

SCORPION NAVIGATION

aka, "Self-Driving Arachnids"

Manuscripts created as a component of
the Scorpion Navigation cornerstone
course at the Department of Biology of
the University of Oklahoma

FALL 2021

Water localization capabilities of the desert grassland scorpion

Cameron Lang, Maggie Bui, D'Oyen Fraser Jr., Greycen Gutierrez, Lucas Hodgson

Instructor: D. Gaffin

ABSTRACT: A common public perception is that scorpions are attracted into buildings by sources of standing water. Previous studies have revealed that scorpions can detect water by contact, but there has been minimal research on scorpions' ability to detect water from a distance. We conducted a Y-maze behavioral assay on desert grassland scorpions, *Paruroctonus utahensis*, to determine if they could detect water at a distance. We predicted that the scorpions would spend more time in the water-containing arm of the maze than in the control arm. Our data showed no statistically significant preference for either condition. However, we observed behavioral patterns that may suggest water-sensing behavior, indicating the need for further research.

Keywords: scorpion, water, humidity, hygrotaxis

Introduction

Scorpions, like other arachnids, are often feared and reviled, especially when they enter our homes. A common public perception asserts that scorpions are drawn into human dwellings by sources of open water or humidity, such as sinks and bathtubs. This claim is widely promoted online, particularly in reference to scorpions in the arid American southwest. However, little scientific evidence is available to corroborate or contradict these anecdotal reports. The purported ability of scorpions to seek out humid environments implies the capacity to perceive humidity and navigate towards it. This behavior is known as hygrotaxis, and is generally understudied in scorpions. In 2020, it was estimated that 1.2 million people were stung by scorpions, and 3000 people died as a result [3]. A study in Arizona, which reports the most scorpion stings of any US state, found that stings frequently occur indoors, specifically within residences [2]. A better understanding of what may attract scorpions into buildings could allow for measures to reduce the risk of scorpions entering homes. This could in turn benefit public health by reducing the frequency of scorpion envenomation, potentially saving lives.

Previous research has indicated that scorpions obtain most of their water from the prey they eat [8], but that they also opportunistically consume mois-

ture from damp substrates as a supplementary source of hydration [8, 7, 5]. When a scorpion comes into contact with water, chemosensory hairs on the tarsi (feet) detect it and elicit various behavioral responses, including water consumption [7]. While scorpions can detect water by physical contact, it remains unclear how they can locate more distant water sources. A potential explanation is hygrotaxis, which has been reported in a variety of arthropods, including crustaceans [10] and insects [12]. Among arachnids, hygrotaxis has been demonstrated in two orders of mites [9, 6] and in at least two species of spiders [11]. However, research on hygrotaxis in scorpions is sparse. A 1964 study presented scorpions with an enclosed humidity gradient and found that the animals generally gravitated towards the more humid region [1], but more recent research into humidity gradient response has been inconclusive [4]. In this study, we seek to address this gap in the literature.

We investigated whether scorpions are capable of perceiving humidity from a distance and whether they can seek out damp environments without directly contacting water. We used a Y-maze behavioral assay to examine preferences for humidity in captive desert grassland scorpions (*Paruroctonus utahensis*). We hypothesized that if scorpions can detect water over a distance, and we allowed them to choose between a dry environment or a humid one, they would have a

statistically significant preference for the humid one. We quantified this preference based on the proportion of time spent in each arm of the maze and compared behavioral responses to the different conditions. Ultimately, we found no statistically significant preference for either condition. However, we suspect that an observed climbing behavior may indicate short-range hygrotaxis.

Materials and Methods

Animal husbandry and preparation

We conducted our study using *P. utahensis*, a vaejoid scorpion native to the American southwest. Adult animals were collected from wild populations found in sandy habitats near Monahans, Texas. When not participating in trials, each scorpion was kept in an individual terrarium constructed from a food storage container (Great Value, 236 ml) with four 5.6 mm air holes drilled into the corners of the lid. To mimic their natural habitat, we placed sand (50 ml) gathered near Monahans in each terrarium. We also placed a small semi-cylindrical PVC plastic shelter approximately 7 cm in length and 3 cm in height into each terrarium, imitating a scorpion's burrow. All terrariums were maintained at ambient temperature and humidity, which we regularly measured using a digital hygrometer and thermometer (ThermPro) and recorded for reference.

