Chapter 8

Thermoregulation in Vertebrates Studied by Telemetry

David W. Osgood

President
Mini-Mitter Co., Inc.
P.O. Box 3386
Sunriver, Oregon 97707

David W. Osgood was born and raised in Oregon, earning a BS in biology from Portland State University in 1963. He was awarded MA and PhD degrees in Zoology by Duke University, the latter in 1968. He spent one year at Duke as a junior faculty member and 14 years as a member of the Zoology Department of Butler University, leaving as a full professor in 1982. His interest in biotelemetry began in graduate school when body temperature data from free-ranging snakes were needed to compare with laboratory results. When these data were published in 1970, the pressure to build similar equipment for others led to the formation of Mini-Mitter. By 1982, Mini-Mitter had grown so much that he gave up teaching and moved the business home to Oregon. He currently oversees the operation of Mini-Mitter, a task which includes acting as a consultant to literally hundreds of teachers and researchers worldwide who use the company's products.

Reptilian Behavioral Thermoregulation Studied by Telemetry

INTRODUCTION

Most vertebrates are incapable of maintaining body temperatures much above that of their surroundings. We speak of these animals as poikilothermic, (variable-temperature) or ectothermic. The mammals and birds do maintain body temperatures above that of the ambient air and these are referred to as homeothermic (constant-temperature) or endothermic. Endothermic animals metabolically generate the heat required to maintain elevated body temperatures while ectothermic ones must depend on external heat sources to warm themselves to higher-than-ambient temperatures. Basking in the morning sun and lying on warm asphalt roadways at night are two of the means employed by different species of reptiles in achieving behavioral thermoregulation. This exercise will provide you with the opportunity to investigate the degree to which ectothermic vertebrates (lizards and/or snakes) are able to maintain a constant body temperature by moving toward or away from a heat source.

Reptiles are found throughout the tropical and temperate portions of the world with the greatest concentrations in the warmer latitudes. Most species are restricted to habitats with characteristic patterns of temperature variation. Desert forms are exposed to surface temperatures as high as 70° C in the daytime while the night temperatures may be near 0°C (even in summer). Those in wetter environments seldom experience temperatures above 40° C. The preferred body temperatures of reptiles reflect the thermal conditions of their natural habitat, and hence can provide clues to the kind of environment in which they are likely to be found. In this laboratory exercise you will determine the preferred body temperature of several different species of lizards, and then you should be able to make some predictions about the natural habitat of each species.

METHODS AND MATERIALS

The lizards will be housed in sand-floored boxes equipped with a light bulb at one end (Fig. 1) providing a photothermal gradient in which the animals will be free to move about, thereby allowing adjustment of their body temperatures. The body temperature of the lizards is monitored with a miniature temperature-sensitive radio transmitter (the **Mini-Mitter**®) that is fed to the animals at the start of the experiment. Before feeding the Mini-Mitter to the lizard, it must be calibrated so that it can be used as a radio thermometer.

Transmitter Calibration

The Mini-Mitter produces a low-strength radio signal that can be picked up on any standard (AM) radio. The effective range varies from receiver to receiver and particularly with the orientation of the receiving antenna. To tune in the signal, turn on the radio and hold it within a foot or two of the Mini-Mitter. Adjust the tuning dial to a position between stations and listen for a clicking sound (the antenna of the radio is quite directional so a stronger signal will be heard for some orientations of the receiver than for others.) The Mini-Mitter signal is a train of clicks with the rate of clicking determined by a temperature-sensitive element in its circuit. Since each Mini-Mitter has its own click rate vs. temperature curve, it must be calibrated before proceeding with the experiment.

