Across the rainbow: assessment of scorpion light responses to different wavelengths of light

An honors thesis by:

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Summary

When exposed to UV light, scorpions fluoresce a neon green color. The reason this happens is still known, because there is confusion regarding scorpion vision and behavioral responses to light. We have modified previous assays to produce a more efficient and automated apparatus. The system is a specially designed, enclosed wooden box with four circular arenas on the top. These arenas are made of a plastic cup with a Plexiglas tube glued in the middle, producing a circular track for the scorpion. In each of the tubes, there is a different wavelength of LED, UV (395 nm), cyan green (505 nm), orange (615 nm) or a no light control, shining across the walking track of the scorpion. In previous assays, there have been strong responses to UV and green lights. The scorpion's movements were tracked in each of the four arenas with a camera from below. We found that the scorpions showed no behavioral responses to the three different wavelengths of light. After testing for biases in our apparatus, we found that the scorpions showed a strong response to the IR light that was coming from the camera below. Although there is no previous literature discussing infrared response in scorpions, we hypothesize this reaction occurred because of a yet to be identified infrared detector. Overall, this new assay reduces the opportunity for human error and is more efficient than previous assays to show the responses of scorpions to different wavelengths of light. This assay will be particularly useful for future studies.

Introduction

To date, there is little known about scorpion vision and behavioral response to light. Scorpions typically have eight well-developed eyes; paired medial eyes that have lenses and can perhaps form images, and three pairs of lateral eyes that are sensitive to very low light levels (Hjelle 1990; Schliwa & Fleissner 1980). Scorpion medial eyes produce neural responses to as low as starlight levels of light intensity (Fleissner 1977b, 1985; Fleissner & Fleissner 2001) and both the medial and lateral eyes show broad physiological sensitivity to a variety of wavelengths (Fleissner & Fleissner 2001). Also, the circadian activity of scorpion eyes has been studied in response to changing intensities of light (Fleissner 1974, 1986) and green light photosensitivity has been recorded in parts of the scorpion tail (Zwicky 1968, 1970a,b). We know that scorpions respond to light—but why and what light?

Some studies have explored scorpion responses to various light wavelengths. In response to steady illumination from above, scorpions moved sporadically under UV and green light compared to other wavelengths (Blass & Gaffin 2008). In this configuration, scorpions responded strongly to both 505-nm (cyan-green) and 395-nm (UV light) but not to 565-nm (yellow-green) wavelengths with their eyes uncovered (Gaffin et al. 2012). In the same conditions, animals with their eyes covered with foil still responded strongly to UV light but not to cyan-green light, suggesting some form of extraocular UV sensitivity. This is curious since scorpion cuticle fluoresces cyan-green under UV light. In a new behavioral assay with light directed from the center across a restricted portion of a circular track, scorpions not only responded strongly to 395-nm, but also to 565-nm and slightly to 630-nm (red) light (Rivera Roldan & Gaffin in press).

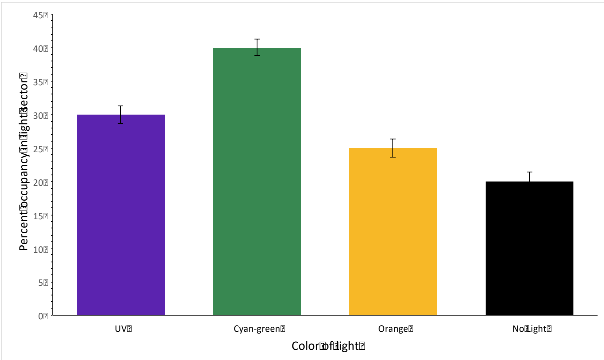

Fig. 1: Predicted behavioral response to light wavelengths. Based on findings in previous studies, we predicted that the scorpions would react most strongly to cyan-green light, then UV light, followed by orange light, and little to no response to the no-light control.

In this study, we sought to resolve some of the adapted and improved the light from the center assay to test scorpion responses to ultraviolet (395 nm), cyan-green (505 nm), and orange (615 nm) wavelengths of light. We predicted that scorpions would respond to each of these wavelengths, but not to the no-light control (Fig. 1); we also expected the strongest response to be to cyan-green. The significant modifications we made to the light from center assay have greatly increased the efficiency of our behavioral tests while decreasing the opportunity for human error. However, we found no significant behavioral responses to any wavelengths of light.