The animals' terrariums were kept in a dark enclosure with a single 60-watt LED light (Ecosmart) inside the chamber to simulate daylight. The light was controlled with a digital timer (BN-LINK) set to illuminate the chamber for fourteen hours per day. Because *P. utahensis* is nocturnal, we artificially inverted the scorpions' day-night cycle to promote higher activity levels during daytime lab hours and to ensure ease of observation. The scorpions were subjected to trials within the first three hours of darkness.

Animals were fed in their terrariums and were not given additional water. Their diet consisted of small crickets (*Acheta domesticus*) measuring ~6.35 mm (Fluker's Cricket Farms, Port Allen, Louisiana). Each animal was fed once, fasted for 14 to 22 days (depending on trial timing), then was fed after participating in a valid trial. To monitor potential negative effects of this fasting schedule, we recorded the scorpions' status (alive or dead) three times per week. To minimize stress that may affect animal welfare or experimental results, we handled the scorpions only when necessary to move them between terrariums and the Y-mazes. We used forceps to hold the animals at the base of the tail for these transfers.

Early observations

We experienced difficulties in the early stages of our project, primarily relating to lethargy and mortality among animals in pilot trials. Twenty scorpions were initially acquired and dehydrated, but only used in pilot trials (another twenty were acquired for actual trials). Contrary to our expectations and anecdotal knowledge of *P. utahensis*'s tolerance to dehydration, we observed significant mortality beginning in the third week of dehydration. During the fourth week of dehydration, mortality of the original sample reached 50 percent, and only 7 of 20 animals survived the full month of dehydration. The animals from the original sample were generally lethargic in pilot trials, frequently refusing to move at all. Physical stimulation with a paintbrush or puff of air were unsuccessful in provoking movement. As a result of this pilot work, we restricted the dehydration period for our experimental animals to two weeks.

Y-maze apparatus

The behavioral assay was conducted in an acrylic Y-maze apparatus (see Appendix A for dimensions) with a vertically-sliding gate enclosing an acclimation chamber at the end of the long arm (Fig 1). We distributed 100 ml of sand on the floor of the maze (during pilot trials we found that animals moved more readily on sand vs bare acrylic). Sand used in the experiments was gathered near Monahans, baked on a hotplate between trials to eliminate moisture and chemical cues, and stored in a sealed container. The two short arms of the maze each contained a round plastic sauce container (DART, 2.5 oz), approximately 4 cm in diameter and 3 cm in height, in a screened-off area at the end; the screen was constructed from fine wire mesh. In the control arm, the sauce container contained 20 ml of paraffin oil. In the experimental arm, the sauce container contained 20 ml of water.

The Y-maze was covered by an inverted plastic trash can (Rubbermaid Brute, 44 gallon) mounted on a wooden turntable, which was then placed on a rubber mat to reduce vibrations. A string was attached to the vertically-sliding gate at the end of the long arm of the maze and threaded through the trash can bottom to allow the gate to be lifted without removing the trash can. Each trial was filmed with an infrared camera (ELP, Megapixel IP CAMERA) fitted on the trash can to give an aerial view of the arena. The filmed trials were processed using MatLab for MacOS (R2021A, Update 5).

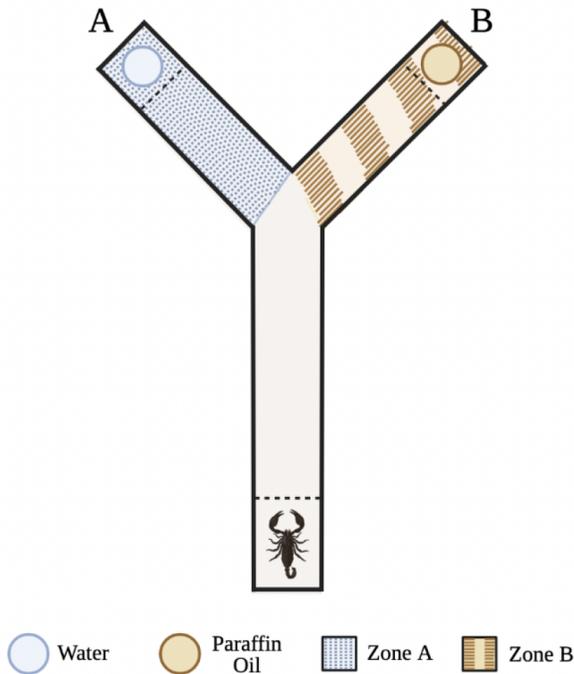


Figure 1: Y-maze. The diagram shows the Y-maze apparatus used in the experiment; note that the locations of the water and paraffin oil sources were switched between trials. The dashed line at the base of the long arm represents the removable gate.

