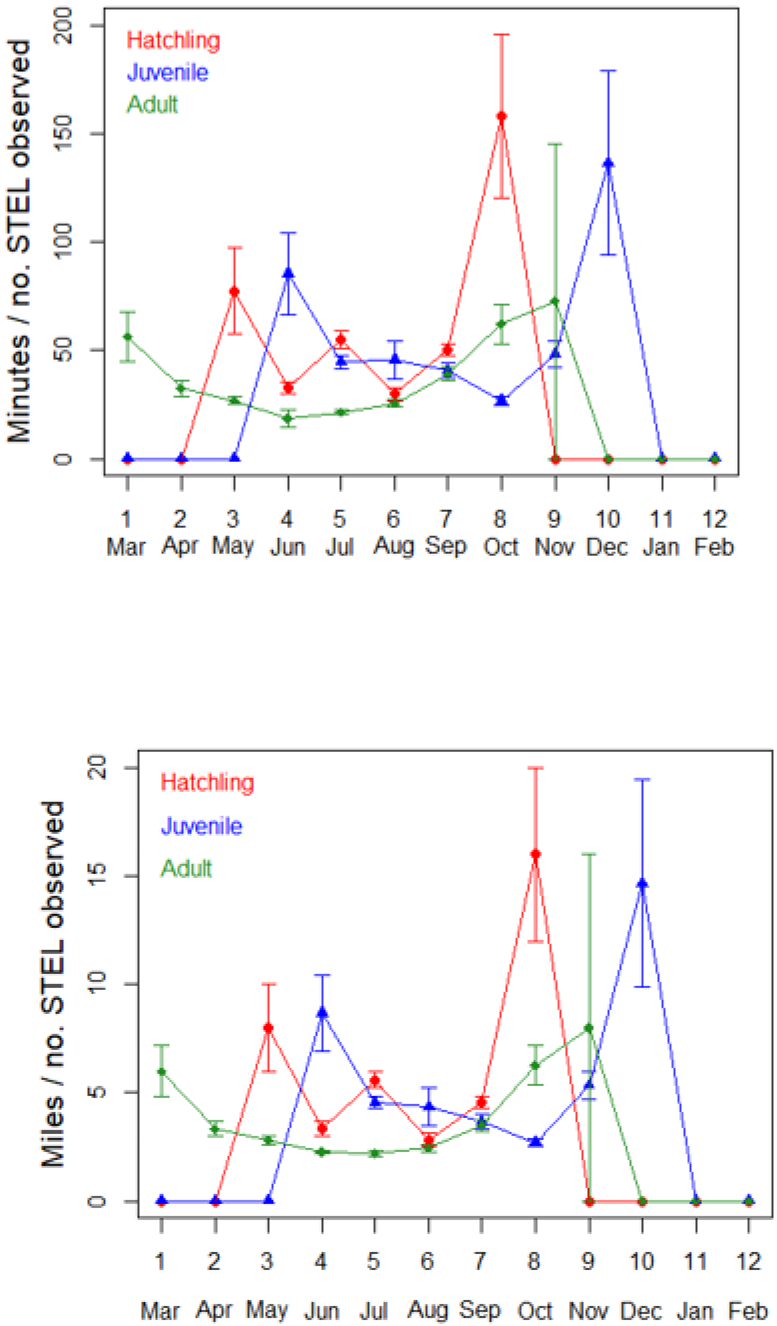


Figure 3.2.3. Average quantity of minutes (A) or miles (B) traveled during three monthly surveys to locate a hatchling, juvenile, and adult Tamaulipan spot-tailed earless lizard (*Holbrookia subcaudalis*; STEL) on the same 38.6 km non-paved road in Nueces County, Texas, during 2022.

June, then by July, September, May, and October, and lastly during the remaining months (Table 3.2.2). Hatchlings were not observed during 6 months (i.e., January – April and November – December) of the year (Figure 3.2.3).

Juvenile STEL were first observed on 28 June 2022 and were last observed on 23 December 2022. Number of minutes driven per juvenile observed ($F_{11,22}$ = 252.3, P = 0.001) and number of km driven per juvenile observed ($F_{11,22}$ = 73.5, P = 0.001) differed by month (Table 3.2.3, Figure 3.2.3). The greatest number of juvenile STEL was observed during October, followed by July – September and November, then June and December, and the remaining months (Table 3.2.3).

Adult STEL were first observed on 25 March 2022 and were last observed on 3 November 2022. Number of minutes driven per adult observed ($F_{11,22}$ = 9.5, P = 0.001) and number of km driven per juvenile observed ($F_{11,22}$ = 5.0, P = 0.002) differed by month (Table 3.2.4, Figure 3.2.3). May–September yielded the greatest number of adult STEL, followed by November, then March, April, and October (Table 3.2.4).

DISCUSSION

We hypothesized that Tamaulipan STEL, being in the same Phrynosomatidae family as Texas horned lizards, would display a similar trend in activity patterns. Texas horned lizards are active from March–October (Henke and Montemayor 1998), as we demonstrated for adult Tamaulipan STEL. However, activity of Texas horned lizards was found to peak in May and subsequently declined each month thereafter until they enter brumation (Henke and Montemayor 1998). In addition, Hibbitts et al. (2021) found activity of Tamaulipan STEL emerged in April, peaked in June, and subsequently declined each month thereafter. However, Hibbitts et al

(2021) did not categorize lizards by age class. In our study, Tamaulipan STEL activity appears more directly correlated with the warmest months during a year, which is typically August in southern Texas (Fulbright and Bryant 2002). These results coincide with a companion study that found that STEL activity is greatest during the diel peak of ultraviolet light (Rangel 2023), which is typically the warmest time of the day.

The number of hatchlings experienced two peaks (i.e., June and August), which agrees with Axtell (1956) that the breeding season for STEL peaks twice annually. Because egg development requires a 2–3 week gestation period and once laid underground require approximately 5–6 weeks to hatch (Axtell 1956), this means breeding likely occurred between early to mid-April. The first adult STEL for this study was observed during the last week of March and adult numbers increased in April; therefore, adult STEL emerge from brumation, seek mates, and breed quickly. An alternative explanation is that adults breed before entering the brumation period, females either retain sperm or experience a diapause to delay egg development, and subsequently emerge during the spring gravid. However, the latter explanation requires further study.

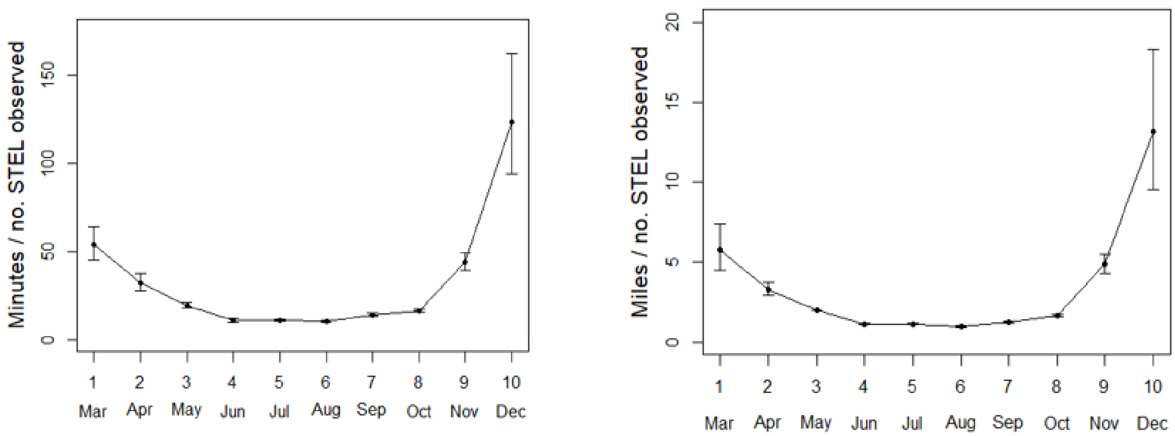


Figure 3.2.2. Graphically, the average quantity of minutes or miles traveled during three monthly surveys to locate a Tamaulipan spot-tailed earless lizard (*Holbrookia subcaudalis*; STEL) on the same 38.6 km non-paved road in Nueces County, Texas, during 2022

Table 3.2.2. *P*-values for the pairwise comparisons via t-tests between months of the log transformed data (log(munites+1) and log(miles+1)) for the average quantity of minutes (black values) and miles (red values) traveled during three monthly surveys to locate a hatchling Tamaulipan spot-tailed earless lizard (*Holbrookia subcaudalis*; STEL) on the same 38.6 km non-paved road in Nueces County, Texas, during 2022.

Month	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
March	**X**[1]	U[2]	0.001	0.001	0.001	0.001	0.001	0.002	U	U	U	U
April	U	**X**	0.006	0.001	0.001	0.001	0.001	0.004	U	U	U	U
May	0.009	0.01	**X**	NS[3]	NS	NS	NS	NS	0.01	0.004	0.006	0.005
June	0.002	0.003	NS	**X**	0.019	NS	0.02	0.038	0.003	0.001	0.002	0.003
July	0.002	0.002	NS	0.024	**X**	0.033	0.044	0.048	0.002	0.001	0.001	0.001
Aug	0.004	0.006	NS	0.036	0.031	**X**	0.034	0.03	0.001	0.002	0.003	0.001
Sept	0.001	0.004	NS	NS	0.003	0.043	**X**	0.031	0.001	0.001	0.001	0.001
Oct	0.007	0.007	NS	0.037	0.05	0.04	0.032	**X**	0.002	0.003	0.002	0.006
Nov	U	U	0.011	0.002	0.001	0.007	0.003	0.008	**X**	U	U	U
Dec	U	U	0.008	0.002	0.002	0.002	0.002	0.008	U	**X**	U	U
Jan	U	U	0.004	0.007	0.003	0.002	0.001	0.004	U	U	**X**	U
Feb	U	U	0.009	0.004	0.002	0.005	0.003	0.008	U	U	U	**X**

[1]Same months cannot be compared for differences in the number of STEL observed.

[2]Average number of STEL observed for the month was 0; therefore, the denominator was 0, causing the *P*-value to be undefined (U).

[3]NS = Not significant.

Table 3.2.3. *P*-values for the pairwise comparisons via t-tests between months of the log transformed data (log(munites+1) and log(miles+1)) for the average quantity of minutes (black values) and miles (red values) traveled during three monthly surveys to locate a juvenile Tamaulipan spot-tailed earless lizard (*Holbrookia subcaudalis*; STEL) on the same 38.6 km non-paved road in Nueces County, Texas, during 2022.

Month	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
March	**X**[1]	U[2]	U	0.003	0.002	0.004	0.003	0.002	0.001	0.006	U	U
April	U	**X**	U	0.006	0.001	0.002	0.001	0.003	0.003	0.002	U	U
May	U	U	**X**	0.001	0.001	0.002	0.001	0.001	0.003	0.006	U	U
June	0.008	0.011	0.007	**X**	NS	NS	NS	0.041	NS	NS	0.006	0.004
July	0.001	0.002	0.001	NS	**X**	NS	NS	0.003	NS	NS	0.001	0.001
Aug	0.012	0.008	0.012	NS	NS	**X**	NS	NS	NS	0.018	0.006	0.004
Sept	0.005	0.003	0.003	NS	NS	NS	**X**	NS	NS	0.04	0.001	0.001
Oct	0.003	0.002	0.004	0.044	0.006	NS	NS	**X**	NS	0.035	0.001	0.002
Nov	0.003	0.004	0.011	NS	NS	NS	0.015	0.05	**X**	0.049	0.002	0.002
Dec	0.009	0.013	0.008	NS	NS	0.019	0.047	0.036	NS	**X**	0.01	0.007
Jan	U	U	U	0.008	0.002	0.007	0.004	0.003	0.003	0.013	**X**	U
Feb	U	U	U	0.007	0.003	0.011	0.003	0.002	0.005	0.014	U	**X**

[1]Same months cannot be compared for differences in the number of STEL observed.

[2]Average number of STEL observed for the month was 0; therefore, the denominator was 0, causing the *P*-value to be undefined (U).

[3]NS = Not significant.

Table 3.2.4. *P*-values for the pairwise comparisons via t-tests between months of the log transformed data (log(munites+1) and log(miles+1)) for the average quantity of minutes (black values) and miles (red values) traveled during three monthly surveys to locate an adult Tamaulipan spot-tailed earless lizard (*Holbrookia subcaudalis*; STEL) on the same 38.6 km non-paved road in Nueces County, Texas, during 2022.

