

Chapter 13

Investigating Animal Respiration with Electronic Probes

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Reprinted From: Hodson, R. C. and J. Acuff. 2003. Investigating animal respiration with electronic probes. Pages 191-210, in Tested studies for laboratory teaching, Volume 24 (M. A. O'Donnell, Editor). Proceedings of the 24th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 334 pages.

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Introduction

The introductory biology course in which this investigation is conducted has segments of plant structure and function, animal structure and function, and ecology. The laboratory component is designed to contain the following elements.

- Emphasis on the process of science rather than specific topics.
- Hands-on, inquiry-based activities.
- Collaboration within and between 3-member student teams.
- Collection of both quantitative and qualitative data using high quality instrumentation.
- Computers to aid data collection and analysis.
- Computer networking to aid collaboration and communication of scientific findings.
- Opportunities for student projects that extend the investigation to asking new questions and testing new hypotheses.

For one of the investigations relating to animal biology we chose to study variables that affect animal respiration without harm to the animal, and sought equipment that would be sufficiently stable and sensitive, but easy enough to learn and rugged enough for inexperienced student use. Instruments manufactured by Qubit Systems, Inc., and software from Vernier Software and Technology, fit these requirements. Some of the equipment may be used for other physiological measurements such as photosynthesis, nitrogen fixation, and water uptake by plants.

The model investigation uses a common green anole readily available in pet stores (or on the campus of a Southern college!) with temperature as the variable. The temperature range of 10°C to 30°C is sufficient to obtain useful responses from a variety of animals including lizard, mouse, frog, baby chick, and insects. Plant material such as germinating seeds, and microbial cultures such as yeast may also be used. Suggestions for student projects are given in the Student Outline.

The investigation has been in use for about 3 years. A variety of organisms and experimental protocols have been tried. Some have been retained and some rejected. We are still discovering new investigations and better ways of doing them. For example, changes have been made to minimize “down” time during data collection and afterwards, to allow enough time for data analysis and sharing of results between student teams. However, we have been limited to the investigations that can be done with animals. Adopters must keep in mind that there may be requirements for having protocols reviewed and approved by an institutional animal use committee, and that some treatments students may devise will not and should not be approved.

Materials

- Computer (PC or Mac)
- Spread sheet software
- *Logger Pro* software
- Low range respiration package (Qubit Systems, Inc.)
- Water vapor trap
- Laboratory pan balance
- Lizard (*Anoles carolinensis*)
- Container for weighing animal
- Crushed ice
- Zip-Lock bags
- Cloth
- Heat lamp

Notes for the Instructor

1. We typically make the following assignments before lab day:
 - Read about animal gas exchange and regulation of body temperature in the course textbook.
 - Read about the principles of equipment operation, particularly the IRGA (infrared gas analyzer), from the handout provided by the Instructor (see below).
 - State an hypothesis and discuss in class before starting the investigation.
2. Use of equipment
 - Operating principles for the electronic probes, calibration procedures for the IRGA (infrared gas analyzer), and instructions for *Logger Pro* software are supplied with the respiration package from Qubit Systems Inc. This material may be reproduced and provided to students. Instructors will need to learn such things as how to customize the *Logger Pro* graph windows for experiment timeout and scales of the X- and Y-axes.
 - A link is placed on the student's computer desktop to the *Logger Pro* file (.MBL file) for respiration.
 - The magnesium perchlorate drying tube must be replaced before the granules become crusty and block airflow, or liquify and corrode the IRGA (infrared gas analyzer). We routinely replace them between 3-hour laboratory sessions.
 - The IRGAs must be running for several hours to charge their internal battery and stabilize. We leave them on for the entire week of the investigation.
 - Some computers are sensitive to processor activity associated with batteries (notebook computers), power management, operating system auto updates, as well as virus scanning, and may drop the serial port connection. Stopping such activity or using the USB port will avoid interruption of data flow between the ULI (universal laboratory interface) and computer.
 - Our students are forbidden to bring removable computer media into the laboratory because of the potential for virus infection. Instead they save their data files to a file server and then access the server via the campus network and transfer the files to their computer.

3. Animals and precautions for handling

- We have successfully used the apparatus with the following animals: anole, mouse (several ages), frog, baby chick (1-21 days of age), and Madagascar cockroach. A small snake and crickets are also possibilities for animals. The animal's size and metabolic rate will influence the chamber size and model of IRGA to use. Information on proper choices of equipment is available at the Qubit Systems Inc web site (URL = <http://www.qubitsystems.com>). Suggestions for housing, feeding, and transporting animals are given in Appendix A. Sample data for a baby chicken are given in Appendix B.
 - No animal should be in an enclosed chamber (cuvette) without airflow.
 - Anoles lack heat sensors in their foot pads and must be protected from excessively warm surfaces. Most species are probably adapted to warm temperatures, so application of cold must be done cautiously. At temperatures approaching 10°C, the animals will drop their respiration rate dramatically and may lie on their backs. Students will think they are dead. If they are kept this way only a few minutes they fully recover.
 - Mice should not be exposed to temperatures much above 30°C. The lethal temperature is very close to this value, and different animals have different sensitivity to heat, so a margin for error is advisable.
4. The cost of the equipment is not trivial. However, it appears to last because in three years none of the IRGAs, ULIs, or other electronic components have had to be replaced. It also is very stable and very sensitive (except for the oxygen sensor; see below) so students obtain valid data. Gone are the days when students devote most of their laboratory reports to explaining what went wrong (artifact) and "what was supposed to happen." Students can proceed with confidence to analyze and interpret data in terms of real biological events and concepts.
 5. It is important to the curriculum at the authors' institution that students be challenged early to go beyond experiments planned by the instructor into territory new to the student. Some projects are suggested in the Student Outline. Other projects are possible. For example, some institutions may have permission to vary the diet given to a mouse. An investigation into the RQ (respiratory quotient) of animals on different diets could be interesting.
 6. The respiration package includes a flow-through oxygen sensor, and we generally include it in the setup. (Though the sample exercise included in the Student Outline section does not include use of the oxygen sensor.) However it is rather sluggish compared to the IRGA and temperature probes, and it needs a stable gas feed to give a meaningful reading. One can monitor the gas stream until equilibrium is reached (5-10 minutes). An alternative is to feed effluent from the animal chamber or IRGA into the gas bag provided with the respiration package. This would average the variable output from the animal and produce a stable reading long enough to collect accurate data for both CO₂ and O₂. Several samples in individual bags may be needed.
 7. Organisms other than animals could be studied. The Qubit manual suggests investigations with respiring yeast cultures. Students could also study the respiration rate of germinating seeds hydrated various times before lab day, or (in a longer term investigation) the climacteric of ripening fruit.
 8. Attention should be given to the conditions present before an animal's respiration rate is determined. We have evidence that temperature equilibration can take several minutes, (*e.g.*,

