

Chapter 18

Energetic Strategies of Terrestrial Vertebrates

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Reprinted From: Winnett-Murray, K., K. G. Murray, L. Hertel, and C. C. Barney. 2000. Energetic strategies of terrestrial vertebrates. Pages 356-379, *in* Tested studies for laboratory teaching, Volume 21 (S. J. Karcher, Editor). Proceedings of the 21st Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 509 pages.

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Introduction

This investigation contrasts the energetic strategies of endothermic and ectothermic vertebrates, using mouse and lizard models, respectively. It has been successfully used in introductory biology courses at Hope College for several years. By increasing the level of mathematical sophistication and/or the number of experimental treatments involved, this lab can be adapted for upper level courses in vertebrate physiology, morphology, or behavioral/physiological ecology. Two parts (one energetic, one behavioral) can be completed in one 3-hour lab. Part A (energetic) allows the students to derive empirical data showing the major difference between ectotherms and endotherms in their metabolic response to temperature changes. Ectotherms typically lack well-developed insulation and have low resting metabolic rates; as a result, body temperature is a direct function of ambient temperature. The metabolic rate of ectotherms is

directly related to body temperature, and hence, is also directly related to ambient temperature. On the other hand, the two major groups of endotherms (birds and mammals) have well-developed insulation and higher resting metabolic rates. Endotherms respond very differently to ambient temperature changes; typically, a minimal amount of energy is expended to maintain a warm body temperature when an endotherm is within an ambient temperature range called the “thermoneutral zone”. When the temperature is below or above this range the animal’s metabolic rate may increase significantly in an attempt to balance the cooling or heating rate. The increase in metabolic rate in response to high temperature and the need for cooling is usually less than that at low temperature. Alternatively, an increase in metabolic rate may include a Q_{10} effect as body temperature rises. While ectotherms must normally “bask” to maintain a warm body temperature and be active, endothermy enables greater production and retention of metabolic heat and the potential to remain active under a wider variety of environmental conditions.

Nonetheless, ectotherms may be at a significant advantage in environments where dietary resources are very limited or unpredictable because their metabolic rates are significantly lower than those of a same-sized endotherm under the same conditions (for further reading see Hairston, 1994, Pough et al., 1996, Townsend, 1987, and Willson, 1984). Most habitats contain a variety of *both* endotherms and ectotherms; students should be prompted by this investigation to explore additional adaptations of animals that compensate for what they may lack in energetic machinery (e.g. food-caching behavior, diurnal activity patterns, etc.; for additional examples see French, 1988, Hainsworth, 1981, Marchand, 1991, McClanahan et al., 1994, Ohmart and Lasiewski, 1971, Prothero and Jurgens, 1986, and West et al., 1999). The evolution of endothermy remains a subject of great fascination and debate, especially as concerns the question of variation in the energetic strategies of dinosaurs (See for example Chinsamy and Dodson, 1995, Desmond, 1976, Fischman, 1995, Gibbons, 1997, and Hurlburt, 1994.), the evolution of flight in independent lines of reptiles, including birds (See Padian and Chiappe, 1998, and Ruben et al., 1997), and the evolution of mammals and mammalian reproductive strategies. (Pough et al. 1996) This fundamental exercise contrasting ectotherms and endotherms should provide students a database from which they can generate many additional questions and hypotheses to explore.

Materials

For each lab of 24-48 students:

- 2 water baths large enough to hold 6, 1-quart Mason™ jars
- 2 balances capable of measuring to the nearest 0.00 g
- A source of ice/ice machine
- First aid kit with alcohol swabs and band-aids (for possible animal bites)
- 12 adult lab mice in 4-6 appropriate holding cages, with food, water, and animal care supplies. Keep different sexes in separate holding cages. Cages should be labeled such that students will return animals to the *same cage* at the end of the exercise. See Appendix B (Animal Use Guidelines).
- 12 anoles (e.g. *Anolis carolinensis*) , large and healthy, in appropriate holding cages. Holding cages should be equipped with food (e.g. live crickets or mealworms), a mister for water (anoles do not drink water from a dish), and a source of heat. (A 60-100 watt desk lamp placed near the cage, but not touching it, is sufficient for the 3-hr duration of this exercise.) The animal holding cages can be misted every few hours with lukewarm water. See Appendix B (Animal Use Guidelines) and Appendix C (Animal Sources).
- 4 desk lamps with 60-100 watt bulbs, to set near anole holding cages
- Live crickets or mealworms (anole food)
- 4 mister bottles (See anoles, above)

- 2 temperature gradient boxes (See Appendix E, Instructions for Construction of a Temperature Gradient Box)
- 2 multi-channel electric thermometers and batteries (See Appendix D, Equipment and Supply Sources)
- 1 Oxygen Analyzer (See Appendix D, Equipment and Supply Sources)
- 2 extension cords, if needed, for temperature gradient boxes
- 2 (extra) 100 watt white light bulbs for gradient boxes
- 1 small jar of vaseline

For each group of 4 students:

- 1 Opaque ice bucket large enough to accommodate 1 or 2 Mason™ jars and ice
- 1 Thermometer
- 1 Kimwipes™ tissue box
- 2 Mason™ jars (1 quart size) with modified lids. Into each lid has been inserted the plastic tip of an 18 gauge syringe needle (in its plastic holder); the needle is cut off so that it is no longer sharp and is pointed down into the jar. The opening through which the syringe tip is inserted is then sealed carefully and completely, inside and out, with epoxy sealant; this must result in an air-tight seal. We check and repair ours after each lab.
- 4 Syringes (60 cc each)
- 2 Flask weight rings (See Appendix D)
- Data sheets from student handout
- 1 Pair of animal handling gloves (leather or heavy garden gloves)
- 1 Mason™ or other 1 quart jar with a mesh or screen lid

Notes for the Instructor

Animal Handling

For many students, this may be their first experience handling live vertebrates in an experimental setting. You will need an approved protocol from your institution's Animal Care and Use Committee prior to conducting this exercise. We furnish every student with a printed copy of two Hope College documents: Hope College Principles for the Care and Use of Laboratory Animals and Hope College Guidelines for Vertebrate Animal Users (Appendix B). A pre-lab for instructors and teaching assistants using the live animals is conducted a week prior to the student lab to ensure that all supervising personnel have been trained in animal use and care and in the use of the electronic thermometers and the oxygen analyzer. It is important that the students be impressed with how much their careful attention to detail is related to their own safety and the safety of their animals. Nonetheless, accidents are possible; we are up-front about this in our pre-lab by fore-warning students of the slight possibility of animal injury or death. To minimize that possibility, we reiterate particular warnings in the printed protocol, demonstrate appropriate handling of both lizards and mice, and discuss possible signs that an animal is distressed. In short, we want students to know we really care about our animals and are prepared to model all precautions. In particular:

