Can scorpions detect the "flash" of an infrared camera? An investigation into scorpion infrared sensitivity.

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Spring 2019

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Summary

Scorpions are known to be sensitive to UV, green, and red light. A previous assay, however, did not show the expected behavioral responses to these wavelengths of light, but rather detected a possible sensitivity to infrared light projected by the video camera onto the arena. A pilot study designed to validate this previous assay found a similar pattern. A new behavioral assay was therefore created to further investigate scorpion infrared sensitivity. The new assay included a new apparatus with infrared cameras placed above and below a circular arena to record scorpion movements and provide infrared light. An infrared gradient was added to determine if scorpions would show a bias towards a particular strength of infrared irradiance. We found no difference in scorpion behavior relative to the gradient. However, the occupancy per sector unexpectedly showed more variability in the control arena compared to the arena with the gradient. Ultimately, a sensitivity to infrared light could not be disproved and more research is needed to reach a conclusion. Scorpion infrared sensitivity has many implications for nocturnal animal research, in which infrared cameras record behavior under the assumption that they are undetectable by the animals being observed and have negligible effects on behavior.

Introduction

Little is known regarding scorpion photosensitivity. Scorpions have one pair of medial eyes that have lenses and likely form images, and three pairs of low-light sensitive lateral eyes (Hjelle 1990; Schliwa & Fleissner 1980). Physiologically, scorpions' medial and lateral eyes are known to be sensitive to a variety of colors, including primary and secondary responses to green light and UV light, but not to light in the red or infrared range (Fleissner & Fleissner 2001). Along with their eyes, some sensitivity to green and shorter light wavelengths, but not heat or infrared light, has been found in the scorpion tail (Zwicky 1968, 1970a,b).

Studies have been conducted to test scorpion behavioral responses to various wavelengths of light. Scorpions moved in rapid bursts when illuminated from above with green and UV light but made slow, deliberate movements under infrared light (Blass & Gaffin, 2008). Rivera Roldan and Gaffin created a new, more sensitive behavioral assay in which green (566 nm), UV (399 nm), and red (630 nm) lights were cast across the behavioral arena rather than from the top. In this case, a strong behavioral response was seen towards green and UV light, with a slight response to red light (Rivera Roldan & Gaffin, 2018).

A follow-up study was conducted by Vanderslice and Gaffin (unpublished), which curiously showed no response to green, UV, or red light. However, the researchers noted there might be a bias towards the infrared light emitted from the camera below (Figure 1). These results have important implications for a field that has been mostly untouched by scorpion research – sensitivity to infrared irradiance. Since previous studies with infrared light directed towards the eyes from above showed no detectable bias in scorpion behavior, it may be possible that the response is due to an extraocular method of detection.

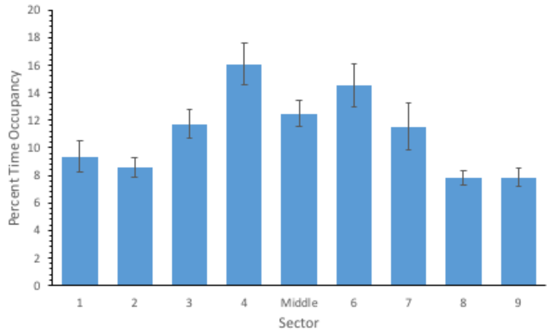

Fig. 1: Scorpion bias to center of apparatus as detected in previous study (Vanderslice & Gaffin, unpublished).

This study was designed to examine the possible detection of infrared irradiance by scorpions. First, I ran a pilot study with the apparatus used by Vanderslice and Gaffin that replicated the behavioral response found in their study and supported the hypothesis that the scorpions may be sensitive to infrared light. I then designed a new behavioral assay and created an apparatus to specifically test scorpion sensitivity to infrared light (Figure 2). In my experiment, scorpions showed no statistically significant difference in their movements within an IR gradient compared to control conditions. However, a chi square analysis showed more variance in the control condition than in the IR gradient. This implies that more research is needed before scorpion IR sensitivity can be ruled out.