Month	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
March	**X**[1]	NS[3]	NS	NS	NS	NS	NS	NS	NS	0.003	0.006	0.005
April	NS	**X**	NS	NS	0.033	NS	NS	NS	NS	0.002	0.001	0.002
May	0.046	NS	**X**	NS	NS	NS	0.008	0.017	NS	0.001	0.001	0.001
June	NS	0.038	NS	**X**	NS	NS	NS	NS	NS	0.007	0.007	0.004
July	NS	0.021	NS	NS	**X**	0.011	0.049	0.043	NS	0.001	0.001	0.001
Aug	NS	0.029	NS	NS	0.02	**X**	NS	NS	NS	0.002	0.002	0.001
Sept	NS	NS	NS	NS	NS	NS	**X**	0.021	NS	0.002	0.002	0.001
Oct	NS	NS	0.015	0.037	0.032	0.049	0.013	**X**	NS	0.001	0.003	0.002
Nov	NS	NS	NS	NS	NS	NS	NS	NS	**X**	NS	NS	NS
Dec	0.015	0.006	0.002	0.001	0.001	0.002	0.007	0.005	NS	**X**	U	U
Jan	0.012	0.003	0.002	0.002	0.001	0.002	0.004	0.006	NS	U	**X**	U
Feb	0.008	0.002	0.001	0.001	0.002	0.002	0.004	0.008	NS	U	U	**X**

[1]Same months cannot be compared for differences in the number of STEL observed.

[2]Average number of STEL observed for the month was 0; therefore, the denominator was 0, causing the *P*-value to be undefined (U).

[3]NS = Not significant.

Juvenile STEL were not observed until midsummer, which was about one month after hatchling STEL were observed. This suggests that STEL potentially have a fast growth rate and are capable of obtaining juvenile and adult size within their hatchling year. However, the number of juvenile STEL observed increased each month and peaked during October, rather than experienced a second peak, as did the number of hatchling STEL. It's possible that food sources for STEL peak during May, which enable a faster growth rate for STEL during this period, and as the summer progresses and conditions become drier, food sources are not as plentiful and growth rates subsequently slows. Typical rainfall patterns of southern Texas include peaks of precipitation during May and September/October (Fulbright and Bryant 2002). Therefore, it is possible that the prevalence of insects increases during or immediate after a wetter period; thus, providing a greater food source for STEL.

Also, juvenile STEL entered brumation later (i.e., December) than their adult counterparts (i.e., majority in October). This strategy to remain aboveground rather than enter brumation like adult STEL could be to continue to forage and take advantage of a second peak of insect prevalence, previously mentioned. An increase in weight may aid survivability during brumation and an increase in SVL size may enhance reproductive capabilities during the next spring. For example, Henke (2013) found that translocated Texas horned lizards needed to obtain a specific fat reserve to survive the brumation period. It is likely that this is also the case for STEL, as they inhabit similar habitat and experience similar environmental and physiological limitations and challenges. Because juvenile STEL were not observed until late June-early July, the above explanation appears plausible; otherwise, juvenile STEL would be expected to emerge from brumation at approximately the same time as adults.

We agree with Hibbitts et al. (2021) that adult STEL emerge in April and enter brumation in October. Our study extends the adult STEL activity by one month on each end of their active period. However, the STEL in our study emerged 1 week earlier and 3 days later, respectively, than reported by Hibbitts et al. (2021), which could have resulted from a yearly difference in weather.

We believe our capture success (i.e., 87%) was very good; however, we were unable to locate any published research for comparison. Our team did have two years of previous experience in detection via road surveys and capture of STEL. We have found that a minimum team of three observers improves capture success; one observer maintains a visual on the STEL and directs the other two observers to its movements, allowing the capture team members to get on opposite sides of the STEL to surround it. However, more observers to surround a STEL is useful, but was not quantified in this study. This capture method also gave the observers a good vantage to estimate the STEL size. We acknowledge the difficulty to categorize an observed STEL to age class if the STEL size was near the cut-off points for an age class. However, based on the accuracy rate of our observers, perhaps 2 STEL that escaped capture [(Hatchlings: 12×0.038) + (Juveniles: 14×0.073) + (Adults: 26×0.024) = 2.1] were incorrectly identified to the proper age class. We believe that such a potential error is nominal and would not affect our results or interpretation. Thus, we believe it unnecessary to reduce the STEL activity to only those individuals that were captured.

Road surveys have been identified as a successful method to survey Tamaulipan STEL (Hibbitts et al. 2021, Rangel et al. 2022), which we concur. This study demonstrates that Tamaulipan STEL are active for a longer period during the year than many reptile species, and that different age classes of STEL display different active periods during a year. Therefore, researchers of Tamaulipan STEL can use this knowledge to develop best periods to survey for Tamaulipan STEL, especially if specific age class data is required. Because STEL are currently under status review for federal listing as a threatened species in the United States (USFWS 2011), greater understanding of the natural history of STEL is needed.

LITERATURE CITED

Axtell, R. W. 1956. A solution to the long neglected *Holbrookia lacerata* problem, and the description of two new subspecies of *Holbrookia*. Bulletin of the Chicago Academy of Science 10:161 – 181.

Axtell, R. W. 1968. *Holbrookia lacerata*. Catalogue of American Amphibians and Reptiles 56:1 – 2.

Dodd, C. K., Jr., K. M. Enge, and J. N. Stuart. 1989. Reptiles on highways in north-central Alabama, USA. Journal of Herpetology 23:197 – 200.

Fulbright, T. E., and F. C. Bryant. 2002. The last great habitat. Special Publication #1 of the Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, Kingsville, Texas, USA. 35pp.

Henke, S. E. 2013. Coping with invasive invaders: Behavoral and morphological modifications

Rangel, E. D., C. Moeller, L. Willard, S. E. Henke, C. B. Eversole, and R. Ayala. 2022of the Texas horned lizard. Pages 159 – 176 *in* W. I. Lutterschmidt, editor. Reptiles in research: Investigations of ecology, physiology and behavior from desert to sea. Nova Science Publishers, New York, New York, USA. 544pp.

Henke, S. E., and M. Montemayor. 1998. Diel and monthly variations in capture success of *Phrynosoma cornutum* via road cruising in southern Texas. Herpetological Review 29:148 – 150.

Hibbitts, T. J., D. K. Walkup, T. J. LaDuc, B. D. Wolaver, J. P. Pierre, M. Duran, D. Neuharth, S. Frizzell, C. S. Adams, T. E. Johnson, D. Yandell, and W. A. Ryberg. 2021. Natural history of the spot-tailed earless lizards (*Holbrookia lacerata* and *H. subcaudalis*). Journal of Natural History 55:495 – 514.

McDiarmid, R. W. 2012. Reptile biodiversity: Standard methods for inventory and monitoring. University of California Press, Berkeley, California, USA. 412pp.

Rangel, E. D. 2023. Efficacy of various survey methods to detect spot-tailed earless lizards. M.S. Thesis. Texas A&M University-Kingsville, Kingsville, Texas, USA. 146pp.

Rangel, E. D., C. Moeller, L. Willard, S. E. Henke, C. B. Eversole, and R. Ayala. 2022. Approach tolerance and escape distances of Plateau, *Holbrookia lacerata*, and Tamaulipan, *Holbrookia subcaudalis* (Reptilia: Phrynosomatidae), spot-tailed earless lizards. Herpetology Notes 15:267 – 270.

Rosen, P. C., and C. H. Lowe. 1994. Highway mortality of snakes in the Sonoran Desert of southern Arizona. Biological Conservation 68:143 – 148.

SAS Institute, Inc. 2012. SAS/STAT user's guide, Version 9.4., SAS Institute Inc., Cary, North Carolina, USA.

USFWS. 2011. Endangered and threatened wildlife and plants; 90-day finding on a petition to list the spot-tailed earless lizard as endangered or threatened. Federal Register 76:30082 – 30087.

WildEarth Guardians. 2010. Petition to list the spot-tailed earless lizard (*Holbrookia lacerata*) under the U. S. Endangered Species Act. Petition submitted to the U. S. Secretary of Interior acting through the U. S. Fish and Wildlife Service. 22pp.

TASK 3 C & D

GIS ANALYSIS OF SPOT-TAILED EARLESS LIZARD HABITAT.

ABSTRACT: This study employed Geographic Information System (GIS) and Species Distribution Models (SDMs) to analyze the habitat of *Holbrookia lacerata* and *H. subcaudalis*. The study encompasses a dataset of 253 field observations across Texas, diligently classified into confirmed sightings and non-detection sites. Methodologically, our study integrated a diverse array of environmental variables, land use data, and anthropogenic factors. This included a detailed examination of soil types via the Soil Survey Geographic Database (SSURGO), and an analysis of land cover using the 2019 National Land Cover Database. These datasets are augmented with information on proximate anthropogenic structures, notably oil and gas wells and other infrastructure components, sourced from the Texas Railroad Commission (TXRRC). This multifaceted approach enables a nuanced understanding of the biophysical and human-influenced factors shaping STEL habitats. We used an exploratory logistic regression and MaxEnt modeling to discern key variables influencing STEL habitat preference. We used an iterative evaluation of variable combinations, allowing for the identification of the most impactful factors across different populations of the species. Notable variables include distance to roads, type of vegetation, soil characteristics, and proximity to active oil wells and pipelines. The study delineates varying habitat preference patterns across the total, southern, and northern populations of STEL, providing insights into the spatial heterogeneity of their ecology and distributions. Our results demonstrate that both natural and anthropogenic factors influence STEL distribution. For instance, the proximity to active oil wells exhibits a contrasting influence on STEL presence between the northern and southern populations, highlighting the species' sensitivity to localized environmental conditions. The results from our logistic regression and

MaxEnt predictive models demonstrate habitat suitability, facilitating targeted conservation and survey planning. The detailed habitat models developed not only guide conservation efforts but also underscore the need for ongoing habitat monitoring and data collection. Due to dynamic environmental conditions, our results demonstrate the need for adaptive management strategies, fueled by continuous data-driven updates to habitat models.

INTRODUCTION

Since the incorporation of new statistical methods and GIS tools, the development of predictive species distribution models (SDMs) has expanded in the field of ecology, biogeography, and conservation (Raes, 2012). SDMs are generally based on describing how climatic and environmental factors relate to occurrence locations in geographic space, in order to delineate suitable habitat over local, regional, and global scales. Common applications for species modeling include forecasting current, past, and future climates, studying relationships between environmental parameters and species richness, mapping invasive species habitat range, and conservation planning (Melo-Merino et al., 2020).

Reptiles and specialist species, in particular, are good candidates for SDMs due to their narrow range of suitable environmental factors and relatively limited geographic extent, this generally leads to higher species model performance (Hernandez et al., 2006). Specialist species are also extremely susceptible to anthropogenic factors like that of climate change, increased agriculture pressure, and urbanization. These habitat alterations can eventually lead to habitat fragmentation for select species, which can result in demographic isolation, population decline, or species extirpation (Ricketts, 2001; Vrba, 1987). Habitat modeling provides a visual representation for the distribution of a species' fundamental niche, often used as a key component in understanding environmental “hot spots”, mitigating habitat fragmentation, and allowing resource managers to adequately plan for current and future climate scenarios. Therefore, SDMs for specialist and at-risk species often possess a large amount of conservation utility.

The spot-tailed earless lizard (*Holbrookia lacerata*) is an elusive, and seemingly rare, lizard that was once separated into two subspecies (*H. l. lacerata* and *H. l. subcaudalis*; Axtell 1968). Today the lizards are considered two distinct species, the Plateau spot-tailed earless lizard (*Holbrookia lacerata*) and the Tamaulipan spot-tailed earless lizard (*Holbrookia subcaudalis*; Hibbits et al. 2019, Maldonado et al. 2020).

Initially the single species was considered to have an historical range from coastal Texas between Baffin Bay and Corpus Christi Bay, north to Austin, extended westward to Midland, Texas, and included northeastern Mexico (Axtell 1968). Today the Balcones Escarpment fault line separates the northern *H. lacerata* from the southern *H. subcaudalis* populations (Maldonado et al. 2020).

Spot-tailed earless lizards live in open areas of low grasslands and shrub vegetation that experience seasonal drought (Axtell 1968, Scott 1996). Spot-tailed earless lizards, like Texas horned lizards (*Phrynosoma cornutum*), appear to prefer areas free of ground litter that provide unobscured travel lanes (Fair and Henke 1997).

Far too few studies have been conducted on either species of spot-tailed earless lizards. The maximum snout-to-vent length is between 65 and 71mm, with the Tamaulipan spot-tailed earless lizard being slightly larger in length and males have a longer tail than females (Axtell 1968). Males of the Plateau spot-tailed earless lizard exhibit a reddish suffusion on the lateral neck and shoulder region during the breeding season; whereas the Tamaulipan populations do not exhibit this coloration pattern (Axtell 1956). A dearth of information exists concerning the ecology of either species.

Both species have experienced sharp declines in their abundance and distribution, but the population of Tamaulipan spot-tailed earless lizards is feared to have been so severe that local extinctions have occurred (Wolaver et al. 2018). Fragmented populations can lead to isolated pockets of remaining populations and result in homozygosity, which in turn, can result in loss of genetic diversity and lead to inbreeding depression (Maldonado et al. 2020). Anecdotal information suggests that Tamaulipan spot-tailed earless lizards are very rare and scattered; whereas, the Plateau spot-tailed earless lizards may occur in concentrations near Kingsville, Del Rio, and San Angelo (iNaturalist.com; accessed 25 November 2020). Hypotheses for the decline of both species include pesticides, invasive fauna, and the invasion of exotic grasses (Duran and Axtell 2010). In addition, agricultural practices and urbanization have been offered as potential factors in the decline (Wolaver et al. 2018); however, Axtell (1968) deemed anthropomorphic habitat modifications as advantageous to the species.