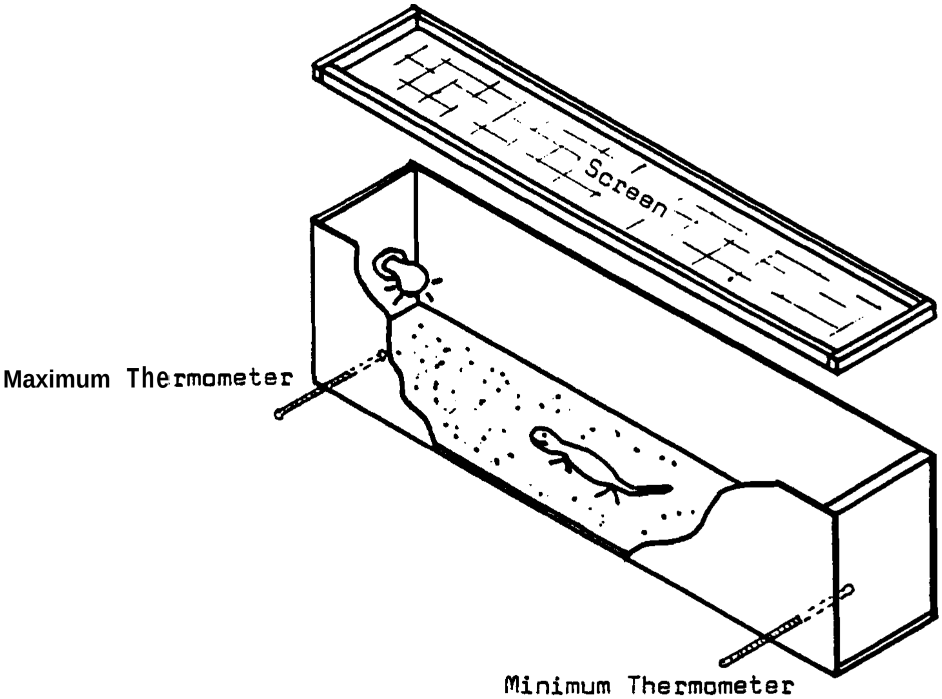


Figure 1. The photothermal gradient box. 1" deep sand on floor.

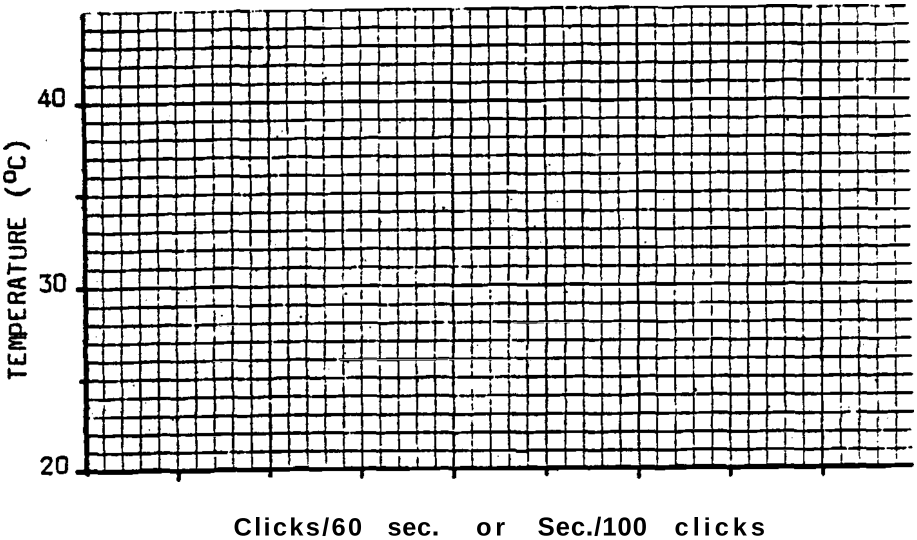
Place the Mini-Mitter and a thermometer in a 1000 mi beaker of water (about 3/4 full) at about room temperature. Allow the transmitter to equilibrate with the water for at least two minutes before taking a reading. Make sure that the water is <u>well stirred</u> during this period so that the temperature of the transmitter and that of the thermometer bulb are the same. Otherwise, you will get a faulty calibration and the entire experiment will not work. After the Mini-Mitter has had time to equilibrate, count the number of clicks in a 60 second interval and record this count on the chart below. Repeat this procedure at about 5-6° C intervals over the temperature range of interest (this will vary depending on the particular animals being investigated). After you have made at least five counts, plot the points on the graph and connect them with a smooth curve. Connecting adjacent points with straight lines will not reduce accuracy noticeably if they are within 5 degrees of each other. Remember, however, that your data can be no better than your calibration curve so make sure that it is as accurate as possible. For very precise calibration of the transmitter, points should be plotted at 3° C intervals over the desired range of temperatures. Accuracy may also be improved by timing 100 clicks with a stopwatch rather than using a wristwatch or wall clock. In that case, data can be converted to clicks/min. or plotted directly as sec./100 clicks. Either method gives good results. By measuring the click rate and using your calibration curve you can now determine the temperature of the transmitter (and hence the deep body temperature of the animal in which you will place it). At this point you are ready to proceed with the experiment.

Animals

The lizards you will be using have been selected to represent a wide range of preferred body temperatures. Your instructor will provide you with a specimen and information on its care and feeding, but not its identify or normal habitat. You will then <u>gently</u> slip the calibrated transmitter down its throat and into its stomach. After inflicting this indignity on your lizard, allow it to escape into its runway where it will spend the next few days. Do not record any data from the specimen until several hours after ingestion of the transmitter. The remainder of the exercise will be done at your convenience during the next several days.

