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Microrespirometers, Mealworms, and Mini-Lab Reports: Observing Respiration in Real-Time Using a Low-Tech Device

Kevin P. Miller

Young Harris College, 1 College St., Young Harris, GA 30582 USA
(**kpmiller@yhc.edu**)

Cellular respiration and other biochemical cellular processes can be difficult topics for students to understand. Having hands-on laboratory activities that cover difficult concepts can help to solidify learning those concepts. This activity revisits a previously presented topic involving the use of microrespirometers to measure the difference in respiration of the mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae) at different temperatures. This publication provides greater detail of the device, discussion about the laboratory exercise, and a suggestion on how to present findings from the exercise using a mini-lab report format.

Keywords: insect, beetle, mealworm, respiration, temperature, microrespirometer

Introduction

The topic of cellular respiration can be challenging to the average student in introductory biology. In an effort to facilitate learning about cellular respiration, a hands-on laboratory exercise was developed using a simple microrespirometer adapted from Lee (1995). This activity uses the respirometer in two different temperature environments to demonstrate the effects of temperature on respiration of the mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae). It is expected that the mealworms respiration rate will decrease with temperature as the beetle is ectothermic. The microrespirometer is composed of a 1-ml disposable syringe that has a capillary tube glued to the needle. There are two wads of cotton in the chamber of the syringe. One wad of cotton is absorbent and has 10 µl of 15% KOH; the other is non-absorbent and serves to protect the living organism from the corrosive KOH. The device is able to measure respiration because the carbon dioxide gas in the chamber is converted to a solid by the KOH. During cellular respiration oxygen is used and carbon dioxide is given off as a waste product. If the container with the test organism is closed, then as oxygen is utilized and carbon dioxide is converted from gas to solid, the pressure will decrease. The respirometer can easily be closed by adding a small drop of manometer fluid to the capillary tube attached to the hypodermic syringe. The manometer fluid is a solution of water, dish soap, and red food coloring. The drop in pressure will then be measurable by the movement of the manometer fluid in the capillary tube.

The test organism, a mealworm, is actually the larval form of a beetle. The mealworm is a grain pest and therefore easy to culture. It can be obtained from pet stores, bait shops, or biological supply companies. The beetle, like its other arthropod kin, is a well-studied ectotherm. As such its rate of cellular respiration should decrease with temperature. This change in respiration can be demonstrated by placing the larval beetles in microrespirometers in either a room temperature (ca. 20ºC) water bath or an ice bath (ca. 0ºC). These water baths work well because the water temperatures are unlikely to fluctuate and the microrespirometer is highly sensitive to changes in temperature.

The assessment for this laboratory activity includes a mini-lab report, see Simmons et. al. (2014). This uses the format of a typical lab report but greatly reduces the amount of material presented by the writer. This can help students write efficiently and can reduce the amount of time instructors spend grading the report.

This laboratory activity is simple, inexpensive, hands-on, and highly engaging. Students often respond well to hands-on activities especially when working with live organisms. The activity can easily be done in 2 hours with 20 minutes for introduction, 30 minutes for data collection, and the remaining time used to work with data.

Student Outline

Objectives

1. Know the basic aerobic cellular respiration chemical equation.
2. Recognize how temperature can be related to cellular respiration.
3. Understand how and be able to explain how a microrespirometer can be used to show that respiration is occurring.
4. Be able to generate a graph from data obtained in lab using Excel™ and be able to interpret that graph.
5. Determine the slope of the data using Excel™ and be able to find how to use the confidence interval of the slopes to determine the relationship between the slopes.
6. Think critically about data and be able to determine what types of data are best used to support hypotheses. (Which variables are best to measure and how do we test those to see changes?)
7. Understand and be able to define the words in bold.

Cellular respiration is an important process for all living things. Simply put, cellular respiration is the process by which chemicals are converted into useable energy. Organisms are not able to take pure energy and use it for most of their daily activities. Even plants must use chemicals to store energy for later use. The energy from cellular respiration is used for a number of cellular processes including: the formation of macromolecules, mitosis, moving substrates into and out of the cell, etc. Next week, we will look at photosynthesis, how sunlight energy is contained to be used for cellular respiration.

There are two main types of cellular respiration that occur in living organisms. The first is **anaerobic respiration** and the second is **aerobic respiration**. Both have to do with the use of oxygen, aerobic requiring oxygen and anaerobic not requiring oxygen. Many organisms, including both prokaryotes and eukaryotes, use aerobic cellular respiration. Because of this, most discussion of cellular respiration indicates the aerobic form.

Please note that cellular respiration should not be confused with breathing, also known as pulmonary respiration. Pulmonary respiration uses organs and organ systems to cause air to move in and out of the lungs for the purpose of gas exchange. Cellular respiration is the metabolic processes associated with energy conversion in the cell.

The Chemistry of Cellular Respiration

Cellular respiration generally starts with glucose, although many different products can be used including fats, proteins, and other carbohydrates. To put it simply, cellular respiration begins with glucose and oxygen which are converted to carbon dioxide and water. Other products of the process include heat and an energy storage molecule such as ATP (adenosine triphosphate). This process can be shown using the following simplified chemical equation.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Heat} + \text{Energy (ATP)}$$

Keep this equation in mind for next week's lab, it will seem familiar! The actual process of how we get from sugar to energy is, as you may already be aware, much more complicated. We will not get into the specifics of glycolysis, the citric acid cycle, or the electron transport chain. However, you will become familiar with them in lecture.