Methods

Animal collection and care

We used all female 24 adult *Paruroctonus utahensis* animals collected from sand dunes Monahans, Texas. Each animal was kept in a 3.8 L glass jar with sand 2.5–5.0 cm covering the floor along with a small piece of clay pot to retain moisture and provide a refuge. Each scorpion was fed one cricket before the experiment began, and to keep the scorpions hydrated, the sand was moistened with 5 mL of water one time per week. Throughout the study, the scorpions were kept in a room held between 21ºC and 23ºC using a small heater; the room was set to a light-dark phase of 20:00-06:00 (dark) and 06:00-20:00 (light).

Arenas

We constructed four identical testing arenas. For each, a Strata 20-ounce clear plastic cup was cut in cross-section 5 cm from its rim to create a cylinder that was 11 cm at one end tapering to 9 cm at the other end. The wide end of the cylinder was then glued to a 12 cm Plexiglas square. We also glued a 5 cm tall and 5 cm wide clear PVC pipe to the square in the middle of the cylinder to create a circular track for the scorpions to walk (Fig. 2).

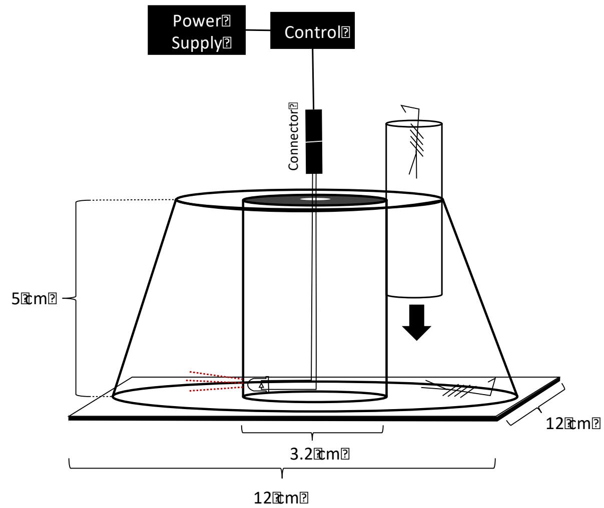

Fig 2: Individual behavioral arena. The arena was a plastic circular track created by a Plexiglas pipe in the middle. The center pipe contained the secured LED light that directed light across the track. The removable tube where the scorpion is held before the beginning of the experiment is pictured at the moment of releasing the scorpion from the tube.

A 5 cm tall removable, plastic tube served as a holding chamber for the scorpions before the trial began. The outer cylinder was covered in two layers of electrical tape to ensure no light would enter from the other arenas. We mounted an LED light (5 mm, 15-degree viewing angle) contained in a small metal lamp housing in the center PVC pipe by drilling four holes in the Plexiglas square and running two short pieces of copper wire through the holes around the LED lamp. We then twisted the strands together at the bottom to hold the light in place. The lamp was mounted with its emitting face flush against the PVC pipe to direct light across the circular track of the arena. The wire from the LED ran up through the central tube and outside the arena to connect to a breadboard.

Each of the four arenas contained a different wavelength of LED: UV (395 nm), cyan-green (505 nm), orange (615 nm), and no light. The no light control arena was identical to the other three in that it still contained a mounted LED, but the light was turned off. A spectrometer (Ocean Optics USB4000 UV-VIS-E) was used to ensure that each light was set to 0.010 ± 0.005 μW/cm^2/nm. This irradiance was chosen based on scorpion responses in previous studies (Gaffin & Barker 2014, Rivera Roldan & Gaffin in press).

Apparatus

Each of the arenas were placed in a square sector on top of a specially designed wooden stand with four square openings (Fig. 3). The arenas were filmed from below with an IR sensitive camera set on a platform 60 cm

