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Chapter 18

Bioenergetics: Energy Flow, Secondary Production, and Ecological Efficiencies of Madagascan Cockroaches

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Contents

Introduction

This laboratory exercise is a small research project that demonstrates ecological principles and is appropriate for a second-semester biology major's course or above. It serves as the ecology portion of the lab, taking about two and one half 3-hour periods. The concepts of energy flow in ecosystems and the role of secondary producers are tested and reinforced. This exercise can be done in the laboratory. Field exercises for ecology are important but they sometimes present problems at certain seasons of the year and for night classes. Students are assigned responsibility for certain portions of the data collection, data analysis, and conclusions. The class is divided into groups responsible for measuring the rate of growth, ingestion, fecal production, and respiration for one of three size categories of roaches over a four-week period. It takes about 1.5 hours the first and last week and about 30 minutes for the intervening weeks. During one of the intervening weeks the three-hour lab also includes respiration measurements by each group. At the end of the data collection, each group shares their data with the others. They now have enough data to produce a collaborative research paper or seminar. I then assign students responsibility for a 5-minute portion of an oral seminar.

Data are relatively easy to collect and do not require much in the way of sophisticated equipment. However, careful manipulation of the data is necessary to make comparisons among size categories and to check the hypothesis that "energy input equals storage plus usage." This lab also serves as an example of trophic level efficiency. It is often stated that each level is only 10% efficient, but this is for an entire level in a functioning ecosystem, not one organism under near optimal conditions. This 10% efficiency can be compared to farm-raised animals and free-range production. It also points out physiological differences between young and mature organisms, and with literature data from insects vs. other organisms.

Student Outline

Introduction

Ecosystems are powered by the energy they capture from the sun. This energy drives photosynthesis, a process in which the atoms in relatively simple, energy-poor compounds are rearranged to produce more complex, energy-rich organic compounds. These energy-rich compounds make up the "stuff" of living organisms, and are passed through the ecosystem when one organism feeds off of another.

The first law of thermodynamics states that energy is not created nor destroyed. Thus we should expect that the amount of energy entering an organism is equal to that leaving. However, the second law of thermodynamics states that when energy is changed from one form to another some will be lost as heat. Thus, we should expect that as energy in the form of organic compounds is passed from one organism to another the transfer will not be perfect and less will be available to the eater than was taken into the eaten. In ecosystems there are photosynthesizing organisms called *producers* and those called *consumers,* which derive their energy by eating producers (and perhaps other consumers). The term *trophic level* is used to denote how many steps away from sunlight an organism is in the pathway of energy transfer. For example the first trophic level is composed of the producers, the second of those that eat the producers, and the third of those that get their energy be eating those in the second trophic

level. It has been estimated that on average only 10% of the energy entering one trophic level is available to the next.

We might look at a single consumer and ask what happens to the energy it ingests. In order for the organism to incorporate the organic compounds it eats into its body they must be absorbed. At this point they are available to be used as building materials to make additional tissues for growth, storage or reproduction. Some of the organic molecules must be burned (oxidized) by the organism so that the energy in the bonds can be released and transferred to ATP to power cell activities.

Based on these laws, we might expect that if we can measure the amount of energy taken into the organism and the amount released (including heat) that the two would be _____. Based on the average efficiency of transfer between trophic levels, we might predict that the amount stored in the tissues and available to the next trophic level would be _____% of that ingested. Conversely, the amount lost as heat should be _____ %. These are our hypotheses.

Methods

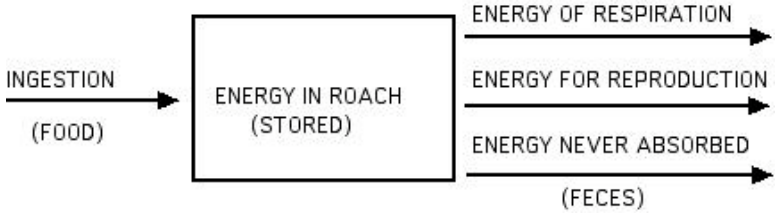
Our Experiment

The study organism will be the Madagascan hissing roach. These are rather large insects that feed by scavenging in warm, moist forests. These insects should be a good model. They grow rather rapidly, but the adults, at least, move fairly slowly. While they molt as they grow, they undergo incomplete metamorphosis so the stages look similar and eat the same food.

How will we measure energy? The energy stored in the food, roach, and feces is stored in the bonds of organic molecules. The average amount of energy in carbohydrates and proteins is 4 kcal/g dry weight, and in fats it is 9 kcal/g dry weight. We can determine how much energy is in the food and body of an organism by using a bomb calorimeter, which burns food completely and measures the energy released, or we can use average values from the literature. If we use literature values, all we need to do is measure the dry weight and convert it to energy. The amount of organic material oxidized by the roach and converted to heat can be determined by measuring respiration rate.

Step 1: Develop a system diagram to model energy flow

Before beginning to make measurements you should determine all of the predicted inputs and outputs of energy for a roach. Any others?



Step 2: Length and biomass measurements at the beginning

a. Measurement of roach weights

Since we can expect growth rates to be different in the young and the adults, we will need to divide them into size classes. Also, since they do not weigh much we will have to do composite samples.

