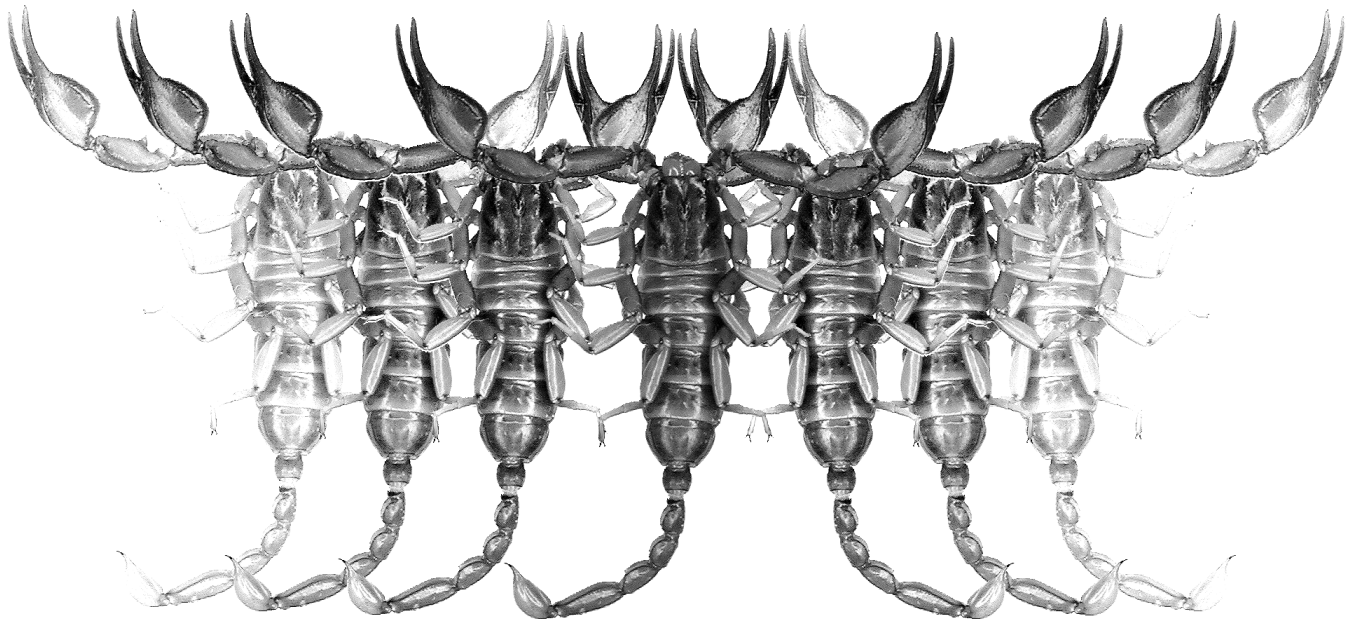


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A Novel Thermal Gradient Design for Small-bodied Ectotherms

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Summary

For ectothermic organisms, environmental temperatures can influence a variety of life history traits. Experimental thermal gradients created in the laboratory are useful tools to examine the thermoregulatory behaviors of these organisms. Here, we provide details on the construction of a novel, cost-effective thermal gradient to study thermoregulatory behaviors in small-bodied nocturnal ectotherms.

Introduction

Environmental factors can play a key role in shaping the life history of organisms, influencing aspects of both their behavior and physiology (Huey, 1982; Patten, 2005; Amiel & Shine, 2012). For ectotherms, environmental temperatures influence a number of life history traits such as adult body size (Partridge et al., 1994), metabolic rate (Heldmaier & Ruf, 1994), locomotor ability (Hertz et al. 1982; Braña, 2000), movement patterns (Avery & Bond, 1989), foraging and feeding rates (Ayers & Shine, 1997), prey-handling performance (Avery & Mynott, 1990), digestive rates (McConnachie & Alexander, 2004), gestation lengths (Beuchat, 1988), reproductive output (Honkoop & van der Meer, 1998), and offspring viability (Shine & Harlow, 1993). As such, ectotherms are sensitive to changes in environmental temperatures, and these organisms use thermoregulation as a means of keeping their body temperatures within tolerable limits. Ectotherms can modify their body temperatures through movements to and from suitable microclimates, increasing or decreasing the duration of basking behaviors, or through alterations in body postures on the substrate (Halliday & Adler, 2002). Understanding the way in which ectotherms use their environment can reveal factors that may have shaped aspects of their ecology.