Methods

Pilot study

*Animal collection and care***.**— The animals used for the study consisted of 20 (19 female and 1 male) desert grassland scorpions, *Paruroctonus utahensis,* that were collected near Monahans, Texas. The animals were housed in 3.8 L glass jars filled with 2.5-5.0 cm of sand, along with one small piece of cork or clay for refuge and moisture retention. They were watered twice weekly and fed a single cricket every three weeks. The testing room was held at a temperature of around 23˚C using a small space heater. The light-dark phase was 7:00-17:00 (light) and 17:00-7:00 (dark).

Arenas.— There were four identical testing arenas, which were constructed by cutting a Strata 20-ounce clear plastic cup in a cross-section 5 cm from the rim to create a cylinder. The cylinder was 9 cm on the narrow end, and 11 cm on the wide end, which was glued to a 12cm x 12cm Plexiglass square. Within the area of the cup, a 5 cm tall x 5 cm wide clear PVC pipe was also glued to the Plexiglass surface, creating a circular track for the scorpions to walk around. The outer side of the cylinder was covered with black

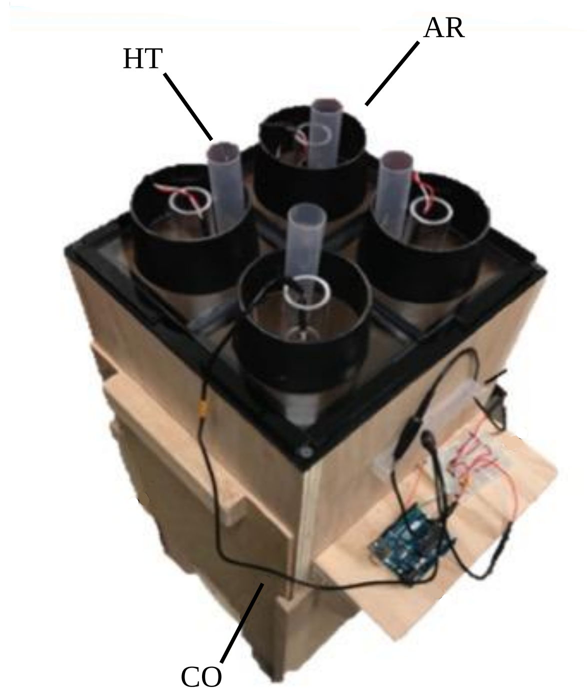

Fig. 2: Experimental apparatus for pilot study. Four individual behavioral arenas (AR) sit on top of a specially constructed wooden box. Each removable holding area is pictured in their positions before the trial initiation with the holding tubes (HT) for each scorpion. An infrared camera is positioned inside the box to capture footage from below the arenas. It is connected to a laptop computer through a notch at the bottom of the apparatus. On the side of the box, there is a camera opening (CO) to allow the camera to be moved if necessary. This opening can be closed during the experiment to eliminate all external light.

electrical tape to ensure no light could enter from the side and the scorpions could not see one another. A removable plastic vial with one end cut off was used as a holding chamber for the scorpions prior to each trial. This holding chamber was positioned in the corner farthest from the center of the apparatus.

Trial protocol.— To set up the experiment, the room had a single red light on, oriented away from the apparatus. Initially, the computer was tuned on, and the scorpions were taken out of their jars and placed in the holding chambers within the arena. The red light was turned off and the scorpions were given five minutes to acclimate. The IR camera was then turned on, and the video capture program was set to record for 60 minutes. Once the plastic holding chambers were lifted from the arena, releasing the scorpions, the recording was started. After the 60-minute period, the recording ended, the video was saved, the red light was turned back on, and the scorpions were returned to their respective jars.

To reduce confounding effects on behavior, each scorpion only experienced the trial protocol once, and the arenas were cleaned with 70% ethanol and Kimwipes between trials. This experiment was conducted over three days (Sunday, Monday, and Wednesday), beginning at 8:00 each morning. There were two consecutive trials on the first two days and one trial on the third day, with an overall total of five trials and 20 scorpions.

Location mapping and analysis.— The laptop computer attached to the IR camera contained a MATLAB program that was used to plot the coordinates of each scorpion every two seconds throughout the trial (using a frame-by-frame subtraction of centroids method). These points were then summed for nine sectors, which were divided equally and arranged according to the center of the apparatus. To generate a percent occupancy value per sector, the non-movement frames were removed and the summed points for each sector were divided by the total number of movement frames. These data were analyzed for all nine sectors. To control for global or room effects, the data were analyzed and processed towards the top of the screen as well as towards the center of the apparatus.