1. You will be in charge of one size class. They have been divided into three groups based on length:
    a. 0 - 2.5 cm (20 roaches)
    b. 2.6 - 5 cm (10 roaches)
    c. >5 cm (5 roaches)

2. In order to monitor growth rate, we need to measure the lengths and produce a histogram (graph of the size distribution at the beginning and end). Transfer the roaches one at a time into the measuring chamber, record their length, and then place them all into the tared weighing container. If you have the larger roaches, note the sex. Males have bumps on the pronotum (head covering).
3. NOTE: THE SMALL ROACHES ARE SOFT SO USE A BRUSH AND SCOOP. THEY ARE ALSO VERY FAST. USE CARE NOT TO LET ANY LOOSE. THE ADULTS WILL HISS, AND THEIR FEET HAVE CLAWS THAT CAN CLING TIGHTLY. TO GET THEM TO LET GO OF YOUR HAND, HOLD YOUR FINGERS DOWN AND GENTLY POKE THEM FROM BEHIND.
4. Weigh the group of roaches to four decimal points and record.
5. Each week for four weeks you will weigh the roaches. At the last weighing measure the lengths again.

b. Weighing the food
   1. Take out two milk bones, weigh them, and put them in the food dish.

c. Setting up the cage
   1. Set up the water container as directed.
   2. Punch holes in the cage as shown in the demo to allow air to be exchanged. CAUTION THESE ROACHES CAN SQUEEZE THROUGH VERY SMALL OPENINGS.
   3. Place the container in a 28^{o}C incubator

Step 3: Weekly measurements

a. Weigh the roaches
   1. Transfer the roaches to a tared weighing container.
   2. Once they are weighed put them in a fresh cage.

b. Weigh the food
   1. Include food in dish and any sorted from feces
   2. Add more food, if needed, and reweigh before putting in clean cage.

c. Weigh the fecal and other material
   1. Separate food particle from fecal material. Brush the fecal material into a vial, dry, and weigh at the end of the experiment.
   2. Note and weigh any exoskeletons.
   3. If there are young, count, measure and weigh them.

d. Clean the water container and cage
   1. Rinse the rocks and scrub the tube (do not use soap). Refill. Place in the new cage.
   2. Clean the old cage for next week.

Step 4: Respiration measurement

The respirometer you will use was developed at the University of Minnesota and presented at an ABLE workshop there. It consists of two major components: the respiratory chamber and the manometer tube (Figure 1). A restraining cage inside the chamber supports the organisms above substances that absorb CO_2 and H_2O (two by-products of respiration).

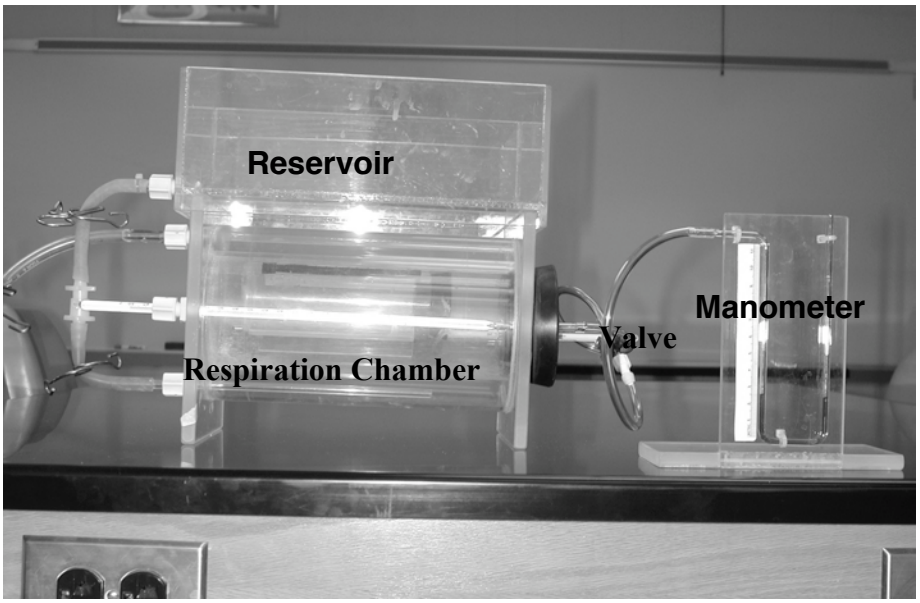


Figure 1. Respirometer

As oxygen is consumed, and the CO_2 and H_2O are absorbed, the gas pressure of the atmosphere inside the chamber is reduced.

If you can measure this decrease in gas pressure, you have actually measured the amount of oxygen consumed in respiration. To detect this change in pressure, a U-shaped tube (manometer) filled with fluid is attached to the respiratory chamber. When equal pressures are exerted on the two ends of the manometer, the fluid levels will be equal. If the pressure inside the chamber decreases, as occurs when oxygen is consumed by the organisms, or if the air cools, the fluid will move toward the chamber. An increase in pressure inside the chamber, as occurs when air inside the chamber is heated, will cause the fluid to move away from the chamber.

A valve between the chamber and manometer tube allows you to connect the manometer and the chamber to begin measurements, or to open them to the air to reestablish equal fluid levels without removing the end of the chamber. A water jacket around the respirometer is used to regulate the temperature inside the chamber. It is fed by a reservoir that sits atop the respiratory chamber.

NOTE: Each respirometer should have three thermometers; one for the water reservoir, another inserted permanently in the water jacket housing, and a third inserted in the chamber stopper. You should seal the chamber in such a manner that the chamber thermometer can be read simultaneously with the water jacket thermometer. Use care in inserting the stopper. You can easily break the attached thermometer.

a. Setting up the respiration chambers
   1. Load all of the roaches from your cage into the chamber and seal it.
      Caution: Whenever you stopper the chamber, the valve between the chamber and manometer **MUST BE OPEN** to the atmosphere! If you fail to open the valve, the manometer fluid will be blown out of the glass U-tube. To obtain an airtight seal, place the stopper in the end of the chamber with the thermometers lined up roughly adjacent to each other. Press firmly and uniformly on the stopper. There

is no need to exert great force. Once the stopper has been pressed firmly into place, give the stopper a light twist. This will have the effect of locking the stopper into place.

2. Allow the water jacket and internal chambers to reach 28^{o}C.

Respiration should be measured at 28^{o}C, because this is the temperature to which the roaches are adapted. In order to achieve a working temperature of 28^{o}C in the chamber, the reservoir should be filled with water at a temperature of 28^{o} plus or minus half the difference between the actual chamber temperature and 28^{o}. With the valve open to the air, run this water through the jacket until the chamber temperature nears 28^{o}C. Then replace it with water of 28^{o}C. The success of this experiment depends largely on how well you can manipulate and maintain an experimental temperature inside the chamber. By keeping the reservoir nearly full of water, you will insure that you have the maximum rate of flow into the water jacket. If you experience difficulty getting water to flow from the reservoir to the water jacket, simply squeeze the hose between the water jacket and reservoir to eliminate air pockets in the hose. To remove the reservoir, clamp the water jacket/reservoir hose above and below the plastic connector, and then separate the connecting junction.