more than 15 minutes for a lizard). Also we have observed that an animal treated to a different temperature and returned to room temperature will not return to its normal respiration rate right away.

9. With animals it makes a difference if temperature is varied from warm to cold or cold to warm. We prefer to take an animal from the temperature of its housing and warm it to 30°C, then record respiration rate as the temperature is decreased to 10°C rather than the reverse. The initial warming treatment seems to have a shorter residual effect on the animal than initial cold.
10. The only complaint we have received from students is that, to some, collecting data is boring. These students want to be doing something with their hands. (Faculty are generally fascinated and want to watch the data be recorded on the computer and try to anticipate what will happen next.) Therefore, it is advised to plan ahead and have some other concurrent activities. Sometimes we have students explore the WWW to learn more about the animal being studied. There is interesting literature on lizard biology, for example. Sometimes we have students produce crossword puzzles of biological terms and challenge each other with them. Students could also be planning their own sequel project.
11. This investigation is particularly well suited for giving students opportunities to struggle with data analysis and presentation. It is not easy to decide what data values to select when an animal's respiration rate is varying considerably over short time intervals. Also, students have to decide how many values to obtain to give a clear picture of respiration rates under varying conditions. Thus, they have to reject the inclination to look for data that support their prediction and really LOOK at the data for a correct analysis.

12. *Rate calculations and data analysis:*

Respiratory rates may be calculated for oxygen consumption (using the oxygen sensor), or for carbon dioxide production (using the infrared gas analyzer, IRGA). If you determine both CO₂ production and O₂ consumption, then respiratory quotient (RQ) can be calculated. (RQ = CO₂ produced ÷ O₂ consumed.) For the model investigation included in the Student Outline section, the oxygen sensor is not used and therefore RQ is not calculated. However, if you want to use the oxygen sensor and calculate RQ, the necessary calculations are given here. The general steps are as follows:

- a. Calculate the change in CO₂ or O₂ concentrations from the air inflow to the outflow (affected by the animal) of the respiration chamber:

$$\Delta\text{CO}_2 = \text{outflow CO}_2 - \text{inflow CO}_2$$

$$\Delta\text{O}_2 = \text{inflow O}_2 - \text{outflow O}_2$$

- b. CO₂ is measured in ppm, and O₂ is measured in %, so these values must be converted to μL per ml of gas. (Remember that 1 ppm = 0.001 μL/ml; 1% = 10 μL/ml)
- c. The respiratory rate (R_{O₂} or R_{CO₂}, as μL/min) is calculated by multiplying the ΔCO₂ or ΔO₂ values by the air flow rate (in ml/min) used in the experiment:

$$R_{\text{O}_2} = \Delta\text{O}_2 (\mu\text{L/ml}) \times \text{flow rate (mL/min)}$$

$$R_{\text{CO}_2} = \Delta\text{CO}_2 (\mu\text{L/ml}) \times \text{flow rate (mL/min)}$$

- d. To make it possible to compare rates between animals of different mass, adjust the result to one unit (gram) of animal mass by dividing by the animal's mass in grams.

e. As an example, a 50-gram animal is used, and the apparatus has an air flow rate of 200 ml/min. The oxygen sensor measures O₂ inflow as 20.90% and O₂ outflow as 20.85%. The IRGA measures CO₂ inflow as 8 ppm and CO₂ outflow as 480 ppm. The following calculations are made:

- calculate the change in CO₂ and O₂ concentrations:

$$\Delta\text{CO}_2 = \text{outflow CO}_2 - \text{inflow CO}_2 = 480 - 8 = 472 \text{ ppm}$$

$$\Delta\text{O}_2 = \text{inflow O}_2 - \text{outflow O}_2 = 20.90 - 20.85 = 0.05\%$$

- convert Δ values to $\mu\text{L}/\text{mL}$:

$$472 \text{ ppm} \times 0.001 = 0.472 \mu\text{L CO}_2 / \text{mL air}$$

$$0.05\% \times 10 = 0.50 \mu\text{L O}_2 / \text{mL air}$$

- multiply by the air flow rate to get respiratory rate:

$$R_{\text{O}_2} = 0.50 \mu\text{L}/\text{ml} \times 200 \text{ mL}/\text{min} = 100 \mu\text{L}/\text{min}$$

$$R_{\text{CO}_2} = 0.472 \mu\text{L}/\text{ml} \times 200 \text{ mL}/\text{min} = 94.4 \mu\text{L}/\text{min}$$

