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Exploring Photosynthesis, Experimental Design and Submerged Aquatic Vegetation with Algae Beads

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Abstract

Algae beads are small alginate beads with green algae (*Chlorella vulgaris*) embedded in them. These beads can be placed in a CO_2 indicator solution and photosynthesis can be monitored based on colorimetric changes of the indicator. The beads are versatile tools that can be used for everything from simple demonstrations of photosynthesis to complex experiments exploring how environmental conditions such as light levels impact photosynthesis. The basic framework presented here can easily be modified to fit in with other examples. In this workshop we used the beads to review basic experimental design principles in the context of the effect of water clarity on photosynthesis in submerged aquatic vegetation. Participants designed and conducted an experiment using algae beads and we discussed how to incorporate them into courses.

Keywords: Photosynthesis, Experimental design, Algae beads

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INTRODUCTION

The opportunity for students to engage in guided inquiry using live organisms is a common goal for lab instructors. Organisms that are inexpensive, hardy, easy to work, and give predictable results are desirable for this type of exercise. Algae beads are small alginate beads that contain living algal cells. They are relatively inexpensive to purchase, easy to maintain, are easy for novices to manipulate, and give predictable and repeatable results.

The procedures presented here are meant to provide an example of how algae beads can be used to allow students to design and carry out their own experiments within a 3-hour lab period.

Context

One of the primary learning objectives of our course is the understanding and use of the scientific process. We frame our examples and exercises in the natural history and ecology of the Chesapeake Bay. This lab activity serves as the first experiment that our students conduct that incorporates all the parts of the scientific process that we cover. In previous weeks students have learned how to

- come up with a research question
- construct a testable hypothesis
- design an experiment with proper replication and controls
- describe their experiment in the format of a Methods Section
- analyze their data using R
- present their results using an appropriate graph and a quantitative comparison statement

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STUDENT OUTLINE

Pre-Lab Exercise – Delivered online

Exploring Submerged Aquatic Vegetation

Exercise 1: Basics of Respiration and Photosynthesis

Breathe in. Breathe out.

Such a simple action is at the heart of one of the most important processes your body is undergoing to sustain life: aerobic respiration.

Aerobic respiration and its counterpart photosynthesis are two main metabolic pathways that help organisms to harvest and store energy for cellular functioning. You might be asking yourself "Isn't this an ecology class? Why are we talking about metabolic pathways?". Don't worry. We're just covering the basics here; you'll learn much more in BSCI 170[1].

For eukaryotes (animals, plants, fungi, etc.) and many bacteria, aerobic respiration is how organisms break down food in order to turn it into energy. Aerobic respiration requires an input of sugar and oxygen to produce carbon dioxide, water, and energy in the form of ATP. The equation looks something like this:

$$\textbf{\textit{Sugar + } O_2 \rightarrow CO_2 + H_2O + ATP}$$

Aerobically respiring organisms (like yourself) are abundant, but their existence is due in large part to the presence of photosynthesizers. Photosynthesizers use light energy from the sun to produce oxygen and store energy that can be used in respiration. Photosynthesis requires the input of carbon dioxide, water, and light and results in stored energy in sugar, with oxygen as a byproduct. The equation for photosynthesis looks something like this:

$$\textbf{\textit{}}CO_2 + H_2O + Light \rightarrow Sugar + O_2$$

Plants respire too!

It likely doesn't surprise you to know that plants undergo photosynthesis. What may surprise you is that they also undergo aerobic respiration. When light levels are reduced, plants will open their stoma (little holes in their tissues) to let in oxygen and utilize the glucose (a sugar) they've created through photosynthesis to undergo aerobic respiration, releasing carbon dioxide in the process.

[1] BSCI170 is our second-semester introductory biology course that focuses on cell and molecular biology topics.

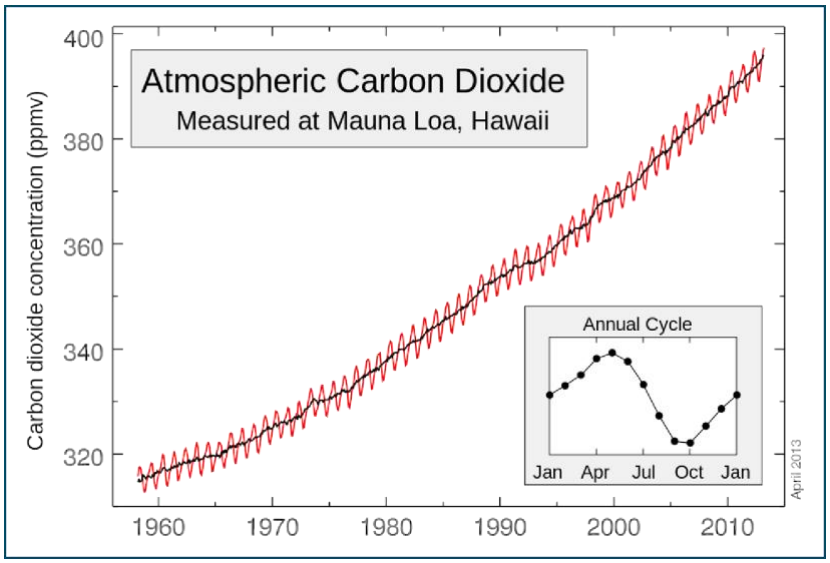

Figure 1. The Keeling curve. The red line shows detailed measurements, and the blue line represents an average over time. The inset graph shows how CO_2 levels fluctuate throughout the year - this is the pattern you see in the red line on the main graph. (*Image courtesy of Narayanese, Sémhur, and the NOAA, CC BY-SA 3.0, via Wikimedia Commons*)

