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Experimental Assessment of Spot-tailed Earless Lizards (*Holbrookia lacerata* and *H. subcaudalis*) as Visual Predators

E. Drake Rangel[1], Scott E. Henke[1], and Cord B. Eversole[2]

[1]Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, Kingsville, Texas 78363, USA (scott.henke@tamuk.edu)
[2]Arthur Temple School of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, Texas 75962 USA

Abstract.—Plateau Spot-tailed Earless Lizards (*Holbrookia lacerata*) and Tamaulipan Spot-tailed Earless Lizards (*H. subcaudalis*) (hereafter STELs) are phrynosomatid lizards that are species of conservation concern. STELs are insectivores and are thought to be visual predators, but predation strategies have not been empirically documented. We randomly assigned 15 Plateau and 15 Tamaulipan STELs to one of three predation-behavior treatments: (1) live cricket, (2) dead cricket glued to monofilament line and moved, or (3) dead crickets. Both STEL species attacked only moving crickets, clearly demonstrating that STELs are visual predators. This has important conservation implications because STELs frequently occupy peripheries of agricultural fields, where pesticides often are used; consequently, feeding on immobile prey, particularly insects, could have negative consequences for these imperiled lizards.

The Plateau Spot-tailed Earless Lizard (*Holbrookia lacerata*) and Tamaulipan Spot-tailed Earless Lizard (*H. subcaudalis*), hereafter collectively STELs, are phrynosomatid lizards inhabiting grasslands, agricultural fields, woodlands, brushlands, and disturbed areas in Texas and northern Mexico (Hibbitts et al. 2021). The Plateau STEL is endemic to central Texas whereas the Tamaulipan STEL occurs in the Tamaulipan Biotic Province of southern Texas and adjacent northern Mexico, with the Balcones Escarpment separating the two species (Hibbitts et al. 2019). Populations of both species have declined in recent decades and both are considered species of conservation concern (Duran and Axtell 2010). Currently, the Tamaulipan STEL is being considered for federal threatened status by the United States Fish and Wildlife Service (Rangel et al. 2024).

Most of what we know about the diet of STELs is based on dissection of 129 museum specimens by LaDuc et al. (2018), who described STELs as opportunistic generalists after finding a variety of arthropods in stomach contents. Grasshoppers dominated (42% by volume, 65% for males), followed by beetles, ants, wasps, termites, caterpillars, spiders, true bugs, and centipedes, and few differences existed between the diets of *H. lacerata* and *H. subcaudalis,* although the latter were less clear due to low sample size.

Axtell (1954) stated that movement was requisite for consumption to occur and that STELs were visual predators. Rangel (2023) agreed but noted that empirical data are lacking to fully support this conclusion. Given the limited empirical evidence, we tested Axtell's (1954) observation that prey movement is necessary for predation.

Material and Methods

We collected 15 Plateau (7M:8F) and 15 Tamaulipan (8M:7F) STELs during collection surveys in May–July 2021 near San Angelo, Tom Green County (31.38153, -100.16043; elev. 812 m asl) and near Bishop, Nueces County, Texas (27.71104, -97.86726; elev. 5 m asl), respectively (Fig. 1). STELs were brought to a laboratory on the Texas A&M University-Kingsville campus where they were housed individually in 37.8-l aquaria equipped with 10 cm of sandy loam soil, heat lamp, UV light, and water provided *ad libitum*. About five live captive-raised crickets (Family: Gryllidae; Rainbow Mealworm, Compton, California 90220) were fed to each STEL each day, with the exception of the day prior to the experiments. STELs were maintained in captivity for at least 30 days to acclimate them to captive conditions. Crickets were withheld for one day prior to the initiation of experiment to motivate them to eat.

