

BIOLOGY 1240: BIOLOGY LABORATORY

SPRING SEMESTER — 2001

BEER-LAMBERT LAW I

October 30, 2001

Student Version

The Beer-Lambert Law of Light Extinction: I.

1 Light in Ecosystems

Light is a central component in almost all ecosystems, since it drives photosynthesis from which nearly all other ecosystem components derive their energy either directly or indirectly. We know of only a few ecosystems (such as those near volcano vents in the deep sea) that can exist in the absence of light. Because of this importance, the physical properties of light affect ecosystem function and structure in profound ways. Three physical facts of light are especially important: (1) Light is composed of a set of different wavelengths each with a specific intensity. (2) Overall light intensity decreases in proportion to the inverse of the distance from the source squared (i.e., $I \propto 1/d^2$). (3) As light passes through a medium that obstructs the passage of a photon, such as water, the intensity of the light is reduced.

In this lab exercise, we will investigate this third phenomenon. We will examine two cases where light intensity (and, therefore, the amount of photosynthesis) is reduced due to the physics of light interception. The two cases are: (a) light in aquatic systems, and (b) light in terrestrial systems.

2 How Much Photosynthesis Occurs in a Lake?

As a motivation for studying the amount of light in aquatic systems, we ask a typical question in ecosystem studies: How much photosynthesis occurs in a lake? In this lab exercise we are going to calculate the amount of photosynthesis that occurs in a lake.

2.1 Who Cares?

There are several reasons why we might want to know the amount of photosynthesis in a lake.

2.1.1 Pure Curiosity

As with a lot of science, it is stubborn, persistent curiosity that drives most scientists. In the case of limnologists (the study of lakes), it is a desire to explain the variability of photosynthesis rates among and within lakes. These rates vary over seasons within lakes and among lakes of different geographical locations. Why? How do lakes work? How many trophic levels (kinds of consumers) can be supported in a lake? Why do there appear to be fewer trophic levels in the ocean than in freshwater streams and lakes? Could it be the rate of photosynthesis in the different systems?

2.1.2 How Many Fish Can We Stock?

Science is also driven by practical problems, such as providing sport fishing for recreational enjoyment. If we wish to stock fish in a lake, how many fish can the lake support? Will it be enough to satisfy demand? The answer might lie in the photosynthetic rate in the lake: the more photosynthesis, the more algae and plants; the more algae, the more herbivores (insects), the more insects, the more fish.

Aquaculture in developing countries is often an important source of protein. If we wish to provide that population with a meaningful existence, then understanding the economics of supplying nutri-

ents for plants that feed fish will increase net human benefit. This requires we be able to quantify the rate of photosynthesis.

2.1.3 Pollution

Too many nutrients in a lake produces a condition of eutrophication (“many nutrients”). This occurs when high nutrient input into a body of water (e.g., dairy cows defecating in rivers) causes high plant growth and in turn large rates of photosynthesis. When the plants die (as they must), they are decomposed by bacteria that consume oxygen causing anerobic (no oxygen) conditions and the production of noxious gases.

Finally, plants do a tremendous amount of good in the conversion of carbon dioxide to oxygen. Too much CO₂ in the atmosphere can be ameliorated by its consumption by phytoplankton (single cell floating plants) in aquatic systems, especially the oceans. Knowing the photosynthesis rate in the ocean is a critical component of our understanding how to control global warming.

3 Refresher on Photosynthesis

All you need to know for today’s class is: 6 molecules of CO₂ and 12 molecules H₂O combine in the presence of light energy to produce 1 molecule of a 6-C sugar, 6 molecules of H₂O, and 6 molecules of O₂.

These identify the key players in factors that control photosynthesis.

3.1 Factors that Limit Photosynthesis

Many biochemical processes such as photosynthesis are involve the break-down and regeneration of chemical molecules producing a cycle like: $A \rightarrow B \rightarrow C \rightarrow D \rightarrow A + S$.

The rate of production of S (sugars, say) is determined by the slowest rate of any of the intermediate steps. If $B \rightarrow C$ is the slowest process, then the production of S can be no faster than this rate, because the next ($C \rightarrow D$) step depends on the rate of production of C. This relationship is sometimes called **Liebig’s Law of the Minimum**.

Many factors control or limit the rate of photosynthesis. All of the chemical constituents of the cellular biochemistry and photosynthetic structures are necessary for photosynthesis. This includes macronutrients like phosphate and nitrate, micronutrients like iron or magnesium as well as water, carbon dioxide, and light.