Experimental protocol

Trials began approximately 30 minutes after the lights went off in the inverted day-night cycle. Scorpions selected for trials had not eaten for between 14 and 22 days. In each trial, a randomly-selected scorpion was transferred from its terrarium to the acclimation chamber behind the gate. Each animal was allowed 30 seconds to acclimate before the gate was lifted to allow the scorpion access to the rest of the Y-maze. Each 10-minute trial officially began once a scorpion's full body emerged from the acclimation chamber into the long arm of the maze. After each trial, the maze was emptied of sand, cleaned with ethanol and dried, and refilled with baked sand. Between trials, we switched which liquid was in each arm and rotated the entire apparatus 90 degrees clockwise to control for possible navigational biases.

Data collection and analysis

Filed trials were blindly reviewed by a team member who did not know which arm corresponded to which humidity condition; reviewers recorded time spent in each arm using video time stamps. A threshold line at

the base of each short arm of the Y-maze defined when a scorpion had entered or exited the experimental and control arm (Fig 1). When the base of the animal's tail crossed this line, it was considered to have entered that arm. Trials in which scorpions never entered either humidity condition were disregarded, and those individuals underwent another trial the next day. No two valid trials used the same scorpion, and scorpions had no more than three opportunities to yield a valid trial. Our data were non-parametric and collected in a repeated measures design. A Wilcoxon Signed-Rank was used to determine if there was a statistically significant ($p < 0.05$) behavioral preference for either humidity condition.

Results

The purpose of the Y-maze experiment was to present scorpions with a choice of two environments that were identical except in their relative humidity. In doing so, we allowed the animals to seek out a water source from a distance, presuming that their state of dehydration would motivate water-seeking behavior. Preference for one arm of the maze over the other, measured as time spent in each arm, served as a proxy for humidity-based water localization capabilities. A statistically significant preference for the water-containing arm over the oil-containing arm would imply the ability to physiologically detect humidity, allowing the animals to locate nearby water sources.

Subjects that participated in the experimental trials were observed behaving normally with no visible signs of distress or agitation. When trials began, most scorpions remained in the acclimation chamber for a few minutes before venturing into the maze. They generally moved at a steady pace, and some stopped to rest for a few minutes. Most scorpions visited both arms of the maze at least once, and usually spent more time in one arm than the other. We also observed scorpions climbing on the mesh screen that prevented access to the liquid media.

We found no statistically significant preference for either the water-containing or oil-containing arm. Scorpions spent a mean time of 194 seconds in the water-containing arm versus 179 seconds in the oil-containing arm (Fig 2A); a Wilcoxon signed-rank test yielded $p = 0.875$ and a value of $W = 37$ with a critical value of $W = 13$ at $n = 12$ ($p < 0.05$). We also compared time spent in the left and right arms. We found no statistically significant difference in time spent in either the left (A) or right (B) arms ($p = 0.209$ and $W = 23$), suggesting no directional preference (Fig 2B).

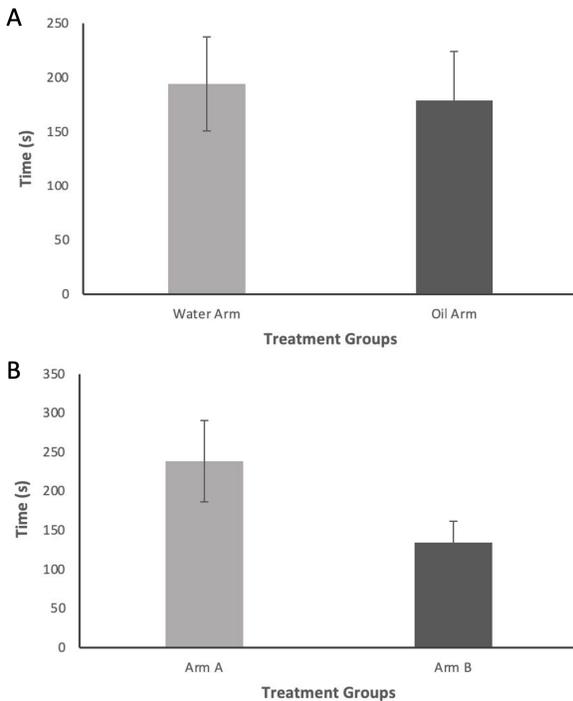


Figure 2: Time spent in water vs oil and A vs B arms. A. Mean time spent by scorpions in the water-containing and oil-containing arms of the Y-maze. B. Mean time spent by scorpions in Arm A (left) and Arm B (right) of the Y-maze. Error bars represent standard error.

