Thermoregulatory Behavior in Diurnal Lizards as a Vehicle for Teaching Scientific Process.

James E. Platz

Department of Biology, Creighton University, 2500 California Plaza, Omaha, NE 68178-0103
Email: jplatz@creighton.edu

Abstract: Field experiments offer the opportunity for hands on experience with the scientific process. While this is true of a wide variety of activities, many have pitfalls both experimental and logistical that reduce the overall rate of success, in turn, influencing student learning outcomes. Relying on small, territorial, diurnal lizards and an array of inexpensive data loggers and lizard models provides a reliable method of testing a hypothesis in the field. This approach virtually guarantees that every student obtains a useable data set within a short period of time. Behavioral responses in lizards are dependent on becoming active only after external, sun driven, heat sources are adequate to permit daily activity on the one hand and avoiding excessive heat uptake above a critical maximum. Activity is dependent upon three primary modes of heat transfer (gain or loss): solar radiation, conductive exchange and convection, concepts that all students seem to understand. Over 11 of the past 15 years all students (more than 80) who undertook this project obtained useable data sets (data log records matched to focal observations). We relied on two different subspecies of *Holbrookia maculata*. Numerous other lizards are amenable to this approach.

Keywords: scientific process, hypothesis testing, behavior, thermoregulation

Introduction

The majority of small iguanine lizards native to the United States are territorial and many have densities high enough in good habitat to expect to find several adults within a grid of 30 m x 70 m and often in a much smaller area. Lizards are best described as ectothermic (Pough et al., 2006) and many are also homeothermic during diurnal activities, often maintaining a body temperature of approximately 35-39 C. Obtaining body temperatures above ambient conditions is generally accomplished by some form of basking behavior. To achieve this, lizards typically take advantage of direct and indirect sources of solar radiation. Herein I outline the major steps in executing a project that carries each student through the complete process of testing and extending a generally accepted paradigm of thermoregulation in small diurnal lizards.

This exercise was conceived and refined over the past 15 years in part to insure that each student would obtain a solid, acceptable data set that would virtually assure that everyone would be able to move through all the phases of the scientific process and present a term paper written in the format that one might use for submitting a manuscript. In our case it is an extension of what is already know in general terms about thermoregulation in small lizards. Each student is expected to achieve a fundamental understanding of what constitutes a valid scientific question; how to carry out a project that tests the validity of a general paradigm; how to analyze and present data graphically and then write a paper based on a set of format criteria. In this case students ask whether they see evidence of behavioral responses to extensive thermal conditions

The process begins when students read a prepared set of published papers. We read papers that lead students from the general to the specific. The first paper considers the physics of heat transfer, then we move to classical (pioneering work) of a general nature, more specific papers that relate to lizard performance and then papers that report results of conspecific subspecies from other locations. Students then write the introduction to their term papers which contains two crucial elements: 1) a distillation of what we know now and 2) a statement of what their papers are going to test. There are no published reports on thermal responses in *Hobrookia maculata thermophila.* Student/faculty discussions have established that we are attempting to test whether this subspecies does in fact show behavior patterns indicative of thermoregulation. data loggers to quantify microclimate, how to explore a variety of ways to present data and use spread sheets as well computer programs that rely on object oriented graphics and how to apply basic statistical methods. These skills are all parts of a coordinated effort culminating in a manuscript that adheres to all of the guidelines required for submission to a particular scientific journal.

Materials and Methods

In our studies in both Nebraska and Sonora, Mexico students laid out grids in areas where lizards were abundant. The long axis ran from south to north. In Nebraska we chose to use wooden stakes 0.5 m x 13 mm x 25 mm to mark the grid; in Mexico we used inexpensive bathroom tiles approximately 100 x 100 mm. Each was labeled with black permanent felt marker pens with letters and numbers large enough to be read at a distance of seven m, the dimensions of each grid quadrat. Thus numbering began at the southwest corner of the grid with A column (A0, A1…A20). The B column was parallel to the A column and again extended from south to north as B0 through B20. Columns C through F were laid out in turn and parallel to the first two.

