BIOL 4405 - Lab 2: Insect Metabolism

In this lab you will:
- hold a cricket
- measure oxygen consumption of crickets
- calculate standard metabolic rate from measurements of oxygen consumption
- compare metabolic rates among crickets of different size and sex and under different environmental conditions.
- plot and analyze metabolic rate data among different experimental groups

After completion of this lab you should be able to:
- calculate metabolic rate from oxygen consumption
- calculate the temperature quotient (Q_{10})
- understand how various factors influence metabolic rate

Assignment: Results Section + At least 1 graph and figure caption

Respiration and Metabolic Rate: Respiration is the process by which organic molecules are broken down to yield ATP. Metabolism refers to the sum total of all catabolic (breaking down) and anabolic (building up) processes that take place in a whole organism. In this lab you will be measuring the amount of oxygen consumed to support aerobic respiration, and you will use this measurement (per unit of time) as a proxy for metabolic rate. As an aside, one could use carbon dioxide produced as a metabolic measurement, but for this lab you will be measuring oxygen consumed. The technique of measuring respiration and using it as a proxy for metabolic rate is called *respirometry.* Respirometry is conducted in an airtight chamber called a *respirometer*.

Crickets and poikilothermy: The house cricket, *Acheta domesticus*, is an insect in the order Orthoptera, which includes crickets, grasshoppers, katydids and other jumping insects. *A. domesticus* is native to Southwest Asia, but has been accidentally introduced around the world by humans because it is commonly bred and sold to feed pets like lizards and frogs. For example, the individuals you will be experimenting with were purchased at Petco. Like most other arthropods, crickets are ectotherms, or poikilotherms, because they're internal body temperatures match that of their surrounding environments. Being a poikilotherm has its advantages; it takes far less energy to sustain a poikilotherm because metabolism isn't diverted toward heat production. This is how snakes can survive for weeks without eating. But, poikilothermy has its drawbacks as well. Temperature affects all biological processes, from the rates of biochemical reactions to the coordinated firing of neurons. Because metabolism is dependent on a whole suite of biochemical reactions, metabolic rate is acutely sensitive to temperature. For this reason, insects and other ectotherms do not sustain metabolic activity in the winter months because biochemical reactions slow down to the point that these organisms go into metabolic quiescence (i.e. diapause). In this lab you will be measuring the effect of temperature on metabolic rates of crickets, as well as comparing the effects of size and sex on metabolic rate.

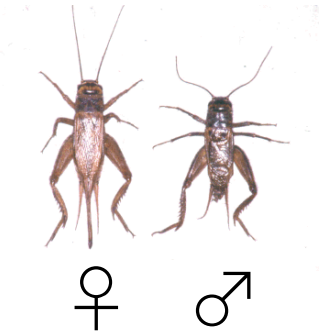

Figure 1. *Acheta domesticus*, the house cricket. On the left is a female with ovipositor protruding from end of the abdomen. On the

right is a male. Males lack the ovipositor. Note also the size difference between the sexes.

Materials:
- scale
- 2 respirometry chambers
- 2 black shields (inside the assembled respirometers)
- 2 calibrated capillary tubes
- 2 rubber stoppers
- cotton balls
- 15% KOH solution
- Water basin half-full with water
- ice
- thermometer
- stopwatch
- pen/pencil + lab notebook
- yourself

I. PROCEDURE:
Measurement of standard metabolic rate:

1. Check to make sure you have all necessary components of both respirometers.
2. Select a cricket from the terrarium. Record the sex of the cricket. Be gentle!
3. Place the the cricket on the scale. Record the mass of the cricket.
4. Place a cotton ball inside the respirometer.
5. Pipet 1 ml of 15% KOH onto the cotton ball inside the respirometer. **DO NOT TOUCH THE KOH SOLUTION WITH YOUR HANDS! WEAR GLOVES. (KOH is a strong base. If skin is exposed wash with running water for several minutes).** KOH absorbs carbon dioxide that is exhaled from the cricket inside the respirometer.
6. Cover cotton ball with the black shield.
7. Place your cricket inside the respirometer and cover with the rubber stopper.
8. Insert calibrated capillary tube into the rubber stopper.
9. Assemble the control respirometer exactly in the same manner as above, except exclude the cricket.
10. Label each respirometer to indicate the experimental treatment or control.
11. Submerge both respirometers in the water basin and place them adjacent to each other in a horizontal position, utilizing the metal tube rack.
12. Leave respirometers for 5 minutes to permit the gas pressure to equilibrate inside the respirometers. Record the temperature of the water in the basin using a thermometer.
13. Note the location where the outer edge of the water reaches on the scale of the calibrated tube. This is the initial reading. For the next 5 min., record the position of the outer edge of the water at 1 min. intervals. Do this simultaneously for both respirometers. *Note: if the water moves so rapidly that it is sucked up the entire length of the calibrated tube in less than 5 minutes, take recordings every 15 or 30 sec. over a shorter time period. If the water moves very slowly, take measurements at longer time intervals. Also Note: It is important to restrict movement of the cricket when measuring metabolic rate because exercise has profound affects on respiration, and thus could skew your results.*