3. Equilibrate for 10 minutes

After achieving the correct temperature in the chambers, close the valve to the air by connecting the chamber and manometer. Wait for 10 minutes before collecting data. If there is no movement of the liquid in the manometer in that time, you probably have a leak and need to open the valve, reseat the stopper, and check for leaks.

b. Measuring oxygen use

1. Collect measurements for at least 2 hours. If the manometer fluid nears the top, you will need to vent it in order to reset the level. This means after taking a reading, open the valve between the chamber and manometer to the air. The new level and time should be noted and the valve closed to the air. By adding the movement for each time after it is reset you will get the total.
2. Convert the distance the fluid moved in the tube into a volume of oxygen used. This can be done by mathematically determining the volume of the manometer tube or you can empirically measure it using a syringe.

Step 5: Converting measurements to energy units - kilocalories

The most accurate way to find the caloric value of an animal is to burn it in a calorimeter and determine the calories released per g dry weight. We will use an average value for insects of 5.4 kcal/g dry weight (Odum, 1971). The food caloric value can be obtained from the box. The caloric equivalent of 1 liter of O_2 respired is 4.9 kcal (Odum, 1971). The caloric value of fecal material is 4.764 kcal/gm from data obtained by bomb calorimetry (courtesy of Lenoir-Rhyne College). Wet weight can be converted to dry weight by multiplying it by the following percentages: Large roaches 41.48%, medium 31.31%, and small 28.84% or you can dry your roaches, weigh them and determine the dry wt to wet weight ratio. See equations below.

Food intake = weight of food x kcal/g

Growth = increase in weight of roaches x 5.4 kcal/g

Respiration = liters of O_2 used x 4.9kcal/l

Fecal output = weight of feces x 4.8 kcal/g

Step 6: Calculations

Now we will see if our model has been supported by our measurements.

a. Units
   1. In order to compare the data you gathered with that of others in the class and other literature values, you must be very aware of units.
   2. Your data were collected for a given number of roaches with a certain weight. You must convert this to 'per roach' units if, for instance, you want to compare how much a single small roach eats in comparison to a large one. However, in order to compare apples to apples, you must compare how much they eat on a per gram basis.
   3. In other words, you might expect a big roach to eat more than a small one, but on a per gram basis the small one would probably eat more because it is growing.
   4. You should also make sure your time units are comparable.

b. Energy balance
   1. Does the energy input equal the output for each age class?
      i.e. Does: Ingestion = Growth + Respiration + Feces
      (where Growth = increased weight + exoskeletons + reproductive products)
   2. Remember that only the absorbed energy enters the organism. This is the assimilated energy:
      Assimilation = Ingestion - Feces
   3. It follows then that:
      Assimilation = Growth + Respiration

c. Calculating efficiencies
   1. Production efficiency = weight gain / ingestion
   2. Assimilation efficiency = assimilation / ingestion

d. Putting it all together
   1. Use length and weight measurements to estimate how much each size class grew /week.
   2. Does energy in = energy out? For all size classes?
   3. Compare the energy data and efficiencies for a roach as it moves through the size classes. Does each size class have the same growth rate, respiration rate, and efficiencies of energy usage? Could you have predicted this?
   4. Compare the average ecosystem efficiency of energy transfer from one tropic level to the next, to the efficiency of energy conversion calculated for the roach.
   5. Compare the values to others for similar organisms.

Notes for the Instructor

As stated in the introduction, this lab is used as a major project for the ecology portion of a first-year, major's biology course. It takes 9-10 hours of lab time spread over 4 lab periods. The first week is the introduction and setup (about 1.5 hours). The next two weeks require that they weigh the roaches, feed and care for them (about 0.5 hours), and during one of these 3-hour labs the rest of the time involves measuring respiratory rate. The fourth week is for finalizing measurements and organizing data (about 1.5 hours). I divide the class into six groups of four students. Each group is responsible for one replicate of the three size categories of roaches, measuring the growth (in length at beginning and end, and weight each week) for four weeks. At the same time they determine the weight of milk bones eaten each week, collect feces to weigh later, maintain the cages, and collect any molts or young produced. We use two cages/group. They can weigh the roaches and transfer them to a new cage, then separate the food and feces for weighing, supply the new cage with food and water, and clean the old cage for use the following week. Respiration measurements are taken by each group. At the end of the data collection, each group shares their data with the others. I then assign each student a part of the study to present during a 5-minute portion of an oral seminar. For example: introduction; methods and materials; measured results of rates (including ingestion, fecal production, growth, and respiration for the different size categories); and discussion (including ecological efficiencies, energy balance, secondary production, and a literature comparisons). Alternate means of data handling and evaluation could be used.

Data are relatively easy to collect but care must be taken with weight measurements, sorting fecal materials, and keeping the chambers dry. Respiration rates need to be carefully made for as long as possible in conditions under which the roaches are living. Careful record keeping and manipulation of the data are necessary to make comparisons in similar units. It is necessary to make sure that the students understand that rates on a per individual basis give one type of information, while rates on a per gram basis (both wet and dry) give another. To compare rates of energy input, expenditure and storage, all units must be converted to units of energy (kcal). Rates have a time component and all rate values need to be in the same units as well. Appendix A gives some student data forms, which might be of help in organizing their data. However, too much help at first in the way of computer spreadsheets is not advised, because students will not understand the conversion process.

A roach colony can be maintained with relative ease (Appendix B). They are good experimental animals because they are large enough and slow enough to handle and measure, they live at a constant temperature of 28^{o}C, they have incomplete metamorphosis, and eat easily measurable food.

The respirometry lab is done using a volumetric respirometer with a water jacket to maintain temperature control that was designed at the University of Minnesota and presented at an ABLE mini workshop in 1988 by Don Anderson and Rick Peifer. Respirometer specifications are included in Appendix C, and they can be built by most college laboratory shops. Other means of measuring respiratory rates would also be acceptable.