1. Lizards should not be handled by the tail, but mice can be handled by the base of the tail. For measurement of anal temperature, the instructor should have a very firm grasp of the mouse by the scruff of the neck while simultaneously immobilizing the anal area while holding the base of the tail. (This can be done with one hand, and the temperature probe can be inserted with the other hand.) The body temperature of the animal must be measured quickly (within

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- a minute) after removing the animal from the temperature gradient as lizards, in particular, will cool rapidly. On the other hand, the temperature must be allowed to stabilize.
2. Lubricate the temperature probe with Vaseline™ before inserting the probe into the animal. Disinfect both the animal and the probe with an alcohol swab after each use.
 3. Do not run mice in sealed metabolism jars for more than 5 minutes or they may begin to run out of oxygen! BE SURE students remove the jar lid and fan fresh air into the mouse jar as soon as the 5 minutes are up. Similarly, watch for signs of heat stress (lots of urination and/or spreading saliva over body with paws) in the mice in the warm (37°C) treatment.
 4. Lizards left in the cold temperature treatment will cool rapidly and become immobile. Green anoles may change color (to dark brown); use your largest, healthiest lizards for this treatment. Keep the time under 50 minutes for the cold treatment and modify this in the metabolic rate equation accordingly. (Simply divide by the number of minutes the animal is actually in the jar.) Cold lizards should be warmed immediately by cupping gently between the hands and blowing warm air on the animal; they should respond by twitching within a few seconds. Once the animal is warmed up enough to move well, place the animal near the heat lamp in the holding cage. To minimize stress on the animals, we keep track of which lizards have been subjected to the cold treatment and do not use them for this treatment again in other lab sections.
 5. Students and instructors should immediately wash their hands thoroughly after handling animals.

Metabolic Rate Calculations

We have learned that the rather lengthy series of conversions involved in determining metabolic rate from oxygen consumption is a limiting factor in the timing of this lab. That is, unprepared students may spend so much time cranking through the calculator operations that they do not complete the animal protocol in a timely fashion. Our remedy for this potential pitfall is a pre-lab worksheet (Appendix A) that is required upon entrance to the lab.

Behavior in a Temperature Gradient (Part B)

There are various options for incorporating this portion into a 3-hr lab. We normally have one student from each 4-person team do the behavior monitoring. Data may be more representative of typical behavior if animals are monitored for a longer period of time; point samples taken once every 10 minutes for 2 hours, for example, may yield better results than once a minute for 30 minutes. At the end of a run, clean out animal waste as this may influence the behavior of subsequent animals. When setting up the gradient box, plug it in and let it come to equilibrium at least 30-40 minutes before the start of lab; we place a frozen ice-pack at the end of the box *opposite* the light bulb to increase the gradient range. The temperature at the mid-point of each box section should be recorded about mid-way through the sampling period. Mice may chew on unprotected temperature probes, which could be hazardous to their health and is definitely bad for the probes. If this is a problem, coat the probes with something distasteful such as quinine solution.

Student Outline

Conceptual Objectives

1. Differences between the terms ectotherm and endotherm, homeotherm and poikilotherm, and warm-blooded and cold-blooded.
2. Consequences of an animal's metabolic strategy (See above) to its behavior and ecology, such as

its activity level under different conditions, the range of habitats it can exploit, its energy consumption, and its strategy for coping with environmental extremes.

3. The relationship between metabolic rate and an animal's energetic strategy, its activity level, its body size and ambient temperature.
4. The relationship between metabolic rate and oxygen consumption.

Skills Objectives

1. Correct handling of live vertebrates, with proper attention to the safety and well-being of investigator and investigatee.
2. Correct use of: electronic thermometer, oxygen analyzer, simple metabolism jar apparatus, and electronic balance.
3. Recording animal behavior in a temperature gradient.
4. Calculation of the mass-specific metabolic rate from raw data on oxygen consumption and animal mass.
5. Use of computer software to construct line graphs and bar graphs.
6. Calculation of the mean, standard deviation, and range of a data set.

Questions

1. Do ectotherms and endotherms differ in the range of temperature regimes they effectively exploit?
2. Do they maintain different body temperatures when occupying micro-environments at their "preferred" ambient temperatures?
3. What are the consequences of these temperature ranges and body temperatures, if any, for the animals' ability to survive in a temporally and/or spatially heterogeneous environment? In environments in which food is scarce and/or predators abundant?

Background

Animals can be divided into groups based upon whether or not they regulate their body temperature within narrow limits or instead let it change along with the surrounding (ambient) environment. Some people call these "warm-blooded" and "cold-blooded," and they correspond roughly to birds and mammals on the one hand, and everything else on the other. Two somewhat better terms to describe these categories are "homeotherms" and "poikilotherms" (or sometimes, "heterotherms"). But not all of the animals that regulate their body temperature (T_b) within narrow limits are birds or mammals. For example, many lizards do so by moving into and out of the shade numerous times during the day. Similarly, not all birds and mammals maintain a really constant T_b over long periods of time. Many rodents and bats hibernate, and other mammals, hummingbirds, and other birds undergo torpor on a daily basis. That is, they sometimes give up trying to regulate T_b at the "normal" level and let it fall to within a few degrees of ambient temperature (T_a) in order to conserve energy. So we need a different pair of terms to describe animals that differ in their approach to regulating body temperature and metabolic rate. *Ectotherms* are organisms that derive most of their body heat from the environment - by basking in the sun, seeking warm micro-habitats, etc. Most invertebrates, fish, amphibians, and reptiles fall into this group, even if they *behaviorally* regulate their T_b within very narrow limits. Lots of plants even do this - many can change the orientation of their leaves to maintain maximal exposure to sunlight over the course of the day. *Endotherms* are organisms that derive most of their body heat from internal metabolism of foodstuffs, especially carbohydrates and lipids. Most birds and mammals fall into this group, even if they don't always regulate T_b closely.

What kind of metabolism are we talking about? Remember the general equation for cellular respiration using glucose (it's basically the same for other carbohydrates, and for lipids as well):



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The energy is used to produce ATP, and it, in turn, is used to drive virtually all of the organism's biochemical reactions, including those that produce the heat for thermoregulation. The rate (i.e., amount per unit time) at which an organism uses energy to drive its metabolic processes is called its *metabolic rate*.