Effects of infrared light.— To determine if scorpion behavior was due to temperature or light detection, measurements were taken around each arena for both temperature and infrared irradiance while the IR camera was on and emitting light but not recording. To measure temperature, a temperature gun was used (Thermaworks IR-Gun-S) and the degrees Celcius were recorded for all nine sectors in each of the four arenas and graphed. The infrared irradiance in irradians (μW/cm^2/nm) was measured using a spectrometer (Ocean Optics USB4000 UV-VIS-E) and graphed, also for all nine sectors for each of the four arenas.

New behavioral assay

Animal collection and care.— The animals used for the study consisted of 16 adult female *Paruroctonus utahensis* that were collected near Monahans, Texas. The animals were housed and maintained as in the pilot study. The testing room was again held at a temperature of around 23˚C using a small space heater. The light-dark phase was 6:00-16:00 (light) and 16:00-6:00 (dark).

Apparatus.— An apparatus was constructed with a black PVC pipe 10.16 cm wide and 40.64 cm tall. A single arena was placed on top of the pipe so that the outer wall of the arena was directly above the walls of the tube. A separate piece of PVC pipe 10.16 cm wide and 26.67 cm tall was placed atop the arena, the tube fitting snugly around the outer wall of the arena and preventing any light from entering. There was one IR camera placed below the arena in the center of the tube, which provided IR light from below. A second IR camera was placed in the center of the upper tube, which functioned to record scorpion movements. The cord connected to the bottom camera was fed through a small notch at the bottom of the PVC pipe and connected to a nearby laptop computer via USB; the top camera was placed within the upper PVC pipe and the cord was connected via USB to a second computer with a MATLAB program (Figure 3).

Arena.— Two of the previously constructed testing arenas were used to create an IR gradient arena and a control arena with homogenous IR coverage. The gradient arena was divided into eight sectors: one sector with no tape to block the IR light from below, two sectors with one layer of tape, two sectors with two layers of tape, two sectors with three layers of tape, and one sector with one layer of white scientific tape and one layer of black electrical tape which almost completely blocked the IR light from below (Figure 4A). The bottom of the control arena was covered with two layers of white scientific tape to create an intermediate level of IR light shining up from the IR camera below (Figure 4B). Measurements were taken with a spectrometer to ensure that the IR value around the gradient was consistent and that the tape applied to the bottom of the control arena created a gradient of IR light.

Trial protocol.— To set up the experiment, the room had a single red light on, oriented away from the apparatus. Initially, the computer was tuned on, and the scorpions were taken out of their jars and placed

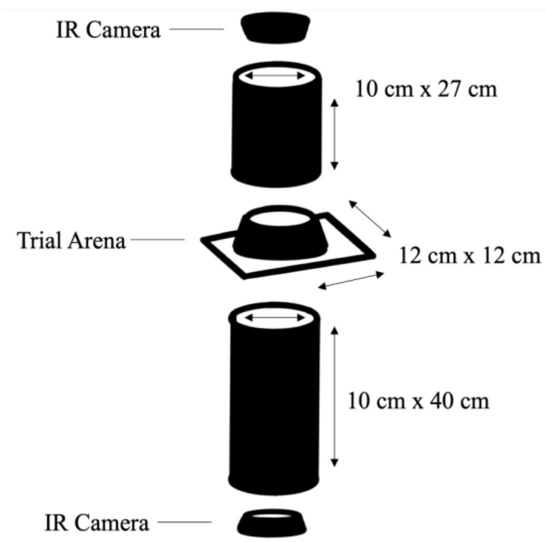

Fig. 3: New behavioral apparatus. Two PVC pipes were cut to create the body of the apparatus, and the removeable arenas were inserted between the two pipes. The lower IR camera provided an IR gradient to the arenas above, and the upper IR camera allowed us to visualize and track scorpion movements.