Calibration Data

Water Bath Temperature	Clicks/ 60 Sec.



DATA TABLE

Time	Clicks/ 60 Sec.	Body Temperature (from curve)	Environmental Temperature	
			Max.	Min.

Computation of Standard Deviation

x* (Body temp.)		$x - \bar{x}$	$(x - \bar{x})^2$
x_1			
x_2			
x_3			
x_4			
.			
.			
.			
x_n			

x [ ]

$\bar{x}$(= mean) = (x)/N [ ]

$\Sigma (x - \bar{x})^2$ [ ]

$$\text{S. D.} = \sqrt{\frac{(x - \bar{x})^2}{N - 1}}$$

[ ]

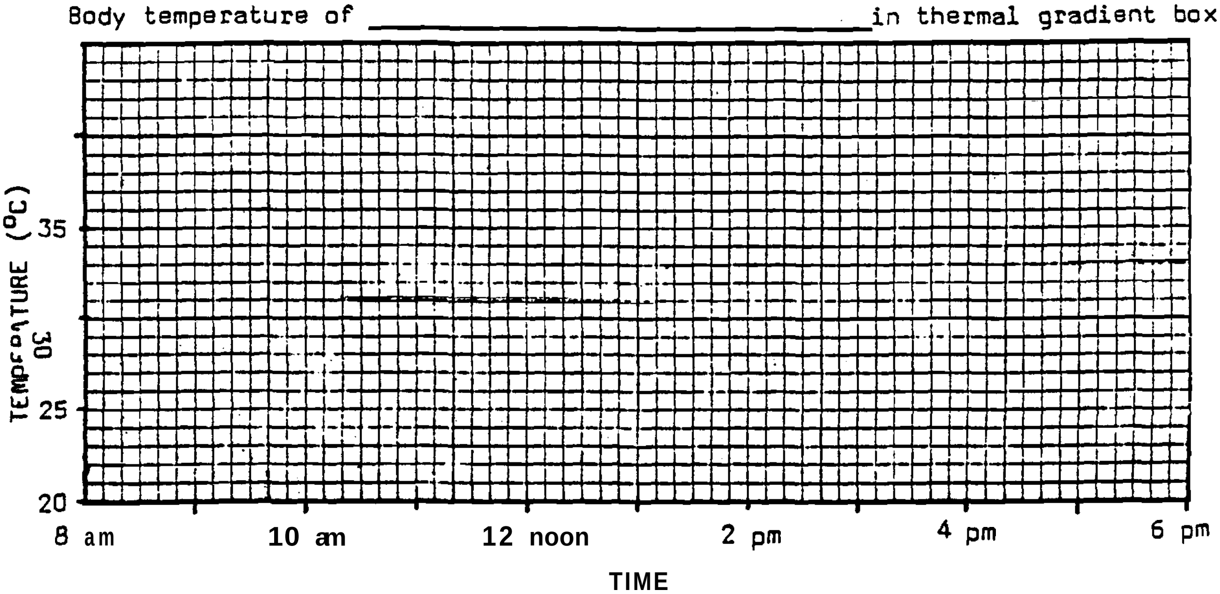
Where:

N = **Number of observations**

Σ = **sum of a series of observations**

* **Same as Body Temperature column in Data Table**

Between now and the next lab period set aside one day to monitor the body temperature of your lizard. Check its temperature once or twice per hour for the entire day (8 a.m. to 6 p.m.). At each reading also record the maximum and minimum gradient temperatures as an indication of the range of temperatures available to the lizard. These data are to be recorded in the table provided. After completing your readings, plot the results on the graph below. The area between the maximum and minimum environmental temperatures can be shaded to aid in visibility.