- Divide rates by mass of animal to get rates per gram:

$$R_{\text{O}_2} \text{ per gram} = 100 \mu\text{L}/\text{min} \div 50 \text{ g} = 2.0 \mu\text{L}/\text{min per gram}$$

$$R_{\text{CO}_2} \text{ per gram} = 94.4 \mu\text{L}/\text{min} \div 50 \text{ g} = 1.89 \mu\text{L}/\text{min per gram}$$

- Now calculate the respiratory quotient:

$$\text{RQ} = \text{CO}_2 \text{ produced} \div \text{O}_2 \text{ consumed} = 1.89 \div 2.0 = 0.9$$

Student Outline

Summary

An experimental animal, a lizard, is humanely contained in a chamber (“cuvette”) into which carbon dioxide-free air flows at a known constant rate. An electronic probe in the chamber monitors temperature, and electronic probes in the outgoing air stream measure oxygen and carbon dioxide concentrations. From the data obtained, respiratory rate is calculated and related to temperature in the chamber. The relationship reveals if the animal responds in a manner more typical of an endotherm or an ectotherm.

Background

This is a quantitative investigation into homeostasis in animals, specifically the influence of temperature on metabolic rate. It tests the validity of certain statements about ectotherms and endotherms. These statements are reproduced below, with modification, from the Instruction Manual supplied by the equipment manufacturer, Qubit Systems Inc. (with permission).

In this model investigation the experimental animal is a lizard (anole, scientific name *Anoles carolinensis*).

Metabolic Rate

Metabolic rate refers to energy metabolism per unit time. The metabolic rate of an animal is affected by the ambient temperature, the time of day and year, its diet, age, level of activity, and many other factors.

Metabolic rate can be calculated from measurements of an animal’s oxygen consumption under a given set of conditions. This is called indirect calorimetry. It is valid to determine an animal’s basic metabolic rate by indirect calorimetry if:

- The animal’s diet is known.
- There is no anaerobic metabolism and ATP and creatine phosphate stores are maintained.
- The change in body oxygen stores over the measurement period is minimal.
- The animal is at rest and under no thermal stress.

In mammals and birds, the stable, fasting, minimum rate of metabolism is called the basal metabolic rate (BMR). The metabolic rate of animals other than mammals and birds is largely dependent on the environment, so there is no metabolic rate that can be called “basal” for these animals. Instead, the minimum metabolism of fasting animals at a given external temperature is called the standard metabolic rate (SMR) for that temperature.

The amount of heat produced for each liter of oxygen consumed in metabolism is relatively constant, whether carbohydrate, fat or protein is being oxidized. The values are 4.5 KCal/L O₂, 4.7 KCal/L O₂, and 5 KCal/L O₂ for protein, fat, and carbohydrate, respectively. The average value of 4.8 KCal/L O₂ is generally used in calculating metabolic rate; however, it is important that the composition of the animal’s diet be taken into consideration. For instance, if an animal had been fed an all-protein or all-carbohydrate diet and the average value was used to calculate metabolic rate, this could result in an error of up to 10%.

Given equivalent conditions of diet and activity, if metabolic rate is considered on a “per unit mass” basis, smaller animals always have higher metabolic rates than larger animals. In general, for each doubling in body weight, standard metabolic rate increases by about 75%. For a wide variety of animals, it has been found that metabolic rate is proportional to (body weight)^{0.75}. This is known

as the “Brody-Kleiber” relationship. In mammals and birds, this relationship may be partly due to smaller animals having larger surface areas relative to their body weights. Since the rate of heat loss is proportional to surface area, small mammals and birds must produce a greater amount of heat per unit of body weight in order to maintain a constant, warm body temperature. For other types of animals, the association between metabolic rate and body weight may be due to the relationship between the rate of oxygen consumption per unit body mass (specific oxygen consumption) and body weight. For diverse types of animals, it has been shown that the log of the specific oxygen consumption is inversely proportional to the log of body mass.

[**Note:** The electronic equipment (probe) available in this investigation is not as sensitive for measuring oxygen concentration as it is for measuring carbon dioxide concentration. Therefore you will use changes in carbon dioxide concentration to determine respiration rate. Carbon dioxide output from an animal is related to oxygen uptake by the Respiratory Quotient. See next paragraph.]

Respiratory Quotient

The respiratory exchange ratio (RER) is the number of CO₂ molecules produced relative to the number of O₂ molecules consumed by intermediary metabolism. In steady state at rest, this is equivalent to the respiratory quotient (RQ).

$$\text{RQ} = \frac{\text{CO}_2 \text{ produced}}{\text{O}_2 \text{ consumed}} = \frac{(\% \text{CO}_2 \text{ expired} - \% \text{CO}_2 \text{ inspired})}{(\% \text{O}_2 \text{ inspired} - \% \text{O}_2 \text{ expired})}$$

The RQ value is directly related to the animal’s diet. In metabolizing carbohydrate alone, one CO₂ molecule is produced for every O₂ molecule consumed, for an RQ of 1.0. In metabolizing fat alone, 0.7 of a CO₂ molecule is produced for every O₂ molecule consumed, for an RQ of 0.7. In metabolizing protein alone, approximately 0.81 of a CO₂ molecule is produced for every O₂ molecule consumed, for an RQ of 0.81. At rest after a period of fasting the RQ will be closer to 0.7 because the animal will be metabolizing stored fat. During exercise, the RER will increase with the level of exercise intensity. This is due to a progressive shift from fat to glucose metabolism.