One common method of assessing the body temperatures of organisms is through the use of experimental thermal gradients (Gatten, 1974; Warburg & Ben-Horin, 1981; Braña, 1993; Refinetti, 1998; Brown & Weatherhead, 2000; Aubret & Shine, 2010; Schuler et al., 2011). Typically these gradients consist of an

enclosure offering two extremes in temperature. One end of the enclosure is maintained above an organism's expected optimum temperature, while the other end is kept below a predicted optimum temperature. The thermal preference of an organism presumably lies between these two extreme temperatures. In combination with field studies, the use of thermal gradients has been helpful in determining the preferred body temperatures of a variety of organisms (Medina et al., 2011; Weatherhead et al., 2011). Although most thermal gradients share the same general design, many differ in the methods used to generate temperature extremes, including incandescent light bulbs (Le Galliard et al., 2003), copper tubing circulating hot water (Warburg & Ben-Horin, 1981), heating pads (Blouin-Demers et al., 2000), and hotplates (Kaufmann & Bennett, 1989). Ice (Huey et al., 1989; Gvozdik & Castilla, 2001) and copper tubing circulating cold water (Blouin-Demers et al., 2000) have been used to construct the cooler end of gradients. However, problems may arise when using these heating and cooling models to investigate thermoregulatory behaviors. For example, the use of incandescent bulbs as a heat source may alter behaviors that typically occur under low light levels or in the complete absence of light. Many organisms may also utilize substrate heat rather than overhead heat to maintain body temperatures, further increasing the difficulty of using an overhead lamp as a source of heat. This is especially true for nocturnal animals. Additionally, the use of a single heating source limits the amount of control over how heat is distributed throughout the substrate within the gradient. The use of coiling systems (e.g., Warburg & Ben-Horin, 1981; Blouin-Demers et

al., 2000) can circumvent some of these problems, but published designs tend to be relatively complex and costly, and some cooling implements such as ice need to be replaced periodically over the course of an experiment. Herein, we provide an easily replicated and cost-effective model for the construction of a thermal gradient to study the thermoregulatory behavior of small-bodied nocturnal organisms. Our experimental design allows for multiple trials to be conducted simultaneously, and our method can be scaled in size to accommodate both smaller and larger sample sizes.

Materials and Methods

All materials used in the construction of the gradient were purchased at a total cost of \$262 USD. Vendors are listed in the Appendix. For enclosures, the following supplies were used: 8 - 71 x 16.5 cm sheets of 3.18 mm thick glass, 4 - 73 x 14.5 cm sheets of 3.18 mm thick glass, 8 - 16 x 14 cm sheets of 3.18 mm thick glass, 2 - 298.6 ml tubes of 100% silicon, and 5 - 2.27 kg bags of clean aquarium gravel (2.5 mm in diameter). For the gradient, we used 2 - 71 x 64 cm sheets of 3.18 mm thick glass, 8 strips of 10.2 cm wide x 70 cm long Flexwatt heat tape, 8 Flexwatt cord attachments, 8 clip sets for Flexwatt heat tape, 8 mechanical dimmers, 1 roll of (19.1 mm x 18.3 m) electrical tape, and 1 roll of (50.8 mm x 45.7 m) foil tape.

To construct our gradient, we first assembled four individual enclosures (Fig. 1). Each enclosure was rectangular in shape, and had the following dimensions: 71 cm (length) x 15.2 cm (width) x 15.2 cm (height). We constructed all enclosures out of glass, and sealed all seams with silicon on the outer surfaces of each corner to both adhere the glass panels and to prevent the potential escape of our study subject, Arizona bark scorpions (*Centruroides sculpturatus* Ewing, 1928, family Buthidae). After enclosures were built, we made a temperature gradient utilizing substratum heat generated by using multiple tiers of Flexwatt heat tape (Fig. 1). We wanted to create a linear gradient of temperatures ranging from 48 °C at the hottest end of the enclosure to 23 °C at the coolest end, based on our predicted upper and lower thermal limits of *C. sculpturatus*. Prior to constructing the gradient, we connected 8 strips of heat tape to mechanical dimmers to allow manual adjustments to the amount of heat produced by each strip of heat tape. We then arranged six rows of dimmer-controlled heat tape latitudinally on top of a lab bench, with the edges of each section of tape abutting the edge of each adjacent strip (Fig. 1). We left one section bare at the lower end of the enclosure equal to the width of the heat tape (10.2 cm). After this initial layer of heat tape was placed, we secured the outer edges of the strips to the lab bench with foil tape. Next, we placed a single piece of glass (71 x 64 cm) over the