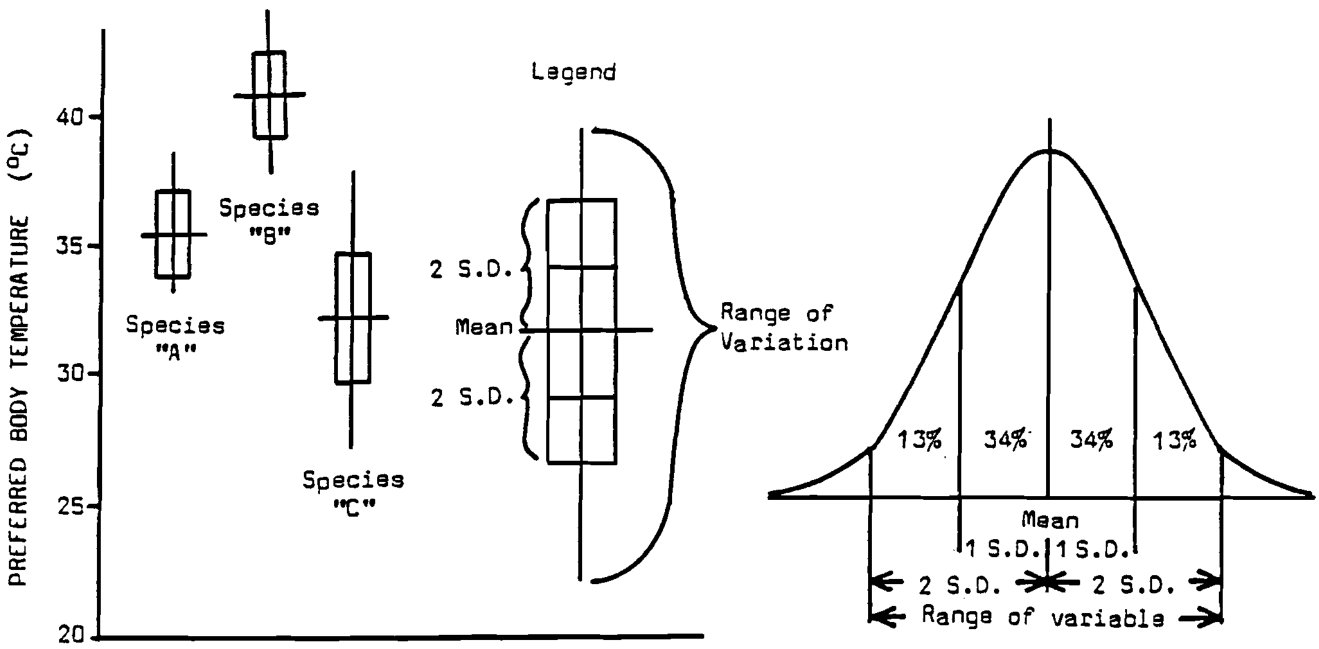
CALCULATIONS

After completing your observations of body temperature of a lizard, you should prepare the results for presentation to the rest of the class. This is best done by computing the mean body temperature and the standard deviation of your observations. The standard deviation table above is designed to assist in making these computations. A desk calculator that accumulates sums of squares makes the job even easier, but it is not a necessity.

The lizard's mean body temperature is simply the average of your observations. It is a measure of the preferred body temperature of your particular lizard. When computed for all available lizards of that species, the mean body temperature estimates the true preferred body temperature of the species. A single individual may have a preferred body temperature somewhat different from that of the majority of animals of the same species, but if observations from several individuals are averaged ,the resultant mean will be a good estimate of the true population mean.

Standard Deviation (S.D.) is a statistic that reflects the degree of variability in a set of observations of a particular characteristic or physiological parameter. Standard deviation provides a measure of the degree of confidence that one can have in his estimate of the true population mean. In all collections of data, it is always true that two thirds of the distribution lies less than 1 standard deviation from the mean (x) and 95% of the distribution lies within 2 standard deviations of the mean. The size of the standard deviation of a group of observations is influenced by the number of observations, with larger sample sizes yielding correspondingly smaller standard deviations for a given character. In the present experiment, standard deviations can be used to determine if two species of lizards have significantly different preferred body temperatures. For example, if species A is found to have a preferred body temperature of 35.4° C with a standard deviation of 1.2° C and species B has a preferred body temperature of 40.6° C with a standard deviation of 0.7° C, the two species differ significantly (in the statistical sense) in body temperature. The intervals enclosed by ± 2 S.D. around their respective mean preferred body temperatures do not overlap [35.4 + (2 x 1.2) = 37.8; and 40.6 - (2 x 0.7) = 38.81.

Calculations of this type are frequently represented graphically as in the figure below. The mean of the observations is represented as a short horizontal line with the range of the observations indicated by a vertical line intersecting it. A hollow box is superimposed over these lines to indicate the interval ± 2 standard deviations. A separate such diagram is constructed for each species or population, and the reader can see at a glance which populations overlap and which are statistically different from each other.



At the next laboratory period we will combine our data with those of other students working with the same lizard species to compute a mean preferred body temperature for the species studied. Compare this mean with those observed for the other species of lizards and make predictions about which species might be desert inhabitants and which would be more likely to be found in wetter (and hence less thermally variable) habitats.

REFERENCES

Brattstrom, B. H. 1965. Body temperatures of reptiles. *Amer. Midland Nat.* 73:376-422.

McGinnis, S. M. 1966. *Sceloporus occidentalis:* preferred body temperature of western fence lizard. *Science* 152:1090-1091.

Mackay, R. S. *Bio-Medical Telemetry*. 2nd ed. John Wiley & Sons, New York. 1970. pp 533.