Five STELs of each species were randomly assigned to one of three treatments. Each STEL was tested once in its assigned treatment, resulting in five trials per treatment per species (i.e., 5 STELs/treatment × 3 treatments = 15 STELs/species). Treatments included: (1) placing one live cricket in an aquarium, (2) gluing a freshly dead cricket onto a 0.15-mm diameter × 60-cm long clear monofilament nylon line (Sunline, Iwakuni, Yamaguchi, Japan) with a 60/100 W hot

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Figure 1. Plateau Spot-tailed Earless Lizards (*Holbrookia lacerata*) (left) and Tamaulipan Spot-tailed Earless Lizards (*H. subcaudalis*) (right) are Species of Concern, the abundance and distribution of which has been greatly reduced in recent decades. We herein demonstrate that Spot-tailed Earless Lizards are visual predators and respond only to prey movement to eat. Photographs by Scott E. Henke.

glue gun (Hobby Lobby, Oklahoma City, Oklahoma 73179) and pulling the dead cricket across the aquarium in a steady 1–2 cm/s motion to mimic live cricket movement, and (3) placing 10 freshly dead crickets evenly distributed on the soil surface of an aquarium (1 cricket/129 cm^2). Maximum allotted time for each treatment was 1 hour (3,600 s). The time elapsed from crickets added to the aquaria to cricket grabbed by a STEL and in its mouth was recorded. We defined consumption or an attempt to consume as 'grabbed by STEL and cricket in STEL's mouth' because we did not want to harm STELs by swallowing monofilament line. Dead crickets attached to monofilament line were pulled from the STEL's mouth once a cricket was in a STEL's mouth.

We used a general linear model (GLM; SAS Institute Inc. 2012) to test the main effects of species and treatment and their interactions based on the time elapsed to attempted consumption by STELs. Attempted consumption was defined as a STEL striking at a cricket, whether successful (i.e., cricket grabbed and in mouth) or not. If a significant interaction was 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 (Shapiro and Wilk 1965). Mean separation using the Tukey's range test were conducted when significant ($P < 0.05$) treatment effects were noted (Tukey 1949). Initially, sex differences in predatory behavior of STELs was not analyzed due to low power of the test caused by insufficient sample size (i.e., 2–3 same sex STELs/treatment/species) (Mascha and Vetter 2018). However, because no species or interactive effects involved species, species were consolidated and sex differences in predatory behavior of STELs were performed post hoc. We used similar statistical methods as described above, but this time we tested the main effects of sex and treatment and their interactions on the time elapsed to attempted consumption. All means are reported ± 1 standard error.

Results

We observed no species difference ($F_{1,24} = 0.07$, $P = 0.79$) between reactions of Plateau and Tamaulipan STELs by treatments, nor was an interactive effect observed ($F_{2,24} = 0.32$, $P = 0.73$) between species and treatment (Table 1). However, a treatment effect was observed ($F_{2,24} = 14.2$, $P = 0.0001$). Both species of STELs consumed prey (i.e., live crickets) or attempted to do so (i.e., dead crickets moved by monofilament line) in an average of about 30 s after gaining access to the prey if the prey moved (Table 1). However, in treatment three (i.e., freshly dead crickets lying on aquaria substrates), neither species consumed or attempted to strike any crickets within the one-hour limit, showing no observable interest in crickets (Table 1). A sex difference ($F_{1,24} = 0.76$, $P = 0.39$) was not observed between the reaction of STELs by treatments, nor was an interactive effect observed ($F_{2,24} = 1.2$, $P = 0.32$) between sex and treatment (Table 1). However, a treatment effect was observed ($F_{2,24} = 20.6$, $P = 0.0001$) similar to above. Both sexes of STELs consumed live crickets or attempted to do so (i.e., dead crickets moved by monofilament line) within ~30 s of cricket placement in aquaria and crickets moving.