strips of heat tape. The glass functioned to secure all heat tape strips, provide a stable surface for the next layer of heat tape, and narrowly but evenly distribute heat. After the sheet of glass was laid, we placed a second layer of heat tape strips directly above the two uppermost rows at the upper end of the gradient (Fig. 1). Both of these strips were connected to dimmers. As before, we secured this second layer of heat tape using foil tape, and covered the strips using a piece of 71 x 64 cm glass. We additionally secured all edges of this second piece of glass, identical in size to the bottom piece of glass, to the lab bench using electrical tape to prevent accidental shifting during substrate changes or during the cleaning of enclosures. When completed, the heat tape was layered in a 2-2-1-1-1-1-0 arrangement (Fig. 1).

Finally, we placed the four enclosures longitudinally on top of the gradient (Fig. 1). Within each of the four constructed enclosures, we placed an even layer of clean gravel substrate 2 cm deep. After plugging in all of the strips of heat tape, we increased temperatures along the gradient by manually adjusting dimmers. We recorded substrate temperatures using a non-contact IR temp gun (Model 42505, Extech Instruments), although any instrument capable of accurately measuring substrate temperatures could be used. We set the coolest end of each enclosure by adjusting the ambient room temperature, which was controlled by a digital wall-mounted thermostat controlling a commercial HVAC unit.

Results and Discussion

The coldest end of the gradient was successfully maintained at 23 °C (+/- 0.5 °C) by setting the room thermostat to 23 °C. The maximum temperature reached within the gradient was 48 °C, although preliminary trials suggested higher temperatures (up to 50 °C) were possible. The maximum temperature was reached within 6 hours of plugging in the heat tape, and temperatures remained constant provided the ambient temperatures of the room remained unchanged. The gradient exhibited a linear increase in temperature from 23–48°C, increasing approximately 3.5 °C every 10.2 cm up from the lower (coolest) end of the enclosure.

There are several advantages to our new design. First, the thermal gradient is relatively cost effective, and all supplies can be purchased for around \$262 (not including the cost of a substrate temperature-measuring device, which varies greatly). Second, the narrow rectangular width of each enclosure, the long linear arrangement of the heat tape strips, and the use of glass sheeting (an efficient heat conductor) to cover tiers of heat tape allow for multiple enclosures to be placed adjacent to one another, thereby increasing the amount of data that can be collected during each timed trial. In