Osgood, D. W. 1970. Thermoregulation in water snakes studied by telemetry. *Copeia* 1970:568-571.

TECHNICAL INFORMATION SHEET

Photothermal gradient boxes: Dimensions not critical. Our design utilized plywood and masonite already available.

Materials

1 - 48" x 8" x 1/2" plywood or board (bottom)

2 - 15-3/8" x 8" x 1/2" plywood or board (ends)

2 - 15-7/8" x 48" x 1/8" masonite (sides) assemble with smooth side in.

1/2" x 1" wood strips (2 - 8-1/2" long, 2 - 49-1/4" long) to form frame for top.

1 - 9" x 50" aluminum window screen (stapled to top frame)

Miscellaneous nails and staples to assemble

Lights: We use gooseneck laboratory lamps with 60 watt bulbs, but any arrangement that provides sand surface temperatures near 50° C immediately under the bulbs is satisfactory.

Photoperiod

For best results a regular photoperiod of between 12 and 15 hours of daylight (=heat) should be maintained for several days prior to and throughout the experiment.

Room temperature

If possible the runways should be set up in a room that will be used for no other purpose. The temperature should be below 22° C to allow the establishment of an adequate thermal gradient in the boxes.

Food and water

Water should always be available in the center of the runways. The animals should be fed on a regular schedule that of necessity will vary from species to species. Consult your animal supplier for specific feeding instructions.

Animals

Reptiles are sold by a number of suppliers in Florida and the Southwestern states. When planning a laboratory that requires reptiles, a dealer should be consulted well in advance of anticipated needs. Most dealers stock relatively few desert forms in the winter months so orders should be placed in the early fall or the experiment postponed until spring. Lizards are more readily accepted by students, but snakes will work equally well as subjects for this experiment. Greater care must be taken to prevent snakes from escaping, but they are able to swallow the transmitters more easily, making them more desirable in this respect.

Recommended lizard genera are *Gerrhonotus*, *Iguana*, *Tupinambis*, *Crotaphytus*, *Basiliscus*, and *Sauromalus*; *Pituophis* and *Natrix* are good snake genera. In general, the larger the individual, the easier it will take the transmitter.

Recovery of the transmitter

This can occur spontaneously as some animals will regurgitate it or pass it on through the digestive tract. Others can be forced to regurgitate it by pressing gently on the abdomen and forcing the transmitter up into the esophagus. Surgical recovery must be used in many cases. Most reptiles take ether anesthesia very well and are quite tolerant of surgical procedures. We recommend that the transmitters not be allowed to remain in the animals more than two weeks without supplementary coatings to seal out moisture. Polyethylene is not a perfect moisture barrier and transmitters intended to be left in animals for extended periods should be dipped in successive layers of Paraffin/elvax. This will provide a good enough seal to allow the transmitter to function for the 2-3 month life of the battery.

