

Lab 3: Photosynthesis *

BIO 3103, Baylor University

Background information

In the presence of light, photosynthetic organisms can utilize light and carbon dioxide (CO_2) to make sugars - the process of photosynthesis. The sugars made are used by these organisms, and the organisms that eat them, as a source of energy. A by-product of photosynthesis is the liberation of oxygen (O_2).

Alongside photosynthesis, these organisms are consuming oxygen via respiration. Though respiration is always occurring, photosynthesis is a light-dependent process and only occurs during certain hours of the day. It is impossible to directly measure gross photosynthesis (or the total amount of O_2 produced), but we *can* measure respiration (R) as the **rate of O_2 consumed** in the dark, and the **rate of O_2 produced** in the presence of light as a measure of net photosynthesis (P_{net}). Combining these direct measurements, we can estimate gross photosynthesis (P_{gross}).

$$P_{\text{net}} = P_{\text{gross}} - R \quad (1)$$

Using the equation above, we can directly measured terms in **blue**, and may use them to calculate **P_{gross}** .

Organisms capable of photosynthesis span an incredible range of morphology, from *unicellular algae* to *vascular plants* (Fig. 1). While every algae cell is photosynthetic, *aquatic macrophytes* (i.e. vascular plants adapted to live in aquatic ecosystems) have large amounts of specialized tissue devoted to the transportation of resources and structural support. These two organisms compete for very similar resources (such as sunlight and dissolved nutrients) - how do their morphological adaptations convey a competitive advantage?

Objectives

You will form hypotheses to test questions about photosynthesis in 2 aquatic organisms - algae and a common aquatic macrophyte.

1. Which organism has the highest rate of *biomass specific* gross photosynthesis?
2. Which organism has the highest rate of *biomass-specific* respiration?
3. Which organism has the highest rate of net primary production (NPP) per day?

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Figure 1: Aquatic algae (left pane, in both meta- and periphyton communities) and aquatic macrophytes (right pane) are photosynthetic organisms that evolved in aquatic ecosystems.

Materials & methods

You will be using a common **oxygen-change method** to determine rates of photosynthesis and respiration. Biological oxygen demand (BOD) bottles use a stopper that prevents gas exchange, which provides a means of isolating processes happening inside the bottle from the outside environment.

Materials

- 10 L photosynthesis solution: 10 L DI Water, 917 mg CaCl₂, 960 mg MgSO₄, 584 mg NaHCO₃, 154 mg KHCO₃
- Samples of algae and macrophyte
- 330 mL BOD bottles
- Dissolved O₂ meter (DO Meter)
- Light source (> 400 µE m⁻²s⁻¹)
- Aluminum foil
- Stir bars / stir plate
- Sieves (fine mesh)
- Forceps
- Drying boats (aluminum)
- Analytical balance (capable of 0.001 g)
- Drying oven

Oxygen production varies with the intensity of light, but reaches saturation as light intensity increases (Fig. 2). Since different organisms display different light-response curves, we need to saturate their photosystems with high light levels to account for a potential confounding variable in our experiment.

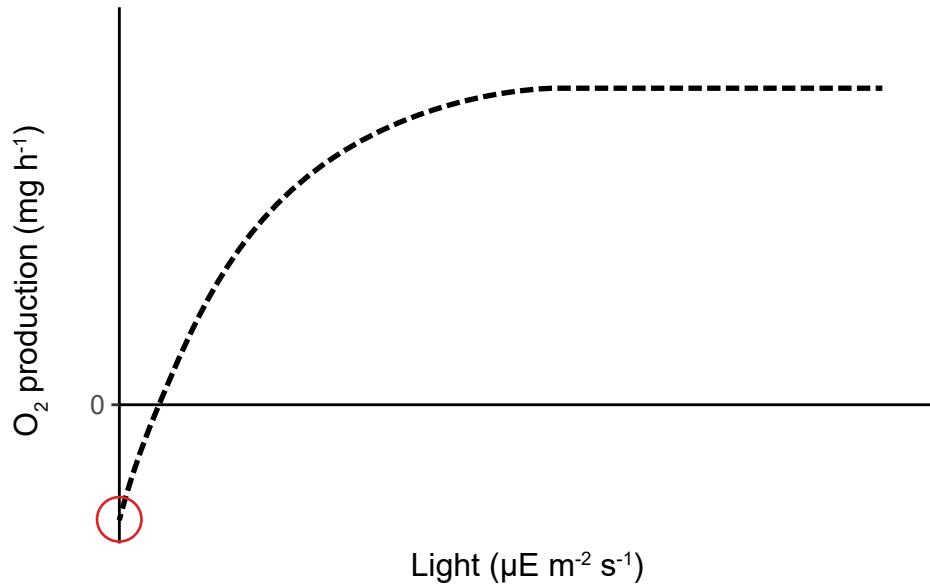


Figure 2: A photosynthesis light-response curve illustrates that as light intensity increases, dissolved oxygen (DO) production eventually becomes saturated. In the dark, photosynthesis shuts off, and respiration causes the rate of DO production to fall below 0 (red circle on the y-axis).

