LAB 10 TROPHIC CHAIN DYNAMICS, 1

Modified from experiments described by Hudon and Finnerty (2013) and Gibson (2018).

OBJECTIVES:

- Distinguish the importance of top-down and bottom-up processes in population regulation
- Hypothesize how initial population densities alter the outcome of trophic interactions
- Estimate algal cell concentration in a culture from a standard curve using spectrometry
- Understand the role of model microcosms in experimental ecology
- Practice using sound experimental design methods, with appropriate controls and replication
- Establish experimental two-species microcosms

PRE-LAB ACTIVITIES: Before coming to lab, view the videos “Establishing an algae-brine shrimp ecosystem” (https://www.youtube.com/watch?v=2Z9RFVk4dCU) and “Using a spectrophotometer” (https://www.youtube.com/watch?v=rmLqEfaTa9k). These provide important background on the procedures and concepts we will use today. After viewing the videos, write down the experimental procedure described in the videos in your lab notebook. Your lab instructor will verify that you have completed this during the first five minutes of lab. In addition, complete the homework in Appendix E (p. 97) involving sample calculations. Your lab instructor will collect these prior to class.

INTRODUCTION

Trophic interactions (i.e., those involving predation or grazing) are among the most important regulators of population size, and their outcome can determine ecosystem stability. For example, when Yellowstone Park was established, in part to protect bison from extinction, wolves and other top predators were not part of the ecosystem, due to historical eradication efforts. With no natural predators, elk and deer populations flourished. These large herbivores decimated saplings of aspen and willow that grow in riparian (riverside) areas, leading to limited recruitment and a dramatic shift in riparian ecosystem structure that negatively affected numerous other species (Ripple et al, 2001).

Although understanding trophic dynamics is important for studying ecosystems, doing so in natural environments, especially with large animals, can be quite challenging and costly. Under such circumstances, many ecologists rely on model ecosystems to test and refine hypotheses about ecosystem dynamics. Model ecosystems allow precise manipulation of limited variables, as well as replication of treatment combinations, at a relatively low cost. The results of such experiments permit a more efficient approach to experimentation when ideas are subsequently tested under natural field settings. In this lab exercise, we will test and refine hypotheses about trophic interactions using a series of two-species microcosms involving *Platymonas* sp. algae and the marine crustacean, brine shrimp, *Artemia salina* (Fig. 1).

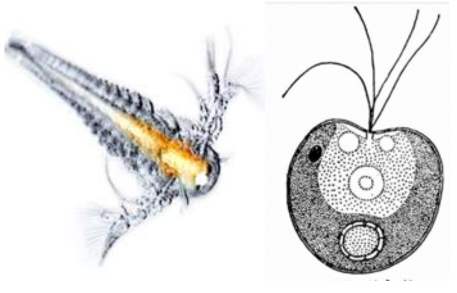

Figure 1. Illustration of a brine shrimp, *Artemia salina* (left), and the single-celled alga, *Platymonas* sp. (right). Note that illustrations are not scaled.

Adult brine shrimp are grazers that feed on a variety of unicellular algae, including *Platymonas* as well as bacteria and yeasts. They reproduce sexually, but over a longer time period than this experiment. They may die if overcrowded, due to insufficient food availability or build-up of waste. *Platymonas* algal cells will rapidly divide mitotically under favorable conditions (i.e., at room temperature when exposed to light).

EXPERIMENT

Working in groups of four students, discuss your plans for the experiment. First identify the **scientific question** you propose to address in your experiment. Next, articulate a **mechanistic scientific hypothesis** for the answer to that question. Finally, state one or more **predictions** stemming from your hypothesis regarding the outcome of the experiment. Along with the predictions, **sketch a graph** illustrating the patterns expected in the data consistent with your predictions. Include all of the above in your lab notebook. Refer to p. 15 for more information about how to state hypotheses and predictions.

Each experimental treatment can have two or three replicates for a total of six jars per group. Your lab instructor will establish one control treatment of algae with no brine shrimp. Your group should consider whether additional control treatments will be needed.

After your group has completed this discussion, complete the Algae-Brine Shrimp Ecosystem Hypothesis and Predictions form and submit it to your lab instructor for approval. Upon approval, proceed with setting up your experimental microcosms.

1. Open the Pasco Spectrometry software program (free download at https://www.pasco.com/downloads/spectrometry/index.cfm), and pair the spectrometer with your device (via Bluetooth or USB cable). You might find a version of the program compatible with your phone on the Apple App Store or Google Play Store – search for Pasco Spectrometry. There are also versions available that are compatible with Windows and Mac computers. Click on the "Analyze Solution" button on the top ribbon. Carefully pipette 2 ml of artificial sea water (with no algae) into a clean cuvette. Wipe the sides of the cuvette with a Kimwipe and place the cuvette in the spectrometer so that the light source passes through the clear sides of the cuvette. Under the 'File' menu, select 'New', and perform a dark calibration, followed by a light calibration. Click the record button, and the absorbance reading should indicate zero (i.e., a thick black line on the graph at Y = zero). See the "Using a spectrometer" YouTube video referenced above for additional information.