The Study of Animal Temperature Regulation by Telemetry

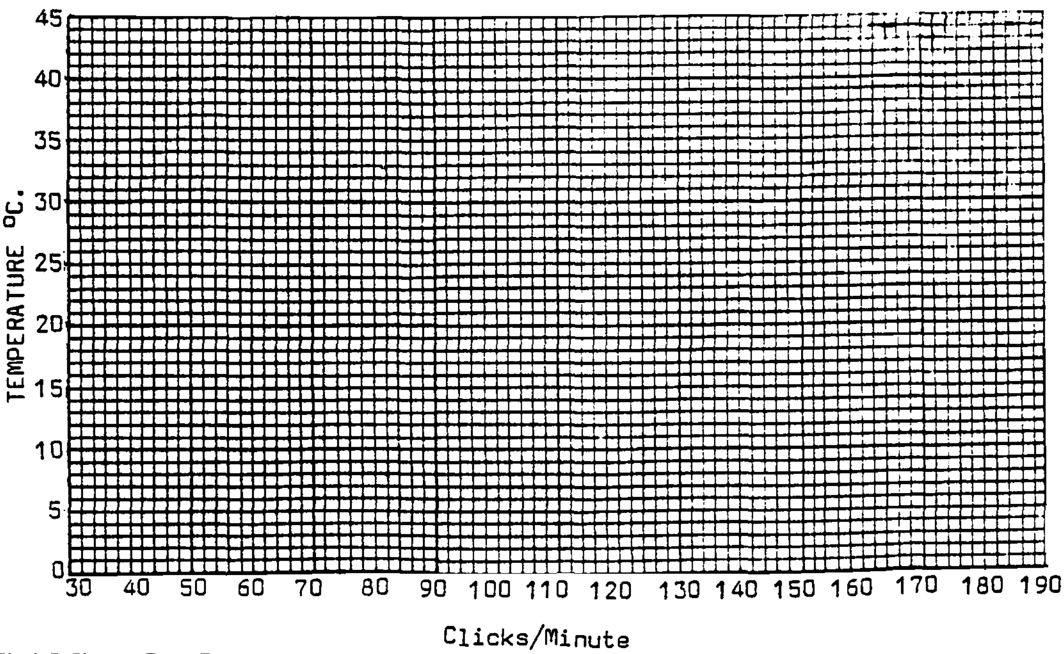
INTRODUCTION

Animals are generally described as being cold-blooded or warm-blooded. The cold-blooded animals include all the invertebrates, the fishes, the amphibians, and the reptiles. Only the birds and mammals are warm-blooded. More correctly, cold-blooded animals should be called poikilothermic (Greek poikilos, various) and warm-blooded ones homeothermic (Latin homeo, same). Poikilothermic means that the animal is primarily dependent upon environmental temperature to determine its own body temperature. Although these animals do produce metabolic heat, they are largely unable to vary heat production or to control heat loss by physiological adjustments. Homeotherms on the other hand are able to regulate their body temperature and can maintain a constant or nearly constant body temperature despite variation in the environmental temperature. In this exercise you will observe that some poikilothermic animals gain or lose heat faster than other poikilotherms. You may also see that homeotherms do not maintain a constant body temperature under all conditions. Try to determine the physical factors that affect the rate of heat loss or gain.

EQUIPMENT

Each group will be provided with a temperature-sensitive radio transmitter (the **Mini-Mitter**®) and an AM radio. (Using the sweep second-hand on a clock or wristwatch to determine accurate time intervals, you will be able to ascertain the temperature of the transmitter and hence that of the animal in which it is placed.) The transmitter emits a radio signal that consists of a series of clicks. The rate of clicking is proportional to the temperature of a thermistor in the circuit. Since each Mini-Mitter has its own click rate vs. temperature curve, the first step in the experiment is the calibration of the transmitter.

Temp. °C.	Clicks per Minute



The temperature signal from the transmitter can be picked up anywhere on the AM radio broadcast band. However, you will find that certain frequencies will give a clearer signal than will others. After you have located a frequency with no interference, you are ready to begin the calibration. This is done by placing the transmitter in a water bath and counting the number of clicks per minute at several temperatures at least 10º C apart. The calibration curves are nearly linear and can be plotted as straight lines between any two adjacent points; but to insure accuracy you should plot at least four points, preferably near 10º C, 20º C, 30º C, and 40° C. The Mini-Mitters have a time constant (time to reach 99% of a new value) of approximately 2 minutes so they should be left in the water baths for at least this long before making a reading. Be sure to stir the water bath adequately so that the temperature is uniform throughout! Plot the points for your transmitter on the graph. Make sure that your calibration is correct; otherwise the data from the following experiments will not be valid.

INSTRUMENTING THE ANIMAL

Introduction of the Mini-Mitter into the animal depends upon the kind of animal involved. Rats must be anesthetized with ether after which the Mini-Mitter is implanted surgically in the body cavity. Rats recover quickly from this type of operation, and only minimal precautions against infection are necessary. After the transmitter has been implanted, the animal should be allowed a couple of days to recover from the anesthetic and surgery. Reptiles and amphibians can be forced to swallow the transmitters without anesthesia. The alligator's jaws can be held open by coaxing it to bite on a soft stick and then the transmitter can be inserted back in the throat with a forceps. Finger pressure from the outside will cause the Mini-Mitter to slide down the esophagus into the stomach.

GENERAL DIRECTIONS FOR TEMPERATURE EXPERIMENTS

After allowing your animal to remain undisturbed for 15 minutes at room temperature, make a test count to determine its body temperature. Record ambient (immediate surroundings) temperature with a laboratory thermometer every time you read body temperature. Be sure the thermometer bulb is dry when making air temperature measurements.

After measuring body and ambient temperatures at normal room conditions, change to different temperature conditions and determine how fast body temperature of the animal changes. As an example, if the animal is at 25º C, place it in a 15º C environment, and follow the body temperature changes at 2 minute intervals. That is, you should make records at 0, 2, 4, 6 minute (etc.) time periods. If the rate is slow, change to measurements at greater intervals (i.e., 0, 5, 10 minutes).

Each student should do the experiment with one poikilotherm and one homeotherm. Be sure that you understand the results of experiments involving the other animals used in the experiments.

Temperature Regulation in the Frog

Determine the change in body temperature when a frog is removed from a room-temperature water bath and placed in a cold water bath. The experiment should continue until equilibrium is reached. Again, place the frog in the room-temperature bath. Is the rate of warming the same as the rate of cooling? Next, place the frog in a 40° C (no hotter) water bath. Determine the rate of change. Graph the changes as a function of time.