Experimental Procedure: Respiration and Temperature

Microrespirometers make a handy instrument for indirectly measuring respiration of small organisms such as plants (yes, they have cellular respiration!) or insects. This experiment involves the use of microrespirometers to indirectly measure the oxygen consumption of mealworms *Tenebrio molitor* (Order Coleoptera, Family Tenebrionidae). The respirometer is made from a 1-ml syringe that has a capillary tube glued in the place of the needle. Additionally, there are two wads of cotton near the capillary tube one of which is standard absorbent cotton containing 10 µl of a 15% KOH (potassium hydroxide) solution. The other is a portion of non-absorbent cotton that protects the test organism from the corrosive KOH. The capillary tube is sealed with a small amount of liquid that is added to the tube. Respirometers work by enclosing organisms or a sample of an organism in a chamber. The KOH, a highly corrosive basic liquid, can take CO_2 (gas) out of the air and convert it to a solid. The respirometer can take advantage of this process as CO_2 is released as O_2 is consumed in cellular respiration. Since liquids and solids take up far less

space than gases, the net result of the O_2 loss and CO_2 conversion from gas to solid is that the pressure decreases in the chamber. This pressure decrease is observed by the movement of liquid, called manometer fluid, in the capillary tube away from the capillary's opening. The movement is measured using a millimeter ruler attached to the capillary tube.

The organisms called meal worms are actually beetles that are grain pests. These beetles make excellent organisms for animal food, bait for fishing, and experiments. Because they are grain pests, they are very easy to raise and take little effort to culture. In this study, the effect of temperature on respiration is observed. The two temperatures are room temperature (ca. 20ºC) and cold (an ice water bath at 0ºC). We could study the respiration of the four main forms of the beetle (the egg, larvae, pupae and adult), the effect of different diets on respiration, the effect of crowding on respiration, the effect of age on respiration, etc. However, as an introduction to the scientific method and respiration, we will look only at the effect of temperature on the rate of respiration.

Materials: Water baths (set at "cold" ca. 0ºC and "room temp." ca. 20ºC), 2 microrespirometers, manometer fluid, 1 larva, thermometer, pipette, stop watch

Procedures

1. Determine which experimental group to test cold or room temp and note the location of the proper water bath. Record its temperature.
2. Record the mass of the larva.
3. Place the larva in one of the microrespirometers tail-first. Place the plunger directly on the 1 ml mark of the syringe. The microrespirometer with the beetle will be the experimental group.
4. Place the microrespirometers in the proper water bath such that the capillary tubes are not under the water but the rest of the device is under water.
5. Wait 10 minutes for the temperature of the respirometers and water to equilibrate.
6. *For the control group - CAREFULLY* place a small drop of manometer fluid (red food coloring mixed with water and dish soap) in the control microrespirometer with a capillary tube. This can be done by taking a capillary tube and dipping it into the manometer fluid, a small drop of the fluid should move into the tube. Carefully place the tips of the capillary tube and the open end of the respirometer's capillary tube together. The fluid in the capillary tube will move into the tube of the respirometer. Note, the pressure shouldn't change in the control, if the manometer fluid moves on its own it is due to either pressure changes or temperature changes.
7. *Now CAREFULLY* adjust the fluid in the control microrespiromters to move to the 50 mm mark. This is very tricky with the cold water and may take trial and error. Place the respirometer back into the water bath and wait for the manometer fluid to stop moving before proceeding. Be careful not to touch the body of the syringe as any change in temperature will affect the respirometer.
8. For the experimental group - *CAREFULLY* place a small drop of manometer fluid into the tube with the larva using the capillary tube as in step 9. Again being careful not to touch the body of the syringe.
9. When the manometer fluid in the experimental group has reached 0 mm on the ruler begin keeping track of time.
10. Record the total distance the manometer fluid has moved at 5, 10, 15, and 20 min. for both the experimental and the control. If at 5 min. the fluid moves from 0 mm to 7 mm you would note that it is at the 7 mm point. If at 10 min. it moves from 7 mm to 13 mm you would note that it is at the 13 mm point. You are not measuring the change in distance at each point, but rather where the fluid has moved to at each time point.
11. Remove the larva from the respirometer when finished and return it to the supply area.
12. Convert mm of fluid movement to volume and then divide the volume by the mass of the larva to get volume/mass. Data can then be shared with the other groups and graphed.

Hypothesis (relate temperature to respiration)

_____

Predict which test variable (cold or room temp.) will have the greater rate of respiration.

_____

Why would that test variable have a greater rate of respiration?

_____

What is the purpose of the control in this experiment?

_____

Exercise 1: Determining Relative Oxygen Consumption

Perform your experiment and place the data in the blanks and table below. Note that you will need the mass of the larvae.

Think about why you need that information.

What is the mass of your larva? _____

Table 1. Manometer fluid movement at 5, 10, 15, and 20 min.

Time (min)	Movement of manometer fluid: Experimental (mm)	Movement of manometer fluid: Control (mm)	Notes: temperature fluctuations, mishandling of respirometer, activity of mealworm before experiment, etc.
5			
10			
15			
20			

Now you need the volume of gas that was displaced (or relatively how much Oxygen was consumed) and to divide that by the mass of the mealworm you worked with. The volume of the tube that corresponds to 1 mm is 0.685 µl. So the conversion of mm to microliters can be done by taking your distance in mm and multiplying that by 0.685 µl/mm. Now take that number and divide it by the mass of the larva. Think about why this data is more useful than just having the time for the manometer fluid to move 50 mm. Then give your data to the class for graphing purposes. Please let them know which test variable you used!

Table 2. Converting your group's mm of manometer fluid movement to µl and then to µl/mg.

Time (min.)	Manometer fluid movement (mm)	µl (your group)	µl/mg (your group)
5			
10			
15			
20			

Table 3. Class data - please note group's test variables below so you can graph them properly! There is no need to place your data below, but make sure to include your own data (above) for your results.

