BIOL 208: Principles of Ecology and Evolution
Lab Week 5, 6, &7

Bioenergetics of Caterpillars

Background

The tobacco hornworm larva (*Manduca sexta*) eats the leaves of a wide range of solanaceous plants (tomato, tobacco). It passes through five, occasionally six, larval instars (stages between molts) before the pupal instar. Most larval growth occurs in the fourth and fifth larval instars, especially the fifth. The tobacco hornworm moth first emerges from the pupal case in early summer (source: Carolina Biological Supply Company "Teamed with Teachers" guide to Carolina Arthropods).

In this lab, you will determine some basic bioenergetics parameters of *Manduca sexta*, including the mass of food consumed, weight gained, assimilated energy, respiration, egestion or excretion, and production. The calculation of these parameters is described later in the laboratory.

The tobacco hornworm, *Manduca sexta* larva (caterpillar), pupa, and adult (moth)

Chapter 54 of your textbook gives an explanation of basic bioenergetics that may help with your interpretation of this study. Further reading in this chapter may also help you to put this study into a greater context.

Experimental Design

In this experiment, we will study the effects of the quantity and quality of food provided to tobacco hornworm larvae on their ingestion, assimilation, and excretion. We will have three treatments (high or low food level and high food with fiber added). Students will work in a group of three and each student will maintain one treatment container with two caterpillars. We will have seven groups per session and thus the lab session as a whole has seven replicates. Caterpillars within a container are *pseudoreplicates* because they are not independent; therefore, we will only use the mean ingestion, excretion, and weight of the two caterpillars within a replicate container.

It is highly recommended that you collaborate with other students in your group in the maintenance of your caterpillars (i.e., feeding and measurement); however, you are entirely welcome to do all measurements on your own caterpillars. Should you choose to share the responsibility of caterpillar maintenance and data collection, be sure each person in your group does their part, or contacts another group member if something will prevent them from performing these actions in a timely fashion. Neglecting caterpillars will mean that pooled class data are less than accurate; in addition, without regular cleaning, the food provided easily molds and the mold overtakes and kills the caterpillars.

Procedure: setup (Day 0 – Lab day 1)

1. <u>Each student does one setup</u>, which consists of:
   - One disposable plastic container with lid
   - Two tobacco hornworm larvae
   - Hornworm larval food at the appropriate level (high, low, or high + fiber)
   - Filter paper (or paper towels)

2. For each setup, moisten the filter paper (<u>it should not be dripping, just moist</u>) and place it in the bottom of the plastic container.

3. Punch <u>small</u> <u>holes</u> in the lid of your container to allow some air exchange. Do not make these holes too big; we want to keep the moisture and the larvae in!!

4. Weigh out the appropriate amount of food, depending upon the treatment you are setting up. For a high food treatment, add 3.0 g of caterpillar food to the container. For a low food treatment, add 1.5 g of caterpillar food to the container. For a high food + fiber treatment, add 3.0 g of fiber-mixed food to the container. Record this as the <u>initial weight of food </u>provided.

5. Weigh your larvae together (all at one time) and add them to the container. Calculate the average weight of each larva and write this into the data table at the end of this document.

6. Mark the container with your lab section ID and your name (yours or your group name if you are sharing responsibilities) and the treatment on a piece of tape.

7. Conditions during the study: tobacco hornworm larvae will grow and pupate quickly with light at about 27°C. We're keeping them in a growth chamber at 23°C with 14L: 10D light condition which will slow their development. Unless the larvae grow so fast they're pupating during the experiment, we will leave them under this condition. (*** tell your instructor if you have any larvae that pupate ***)

8. Place the containers in the incubator in the lab with the rest of the class' containers. Check at least every day to be sure the food has not been used up. Follow the instructions below for what to do over the duration of the experiment.

9. Discuss as a group possible outcomes and generate your hypothesis on each of the efficiencies that you learned in the lecture before the lab. Complete the table below by filling each cell with your predicted ranking (i.e, 1, 2, or 3). If you think two or three treatments are the same, put the same numbers for both cells.

Efficiency	High food	Low food	High + fiber
CE			
AE			
PE			
TLTE			

Procedure: Maintenance for the duration of the experiment

1. For the first week, check your larvae daily. If the paper towel is completely dry, continue checking them every day to ensure the filter paper does not dry out entirely and the larvae do not run out of food. If the paper towel doesn't get dry out after 24 hrs, you can check them every two days.