To illustrate this point, look at the Keeling curve (***Figure 1***), which shows the increase in carbon dioxide in our atmosphere over time. Notice that although there is an upward trend of carbon dioxide concentration in the atmosphere, the line looks jagged, with carbon dioxide concentration increasing and decreasing every year. Like the breath you took at the beginning of this reading, the earth breathes on an annual basis. The northern hemisphere has a greater coverage of land and therefore a greater abundance of photosynthesizers. When it experiences greater amounts of light in summertime, atmospheric carbon dioxide decreases because the photosynthesizers are utilizing it. During the wintertime, atmospheric carbon dioxide increases because respiration by photosynthesizers dominates.

PHOTOSYNTHESIS AND pH

Let's look at what happens with the products of photosynthesis in an aquatic system.

When carbon dioxide is dissolved in water, a series of chemical reactions occur. These reactions cause the carbon dioxide and water molecules to break down and recombine. The result of this chemical reaction is carbonic acid (HCO_3^-) and hydrogen ions (H^+). The chemical equation looks something like this:

$$\mathbf{CO_2 + H_2O \rightarrow HCO_3^- + H^+}$$

Recall the amount of hydrogen ions in a solution is what dictates the pH. A greater concentration of hydrogen ions means a solution is more acidic (i.e., a **low pH**). So, a solution with a greater concentration of carbon dioxide would be more acidic and have a lower pH.

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How does this relate to photosynthesis?

When light levels are high and photosynthesis is occurring, carbon dioxide concentrations decrease, and the pH of the system increases. When light levels are low and plants instead undergo respiration, carbon dioxide concentrations increase, and the pH of the system decreases.

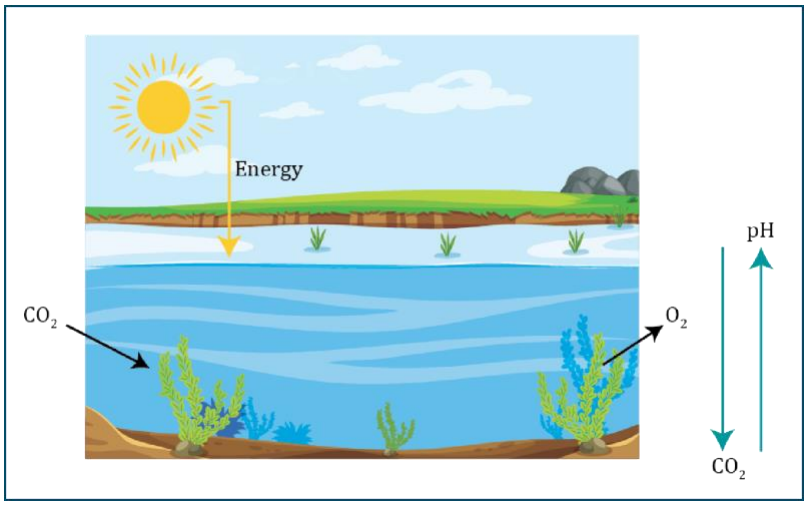

Figure 2. As aquatic plants photosynthesize, CO_2 levels in the water decrease and the pH of the water increases. © Van-Griner, LLC.

Perhaps you've heard about **ocean acidification** as the other great threat, along with global warming, associated with increasing greenhouse gases in our atmosphere. Ocean acidification is based on this same premise: as atmospheric carbon dioxide increases, more carbon dioxide becomes dissolved in ocean waters, resulting in decreased pH. This has a detrimental effects on many marine organisms (if you're interested in reading more, visit *https://go.umd.edu/ocean-acidification)*.

Exercise 2: Submerged Aquatic Vegetation

Submerged aquatic vegetation (also known as **SAV**) are flowering plants that spend their life in aquatic environments. While their aquatic life cycle makes SAV unique among plants, they are photosynthetic organisms that require carbon dioxide and light to thrive. They are also ecosystem engineers, meaning that they alter the ecosystem in a beneficial way for other species.