Materials

- Roaches –Madagascar hissing (5 large/group, 10 medium/group, 20 small/group)
- Incubator set at 28^{o} C (about 3 X 3 X 2 feet) for housing roaches
- 2 scales that weigh to 4 decimal points
- Measuring chambers - Petri dishes and ruler (1/group)
- Brushes (1-2/group)
- Weigh boats
- Weighing container (2)
- Housing chambers – Glad disposable containers (1.89 liter storage containers 9.5cm high) with pin holes (2/group)
- Milkbones
- Food dishes – small weigh boats (2/group)
- Water containers - Appendix B (1/group)
- Respirometers - Appendix C (1/group)
- Thermometer (1/group)
- 500ml beakers (2/group)
- Marker for glass and plastic (1/group)
- 1ml methylene blue diluted for manometer or manometer fluid
- Bags for absorbants – tobacco bags from hardware store (2/respirometer)
- 200 gm anhydrous $Ba(OH)_2$) (25-30 gms/bag) **Caution caustic powder!!**
- 200 gm anhydrous $CaCl_2$ (25-30 gms/bag)

Acknowledgements

Thanks go to Mike Stone of Georgia Perimeter College, who is extremely adept at rearing the roaches. He contributed by designing the watering containers, and as general partner in the logistics of the lab equipment design. I would like to also acknowledge Don Anderson and Rick Peifer of the University of Minnesota for the design of the respirometers. This was presented at a Mini-workshop at the 1988 ABLE conference. Their handout is reproduced in Appendix C with only the venders omitted. Marsha Fanning of Lenoir-Rhyne College helped by measuring the caloric value of the fecal material. .

Literature Cited

Odum, E. P. 1971. Fundamentals of ecology. Third edition. Saunders. 574 pp.

Appendix A: Student Worksheets

WEIGHT GROWTH CHART FOR ROACHES

Group #/Name _____

Size category of Roaches _____

Number of Roaches _____

	Weight (grams wet weight)	Weight produced (gm)
Beginning Weight Roaches		
Weight after ___ week		
Weight after ___ week		
Weight after ___ week		
Weight after ___ week		
Total weight gain		
Average weight gain/week		

FOOD CONSUMPTION OF ROACHES

Group #/Name _____

Size category of Roaches _____

Number of Roaches _____

	Weight (grams wet weight)	Weight eaten (gm/week)
Beginning Weight Food		
Weight after ___ week		
Beginning Weight Food		
Weight after ___ week		
Beginning Weight Food		
Weight after ___ week		
Beginning Weight Food		
Weight after ___ week		
Total weight eaten		
Average weight eaten/week		

FECES PRODUCTION CHART OF ROACHES	
	Group #/Name
Size category of Roaches	
Number of Roaches	
	Weight (grams wet weight)
Beginning Weight of Feces	
Total weight produced	
Average weight/week	

OXYGEN USE CHART FOR ROACHES			
		Group #/Name	
Size Category of Roaches			
Number of Roaches			
	Millimeters	Displacement (mm)	Time in minutes
Beginning Manometer Reading			
Manometer Reading after ___ min			
Beginning Manometer Reading			
Manometer Reading after ___ min			
Beginning Manometer Reading			
Manometer Reading after ___ min			
Beginning Manometer Reading			
Manometer Reading after ___ min			
Total Manometer Displacement			
Total time in hours			
Average displacement/hr			
Oxygen Use in ml/hr			
Oxygen Use in ml/week			

Appendix B: Rearing Directions for Roaches

Colony setup and maintenance

Materials

- 10 or 20 gallon aquarium
- tight fitting lid with mesh (the lid for housing reptiles was modified by attaching a strip of adhesive foam to the inside of the lid to prevent the small roaches from escaping)
- watering containers (the one shown below for the student setup is a smaller version of the one for the colony which made from a whole fruit fly vial and the bottom of a standard-sized Petri dish)
- reptile bark for the bottom
- reptile heating pad
- .paper tubes for them to hide in
- dog or cat food
- fresh vegetable slices several times a week

Comments

We started with one pregnant female, and within a year we had plenty for this lab. We now have to euthanize them to keep the population under control.

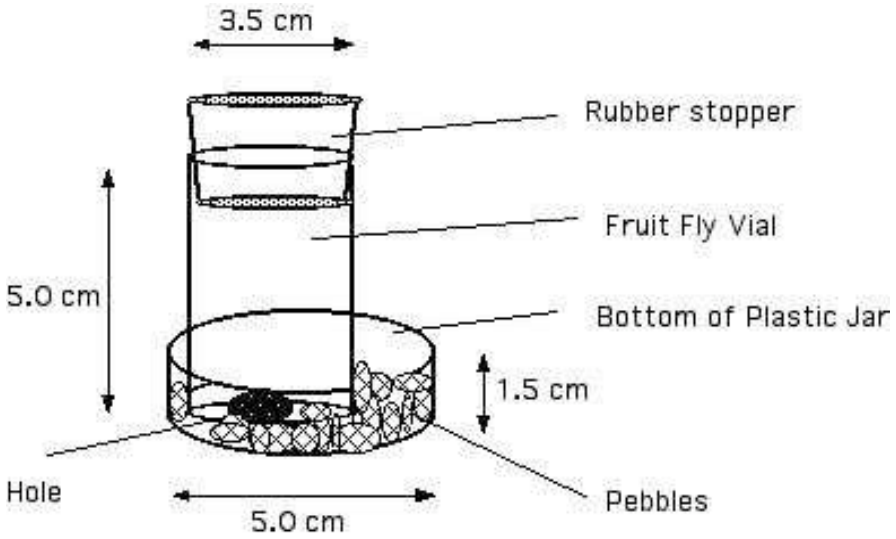


Figure B. Water dispensers made for student cages. Made from a cutoff fruit fly vial and a cutoff plastic jar glued with Weldon #3 acrylic cement or airplane glue. The hole needs to be large enough that water will run out slowly, but not too large or it will all run out.