Time (min.)	µl/mg	µl/mg	µl/mg	µl/mg	µl/mg
5					
10					
15					
20					
Variable - RT or Cold					

Exercise #2 Graphing the Results

Generate a graph using the data from tables 2 and 3 in Excel™ following the directions given to you. These directions will also include directions for determining confidence intervals for the average slopes of the RT and cold data. For your graph remember to include a short title, x and y axis labels (include units!), and a legend. Make sure your graph has the following components.

Checklist for graph:

_____Title
_____Is the graph easy to understand?

_____Units for x and y axis
_____Does the graph have "trend lines" for each group?

_____Legend
_____Does the graph have a description?

Table 4. Grading Rubric for Mini Lab Report (25 pts.)

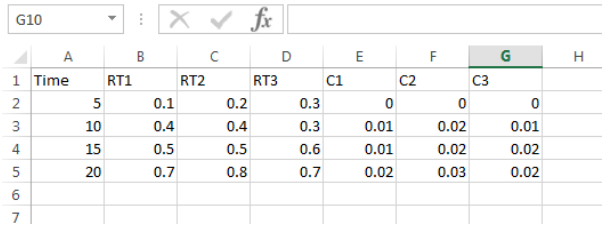
Sections	Criteria	Excellent	Competent	Needs work	Very poor	Comments
Title (2 pts.)	Title is informative, but not too long.	2 pts.	1.6 pts.	1.3 pts.	1 pt.	
Introduction (6 pts.)						
Background information (4 pts.)	Student used 2 sources, including a primary source (cited in text). Background was discussed with enough depth.	4 pts.	3.2 pts.	2.5 pts.	2 pts.	
Objective/Hypothesis (2 pts.)	Objective/ Hypothesis were clearly stated.	2 pts.	1.6 pts.	1.3 pts.	1 pt.	
Methods (4 pts.)						
Experimental design (2 pts.)	Procedure was written with enough detail to repeat experiment, but not too much detail.	2 pts.	1.6 pts.	1.3 pts.	1 pt.	
Correct format (1 pt.)	Passive voice was used. Written in paragraph format (no lists!)	1 pt.	0.8 pts.	0.6 pts.	0.4 pts.	
Variables correctly stated, controls stated (1 pt.)	IV and DV and controls were clearly identified.	1 pt.	0.8 pts.	0.6 pts.	0.4 pts.	
Results (4 pts.)						
Description of results (2 pts.)	Results were described in paragraph form. Confidence interval for slopes are noted.	2 pts.	1.6 pts.	1.3 pts.	1 pt.	
Figure/Table neatness (1 pt.)	Figure/table is easy to understand and neat.	1 pt.	0.8 pts.	0.6 pts.	0.4 pts.	
Figure/Table labels and title (1 pt.)	Good title is on the figure/table.	1 pt.	0.8 pts.	0.6 pts.	0.4 pts.	

Discussion (6 pts.)		Excellent	Competent	Needs work	Very poor	Comments
Comparison of previously published information (3 pts.)	Student compared data using two sources. Sources were cited in text. At least one primary source used.	3 pts.	2.5 pts.	2 pts.	1.5 pts.	
Hypothesis and Interpretation of results (1 pt.)	The student explained if and how the hypothesis was supported by the data.	1 pt.	0.8 pts.	0.6 pts.	0.4 pts.	
Unexpected results noted (1 pt.)	The student explained any unexpected results.	1 pt.	0.8 pts	0.6 pts	0.4 pts	
New experiments stated (1 pt.)	Student suggested new experiments that could be done.	1 pt.	0.8 pts.	0.6 pts.	0.4 pts.	
Overall style and grammar (2 pts.)						
Style (1 pt.)	Writing was. concise. Enough transitions between ideas. Writing flowed well. Report was about 1 page (graph on a second page).	1pt.	0.8 pts.	0.6 pts.	0.4 pts.	
Grammar and spelling (1 pt.)	Few grammar. and spelling mistakes.	1 pt.	0.8 pts.	0.6 pts.	0.4 pts.	
Literature cited in text and after (1 pt.)	The literature. was cited correctly (in text and in this section). Two sources were used (one primary source).	1 pt.	0.8 pts.	0.6 pts.	0.4 pts.	

Notes:

Procedures for Generating a Graph Using Excel™

1. Open Excel™ and set up a spreadsheet with the data like figure 2.



	A	B	C	D	E	F	G	H
1	Time	RT1	RT2	RT3	C1	C2	C3	
2	5	0.1	0.2	0.3	0	0	0	
3	10	0.4	0.4	0.3	0.01	0.02	0.01	
4	15	0.5	0.5	0.6	0.01	0.02	0.02	
5	20	0.7	0.8	0.7	0.02	0.03	0.02	
6								
7								

Figure 2. Excel spreadsheet with example data.

2. Select the Insert Tab and look for Charts as in figure 3. Choose the highlighted chart type and select Scatter with no line connecting the data points (the first choice)

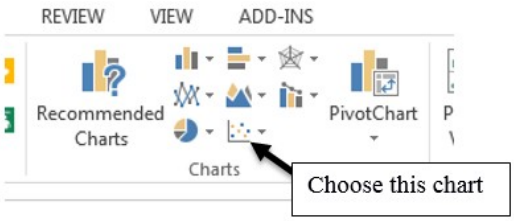


Figure 3. Chart menu in Excel with arrow pointing toward the scatterplot option.

3. This will open the Chart Tools feature, see figure 4. You will have a graph automatically generated. It will be wrong. You must now select the Select Data function as shown in figure 4.