Temperature and Metabolic Rate

The body temperature of ectotherms (such as a frog and other amphibians, and a cricket and other insects) is largely determined by the temperature of their surroundings. Ectotherms rely almost entirely on environmental sources of heat. The metabolic rate of an ectotherm is therefore linked closely to the external temperature. This effect of temperature on metabolic rate can be attributed to changes in enzyme activity. The reactions of both anaerobic and aerobic cellular respiration are catalyzed (facilitated) by enzymes. Enzyme-catalyzed reactions are extremely sensitive to small changes in temperature.

Ectotherms control their body temperatures primarily through behavioral adaptations. They actively select the most appropriate environment. When breathing air with a normal concentration of O₂, some ectotherms will select temperatures of 37° C or higher in a thermal gradient. Exposure to hypoxia causes the animals to select temperatures below 30° C. The lower metabolic rate which results from the selection of the lower ambient temperature helps the animals to survive in hypoxic conditions.

Some of the more highly evolved ectotherms use cardiovascular control, evaporative cooling, and endogenous heat production to help control their body temperatures. Of the ectotherms, reptiles such as snakes generally have the most sophisticated mechanisms for thermoregulation. Their

strategies for temperature regulation are quite similar to those used by endotherms, although they have no insulated body covering, and a much lower metabolic rate.

Endotherms, such as birds and mammals, regulate their own body temperatures. In most mammals, the normal physiological range for core body temperature is from 37° C - 38° C. For birds, core body temperature is closer to 40° C. Endotherms regulate temperature through a high rate of endogenous heat production and by controlling the rate of heat exchange. Because of the endogenous heat production, an endotherm usually has a metabolic rate at least five times that of an ectotherm of equal size and body temperature.

An endotherm can maintain a constant body temperature within a particular range of external temperatures called the thermal neutral zone. In this zone, external temperature has very little effect on an endotherm's metabolic rate, because it is able to compensate for changes in ambient temperatures by varying its thermal conductance. It can do this by changing the supply of blood to superficial areas, increasing or decreasing the degree of insulation afforded by fur or feathers, or making changes in body orientation. The temperatures at either end of the thermal neutral zone are called critical temperatures. Temperatures hotter and colder than the critical temperatures cause an increase in metabolic rate because of the amount of energy required to operate the endotherm's more complicated temperature-regulation mechanisms such as shivering and sweating.

Below the lower critical temperature, the animal can no longer decrease its thermal conductance enough to compensate adequately for the rate of heat loss. It must therefore increase its rate of heat production in order to prevent a decrease in core temperature. At the upper critical temperature, which is generally slightly below the operational body temperature, thermal conductance is maximal. When the ambient temperature exceeds body temperature, an animal can no longer lose heat to the environment through conduction, convection, and radiation. It has to lose heat through water evaporation or sweating. An increase in metabolic rate is required to mobilize the water used in evaporative cooling.

Endotherms are well adapted to coping with changes in external temperature. Unlike ectotherms, endotherms tend to be both heavily and variably insulated. They typically have layers of adipose tissue and skin covered with hair or feathers. The fluffing or compression of the fur or feathers allows an endotherm to alter its thermal conductance. Some animals add significant bulk to their adipose tissue during the fall. This serves as both additional insulation and additional fuel. Endotherms also compensate for heat loss through increased heat production and increased muscular activity either by locomotion or shivering. Some mammals use nonshivering thermogenesis as a means of heat production. Generally, deposits of brown fat are used as fuel. Some endotherms are also capable of hibernating in harsh weather conditions. For example, when ground squirrels enter hypothermia-induced hibernation, the resulting hypometabolism decreases their energy requirements by 88%.

Endotherms' complex temperature regulation mechanisms are dependent on accurate feed back from peripheral heat sensors. Impulses from surface temperature receptors are integrated with information about internal body temperature in the hypothalamus. Regulation of body temperature through sweating or shivering is initiated by the action of peripheral chemoreceptors.

Objectives

1. Learn about the physiology of ectotherms and endotherms.
2. Continue developing your understanding of the scientific process.
3. Learn to use and care for research-grade electronic equipment for data collection and analysis.

4. Enhance computer skills.

Scientific Question

Is there a change in the metabolic rate of a lizard over the temperature range of 10°C-30°C?

Methods**A. Setting Up the Animal Respiration Apparatus**

1. Consult Figures 1 and 2 below for the apparatus setup. Connect components with plastic tubing in the order given in Table 1 below (the Luer connectors are mated in a particular way to prevent incorrect couplings). The slide on the IRGA should be set to 500 ppm.
2. Turn on the air pump. With the animal chamber in the system and closed, check for air leaks by dipping into water the end of one plastic tube at a connection downstream of the chamber. If you have a precision air flow meter available, determine the actual air flow rate rather than accepting the given rate of 200 ml/min. Determine the flow rate just downstream from the flow restrictor (before the animal chamber). Reconnect tubing and leave the pump running.
3. Connect the leads from the electronic sensors to the ULI in the following order:
 - Temperature probe to DIN 1
 - IRGA to DIN 2
 - Oxygen sensor to DIN 3
 The ULI lead is attached to a serial port of the computer.
4. Turn on the ULI and computer. If the ULI is not turned on, the computer will not detect attachment to a serial port when you execute the next step.