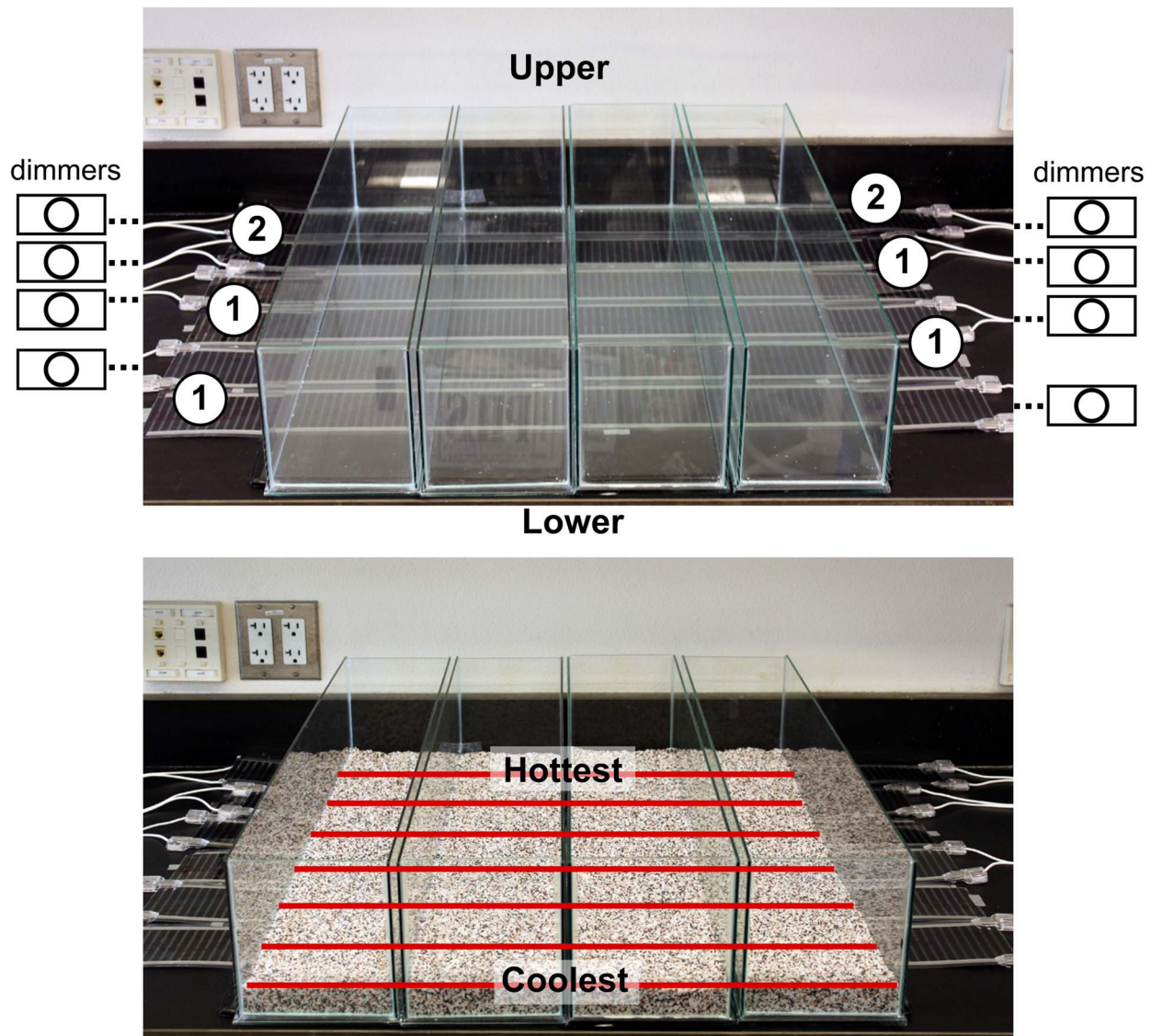


Figure 1: Fully assembled thermal gradient with enclosures. For scale, each enclosure measures 71 cm (length) x 15.2 cm (width) x 15.2 cm (height). Numbers refer to number of Flexwatt heat tape strips in each of the six horizontal heated layers. Hottest and coolest ends of enclosures and orientation of design are noted.

our study, we were able to measure thermal preferences of four scorpions at a time. Simply using longer strips of heat tape would allow more enclosures to be added. Additionally, because substratum heat was used rather than heat from an incandescent bulb, it allowed for experimental trials to be conducted in the absence of light which is likely beneficial when studying the thermoregulatory behavior of nocturnal organisms. Finally, our design does not rely on items such as ice (which require replacement) or a constant source of water (such as the case with copper tubing), allowing for the gradient to operate for an extended period of time following its initial setup.

Our thermal gradient design, however, is not without limitations. All enclosures were made of glass, and

so care must be taken when moving the gradients and when replacing substrate. Also, our enclosures used an open-top design for increased visibility and to prevent heat buildup inside the enclosures. This lidless design will not contain flying or agile organisms capable of climbing sheer glass walls. Further, our use of small diameter gravel as a substrate may not work for all study organisms. For example, some organisms may alter their thermal environment by burrowing within the substrate, and so the appropriate substrate should be selected carefully. Experimental design may also be improved by constructing the gradient within an environmental chamber to control additional environmental factors such as humidity. Because the electric currents we used to generate heat also create magnetic fields, animals may

respond to both temperature and magnetic gradients. To control for magnetic inference, a polymer film insulation could be placed over each section of the heat tape.

As noted by the vendor of the heat tape: “The manufacturer states that a qualified electrician should install it. Use common sense when using this or any other electrical heat element.” Improper installation of electrical components used in our thermal gradient design may cause electrical hazards, and we the authors take no responsibility for any damages incurred.

Acknowledgments

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APPENDIX

Flexwatt heat tape, Flexwatt cord attachments, and clip sets for Flexwatt heat tape were purchased from The Bean Farm (Carnation, Washington, USA). Glass sheets and foil tape were purchased from The Home Depot. Dimmers (rotary single-pole for incandescent lights; Lutron Electronics Co., Coopersburg, Pennsylvania, USA), electrical tape, and silicon were purchased from Lowe's. Aquarium gravel was bought at a local pet store (Trop Aquarium, Las Vegas, Nevada, USA) in sterile prepackaged bags.