Appendix C: Respirometer Specifications

(The following pages are scanned from the handouts of a Mini-workshop presented at the 1988 ABLE Conference by Don Anderson and Rick Peifer.) *

How to Construct an Affordable and Workable Plexiglass Respirometer

Don Anderson
Rick Peifer

Introduction

Accurately measuring the respiration rate of any homoiotherm over a range of ambient temperatures has always been a difficult task in a general biology laboratory setting. This has been partially due to the nature of the students, i.e. inexperienced at experimental procedures, although the principle reason, in our opinion, for widespread failure has been poor design of the respiratory equipment. If your experience is anything like ours, you probably at sometime used sealed fruit jars immersed in water baths. Leaking respirometers and exothermic reactions due to water mixing with the gas absorbent substances were usually standard outcomes. In addition, it always seemed to take the students ten minutes longer than the lab period to reach a specific experimental temperature.

In the wake of these frustrations, we designed a plexiglass respirometer that solved the problem of gas leaks, and the mess usually associated with open water baths. It is durable (we have used our equipment for over five years without replacement), and has few parts that are subject to breakage. The apparatus also allows the students to reach their experimental temperatures more quickly, and to more easily reach temperature equilibrium. By reducing the diameter of the manometer glass tube, the system can be also made more sensitive to pressure changes, and therefore be used to measure the respiration rates of poikilotherms, such as scorpions.

The respiration apparatus we use consists of 1. respiration chamber, 2. water reservoir, 3. restraining cage, and 4. manometer, Figure 1.

Materials

Respiration Chamber: Cost approximately $15.50, (Figure 4).

Qty	Description	Dimensions
1	Jacket Wall (Outer), clear cast tube.	5" O.D. X 8 1/2" long
1	Chamber Wall (Inner), clear cast tube.	3 1/2" O.D. X 9" long
1	Open End Plate, 3/8" clear plastic sheet.	6" X 6 1/2" with a 3 1/2" hol Figure 2.
1	Closed End Plate, 3/8" clear plastic sheet.	6" X 6 1/2" with four drilled and tapped holes. Figure 3.

Water Reservoir: Cost approximately $ 5.75, (Figure 5).

Qty	Description	Dimensions
1	Bottom, 3/16" clear plastic sheet.	6 3/16" X 9 1/2"
2	Sides, 3/16" clear plastic sheet.	3 3/4" X 9 1/2"
1	End Plate, 3/16" clear plastic sheet.	3 3/4" X 6 1/2"
1	End Plate with Drain, 3/16" clear plastic sheet.	3 3/4" X 6 1/2" with one drilled and tapped hole
1	Reinforcing Plate for drain hole, 3/16" clear plastic sheet.	1 1/2" X 1 1/2" with a drilled and tapped hole

Restraining Cage: Cost approximately $3.50, (Figure 6).

Qty	Description	Dimensions
1	Cage Tube, 2" O.D. clear plastic tube.	5 1/8" long with three 1/2" holes spaced equally apart along the top
1	Cage End Plate, 3/16" clear plastic sheet.	2" diameter circle with five 1/4" holes
2	Cage Legs, 3/16" clear plastic sheet.	7/8" X 5"

Manometer: Cost approximately $1.00, (Figure 7).

Qty	Description
1	14" X 4 mm O.D. glass tube, bent according to Figure 7, mounted in a wood frame (5/8" X 4" X 6 1/4") attached to a wood base (5/8" X 4" X 6 1/4").
1	Brodie Manometer Fluid, Carolina #68-2256
1	Plastic Ruler

Additional Parts: Cost approximately $25.00 per respirometer system.

Qty	Description
4 tsp	Absorbent: BaraLyme granules for CO_2 absorption (Barium Hydroxide Lime, U.S.P.), in a 2 1/2" X 3" cloth, drawstring bag.
4 tsp	Absorbent: Calcium Chloride 4 Mesh for H_2O absorption, in a bag as above.
3	Clamp: pinchcock (Day).
1	Clamp: screw, size B (Hoffman).

Additional Parts: continued.

5 Connector: Jaco #10-4-2-P male (port connectors for respirometer and reservoir).

5 Connector: Jaco #0-4-P ferrule nuts (companion to connectors listed above).

1 Connector: Nalgene T, 3/16" (vented respirometer to manometer connection).

1 Connector: Nalgene twist, 1/4" (reservoir disconnect).

1 Rod: glass 1/4" X 2 1/2" (plug for unused port).

1 Stopper: rubber #10.

1 Stopper: rubber #14, single hole.

2 Thermometer: -10 to 110°C, red alcohol.

1 Tube: 2 1/2" X 1/4" I.D., glass (chamber vent through stopper).

1 Tube: 2 1/2" X 1/4" I.D., plastic (respirometer exhaust port).

1 Tube: 10" X 1/4" I.D., plastic (respirometer inlet port tube).

2 Tubing: amber (surgical) 1/16" wall, 1/4" I.D. X 3 1/2" (reservoir to respirometer fill).

1 Tubing: amber (surgical) 3/64" wall, 1/8" I.D. X 30" cut to 14", 12", and 4" lengths (respirometer to manometer and vent lines).

1 Tubing: Tygon, 1/16" wall, 1/4" I.D. X 11" (respirometer exhaust).

Cutting Procedures

General

The first step in the construction of the respiration apparatus is that of cutting the plastic. While the sheet material can be cut on a home circular saw, the tubular material is best cut on a band saw. (If a band saw is not available, the tubing can be cut on a table saw, but one must support the tubing in a jig and rotate the plastic after each 1" to 2" cut to prevent cracking.) After cutting the plastic (both sheet and tube), finish the edges smooth and flat to provide an adequate cementing surface. A home disk or belt sander can be used.

Respirometer

The open end plate is a 3/8" thick clear plastic piece measuring 6" x 6 1/2". The 3 1/2" hole in the open end plate is located 3 1/2" down from the midpoint of the 6" wide top (see Figure 2). The hole is cut with a woodworking hole cutter mounted in a drillpress.

The closed end plate is also 3/8" thick plastic cut to 6" X 6 1/2". Four pilot holes (1/8") are drilled in this plate. They serve to guide the placement of

the 11/32" drilled holes, which are in turn threaded with a 1/8" pipe tap. Drill and tap these holes at the positions indicated in Figure 3. These holes will dictate the position of both tubes, hence the drilling must be done accurately.