To capture lizards for focal observations, each student used white No. 50 carpet thread in the form of a small noose attached to one end of a 1 m x 8 mm diameter wooden dowel purchased at a hardware store. Most lizards were readily noosed during the early part of their daily activity period. We then measured the snout vent length (SVL) and tail length to the nearest mm to obtain body size. Each lizard was weighed and sexed at the point of capture. To make it easier to identify focal lizards on successive days, each was identified with a small dab of fingernail polish applied to the neck, mid body, a shoulder, or a thigh. When additional codes were needed, we substituted fingernail polish for a small dot of airplane dope obtained at a hobby store. This permitted the use of the same codes but in different colors. If a lizard paint code began to slough off during the study, the individual was noosed and repainted. After data were obtained from a lizard, we recorded where the lizard was first seen and returned it to that location.

Students spent about two hours in preliminary observations cataloguing what lizards did during the day in terms of changes in posture and exposure to the sun. This resulted in identifying five postures in which less and less contact was made with the substrate (Figures 1 and 3). In addition to this, students quickly noticed that lizards spent more time facing west in the cool of the morning so we incorporated not only posture, but also compass orientation to the sun. During mid day when the sun bore down almost vertically on the study area, lizards shuttled in and out of the sun. At times *Holbrookia* also moved off of solid substrates (in the sun at times and shade as well) so we noted substrate in addition to posture and body orientation to the sun. Students also grasped the heat transfer implications of lizards that chose a posture when it was quite warm in which the body was more or less parallel to the light, body inclined, so that only the head was receiving direct incident light.

In discussion of the three primary modes of heat transfer (direct solar radiation, conduction and convection) we explored the solar consequences and decided that east and west orientation (especially if the lizard adopted an elevated body attitude parallel to the sun's rays) permitted scoring compass orientation as head to sun (east) or tail toward sun (west). Lizards facing north or south received the same incident solar radiation at a given moment, so we had effectively three categories for orientation, even though students noted north and south in their focal observations records.

To determine the heat transfer conditions of lizards, we deployed an array of small battery operated data loggers from Onset Computers (PO Box 3450, Pocasset, MA 02559-3450). To estimate the body temperature a lizard would experience in full sun or full shade, we constructed two lizard models (Figure 2). Each consisted of copper pipe 10 mm in dia. and 52 mm in length; available in most hardware stores as 3/8 inch dia. water pipe. To provide a realistic level of absorbance of natural light, we did several tests of actual lizards in full sun. Adult lizards (55 mm SVL) were temporarily tethered to a block of Styrofoam and recordings of body temperature were made at 5 second intervals until the lizard showed signs of discomfort; no tests were conducted above 39C which is near the upper thermal limit for most lizards. We did this in a container that eliminated wind. All lizards were maintained at a temperature of 30 C before the tests began. The air temperature for tests was 34C. This produced a heat up, cool down curve that we incorporated into a spreadsheet (See Appendix, Table 1). Tests of various lengths of pipe and paint color resulted in a close approximation to real lizard responses to solar uptake when we used flat gray, car primer. A cork was placed in each end of the pipe. One cork accepted the temperature sensor probe and the other accommodated the wire stand used to hold the pipe about 1 cm above the substrate (Figure 2). By placing one model lizard in open sun and the other in full shade, provided students with realistic estimates of body temperatures under those two extremes. At both locations and over multiple years we also used the noosing technique to sample lizards using a fast reading cloacal thermometer (Model T-6000, Miller and Weber, 1637 George St., Ridgewood NY, 11385) to the nearest 0.2 C in both open sun and in full shade. Body temperatures agreed well with estimates from the model lizards.

To further enhance the student's ability to comprehend the thermal regime of lizards, we placed a bare probe in open sand 3 mm below the surface and another in the sand in full shade. The latter we found useful when it became apparent that part of the thermoregulatory repertoire of *Holbrookia* occasionally involved burying in the sand in the shade for substantial intervals. Another data logger recorded burrow temperature 10 cm into the den. Onset computers also make data loggers that record direct solar radiation, relative humidity and barometric pressure. Of those, sunlight was deemed a valuable addition. Days when clouds come and go were among the most interesting. Having sunlight variation records at five min intervals synchronized to focal observations allowed students to look for changes in posture and orientation that were either consistent with or at odds with the hypothesis.