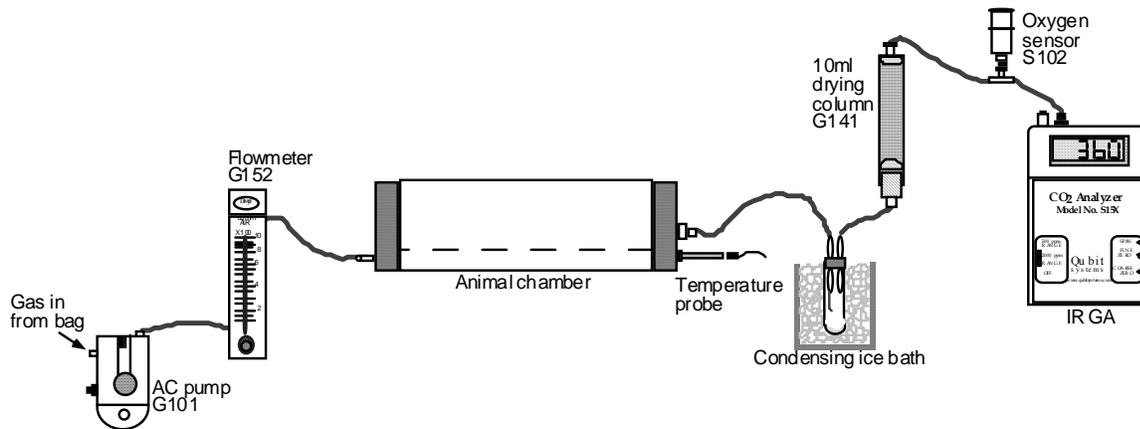


Figure 1. Diagram of respiration equipment from Qubit Systems manual. A calibrated gas flow restrictor is used in place of the flowmeter shown. Also the “Gas in from bag” in the diagram is replaced by a soda lime tube (carbon dioxide absorber) and air filter.



Figure 2. Photograph of setup for animal respiration study showing components in the order of gas flow.
[Not shown: soda lime tube, air filter]

A - Air pump

B - Air flow restrictor

C - Animal cuvette. Cuvettes are sized to the animal. Large cuvette for mouse shown.

D - Condenser tube in ice bath to remove some of the water vapor in gas stream.

E - Drying column (magnesium perchlorate) to remove all water vapor in gas stream.

(Note that if magnesium perchlorate absorbs too much water vapor it will liquefy and may cause serious damage to the analyzers. For this reason, you should always check the perchlorate before using the apparatus. You should replace it if it forms a solid plug or begins to liquify.)

F - Oxygen sensor (output not used in the model investigation)

G - Carbon dioxide sensor (IRGA = infrared gas analyzer)

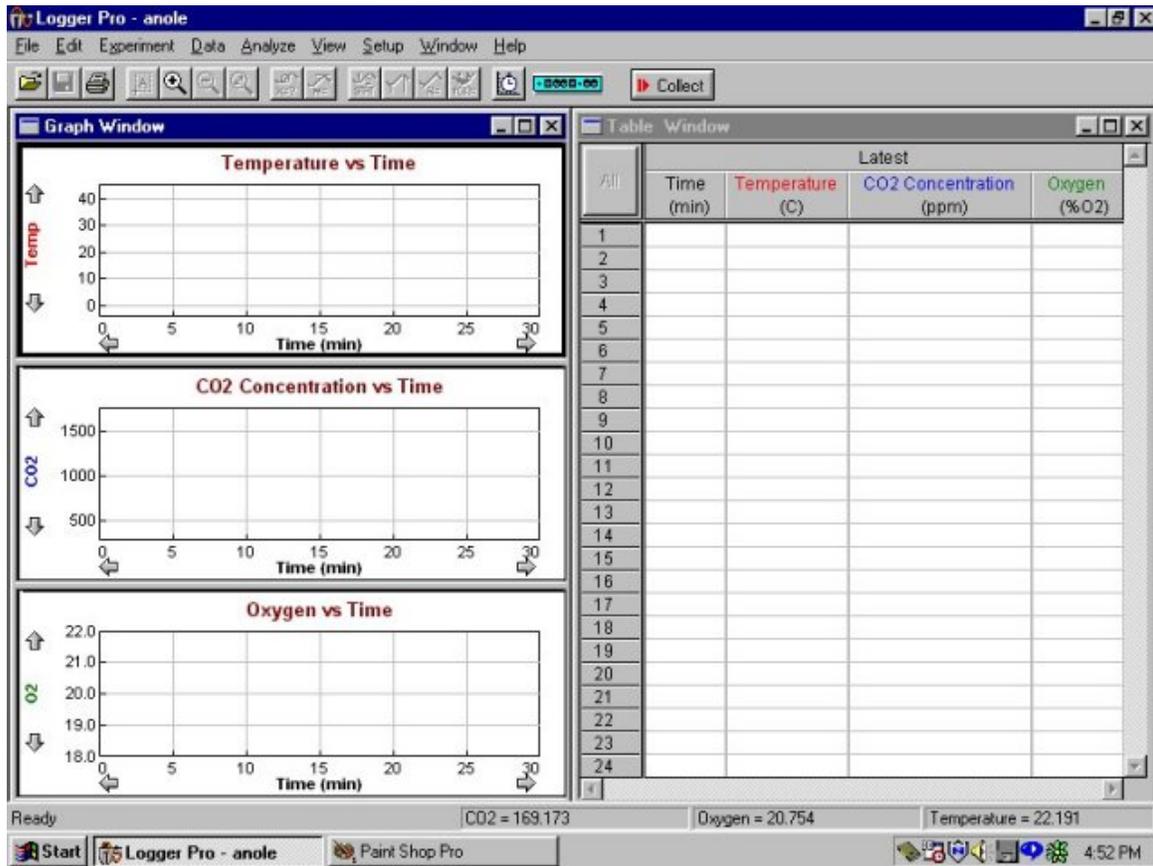
H - Universal laboratory interface (ULI) converting analog to digital signal.