The Plateau spot-tailed earless lizard was petitioned by WildEarth Guardians for federal protection in January 2010 (Ingram 2018). The USFWS in 2011 produced a '90-day finding' report that suggested that listing the spot-tailed earless lizard as threatened may be warranted, placing much emphasis on the distributional overlap between spot-tailed earless lizards and red-imported fire ants (*Solenopsis invicta*). Critics of the USFWS findings argue that virtually nothing is known about the species; hence it is impossible to identify vulnerabilities for the species. Critics have suggested that federal listing of the Plateau spot-tailed earless lizard would hamper crude oil production of the Eagle Ford Shale (Ingram 2018). Unfortunately without actual data of the species' abundances, distributions, and ecologies, such criticisms can continue.

The USFWS is expected to render a listing proposal in 2022 as to the status of spot-tailed earless lizards.

Because spot-tailed earless lizards are a poorly known species of conservation concern, species distribution mapping is imperative in decision-making processes. Therefore, our objectives were to 1) map areas of known STEL occurrences, 2) determine habitat features that can predict potential STEL occurrences and be used in future survey planning, and 3) rank habitat characteristics and create predictive variables.

METHODS

Data acquisition and preparation

We used 253 STEL observations for the analysis. Of these, 141 were confirmed presence of lizard sightings and 112 were sites where surveys were conducted but no lizards were found. In later steps, these points were split into southern and northern populations. The southern population was comprised of sites in Jim Wells, Nueces, Kinney, and Val Verde counties. This subgroup had 37 confirmed lizard points and 48 points with no lizards. The northern population was comprised of sightings in Kimble, Crockett, Schleicher, San Angelo, Tom Green, Reagan, Glasscock, and Midland counties. The northern subset had 104 points with lizard sightings and 64 points with no lizards observed. A table with all observed points used in the analysis is attached in the appendix. Buffers of 1000m were created around each field data point as an analysis zone for detailed habitat and environmental data.

Once field data points were converted and checked, we gathered data from publicly available GIS data sources (Table 1). All data was projected into NAD 1983 UTM Zone 14N for optimal processing and accuracy. DEMs derived from this data were obtained from the Texas Natural Resources Information System (TNRIS) to use for site elevation and to calculate aspect.

Land use/ land cover data was downloaded from TNRIS in the form of the 2019 National Land Cover Database. The 16 land cover classes were examined and reclassified into broader categories- water (including wetlands), barren, cultivated, developed, forest, grassland/ pasture, and shrub/ scrub types. All raster data representing these land cover types were converted into individual polygon layers for distance calculations and clipped by the 1000m point buffers around field points. Rivers and waterbodies from the Texas Railroad Commission (TXRRC) were merged with the output "water" land cover to capture smaller waterways not covered in the NLCD layers.

Soil data was obtained from Soil Survey Geographic Database (SSURGO) through the ArcGIS Living Atlas (USA Soils Map Units). A pdf from USDA explaining all the column description types and classifications can be found here: https://www.nrcs.usda.gov/sites/default/files/2022-08/SSURGO- Metadata-Table-Column-Descriptions-Report.pdf. Soil data was reprojected and cut to the area of interest.

We downloaded oil/gas data from TXRRC for each county of presence/absence including well data, underground pipe, rail, airport, roads and water polygons/lines. For oil and gas surface wells, points denoted as "canceled location" and "permitted location" were removed. Points noted as Dry Hole, Plugged, Shut-In were combined and classified as "Surface Wells Inactive" (n=22,059) indicating that these sites would have less regular human activity around them and not be kept clear of vegetation continuously. All other wells were considered "Surface Wells Active" (n= 26,425) with regular visits by workers and maintained to be clear of vegetation. We did attempt to separate out brine well sites to see if they were significant, but there were only 5 classified as brine in a dataset of 48,000+ points. Railroad data was removed from the project as they did not cover a significant portion of the study area. Oil and gas pipelines were examined and 15 lines were removed from the analysis for having a "revoked status." Pipelines were separated into active (n= 19,957) and inactive (n= 2,026) types based on TXRCC information.

Table 1. Data and data sources for STEL GIS analysis.

Description	**Layer type**	**Source**	**Modifications/reasoning**	**Source link**
STEL locations	Point	TAMUK	Presence/absence	
LiDAR	las/lasd	USGS earth explorer	Used to calculate elevation, aspect	https://earthexplorer.usgs.gov/
Land Use, Land Cover	raster	NLCD/TNRIS	Modified land cover classes to create broad Categories	https://data.tnris.org/collection?c=97a6ce2e- 8a4c-4570-a3ed-983ef1a4554b#5.75/31.32/-100.077
Soil Data	polygon	USDA/ SSURGO	Clipped to study area	https://www.nrcs.usda.gov/resources/data-and-reports/soil-survey-geographic-database-ssurgo
Roads	line	TXDOT, TXRRC	Merged various sources of roads - txdot, txrrc	https://gis-txdot.opendata.arcgis.com/
Pipelines (Oil and Gas)	line	TXRRC	Data available per county, merged everything in AOI	https://www.rrc.texas.gov/resource-center/research/data-sets-available-for-download/

We created outlines of 1000 m buffers around all field points. Each 1000 m buffer was examined and polygons drawn around any structure- houses, barns, sheds, large tanks, etc. This did not include most oil/ gas well related anthropogenic features like pumpjacks, wellheads, or Christmas trees (a piece of equipment that provides flow control on an oil or gas well), as these would be captured in the active well layer. The final buildings layer included 1,002 individual features.

Once all GIS layers and rasters were gathered and processed, information was assimilated into the attribute table to join that information with each individual field data point. To calculate distances to these many landscape features, the Near tool in ArcPro was used. Near was used to obtain distances for each point to nearest: active well, inactive well, active pipeline, inactive pipeline, habitat polygons (water, barren, cultivated, developed, forest, grassland/ pasture, and shrub/ scrub), and building. The Extract Values to Points tool was used to assign values from associated rasters (elevation, aspect) to columns in the attribute table for all field data points.

As the next step in the project required all quantitative data, any qualitative data in the attribute table was assigned numerical values for analysis. Numerical columns were added to represent soil characteristic text for drainage class, hydrologic group, runoff class, taxonomic order, suborder, great group, and particle size.

All final GIS metadata records were created in accordance with the FGDC Content Standard for Digital Geospatial Metadata (FGDC-STD-001-1998).

Table 2. Relevant SSURGO soil tabular data and definitions

Label	Column Name	Description
Drainage Class	drainagecl	Identifies the natural drainage conditions of the soil and refers to the frequency and duration of wet periods. An example of a drainage class is “well-drained”.
Great Group	taxgrtgroup	The third level of Soil Taxonomy. The category is below the suborder and above the subgroup.
Hydric classification	hydclprs	An indication of the proportion of the map unit, that is "hydric." Hydric soils form under conditions of saturation, flooding or ponding long enough during the growing season to develop anaerobic conditions in the upper part.
Hydrologic Group	hydgrp	A group of soils having similar runoff potential under similar storm and cover conditions. Examples are A and A/D.
Irrigated Capability Class – Dominant Condition	iccdcd	The broadest category in the land capability classification system for soils. This column displays the dominant capability class, under irrigated conditions, for the map unit based on composition percentage of all components in the map unit.
Particle Size	taxpartsize	Particle-size classes are used as family differentiae. Particle-size refers to grain-size distribution of the whole soil and is not the same as texture.
Runoff Class	runoff	Runoff potential class for the soil.
Suborder	taxsuborder	The second level of Soil Taxonomy. The suborder is below the order and above the great group.
T Factor (Soil Loss Tolerance)	tfact	The maximum amount of erosion at which the quality of a soil as a medium for plant growth can be maintained.
Taxonomic Order	taxorder	The highest level in Soil Taxonomy.
WEI	wei	A value in tons/acre/year that is a factor in calculating soil loss by wind.

Logistic Modeling

The layer file and associated attribute table with all habitat/ landscape features were run through Exploratory Logistic Regression analyses to determine best explanatory variables. Using the Exploratory Regression tool in ArcPro, all variables were tried in various combinations to determine the best models to take to MaxEnt. Dependent variable in all cases was set as the presence/ absence of STEL for each field data point. Search criteria were set with a maximum number of 7 and a minimum number of 1 explanatory variables, minimum adjusted R-Square value was set at 0.05, maximum coefficient p-value cutoff was set at 0.05, and maximum VIF value cutoff was set at 7.5. Minimum acceptable Jarque Bera p value was 0.1 and Minimum acceptable spatial autocorrelation p value was set for 0.05. Three sets of data were run in these analyses: the total population including all points, the southern population, and the northern population.

When all points were run together as one large population, we could not achieve a model that passed all of the above criteria. No model was generated that passed the Jarque-Bera and Spatial Autocorrelation (Global Moran's I) tests. These tests examine for normally distributed residuals or residuals that are free from statistically significant spatial autocorrelation. All of the p-values for the Jarque-Bera summary were close to 0.000000, indicating that the data is far away from having normally distributed residuals. Likewise, all Spatial Autocorrelation p-values were 0.00000 indicating there is some significant autocorrelation.

Considering the small sample size and opportunistic characteristics of the field data observations, this is not surprising. The addition of more field data points in the future will allow for more robust models and these conditions may be met. This occurred in the analyses for the entire population and the northern population.

The southern population data subset passed all of these conditions, resulting in multiple passing models. The model with the highest adjusted R Square value included distance to road, distance to shrub/ scrub, hydric classification, hydrologic group, and runoff value.

Variables that continually ranked in the highest adjusted R-Squared results are shown below. These variables were chosen to be used in the next step, Max-Ent modeling. Columns indicate whether this variable was significant in the total population, southern, northern, or multiple models.

Table 3. Variables identified during exploratory regression as important and or significant.

Variable	**+/-**	**Total Population**	**Southern**	**Northern**
Distance to road	+ indicating STEL were more likely to be found farther from the road	X	X	
Distance to shrub/ scrub	+ indicating STEL were more likely to be found farther from shrub/ scrub habitat	X	X	
Drainage class	+ well drained soils were more likely to have STEL present	X		
T Fact (Soil loss tolerance)	- Smaller values were more likely to have STEL (maximum amount of erosion at which the quality of a soil as a medium for plant growth can be maintained)	X		X
Particle Size	- smaller particle sized soils were more likely to have STEL	X		X

Distance to active surface well	- STEL were more likely to be found closer to active surface wells in **the total population** + STEL were more likely to be found farther from active surface wells in t**he northern population**	X		X
Hydric classification	- less hydric soils were more likely to have STEL (An indication of the proportion of the map unit, that is "hydric")	X	X	
Soil Great Group	STEL were most often found in Calciustolls, Haplusterts, and Haplocalcids soils (third level of Soil Taxonomy, category is below the suborder and above the subgroup)	X		
Hydrologic group	+ C and D value groups were more likely to have STEL, (classification for runoff		X	

RESULTS AND DISCUSSION

Exploratory regression analyses showed a number of important variables. We used those variables, with some caveats, to plug into the Presence-only Prediction model (MaxEnt). We predicted range over the Texas counties that spanned the general observation points. We ran one analysis for the total dataset, one for the southern, and northern population.

For the overall range, the inputs included distance to roads, distance to shrub/scrub, type of drainage, soil loss tolerance (T Fact), particle size, distance to active surface oil wells, and hydric classification were significant.

For the Southern population, the inputs included to distance to shrub, distance to road, hydrologic group, hydric classification, and runoff classification.

For the northern population, we inputted distance to active oil wells, distance to active pipelines, distance to water, soil loss tolerance (T Fact), Irrigated Capability Class, WEI, and particle size.

To create a predictive raster, all variables needed to be converted into raster form from point, line or polygon. For variables such as surface well, active pipes, and water, where we needed to measure distance from STEL points, we used the distance accumulation tool to build a raster on a 24m2 grid. All soil data was converted into raster data from polygons, and each value field populated the raster accordingly.

To run the analysis, we chose original (linear), squared (quadratic), pairwise interaction (product), and discrete step (threshold). By choosing multiple basis functions, the tool produces multiple transformed variables and attempts to use them in the model. This informs the final raster, and the statistical reports show how well the model explains the data. The number of knots controls how many thresholds are created, which are used to create multiple explanatory variable expansions using each threshold. For this study, we chose 10 knots, which is the default. We applied spatial thinning to reduce sampling bias by removing any points that may have been sightings of the same specimen. This ensures remaining points are at minimum 150 meters apart, based on previous conversations about possible range. Spatial thinning is also applied to background points (absence data) whether they are provided in input point features or generated by the tool. No points were spatially thinned, so all points were used to train the model. Relative weight of presence to background was set at 100, on a scale from 1-100, where 100 is when the presence points are the primary source of information. When the value is close to 100, the model penalizes each misclassified presence point 100 times more than each misclassified background point (assuming that the correct classification of background is absence) and the traditional MaxEnt approach is applied.