Now in order to forage actively, or to be able to escape from predators, an animal has to maintain a pretty high metabolic rate, since the chemical reactions involved in muscle contraction, digestion, nerve impulse conduction, etc. all require energy. But, like all chemical reactions, these are *temperature-dependent*: their rate (hence, the rate at which the animal can run, digest its food, etc.) is a function of temperature. So we might expect animals to maintain T_b (and, therefore, metabolic rate) at a fairly high level when possible. When an animal can't do so in its present surroundings, it has three options: 1) Move somewhere else, 2) Give up regulating T_b at a high level and either let it vary with the environment or just regulate it at a lower level, or 3) Die.

In this lab, we will compare representative ectotherms and endotherms (a lizard, *Anolis carolinensis*, and the house mouse, *Mus musculus*), in terms of: 1) Their "preferred" body temperature, 2) The ranges of ambient temperatures they exploit, 3) Their metabolic rates at different temperatures, and 4) The consequences of those metabolic rates.

Before you get started, decide on one group member who will be responsible for part B: behavior in a temperature gradient. The rest of you will work on part A.

Part A: Metabolic rates of lizards and mice at different temperatures

In this part of the lab, we'll determine the metabolic rates of *Mus musculus* and *Anolis carolinensis* at three ambient temperatures: One at room temperature, one in an ice bath, and one in a warm water bath. Strictly speaking, metabolic rate is measured in joules (or calories) of energy used per unit time, and since virtually all of the energy used by an animal is eventually dissipated as heat, metabolic rate is best measured using a technique called *direct calorimetry*. In this method, the animal is placed inside a chamber immersed in water (a calorimeter), and the amount of heat that it produces is measured by the increase in the temperature of the water surrounding the chamber. Although this method is accurate, it's very complicated and expensive to do. Fortunately, we can take advantage of that basic equation for cellular respiration to measure metabolic rate indirectly: It turns out that *it takes 1 liter of oxygen to produce 4.825 kilocalories of energy from metabolic breakdown of a mixture of carbohydrates and fats*. So we can measure metabolic rate indirectly as oxygen consumption! Luckily, we have an oxygen analyzer for this purpose. We'll place our animals in enclosed chambers maintained at the different temperatures for a specified time, and then determine oxygen consumption as the difference between the oxygen content of the chamber and that of room air.

Part B: Behavior of representative ectotherms and endotherms in a thermal gradient

In this part, we'll determine the *ranges of ambient* temperatures used by mice and lizards, the ambient temperatures they *prefer*, and the *body temperatures* they maintain when at those preferred ambient temperatures. We'll do this by allowing the animals to move freely within a temperature gradient that we create in the lab. We'll measure their body temperatures by...well, you'll see later...

But before you get started, take a few minutes to pose some hypotheses:

1. Which would you expect to exploit the widest range of ambient temperature, an ectotherm or an endotherm? Why?
2. Which animal would you expect to have the highest preferred ambient temperature? Why?

- When both are allowed to seek out their own preferred ambient temperature, would you expect body temperature to be highest in a representative ectotherm or a representative endotherm? Why?

Procedure

Part A: Metabolic Rates of lizards and mice at different temperatures.

Examine one of the metabolism chambers and one of the 60 cc (60 ml) syringes at your lab table. Note that the chamber consists of a Mason™ jar with a hypodermic needle inserted through a hole in the lid and glued in place. When the two-part lid is screwed on the jar and a syringe is twisted onto the hypodermic needle, an air-tight seal is formed. *The seal must be absolutely airtight* if accurate measurements are to be made. Screw the lid onto the Mason™ jar and then practice placing the syringe on the needle. The syringe should be screwed on firmly, but not so tightly that you break the epoxy seal holding the needle in place. It's best to hold onto the hub of the needle when attaching the syringe, in order to minimize the chance of breaking the epoxy seal.

- First, determine the oxygen content of room air. Remove the lid from one of the Mason™ jars. Remove the syringe from the needle, if attached, and pull the plunger back to the 60 cc mark. Attach the syringe to the needle of the lid. Then screw the lid onto the Mason™ jar. Slowly push the plunger of the syringe in all the way. Then slowly pull the plunger of the syringe back to the 60 cc mark. You have now taken a sample of the chamber air. Adding the 60 cc of air first allows your final sample of air to be at normal barometric pressure. Next carefully remove the syringe from the needle and quickly cover the hole at the end of the syringe tightly with your finger. Take your air sample to the teaching assistant who is running the oxygen analyzer. The TA will slowly inject your air sample into the oxygen analyzer. For the next 30 seconds record the *lowest* reading which appears on the digital output of the oxygen analyzer. This is the oxygen content of your chamber when it contains room air. Record the oxygen content of room air in your data sheet.
- Next set up the lizard experiments. Your group will be assigned to two of the three temperature treatments for lizards. Remove the covers from two of the Mason™ jars and place the jars inside separate ice buckets. For each jar, withdraw the plunger of a 60 cc syringe to the 60 cc mark and attach the syringe to the needle glued to the jar lid. Obtain lizards and weigh each one to the nearest 0.1 gram. Be sure to tare the weight of the beaker and cover in which you weigh the lizard prior to weighing. Record the weights of the lizards in the data sheet. *Be sure not to confuse the lizards during the rest of the experiment - they look about the same!*

If you're assigned to the ice bath treatment: The jar in the ice container should be inserted such that ice, and/or cold water which has been adjusted to a temperature of 15° C, surrounds the bottom two thirds of the jar. Place one lizard in the jar and screw on the lid with the syringe attached. *Be sure the lid and syringe are attached so that the seal is air-tight.* Record the time when you placed the lid on the jar on your data sheet. Monitor your lizard's health by checking it every 5-10 min. This experiment will not exceed 50 minutes. If your lizard appears distressed or is not moving at all, report this immediately to your instructor or TA.

If you're assigned to the room temperature treatment: Place the lizard in the jar within an empty ice bucket and screw on the lid with the syringe attached. *Be sure the lid and syringe are attached so that*

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the seal is air-tight. Record the time when you placed the lid on the jar on your data sheet and room temperature, which should be about 25° C. If your lizard appears distressed or is not moving at all, report this immediately to your instructor or a TA.

If you're assigned to the warm temperature treatment: Place the lizard in the jar and screw on the lid with the syringe attached. *Again, be sure the lid and syringe are attached so that the seal is air-tight.* Then immerse the whole jar into the circulating warm water bath (at 35°) and record the time when you placed the lid on the jar. The water level should be about 2/3 - 3/4 up the side of the jar, but well below the lid. If your lizard appears distressed or is not moving at all, report this immediately to your instructor or a TA.