4 Concept Map

4.1 Concept Maps Organize Our Ideas

A concept map is a picture of what concepts and variables are involved in a process. Entities are circles and connections or influencing effects are arrows. Here is a general idea of what we mean.

Applied to the growth of tomatoes, a concept map looks like the following:

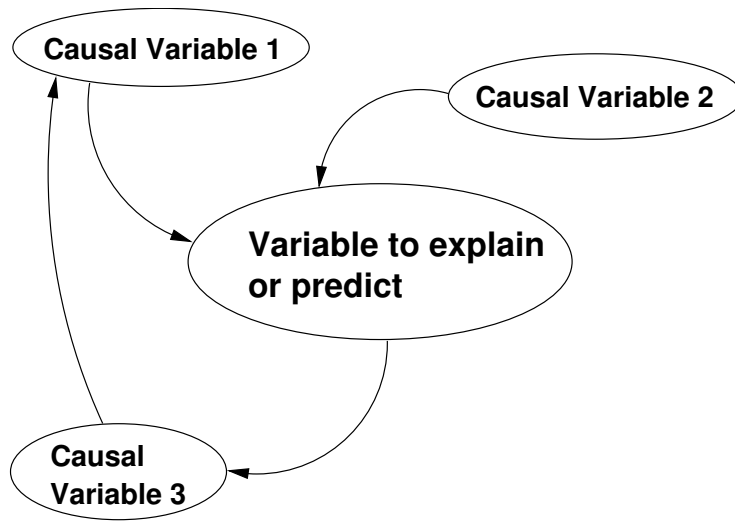


FIGURE 1: A concept map.

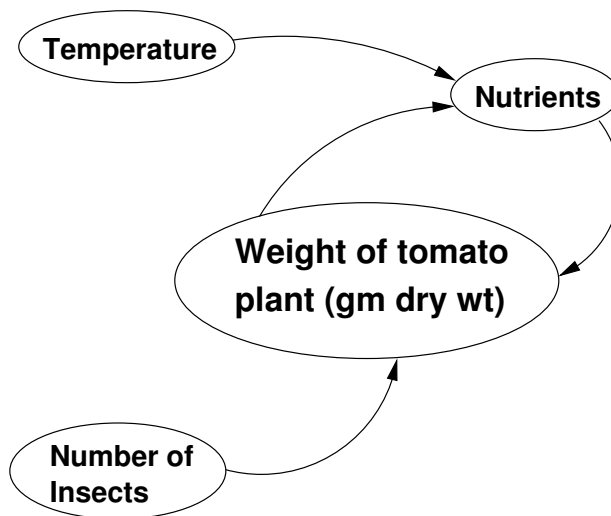


FIGURE 2: A concept map for tomato growth.

4.2 Concept Map of Photosynthesis

Draw a concept map of the environmental factors that limit photosynthesis. Draw it on a separate piece of paper and be prepared to discuss it with the class. To get you started, address these questions:

1. What do plants require for photosynthesis?
2. Which of these can possibly limit the rate of photosynthesis if they are present in the environment in low quantities?
3. Is there always only one (which might change) limiting factor?
4. How do the factors interact?

5 The Relation of Light and Depth

Our goal is calculate the amount of photosynthesis in an entire lake. Because we all know that lakes have depth, it seems clear that the model should consider the relationship between depth and light. In an actual ecological study, it would include these other limiting variables as well, but we will simplify the problem and consider only light.

5.1 Your Job

Guess a relationship between light intensity and depth. Plot 3 curves showing possible relationships between light and depth. We don't know the intensity of light at the surface of the lake, so let's assume that whatever that value is, we will divide **all** the values in the lake by that amount. By doing this, we will **transform** the light levels to be fractions of the surface intensity.

Mini-quiz: After doing this transformation, what will be the possible ranges of light intensity in the lake? Here are some possibilities: minus infinity to positive infinity? 1.0 to positive infinity?, 1.0 to 0.0?, 1.0 to minus infinity? 0.0 to positive infinity? Which do you think it is?

6 Data Collection

We've made a prediction of the qualitative behavior of light in a lake over depth. It's time to test the prediction.