Our presence probability transformation was set to C-log-log, which best explains more stationary species. The C-log-log link function converts the predictions to probabilities, and is recommended when the presence and location of a species is unambiguous. Presence locations were confirmed sightings, and because this species is not migratory, these sightings are considered unambiguous presence. The presence probability cutoff establishes which probabilities correspond with presence in the resulting classification. The cutoff value is used to help evaluate the model's performance using training data and known presence points and it was set to 0.8.

We used random resampling (in three groups) to validate the prediction model, which excludes a portion of the data during training of the model and uses it to test the model's performance after it has been trained.

Due to the small sample size, some variables that were identified as important in the exploratory regression stage were not able to be included in the MaxEnt analysis. MaxEnt requires a minimum of 8 total points to fall in a category of a variable in order to have an adequate sample size. This happened with the Irrigated Capability Class and the soil great group variables.

Output charts are different for continuous and categorical variables.

Continuous Variables

MaxEnt produces a Partial Response of Continuous Variables chart composed of multiple charts; each chart visualizes the effect of changing values in each explanatory variable on presence probability, while keeping all other factors the same. Below are the Partial Response of Continuous Variables charts for the three analyses.

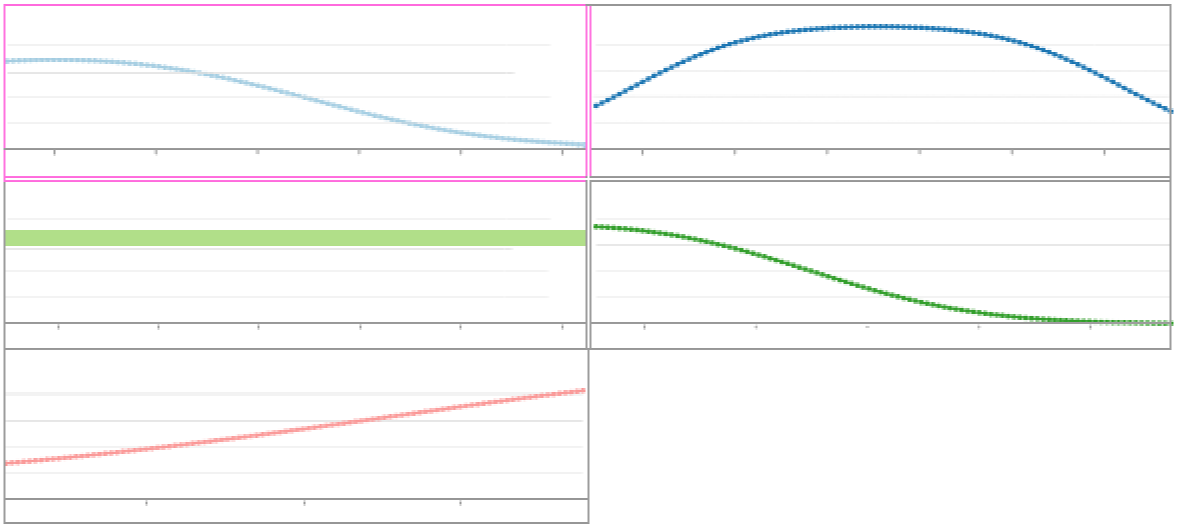


Figure 1. Partial response of continuous variables chart—Overall population.

By examining the shapes of the curves for each variable, we can see relationships between STEL presence and variable values. For example, in the middle top graph, DISTANC_ROAD1, peak probability of STEL occurs roughly 800 to 1,800 m from roadways. In the DISTANC_SURF1 graph, the relationship shows a more gradual downward slope; as distance from wells increases, STEL probability decreases. Interestingly the DISTANC_SHRUBSCRUB line is nearly horizontal, indicating that the change of this variable can't really impact the probability of presence much, holding the other variables at their average values.

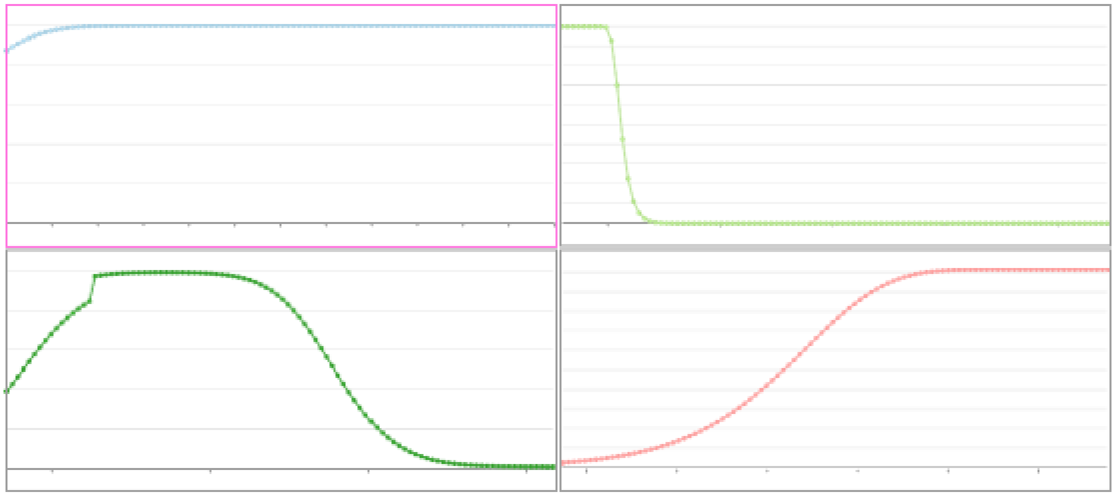


Figure 2. Partial response of continuous variables chart—Southern population.

Distance to road in the southern population was a strong variable in this model, as well as distance to shrub scrub.

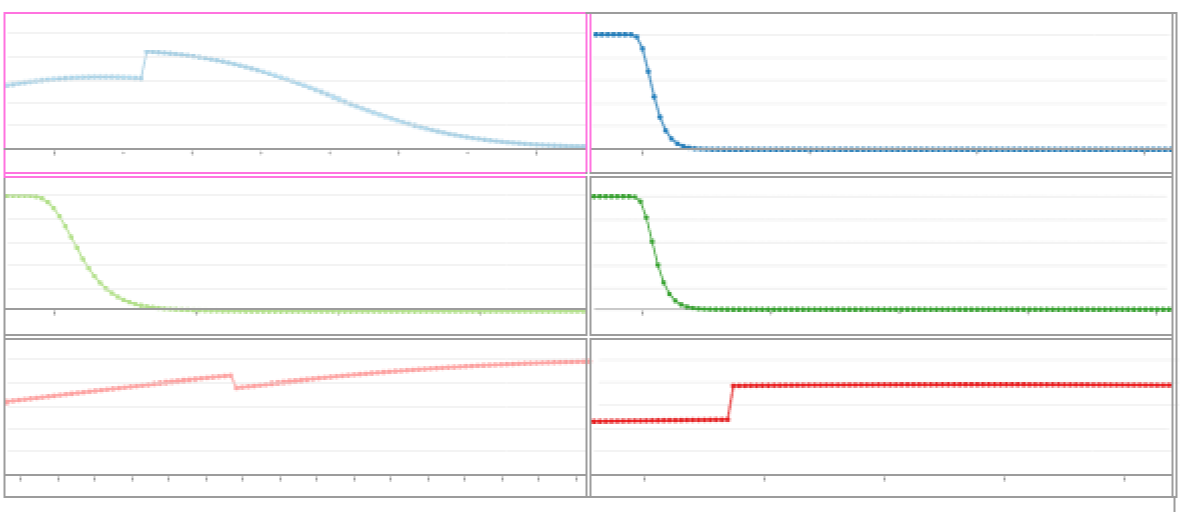


Figure 3. Partial response of continuous variables chart-Northern population

Distance to active pipeline, active surface wells, and water habitat had strong responses for STEL probability in the northern population with higher probabilities of presence closer to these features.

Categorical Variables

Categorical variables were only actively used in the southern population model. Other categorical variables were attempted, but due to lack of enough points in each category, they were removed from the analysis.

Below is the Partial Response of Categorical Variables chart for the southern analysis, showing the probability of the runoff potential variable. Areas classified as medium (1) had a slightly higher probability for STEL presence than high (3) and negligible (5). The least probability was found in the category “low” (2).

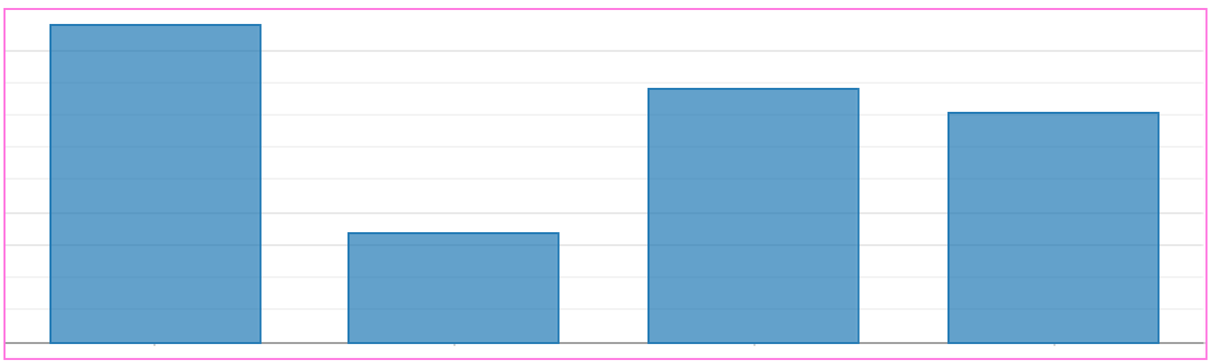


Figure 4. Partial response of categorical variables chart for runoff values in the southern population. Categories for columns left to right are medium, low, high, and negligible.

Conclusions and Future Work

Additional points will allow the model to become more precise and robust in its predictions. Once additional points are gathered, we suggest rerunning the MaxEnt analysis to refine results

Some outcomes of the exploratory regression analyses were unexpected. When all points were run together for the entire population, closer distances to active surface wells were more likely to have STEL. However, when the northern population points were separated out and run, this variable reversed- STEL were more likely to be found farther from active surface wells. In this same northern population, STEL were more likely to be found closer to active pipelines. This could indicate that the act of clearing and maintaining these pipelines are still important factors to STEL, but the high density of wells in the area could be adversely affecting STEL sightings. This is likely driven by the STEL points found southeast of San Angelo and those near Eldorado as both areas have a less dense concentration of wells than sites surveyed in Reagan County. Further data points of both STEL sightings and sites with no STEL are needed to draw a definite conclusion.

If possible, soil samples in areas of high STEL abundance should be taken to confirm the findings of the SSURGO soil database variables. Irrigated Capability Class continually appeared as an important variable, but the spatial nature of the data and coverage for that part of the state caused problems when added to the MaxEnt model. There is a broad zone through the middle of the study area that has no data for the Irrigated Capability Class raster. Due to this zone of null data, models run including this variable were misshapen and showed STEL presence in strange areas (outside of known range). Likewise, the soil great group variable was important in exploratory regression analyses, but when used in MaxEnt, there were not enough points (8 minimum) in each of the categories present. Due to this, the variable was excluded from final models. The addition of new points in a future analysis may enable this variable to be rerun and used.

MaxEnt does not assume nor require absence. MaxEnt is a general-purpose method for making predictions or inferences from incomplete information. Given a set of known presence locations and given explanatory variables that describe the study area, MaxEnt contrasts the conditions between presence locations and the study area to estimate a presence probability surface. The final MaxEnt products show three zones of probability for STEL presence. These zones are 25-49%, 50-75%, and 76-99% probability that STEL are present using the set of variables given. Maps showing TAMUK-provided field points over these zones can be found in the appendix. These maps should be used to explore future locations for potential STEL presence. There are versions of each location showing outputs from the overall, southern and northern population models. Each model shows slightly different potential probabilities due to different variable inputs, and can be viewed as collective suggestions. Further studies that differentiate district STEL populations will improve predictive capabilities as there are genetic unknowns in population relationships. Again, the addition of new field data points will enhance and create more accurate MaxEnt models.

LITERATURE CITED

Axtell, R. W. 1956. A solution to the long neglected *Holbrookia lacerata* problem, and the description of two new subspecies of *Holbrookia*. Bulletin of the Chicago Academy of Science 10:163-179.

Axtell, R. W. 1968. *Holbrookia lacerata*. Catalogue of American Amphibians and Reptiles 56:1-2.

Duran, M., and R. W. Axtell. 2010. A rangewide inventory and habitat model for the spot-tailed earless lizard, *Holbrookia lacerata*. Report submitted to Texas Parks and Wildlife Department, Austin, Texas.

Fair, W.S., and S.E. Henke. 1997. Effects of habitat manipulations on Texas horned lizards and their prey. Journal of Wildlife Management 61:1366-1370.

Hernandez, P. A., C. H. Graham, L. L. Master, and D. L. Albert. 2006. The Effect of Sample Size and Species Characteristics on Performance of Different Species Distribution Modeling Methods. Ecography. 29(5): 773–785.