Results

With the advent of compact digital projectors we now routinely review lizard postures with students prior to making focal observations. We practiced scoring lizard posture based on photos (Figures 3 A-C). Data sheets contained a small series of stick figures (Figure 1) labeled A-D depicting posture on the basis of the amount of substrate contact. Posture A (Figure 3A) represented full contact with the substrate and posture D (Figure 3B) indicated a lizard with only toes and heels touching the substrate. Postures in between A and D are fairly stereotypic and involved intermediate substrate contact while posture E entails a strategy off of hard, high thermal heat solid substrates (Figure 1, Legend). Data sheets contained columns to the right of the defined postures that permited students to record grid location, posture, compass orientation and substrate as well as activities such as foraging, territorial defense or courtship**.** Each row constituted a record that was synchronous with the data logger array records. Before students began focal observations, we synchronize our watches with each other and with the data loggers. All loggers were armed to record on the hour and at 5 min intervals thereafter. On a typical day in the field after the grid has been laid out and marked and the data logger array had been deployed, students searched, located, noosed and painted lizards. After a student had marked his or her lizard, focal observations began. If students entered the field shortly before lizards became active in the morning, the data logger array provided information useful in determining the initial thermal conditions (sand temperature minima) required before lizard activity commenced. A single data sheet contained 12 focal observations (one hour). Four hours of observations thus provided a long enough period to see lizards initiate day time activities, and if the air and substrate temperatures were high enough by noon, too see a shift from uptake of heat to reach operating temperatures to conditions in open sun that exceeded a critical thermal maximum for activity. When sand temperatures exceeded voluntary operating temperatures, lizards either shuttled in and out of the sun, adopted postures and compass orientation that minimized radiant and conductive energy uptake, or adopted perches above the sand (Figure 3C) or moved into partial or full shade.

Figure 1. Depicts all the postures that *Holbrookia maculata* adopted. In posture A the venter was in contact with the substrate; in posture B the front limbs were fully extended raising the anterior part of the body off of the substrate; in posture C all four limbs were extended, minimizing body contact with a hard substrate. Posture D differed from C because the lizard is on an incline. Posture E depicted a lizard that had adopted an elevated stance off of the sand substrate. Figure 2F indicated that a lizard had either entered a burrow or (most often) buried in loose sand in the shade at the base of a plant.

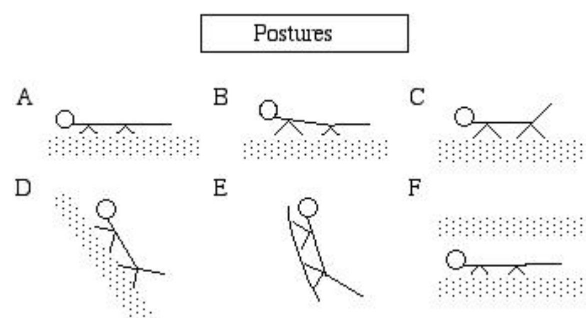


Figure 2. Represents a Hobo, four channel temperature data logger connected to a model lizard made from copper pipe.

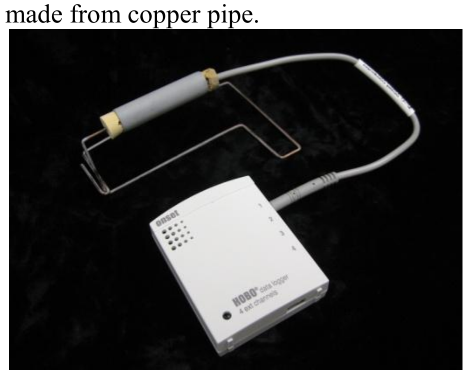


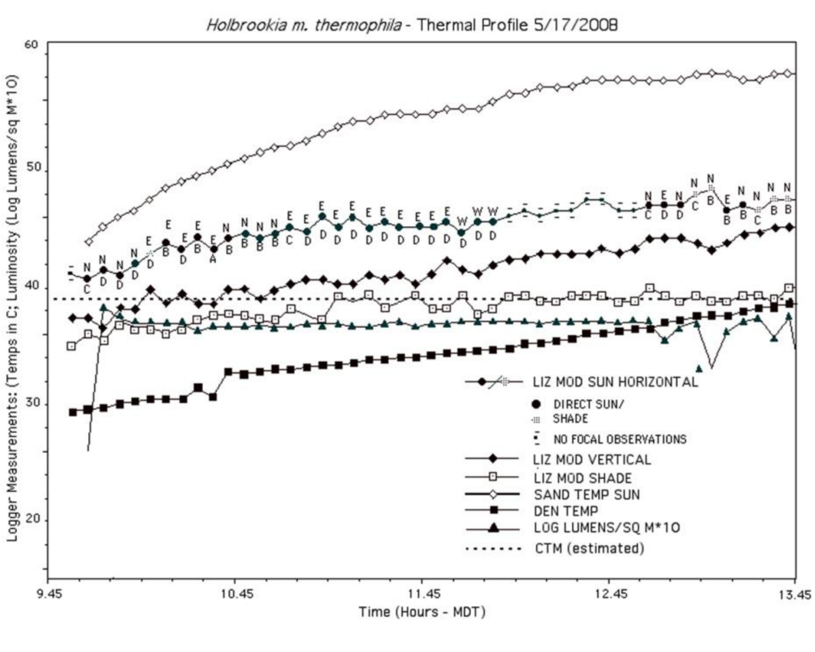
Figure 3. Depicts three of the postures *Holbrookia maculata* adopted to modulate conductive heat exchange. Figures 3 A and B were on hard, high thermal substrates such as rock or sand. In 3A the lizard was in posture A with full body contact with the substrate; In 3B all four limbs were extended as in posture D. Figure 3C depicts a lizard that was in contact with a low thermal mass substrate in an inclined position and without contact with the surrounding sand. **3A**