After the first lizard has been in its jar for 70 to 80 minutes (50 minutes or less for cold lizards), obtain an air sample as before; push the plunger down all the way to expel all air from the syringe into the jar, and then withdraw the plunger back to 60 ml in order to get a sample of air from the chamber. Record the time you take the air sample on your data sheet. Remove the syringe, cover the opening, and take the air sample to the TA. After the TA has injected the air sample, record the *lowest* oxygen concentration reading that occurs. While one member of the group is obtaining the oxygen concentration the other members should obtain the air sample for the lizard at the other temperature, again remembering to record both the time at which the sample was taken and the lowest oxygen concentration reading. Then return both lizards to their cages and wash and dry the metabolism jars. Lizards run in the ice bath should be warmed up according to your instructor's directions before going back into their cages.

3. During the 70 to 80 minutes you're waiting to gather data on the lizards, you should measure the metabolic rate of a mouse at *three* different temperatures. Prepare a jar by removing the lid and attaching a syringe to the needle after withdrawing the plunger to the 60 ml mark. Obtain a mouse and weigh it to the nearest 0.1 gram. Your instructor will demonstrate proper handling of live mice, sample expletives that diminish the effects of painful bites, etc. Anyone who handles mice does so at their own risk. Record the weight of the mouse on your data sheet. Place the mouse in the metabolism jar and immediately screw on the lid with its attached syringe. Record the time on the data sheet. First, place the mouse in an empty ice bucket to measure its metabolic rate at room temperature (25° C). Be sure to place sheets of paper between metabolism jars so that animals in the same ice bucket can't see each other. After *five minutes* obtain the air sample and determine the oxygen concentration as described previously. Temporarily remove the mouse from the jar, clean the jar of urine and feces if necessary, and fan fresh air into the jar with your hand. *Never leave your mouse in a sealed jar for longer than the 5-minute run!*

Following the experimental run at room temperature, do one with the same mouse while it is in the jar in the ice bath at 15° C. Pre-chill this mouse in the jar with mesh lid for 10 minutes; this will allow fresh air to enter the jar. After pre-chilling, place the mouse into the metabolism jar and immerse into the ice bath such that the bottom two thirds of the jar are immersed in ice and wait five minutes for the jar to cool. Then place the mouse in the metabolism jar and determine the oxygen concentration after five minutes as before. Record your data on the data sheet.

After the run in the ice bath, clean the Mason™ jar, fan fresh air into it and do one experimental run on the same mouse in the warm water bath at 35° C. Pre-warm the mouse, with the jar lid on, but without the syringe on for 10 minutes. Then connect the syringe and adjust the pressure as described

above for the 5-minute run in warm water.

Part B: Behavior of ectotherms and endotherms in a thermal gradient.

The light (or heating element) in the gradient box should have been turned on before your lab period started. Place a mouse or lizard in the box and replace the lid. Wait 5 minutes before collecting data, so that the temperature gradient can be re-established. Let the animal move about and seek out the part of the gradient where it can attain its "preferred" T_b , if any. After the 5-minute waiting period, record the animal's position in the box (box section 1-7) *every minute for the next 30 minutes (or per instructions)*. If the animal overlaps two sections, count it in the section that contains most of its body (not its tail). You should also record the ambient temperature in each of the 7 sections of the box 20 minutes after the end of the 5-minute waiting period (i.e., 5 minutes before the end of the run).

After the 30 minutes is up, quickly remove the animal from the box and let the instructor record its colonic (mouse) or cloacal (lizard) temperature. Record the body temperature on your data sheet. Repeat this whole experiment with the other animal (mouse or lizard).

Data Analysis

Part A. For each measurement of oxygen consumption, calculate the energy usage as calories per gram of body weight:

1. First determine how many ml of oxygen were used and record this value on the data sheet. To calculate the amount of oxygen used, first subtract the oxygen concentration of the sample from the oxygen concentration of room air and convert from a percentage to a decimal.

$$\text{Example: } 20.95\% \text{ (room air)} - 20.15\% \text{ (sample)} = 0.80\% = .0080$$

Then multiply the decimal change in oxygen concentration by the total volume of the system. The total volume of the system is the volume of the jar (470 ml) plus the volume of the syringe (60 ml) minus the volume taken up by the animal. *The volume taken up by the animal will be different for each animal!* Estimate the volume taken up by the animal as 1 ml for each 1 gram of body weight.

$$\begin{aligned} \text{Volume} &= 470 \text{ (jar)} + 60 \text{ (syringe)} - 5 \text{ (for an animal weighing 5 grams)} = 525 \text{ ml} \\ \text{Oxygen used} &= 525 \text{ ml} \times .0080 \text{ (for this example)} = 4.200 \text{ ml} \end{aligned}$$

2. Next convert the ml of oxygen used into calories of heat produced by multiplying the ml of oxygen used by 4.825 calories per ml of oxygen. Record these data on your data sheet.

$$\text{Example: } \text{calories produced} = 4.200 \text{ ml} \times 4.825 \text{ calories/ml} = 20.265 \text{ calories}$$

3. To determine the rate of heat production divide the calories produced by the number of minutes over which the measurement was taken to get calories/minute.

$$\text{Example: } \text{calories/minute} = 20.265 \text{ calories/100 minutes} = 0.20265 \text{ calories/minute}$$

4. Finally, in order to take into account differences in the weights of the animals, divide the calories/min by the *weight of your animal in grams* to obtain calories per gram per minute. Record this on your data sheet.

$$\text{Example: } (0.20265 \text{ calories/minute}) / 5 \text{ grams} = 0.04053 \text{ calories/gram-minute}$$

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As soon as you have completed each measurement/calculation of metabolic rate, put your data (calories/gram-minute) on the board. When the other groups have also done so, calculate the mean value for each species at each temperature.

For your lab report, you'll need to make a graph of your lab section's data on metabolic rate vs. ambient temperature for lizards and mice using the assigned software. Since we'll have data on several individuals of each species per temperature, we'll also have variation in metabolic rate. So we'll also put error bars on our graph.

Your graph should have a line connecting the 3 values for mice and another line connecting the 3 values for lizards, and each point will represent a mean. The standard error bars represent the amount of variation around each mean. How does metabolic rate of lizards and mice vary with ambient temperature? How do mice and lizards compare with each other at the same ambient temperatures? Why do lizards attempt to maintain such a high T_b if this also means a high rate of energy expenditure? Under what conditions should lizards do the opposite, that is, maintain a low T_b ? Which "strategy" (endothermy or ectothermy) is "better"? Think about your results in terms of advantages and disadvantages in different environmental conditions.