6.1 Using the Traceable Light Meter

1. Push the right slider switch all the way to the top so that it rests on **Lux/Fast**.
2. When not in use, the left slider switch should be in the **OFF** position: all the way to the top.
3. The left slider switch also controls the range of light intensity being measured; the range increases from top to bottom of the switch. To insure maximum precision, when measuring light, use the smallest range possible. If you see a single "1" in the LCD display, you need to increase the range by sliding the switch down to the next interval.
4. Taking a measurement:

- (a) Hold the Light Sensor (the white dome on the black thingey at the end of the coiled cable) perpendicular to (facing) the light source.
 - (b) At high light intensities, the LCD display will fluctuate, especially when the sensor is hand-held. Hold the sensor as still as possible for several seconds and note the readings mentally. After several seconds, report your best estimate of the average reading.
 - (c) The light sensor dome collects light from the sides as well as directly in front of the dome. Stand aside from the sensor so that you do not block the light.
 - (d) Turn the unit off when not in immediate use.
5. Please do not leave the light sensor near the hot light source. It will melt!

6.2 Measuring Light in Water

CAUTION: The lights we will use in this exercise are very BRIGHT and very HOT! Avoid looking directly at the light. Do not touch the light when on and do not splash water on the hot bulb.

Figure 3 shows the basic set-up. The “X” marks the location of the light meter.

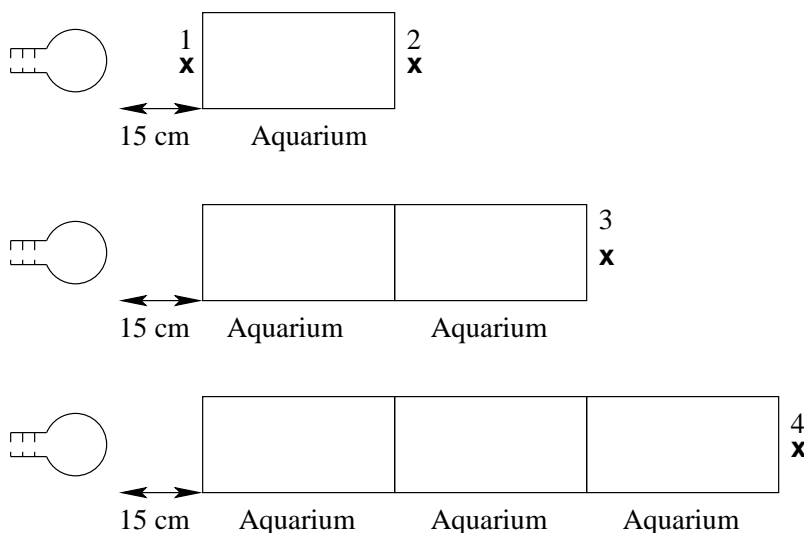


FIGURE 3: Experimental Setup to Measure Light Through Water. Make 4 measurements as indicated by the numbers and \times . Repeat with clear water and 2 amounts of dye.

1. Students from two lab benches will work together to measure the effect of water and dye on light attenuation. On one of the 2 benches, you will find 3 aquaria filled with water and with black paper lining the inside. [The paper is needed to prevent light from traveling down the glass sides of the aquaria, thereby producing more light far from the source than would occur in water that was not artificially contained in glass.] Nearby, additional pieces of paper are available to be placed on the top of the aquaria to prevent reflection from the underside of the water surface.
2. Assign members of the group to be (1) the reader of the light meter, (2) movers of the water-filled aquaria, (3) mixers of the dye, (4) data recorder. Other students should copy the data onto their own datasheets.
3. For the first measurement of **incident** radiation, make sure that no aquaria is in front of the light. Then, position the light meter 15 cm from the light source and oriented to point

directly at the middle of light bulb. This is measurement number “1” in the above figure and will be the *incident light*. Record this value on the attached datasheet (Fig. 4).

4. Place the aquarium with pure water (no dye) 15 cm from and long-ways to the light source. Measure the light level behind the glass furthest away from the light source. This is measurement number “2” in the above figure. Record this value on the datasheet (Fig. 4) below in the column “Clear Water”. [For the moment, ignore the column that says “Transformed Clear.”]
5. Repeat the above step after adding a second aquarium (measurement #3) and then a third aquarium (measurement #4) behind the first aquarium. Each time, measure the light at the end of the “water column.” Also measure the distance between the light sensor and the light source.
6. After you have made 4 measurements with pure water, repeat the procedure after adding 6 drops of the supplied dye to each aquarium. Thoroughly mix the dye in each aquarium and measure the light at the end of the chain of aquaria. You will have another set of 4 observations. Record these in the column labeled “6 Drops Dye.” Again, ignore for now the column “Transformed 6 Drops.”
7. Repeat the procedure again after adding an **additional** 44 drops of dye (for a total of 50 drops per aquarium). Record these in the column labeled “50 Drops Dye.” Again, ignore for now the column “Transformed 50 Drops.”