Table 1. Arrangement of components in the gas stream for respiration rate determination.

1	Soda lime tube	Absorbs all carbon dioxide in the incurrent air so its initial concentration is not a variable.
	↓	
2	Particle filter	Removes small soda lime particles so they do not harm the animal.
	↓	
3	Air pump	Maximum output is about 1400 ml per min.
	↓	
4	Flow restrictor	Reduces air flow rate to promote accumulation of carbon dioxide in animal cuvette to measurable level. Red (200 ml/min) for anole.
	↓	
5	Animal cuvette with temp probe	Chamber for the experimental animal. It is fitted with a temperature sensor which is already calibrated.
	↓	
6	Water vapor condenser (tube in ice bath)	Removes bulk of water vapor from gas stream.
	↓	
7	Magnesium perchlorate column	Removes last traces of water vapor from gas stream. The IRGA requires dry air.
	↓	
8	Oxygen sensor	Measures oxygen concentration in percent. It is already calibrated. Data not used in the model investigation.
	↓	
9	Carbon dioxide sensor (IRGA)	Measures carbon dioxide in ppm (parts per million). Normal air has about 350 ppm. It is pre-calibrated but the calibration drifts with time and needs to be adjusted periodically by laboratory personnel. It must be turned on several hours before lab starts.

B. Using the Animal Respiration Apparatus

1. Click on the “Anole.MBL” icon on the computer desktop. The computer screen should show three graph windows as follows, from top to bottom: Temperature vs. Time, CO₂ Concentration vs. Time, and O₂ vs. Time (Fig. 3). At the bottom of the screen there are three little windows showing probe outputs in real time. When collecting data, the upper graph window displays the temperature readings in degrees Celsius, the middle graph window displays the carbon dioxide concentration in ppm, and the lower window displays oxygen concentration in percent. The table window displays numerical values from all three probes and is useful when analyzing data or saving it to a spreadsheet program in your computer. The data collection time should be preset to 90 or 120 minutes (30 minutes is shown in Figure 3).

Figure 3. Sample screen from computer running *Logger Pro* for respiration data collection.

2. Take time now to become familiar with basic functions of *Logger Pro*. There are two that you will use most often; others such as changing the range on the X- and Y-axes you can learn from the instruction manuals provided by the software manufacturer or your Instructor.
 - Analyze data. Click on "Analyze" and then "Examine." Note that when you place your cursor in the graph field, a vertical black line appears and there are values in little boxes, one in each graph. The values give probe readings for the times selected on the X-axis.
 - Stop and restart data collection without losing data from the previous run(s). Click on "Data" and then "Store Latest Run." When you restart data collection the previous lines are dimmed and a new, heavier line begins to form.

3. Before proceeding you need to be aware that you are using quite sophisticated equipment and it is not immune from the occasional glitch. We can list some things to watch out for, but the list will never be complete; something new is always popping up.
 - The air pump is worn out and pumping little or no air.
 - The animal chamber is not sealed so airflow does not get to the IRGA.
 - The ULI is not turned on.
 - The IRGA is not turned on or not well warmed up, or the range switch is set to 2000 ppm instead of 500 ppm.
 - Cables to the ULI are not connected properly.

- The soda lime or magnesium perchlorate absorber tube is exhausted.
 - The computer is connected to an AC outlet loosely so someone could disconnect it unintentionally.
4. You are now ready to collect data. To do so, click on the “Collect” button. Click on this button again (its name will have changed to “Stop”) to stop data collection. Plan to stop data collection periodically and save it on your computer as insurance against computer failure and for later analysis.

IMPORTANT: If you attempt to exit the application without storing data in a file with a new name, you may get a dialog box asking if you want to save the setting. Always say no to prevent overwriting the calibration file.

5. Collect data for about 5 minutes. The readings will give baseline values for the three parameters. Most important is the reading for carbon dioxide concentration. Its value should be within approximately 20 ppm of zero. [Note that negative values are preceded by a colon in the IRGA meter.] If it is outside this range, suggest to your laboratory instructor that the IRGA needs adjustment. Continue collecting data during the next step.
6. Weigh a lizard. Handle gently but firmly so it does not lose body parts (tail) or get away. It won't bite!!
7. Place the animal in the chamber. Use the heat lamp (or hair drier) to raise the recorded temperature to 30°C over a period of 15 to 30 minutes. Note and interpret the animal's behavior. Hold the temperature at 30°C until an approximately stable carbon dioxide concentration reading is obtained. This should take no more than 15 minutes. If the animal appears to be agitated by movement in its surroundings, cover the chamber loosely with the cloth provided.
8. Pack cracked ice, held in zip-lock bags, around the animal chamber such that the temperature gradually falls continuously to 10°C over a minimum of 60 minutes. Alternatively drop the temperature more quickly but in stages, *e.g.*, to 25, 20, 15, and 10 °C, and hold at each stage for 5 to 10 minutes. Consult the laboratory instructor if you think the animal is being harmed at the lower temperatures.
9. Raise the chamber temperature to ambient by removing the ice packs and heating gently with the lamp or hair drier. Return the animal to its container.

C. Saving Data

There are three main ways data may be saved. Your choice will be influenced by the amount of time you have left in the laboratory period and the software available to you.

1. Save the Logger Pro file (MBL file name extension) on a floppy or Zip disk and take it with you, or transfer the file to a laboratory server and download it to your computer via the campus network. You must be able to run the Logger Pro software on your computer in order to open the MBL file and work with the data. A read-only version of *Logger Pro* is available from the vendor, Vernier Software and Technology (<http://www.vernier.com>).
2. Analyze the data right away and transfer values for the variables to your notebook, or to an electronic spreadsheet.