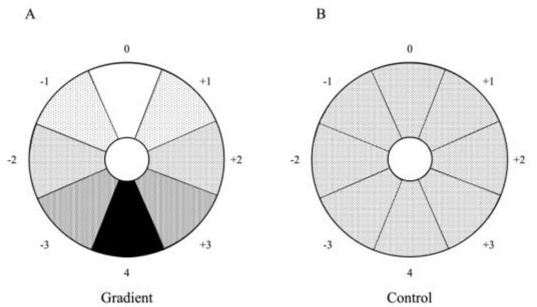

Fig. 4: Depiction of gradient and control arenas and sector labeling format. A. Gradient arena for new behavioral assay. The 0 sector represents a clear plexiglass surface with no shading. The shading increases towards the opposite end of the arena, and sector 4 represents the highest level of shading with almost no infrared irradiance penetrating to the surface. B. Control arena for new behavioral assay. The entire surface is shaded, and a medium level of infrared irradiance penetrates to the surface. The shading in the control arena is equivalent to the +/- 2 sectors of the gradient arena.

within the arena. The light was turned off and the scorpions were given five minutes to acclimate. The IR camera was then turned on, and the video capture program was set to record for 30 minutes, which was determined to be sufficient time for the scorpions to choose a comfortable sector within the gradient. After the 30-minute period, the recording ended, the video was saved, the red light was turned back on, and the scorpions were returned to their respective jars. The arenas were cleaned with 70% ethanol and Kimwipes between each trial. This experiment was conducted over a period of two weeks, with two consecutive trials daily starting at 7:00 am. Overall, there were 32 trials and 16 scorpions. A repeated measures design was used in which each scorpion experienced the gradient once and the control arena once.

Location mapping and analysis.— The laptop computer attached to the IR camera contained a MATLAB program that was used to plot the coordinates of each scorpion for every two seconds throughout the trial (using a frame-by-frame subtraction of centroids method). These points were then summed for eight sectors, which were divided equally and arranged with sector zero in the center of the clear end of the arena. To generate a percent occupancy value per sector, the non-movement frames were removed and the summed points for each sector were divided by the total number of movement frames. The data were kept only if the scorpions traveled across at least three sectors to ensure they experienced an IR gradient. These data were analyzed both for all eight sectors and by reducing the sectors to five regions by summing the percent occupancy of the sectors that were equidistant from the center. To control for global or room effects, the data were analyzed and processed towards the top of the screen as well as towards the clear sector of the arena.

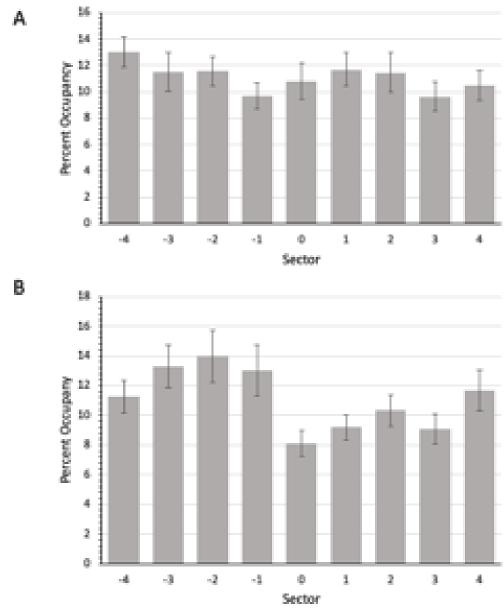

Fig. 5: Summary of animal locations relative to center of apparatus and top of video frame (pilot study). The average percent occupancy (+/- SE) of the twenty scorpions is shown based on nine sectors plotted relative to the top of the video frame (A) and relative to the center of the apparatus (B).

Results

Pilot study

The average percent time occupancy of the scorpions in each of the nine sectors was plotted to determine if there was a bias in scorpion behavior relative to the center of the apparatus. There was no significant difference in percent time occupancy in the control, when the behavior was plotted relative to the top of the screen (Figure 5A). However, a difference was found when the behavior was analyzed towards the center of the apparatus – the scorpions showed a distinct reduction in the time spent in the 0 sector relative to the other sectors (Figure 5B). The average temperature values were consistent around the arena (Figure 6A), while the IR measurements revealed a distinct gradient with the highest value towards the center of the apparatus for all four arenas (Figure 6B).