The small sample size of the experiment ($n=12$) was a consequence of unexpected lack of behavioral response to the Y-maze in many scorpions. From a study population of 20 individuals, we obtained 12 valid trials according to our criteria. We were forced to exclude a number of trials because the scorpion in question never entered either arm. Due to limitations of time and animal health, we were unable to obtain more valid trials.

Discussion

Our investigation found no statistically significant evidence of humidity preference or hygrotaxis in *P. utahensis*. As such, we cannot reject the null hypothesis that scorpions do not navigate to water by perceiving humidity gradients. The lack of statistically significant preference for the water-containing arm of the Y-maze is consistent with the similar data gathered by Bowman et al [4]. Our methodology addressed several experimental design factors that may have influenced the results of that study: We dehydrated our scorpions more extensively, inverted their day-night cycle

to increase activity levels, and conducted trials with the apparatus in complete darkness. Despite these adjustments, our scorpions showed no preference for the water-containing arm of the maze.

The lack of statistical significance in our findings does not necessarily indicate that scorpions are incapable of humidity-based orientation; a variety of explanations for the approximately equal preference for water and oil arms are possible. We suspect that the water source used may not have provided sufficient airborne moisture to permit hygrotaxis. Future experiments could implement several methods to increase the number of water molecules available to the scorpions in the maze. For example, larger volumes of water could be added, potentially in a container with more water-air surface area to increase evaporation. Warming the liquid sources or using small fans to move air from the maze arms towards the acclimation chamber could also increase accessibility of airborne water molecules.

The most intriguing post-hoc behavioral observation from the experiment was the scorpions' behavior near the liquid source after entering a particular arm. Scorpions frequently used their pedipalps to investigate the mesh screen impeding their access to the liquid medium; in most of those cases, the scorpion proceeded to climb onto the mesh, frequently walking in circles on the screen. Scorpions climbed an average of 1.08 times per trial in the water-containing arm, compared to 0.58 times in the oil-containing arm. As this behavior was observed more frequently near water, it may suggest short-range hygrotaxis; further experimentation could characterize scorpion behavior in close proximity to an inaccessible water source. Scorpions possess a small tarsal organ, located on the dorsal side of each tarsus; previous research has shown that this organ responds electrophysiologically to sources of water brought within 1 cm of the structure [7]. It is possible that this organ could allow perception of humidity as the animal climbed the screen near to the water source. Future modifications of this experiment might examine this near range response both with the tarsal organs in tact or covered.

We have some additional suggestions for future studies. First, due to the animal mortality we experienced during our pilot studies, we strongly recommend dehydrating scorpions for no more than 15 days. Second, we found that an acclimation period of 30 seconds in the Y-maze was optimal to ensure the animals were calm but active in the maze. We also observed that the scorpions moved more slowly and less erratically when the Y-maze substrate was sand, as opposed to bare acrylic. Finally, we feel that the near-complete

darkness provided by the trash can over the maze was useful in ensuring that the scorpions were exhibiting exploratory behavior, rather than a stress response. We believe that these successful methods, along with the modifications noted above, will allow future researchers to better investigate scorpions' potential ability to locate water using hygrotaxis.

Acknowledgments

We are indebted to George Martin for constructing our Y-maze apparatus and to Dr. Ingo Schlupp for invaluable statistical consultation and general advice. We also thank Dr. Mariëlle Hoefnagels for her assistance in preparing and editing our manuscript, Dr. Amanda Schilling for LaTeX assistance, Dr. Claire Curry for Zotero advice, and Liz Cooley for ordering our supplies.