7 Analyze the Aquaria Data

The students at each lab bench should work together to analyze the data.

Here are the steps to begin to analyze the data.

1. Plot the data using the attached graph paper.
2. Which of the curves that you guessed earlier was closest to the data?
3. Try describing the data mathematically using the equations and graphs that you’ve seen in high school and elementary college math classes. Use this background to answer these questions:
 - (a) Are the curves straight lines? [**Noooooo!**] So the equation is **not** like $y = mx + b$
 - (b) What curves do you know that look even a little like the data? Think back to earlier math classes: the equation must decrease sharply at first, then more gradually. Many equations do this, but you’ve seen two in particular that might work. [Hint: For one of the equations, only part of its graph looks like your data.]

Stuck? Keep trying! Take a wild guess! **Any** guess is better than none.)

If, after you’ve really tried to recall some earlier math, and you’re still stuck, beg for a really big hint out of your instructor. But be warned, we want you to think hard about this problem.

Write the two equations in the boxes below:

Light Values Through 3 Aquaria

Position #	Distance from bulb	Clear Water	Transformed Clear	6 Drops Dye	Transformed 6 Drops	50 Drops Dye	Transformed 50 Drops
1	15 cm						
2	46 cm						
3	77 cm						
4	108 cm						

FIGURE 4: Datasheet for the aquaria light experiment.

