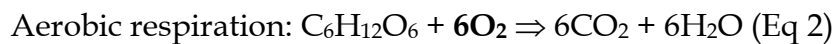
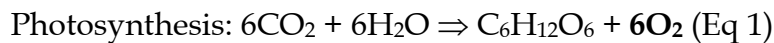


## Aquatic Ecology Lab Exercise 3: Aquatic Primary Production & Metabolism

### I. Introduction

Lake productivity is perhaps the most widely measured limnological variable. It is important for determining whether lakes are eutrophic as a result of anthropogenic nutrient loading as well as for determining the primary productivity of the autotrophs which generally form the energy base of aquatic food webs and ecosystems. In this exercise, we will examine the productivity of the greenhouse pond using changes in dissolved oxygen as a measure of the amount of carbon taken up by photosynthesis:



Recall that in most ecosystems, photosynthesis and respiration are occurring simultaneously. Photosynthesis adds  $\text{O}_2$  to the water, and respiration removes it. These familiar photosynthesis and aerobic respiration equations show that by measuring changes in  $\text{O}_2$ , we can determine the rates of photosynthesis and respiration in an ecosystem.

The technique we will use is called the light/dark bottle method. In clear-glass "light" bottles, we can incubate lake water over a period of time to indirectly measure photosynthesis, or gross primary productivity (Eq 1). If photosynthesis is greater than respiration in the light bottle, then  $\text{O}_2$  will increase over time. However, whole-community respiration ( $\mathbf{R_T}$ ) is still occurring in the light bottles, because all organisms living in the water are respiring. This means that a light bottle gives you an estimate of net ecosystem productivity ( $\mathbf{NEP}$ ).

Using another set of "dark" bottles (painted to prevent light from entering), we can fill them with lake water and let them incubate over time. Since photosynthesis is blocked in these bottles,  $\text{O}_2$  declines through time solely as a result of decomposition and respiration of carbon compounds floating in the water (by bacteria, plankton, macroinvertebrates) (Eq 2). Thus, the dark bottles give us a rate of total ecosystem respiration through time ( $\mathbf{R_T}$ ).

The difference between the final oxygen concentrations between the light and dark bottles is thus a measure of total photosynthesis, or gross primary production ( $\mathbf{GPP}$ ). Therefore, with some relatively simple measurements of  $\text{O}_2$ , we are capable of teasing apart the individual components of lake metabolism.

In addition, we will take measurements of  $\text{O}_2$  as a function of depth in the pond during mid-day to determine if we can detect whether the Production:Respiration ( $\mathbf{P:R}$ ) ratio might vary with moderate depth differences.

## II. Equipment

Oxygen Meter	Ropes or BOD bottle trapeze (+sinkers & float)
Self-stirring BOD Probe	Van Dorn sampler
Clear BOD bottles	Labels for bottles
Dark BOD bottles	Clips or string for bottles (knife/scissors),
Tape measure/meter stick	Depth sounder
Aluminum foil	Secchi disk and %Irradiance meter
Boat (?)	Transect line

### Material options for experiment:

Colored cellophane	Fish
Filtering apparatus	Plankton net
Organic sediment	Mineral sediment

## III. Procedure

### A. Productivity using light and dark BOD bottles

- 1) Using the Van Dorn sampler, collect water samples from known depths. Start at the deepest depth first.
- 2) For each sample depth, remove the sample from the Van Dorn bottle immediately upon retrieval. Transfer the water to a 'dark' bottle by placing the delivery tube to the bottom of the bottle. Allow the water to flow continuously through the bottle to prevent splashing and bubble formation, until at least three times the capacity of the bottle has overflowed (count the seconds needed to fill the BOD bottle initially, then repeat twice). Withdraw the delivery tube gently at the end of the sample flushing, without stopping flow, so the bottle is **completely filled**.
- 3) Insert the BOD probe and take an initial DO measurement. Record this measurement in the data table in the handout.
- 4) Cap the bottle, and make sure no light enters the bottle. Attach the dark bottle onto the rope or trapeze using the clip or string. Place at the deep depth.
- 5) Repeat steps 2-4 for a 'light' bottle.
- 6) Repeat steps 2-5 for the other depth(s).
- 7) Secure the vertical rope holding the bottles or the bottle trapeze.
- 8) Allow the bottles to incubate for a minimum of 2hrs. In the meantime, record % irradiance profile and secchi depth (if light penetration is not to bottom. Also take DO and temperature profiles.
- 9) Retrieve the bottles to end the incubation. Wrap 'light' bottles in aluminum foil. Use the BOD probe to determine DO in the 'light' bottles first, then the dark bottles.