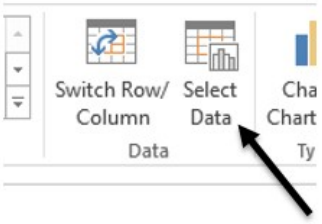


Figure 4. Select Data function with arrow pointing to select data feature.

4. A dialog window, see figure 5, will pop up and you will click on Switch Row/Column to get variables in the proper location on the graph. The window will look like figure 5 after clicking the Switch Row/Column button.

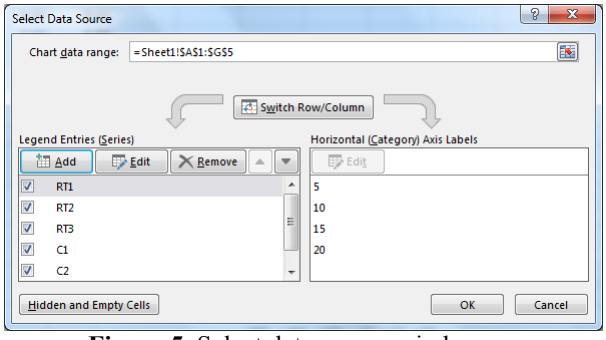


Figure 5. Select data source window.

5. Your graph should look something like figure 6. Notice that there are no labels for the x or y axes, nor is there a title for the graph. You will need to add these later.

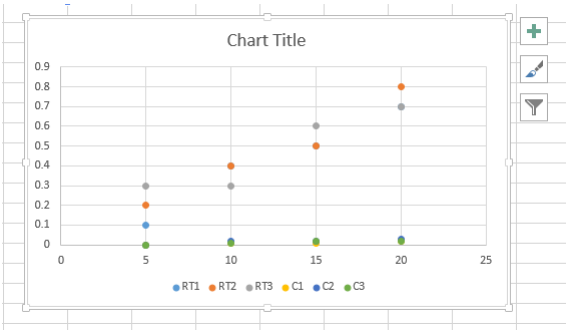


Figure 6. Graph of example data.

6. Now move your chart to a chart page by right clicking the mouse and selecting Move Chart and the dialog window as in figure 7 will show. Select New sheet and click OK. The chart will now be on its own sheet.

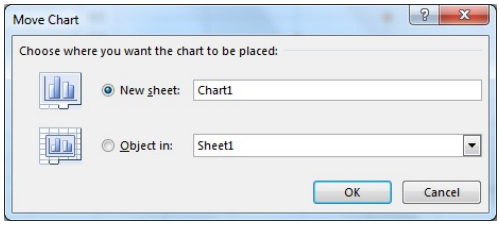


Figure 7. Move chart window.

7. Click on the chart (near the title is best) and you should see a list of choices called chart elements as in Figure 8. This brings up a set of tools you can use to adjust your chart. Select Axis Titles to allow you to add X and Y axis titles. You will need to manually enter the titles of the X and Y axes.

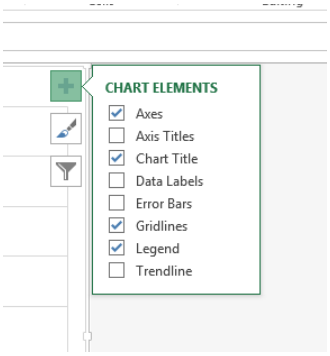


Figure 8. Chart elements tools.

8. You can now select Trendline from the above menu and this dialog window will display as in figure 9.

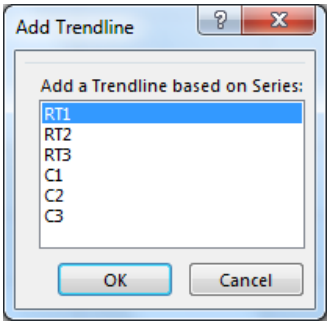


Figure 9. Add trendline window.

9. Select RT1 and OK and a trend line will appear on the chart. Now double click on the trend line to access formatting of the trend line, see figure 10. Here you can change the color and width of the line. It is recommended that you change the width of the line as the default is fairly thin. Note you will unfortunately need to do this for all the trend lines.

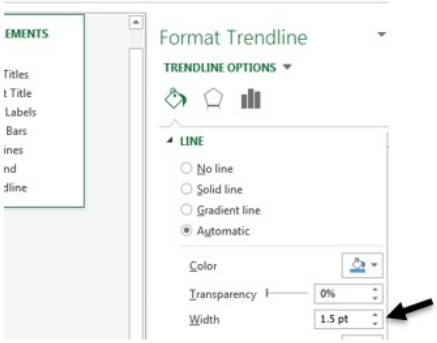


Figure 10. Format trendline options with arrow pointing to the width change feature.

10. To add more trend lines you will need to access the Chart Elements tool kit again, but you will need to specify Linear as in figure 11. Make sure to do this for all groups. Make sure not to unselect Trendline, or you will lose all the trend lines generated.

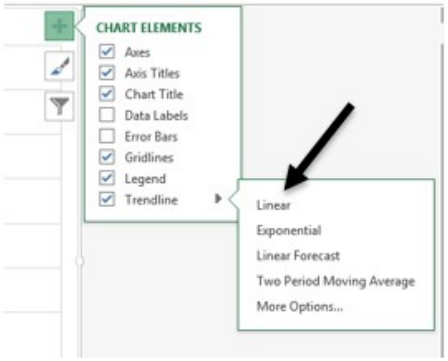


Figure 11. Chart elements options showing trendline options. The arrow is pointing toward the linear trendline option.

11. Now, if you haven't already changed your axis titles and chart title, manually click on them and change them to make them appropriate for your graph.