14. Calculate the amount of oxygen consumed per time (i.e. the metabolic rate) from your average number of units moved per time period by the water on the calibrated tubes using the following equation:

$$Metabolic\ rate\ (M.R.) = \frac{ml\ Oxygen}{h} = \frac{(No.\ units\ on\ tube \times 0.01\ ml)}{Time\ in\ sec.} \times \frac{60\ sec.}{1\ min.} \times \frac{60\ min.}{1\ h} = \frac{ml\ Oxygen}{h}$$

The **mass-specific metabolic rate** can then be calculated with the following equation:

$$Mass\text{-}specific\ M.R. = \frac{ml\ Oxygen}{h} \div mass\ (g) = \frac{ml\ Oxygen}{g \cdot h}$$

15. Remove the respirometers from the water and open the respirometers to let fresh air in. Close the respirometers. Repeat steps 11 - 14 above to take another measurement of respiration of the same cricket. Do this again to take 3 total measurements of a single cricket. Average the three measurements for both respirometers. Subtract the background rate (i.e. the avg. of the control respirometer) from the observed cricket rate (i.e. the avg. of the cricket's respirometer) to calculate the true metabolic rate of your cricket.

Do females and males have the same metabolic rate?

16. Place your cricket into the plastic bin next to the cricket terrarium. Select another cricket from the cricket terrarium that is of the opposite sex from the one that you made your first metabolic measurements.
17. Repeat steps 2 - 15 to measure the metabolic rate of your new cricket.
18. Repeat steps 2 - 15 for at least 3 individuals of each sex. Try to select crickets of different sizes so as to also measure the potential affect of size on metabolic rate.

What is the effect of size on metabolic rate?

19. Now, look at your distribution of sizes of individuals measured. If your crickets were all of a similar mass, take metabolic measurements (repeating steps 2 - 15) of 4 more crickets that are of different size than those you previously measured (2 bigger and 2 smaller).

What is the effect of temperature?

20. Repeat steps 2 - 15 on 3 more crickets, but instead add ice to the water in the water basin to make an ice bath. Allow the respirometers to equilibrate for 10 minutes prior to measurement. Check the ice bath temperature with a thermometer. Record this temperature at the beginning of each metabolic measurement. Repeat again on 3 more crickets with warm water from the tap.

II. Data analysis in Prism:

Use Prism to analyze your data by comparing the metabolic rates among your experimental treatments of sex, size, and temperature. You will want to do 6 analyses and plot the 6 corresponding graphs. These six analyses will allow you to determine the main effects driving the differences in metabolic rate—to basically answer the following 6 questions:

- What is the effect of sex on metabolic rate?
- What is the effect of size on metabolic rate?
- Is there a combined effect of sex and size on metabolic rate?
- What is the effect of temperature on metabolic rate?

BIOL 4405 - Lab 2: Insect Metabolism

- Is there a combined effect of sex and temperature on metabolic rate?
- Is there a combined effect of size and temperature on metabolic rate?

The last question at the bottom of each section are prompts for your discussion. Try to synthesize the data to explore major trends.

Sex and Metabolic Rate

1. When the first window pops up after starting the Prism program, choose "Column" on the left menu. Select "Enter replicate values, stacked into columns."

2. Click "Create." Enter your calculated mass-specific M.R.s in separate columns for each sex (pool all male data regardless of temp. and all female data regardless of temp). Group A should be one sex and Group B the other sex. Label which sex is which.

3. With your data table selected on the left menu, click the "= Analyze" button. Select "t-tests" under Column analyses. Click OK.

4. Select Unpaired. Select Yes for assuming Gaussian distribution. Select to assume the same S.D. Click OK.

5. Review the results of your analysis by clicking on the "Unpaired t test.." under the Results tab on the left. Look at the corresponding graph (choose any graph type that you feel best fits) by clicking under the Graphs tab on the left. Label your axes appropriately.

6. Is there a significant difference between the sexes?

Size and Metabolic Rate

1. Create a new data table + graph... Choose XY from the menu on the left. Select Enter and plot a single Y value for each point. Click Create.