Figure 3. Numerous species fall within the category 'submerged aquatic vegetation', some more abundant in saltwater (like A, eelgrass, *Zostera marina*), some more abundant in brackish water (like B, widgeon grass, *Ruppia maritima*), and some more abundant in fresh water (like C, wild celery, *Vallisneria americana*). (*Images courtesy of: A: Nozères, Claude, CC BY 4.0; B: Edward G. Voss @ USDA-NRCS PLANTS Database / USDA NRCS. 1992. Western wetland flora: Field office guide to plant species. West Region, Sacramento, CA., Public domain; C: Fredlyfish4, CC BY-SA 4.0, all via Wikimedia Commons*)

Throughout the Chesapeake Bay and its tributaries, SAV has suffered substantial losses in abundance, which has important consequences to the Chesapeake Bay ecosystem as a whole.

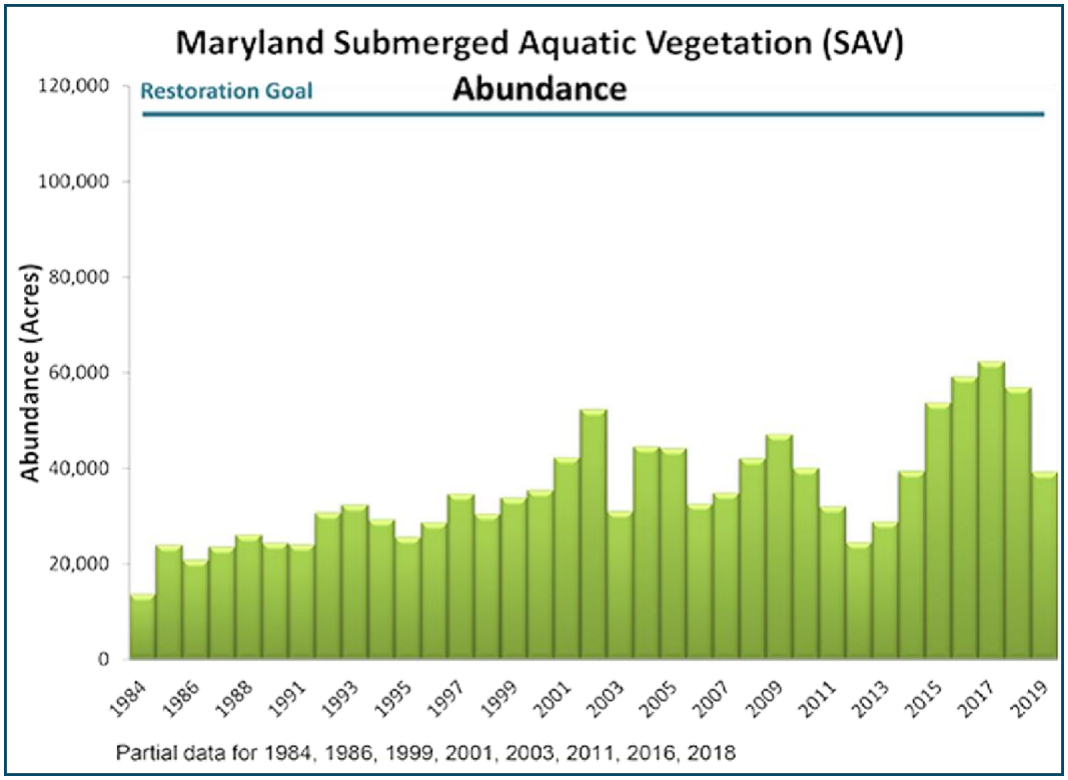

Figure 4. The coverage of seagrass in the Chesapeake has fluctuated dramatically since monitoring began in 1984, but it has not come close to the restoration goal set by agencies in the Bay's watershed (*Image courtesy of Maryland DNR*)

Watch the following video to learn more about SAV: *https://go.umd.edu/SAV*

Water clarity

As you saw in the previous video, water clarity is one important factor that affects how well SAV can grow. What leads to poor water clarity?

Watch the following video to understand more about water clarity in the Chesapeake Bay:

https://go.umd.edu/clarity

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In-lab Activities

Investigating Submerged Aquatic Vegetation

During your pre-lab, you learned that submerged aquatic vegetation (SAV) in the Chesapeake Bay is negatively affected by decreases in water clarity. As water clarity declines, the ability of light to effectively penetrate the water column and provide enough light energy to allow SAV to properly photosynthesize is threatened. The task of your research team for today is to design and conduct an experiment to test the effect of light quantity or quality on SAV.

Figure 1. Eel grass (*Zostera marina*) is one of the most abundant seagrasses in the Chesapeake. The only species of seahorse that calls the Bay home is the lined seahorse (*Hippocampus erectus*). They spend most of their life in these grass beds. (*Image courtesy of Maryland DNR)*

It would be impractical for us to conduct experiments with actual SAV, so we will be using Algae Beads. These are little gel beads with the green alga *Chlorella vulgaris* embedded within them. When exposed to light, the algae undergo photosynthesis and cellular respiration, similar to SAV in the natural environment. You will place the beads in water that contains an indicator dye that changes color in relation to pH. Recall that as photosynthesis occurs and CO_2 is removed from the water, the pH will change.

How will the pH change when photosynthesis and respiration are both occurring together?

How will the pH change when only respiration is occurring?