## IV. Whole-ecosystem metabolism (FYI)

In addition to measuring **GPP**, **NPP**, and **R<sub>T</sub>** using light and dark bottle incubations, it is possible to measure ecosystem metabolism for the entire lake ecosystem. Whole-lake ecosystem studies are becoming more popular because they integrate over a much larger area, they are an *in situ* measurement, and they avoid some of the artificiality of using bottles. Here's how to measure **GPP**, **NEP** and **R<sub>T</sub>** for the entire pond.

- 1) **Respiration:** One way to measure respiration is to measure DO in the lake over a 24-hour period. At night, when no photosynthesis is occurring, we can estimate decreases in O<sub>2</sub> to estimate **R<sub>t</sub>**. The rate of O<sub>2</sub> consumption can be converted rate of carbon release during respiration. Since we don't have the time to do this, we can estimate the rate of O<sub>2</sub> consumption using a dark bottle and techniques (steps 2-9 above). The rate of CO<sub>2</sub> produced over this interval is the respiration rate. This calculation is described below.
- 2) **NEP:** Dropping an oxygen probe into the water at the mixed layer for an interval of time (such as a 24hr period) and measuring the change in DO concentration will give you an estimate of daily whole-system net carbon metabolism because both primary productivity and respiration are occurring. **If DO increases over this interval, NPP is positive.** We can thus consider the system to be net autotrophic.
- 3) Because **NEP = GPP - R<sub>t</sub>**, Adding (1) and (2) together yields an estimate of **GPP**.

## Aquatic Ecology: Pond PP & Metabolism Lab Datasheet (Spring 2003)

DO Profile:		Temperature Profile	General Data
Depth (m)	DO (mg/L)	°C	Date: _____
0.25			Air Temp: _____
0.50			Clouds: _____
0.75			
1.00			
1.25			
1.50			

### Incubation Period

7. Time Initial \_\_\_\_\_

8. Time Final \_\_\_\_\_ Incubation Period =  $T_f - T_i =$  \_\_\_\_\_ hrs

### DO Measurements

9. <i>Bottle Depth 1</i> = _____ (m)	DO (mg/L = ppm)			
	Rep 1	Rep 2	Rep 3	AVERAGE
10. Light bottle initial ( $I_L$ )				
11. Dark bottle initial ( $I_D$ )				
12. Light bottle final ( $F_L$ )				
13. Dark Bottle final ( $F_D$ )				

14. <i>Bottle Depth 2</i> = _____ (m)	DO (mg/L = ppm)			
	Rep 1	Rep 2	Rep 3	AVERAGE
15. Light bottle initial ( $I_L$ )				
16. Dark bottle initial ( $I_D$ )				
17. Light bottle final ( $F_L$ )				
18. Dark Bottle final ( $F_D$ )				

### Calculations

9. <i>Bottle Depth 1</i> = _____ (m)		$gC / m^2 / hr$
19. <i>Length of water column represented</i> = _____ (m)		((row 20 * row 19) / 1000)
20. Ecosystem Respiration ( $R_T$ ) (see next page) ( $375 * (I_D - F_D) * 1.0$ ) / hours	$mgC / m^3 \text{ water} / hr$	
21. GPP (see next page) ( $375 * (F_L - F_D)$ ) / 1.2 / hours		
22. NEP: $NEP = GPP$ (line 21) - $R_T$ (line 20)		
23. P:R for depth 1 ( $GPP: R_T$ )		

14. <i>Bottle Depth 2</i> = _____ (m)		$gC / m^2 / hr$
24. <i>Length of water column represented</i> = _____ (m)		((row 20 * row 19) / 1000)
25. Ecosystem Respiration ( $R_T$ ) (see next page) ( $375 * (I_D - F_D) * 1.0$ ) / hours	$mgC / m^3 \text{ water} / hr$	
26. GPP (see next page) ( $375 * (F_L - F_D)$ ) / 1.2 / hours		
27. NEP: $NEP = GPP$ (line 26) - $R_T$ (line 25)		
28. P:R for depth 2 ( $GPP: R_T$ )		