2. In the X column, enter the masses (g) of each cricket that you measured.

3. In the Group A column, enter the mass-specific metabolic M.R. for each cricket.

4. Click "=Analyze" and choose Transform under Transform, Normalize analyses. Click ok.

5. Check "Transform X values using", select "X=Log(X)" from drop down menu. Check "Transform Y values using", select "Y=Log(Y)" from drop down menu. Click ok.

**NOTE: Transforming the data allows us to do a linear regression on data that originally was logarithmic (curved). This doesn't change the data, just allows us to see a better relationship.

6. Under Results section, select the new Transformed data table. Click “=Analyze” choose Simple Linear regression under XY analyses. Click OK.

7. Check to test whether slopes and intercepts are significantly different. Leave the rest of the default options. Click OK.

8. Review the results of the analysis by clicking on the “Linear reg...” under the Results tab on the left. Record the R squared value under “Goodness of Fit” and the p-value under “Is the slope significantly none zero?”

9. Look at the corresponding graph (Transform of...) select by clicking under the Graphs tab on the left. Label your axes appropriately.

10. Is there a significant effect of size on metabolic rate? (R-square and P-value tell you this but also look at the trend of the line on the graph)

Sex and Size and Metabolic Rate

1. Create a new data table + graph... Choose XY from the menu on the left. Select Enter and plot a single Y value for each point. Click Create.

2. In the X column, enter the masses (g) of each cricket that you measured.

3. In the Group A column, enter the mass-specific M.R. for each female cricket that corresponds to the entered mass in the X column. In the Group B column, enter the mass- specific M.R. for each male cricket that corresponds to the mass entered in the X column. Each box in Group A with a value should have an adjacent box in Group B that is blank, and vice versa.

4. Click “=Analyze” and choose “Transform” under Transform, Normalize analyses. Click ok.

5. Check “Transform X values using”, select “X=Log(X)” from drop down menu. Check “Transform Y values using”, select “Y=Log(Y)” from drop down menu. Click ok.

6. Under Results section, select the new Transformed data table. Click “=Analyze” choose Simple Linear regression under XY analyses. Click OK.

7. Check to test whether slopes and intercepts are significantly different. Also check to show the 95% confidence band of the best-fit line. Leave the rest of the default options. Click OK.

8. Review the results of the analysis by clicking on the “Linear reg...” under the Results tab on the left. Record the R squared values under “Goodness of Fit” and the p-values under “Is the slope significantly none zero?”

9. Click the tab next to the results line. Click on “Are the lines different?” Record the p-value for “Are the slopes equal” and the p-value for “Are the elevations or intercepts equal?” and read the explanations.

10. Look at the corresponding graph by clicking under the Graphs tab on the left (Transform of...). Label your axes appropriately.

11. Is there a combined effect of sex and size on metabolic rate? (Is the trend the same or different for male and female?)

Temperature and Metabolic Rate

1. Create a new data table + graph...Choose Column. Enter your calculated mass-specific M.R.s for Room temperature in Column A. Enter your calculated mass-specific M.R.s for Ice bath in Column B (regardless of sex). Label which temperature is which.

2. With your data table selected on the left menu, click the “= Analyze” button. Select “t-tests” under Column analyses. Click OK.

3. Select Unpaired. Select Yes for assuming Gaussian distribution. Select to assume the same S.D. Click OK.

4. Review the results of your analysis by clicking on the “Unpaired t test..” under the Results tab on the left.

5. Look at the corresponding graph (choose any graph type that you feel best fits) by clicking under the Graphs tab on the left. Label your axes appropriately.

6. Is there a significant difference between the temperatures?

7. Calculate the Q_{10} effect using the following equation, where $M.R._2$ is the average mass-specific M.R. at the higher temperature, $M.R._1$ is the average mass-specific M.R. at the lower temperature, T_2 is the value of the higher temperature, and T_1 is the value of the lower temperature.

$Q_{10} = (M.R._2/M.R._1)^{(10/(T_2 - T_1)}$.

8. What is the Q_{10} for crickets? What does this suggest for metabolic rate?

Sex and Temperature and Metabolic rate

1. Create a new data table + graph... Choose grouped. Select “enter __ replicate values in side by side subcolumns” Click create.