12. Click on the legend, most likely located at the bottom of the chart, and adjust it to move it to the right side of the graph and add a text box to the bottom of the chart to add a descriptive sentence (or two) about the chart. For instance you will want to note that RT was the room temperature group and that C was the cold group. Your chart should look something like figure

13. You will need to add the titles and units for the axes as well as a title for the chart.

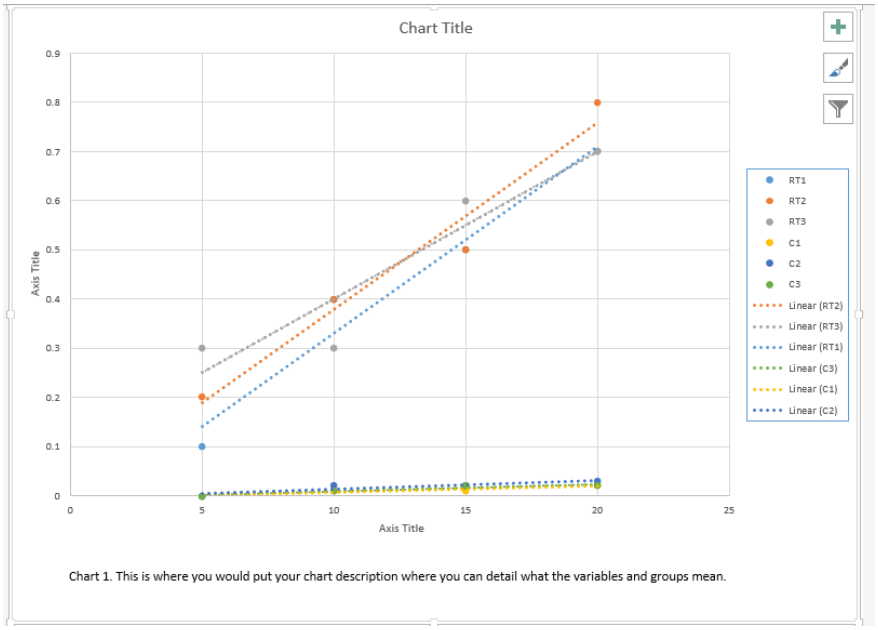
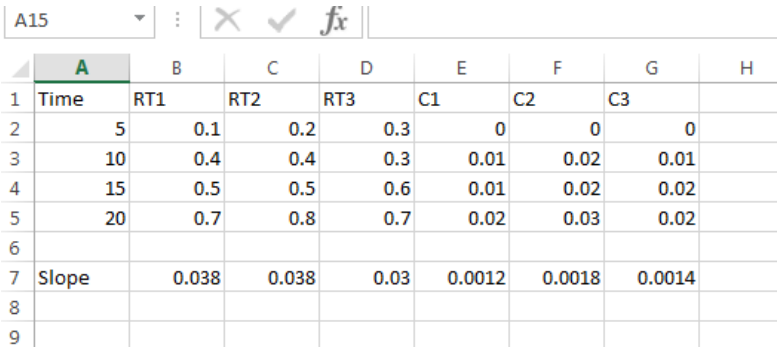


Figure 12. Graph of example data showing trendlines for each experimental group.

Determining Confidence Intervals for Slopes Using Excel™

Now that we have a graph of our data, it is time to analyze the data. This can be done by looking at the slope of our trend lines and generating confidence intervals for those slopes. This can be done in Excel™.

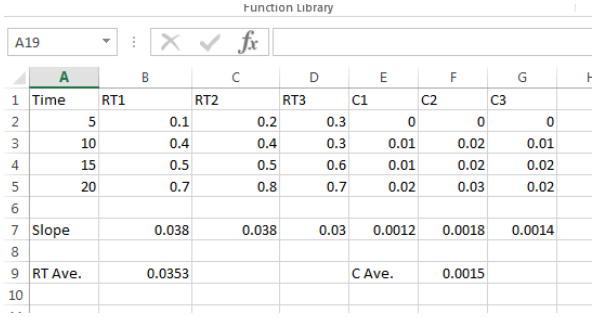
1. Go back to your sheet with your group data, see figure 2. Use the formula "=SLOPE(B2:B5,$A$2:$A$5)" to determine the slope of the RT1 data. You can now copy and paste that for the rest of the groups (notice that adding a $ before the cell column and cell row "fixes" the field so it will not change when pasted to a new location!). This should look like the example on figure 13. You now have the slope for each group.



	A	B	C	D	E	F	G	H
1	Time	RT1	RT2	RT3	C1	C2	C3	
2	5	0.1	0.2	0.3	0	0	0	
3	10	0.4	0.4	0.3	0.01	0.02	0.01	
4	15	0.5	0.5	0.6	0.01	0.02	0.02	
5	20	0.7	0.8	0.7	0.02	0.03	0.02	
6								
7	Slope	0.038	0.038	0.03	0.0012	0.0018	0.0014	
8								
9								

Figure 13. Example data with values for slopes.

2. To calculate average slope for each variable use the formula "=AVERAGE(B7:D7)", this will give you the average slope for the room temp groups. Use the formula "=AVERAGE(E7:G7)" will give you the average slope for the cold temp groups. It should look like figure 14.



	A	B	C	D	E	F	G
1	Time	RT1	RT2	RT3	C1	C2	C3
2	5	0.1	0.2	0.3	0	0	0
3	10	0.4	0.4	0.3	0.01	0.02	0.01
4	15	0.5	0.5	0.6	0.01	0.02	0.02
5	20	0.7	0.8	0.7	0.02	0.03	0.02
6							
7	Slope	0.038	0.038	0.03	0.0012	0.0018	0.0014
8							
9	RT Ave.	0.0353			C Ave.	0.0015	
10							

Figure 14. Example data with slopes and averages of slopes.