Temperature Regulation in the Alligator* (or lizard)

This experiment can be done in either air or water. If done in air, make sure the animal is dry before making any measurements. (Why?)

1. Water: Follow the same procedure as given for the frog. Remember that the alligator, unlike the frog, is exclusively an air-breather. Don't drown the beast!

2. Air: Dry the alligator and allow the animal to equilibrate with the room temperature. Then place him in a cold, dry environment to determine the rate of temperature change. A refrigerator, normally ranging from 3-5° C, is satisfactory. (Don't keep the refrigerator door open any longer than necessary since the cooling system is not overly efficient and may take some time to return to its normal temperature.) The air temperature in the refrigerator should be recorded. After reaching equilibrium at the cold temperature, return the alligator to room temperature and determine the rate of increase in body temperature. Is the increase linear? Assuming that the alligator and frog were the same size, would their rates of change be the same under similar conditions? Why? What factors are involved here?

   * South American Caiman

Temperature Regulation in the Snake

Determine the ambient and body temperature.

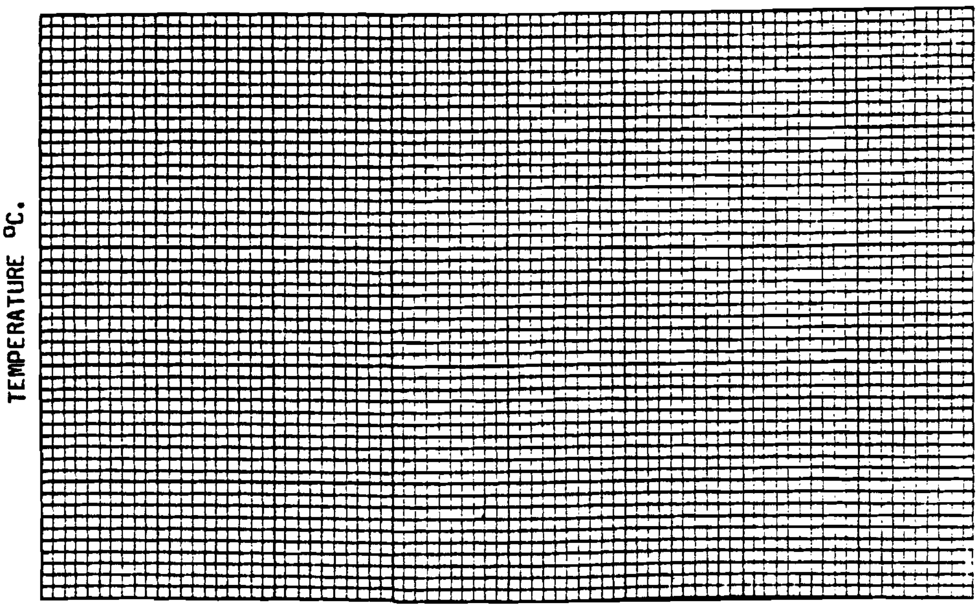
1. Place the snake in a cold, dry environment to determine the rate of temperature change. Follow the same procedure as outlined in B-2 above.

2. Place the snake in an incubator or under an infra-red light (in a cloth bag, if necessary) to determine the rate of increase in body temperature above room temperature. With infra-red light, only the body surface is warmed - the air temperature does not increase. (Infra-red sources can generate very high temperatures; don't broil the animal.) What factors are important in determining how rapidly the body temperature of a snake will change? Why would an inanimate object of the same shape not change at the same rate as does the snake? Explain the physical principles involved.

RECORD DATA HERE:

POIKILOTHERM USED ______________________

Time	No. Clicks	Body Temp.



Temperature Regulation in Mammals

Here we are measuring the core (deep body) temperature. Will it be the same at room temperature as at low temperature? At high temperature? The transmitter has been previously implanted so you need only to make the measurements.