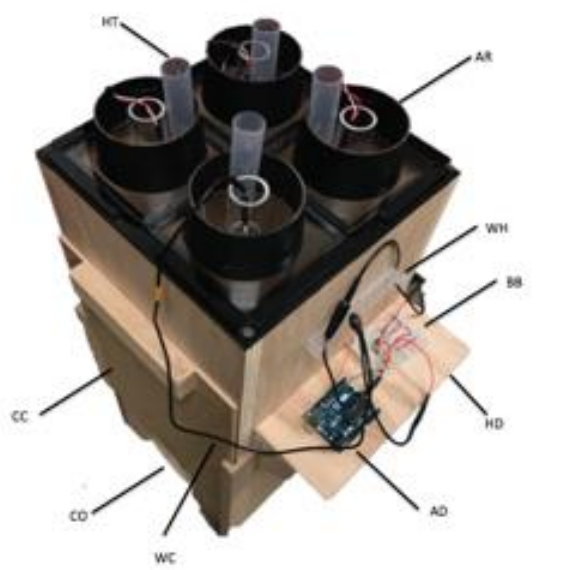

Fig 3: Experimental apparatus. Four individual behavioral arenas (AR) sit on top of a specially constructed wooden box. Each removable holding area is pictured in their positions before the trial initiation with the holding tubes (HT) for each scorpion. An infrared camera is positioned inside the box. It captures footage from below the arenas and is connected to a laptop computer. On the side of the box, there is a camera opening (CO) to allow us to move the camera if necessary. This opening can be closed during the experiment by the camera closer (CC) to eliminate all external light. There is also a wire holder (WH) and a holding desk (HD) on the side of the box. The wire holder helps keep the wires in place and the desk holds the necessary equipment for the LED lights. The wires (WC) shown connect the LEDs from each arena to the breadboard (BB), which is connected to the Arduino (AD) that regulated the frequency of each light.

beneath the bottom of the arenas. To further ensure no outside light, the area underneath the arenas was surrounded with wooden panels to form a closed box. The cord from the camera exited the box through a small notch at the bottom and connected to a laptop computer stationed 40 cm away from the apparatus. The laptop contained a specially written MATLAB program to acquire and process the video information. A holding desk was attached to the side of the box to house the breadboard and an Arduino. The LED wires connected to a 10-ohm, in-series resistor on the breadboard before running to the Arduino. The Arduino was then connected to the laptop computer and was used to control the intensity of each LED.

To mitigate against room and/or global cues that could affect scorpion behavior, the arenas and lights were oriented in new directions between trials based on a randomization process. Each scorpion had a two-night break between trials, and the scorpions were placed in the arenas in a different order to create a similar experience among the animals. Each scorpion experienced each light wavelength once, each of the four positions on the arena support box once, and light directed toward each of the four orientations once.

Trial protocol

We used a dim red light directed away from the apparatus to allow us to see during trial preparations. Before initiating trials, each arena was cleaned with a Kimwipe and 70% ethanol. The arenas were shifted to the proper section and direction. While the arenas were drying, the computer was turned on and the MATLAB program was opened. The scorpions were taken out of their jars and placed into the respective holding chamber of their arena, opposite of the LED. After the scorpions were placed in their arenas, the video capture program within MATLAB was set to record for 60 minutes. All lights in the room were turned off and the scorpions were released by lifting the holding tubes. We started the video recording immediately after releasing the animals. After the 60-minute recording, the red light in the room was turned on and the data were analyzed through MATLAB. The scorpions were placed back in their jars and the arenas were again cleaned and positioned atop the support box according to the randomized schedule. Then, the next four scorpions were placed in the waiting areas of the arenas and the procedure was repeated. The experiment was conducted over twelve consecutive days with two consecutive trials recorded each day. Each trial used four scorpions, one in each arena. The trials were conducted within 15 minutes of each other and all trials were conducted within a four-hour time period, between the hours of 21:00 and 01:00, of the 24-hour day.

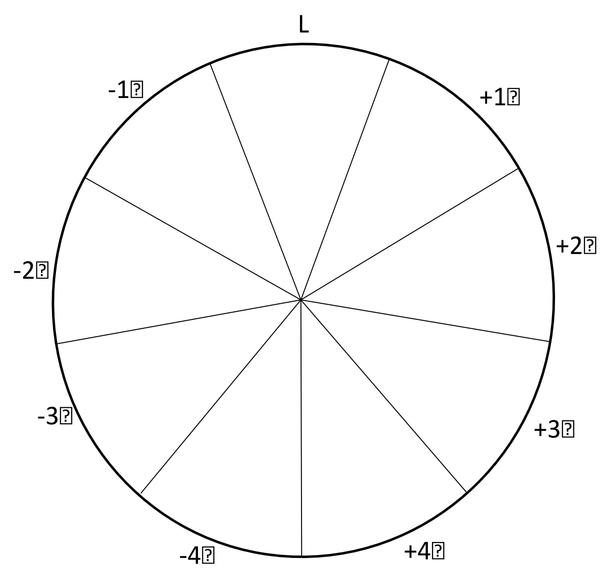

Fig 4: Arena scoring system. Each arena was divided into nine equal different sectors starting with the light sector (L) centered on the LED.