Activity 1: Basic Algae Bead Procedures

Although you will get to design much of your experiment, we will start with a quick experiment so that you can see how the algae beads work.

1. Set your spectrophotometer to **550 nm** and zero it with distilled water.

2. Label an empty cuvette for your experiment. Be sure that the label doesn't disrupt light reaching the algae beads.

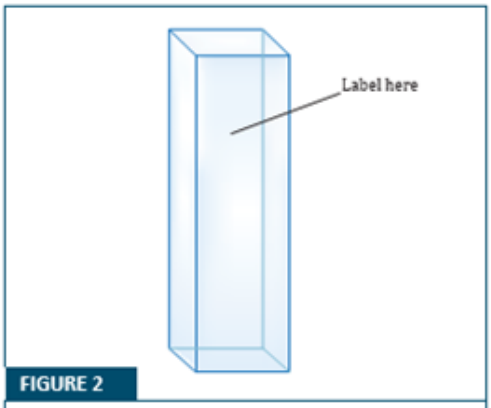

FIGURE 2

Adding a label to the cuvette. ©Van-Griner, LLC

3. Label a transfer pipette **Algae** and convert it into a scoop by cutting the transfer pipette across the bulb diagonally. Use the algae transfer pipette to transfer **5** algae beads into the of the cuvette.

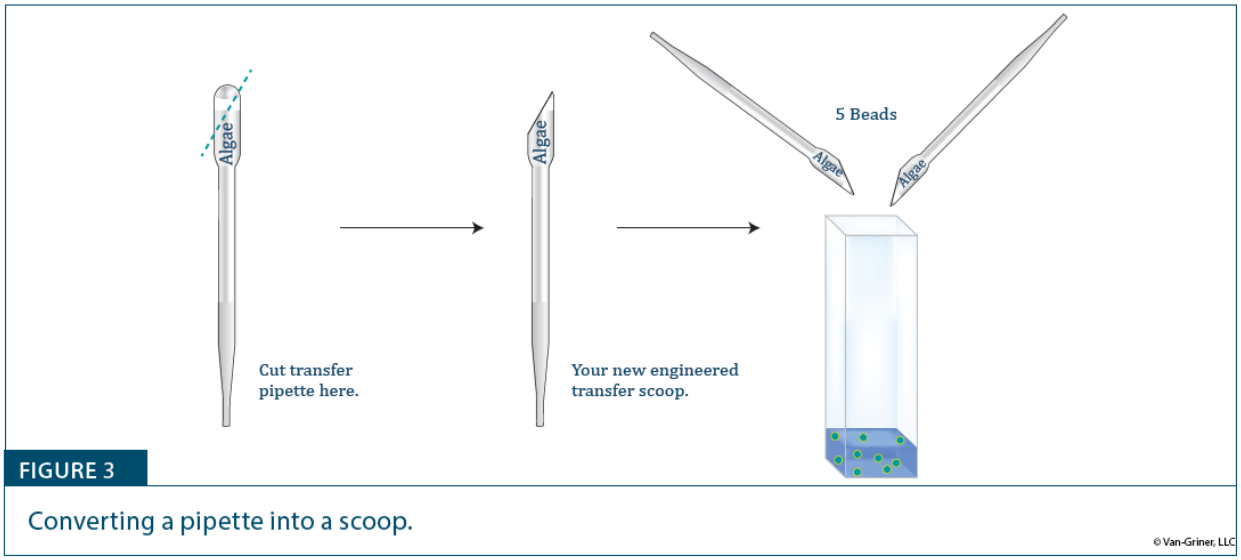

FIGURE 3

Converting a pipette into a scoop.

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4. Label a new transfer pipette **Excess** and use it to remove and discard the liquid that transferred along with the beads.