2. Label “group A” as female and “group B” as Male. Label Row 1 as Ice and Row 2 as Room temperature.

3. Copy female ice bath mass-specific M.R. and **PASTE TRANSPOSE** the values into the first row in group A.

4. Copy the male ice bath mass-specific M.R. and **PASTE TRANSPOSE** the values into the first row in Group B.

5. Do the same thing for the Room temperature values for male and female.

6. Click "=Analyze" select 2-Way ANOVA under grouped analyses. Click Ok.

7. Under the "Factor Names" tab rename the column factor as Sex and rename the row factor as Temperature. Click Ok.

8. Review the Results. Record the 3 p-values (sex, temperature and interaction)

9. Look at the corresponding graph by clicking under the Graphs tab on the left (select the type of graph that you think best fits the data). Label your axes appropriately.

10. Is there a combined effect of sex and temperature on metabolic rate? (interaction p-value)

Size and Temperature and Metabolic Rate

1. Create a new data table + graph... Choose XY from the menu on the left. Select Enter and plot a single Y value for each point. Click Create.

2. In the X column, enter the masses (g) of each cricket that you measured.

3. In the Group A column, enter the mass-specific M.R. for each Room temperature cricket that corresponds to the entered mass in the X column. In the Group B column, enter the mass- specific M.R. for each ice bath cricket that corresponds to the mass entered in the X column. Each box in Group A with a value should have an adjacent box in Group B that is blank, and vice versa.

4. Click "=Analyze" and choose "Transform" under Transform, Normalize analyses. Click ok.

5. Check "Transform X values using", select "X=Log(X)" from drop down menu. Check "Transform Y values using", select "Y=Log(Y)" from drop down menu. Click ok.

6. Under Results section, select the new Transformed data table. Click "=Analyze" choose Simple Linear regression under XY analyses. Click OK.

7. Check to test whether slopes and intercepts are significantly different. Also check to show the 95% confidence band of the best-fit line. Leave the rest of the default options. Click OK.

8. Review the results of the analysis by clicking on the “Linear reg...” under the Results tab on the left. Record the R squared values under “Goodness of Fit” and the p-values under “Is the slope significantly none zero?”

9. Click the tab next to the results line. Click on “Are the lines different?” Record the p-value for “Are the slopes equal” and the p-value for “Are the elevations or intercepts equal?” and read the explanations.

10. Look at the corresponding graph by clicking under the Graphs tab on the left (Transform of...). Label your axes appropriately.

11. Is there a combined effect of size and temperature on metabolic rate? (Is the trend the same or different for room temperature and Ice?)

I. PROCEDURE:

Measurement of standard metabolic rate:

1. Check to make sure you have all necessary components of both respirometers.
2. Select a cricket from the terrarium. Record the sex of the cricket.
3. Place the cricket on the scale. Record the mass of the cricket.
4. Place a cotton ball inside the respirometer.
5. Pipet 1 ml of 15% KOH onto the cotton ball inside the respirometer.
6. Cover cotton ball with the black shield.
7. Place your cricket inside the respirometer and cover with the rubber stopper.
8. Insert calibrated capillary tube into the rubber stopper.
9. Assemble the control respirometer exactly in the same manner as above, except exclude the cricket.
10. Label each respirometer to indicate the experimental treatment or control.
11. Submerge both respirometers in the water basin and place them adjacent to each other in a horizontal position, utilizing the metal tube rack.
12. Leave respirometers for 5 minutes to permit the gas pressure to equilibrate inside the respirometers. Record the temperature of the water in the basin using a thermometer.
13. Note the location where the outer edge of the water reaches on the scale of the calibrated tube. This is the initial reading. For the next 5 min., record the position of the outer edge of the water at 1 min. intervals. Do this simultaneously for both respirometers.

    Note: if the water moves so rapidly that it is sucked up the entire length of the calibrated tube in less than 5 minutes, take recordings every 15 or 30 sec. over a shorter time period. If the water moves very slowly, take measurements at longer time intervals. Also Note: It is important to restrict movement of the cricket when measuring metabolic rate because exercise has profound affects on respiration, and thus could skew your results.
14. Calculate the amount of oxygen consumed per time (i.e. the metabolic rate) from your average number of units moved per time period by the water on the calibrated tubes using the following equation:

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The **mass-specific metabolic rate** can then be calculated with the following equation:

$$Mass\text{-}specific\ M.R. = \frac{ml\ Oxygen}{h} \div mass\ (g) = \frac{ml\ Oxygen}{g \cdot h}$$

15. Remove the respirometers from the water and open the respirometers to let fresh air in. Close the respirometers.

Repeat steps 11 - 14 above to take another measurement of respiration of the same cricket. Do this again to take 3 total measurements of a single cricket. Average the three measurements for both respirometers. Subtract the background rate (i.e. the avg. of the control respirometer) from the observed cricket rate (i.e. the avg. of the cricket's respirometer) to calculate the true metabolic rate of your cricket.