- Highlight the Table Window (right side of computer screen). Click on the button “All” at the upper left corner and then click “Edit” and “Copy” on the task bar. Open a spreadsheet program and “Save” or “Paste” the data into the spreadsheet.

D. Analyzing and Presenting Data

Your Instructor will help you with the first steps, (a) deciding how many data points to extract from the data stream, and (b) doing the extraction (*i.e.*, averages over time or values at specific times). Below are instructions on the second step, how to convert the raw data (ppm carbon dioxide) to values that allow valid comparisons between animals.

For this model investigation, the oxygen sensor output is not being used and therefore the RQ (respiratory quotient) is not being determined. Your Instructor can help you use the oxygen sensor data if it is needed for your investigation.

1. Respiratory rate calculation for carbon dioxide production.

The goal is to calculate the rate of respiration based on carbon dioxide production at each selected temperature. The rate of respiration, R_{CO_2} , is calculated using values for the terms in the following equation:

$$R_{CO_2} = CO_2 \text{ output rate } (\mu\text{L}/\text{min}) = \text{air flow rate (mL}/\text{min}) \times \Delta CO_2 (\mu\text{L}/\text{mL})$$

where

- CO_2 output rate = μL (microliters) CO_2 produced by the animal per minute
- air flow rate = mL (milliliters) per min passing through the animal chamber
- ΔCO_2 = difference in CO_2 concentration between incoming and outgoing air expressed as microliters CO_2 per milliliter air

Here is an example of a calculation. Refer to the 9 rows in Table 2 for the sample values used in the calculation.

- First, determine the change in CO_2 concentration (Δ ppm CO_2) from the air flowing into the animal chamber (before the animal has affected its carbon dioxide concentration) to the outflow from the chamber.

$$\Delta \text{ ppm } CO_2 = \text{outflow ppm } CO_2 - \text{inflow ppm } CO_2$$

where

- the value for inflow is from row 4 of Table 2.
- the value for outflow is from row 5.
- the result is in row 6.

Convert ppm CO_2 to μL (per mL air volume) using the factor:

$$1 \text{ ppm} = 0.001 \mu\text{L},$$

thus $\mu\text{L } CO_2 = \text{ppm } CO_2 \times 0.001$ as shown in row 7.

Use these μL values and the air flow rate (row 1) to calculate R_{CO_2} as given in row 8.

To make it possible to compare rates between animals of different mass, adjust the result to one unit (gram) of animal mass by dividing by the animal's mass in grams (row 2) as given in row 9.

Table 2. Sample raw data and results of calculations. Raw data are indicated as [RD].

1	Air Flow Rate (ml/min) [RD]	200
2	Animal Mass (gm) [RD]	50
3	Temperature ($^{\circ}\text{C}$) [RD]	22
4	CO_2 In (ppm) [RD]	8
5	CO_2 Out (ppm) [RD]	480
6	ΔCO_2 (ppm)	472
7	ΔCO_2 ($\mu\text{L}/\text{mL}$)	0.472
8	R_{CO_2} ($\square\text{L}/\text{min}$) (equation 1)	94.4
9	Specific CO_2 Rate ($\mu\text{l}/\text{min} \times \text{gm}$)	1.89

2. Thermal Quotient (Q_{10})

The Q_{10} may be calculated for an exotherm. Select respiration rate values at two temperatures differing by 10°C . The equation is:

$$Q_{10} = \text{respiration rate at } T+10 \div \text{respiration rate at } T$$

3. Calculate the Metabolic Rate in KCal and KJoules

The average amount of heat produced for each liter of oxygen consumed in metabolism is about 4.8 KCal or 20.1 KJoules. You can therefore obtain the metabolic rate in Cal per gram per minute by multiplying its oxygen uptake in $\square\text{L}$ per gram per minute by 0.0048. To obtain metabolic rate in Joules per gram per minute, multiply its oxygen uptake by 0.0201 instead.

Data presentation is best done with a bar chart or preferably X-Y scatter graph plotting respiration rate as a function of external temperature.

Scientific Questions for Projects

1. Do dark-furred and white-furred mice respond differently to heat and cold?
2. Do baby and full-grown mice respond differently to heat and cold?
3. Do baby and full-grown mice have different basal metabolic rates?
4. Do anoles or insects have circadian rhythms in respiration rate?
5. Does the RQ vary with temperature?
6. Does the metabolic rate at different external temperature have the same trend for lizards compared to baby chickens?
7. Does the metabolic rate at different external temperature have the same trend for mice compared to baby chickens?
8. Do different species have different RQ's?

Literature Cited

Student Instruction Manual, Respiration Package. 1999. Qubit Systems, Inc. Kingston, Ontario. 97 pages.

Acknowledgements

This investigation is based on work published by Qubit Systems, Inc. Permission to quote from the Introduction to the Student Manual is gratefully acknowledged. Funding was provided by the Howard Hughes Medical Institute and the National Science Foundation, along with the Center for Teaching Effectiveness and the Department of Biological Sciences at the University of Delaware. Assistance from Dr. Stephen Hunt, Qubit Systems, Inc. and representatives of Vernier Software and Technology are gratefully acknowledged.