3B

3C

Once out of the field, all data logger records were downloaded as tab delimited data matrices and imported into a spreadsheet. Students then began the process of building graphs that permitted viewing all of the logger records for the day. Together we used a digital projector to examine graphs of microclimate conditions. Figure 4 contains a typical, minimal array of data logger records. At this point I requested that students each write up a general summary of the changes in microclimate for the logger records for that day. The next process involved examining what a given lizard did under a particular set of climate conditions. The goal at this point was to test the following hypothesis: Was there evidence to support or refute the proposed hypothesis that *H. maculata thermophila* used behavior to regulate body temperature?

Figure 4. Represents a typical composite graph of environmental conditions recorded by the data logger array with posture, compass orientation and sun conditions experienced by a single focal lizard.



We typically made focal observations over a span of three days but two are sufficient. Three increased the likelihood of different weather conditions that could result in behaviorally driven thermoregulatory differences between days. Once students had two or three days of observations, we spent a considerable amount of time graphing the results. I encouraged each to experiment with presentation methods. This worked better as a one-on-one activity than as a group using projected graphs. Once the data were graphed, each student was required to examine the behavior of his or her focal lizard. Four hours of observations synchronized to the data logger records for a day provided 48 records of orientation, posture and shade choices. We operated under the assumption that body temperatures close to 39 C or higher (Figure 4) represented an approximate upper limit for activity. As the process of presenting data matured, students used an object oriented drawing program to place a small circle on the graph line depicting the model lizard in full sun (Figure 4). We used symbol color to designate whether the lizard was in full sun (yellow), partial sun (gray) or full shade (black). For publication purposes we made sure that the symbols we chose worked in black and white. Just above each circle students placed a small capital letter to designate compass orientation at that moment. Just below each circle, an upper case letter (A-F) indicated posture at each focal observation. I lead students in a discussion as to how best to present the data in such a way that they could identify all of the conditions on a single figure. We found that this facilitated the discovery of patterns in behavior. These could then be used to provide evidence that lizards are indeed using posture and orientation (behavior) to avoid temperatures that exceed the maximum or in fact dropped below a body temperature that permitted lizards to function efficiently, or in fact did not support our hypothesis. From the required readings (Huey and Kingsolver, 1989) students are familiar with the concept of optimal performance including sprint speed (for predator avoidance) as well as digestion rates, hearing and reproductive activities. In warm climates most lizards in full sun do not tolerate solid substrates above 40 C for more than about 5 min if they are already at or near the upper end of thermal tolerance. Warm lizards on substrates in the 40 to 60 C range were expected to be there for less than 5 min (Table 1). Lizards generally responded in less than 2 min. This time worked well because we took focal observation at 5 min intervals. When air and sand temperatures exceeded 40 C and the sun was out, lizards on solid substrates retreated to partial or full shade and buried in shady sand at times. Focal lizards tolerated full sun at slightly lower air temperatures (less than 40 C) for prolonged periods if they are off of a solid, high thermal substrate (Figure 3, Posture E). Students soon realized that by using this simple color-coding, depicting orientation and posture, all in one part of the graph, allowed them to look for sun-shade shuttling and long stretches of time during which a lizard stayed in one posture and oriented in the same direction. We plotted colored circles on graphs (Figure 4) on the logger curve for model lizards in open sun for two reasons. One is practical. The graph was less crowded there. The second reason was to remind students that occupying a solid substrate in open sand is not a viable option for lizards unless they behave in such a way that they remove themselves from the substrate. Lizards that move into vegatation off of the sand virtually eliminated conductive heat uptake. This was almost always coupled with posture oriented parallel to solar input reducing direct solar heating to a minimum.