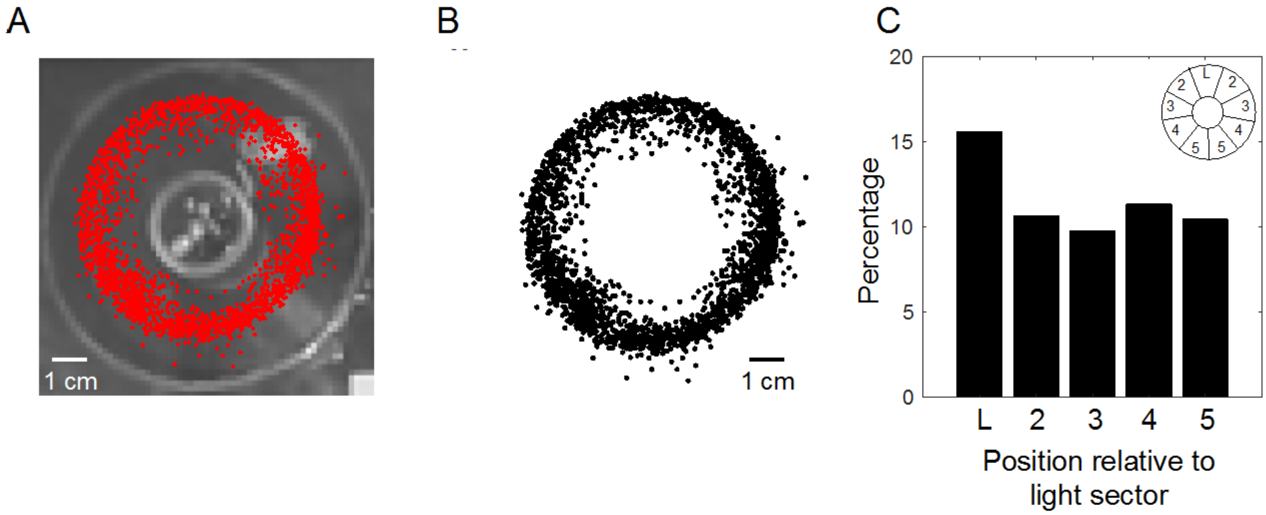

Fig 5: MATLAB plotting. **A.** After completion of a 60-minute trial, MATLAB produced a visual to show the scorpion's position throughout the trial. Each red dot represents the position of the scorpion during each second throughout the trial in the arena. **B.** Shown is the graph of the animal's x-y positions during the trial. **C.** Percent occupancy of the scorpion in each of the five regions of the arena.

Location mapping / analysis

After completing a 60-minute trial, MATLAB plotted the coordinates of each scorpion (using a frame-by-frame subtraction of centroids method) for each second of the trial. Then these points were summed for each of the nine equal sectors arranged relative to the direction of the light (Fig. 4). All non-movement frames were removed and the summed points for each sector were divided by the total number of movement frames to generate a percent occupancy value per sector. These data were further reduced to five regions by averaging the percent occupancy of the sectors that were equal distances away from the light sector. Only scorpions that entered the light-containing sector of the arena were used in our analyses. The percent occupancy in each of the light sectors for the four conditions was compared using an ANOVA, repeated measures test to assess statistical significance at a $P<0.05$ level.

Results

Most scorpions moved around the arenas, moving in quick sporadic motions followed by long pauses in a single spot. In general, the scorpions walked around the outer portion of the arena. Those animals that seemed responsive to the LED light would turn toward the LED, repeating this movement as if wanting to find the light. Figure 5 is a representative example of a scorpion's response to orange light. Of the 24 animals tested, 16 met legitimacy of entering the light sector during all four light conditions. Figure 6 shows the mean occupancy of the scorpions in each light sector across all four light conditions. There was no significant bias in the behavioral response to any of the four conditions.