Do females and males have the same metabolic rate?

16. Place your cricket into the plastic bin next to the cricket terrarium. Select another cricket from the cricket terrarium that is of the opposite sex from the one that you made your first metabolic measurements.
17. Repeat steps 2 - 15 to measure the metabolic rate of your new cricket.
18. Repeat steps 2 - 15 for at least 3 individuals of each sex. Try to select crickets of different sizes so as to also measure the potential affect of size on metabolic rate.

What is the effect of size on metabolic rate?

19. Now, look at your distribution of sizes of individuals measured. If your crickets were all of a similar mass, take metabolic measurements (repeating steps 2 - 15) of 4 more crickets that are of different size than those you previously measured (2 bigger and 2 smaller).

What is the effect of temperature?

20. Repeat steps 2 - 15 on 3 more crickets, but instead add ice to the water in the water basin to make an ice bath. Allow the respirometers to equilibrate for 10 minutes prior to measurement. Check the ice bath temperature with a thermometer. Record this temperature at the beginning of each metabolic measurement. Repeat again on 3 more crickets with warm water from the tap.

Data Analysis R

Sex and Metabolic Rate

- **Import your data into RStudio.** You can do this by creating a comma-separated value (CSV) file with your data and then importing it into RStudio using the read.csv() function. Make sure that your data is formatted correctly in the CSV file. Each column should represent a different variable (e.g., sex, metabolic rate), and each row should represent a different observation (e.g., a different cricket).
- **Perform a t-test to compare the means of the two groups.** In this case, you want to compare the mass-specific metabolic rates of male and female crickets. You can do this in RStudio using the t.test() function. For example:
- t.test(mass_specific_MR ~ sex, data = your_data)
    - Replace mass_specific_MR with the name of the variable that contains your mass-specific metabolic rate data.
    - Replace sex with the name of the variable that contains your sex data.
    - Replace your_data with the name of your data frame.
- **Create a boxplot to visualize the results of your t-test.** You can do this in RStudio using the boxplot() function:
- boxplot(mass_specific_MR ~ sex, data = your_data,
- xlab = "Sex", ylab = "Mass-Specific Metabolic Rate")

You can customize the appearance of your boxplot using the various arguments that are available for the boxplot()function.

Size and Metabolic Rate

- **Create a scatter plot to visualize the relationship between size and metabolic rate.** You can do this using the plot() function:
- plot(mass, mass_specific_MR, data = your_data,
- xlab = "Mass (g)", ylab = "Mass-Specific Metabolic Rate")
- **Perform a linear regression analysis to determine if there is a significant relationship between size and metabolic rate.** You can use the lm() function to perform the linear regression analysis and the summary() function to view the results:
- model <- lm(mass_specific_MR ~ mass, data = your_data)
- summary(model)

- **Add the regression line to your scatter plot.** You can use the abline() function to add the regression line:
- abline(model)

Sex, Size, and Metabolic Rate

- **Create a scatter plot with two regression lines, one for each sex.** You can do this by first creating a scatter plot of all of your data:
- plot(mass, mass_specific_MR, data = your_data,
- xlab = "Mass (g)", ylab = "Mass-Specific Metabolic Rate")
    - Then, use the abline() function to add the regression lines for each sex:
    - abline(lm(mass_specific_MR ~ mass, data = subset(your_data, sex == "Female")), col = "red")
    - abline(lm(mass_specific_MR ~ mass, data = subset(your_data, sex == "Male")), col = "blue")
- **Perform an analysis of covariance (ANCOVA) to determine if the slopes or intercepts of the regression lines are significantly different.** You can use the aov() function to perform an ANCOVA. For example, to test for differences in slopes:
- model <- aov(mass_specific_MR ~ mass * sex, data = your_data)
- summary(model)

Temperature and Metabolic Rate

- Repeat the steps for **Sex and Metabolic Rate** using temperature as the grouping variable instead of sex.

Sex, Temperature, and Metabolic Rate

- **Create a boxplot to visualize the interaction between sex and temperature.** You can use the boxplot()function. For example:
- boxplot(mass_specific_MR ~ sex * temperature, data = your_data,
- xlab = "Sex and Temperature", ylab = "Mass-Specific Metabolic Rate")
- **Perform a two-way ANOVA to determine if there is a significant interaction between sex and temperature.**You can use the aov() function to perform a two-way ANOVA. For example:
- model <- aov(mass_specific_MR ~ sex * temperature, data = your_data)
- summary(model)

Size, Temperature, and Metabolic Rate

- Repeat the steps for **Sex, Size, and Metabolic Rate**, using temperature as the grouping variable instead of sex.