Part B. First, be sure to get your raw data up on the board as soon as it's available. We'll only be running one lizard and one mouse per room for this part, so we'll combine data from the two sections. After the data from both individuals of a particular species are up on the board, determine the mean (average) PROPORTION of point samples at which the animal was in each of the seven box sections. You can use the data sheet provided to record these class data from the board, since you'll need them to do your analysis. Construct a bar graph of the proportion of point samples in which mice and lizards were in the various sections of the thermal gradient boxes. Your graph represents the ways in which the lizards and mice used the thermal environment. Did both species seem to use this variable environment similarly, i.e., did they move around a lot or did they seek out a particular temperature and remain there? If the latter, did they prefer different ambient temperatures? What do the similarities and differences between the behavior of these representative ectotherms and endotherms tell you about the ecological conditions to which the two energetic strategies are best suited? Is one always "better" than the other, or are there likely to be sets of conditions under which each strategy might be more advantageous?

Part A: DATA SHEET FOR LIZARDS (your group's data)

a) Oxygen concentration of room air

b) Lizard data

	Temp: _____ °C	Temp: _____ °C
Wt. of Lizard (g)		
Time lid placed on jar		
Time air sample taken		
Elapsed time (minutes)		
O ₂ concentration of sample		
ml O ₂ consumed		
Energy produced (calories)		
Rate of energy produced (calories/minute)		
Mass-specific metabolic rate (calories/g-minute)		

Energy Strategies of Vertebrates

Part A: DATA SHEET FOR MICE (your group's data)

a) Oxygen concentration of room air

b) Mouse data

	Temp: ___ °C	Temp: ___ °C	Temp: ___ °C
Wt. of Mouse (g)			
Time lid placed on jar			
Time air sample taken			
Elapsed time (minutes)			
O₂ concentration of sample			
ml O₂ consumed			
Energy produced (calories)			
Rate of energy produced (calories/minute)			
Mass-specific metabolic rate (calories/g-minute)			

2. Compiled data, averaged for the entire class.

Lizards in ice bath _____

Lizards at room temperature _____

Lizards in warm bath _____

Mice in ice bath _____

Mice at room temperature _____

Mice in warm bath _____

Part B: Lizard & Mouse Behavior Data (Your Group's Data)

Lizard

Minute	Section
0	
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	

Body Temperatures

Lizard= _____

Mouse= _____

Box Temperatures

Lizard Run

Section	Temp
1	
2	
3	
4	
5	
6	
7	

Section	Temp.
1	
2	
3	
4	
5	
6	
7	

Mouse

Minute	Section
0	
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	

Energy Strategies of Vertebrates

Part B: Means for Lizard and Mouse Behavior and Temperature Data (Class Data)

Mean body temperature for mice:

Mean body temperature for lizards:

Section	Number of point samples	Proportion of samples	Number of point samples	Proportion of samples
1				
2				
3				
4				
5				
6				
7				

Student Report

Energetic Strategies of Terrestrial Vertebrates

GROUP MEMBERS:

LAB INSTRUCTOR:

LAB DAY AND TIME:

Part A. Metabolic Rates

1. Attach a copy of your line graph showing the metabolic rates of mice and lizards at the three temperatures we used. Use the class averages to construct the graph and to interpret all questions. Be sure to record the temperature (independent variable) along the x-axis and the units of metabolic rate (dependent variable) along the y-axis. Use two separate lines on the SAME SCALE for the two animals. Provide a legend for the lines. (4 pts)
2. The middle area of the mouse graph, close to the animal's preferred body temperature, represents the animal's thermoneutral zone. Within this temperature range, the animal does not have to expend much energy to regulate its body temperature. Explain the relationship between metabolic rate of the lizard (an ectotherm) and ambient temperature, and contrast this with that of the mouse. (2 pts)
3. Metabolic rate is a direct reflection of how much food is being broken down per unit time. What price must an endotherm pay to maintain a nice warm body temperature (fueled by a high metabolic rate) over a wide range of temperatures? (2 pts)
4. Based on your answer to #3, EXPLAIN IN DETAIL the types of environmental conditions that would favor *ectothermic* energetics rather than *endothermic* energetics. In what types of habitats might these environmental conditions exist? (Think about where you find lots of ectotherms, but few endotherms!) (2 pts)

Part B. Behavior in a temperature gradient

1. Attach your bar graph showing the *class means* for proportion of point samples (out of 30) in which lizards and mice were in each of the seven box sections. (4 pts)
2. Referring again to the *class mean* behavior data, compare the RANGE of temperatures over which these two animals were active. Which animal can be active over a wider range of temperatures, and why? (2 pts)
3. In the real world, this might be one *disadvantage* of being an ectotherm. What kinds of animals are most common in the arctic and antarctic -- endotherms or ectotherms (give examples)? Why? (2 pts)
4. Refer to your *class means* for the behavior data and compare the average lizard and mouse body temperatures at the conclusion of this experiment.

Mouse: _____ Lizard: _____

Is this what you expected? Explain: (2 pts)

Acknowledgments

The faculty and students of the Biology Department of Hope College and the 1999 participants of the ABLE Workshop/Conference in Lincoln, Nebraska provided invaluable comments during many phases of the evolution of this lab. Financial support for continued development of this lab in particular, and the curricular integration of organismal and ecological levels of organization in general, is provided by NSF-ILI Grant No. DUE 9851665 to K.G. Murray and K. Winnett-Murray.