Prepare the jacket tubing by cutting and finishing a 8 1/2" length of 5" diameter clear cast tube. Cut and finish a 9" length of the 3 1/2" diameter tubing to be used as the chamber wall.

Assembly

Respirometer

First the 9" length of narrow tubing is solvent cemented to the inside surface (opposite the tapped holes) of the closed end plate. The outside diameter of the tube must be positioned just inside the tapped holes when cemented. One may wish to construct a jig to aid in the positioning, since free hand cementing is difficult to control. Dunk the tube end in methylene chloride (1/8" deep) for 20-30 seconds three times. When the end is slightly soft, it is ready to be cemented. Press the piece firmly onto the end plate and weight it. The weight should remain in place for at least one hour. After the weight is removed, observe the joint to see that it is uniformly clear.

The next piece to be assembled is the 5" diameter tube. The 8 1/2" length is also cemented to the closed end plate. Soften one end of the tube by repeated dunking in methylene chloride. The 5" diameter tube is positioned with its inside diameter just outside the tapped holes. Weight the tube and allow the assembly to dry overnight. At this point the assembly is a 9" length of 3 1/2" diameter tube inside a 8 1/2" length of 5" diameter tube, both solvent cemented to the closed end plate with the tapped holes in the space between the two tubes.

The last piece can now be added. The open end plate is positioned so that the 1/2" and 1" vertical offsets (relative to the jacket wall) are opposite those of the closed end plate. This offset provides the pitch required to remove the air in the apparatus when it is filled with water. The cementing of the open end plate is handled differently than the closed end plate. In this case the solvent is brushed on with a small paint or acid brush. Brush on the solvent, allow it to set for 20 to 30 seconds, and brush it on again. Repeat this process 4 to 5 times or until the end becomes slightly soft. Wet the end one final time, orient the open end plate, and press the end plate onto the 5" tube. (The 3 1/2" tube will pass through the 3 1/2" hole in the end plate.) Pressure should be applied, through the use of weight as described above, for about one hour. In order to cement the inside tube to the plate it passes through, a thickened methylene chloride solution is used. Thicken the solvent with fine acrylic shavings, filings or dust. The cement should remain liquid enough to be used with a pasteur pipette. Apply the cement to the junction of the tube and end plate. Repeat solvent applications until the joint appears clear, and allow the apparatus to dry overnight. After the cementing is complete, the Jaco connectors can be threaded into the closed end plate. One should not apply great pressure when tightening the connectors as the threads are shallow and strip easily. Solvent cement (methylene chloride) may be brushed on the connector and respirometer threads to secure the seal. Finally install the 2 1/4 and 8" pieces of clear plastic tube, the alcohol thermometer and the glass plug This completes the construction of the respirometer.

Reservoir

The reservoir is a rectangular open box with its floor elevated 1/2" (Figure 5). The skirt effect of the end and side pieces serve to hold the reservoir on top of the respirometer. Use a corner jig to aid in construction of the reservoir. As with the tube material, the pieces cut from the sheet stock must follow the solvent dunking procedure mentioned above to insure well cemented joints. With the aid of the jig attach a side piece to an end piece. Next add the floor piece. (This is elevated by blocks which are part of the corner jig.) Turn the assembly 180° and cement the other side and end into place. At the bottom center of one end of the box cement a 1 1/2" square piece of 3/16" sheet material. Drill (11/32") and tap (1/8" pipe tap) it for a Jaco connector. Finally, insert a 2 1/2" length of plastic tube into the Jaco connector.

Restraining Cage

The body of the restraining cage is made of clear acrylic tube 2" in diameter and 5 1/8" long (Figure 6). Drill three 1/2" air holes in the top of the tube and cement a 2" circular end plate (1/8" sheet with 1/4" air holes) to one end. Legs for the cage are made of strips of 3/16" sheet (scrap) 7/8" X 5". Solvent cement the strips at approximately a 45° angle to the restraining cage. (A jig will help to control the angle until the cement is set.) A #10 rubber stopper is the final component of the restraining cage.

Manometer

Construct an L-shaped manometer frame with 2, 5/8" X 4" X 6 1/4" pieces of wood. Mount the U-shaped 4 mm O.D. glass manometer tube on a face of the frame (see Figure 7). Fasten a plastic ruler along the connection side of the manometer tube.

Final Connections

Prepare the #14 single hole rubber stopper by boring a second hole 1" from the center hole. Using glycerin, insert a 2 1/2" length of 1/4" diameter glass tube into the center hole until it is flush with the inner cork surface. Next, slide an alcohol thermometer into the bored hole, and orient it so that it can be read through the respirometer walls.

The manometer is connected to the chamber with surgical tubing (1/8" I.D., 3/64" wall), which is vented with a Nalgene T (3/16"). Connect 14", 12", and 4" lengths of tubing to the T. Further connect the 14" length to the glass tube inserted in the rubber stopper. Lastly connect the 12" length to the bent arm of the manometer.

Two 3 1/2" lengths of surgical tubing (1/16" wall, 1/4" I.D.) joined with a Nalgene twist connector attach the reservoir drain to the respirometer inlet. Clear Tygon tubing is attached to the respirometer exhaust port.

Modifications made to original design

Mike Stone and I have modified the design slightly by:

- replacing the rubber tubing between the chamber and manometer with polyethelene airline tubing (1/4 inch od, 3/16 id)
- replacing the T-tube and clamp with a 3-way stopcock (argyle HRI8888-173500) connected with luer-lock to hose barb adapter

This makes sealing the respirometer much easier.