New behavioral assay

A spectrometer was used to ensure that the tape on the bottom of the arena provided a gradient of infrared light (Figure 7), while the control had a consistent level of infrared irradiance throughout all sectors. The summed percent time occupancy of the scorpions in corresponding sectors around the gradient were plotted and compared to both the control and expected values; no difference was found in the behavior relative to the gradient (Figure 8). Out of the 16 total scorpions, 2 did not cross over 3 sectors and

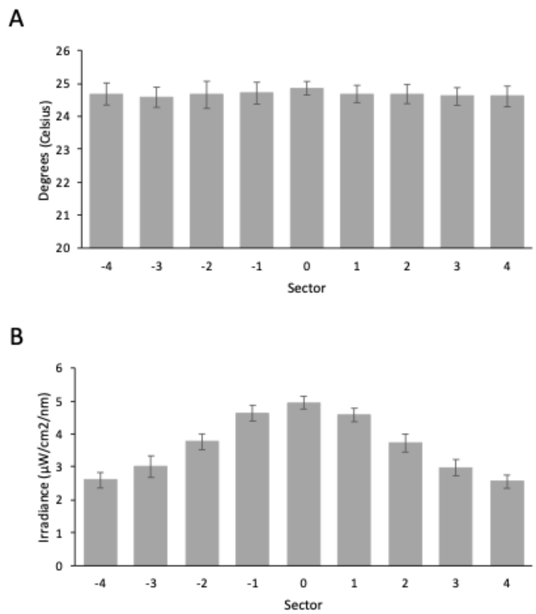

Fig. 6: Temperature and infrared gradients relative to the center of apparatus (pilot study). For each arena, sector 0 was oriented towards the center of the apparatus. A. Temperature values (ºC) for the four testing arenas (mean +/- SE) were similar in all sectors of the arena. B. Infrared values (in irradians; mean +/- SE) by sector for the four testing arenas were strongest near the center of the apparatus and decreased towards the exterior of the apparatus.

experience a gradient and 1 out of the 16 died before a second trial could be performed, so their trials were excluded from analysis of the data. Analysis of the data revealed that the chi square values for the percent occupancy in the control arena showed a higher level of variance than the gradient (Figure 9).

Discussion

Ultimately, the results of the pilot study and new behavioral assay did not show a clear bias in scorpion behavior towards IR light. However, scorpion IR

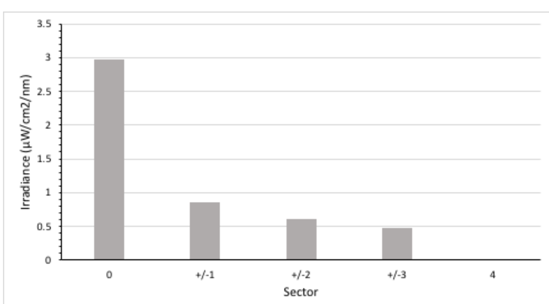

Fig. 7: Infrared intensity in the gradient arena. A spectrophotometer was used to measure infrared values (µW/cm^2/nm) around the gradient arena to ensure a gradient existed. The control arena was calibrated to a value equivalent to sectors +/- 2 in the gradient arena.

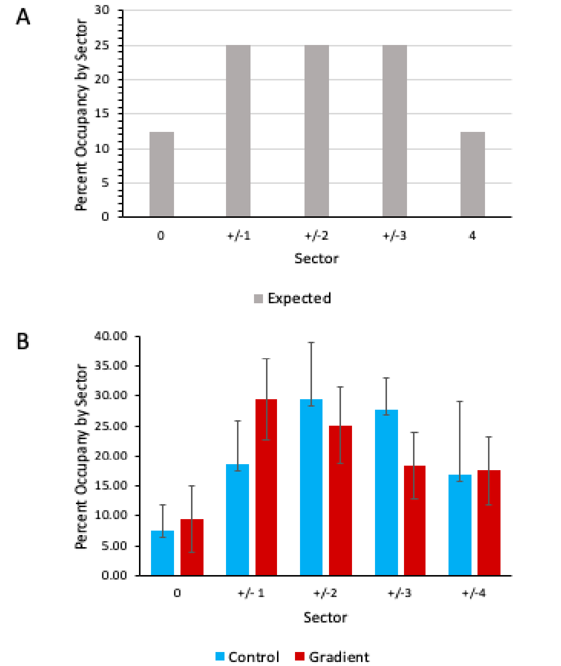

Fig. 8: A. Expected values for the percent time occupancy per sector based on the size of the sector. B. Average percent time occupancy per sector values for both the control and gradient arenas (mean +/- SE).

sensitivity cannot be completely ruled out based on these results. The slight bias towards IR light and the variance in the chi square results for the new assay warrant more research on the topic of scorpion IR sensitivity.