Hibbitts, T. J., W. A. Ryberg, J. A. Harvey, G. Voelker, A. M. Lawing, and C. S. Adams. 2019. Phylogenetic structure of *Holbrookia lacerata* (Cope 1880) (Squamata: Phrynosomatidae): one species or two? Zootaxa 4619:1.6.

Ingram, M. 2018. The Endangered Species Act in Texas: a survey and history. Texas Public Policy Foundation, Austin, Texas.

Maldonado, J. A., T. J. Firneno, Jr., C. E. Roelke, N. D. Rains, J. Mwgiri, and T. K. Fujita. 2020. Transcriptome sequencing reveals signatures of positive selection in the spot-tailed earless lizard. Plos ONE: https://doi.org/10.1371/journal.pone.0234504.

Melo-Merino, S. M., H. Reyes-Bonilla, and A. Lira-Noriega. 2020. Ecological Niche Models and Species Distribution Models in Marine Environments: A Literature Review and Spatial Analysis of Evidence. Ecological Modelling. 415.

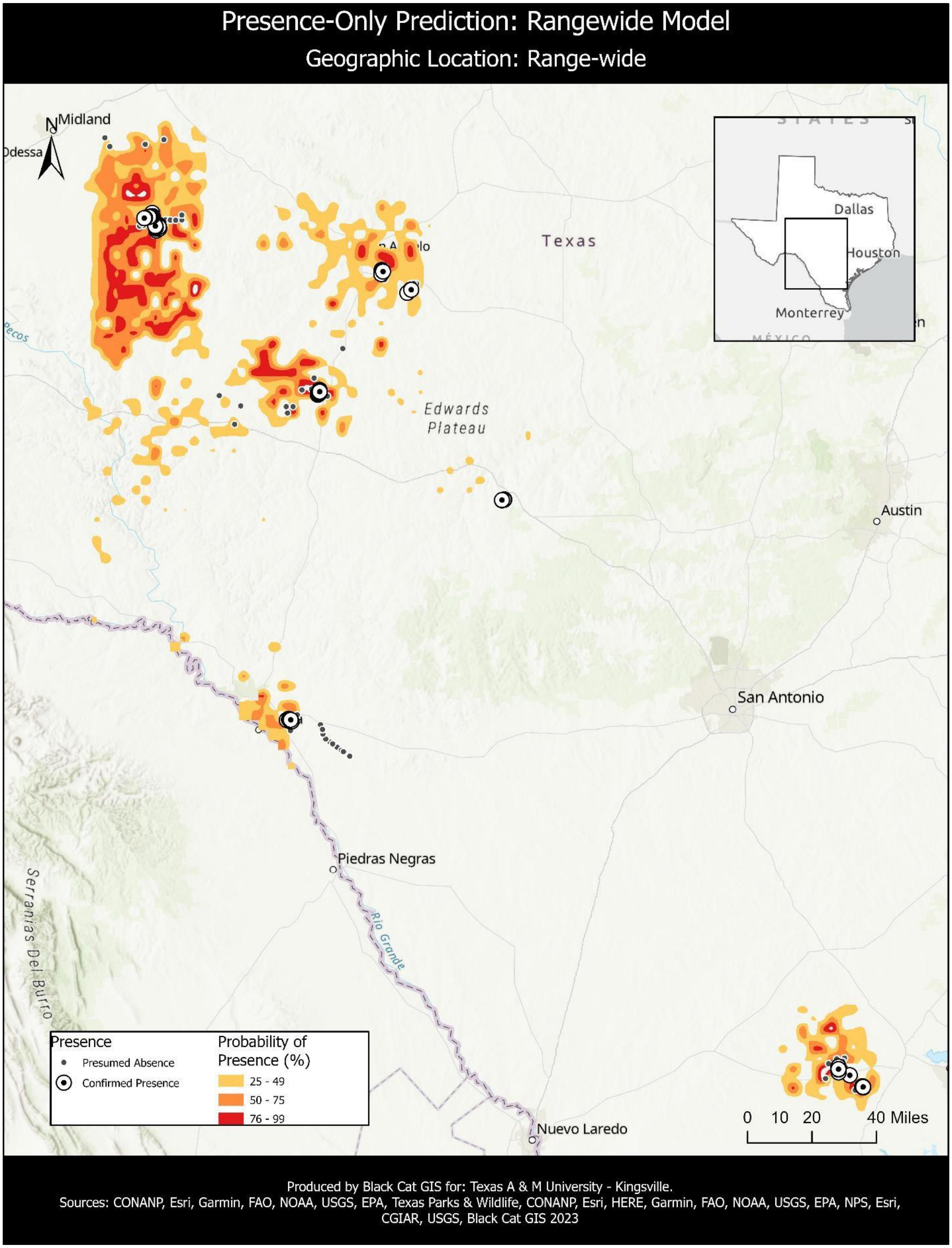
Raes, N. 2012. Partial Versus Full Species Distribution Models. Natureza & Conservação. 10(2): 127–38.

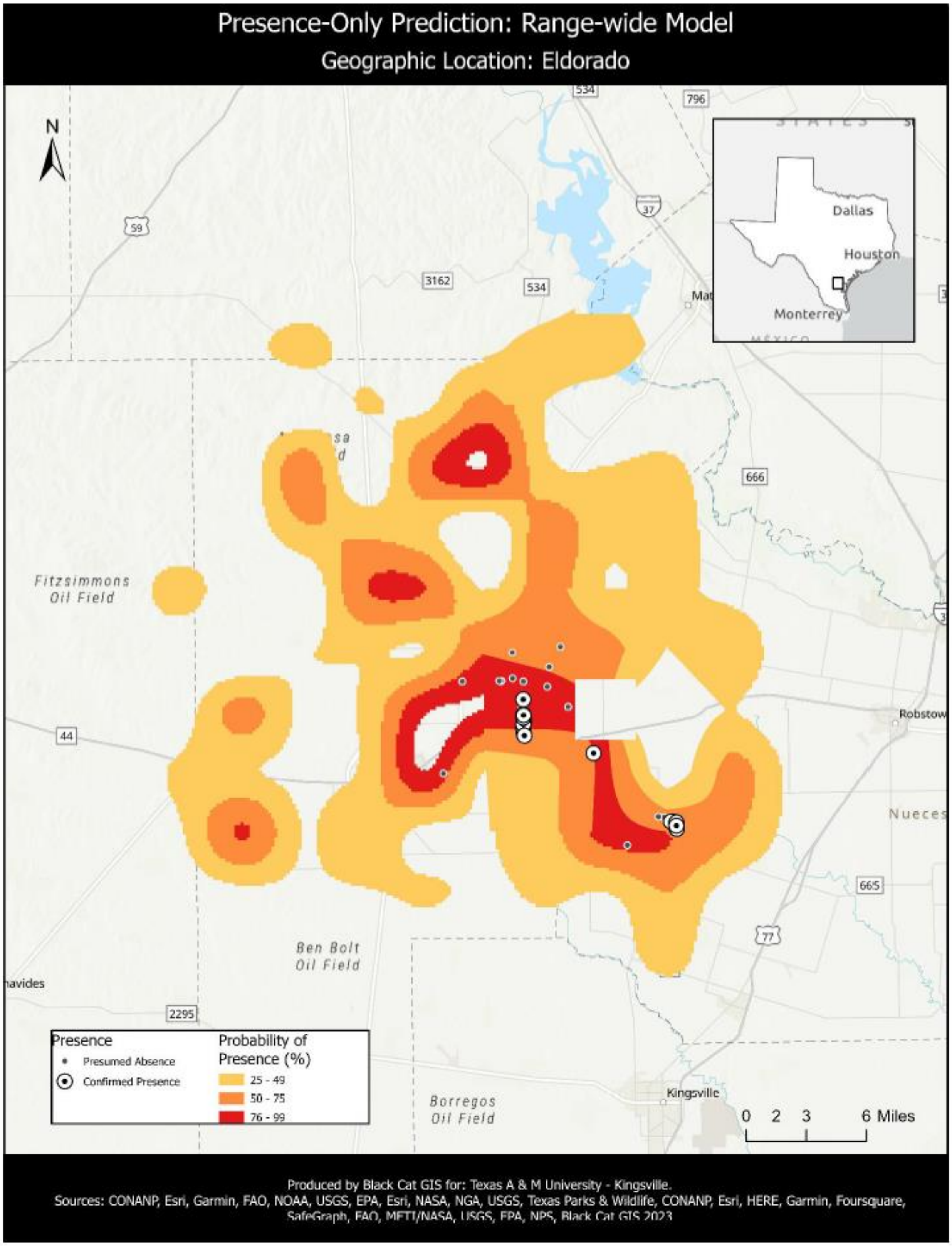
Ricketts, T. H. 2001. The Matrix Matters: Effective Isolation in Fragmented Landscapes. American Naturalist. 1(158): 87-99.

Scott, N. J., Jr. 1996. Evolution and management of the North American grassland herpetofauna. Pages 40-53 *in* D. M. Finch, editor. Ecosystem disturbance and wildlife conservation in western grasslands. Symposium Proceedings; 22-26 September 1994; Albuquerque, NM. General Report RM-GTR-285.

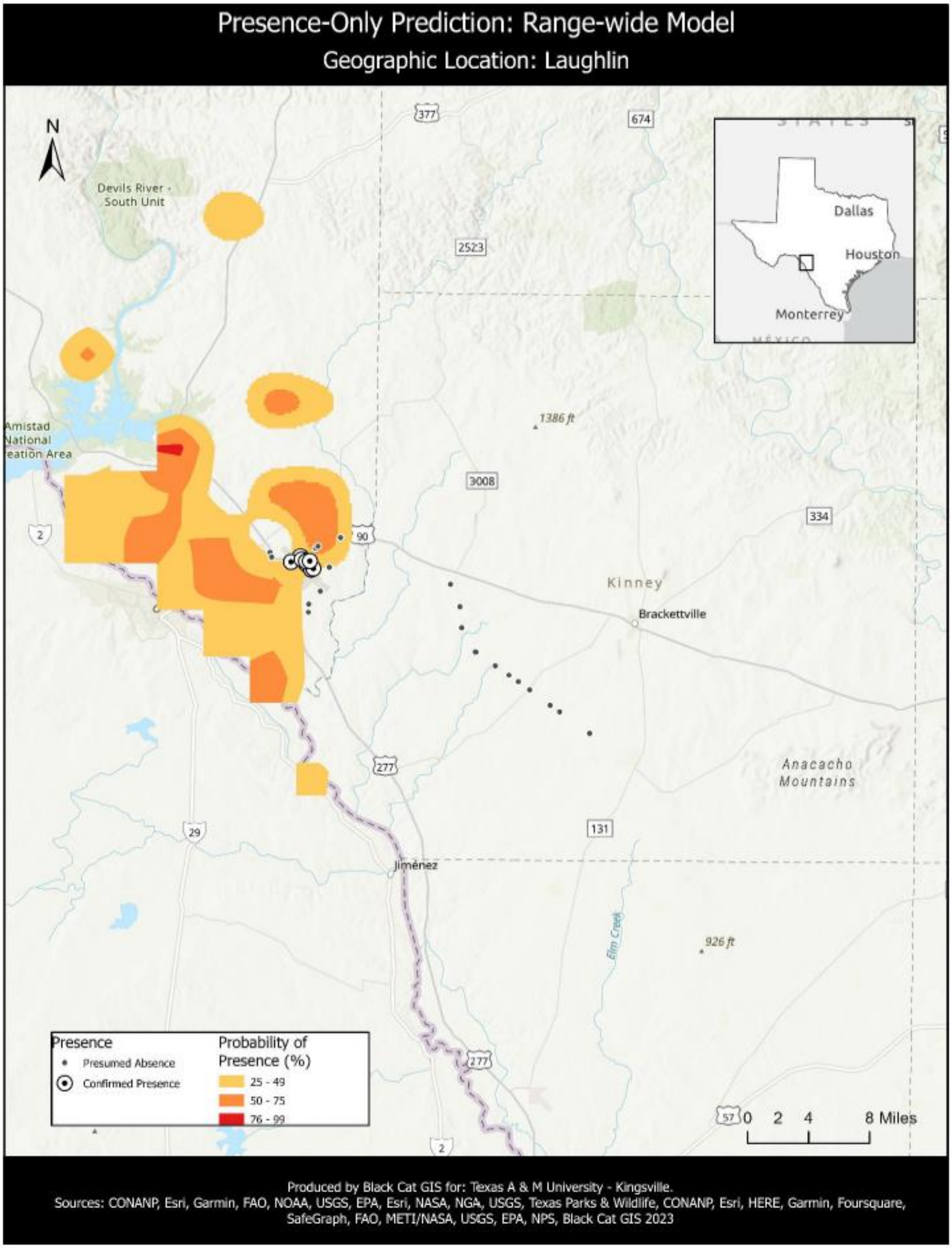
Vrba, E. S. 1987. Ecology in Relation to Speciation Rates: Some Case Histories of Miocene-Recent Mammal Clades. Evolutionary Ecology 1: 283–300.