References

- [1] ABUSHAMA, F. T. On the behaviour and sensory physiology of the scorpion *Leiurus quinquestratus* (H. & E.). *Animal Behaviour* 12, 1 (Jan. 1964), 140–153.
- [2] BENNETT, B. K., BOESEN, K. J., WELCH, S. A., AND KANG, A. M. Study of factors contributing to scorpion envenomation in Arizona. *Journal of Medical Toxicology* 15, 1 (Jan. 2019), 30–35.
- [3] BOUBEKEUR, K., L'HADJ, M., AND SELMANE, S. Demographic and epidemiological characteristics of scorpion envenomation and daily forecasting of scorpion sting counts in Touggourt, Algeria. *Epidemiology and Health* 42 (July 2020), e2020050.
- [4] BOWMAN, M., RILEY, D., AND USMAN, M. Some like it humid: hygrotaxis in the striped bark scorpion. 5.
- [5] CRAWFORD, C. S., AND WOOTEN, R. C. Water relations in *Diplocentrus spitzeri*, a semimontane scorpion from the southwestern United States. *Physiological Zoology* 46, 3 (July 1973), 218–229. Publisher: The University of Chicago Press.
- [6] GAEDE, K. On the water balance of *Phytoseiulus persimilis* A.-H. and its ecological significance. *Experimental & Applied Acarology* 15, 3 (Sept. 1992), 181–198.
- [7] GAFFIN, D., WENNSTROM, K., AND BROWNELL, P. Water detection in the desert sand scorpion, *Paruroctonus mesaensis* (Scorpionida, Vaejovidae). *Journal of Comparative Physiology A* 170, 5 (June 1992).
- [8] HADLEY, N. F. Water relations of the desert scorpion, *Hadrurus arizonensis*. *Journal of Experimental Biology* 53, 3 (Dec. 1970), 547–558.
- [9] MORI, H., AND CHANT, D. A. The influence of humidity on the activity of *Phytoseiulus persimilis* athias-henriot and its prey, *Tetranychus urticae* (c. l. koch) (Acarina: Phytoseiidae, Tetranychidae). *Canadian Journal of Zoology* 44, 5 (May 1966), 863–871. Publisher: NRC Research Press.
- [10] PIERSANTI, S., PALLOTTINI, M., SALERNO, G., GORETTI, E., ELIA, A. C., DÖRR, A. J. M., AND REBORA, M. Resistance to dehydration and positive hygrotaxis in the invasive red swamp crayfish *Procambarus clarkii*. *Knowledge & Management of Aquatic Ecosystems*, 419 (2018), 36. Number: 419 Publisher: EDP Sciences.
- [11] SAVORY, T. H. Environmental differences of spiders of the genus *Zilla*. *Journal of Ecology* 18, 2 (1930), 384–385. Publisher: [Wiley, British Ecological Society].
- [12] SUN, Y., GAO, S., JI, F., AND ZHU, Y. A novel hygrotaxis assay for assessing thirst perception and water sensation in *Drosophila*. *Bio-protocol* 9, 3 (Feb. 2019), e3154.

Appendix

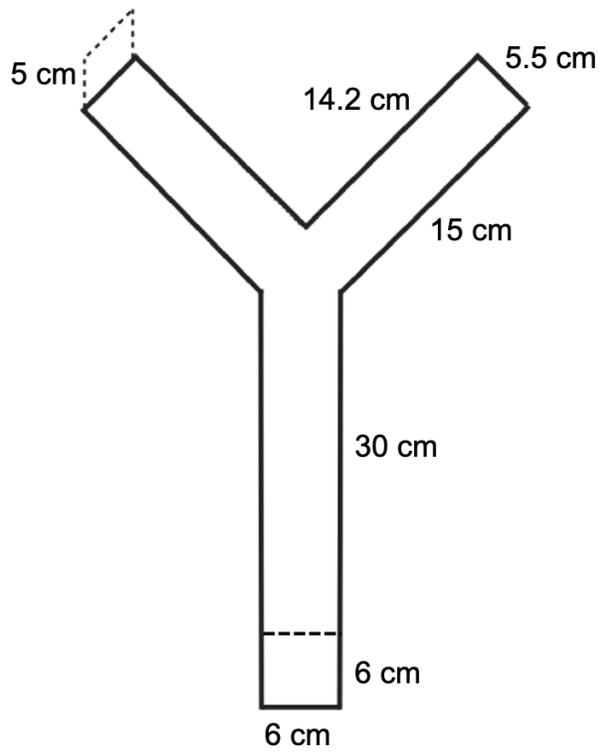


Figure 3: Y-Maze dimensions. Approximate measurements of Y-maze, based on initial sketches of the apparatus.