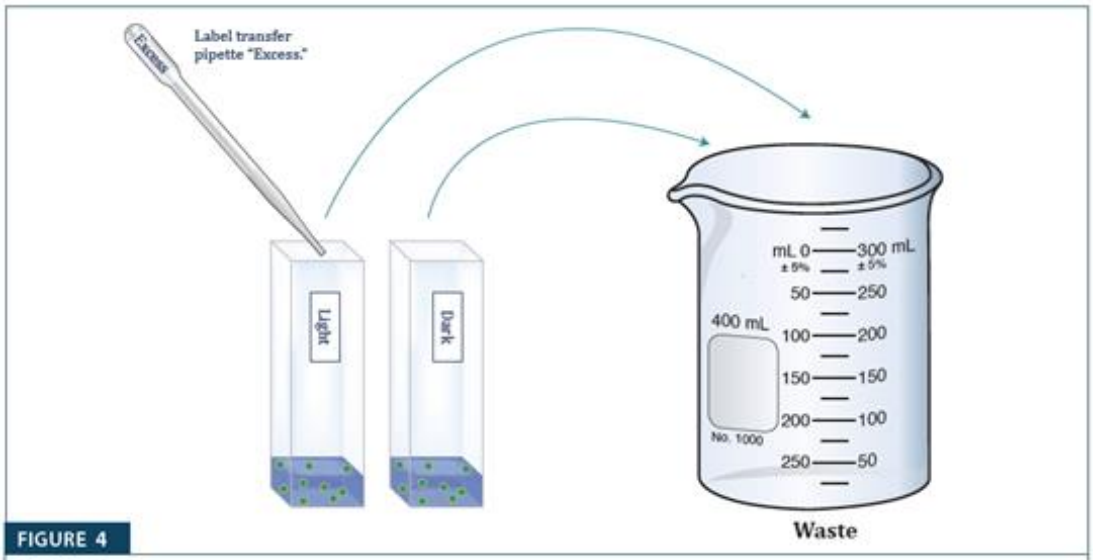

FIGURE 4

Transferring excess to a waste container.

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5. Use a micropipette to add 1 mL of distilled water to each of the cuvettes. Let the algae beads incubate in the water for 5 minutes to allow indicator within the bead to wash out.

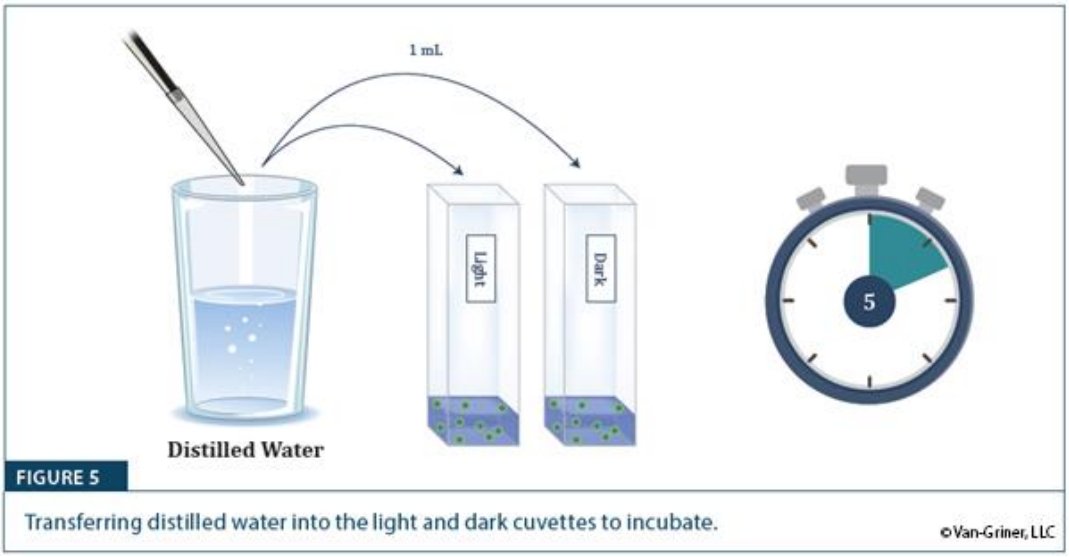

FIGURE 5

Transferring distilled water into the light and dark cuvettes to incubate.

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6. Label a new transfer pipette **Wash.**

7. Use the **Wash** transfer pipette to remove the water from the cuvette. Discard the water into the waste container.

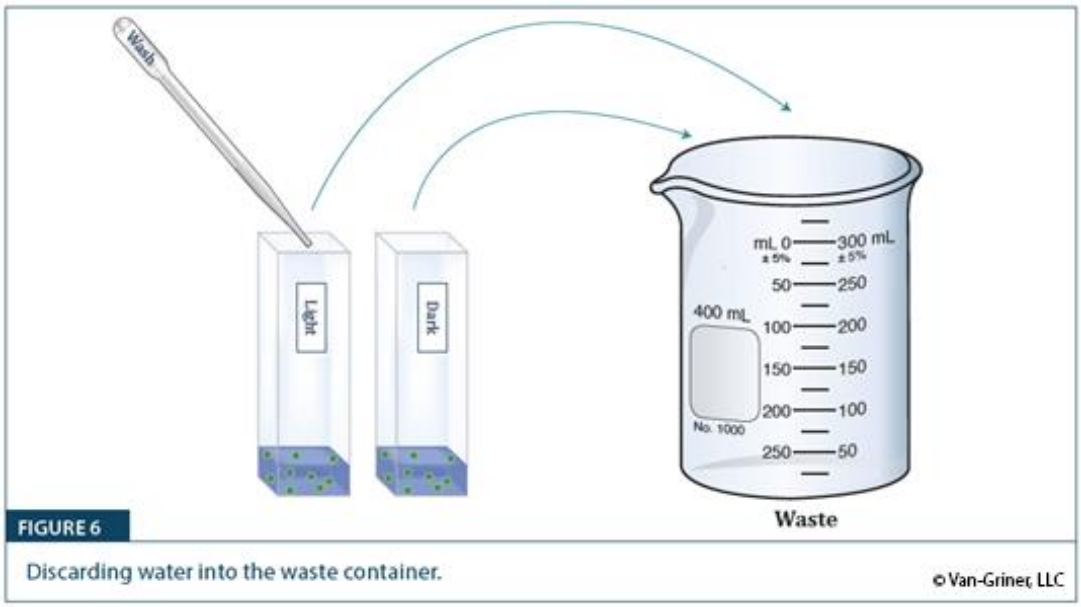

FIGURE 6

Discarding water into the waste container.