After analyzing our trials, we checked for extraneous biases in the system by examining the control group of no light arenas. Of these 24 trials, 18 scorpions met our criteria of entering the light sector. We plotted this group of data in two ways: 1) relative to the top of the apparatus to check for global, geomagnetic, or room cues and 2) relative to the middle of the apparatus to test possible influences below animals. Figure 7A shows that there were no biases in the apparatus or the room by pointing the light section to the top of the apparatus (ANOVA, repeated measures, P=0.7178). Figure 7B shows a significant bias to the sectors one away from the sector

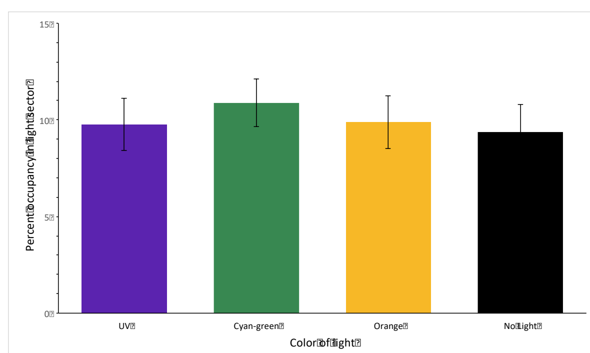

Fig 6: Summary of responses to light. Plots compare average percent occupancy (+/- SE) in the light sector for the four light conditions. There was no significant behavioral response across the four conditions.

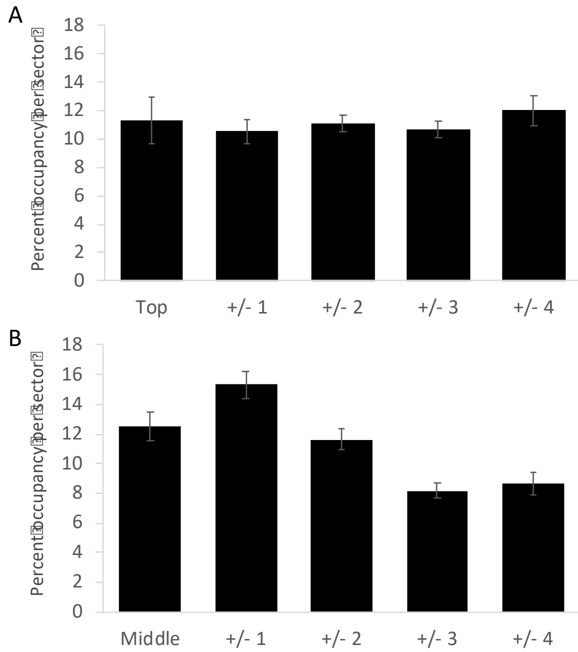

Fig. 7: Plots of control trials relative to top of video frame and middle of apparatus. **A.** When controls were plotted relative to the top of video frame, there was no significant bias was found in the arena (repeated measures ANOVA test, *P*=.7178). **B.** When controls were plotted to the middle of the apparatus control, there was a significant bias found in the arena (repeated measures ANOVA test, *P*<.0001).

oriented to the middle of the arena (ANOVA, repeated measures, P<0.0001). Furthermore, there was a detectable difference in IR values on the inside and outside of each arena (Fig. 8).

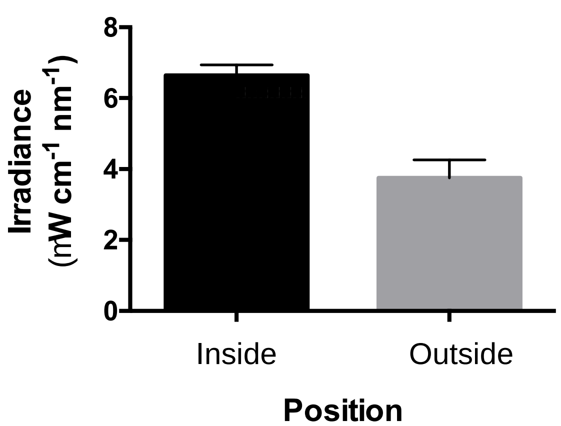

Fig. 8: Differences in IR levels from center to periphery of individual arenas. The irradiance on the inside of the arenas, closest to the middle of the apparatus, was larger than the irradiance on the outside of the arena.

Discussion

In this assay, we found that scorpions showed no bias to UV, cyan-green, or orange wavelengths of light. There was no bias in the apparatus, but there was a bias found relative to the IR light coming from below the arenas. The greatest triumph of this experiment was the improved efficiency of the assay along with the decreased opportunity for human error. The efficiency was greatly increased due to the locked positioning of our LED lights, adding an Arduino, mounting our camera in the apparatus, adding a holding area for the preparation stage of the trials, and the MATLAB code.