Discussion

As STELs only attacked moving crickets within one hour, we clearly demonstrated that they are visual predators. STELs did not consume non-moving crickets in our trials, suggesting a strong reliance on visual cues for predation. If other senses (e.g., olfactory or chemosensory) were capable of eliciting an attack, we would have expected STELs to have consumed

Table 1. Average time elapsed (seconds) from introduction of prey item (i.e., live cricket, freshly dead cricket glued to monofilament line, freshly dead crickets) into aquarium with a captive individual spot-tailed earless lizard Group A (STEL; *Holbrookia lacerata* and *H. subcaudalis*; N = 5/treatment/species) or Group B (sex; N=5/treatment/sex) to cricket in STEL's mouth after a 1-day withholding period of food. [1]Treatments included 1) placing one live cricket (Family: Gryllidae) in a 37.8-l aquarium, 2) gluing a freshly dead cricket onto monofilament line and pulling the cricket across the aquarium at ~1-2 cm/s, and 3) placing 10 freshly dead crickets evenly distributed onto the soil surface of a 37.8-l aquarium (1 cricket/129 cm^2). Maximum allotted time for each treatment was 1 hour (3600 s). [2]Means with the same capital letter are not different ($P > 0.05$) between treatments within a species (Group A) or sex (Group B). Means with the same lowercase letter within a treatment are not different ($P > 0.05$) between species or sex.

		Treatments[1]		
Group	**Species**	**Live cricket**	**Monofilament line**	**Dead crickets**
A	*H. lacerata*	28.8 ± 4.4 Aa[2]	31.6 ± 4.0 Aa	3600.0 ± 0.0 Ba
	H. subcaudalis	31.8 ± 5.0 Aa	31.6 ± 3.8 Aa	3600.0 ± 0.0 Ba
B	Male	30.3 ± 4.7 Aa[2]	30.9 ± 4.1 Aa	3600.0 ± 0.0 Ba
	Female	30.8 ± 4.3 Aa	31.8 ± 3.7 Aa	3600.0 ± 0.0 Ba

dead crickets within the 60-minute period. If freshly killed crickets have a different odor than live crickets, and if sense of smell was the main factor in eliciting an attack, then we would not expect STELs to respond to dead crickets glued to monofilament lines. We ruled out the sense of touch as a possible factor in locating prey because the densities of dead crickets were such that STELs often stepped on dead crickets during the 60-minute period, but contact with a dead cricket never elicited an attempt to strike. STELs might be capable of hearing prey movement, but they did not react to other auditory stimuli (i.e., tapping on aquarium glass) during experiments. We found that STELs did not appear to react to auditory stimuli, but instead, focused on prey movement. Thus, STELs almost certainly are visual predators.

This concept is important because STELs are commonly found on the periphery of agricultural fields exposed to various pesticides to reduce crop loss to insects; however, such a practice presumably decreases prey availability for STELs, especially when pesticides are fast-acting and insects die quickly. A reduction in moving prey, such as insects killed by pesticides, could potentially limit food availability for STELs, which could affect growth and reproduction, although further research is needed to confirm those assumptions. Axtell (1954, 1956) noted that STELs have the capability of reproducing within their hatchling year and can produce two clutches of eggs in a given reproductive season if they acquire sufficient body size. Eversole et al. (2024) conducted population viability analyses of STELs and concluded that hatchling survival, age at first reproduction, and percent of females breeding within the population had the strongest effects on STEL population growth rates and extinction risks. Therefore, maintaining a diverse food supply as outlined by LaDuc et al. (2018) can be a critical albeit indirect factor in maintaining stable STEL populations.

LaDuc et al. (2018) found sex-specific dietary differences in STELs. Although we did not investigate this factor, we found no apparent sex-related differences in predation behavior. Male and female STELs potentially select different prey items, but both sexes appear to hunt visually based on movements of prey.

Because STELs do not appear to consume non-moving prey (i.e., dead insects), insects killed by pesticides likely will not be consumed; therefore, the odds of pesticides bioaccumulating in STELs appear low. However, STELs might nevertheless be exposed to pesticides if they are directly sprayed during the application process or if pesticides are absorbed through the skin. Therefore, further research into pesticide use is needed to determine effects of pesticides on these species of conservation concern.

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