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8. Use a micropipette to transfer 1 mL of CO_2 indicator to each cuvette. Cap cuvettes tightly.

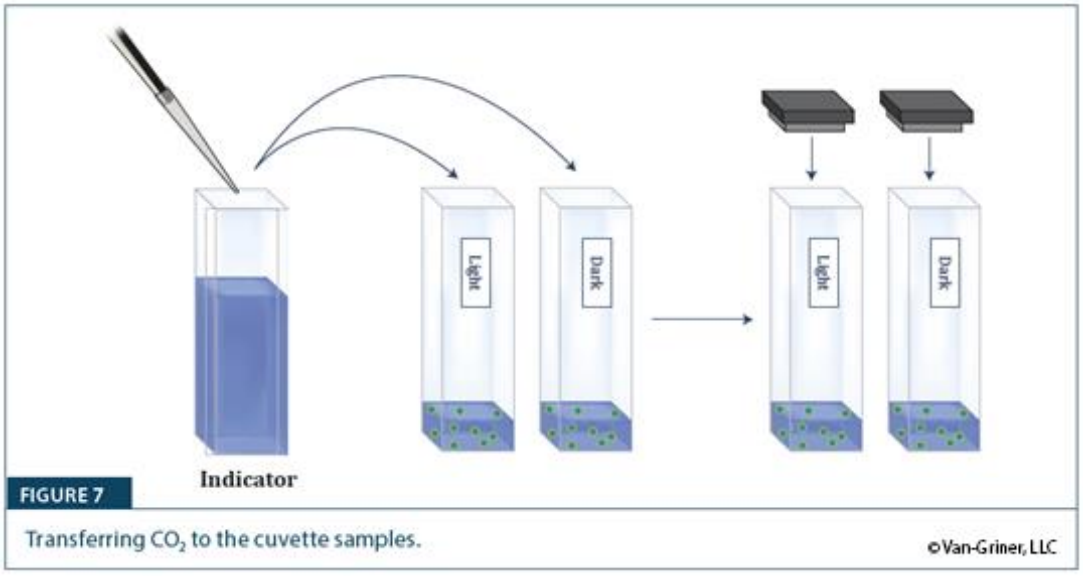

FIGURE 7

Transferring CO_2 to the cuvette samples.

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9. Place your cuvette under the light.
10. Collect data starting at time = 0 minutes. Every 5 minutes thoroughly mix the CO_2 indicator in the cuvette by inverting it 3 times. Gently tap the top until all the algae beads are at the bottom of the cuvette. Use the spectrophotometer to record the absorbance at 550 nm (be sure to zero the machine with distilled water). Be quick about taking this reading and immediately return the cuvettes to the experimental conditions.
11. Collect data for 15 minutes.

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Cleanup Procedures

1. Use your pipette labeled EXCESS to remove the indicator from the cuvettes and put it in the Liquid Waste.
2. Pipette ~1 mL of distilled water into each cuvette.
3. Place the cap on the cuvette and mix to rinse the beads.
4. Use the WASH pipette to remove the water from the cuvette and discard into the Liquid Waste.
5. Repeat the distilled water wash until the water is clean (2-3 times).
6. Use the WASH pipette to move remaining water into Liquid Waste.
7. Move the algae beads back to their container.
8. Put the cuvettes upside-down on the rack to dry.

Activity 2: Investigating the Effect of Light Levels on Photosynthesis

Experimental design

Your group needs to design and carry out an experiment. The research question that you are trying to answer is how light level or quality affects photosynthetic rates of SAV.

There are a bunch of different materials available for you to set up this experiment. You do not need to use them all.

MATERIALS AND EQUIPMENT AVAILABLE

- LED light banks: These lights are optimized to produce light in the spectrum that plants use for photosynthesis.
    - Light meters
- Shade cloth: There are pieces of shade cloth in varying degrees of light blocking. You can layer pieces to get even more variety.
    - Frames
    - Assorted binder clips and other stuff you might find handy

LIMITS

- Two treatments
- 10 cuvettes
- 50 beads
- Only 1 light
- Time course ≥ 45 minutes

With all of this in mind, work with your team to design your experiment. Write everything down on the ***Experimental Design Form.***

Your group needs to have your experimental design checked by your TA before you can start your experiment.

Conduct the experiment

Record your data and any notes about your experiment.

Analyze data

For our course we have students use R to analyze their data and create an appropriate graph. For this workshop, make a graph using whatever you feel comfortable with.