Literature Cited

- Chinsamy, A. and P. Dodson. 1995. Inside a dinosaur bone. *American Scientist* 83: 174-180.
- Desmond, A.J. 1976. *The hot-blooded dinosaurs: a revolution in paleontology*. The Dial Press/James Wade, New York.
- Fischman, J. 1995. Were dinos cold-blooded after all? The nose knows. *Science* 270:735-736.
- French, A.R. 1988. The patterns of mammalian hibernation. *American Scientist* 76:568-575.
- Gibbons, A. 1997. Lung fossils suggest dinos breathed in cold blood. *Science* 278:1229-1230.
- Hainsworth, F.R. 1981. Energy regulation in hummingbirds. *American Scientist* 69:420-429.
- Hairston, N.G., Sr. 1994. *Vertebrate zoology: an experimental field approach*. Cambridge University Press, New York, New York, 347 pages.
- Hurlburt, G.R. 1994. Were dinosaurs cold- or warm-blooded?: An exercise in scientific inference. Pages 181-214 *in* *Tested studies for laboratory teaching*, Volume 15 (C.A. Goldman, Ed.). Proceedings of the 15th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 390 pages.
- Marchand, P.J. 1991. *Life in the cold: an introduction to winter ecology*. 2nd Edition. University Press of New England, Hanover, New Hampshire, 239 pages.
- McClanahan, L.L., R. Ruibal, and V.H. Shoemaker. 1994. Frogs and toads in deserts. *Scientific American*, March 1994: 82-88.
- Ohmart, R.D. and R.C. Lasiewski. 1971. Roadrunners: energy conservation by hypothermia and absorption of sunlight. *Science* 172: 67-69.
- Padian, K. and L.M. Chiappe. 1998. The origin of birds and their flight. *Scientific American*, February 1998: 38-47.
- Pough, F.H., J.B. Heiser, and W.N. McFarland. 1996. *Vertebrate Life*, 4th Edition. Prentice Hall, Upper Saddle River, New Jersey, 798 pages.
- Prothero, J. and K.D. Jurgens. 1986. An energetic model of daily torpor in endotherms. *J. Theor. Biol.* 121: 403-415.
- Ruben, J.A., T.D. Jones, N.R. Geist, and W. Jaap Hillenius. 1997. Lung structure and ventilation in theropod dinosaurs and early birds. *Science* 278: 1267-1270.
- Townsend, C.R. 1987. Bioenergetics: linking ecology, biochemistry, and evolutionary theory. *Trends in Ecology and Evolution* 2: 14-15.
- West, G.B., J.H. Brown, and B.J. Enquist. 1999. The fourth dimension of life: Fractal geometry and allometric scaling of organisms. *Science* 284: 1677-1679.
- Willson, M.F. 1984. *Vertebrate Natural History*. Saunders College Publishing, New York, New York, 621 pages.

Appendix A Pre-Lab Student Worksheet

Name: _____

Instructor: _____

Lab Day/Time: _____

**Pre-lab Worksheet for Energetic Strategies of
Terrestrial Vertebrates (10 pts.)**

To ensure that everyone knows beforehand how to compute metabolic rate from data on oxygen consumption in the experimental protocol we will use next week in lab, calculations on this worksheet must be done correctly before you come to lab next week. In fact, this sheet will be collected at the beginning of lab, and you won't be able to participate without it! Follow the example given in the lab handout to learn how to compute metabolic rate.

Assume that you have an experimental apparatus set up as described in the lab handout: The chamber volume is 470 ml, and the syringe volume is 60 ml. First, you measure the oxygen concentration of room air as 20.95%.

Your animal weighs _____ grams, and after it has been in the chamber for 5 minutes you measure the oxygen concentration of the chamber air as _____. Using these data, compute the mass-specific metabolic rate of the animal and report it below. Be sure to: 1) Show your work, and 2) Report your answer in correct units.

Instructor's Answer Sheet

We usually construct several versions of the pre-lab worksheet, and distribute them at random. Examples of versions and answers appear below:

Version No.	Animal Weight	Chamber O ₂ concentration	Mass-specific metabolic rate in calories/g-minute
1	16.3	20.2	0.2281
2	18.3	19.9	0.2833
3	20.3	19.7	0.3029
4	22.3	19.5	0.3186
5	24.3	19.3	0.3314
6	26.3	19.1	0.3419

Appendix B *Animal Use Guidelines*

Included below are 3 documents currently in use by the Hope College Animal Care and Use Committee, chaired by Dr. Dan A. Gerbens (Department of Biology, PO Box 9000, 35 E. 12th Street, Holland, MI 49422-9000; email: gerbens@hope.edu).

1. Statement of Policy on the use of Animals in Research and Teaching; Hope College, May, 1998; by Dr. Dan A. Gerbens.

Hope College recognizes the value of the use of animals in research and teaching. Advances in scientific and medical knowledge have improved human and animal health, have led to the alleviation of pain and suffering, and have saved countless lives. The College endorses the judicious use of animals in research and teaching, and ensures the humane and ethical treatment of all animals used in this manner.

The College accepts its legal and ethical responsibility to ensure that animals are spared unnecessary pain and distress and are not used needlessly. To this end, the College adheres to and enforces all applicable federal, state, and local regulations and guidelines pertaining to the use of animals in research and teaching. The Hope College Animal Care and Use Committee was constituted to provide supervision and review of all College projects involving the use of animals. This Committee is comprised of scientists, non-scientists, a member of the community, and a veterinarian. All project proposals for the use of animals are reviewed by the entire Committee to ensure that humane care and use guidelines are followed. The Committee has the responsibility to approve, require modifications of, or to prohibit a project's use of animals. The Committee also receives and investigates concerns expressed regarding the use of animals on the Campus.

The College acknowledges that vertebrate animals warrant moral concern and the use of animals requires responsibility for the stewardship of life that goes beyond immediate research or educational needs to include the acquisition, care, and disposition of the animals. Stewardship also involves sensitivity to the scientific requirements and community attitudes toward the use of animals. To this end, the College endorses the following basic principles as overarching guidelines for the use of animals in research and teaching:

Living creatures deserve respect. Research and teaching should make use of appropriate species and involve the minimum number required for valid scientific results. The use of computer simulations and modeling is encouraged, provided educational objectives are not compromised or weakened.

When animals are used, the assessment of the overall value of the investigation or exercise should include consideration of the range of societal good to be derived from the use of animals vs. the potential for pain and/or distress in the animal(s).

Minimization of pain and distress are moral imperatives. Vertebrate animals are sentient and it should be considered that procedures that can cause pain or distress in humans have the potential to cause similar pain and distress in animals.

Hope College, through the Hope College Animal Care and Use Committee, maintains training programs for researchers, students, and animal care workers to ensure that any individuals working with animals are qualified to perform their tasks in a humane and scientifically appropriate manner and to recognize and report any compromise in the care and use of animals.

2. Hope College Principles for the Care and Use of Laboratory Laboratory Animals

(Adapted from US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training).

- I. The transportation, care and use of animals should be in accordance with the Animal Welfare Act and other applicable federal and state laws, guidelines, and policies.
- II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.
- III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer stimulation and **in vitro** biological systems should be considered.
- IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain and distress in other animals.
- V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by neuromuscular blocking agents.
- VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.
- VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided.
- VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.
- IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal research committee. Such exception should not be made solely for the purpose of teaching or demonstration.

3. Hope College Guidelines for Vertebrate Animal Users

(This 4-page document modified in part from the NIH guidelines for animal research and detailing animal care guidelines for research and teaching, forms required to be on file in the animal facility office, and other animal use information may be obtained by emailing or writing to the authors).