Methods

1. Each group will be responsible for **one** of the two species. Fill 12 BOD bottles with photosynthesis solution (fill to brim - the idea with BOD bottles is for the glass stopper to push any excess water out of the seal the stopper creates).
2. Carefully transfer a representative sample of your organism into 6 of the BOD bottles. Add a small stir bar to each bottle, and place the glass stopper and plastic cap on the bottle to seal. Use the DO probe to measure oxygen concentration in the remaining bottle (your control bottle).
3. You will have 6 replicates for each organism. Wrap the bottles in aluminum foil to block light, and record the initial times for these samples.
4. While you wait (at least 2 hours)...
 - Observe samples of these organisms under a dissecting/compound microscope and note differences in morphology. Note differences in the proportion of support tissues (i.e. stems) vs. photosynthetic tissues for each organism.
5. After at least 2 hours, remove the foil and measure DO concentrations in the bottles. Make sure to record the end time each time you take a DO measurement.
6. Place all bottles under a light source.
7. Wait at least 1.5 hours.
8. Measure the DO concentrations in the bottles.
9. Carefully empty the BOD bottle into a fine-mesh sieve to separate the sample from the photosynthesis solution. Collect/scrape the sample into a labeled aluminum drying tin, and place tins into a drying oven for 24 hrs at 105°C.

Data analysis

You can directly determine P_{net} (from the light treatment bottles) and R (from the dark treatment bottles) by calculating the change (Δ) in dissolved oxygen (DO) concentrations:

$$\Delta DO = DO_{final} - DO_{initial} \quad (2)$$

You also recorded the elapsed incubation time (Δh), the volume of the BOD bottles (0.330 L), and the mass of the sample (in dry weight, g). You can then normalize ΔDO to volume (L) and dry weight (g), and use that to then calculate P_{gross} , or the total oxygen produced by photosynthesis per unit biomass.

For the 1st, and 2nd questions, a two-sample t-test comparing the rates of each process (P_{gross} and R) will tell you if there are significant differences between each organism. Bar-graphs (with error-bars display the standard error of the mean) are a good way of displaying this data visually.

The 3rd question requires you to construct a simple **model**. A model is a way to represent a natural process using mathematics. Some models are simple (like the one you will construct), and some are incredibly complex. What assumptions can you make to tackle question 3? If we assume that these organisms respire for 24 hours a day, and only photosynthesize when the sun is out (10 hours a day), we can use these values as a foundation for our model (Fig. 3). While this makes the maths significantly easier, what are the limitations of this assumption?

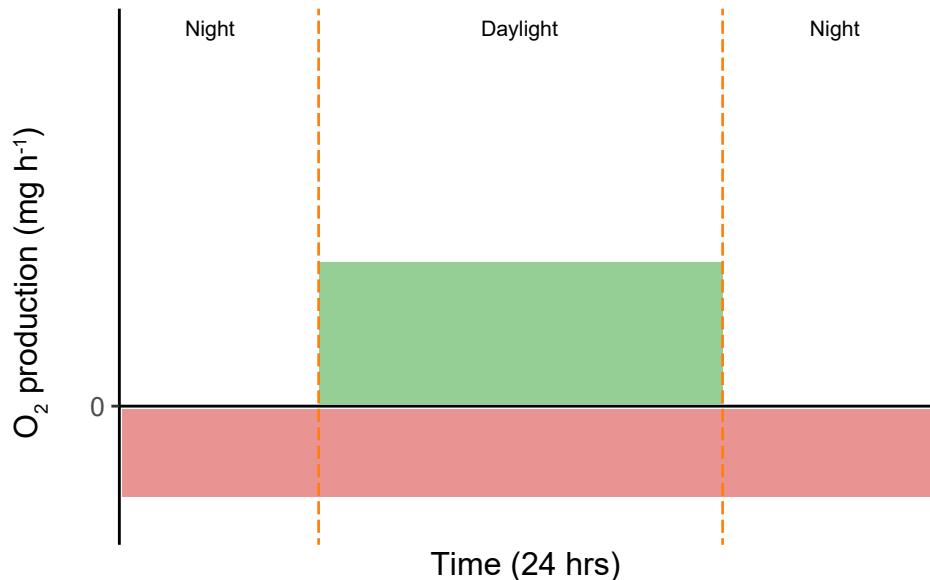


Figure 3: A photosynthesis light-response curve illustrates that as light intensity increases, dissolved oxygen (DO) production eventually becomes saturated. In the dark, photosynthesis shuts off, and respiration causes the rate of DO production to fall below 0 (red circle on the y-axis).

There are no associated statistical tests for question 3, since you should calculate daily values using mean P_{gross} and mean R (no replicate values for this question). You can present the results of your model in the text of your results section.

Lab report specifics

1. Introduction

- Importance of photosynthesis
- Morphological adaptations of aquatic photosynthesizers
- Objectives
- Hypotheses

2. Methods

- Oxygen change method (light/dark treatments)
- Experimental design
- Calculations / statistics / model explanation

3. Results

- Results/graphs/statistics for questions 1 and 2
- Results for question 3

4. Discussion

- Hypotheses rejected/supported?
- Provide a coherent explanation for the patterns you see in the photosynthesis and respiration data (use your observations of morphology and the information you covered in your introduction to tie everything together)

Table 1: Datasheet for BOD experiment.

Table 2: Calculation sheet for BOD experiment.