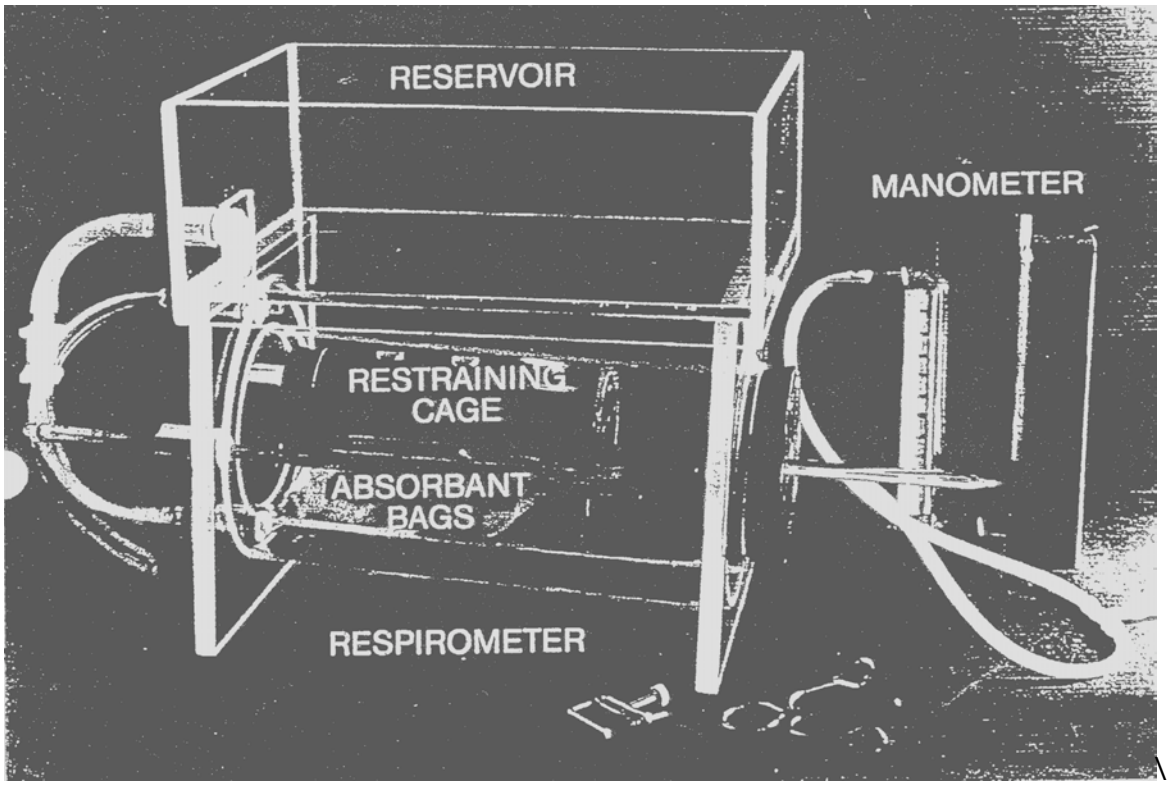
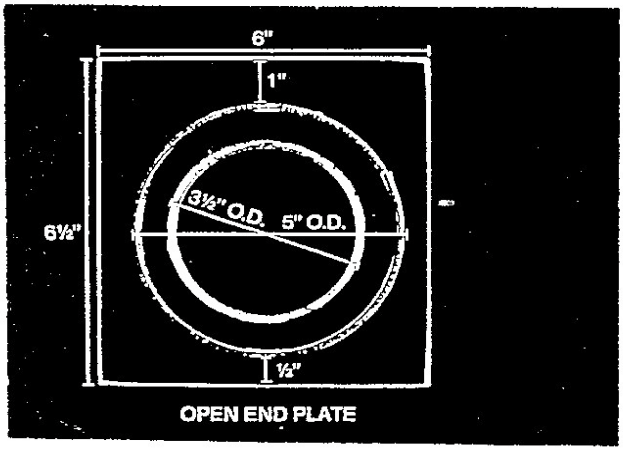


Figure 1. Respiration apparatus.



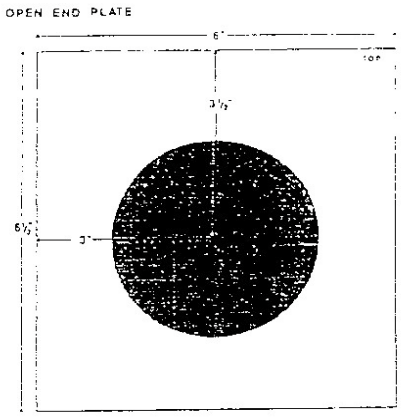
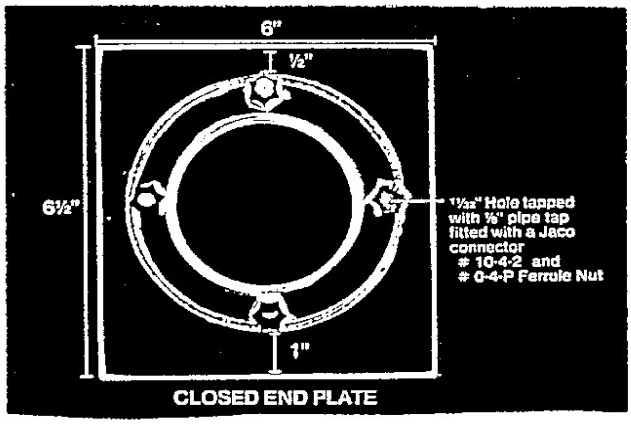


Figure 2, A and B: Open end plate.



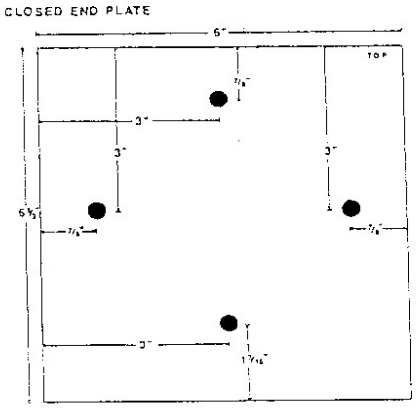


Figure 3, A and B: Closed end plate.