Appendix C Animal Sources

Mice are usually purchased from local, reputable pet stores and kept in the animal facility for a few weeks prior to this laboratory to ensure that they are free of disease.

Anoles can be obtained from:

Amphibians of North America
Charles D. Sullivan Company, Inc.
6685 Holt Road
Nashville, TN 37211
Phone: 615-832-0958; FAX: 615-833-5286
E-MAIL: Sullivan@bellsouth.net

Appendix D Equipment and Supply Sources

Ametek Dual Channel Oxygen Analyzer, Model S-3A

Purchased from Ametek, Thermo Instruments Division (original purchase price was \$14,000; for a single channel analyzer the purchase price was \$11,000). Columbus Instruments, Columbus OH 43204-2121 (614-276-0861) makes the OxyMax Metabolic System for Educational Use; total price for a 16-channel gas monitoring system with accessories is approximately \$47,400.

Electronic thermometers

Cole-Palmer Digi-Sense Thermistor Thermometer Cat. No. P-08525-00 (\$300).

Benchtop Switchbox (10 inputs; allows instructor to switch among temperature probes in different sections of the temperature gradient box)

Cole-Palmer Cat. No. C-08401-12 (\$107).

Probes

YSI 401 general purpose thermistor probe (7 needed for gradient box); Cole-Palmer Cat. No. P-08430-00 (\$50 each).

YSI 402 small flexible thermistor probe (for animal anal temperature; 1 needed); Cole Palmer Cat. No. P-08432-00 (\$70 each).

Lead rings for 250-1000 ml flask (1 for each Mason Jar optional to weight jars in water baths) Fisher Cat. No. 05-869-2 (1-800-766-7000)

Appendix E Instructions for Construction of a Temperature Gradient Box

Materials list

- (2) 1"x6"x6' boards (pine or other soft wood suitable)*
- (2) 1"x8"x8' boards (pine or other soft wood suitable) *
- (4) 4.5" x 13" plexiglas pieces, each 3/32" or 1/8" thick
- (1) Ceramic light socket
- (1) 6' two-conductor electrical cord with plug (gauge suitable for 110vac lamps ok)
- (1) 5"x8" sheet of 1/2" mesh hardware cloth
- (40) #4 x 1/2" pan head or round head wood screws
- (1) 60w, 110 vac ceramic heating element (ZooMed Company)

- (2) Metal handles for lid
- (1 lb.) 6d (2" length) common nails
- (2) #10 x 1 1/4" pan head or round head wood screws (for lamp socket)
- (2) Handles

*Note that board dimensions for width and thickness have historical significance only - as of this writing, "1x6's" are actually 3/4" x 5 1/2" and "1x8's" are actually 3/4" x 7 1/4".

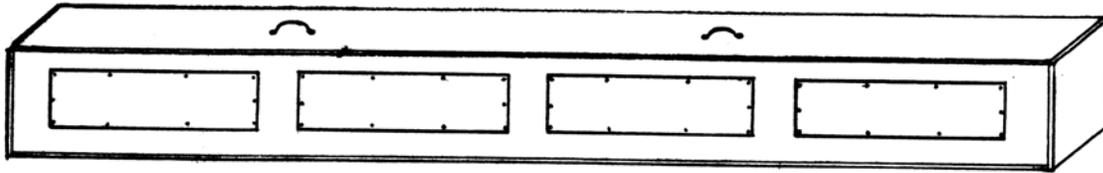


Figure 18.1A. Diagram for temperature gradient box. Side view, showing viewing windows cut into one side only.

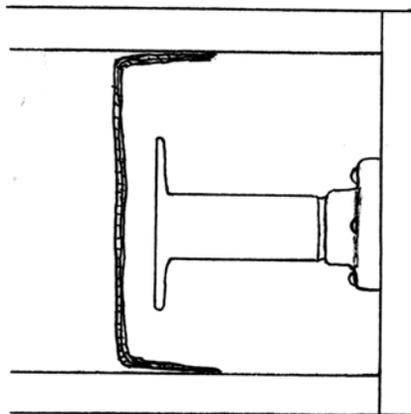


Figure 18.1B. Top view, looking down into one end of the inside of the gradient box. This end has the ceramic heating element protected by wire screening.

Instructions

The dimensions below and in Figure 18.1 are for the particular gradient boxes we made, and are not critical. The instructions are offered as a security blanket and not as an insult - it's just a wooden box.

1. Cut the two 1x6's to 4' 11 3/8" long. Into one of these (to be the front), cut four equally-spaced 12" x 3 1/2" openings with a saber saw (Figure 18.1A).
2. Cut one of the 1x8's to make one piece 4' 11 3/8" long (the bottom) and two pieces 6 1/4" long (the ends).
3. Nail through the end pieces into the bottom piece, such that the end pieces extend 5 1/2" above the upper surface of the bottom piece. Use wood glue for stronger (and better sealed) joints.
4. Nail through the bottom piece into the front and back pieces, such that the top edges of the front, back, and side pieces are all flush with one another.
5. Nail through the end pieces into the front and back pieces.

Energy Strategies of Vertebrates

6. Use a router with a 1/4" rabbet bit to make a recess 1/2" deep along the top inside edges of the box, into which the lid can fit.
7. Cut the other 1x8 to fit the opening in the top of the box as a lid (approx. 4' 11 3/8" long x 6 1/4" wide). Attach the two handles to the top surface of the lid as shown in Figure 18.1A.
8. Drill a 1/4" hole into one of the end pieces near the rear bottom corner of the box, and feed the bare end of the electrical cord through it from the outside to the inside.
9. Connect the two wires to the terminals of the lamp socket, and then attach the socket to the center of the end piece with wood screws. Screw the heating element into the socket.
10. Bend the hardware cloth and insert it into the box as shown in Figure 18.1B, to prevent animals from contacting the heating element directly. Divide the remainder of the box interior (beyond the hardware cloth) into 7 equal-length zones and mark their boundaries on the box bottom with a permanent marker.
11. Number the sections 1-7 with permanent marker, with section #1 farthest from the heating element.
12. Drill a 3/16" hole through the back piece into the center of each of the sections, just above the bottom piece. These holes will accommodate the temperature probes.
13. Drill approximately 10 equally spaced 3/32" diameter holes around the periphery of each of the four plexiglas pieces, and cover the four openings in the box front with these. Attach them to the box front with the pan head screws.

Finished box dimensions are 5' 7/8" long x 6 3/4" high (with lid) x 7 1/4" deep.