Final Average Data across the entire water column (Assumptions?)	$mgC / m^3 \text{ water} / hr$	$gC / m^2 / hr$
29. Ecosystem Respiration ( $R_T$ ) = Avg of row 20 and 25		
30. GPP = Average of rows 21 and 26		
31. NEP = Average of rows 22 and 27		
32. P:R = Average of rows 23 and 28		

## V. Explanation of Calculations (From Cox 1996)

It is easy to convert the measurements of oxygen into an estimate of lake productivity based on the equation for photosynthesis.



The goal is to determine **GPP** in units of  $\text{mg-C} \cdot \text{m}^{-3} \text{ water} \cdot \text{hour}^{-1}$ . Since we are measuring  $\text{O}_2$  production as a surrogate for C fixation (see equation 1), we need to convert the mass of  $\text{O}_2$  that we measure to the mass of  $\text{CO}_2$  fixed. Note that photosynthesis generates 12 atoms of oxygen ( $6\text{O}_2$ ) to the 6 atoms of C ( $\text{C}_6\text{H}_{12}\text{O}_6$ ) fixed by photosynthesis.

- 1) The values of oxygen concentration in  $\text{mg/L}$  (ppm) for the initial ( $I_L$  &  $I_D$ ), the final light bottle samples ( $F_L$ ), and the dark bottle samples ( $F_D$ ) are used to calculate measures of community metabolism in  $\text{mgC} \cdot \text{m}^{-3} \text{ water} \cdot \text{hour}^{-1}$ .

<b>Respiration</b> ( $\text{mgC} \cdot \text{m}^{-3} \text{ water} \cdot \text{hour}^{-1}$ )	$= 375 \cdot (I_D - F_D) \cdot (\text{RQ}) / \text{hrs}$	where RQ=	<b>Respiratory Quotient</b> (molecules of $\text{CO}_2$ produced/ molecules of $\text{O}_2$ taken up)
<b>GPP</b> ( $\text{mgC} \cdot \text{m}^{-3} \text{ water} \cdot \text{hour}^{-1}$ )	$= (375) \cdot (F_L - F_D) / \text{PQ} \cdot \text{hrs}$	where PQ=	<b>Photosynthetic Quotient</b> (molecules of $\text{O}_2$ produced/ molecules of $\text{CO}_2$ taken up)

$$\text{NEP} = \text{GPP} - \text{R}_T$$

- The value of 375 represents a conversion factor from the mass of the 12 atoms of oxygen ( $6 \text{O}_2 = 192$ ) to that of the six atoms of carbon ( $6 \text{CO}_2, \text{C} = 72$ ) in the photosynthesis equation ( $72/192 = 0.375$ ) and the conversion of 1L to  $1 \text{m}^3$  (1000x).
  - Appropriate **PQ** and **RQ** values must be selected for calculation of respiration and photosynthesis. Values of 1.0 may be used for both on the assumption that only carbohydrates are being produced by photosynthesis or broken down in respiration. Where the bulk of the ecosystem metabolism is due to phytoplankton, however, a **PQ** of 1.2 and **RQ** of 1.0 is probably more accurate.
- 2) To obtain total metabolism beneath a square meter of lake surface ( $\text{g-C} \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$ ), multiply the values for **GPP**, **NEP**, and **R<sub>t</sub>** by the depth range of the water column they represent (in meters), and these values are totaled for samples from all depths. Divide by 1000 to convert mg to grams C assimilated.

## VI. Questions and items to turn in:

- 1) Turn in your data sheet and additional calculations
- 2) Was there a difference in the P:R between the surface and bottom of the pond?
- 3) How might a whole-system change in  $\text{O}_2$  differ from this light-dark bottle method?
- 4) (i.e. Are there other sources of oxygen change that are not accounted for in the bottles?)
- 5) Note that your final calculations (#29-32) have you averaging the depth-specific rates in order to estimate whole system metabolism. What is the major assumption of this calculation? How would it be corrected? (hint: see Lecture 2)