The unexpected results found in Vanderslice and Gaffin's study initially indicated that the lack of IR light flooding the arenas and the IR emanating from the video camera at the bottom of the apparatus may have had an influence on scorpion behavior. When the percent time occupancy graph (Figure 5B) was compared with that in Vanderslice and Gaffin's study (unpublished) a similar pattern was found; the

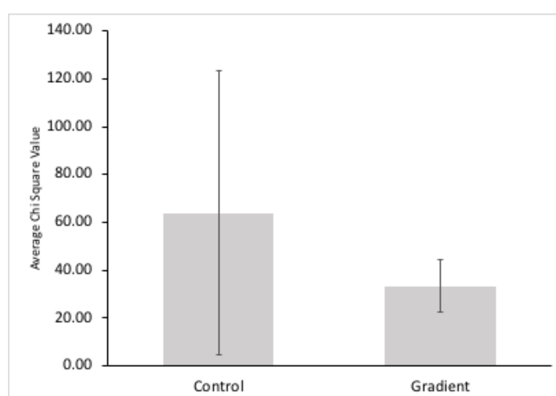

Fig. 9: Chi square values for the percent time occupancy per sector in the control and gradient arenas (mean +/- SE).

scorpions were biased to certain sectors relative to the IR camera below (Figure 1). The pattern found in the pilot study indicated that a different apparatus was required to determine if the scorpions were biased towards the IR light or to a different confounding variable. Furthermore, the measurements of temperature and infrared irradiance in the pilot study suggested that IR (and not temperature) was likely the most important influence on the behavior (Figure 6).

Scorpion behavior in the new apparatus did not show a significant bias relative to the gradient of IR light, but the chi square values for the control showed more variability than those for the gradient. This unexpected result may be due to a new element introduced to the apparatus – cameras positioned not only below, but also above the arena. The camera's light sources provided infrared light from both the top and the bottom, which may have influenced the behavior of the scorpions. However, the addition of a second camera became necessary to visualize scorpion movements because the tape on the bottom of the testing arenas created a barrier between the lower camera and the test subjects. Future improvements on this design might include a pressure plate, which would allow us to detect scorpion movements along an IR gradient without flooding the arena with additional IR light from a camera.

While little is known about scorpion vision, multiple scorpion organs have the potential to detect infrared irradiance, including the tarsal organs, constellation array, and pectines. The tarsal organs of the scorpion have been shown to be excited by humidity (Gaffin et al. 1992), and their placement on the perimeter of the body make them a good candidate organ. Few insects are sensitive to infrared radiation, but those that are, including *Melanophila acuminate* (the fire beetle) and *Rhodnius prolixus* (a bloodsucking bug), have a similar morphological feature for detecting infrared irradiance: many peg sensilla within a pit organ (Schmitz & Bleckmann 1998; Zopf et al. 2014). The constellation array on the first fixed finger of the pedipalp (Fet et. al 2006) could also be in an optimal position to sense infrared irradiance. While so far scorpion pectines have only been shown to detect chemosensory and tactile stimuli, they are also a prime organ for gathering ground-based information.

This study has many implications for nocturnal animal research, in which the observer's goal is to preserve and measure natural behavior. If infrared light can be sensed by scorpions, it may have a large effect on their behavior and on our current understanding of scorpion biology. Additionally, other organisms may be sensitive to infrared light, so the results of this research may call into question the results of many previous behavioral studies using IR cameras.

Acknowledgments

I thank Dr. Doug Gaffin for his exceptional counsel, encouragement, and edits, Dr. Matthew Rowe for his advice and inspiration, Brad Brayfield for his technical instruction, Tanner Ortery for caring for the animals, Dr. Mariëlle Hoefnagels for her constructive criticism and feedback, and other members of the scorpion lab for their suggestions and support. I also thank the OU Honors College, the OU Biology Department, and the Undergraduate Research Opportunities Program for the funding and opportunity to conduct our research. Finally, I thank the Sealy Smith Foundation in Monahans, TX for allowing us to collect animals on their land.

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