The data from figure 14 tell you the relationship between respiration and time. Typically, we see in our data that when one goes up (respiration) so does the other (time). There are three types of relationships we see when looking at slopes: a positive relationship (as above), a negative relationship (one goes up and the other down), or no relationship (neither variable moves with the other). You will want to note what type of relationship is seen in each test variable when writing up your results. Note also how each slope relates to the average as well.

Procedures for determining the 95% confidence interval for your slopes.

Now that we have slopes and average slopes for your two variables, we can estimate a confidence interval, or C.I., for the average slopes. This will give us a range of values where the true value of our slopes most likely are. The formula for calculating the C.I. of a linear slope is $b_1 \pm t_{(n-2)} * \sigma_{b1}$. Where b_1 is the slope of the line, $t_{(n-2)}$ is the t statistic with the degrees of freedom or the number of groups (n) minus 2, and σ_{b1} is the standard error of the slope. This interval will give us evidence that our slopes are either different or similar to each other.

1. First you will need to open a new sheet by clicking on the plus sign in the lower left-hand corner of your spreadsheet as in figure 15.

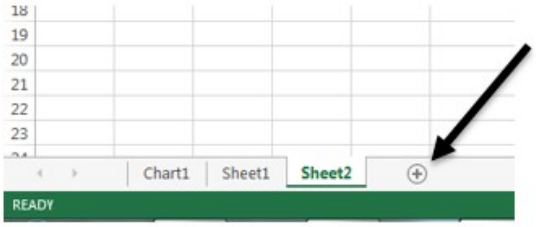


Figure 15. Arrow shows where to click to generate a new sheet.

2. Now copy and paste your data from Sheet1 onto Sheet2. Then move the data so that they are in 3 columns as in figure 16. This can be done with formulae or by cutting and pasting, it's your choice.

F19

	A	B	C	D
1	Time	RT	C	
2	5	0.1	0	
3	10	0.4	0.01	
4	15	0.5	0.01	
5	20	0.7	0.02	
6	5	0.2	0	
7	10	0.4	0.02	
8	15	0.5	0.02	
9	20	0.8	0.03	
10	5	0.3	0	
11	10	0.3	0.01	
12	15	0.6	0.02	
13	20	0.7	0.02	
14				

Figure 16. Format for data to calculate C.I. of slopes.

3. Now set up columns like what is shown in figure 17. SE is standard error and T score is the T statistic for our data. You will need both to obtain the 95% C.I.

14					
15	SE slope RT				
16	SE slope C				
17	T score				
18					
19	Confidence interval for slopes			High	Low
20	RT		plus/minus		
21	Cold		plus/minus		
22					

Figure 17. Set up for determining C.I. of slopes.

4. Next to the cell marked SE slope RT (B15) as in figure 17 use the formula "=INDEX(LINEST(B2:B13,A2:A13,TRUE,TRUE),2,1)" to determine the standard error of your room temperature slope. You need to make sure to follow this formula exactly. When you start typing it will give you prompts of where to place the information in your formula.

5. Now use a similar formula to determine the standard error of your cold temperature slope (in B16). That formula is "=INDEX(LINEST(C2:C13,A2:A13, TRUE,TRUE),2,1)".

6. Next to the cell marked T score (B17) use the formula "=TINV(0.05,10)" this will give you the T statistic for our data. Steps 4 to 6 should look like figure 18.

14		
15	SE slope RT	0.0033
16	SE slope C	0.0003
17	T score	2.2281

Figure 18. Standard error and T score for the slopes.

7. In the cell next to the cell marked RT (B20) as in figure 18 use the following formula "=SLOPE(B2:B13,A2:A13)" to get the slope for the room temperature group and then next to the cell marked Cold (B21) use "=SLOPE(C2:C13,A2:A13)" to get the slope for the cold temperature group.

8. Now in cell D20 as in figure 18 use the formula "=B17*B15" and "=B17*B16" in cell D21. These values are the intervals that you can add or subtract from the slopes to determine where the actual value of the slopes most likely are.

9. Now it will likely be helpful to you to see what the values of the 95% C.I. for each slope are to determine if the values overlap or not. If they overlap it gives evidence that the slopes are similar to each other. If the confidence intervals do not overlap, this may indicate that your slopes are different. You can do this by using the following formula for the room temperature slope in E20 "=B20+D20" for the high, and in F20 "=B20-D20" in for the low value. To determine the values for the cold temperature slope, use the following formula in E21 "=B21+D21" for the high, and in F21 "=B21-D21" for the low value. It will look something like figure 19.

18						
19	Confidence interval for slopes				High	Low
20	RT	0.0353	plus/minus	0.0074	0.0427	0.0280
21	Cold	0.0015	plus/minus	0.0006	0.0021	0.0009

Figure 19. Slopes of lines with C.I. for slopes.

Notice that the C.I.'s do not overlap in the example above, the RT slope is 0.0427 to 0.0280 and the Cold slope is 0.0021 to 0.0009. This would **indicate** that the two slopes are different. You may not get this result and you should be careful when noting your result. They give evidence for a conclusion, they do not prove the result!

Materials

The following list of materials is intended for one lab section of 24 students. Microrespirometers can be reused within a short time, but will become difficult to read due to manometer fluid buildup after a few uses.