Appendix F Sample Data Set

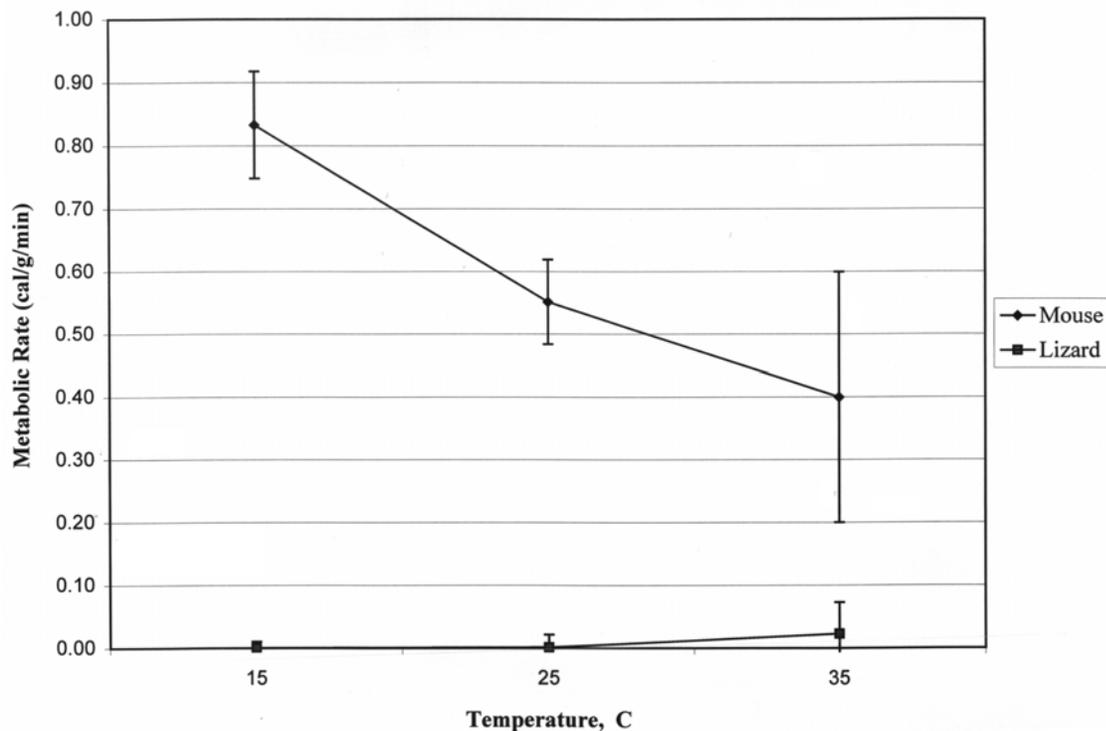


Figure 18.2. Sample data set showing metabolic rate as a function of ambient temperature for lizards vs. mice. Data collected by participants in the 1999 Workshop/Conference of ABLE at Lincoln, Nebraska.

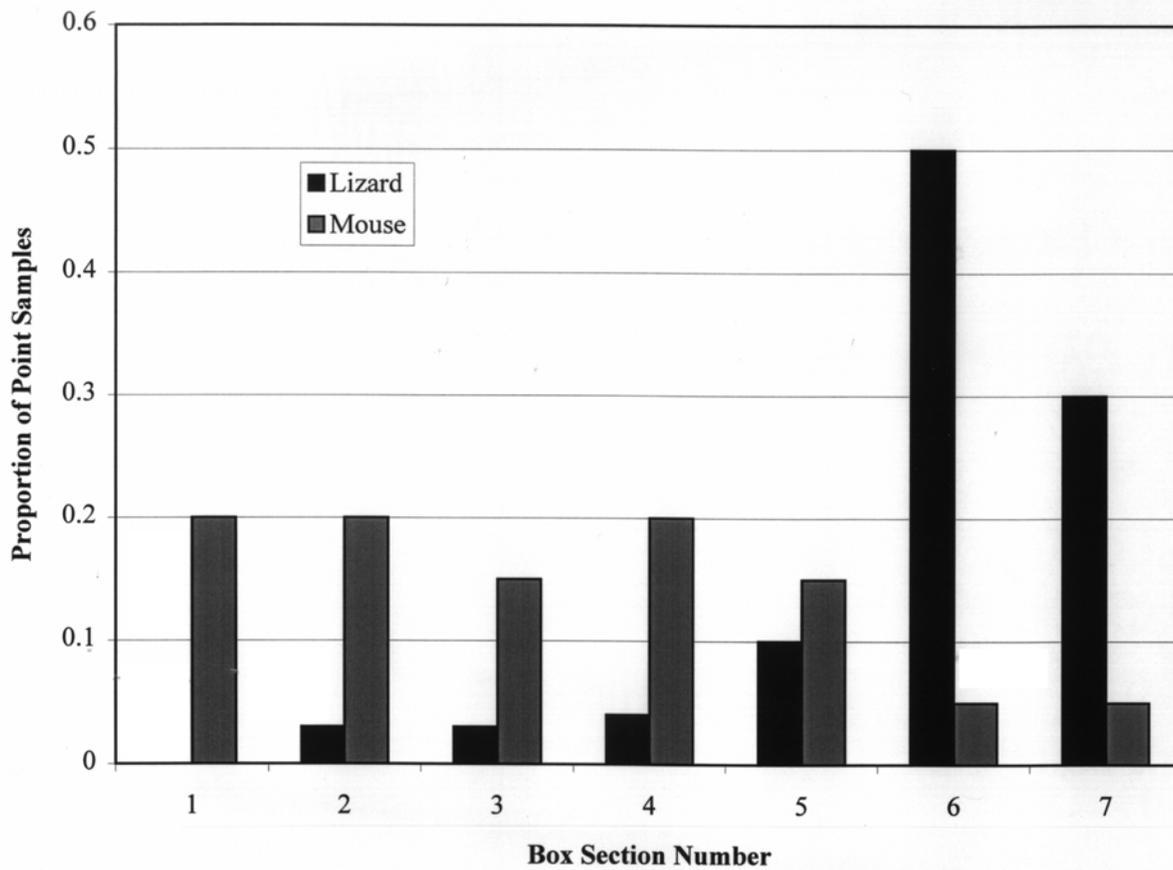


Figure 18.3. Sample data set for lizard vs. mouse behavior in a temperature gradient. Gradient box sections are numbered from 1 (coolest) to 7 (warmest). Typically, ectotherms (e.g. lizards) will spend most of their time in the warm end of the box and exhibit little variation in choice of box section. Endotherms (e.g. mice) move round considerably more, resulting in a lower mean and a higher variance in box section choice.