EXPERIMENTAL DESIGN FORM

Research Question	
VARIABLES	
Dependent	
Independent (or 2nd dependent if correlation experiment)	
Controls	
Hypothesis	
Statistical Test You Plan to Use to Analyze Results	
Graph Type	
Data Table	

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MATERIALS NEEDED

METHODS

MATERIALS

Equipment

Per group

- Spectrophotometer – Visible light spectrophotometer with a cuvette holder that is capable of holding 1 cm^2 cuvettes
- Cuvette – plastic 1 cm square – 12
- Cuvette cap - 12
- Micropipette capable of delivering 1 mL
- Liquid Waste beaker (400 mL)
- Frame for holding grow light
- Grow light – Many options for this. We use a 5” x12” LED full spectrum grow light. An incandescent bulb in a desk lamp will work as well.
- Frame for shade cloth
- Shade cloth – We purchase knitted shade cloth from *shadeclothstore.com* in a range of weights from 30-90% light blocking and cut it into 12”x24” pieces.
- Light meter – any simple light meter will do for this lab. You really only need 1 or 2 for the whole classroom.

Supplies

- Algae Beads – 50 (see Instructor Notes for more information)
- Distilled water
- pH Indicator solution (see Instructor Notes for more information)
- Kim wipes
- Disposable transfer pipette – 3
- Micropipette tips
- Label tape
- Marker

Frames

Grow Light Frame

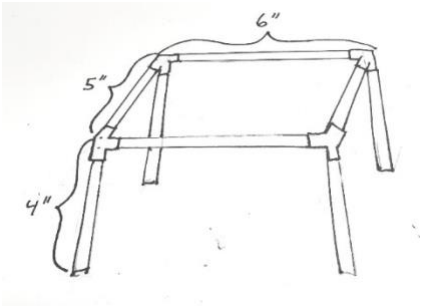
These dimensions work for our grow lights which hang from a chain. Your dimensions and design will likely need to be adjusted for your lights.

- 24”L x 14”W x 32”H
- 18’ ½” PVC pipe
- 4 3-way connectors
- 2 T connectors

Shade Cloth Frames

These provide plenty of room for this experiment. You may need to adjust the setup for your individual experiment.

- 6”L x 5”W x 4”H
- 3.5’ ½” PVC pipe
- 4 3-way connectors



NOTE: The 3-way PVC connectors are not always available at big-box hardware stores. There are a multitude of online suppliers for them.

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NOTES FOR THE INSTRUCTOR

Algae Beads

Algae beads are small alginate beads that have a green alga encapsulated in them. The beads are placed in a CO_2 indicator solution for conducting experiments. When exposed to light, the algae photosynthesize and the pH increases, causing a color change in the indicator. This allows the experimenter to do things like manipulate light levels to examine the effect that this has on photosynthesis. The algae will, of course, undergo respiration if the beads are put in the dark, allowing for the demonstration of respiration.

There are numerous suppliers of the beads. The instructions provided for this workshop are based on beads from Algae Research and Supply (*https://algaeresearchsupply.com*). We have also used the beads available from Bio-Rad (*https://www.bio-rad.com*). Both worked well. The beads available from Bio-Rad are smaller and contain a different algal species, *Scenedesmus obliquus.* The only change in the procedures was that we used 10 beads instead of 5. We have noticed that there is more variation in the size of the beads that are supplied by Algae Research and Supply, but we have done experiments and found that 5 of the smaller beads and 5 of the larger beads do not produce significantly different results. There are other suppliers of beads, but we have not had any experience with them.

Indicator

The CO_2 indicator is a hydrogen carbonate indicator and is available from the bead suppliers as a 10X concentrated solution. It contains water, ethanol, sodium hydrogen carbonate, thymol blue and creosol red. If you don't have access to spectrophotometers, or don't want to use them, the bead suppliers also provide colorimetric charts that you can use to visually determine the pH of the CO_2 indicator.

One temptation when working with this system is to try to convert the absorbance value to an exact pH or to use it to determine a photosynthetic rate. In our experience, this does not go well. We have students compare and report absorbances, focusing on relative changes in the amount of photosynthesis that is occurring.

Assessment

This Research Report is a group assignment in our course and is turned in one week after the lab session.

Part 1 – Google Sheet (15 pts.)

- Create a Google Sheet that contains the data from your experiment and is set up to be imported into R.
- Name it GROUP #_Section #_SAV Report (Replacing Group # and Section # with your info)
- Share the Sheet with all the members of your group and your TA

Part 2 – Written Report (65 pts.)

Answer the following questions (do not include the questions, just provide the answers):

1. What was your research question? (5 pts.)
2. What was your hypothesis? (8 pts.)
3. Write out your Methods. Don't include any preliminary experiments, only your final experiment. (10 pts.)
4. Write a short summary of your results. Be sure to include:
    a. A description of the reasoning behind your experiment. (5 pts.)
    b. A QC statement that includes the results of your statistical analysis. (8 pts.)
    c. An appropriate table that contains the data from your experiment. (5 pts.)
    d. A figure, including a caption, that illustrates the results of your experiment. (10 pts.)
    e. The conclusions that you made from your experiment. Be sure to refer to your results. (5 pts.)
5. Based on what you learned from your experiment, what would you do next if you had the chance to work with this system some more? (5 pts.)
6. Prepare and include an author Contribution Statement. (4 pts.)