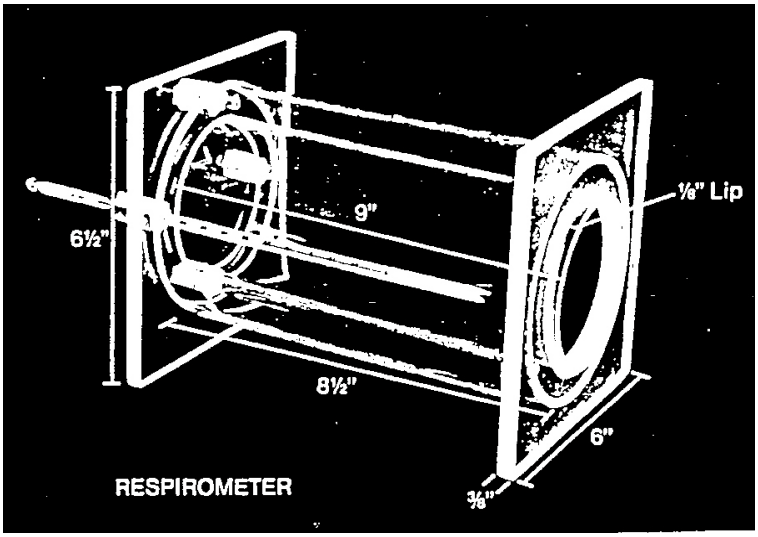


Figure 4. Respirometer

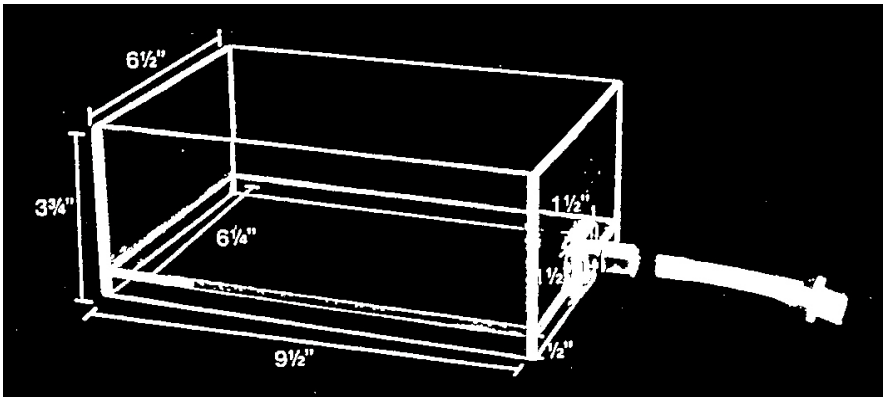


Figure 5. Reservoir.

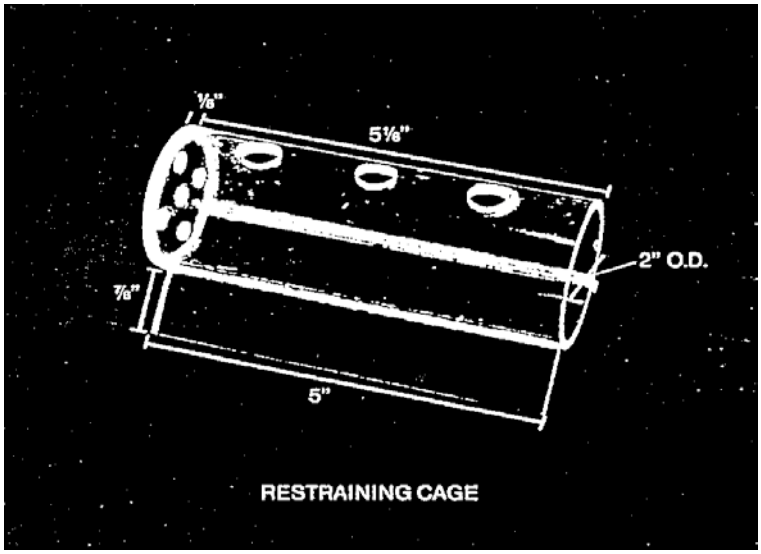


Figure 6. Restraining cage.

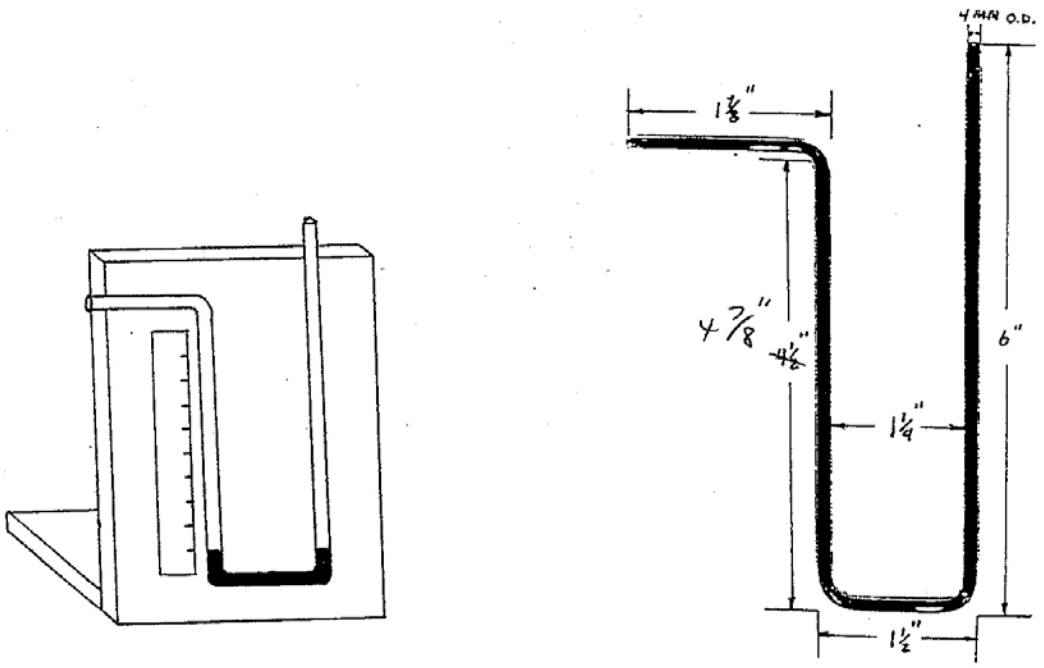


Figure 7. Manometer and manometer tube.