- Two large deep water tubs (at least 7 inches or 18 cm deep). One for ice water and the other with room temperature water.
- An active culture of mealworms. You will need 1 mealworm larva per group.
- Two thermometers – or one per water bath.
- Crushed ice for an ice water bath (a large volume is needed for a large tub).
- Eight to twenty-four microrespirometers per lab section (You will need the larger amount if you have the students utilize their own control microrespirometer and the lesser amount if the instructor sets up the control microrespirometers for the class).
- Manometer fluid – red food coloring, dish soap, and water. Add enough drops of food coloring to saturate the solution (about 10 drops per 20 ml of water), as for the soap – only a small drop is needed for 20 ml.
- Capillary tubes.
- Stopwatches – 6 to 12 stopwatches, although most students have access to chronometers on their cell phones.
- Balances for determining mealworm mass – a scale that measures to the tenth of a mg is best.
- Test tube racks – one per water bath. These are optional and can be used to hold up the respirometers.

Notes for the Instructor

Construction of the respirometer is simple, and the materials are inexpensive. Begin assembly with a 1-ml disposable syringe that has had a 50-µl disposable glass calibrated micropipette hot-glued in place of a needle. The pipette is glued into place with the use of a hot glue gun being sure to completely surround the opening with glue to form a proper seal. It is best to allow the glue to cool and solidify before moving on to the next steps. When the glue is cool and well solidified, the plunger of the syringe can be carefully removed and a small wad of absorbent cotton added just inside the opening of the syringe. Then 10 µl of 15% KOH are added to the absorbent cotton using a micropipette. The wad of absorbent cotton with KOH can be moved a centimeter or two up the syringe using a small rod, care should be taken using the KOH to protect the user (gloves, googles, and a fume hood should be used to work with the corrosive KOH). The wooden portion of a sterile cotton-tipped applicator, the opposite end of a Crayola size 2 paint brush, or a straightened paperclip can be used to push the cotton wad with KOH up the syringe. In order to ensure that your mealworms will not come in direct contact with the corrosive KOH, a small wad of non-absorbent cotton is then placed in the opening of the syringe and both wads of cotton moved closer to the glass micropipette using the small rod again. A millimeter ruler can then be taped to the capillary tube using invisible tape so as to be able to see the ruler. This ruler should be placed with the 0 mm mark near the opening of the capillary tube so that readings can be made from smaller units to larger units (from 0 mm to 100 mm). It is best to leave just a few mm of space from the opening of the capillary tube to the 0 mm mark on the ruler. It is recommended that the instructor allocate at least 3 hours for the construction of fifty microrespirometers, especially as the application of the capillary tubes using hot glue does require cooling time. Construction can be done in two days, the first day being the application of the capillary tubes using hot glue. The next day is used to complete the rest of the respirometer and allow for maximum hardening/cooling of the hot glue. Please note that the devices can be broken very easily and must be handled carefully by the user as they contain corrosive KOH and a glass capillary tube. If the glass capillary tube breaks, take care to clean the glass carefully and don't handle the glass with bare hands. Rather sweep it up and dispose of it in a proper broken glassware disposal container. The microrespirometers can be reused within a short period of time (1 hour). However, reuse is limited by manometer fluid buildup around the opening of the capillary tube. It is best to remove the capillary tubes after they have been used if you wish to save the syringe and reconstruct the respirometer. The cotton wads should also be removed. This can be done with a straightened paper clip. Cotton wad removal can take some practice.

As for the control respirometers, they are tricky to set up for the inexperienced user. It is highly recommended that the instructor set them up instead of having students do it. For the cold water control, the manometer fluid can be added just after the respirometer has been added to the water bath. The drop in temperature and resulting drop in pressure will draw the manometer fluid down close to the half-way point of the capillary tube. The room temperature water bath will need to be manually adjusted to the 50 mm mark. This can be done while the respirometer is under water by adjusting the plunger of the syringe. The purpose of the control is to note any changes in pressure in the room and/or changes in temperature in the bath. However, since the baths are very temperature stable there should be little change in pressure due to temperature.

The water baths should be able to hold the microrespirometers vertically. I have found that that more water/ice allows for better temperature control, and this setup requires the temperature be very stable. It is advisable to fill your room temperature tub about an hour or more before the experiment begins in order to ensure stable room temperature water. For the ice water bath, it is advisable to fill the tub with crushed ice and then fill with room

temperature water until the proper water level is reached. The room temperature water will melt some of the ice, but will leave enough to keep the temperature at 0°C.

The beetles used in this test are called mealworms because they are grain pests. They will eat any type of grain given to them and are easy to culture. Beetles can be purchased from biological supply companies or from your local bait shop. Please note that many of the mealworms that you see in bait shops have been treated with juvenile hormone to increase their size (they may fail to pupate and therefore fail to grow to adults). It is recommended you not use these larvae but rather allow them to go to adulthood and produce larvae free of the hormone, which does not persist in the culture. Their life cycle is around 2 months and can be sped up by increasing the temperature of their environment or slowed down by lowering the temperature. Beetles will continue to mate and reproduce continuously so larvae can be collected at any time. Cultures can be maintained by placing them in an open smooth-sided plastic container with oats for food and soaked paper towels for water. Cultures should be watered regularly, and they can be supplemented with raw fruit, potatoes, carrots, cat or dog food, bone meal, and multivitamins if you wish to have a vigorous colony. However, regular watering and oats allows for a smaller, less difficult to culture colony. It is best to restart the colony and/or remove waste from the colony at least once a year. Multiple colonies can be keep in different containers to allow for staggered colony restarts since it takes about 2 to 3 months to restart a colony from adults.