Discussion

Before fieldwork commenced, I spent some time in discussion with the class to be sure that they understand the limits of science. To be scientific, a hypothesis was restricted to the real, measurable world and hypotheses had to be vulnerable to falsification. We explored the utility of using hypotheses in studying biological systems. Because we are often in remote settings, I provided a list of papers to read and copies of key papers that introduce students to life as ectotherms; first with a classic paper (Cowles and Bogert, 1944) and then with a more modern treatment of heat sources available to lizards, both direct and indirect. Pough et al. (2006) is a fine source of first principals. For instructors and advanced students, Pough et al. (2004) is exceptional and much more detailed. The next required reading (Huey and Kingsolver, 1989) provided students with a nice treatment of the concept of thermal optima. From this point readings contained information that more specific for the species we studied (Stebbins, 2003) and finally published field studies germane to the subspecies we chose for our thermal studies (Hager, 2006). For those in the eastern half of the U S, Conant and Collins (1998) is a useful general reference for students and instructors. After the students had read the assigned papers we engaged in a group discussion before the introduction was written. Once focal observations were completed we spent time examining the best ways to present the results. Students generally converged on a best suitable way to render the results in graphical form. Students had the most difficulty with a tendency to combine results and an explanation fo them in that section rather than waiting until the discussion section. In the discussion section, the greatest challenge was to fully utilize the results to support a general conclusion and to actually come to grips with the heat transfer modes that were operating at a given point in time. Students were encouraged to look for long bouts of the same posture and orientation and then consider what the lizard body temperature would be, given the external heat conditions. The short period of time required for a lizard to over heat (Table 1) was a powerful tool for students as they began to present results in favor of their argument. Identifying long bouts of stereotypic behavior could be treated with simple statistics. We spent a good deal of time with the logic of long bouts in one posture considering whether it was random or not. Because the student had a full set of graphs on computer, students can also do oral presentations as reinforcement to the written work. The sequence, oral presentation of data first has the advantage of refining or at times revising explanations that would go into the discussion section. There is also merit in having them do the oral presentation once the term paper is complete.

Alternative Enrichment Exercises:

For students who become curious during the execution of this exercise, there are a number of questions that can be addressed with the existing data and these include: (1) What is the drop in temperature that a lizard (or model) undergoes if oriented parallel to the sun? (This is a simple experiment and we now put a third model lizard in the field next to the horizontal one in open sun and plotted both on graphs (See Figure 4.) (2) Is there a difference in distances traveled on days that differ in weather? (3) Is there a difference in the size of the area (territory) that females vs. males cover in a day or as a composite over several days? (4) Do students see evidence of differences in behavior at a given time of day between males and females? (5) What is the sex ratio and density of adults on the study area? (6) Where do lizards spend the night?

Data sheets, a complete checklist of guidelines we use for the construction of a paper, a more extensive list of suitable papers relevant to thermoregulation in reptiles, heating curves and sample graphs are available in PDF format and free to download at: http://biology.creighton.edu/faculty/platz/

Acknowledgements

The author thanks anonymous reviewers for constructive comments made during revision of this manuscript as well as the Creighton University Computer Center for funding the wireless laptop network.

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Appendix

Table 1. Provides a heat/cooling curve for a 55 mm SVL, male *Holbrookia maculata*, from an initial body temperature of 30 C exposed at a 90 degree angle to full sun. Ambient air temperature was 34 C. Heating beyond 39 C was extrapolated from lower temperature rates of heating.

FUTURE Tb (C)		30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
DELTA Tb (C)		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
BODY T. (C)																
30	0	28	55	83	110	138	165	193	220	248	275	303	330	358	385	
31		0	28	55	83	110	138	165	193	220	248	275	303	330	358	
32			0	28	55	83	110	138	165	193	220	248	275	303	330	
33				0	28	55	83	110	138	165	193	220	248	275	303	
34					0	28	55	83	110	138	165	193	220	248	275	
35						0	28	55	83	110	138	165	193	220	248	
36							0	28	55	83	110	138	165	193	220	
37								0	28	55	83	110	138	165	193	
38									0	28	55	83	110	138	165	
39										0	28	55	83	110	138	
40											0	28	55	83	110	
41												0	28	55	83	
42													0	28	55	
43														0	28	
44															0	