Wolaver, B. D., J. P. Pierre, S. A. Ikonnikova, J. R. Andrews, G. McDaid, W. A. Ryberg, C. M. Duran, and T. J. Hibbitts. 2018. An improved approach for forecasting ecological impacts from future drilling in unconventional shale oil and gas plays. Environmental Management 61:805-818


Presence-Only Prediction: Rangewide Model
Geographic Location: Range-wide
Midland
Odessa
N
Texas
Edwards Plateau
Austin
San Antonio
Piedras Negras
Rio Grande
Serranías Del Burro
Nuevo Laredo
Pecos
Dallas
Houston
Monterrey
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 10 20 40 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NPS, Esri, CGIAR, USGS, Black Cat GIS 2023



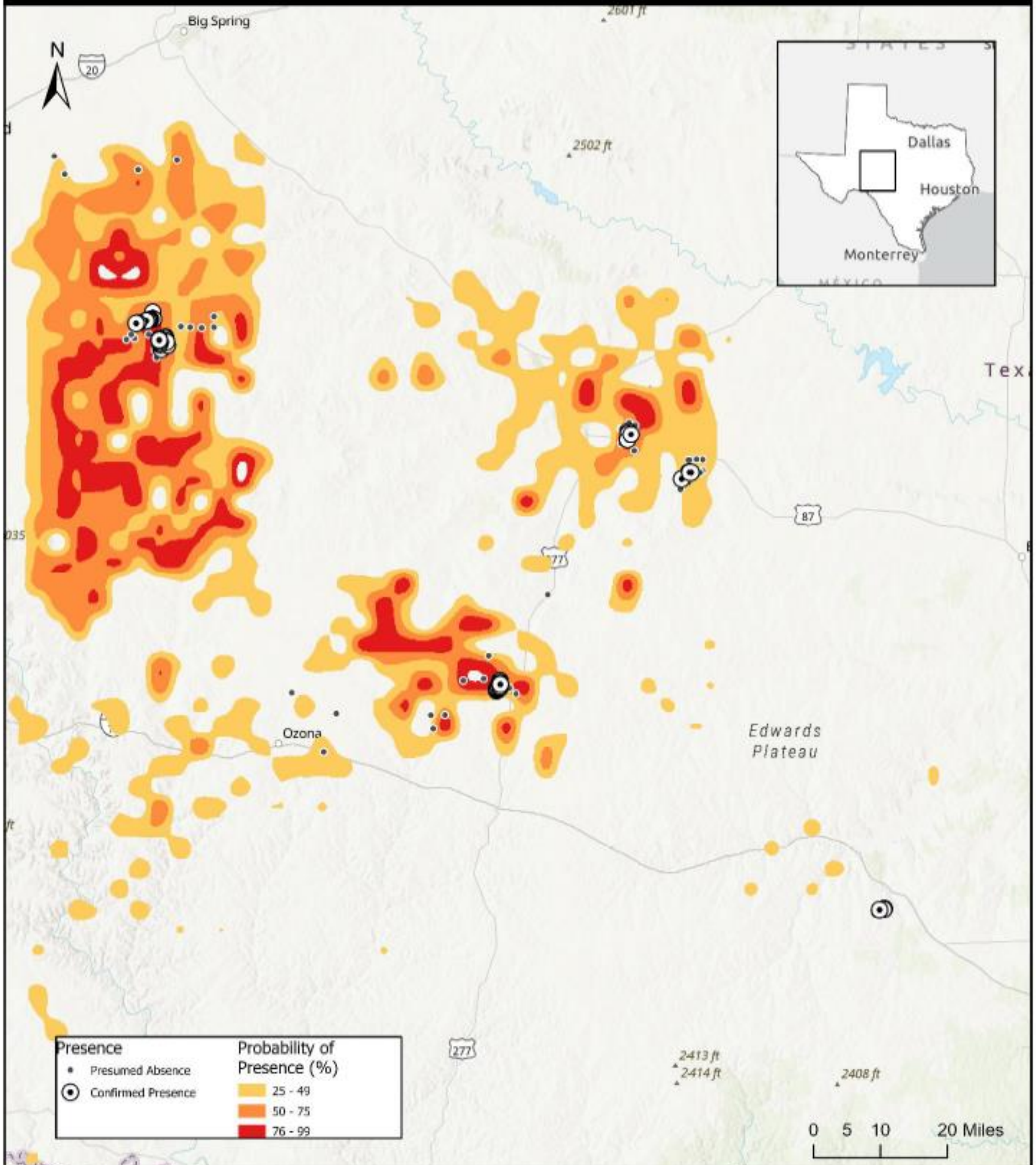
Presence-Only Prediction: Range-wide Model
Geographic Location: Eldorado
N
Dallas
Houston
Monterrey
Fitzsimmons Oil Field
Ben Bolt Oil Field
Borregos Oil Field
Kingsville
Robstown
Nueces
3162
534
796
666
665
2295
44
59
37
77
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 2 3 6 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Esri, NASA, NGA, USGS, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, Foursquare, SafeGraph, FAO, METI/NASA, USGS, EPA, NPS, Black Cat GIS 2023



Presence-Only Prediction: Range-wide Model
Geographic Location: Laughlin
N
Devils River - South Unit
Amistad National ...eation Area
Dallas
Houston
Monterrey
1386 ft
Kinney
Brackettville
Anacacho Mountains
Jiménez
Elm Creek
926 ft
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 2 4 8 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Esri, NASA, NGA, USGS, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, Foursquare, SafeGraph, FAO, METI/NASA, USGS, EPA, NPS, Black Cat GIS 2023

Presence-Only Prediction: Rangewide Model

Geographic Location: Northern



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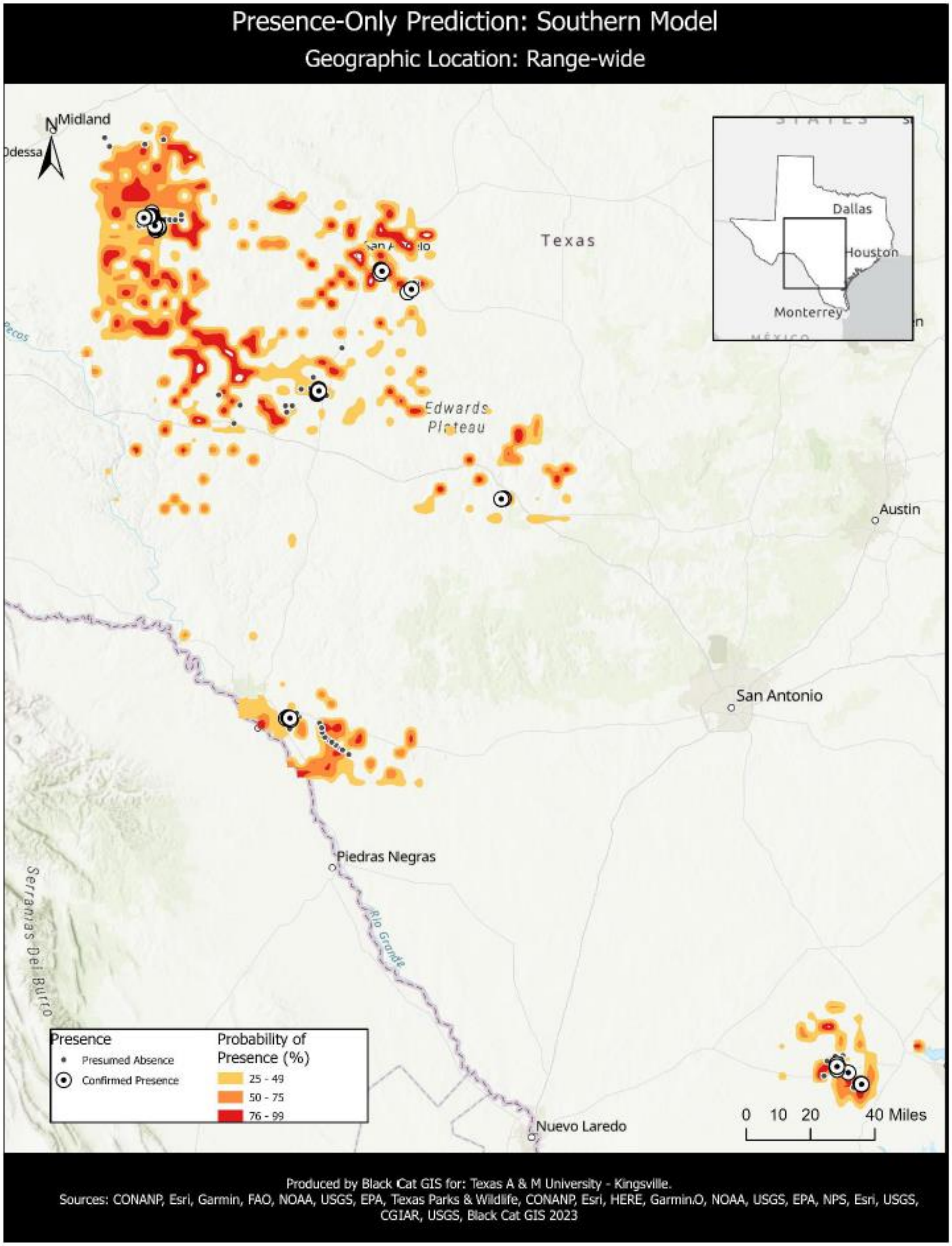
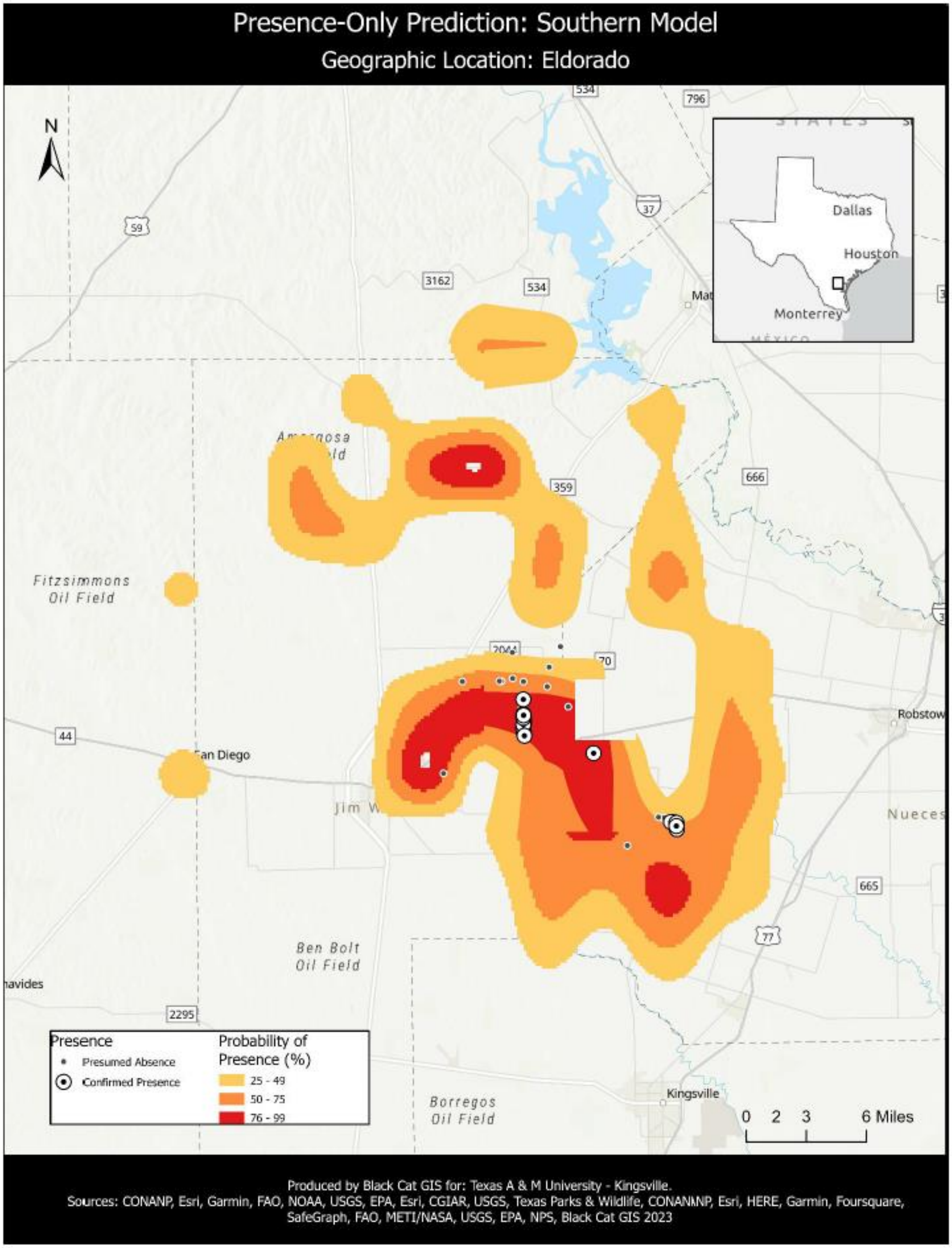
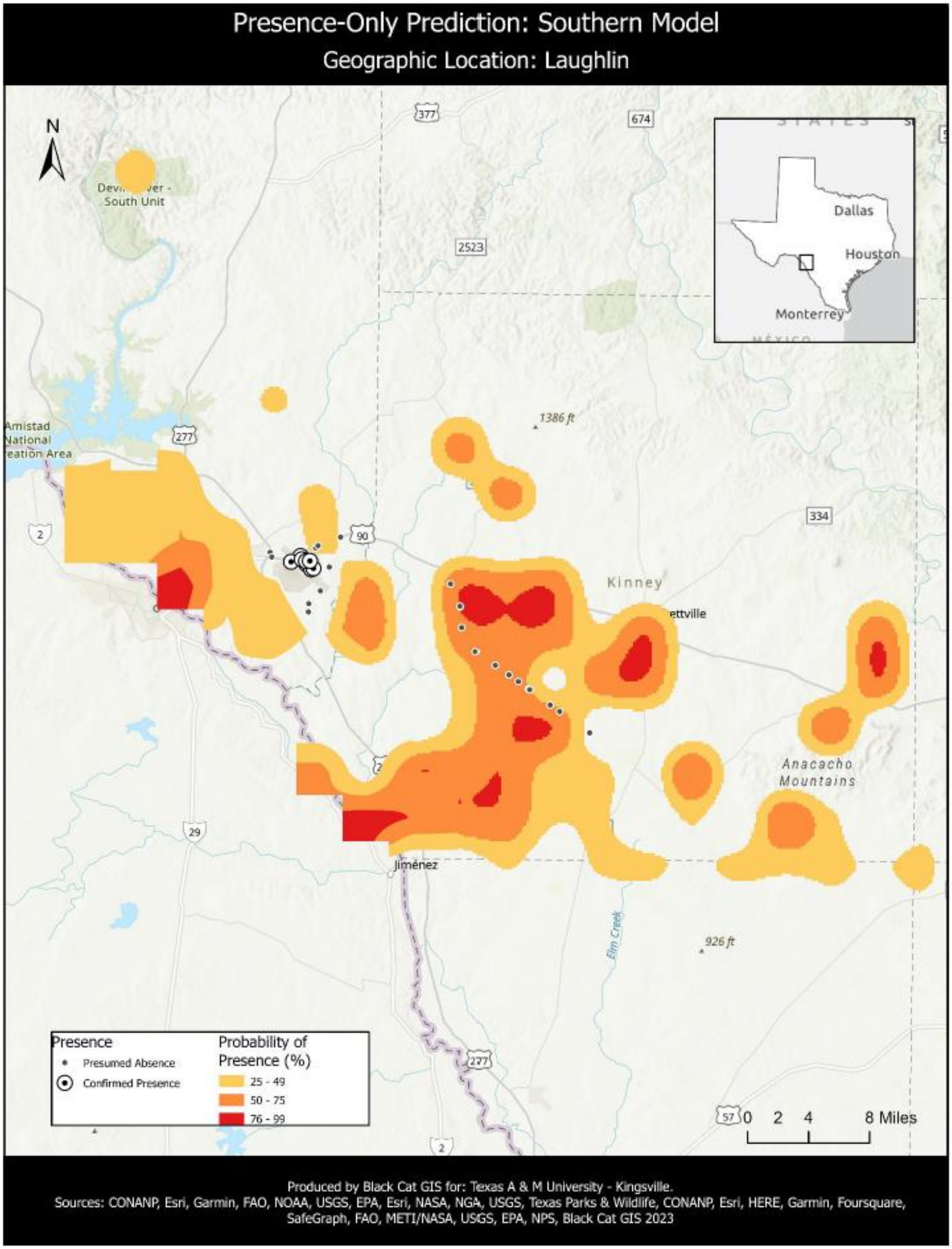
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NPS, Esri, USGS, Black Cat GIS 2023