I have found the mini lab report assessment has had a positive effect on improving time of grading and easing students into scientific writing. The mini lab report can be modified to utilize parts of a typical lab report in order to emphasis the objectives of a laboratory exercise. I have found that students often start introductory biology with little scientific writing skills. I therefore introduce them to scientific writing by having them work on the various parts of a lab report in pieces. I do this by having them write an introduction and methods for an osmosis lab that occurs earlier in the semester. Then for this lab, looking at respiration, we have had them write the results and discussion portions of a lab report. This gives more time in lab to discuss lab reports, and there is no need to discuss all the parts of the lab report at one time. As well, when looking at lab reports in parts, we can emphasize the aspects of the scientific process that match our learning objectives for the lab. For instance, with our respiration lab, we would like them to be able to generate a graph in Excel and learn how to use it to generate confidence intervals for slopes in an effort to improve their data analysis skills. I have included the full lab report rubric with this document, but this can be modified to include only the aspects you would like to use for the assessment.

Cited References

Lee RL. 1995. Using microrespirometers to measure O_2 consumption by insects and small invertebrates. The American Biology Teacher, 5: 284-285.

Simmons AD, Larios-Sanz M, Shivas A, and Rosemarie CR. 2014. Using mini-reports to teach scientific writing to biology students. The American Biology Teacher, 76(8): 551-555.

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About the Author

Kevin P. Miller has been a member of ABLE since 2010. Kevin's current position is Laboratory Instructor/Technician in the Young Harris College Department of Biology and Associate Director of the Biopredatory Beetle Facility at YHC in Young Harris, Georgia. Kevin teaches introductory biology labs for majors and entomology courses. Kevin graduated with a BA in biology from Goshen College and his M.S. in entomology from The Ohio State University. Kevin's role at the college has recently expanded to include an Associate Directorship of YHC's Biopredatory Beetle Facility raising beetles to combat the introduced hemlock wooly adelgid pest infesting hemlocks in the local area. His other interests include aquatic entomology looking at impacts of development and hemlock loss on aquatic macroinvertebrate diversity.

Appendix A Sample Results

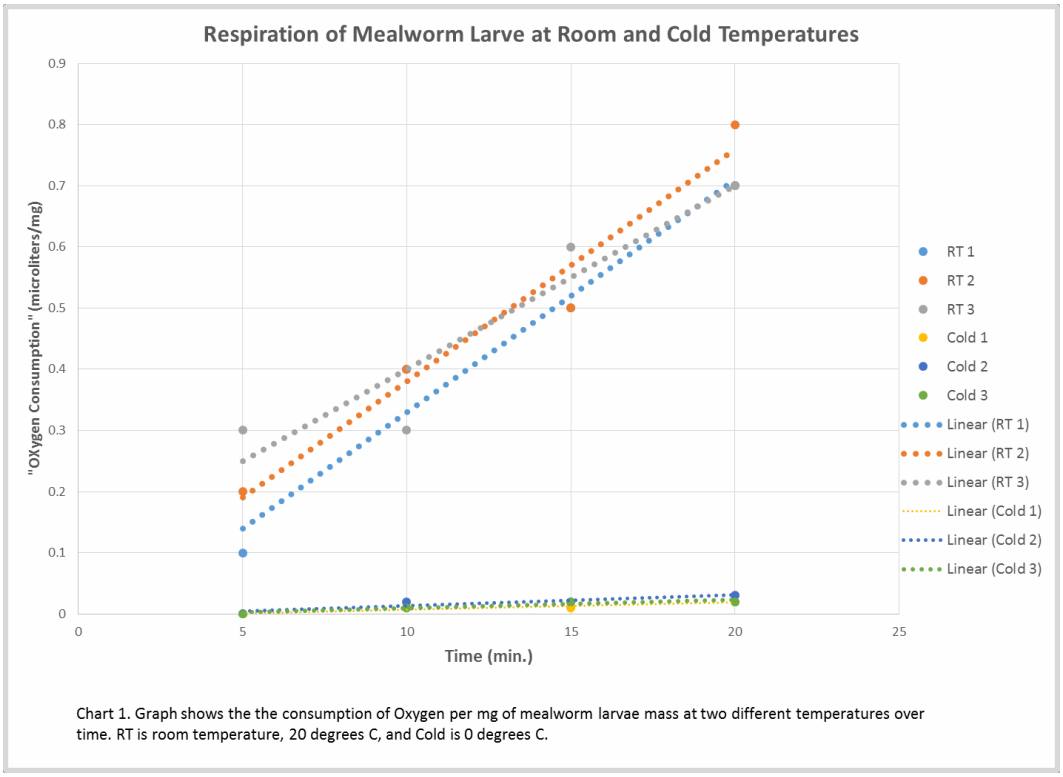

Figure 1. The chart above is sample data based on typical results, not actual results.

Appendix B
Supplies for the Microrespirometers

1. Calibrated micropipettes, 50-µl - Drummond Scientific Co. which can be purchased from Fisher Scientific Supply item number 21-180-16 for $72.37 US/pack of 250.
2. Disposable hypodermic syringes, 1-cc – Carolina Biological Supply item number 697765 for $12.95 US/pack of 25.
3. Non-absorbent non-sterile cotton - Ward's Scientific Supply item number 153828 for $13.80 US/1 pound pack - you won't ever need more!
4. Absorbent cotton – a little goes a long way.
5. Printed mm ruler from - http://www.vendian.org/mncharity/dir3/paper_rulers/UnstableURL/rules_mm.pdf.
6. 15% KOH solution.
7. Hot glue gun with glue.
8. Flat washers, 3/8 inch (inside diameter is 3/8 inch or 1 cm) – one per microrespirometer.
9. Size 2 Crayola paintbrush, sterile cotton-tip applicator, or straightened paperclip to push cotton wads up the syringe.
10. Capillary tubes (for manometer fluid) – Carolina Biological Supply item number 700702 for $14.00 US/pack of 100 tubes.

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