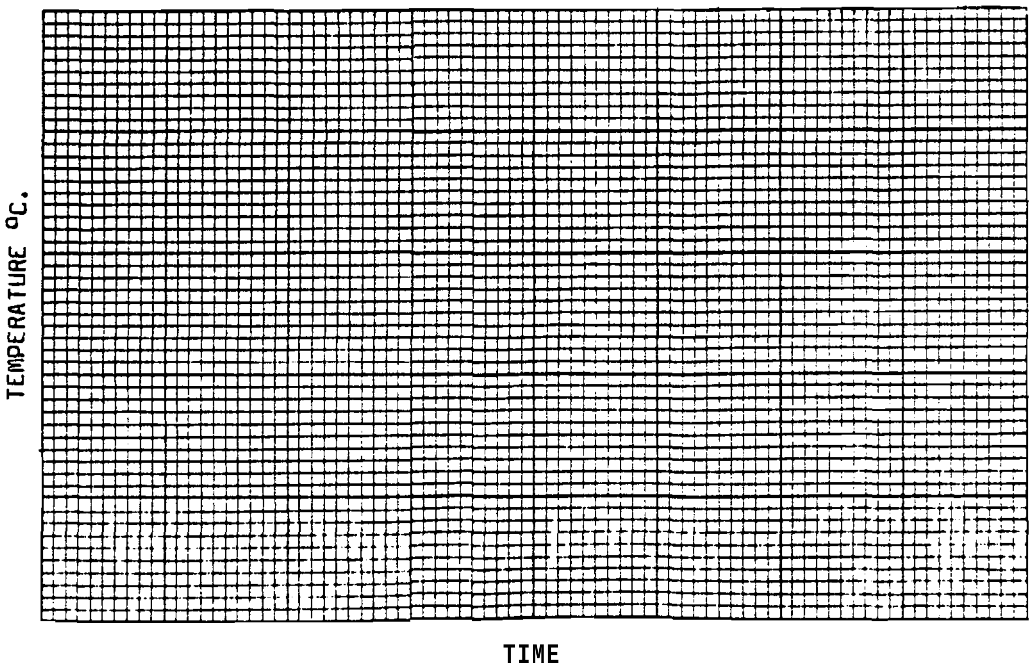
Since the radio signal will not pass through sheet metal, provisions must be made to pick up the signal from inside the refrigerator or incubator for the corresponding cold or warm environment experiments. One satisfactory method for doing this is to place a portable radio inside the refrigerator near the transmitter and use the earphone or a small accessory speaker to carry the signal to the outside. In an alternate method you can loop an antenna (made from any type of wire) around the animal cage and run this outside the refrigerator to the antenna rod of the radio (see Mini-Mitter Technical Data Sheet for further information).

Place the animal in the freezer compartment of the refrigerator. Do NOT put it on a cold, hard surface (frost-bite). Line the container with a cloth or paper. Follow the temperature change. How does the rate of change compare with that of the poikilothermic (non-regulating) animals? Which is faster? Which is greater? What behavioral changes do you notice in the mammal at low temperature? Do not leave the animal in the freezer for more than 15 minutes. Graph the body temperatures as a function of time.

Place the mammal in an incubator at 45° C. Determine the rate at which the body temperature changes. Observe the animal closely to make sure it is not in pain. If it begins to behave peculiarly, remove it from the high temperature immediately. Do not leave the animal in the incubator for more than 15 minutes. Does the body temperature increase? Why? What mechanisms do homeothermic (regulating) animals have to control their body temperatures at both high and low ambient temperatures?

HOMEOTHERN USED ______________________________

Time	No. Clicks	Body Temp.



APPENDIX 1

EQUIPMENT LIST FOR TELEMETRY LABORATORIES

1. Copies of lab exercises
2. Mini-Mitters with batteries
3. Thermometers
4. 1 or 2 liter beakers
5. 8-12 in. pieces of solid copper wire 18-26 ga. (for holding transmitters in beakers)
6. Magnetic stirrers or stirring motors
7. Stopwatches
8. Portable AM radios (may be supplied by students)
9. Animals, as needed. Frogs are generally available from standard sources. Among reptiles, snakes are best choices and they are readily available locally in most areas. You may have to plan ahead to get them for a winter lab. Local kids know where and will usually catch all you can use for a dollar or two apiece. Rats, hamsters, gerbils, mice are all good subjects, with a large and a small species allowing comparison of surface/volume considerations.

ANIMAL SUPPLIERS

Mammals

Ancare Corp.
47 Manhasset Ave..
P.O. Box 354
Manhasset, NY 11030
(516) 627-9292

Buckshire Corp.
2025 Ridge Rd.
Perkasie, PA 18944
(215) 257-011

Charles River Labs., Inc.
251 Ballardvale ST.
Wilmington, MA 01887
(617) 658-6000

Taconic Farms, Inc.
33 Hover Ave.
Germantown, NY 12526
(518) 537-620

Reptiles & Amphibians

Boreal Labs, Ltd.
1820 Mattawa Ave.
Mississisauga, ON L4X 1K6
Canada
(800) 387-9379
in US (800) 828-7777

Kons Sci. Co., Inc.
P.O. Box 3
Germantown, WI 53022-0003
(414) 242-3636

Wm A. Lemberger Co., Inc.
P.O. Box 2482
2500 Wausau Ave.
Oshkosh, WI 54903
(414) 231-8410