2. After resuspending the stock algal culture, carefully pipette 2 ml of the suspension into a clean cuvette, wipe the clear sides with a Kimwipe, and place the cuvette into the spectrometer, being careful to align the clear sides of the cuvette with the light source. Use the coordinates tool to set the wavelength to $\lambda = 680$ nm, and record the absorbance in your lab notebook. See the YouTube video, PASCO Spectrometer: Quick Start (https://www.youtube.com/watch?v=i5BexMng2WY) from 4:33 to 6:47 for a video demonstration of this process using the Pasco PS-2600.

3. Using the absorbance value measured above, determine the concentration (cells/ml) of *Platymonas* algae in the stock culture using the following formula, which was derived from a standard curve (Fig. 2):

$$\text{Absorbance} = [4*(10^{-7})x] + 0.0026$$

Where x = number of algal cells per milliliter. Enter this concentration into your lab notebook. Note that by solving for x, the formula becomes:

$$x = (\text{Absorbance} - 0.0026)/(4 * 10^{-7})$$

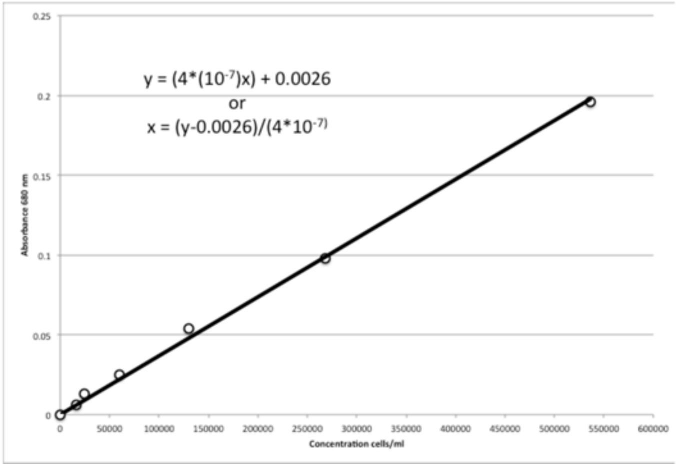

Figure 2. Empirically derived standard curve of Absorbance at λ = 680 nm and *Platymonas* concentration in cells per ml.

4. Next, calculate the amounts of algal stock culture and artificial sea water needed for each of your experimental microcosms, using the methods from your homework exercise. Write these down in your lab notebook and verify your calculations with your lab instructor. Although this may seem like a time-consuming step, the success of the experiment depends on correct calculations. Be sure each member of the group is comfortable performing these calculations because they will certainly appear on exams.

5. Using tape and a wax pencil, label your six microcosm jars with your name, lab section, and experimental treatment.

6. Use the graduated cylinder to measure the appropriate amount of artificial sea water into each jar. Remember to read the volume of sea water in the cylinder from the meniscus. Next, carefully pipette the appropriate volume of stock algal culture into each of the microcosm jars. Remember to resuspend the algae prior to pipetting.

7. Obtain a subsample of the brine shrimp culture in a Petri plate and, using a dissecting microscope, select the appropriate number of adult brine shrimp to add to each of your microcosm jars. This can be a little tricky. Be sure you choose healthy (swimming) adults and not eggs or larvae. If the culture is too dense to reliably select one at a time, dilute your sample with a little artificial sea water. When transferring shrimp into your experimental microcosms, minimize the amount of additional sea water added. Be sure you write down the number of brine shrimp actually added into each jar.

8. The jars will be stored under lights at room temperature for the next two weeks, after which we will record shrimp survival and measure algal concentration using spectrometry as above.

9. Place a couple of brine shrimp in the well of a depression slide. Add a few drops of the *Platymonas* stock solution, and place a cover slip over the well. Add more solution under the cover slip if needed to remove bubbles. Examine the shrimp feeding behavior under a light (compound) microscope using the scanning objective. Describe what you observe in your lab notebook, and include at least one sketch of what you see.

QUESTIONS

1. What is the main question addressed in your experiment?

2. What is your mechanistic scientific hypothesis that answers the question?

3. How does your experimental manipulation test your hypothesis?

4. What do you predict will be the outcome of the manipulation?

5. Why do you predict that – i.e., explain the dynamics inside the microcosm that lead to the predicted outcome.

6. What data will you collect? How will you analyze the data? What will be compared to evaluate support for your hypothesis?

7. How will the data be presented, graphically?

LITERATURE CITED

Gibson, J.P. 2018. Food chain dynamics in a simple ecosystem. Botanical Society of America, QUBES. doi:10.25334/Q4P419.

Hudon, D. and Finnerty, J. R. 2013. To build an ecosystem: an introductory lab for environmental science and biology students. The American Biology Teacher 75: 186-192.

Ripple, W. J., Larsen E. J., Renkin, R. A., and Smith, D. W. 2001. Trophic cascades among wolves, elk, and aspen on Yellowstone National Park's northern range. Biological Conservation 102: 227-234.