These results are different than what we expected based on previous studies. Rivera-Roland (in press) found strong responses to UV and yellow-green wavelengths of light and a slight response to red light in a similar assay. We found no overall bias toward any of the wavelengths, but a clear orientation relative to the IR light emanating from the camera below the arenas. Other than a head aversion response (Abushama 1964), we found no literature related to IR sensitivity in scorpions. Some beetle species have IR receptors used for fire detection (Schmitz & Bousack 2012). Future research should be directed at characterizing the nature of this IR response and pinpointing the sensory receptors responsible.

Moving forward with understanding scorpion light sensitivity, it is necessary to re-run this experiment with scattered IR light from below to further understand the attraction of scorpions to different wavelengths of light. It will be interesting to fill in the other colors of the rainbow, such as blue and pink, to test scorpions' responses to different wavelengths of light. Additionally, testing scorpions' responses to IR light opens a new door to exploring scorpion behavior. This new finding seems to be an interesting development in the investigation of scorpion sensory biology.

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References

Abushama, F.T. 1964. On the behavior and sensory physiology of the scorpion *Leirus quinquestriatus*. Animal Behaviour 12: 140–153.

Blass, G.R.C. & D.D. Gaffin. 2008. Light wavelength biases of scorpions. Animal Behaviour 76: 365–373.

Fleissner, G. 1974. Circadiane Adaptation und Schirmpigmentverlagerung in den Sehzellen der Medianaugen von *Androclonus australis* L. (Buthidea, Scorpiones). Journal of Comparative Physiology 91:399–416.

Fleissner, G. 1977. The absolute sensitivity of the median and lateral eyes of the scorpion, *Androctonus australis* L. (Buthidae, Scorpiones). Journal of Comparative Physiology, 118:109e120.

Fleissner, G. 1985. Intracellular recordings of light responses from spiking and nonspiking cells in the median and lateral eyes of the scorpion. Naturwissenschaften, 72:46e48.

Fleissner, G. 1986. Die innere Uhr und der Lichtsinn von Skorpionen und Ka ̈fern; zur neurobiologischen Analyse der Circadianen Uhr der Arthropoden. Naturwissenschaften, 73:78e88.

Fleissner, G. & G. Fleissner. 2001. Night vision in desert scorpions. In: Scorpions 2001; In Memoriam Gary A. Polis (Ed. by V. Fet & P. A. Selden), pp. 317–324. Burnham Beeches, Bucks: British Arachnological Society.

Gaffin, D.D., et al., 2012. Scorpion fluorescence and reaction to light. Animal Behaviour. doi:10.1016/j.anbehav.2011.11.014.

Gaffin, D.D. & T.N. Barker. 2014. Comparison of scorpion behavioral responses to UV under sunset and nighttime irradiances. The Journal of Arachnology 42:111–118.

Gaffin, D.D., L.A. Bumm, M.S. Taylor, N.V. Popokina & S. Mann. 2012. Scorpion fluorescence and reaction to light. Animal Behaviour 83:429–436.

Hjelle, J.T. 1990. Anatomy and morphology. In: The Biology of Scorpions (Ed. by G. A. Polis), pp. 9-63. Stanford, California: Stanford University Press.

Rivera Roldan, N. & D. D. Gaffin. 2018. New, sensitive behavioral assay shows scorpions are attracted to multiple wavelengths of light. Journal of Arachnology *in press*.

Schliwa, M. & Fleissner, G. 1980. The lateral eyes of the scorpion. *Androctonus australis*. Cell and Tissue Research, 206:95–114.

Schmitz, H. & H. Bousack. 2012. Modeling a historic oil-tank fire allows an estimation of the sensitivity of the infrared receptors in *Pyrophilous Melanophila* beetles. PLoS ONE, 7(5):e37627.

Zwicky, K.T. 1968. A light response in the tail of *Urodacus*, a scorpion. Life Sciences, 7:257e262.

Zwicky, K. T. 1970a. The spectral sensitivity of the tail of *Urodacus*, a scorpion. Experientia, 26:317.

Zwicky, K. T. 1970b. Behavioral aspects of the extraocular light sense of *Urodacus*, a scorpion. Experientia, 26:747e748