Part 3 – R Code (20 pts.)
Copy your script at the end of your written report. Be sure that there are no errors and that you have an **appropriate #comment line before each line of code**. Include the link to your Google Sheet containing your data. Your TA will be copying this into R and running it.

ACKNOWLEDGEMENTS

I would like to thank the countless teaching assistants who gave us feedback as they used this exercise in the classroom. I would also like to express my appreciation for the support received from the Biological Sciences Program at the University of Maryland for the development of new lab exercises. Thanks to Van-Griner Learning for the use of their illustrations in this publication. Thanks to Algae Research and Supply for providing materials for use in the workshops.

About the Author

Hans is the Lab Coordinator for the Principles of Ecology and Evolution Lab (BSCI161) at the University of Maryland. He holds a B.A. in Biology from St. Mary's College of Maryland, an M.S. in Entomology from the University of Maryland, and an M.D.E. in Distance Education from University of Maryland, University College. His current research focuses on educational outcomes in laboratory settings and a survey of tiny Miocene shark and ray fossils found along the Chesapeake Bay.

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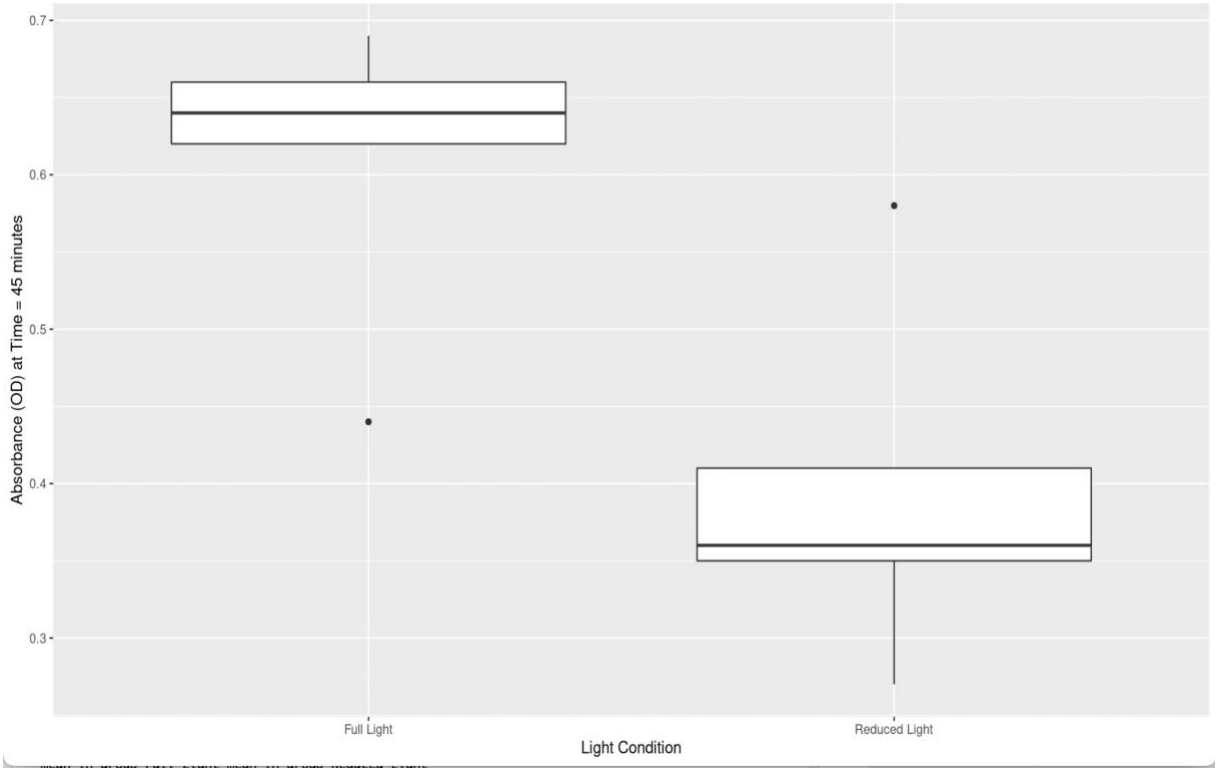
APPENDIX A

SAMPLE RESULTS

From a student research report, Fall 2023:



Algae beads were either placed in a full light condition (1600 lux) or reduced light condition (320 lux). Absorbance of the indicator was measured every 5 minutes and the graph has the absorbance of 5 trials from each light condition after 45 minutes in the light. Graph shows absorbance (OD) as a function of light condition. The mean absorbance at t = 45 minutes obtained from the indicator until the full light condition was 0.610 OD, while it was 0.394 for the partial light condition. The degrees of freedom is 7.8061, the t-Value is 3.1827, and the p-Value is 0.01337. As the p-Value is less than 0.05, the data is significant.

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