2. On the designated days (see table below), do the following:
   - remove all the fecal pellets and weigh them together; record this weight
   - weigh any uneaten food, record this value, and discard the old food
   - rinse out the container and dry it if it is dirty or if any mold has developed
   - replace the filter paper with new, moistened filter paper
   - on the designated feeding days (see table below), weigh out the appropriate amount of food (high or low) and provide this to the larvae. Be sure to record the weight of the food added.
   - weigh both larvae at one time, calculate the average larval weight, and add these values to the data table below
   - **If one of your larvae dies, let your instructor know promptly. Now you need to feed them a half of the original food**

Procedure: Break down experiment (see table below for date)

1. Collect the fecal pellets, weigh them and record the value.

2. Weigh both of your larvae at one time, record this weight, then calculate the average weight of each larva. Put these data into the table below.

3. Weigh any remaining (unconsumed) food and record this value.

4. Clean up: put the larvae into the container provided for this purpose. Throw away any remaining uneaten food. Wash out each container and set it out to dry. Clean up any remaining materials from the lab, if any.

5. Enter your data into the Google spreadsheet that your instructor provides you. The summary data will be posted on Moodle once all groups have entered their data.

Caterpillars fed <u>low</u> food level (~ 1.5 g when fed)					
Day	Food added (g)	Unconsumed food (g)	Fecal pellets (g)	Both larvae together (g)	Average larval weight (g)
0		---	---		
2					
4					
6					
8					
10					
12					
14					

Caterpillars fed <u>high</u> food level (~ 3.0 g when fed)					
Day	Food added (g)	Unconsumed food (g)	Fecal pellets (g)	Both larvae together (g)	Average larval weight (g)
0		---	---		
2					
4					
6					
8					
10					
12					
14					

Caterpillars fed <u>high</u> food WITH FIBER (~ 3.0 g when fed)					
Day	Food added (g)	Unconsumed food (g)	Fecal pellets (g)	Both larvae together (g)	Average larval weight (g)
0		---	---		
2					
4					
6					
8					
10					
12					
14					

Calculations

Perform the following calculations for the data collected over the entire week, using the Excel spreadsheet. **<u>Make sure to convert all the values into per-worm values</u>**.

- o **Food provided (g)** = sum of all the food you added to your container from Day 0 to the end of the experiment (per worm)
- o **Food unconsumed (g)** = sum of all the uneaten food during the experiment, including any uneaten food weighed at the end of the experiment (per worm)
- o **Consumption (g)** = Food provided – Food unconsumed (per worm)
- o **Egestion (g)** = sum of all the feces produced in the container for the entire experiment (per worm)
- o **Assimilation (g)** = Consumption - Egestion
- o **Weight gained (g)** = Final average larval weight (within each container) – Initial average larval weight (within each container)

<u>Calculations of efficiencies on the class data (posted on Moodle):</u>

*** *First do these calculations for each container separately, then calculate a mean and standard deviation for each variable (i.e. across all data)* ***

- o **Consumption efficiency (CE)**: (Consumption / Food provided) * 100

- o **Assimilation efficiency (AE)**: ((Consumption – Egestion)/ Consumption) * 100

- o **Production efficiency (PE)**: (Weight gained/(Consumption-Egestion)) * 100

- o **Trophic level transfer efficiency (TLTE)**: (CE/100 * AE/100 * PE/100) * 100

Assignment: due in lab after Spring Break

1. Calculations of *means and standard deviations* from class data:

    - Weight gained   - Assimilation

    - % Weight gained   - Consumption efficiency (%), CE

    - Food provided   - Assimilation efficiency (%), AE

    - Food unconsumed   - Production efficiency (%), PE

    - Consumption   - Trophic level transfer efficiency (%), TLTE

    - Egestion

2. Make a bar graph for each of the four transfer efficiencies (CE, AE, PE, TLTE), plotting treatment (food level) on each graph. Be sure to include standard deviation error bars on your graphs to display the variability in the data.

3. Run an ANOVA on the class data for CE, AE, PE, and TLTE. Do post-hoc comparisons to determine which means differ from the others. If there are differences among groups, which of the three efficiencies is the most likely source of the differences? Make sure you report all relevant statistics (F values, critical F values, p values, Q values). Did your results support or reject your hypotheses? Why or why not? Interpret your results from an ecological standpoint.

4. In our experiment, the caterpillars were fed on a diet of wheat germ. How do you think the various efficiencies would have changed if we had fed them on a more natural diet of tobacco leaves? Why might they have changed in these ways?

5. The temperatures in our study were probably lower than those the caterpillars normally experience in their normal summer growing season. Predict how <u>each efficiency</u> (deal with each separately) might have changed if the temperature had been higher and <u>give reasons for your predictions</u>. (Remember that the caterpillars are ectothermic).

6. Explain what these calculations mean for food webs, trophic levels, and types of organisms in the food web. For example, why does it matter how much a predator eats, excretes, assimilates, etc.? Why do some ecosystems have a greater number of trophic levels than others? Put this study into a greater context.