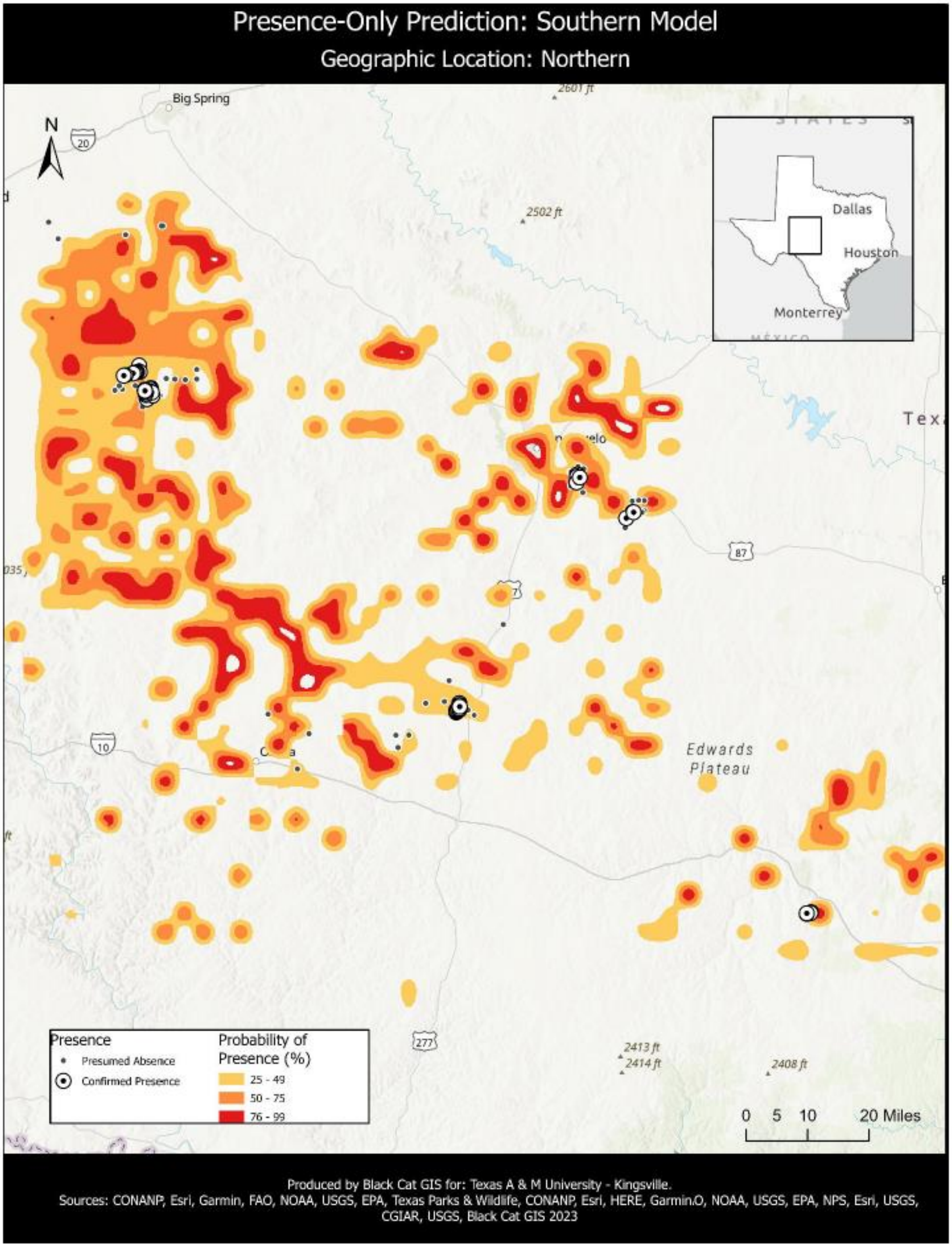


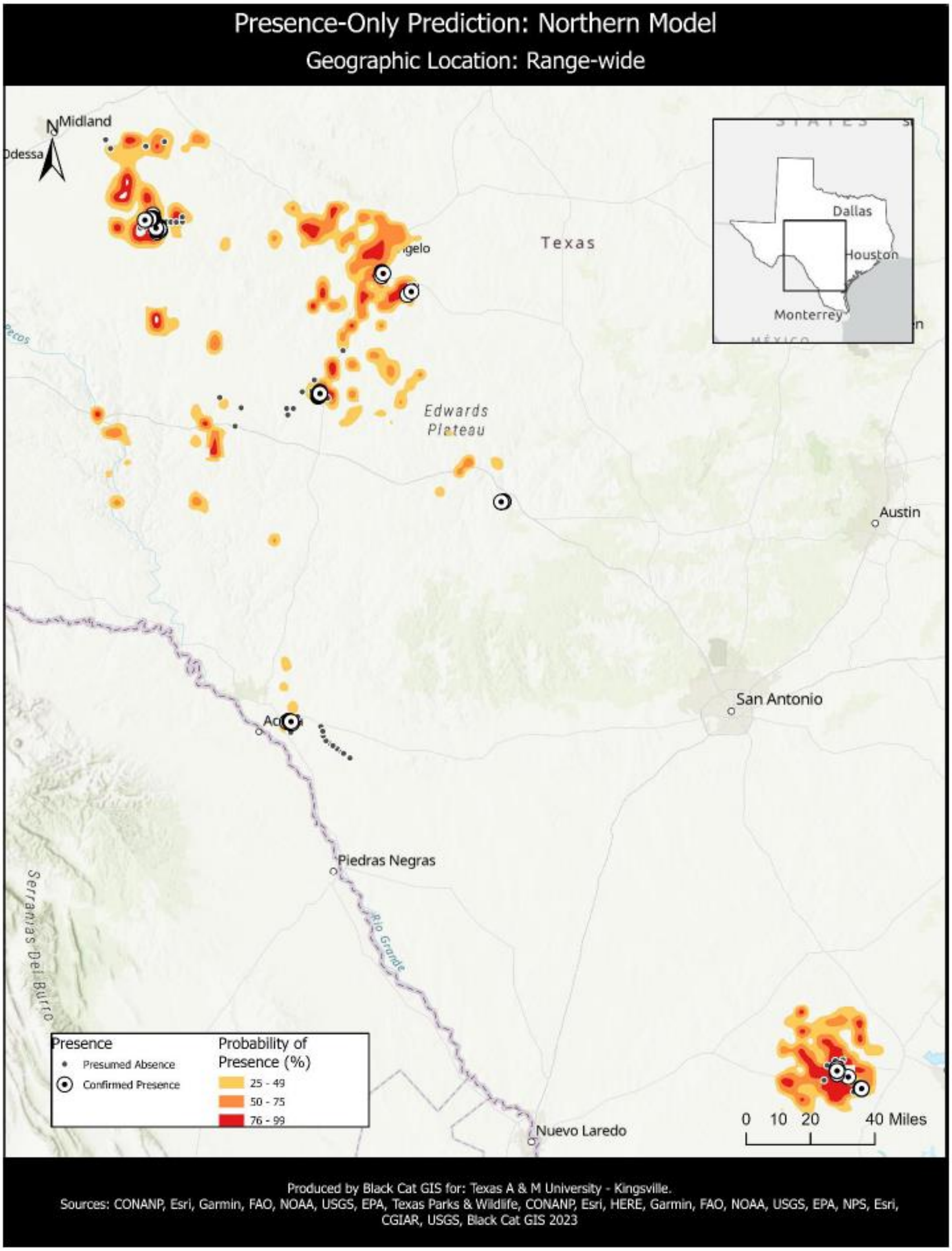
Presence-Only Prediction: Southern Model
Geographic Location: Range-wide
N
Midland
Texas
Dallas
Houston
Monterrey
Edwards Plateau
Austin
San Antonio
Piedras Negras
Rio Grande
Serranias Del Burro
Nuevo Laredo
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 10 20 40 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin.O, NOAA, USGS, EPA, NPS, Esri, USGS, CGIAR, USGS, Black Cat GIS 2023


Presence-Only Prediction: Southern Model
Geographic Location: Eldorado
N
Dallas
Houston
Monterrey
Fitzsimmons Oil Field
Ben Bolt Oil Field
Borregos Oil Field
Kingsville
Robstow
Nueces
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 2 3 6 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Esri, CGIAR, USGS, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, Foursquare, SafeGraph, FAO, METI/NASA, USGS, EPA, NPS, Black Cat GIS 2023