Appendix A. Annotated Materials List

1. Computer with *Logger Pro* software from Vernier Software and Technology and a spread sheet program (Microsoft Excel suggested). There are *Logger Pro* versions for the PC and Mac, and it ships with Qubit Systems Inc. equipment or may be obtained separately. The Vernier website offers a free reader version enabling students to open and work with *Logger Pro* files away from the teaching laboratory.
URL=<http://www.vernier.com>
2. Qubit Systems Inc. Low Range Respiration Package (URL=<http://www.qubitsystems.com>) which includes:
 - a. Universal lab interface (ULI)
 - b. ULI cable
 - c. ULI power supply (AC adapter)
 - d. S151 low range carbon dioxide analyzer, 0-500 and 0-2000 ppm ranges (IRGA, infrared gas analyzer) Should be running at least 1.5 hours before lab starts. Other models cover higher carbon dioxide concentrations.
 - e. Flow restrictor, red, 200 ml/min. Restrictors for 100 and 500 ml/min also available.
 - f. Medium size animal chamber (Fig. 4) with temperature probe. Smaller and larger chambers (Fig. 5) are available.
 - g. Carbon dioxide scrubbing tube (soda lime in a 60-mL syringe barrel). Used to calibrate IRGA at the zero carbon dioxide concentration, and to remove all carbon dioxide from air supplied to animal if desired.
 - h. Drying column (magnesium perchlorate in a 10-mL syringe barrel)
 - i. Oxygen sensor
 - j. AC gas pump
 - k. Equipment stand

Also available by special request is a particulate filter to remove soda lime particles from air supplied to animal.

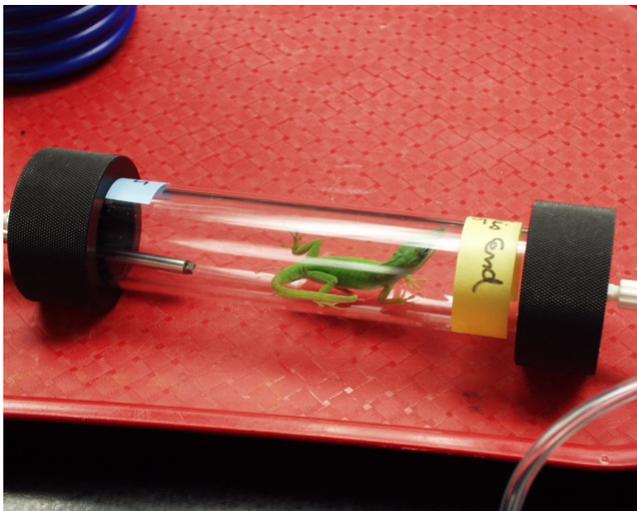


Figure 4. Medium size animal cuvette. Suitable for cockroach and lizard.



Figure 5. Large size animal cuvette. Suitable for mouse and similar size animals. The enclosed air is stirred by an electric fan.

3. Plastic insulated container, one quart. Available at grocery, drug, or house wares store.
4. Water vapor trap. This is fashioned from a large glass test tube or plastic graduated cylinder with a 2-hole rubber stopper and glass tubing.
5. Laboratory balance. A top loading balance with accuracy to 0.01 gram is suggested.
6. Animal weighing container. For larger animals we use plastic beakers, and for smaller animals we use the transport chamber (*e.g.*, Fig. 6).
7. Anole (lizard). They are purchased from a local pet shop or Carolina Biological Supply Company; institutions in warmer climates may be able to collect them from a local habitat. They are kept in a teaching laboratory in a large glass aquarium tank with moisture, foliage and other hiding places and fed live crickets also available from the pet store. Our university requires a permit that is in accordance with NIH animal use guidelines. Individual animals are provided to students in a special container (Fig. 6).



Figure 6. Lizard (and other animal) transport chamber

8. Other animals we have tried are the following:
 - Madagascar hissing cockroaches. They were first purchased from Carolina Biological Supply and then reared in a modified glass aquarium tank (Fig. 7) with dog chow for food. They also eat fresh apples and carrots and other fresh produce. They are provided to students in a smaller chamber (Fig. 8) manufactured by Penn-Plax, Inc. (<http://www.pennplax.com>). Not only are cockroaches nocturnal but they can close the spiracle openings to their gas exchange tracheal system to conserve water and therefore at times show little or no external respiration. Students may be able to detect periodic breathing.



Figure 7. Cockroach rearing cage.



Figure 8. Cockroach transport chamber.

- Mice. They are purchased from Harlan (<http://www.harlan.com/>) or may be available from research laboratories in the university. They are kept in holding cages in a special animal care facility and provided to students in the same container. Our university requires a protocol permit that is in

accordance with NIH animal use guidelines. Their respiration rate can be highly variable as they periodically explore the chamber and then sit grooming themselves. Usually they show a nice increase in respiration rate at the colder temperatures. We have not reliably seen an increase at elevated temperature.

- Baby chicken. We have tried animals 1-2 days old and 14-21 days old. The older animals have given an obvious increase in respiration rate at lower ambient temperatures (see Appendix B). Younger animals in one trial did not exhibit a change in respiration rate over the 30°C-10°C range.

Alone this is not very interesting to students, but it is interesting when compared to the lizard's response. They are fed a recommended diet. During the first week they must be kept at about 38°C, and this would be a good starting temperature for the investigation.

- Frog. They can be obtained from Carolina Biological Supply Company and fed mealworms or fly larvae.
9. Crushed ice (if doing temperature change variable)
 10. Zip-Lock bags
 11. Cloth, preferably black (for covering animal chamber)
 12. Heat lamp. A hair dryer may be substituted. Students need to be cautioned that heating can cause the temperature of surfaces that the animal will contact to be much greater than the air temperature which is the parameter being recorded.

Appendix B. Sample Data

Figure 12. Sample *Logger Pro* graph window for baby chicken respiration. Initial temperature of approximately 30C was slowly decreased to 10C. Experiment duration was 90 min. Compared to a mouse (data not shown) there was much less variation in CO₂ output and a thermal neutral zone was not apparent.