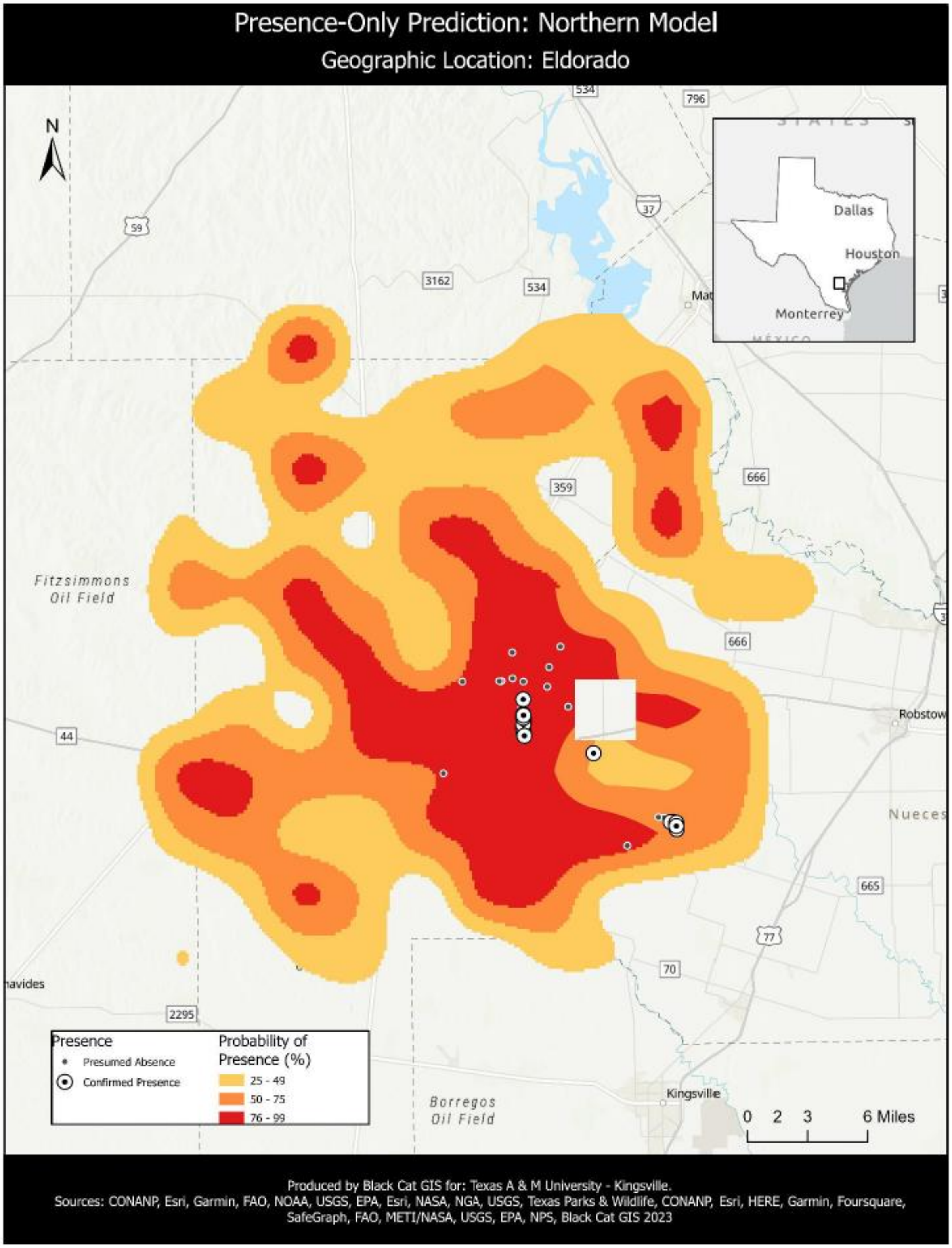


Presence-Only Prediction: Southern Model
Geographic Location: Laughlin
N
Dallas
Houston
Monterrey
Kinney
Anacacho Mountains
Jiménez
1386 ft
926 ft
Elm Creek
Amistad National
South Unit
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 2 4 8 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Esri, NASA, NGA, USGS, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, Foursquare, SafeGraph, FAO, METI/NASA, USGS, EPA, NPS, Black Cat GIS 2023

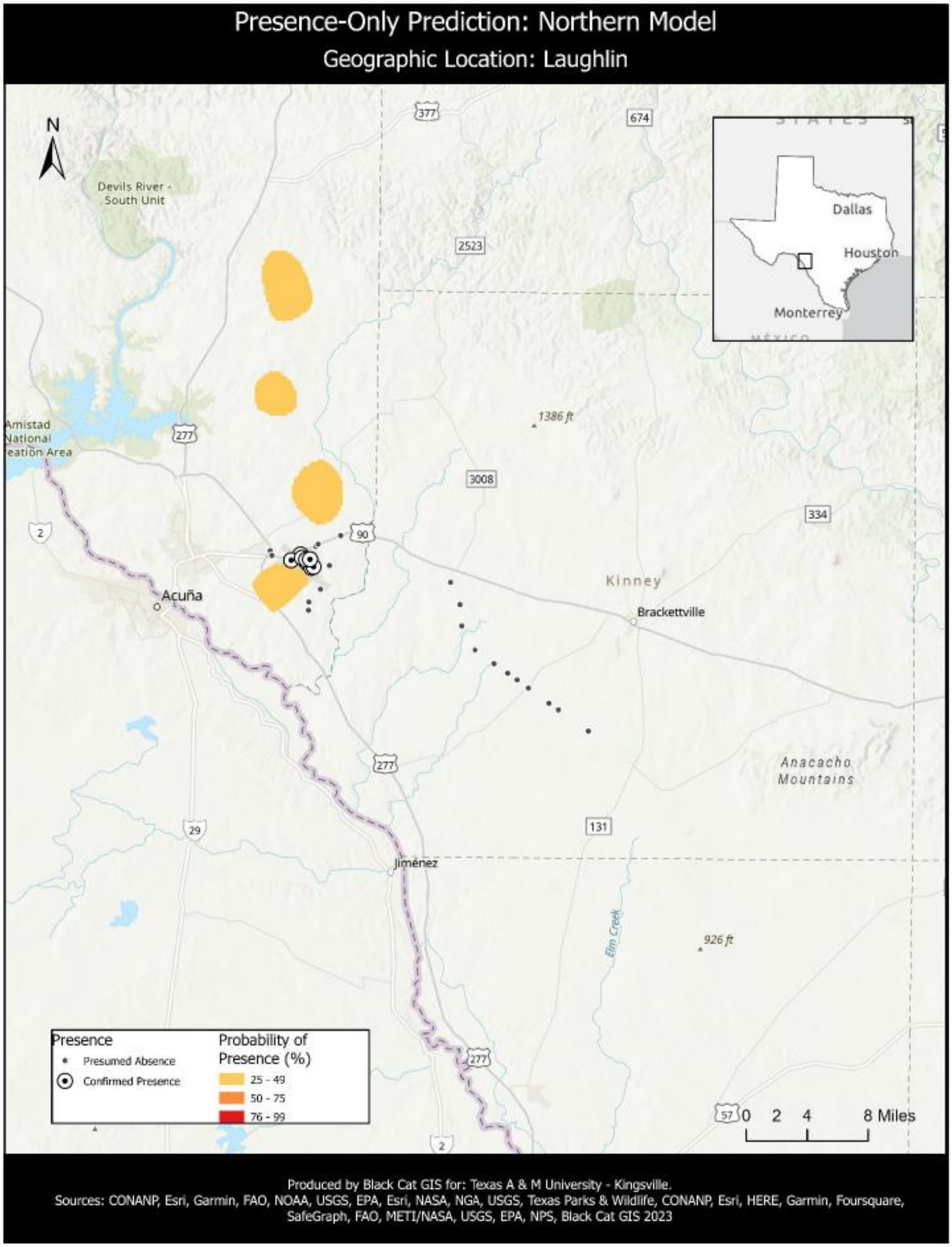

Presence-Only Prediction: Southern Model
Geographic Location: Northern
Big Spring
2601 ft
2502 ft
Dallas
Houston
Monterrey
Edwards Plateau
2413 ft
2414 ft
2408 ft
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 5 10 20 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin.O, NOAA, USGS, EPA, NPS, Esri, USGS, CGIAR, USGS, Black Cat GIS 2023



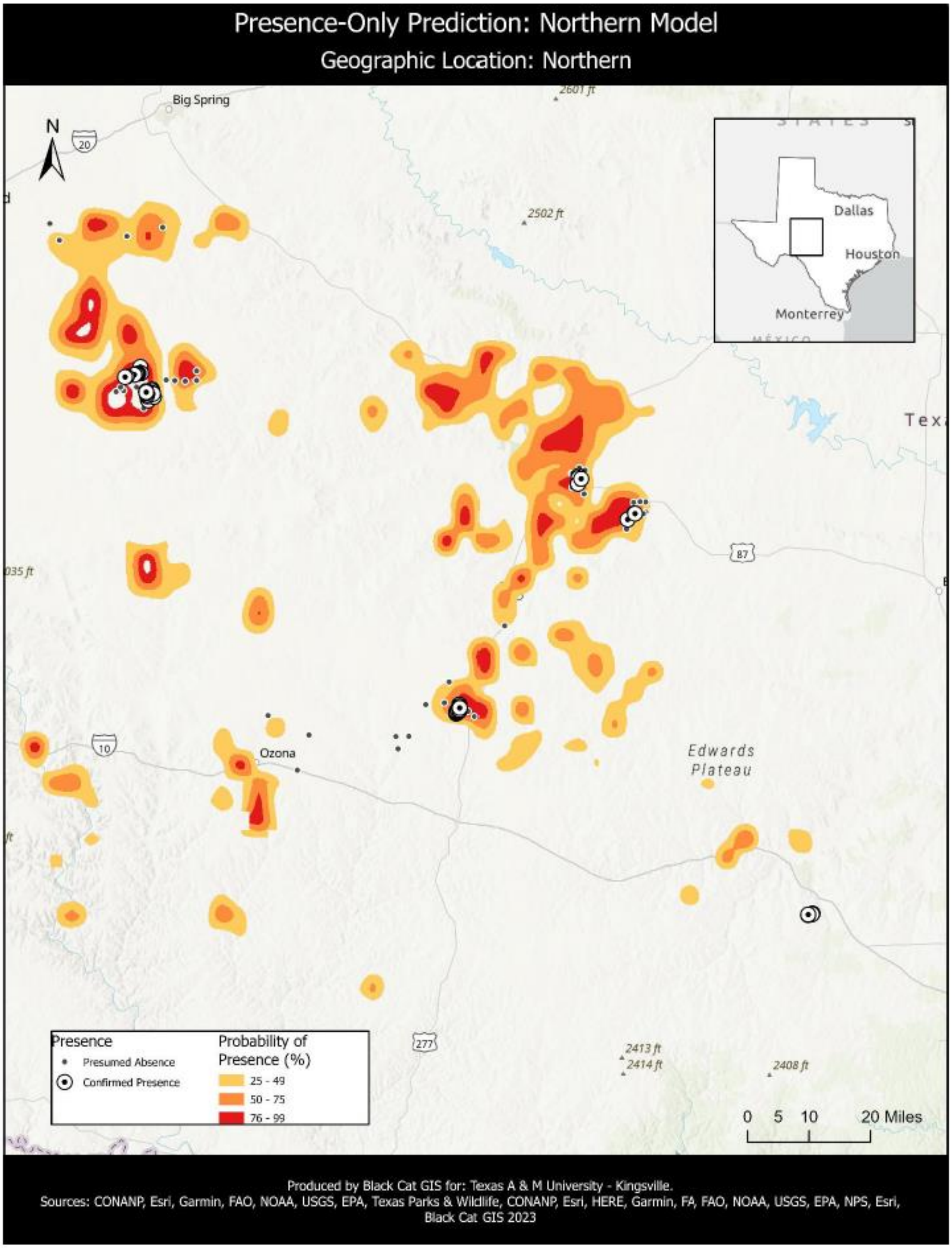
Presence-Only Prediction: Northern Model
Geographic Location: Range-wide
Midland
Odessa
Texas
Edwards Plateau
Austin
San Antonio
Piedras Negras
Rio Grande
Nuevo Laredo
Serranias Del Burro
Dallas
Houston
Monterrey
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 10 20 40 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NPS, Esri, CGIAR, USGS, Black Cat GIS 2023



Presence-Only Prediction: Northern Model
Geographic Location: Eldorado
Dallas
Houston
Monterrey
Fitzsimmons Oil Field
Robstow
Nueces
Kingsville
Borregos Oil Field
avides
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 2 3 6 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Esri, NASA, NGA, USGS, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, Foursquare, SafeGraph, FAO, METI/NASA, USGS, EPA, NPS, Black Cat GIS 2023



Presence-Only Prediction: Northern Model
Geographic Location: Laughlin
N
Devils River - South Unit
Amistad National eation Area
Acuña
Kinney
Brackettville
Anacacho Mountains
Jiménez
Elm Creek
1386 ft
926 ft
Dallas
Houston
Monterrey
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 2 4 8 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Esri, NASA, NGA, USGS, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, Foursquare, SafeGraph, FAO, METI/NASA, USGS, EPA, NPS, Black Cat GIS 2023



Presence-Only Prediction: Northern Model
Geographic Location: Northern
Big Spring
N
20
2601 ft
2502 ft
Dallas
Houston
Monterrey
Tex
87
10
Ozona
Edwards
Plateau
277
2413 ft
2414 ft
2408 ft
Presence
Presumed Absence
Confirmed Presence
Probability of Presence (%)
25 - 49
50 - 75
76 - 99
0 5 10 20 Miles
Produced by Black Cat GIS for: Texas A & M University - Kingsville.
Sources: CONANP, Esri, Garmin, FAO, NOAA, USGS, EPA, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, FA, FAO, NOAA, USGS, EPA, NPS, Esri, Black Cat GIS 2023