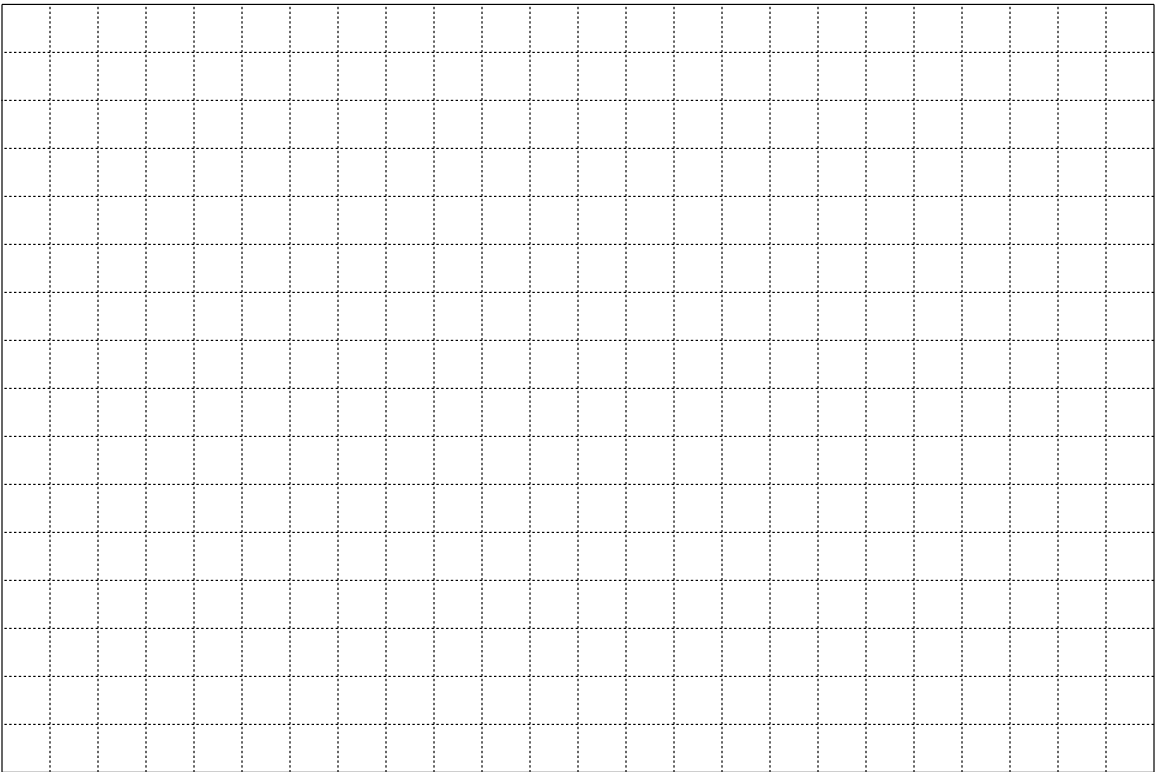
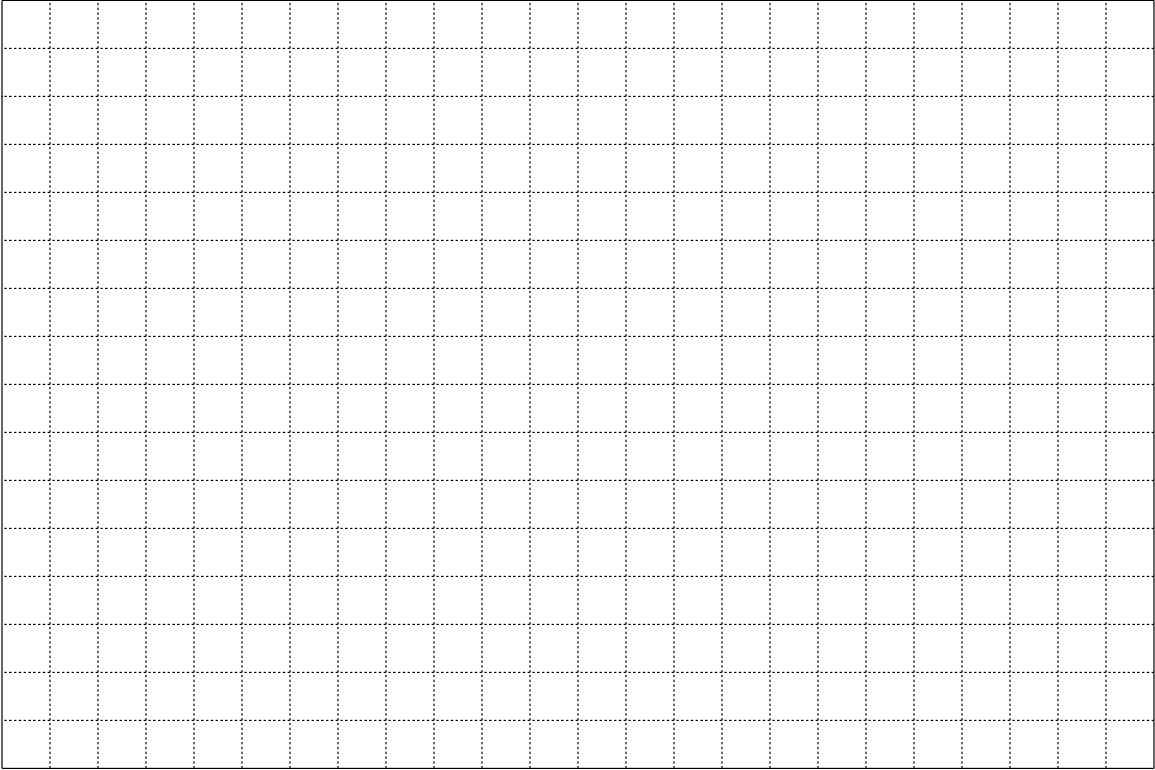


FIGURE 5: Graph paper

Your Equation 1:

Your Equation 2:

Can these two equations approximate the data? In other words, will it be possible to pick **some** values for the constants that will make the graph of the equation look like the data?

When you've convinced yourselves that the two equations in the hint **could** be good candidates, go on to the next section.

8 Class Candidate Models

Your instructor will lead a discussion with the entire class to reach an agreement on 2 possible equations. You should contribute to the discussion your equations. In any case, after this class discussion, you now have two candidate models for your data. You can write them below:

Class Equation 1:

and

Class Equation 2:

For the moment, we won't worry about what these constants mean. Our main worry now is that maybe one or both of the equations are inadequate to represent our data. We want to use a little mathematical reasoning to see if we can eliminate one or the other (**or both!**).

8.1 Your Job

Decide if either of these equations is better than the other.

1. Can both equations be made to look like the data?
2. Can both equations be made to look completely **unlike** the data? (“Unlike” means reverses the basic, qualitative relations.)
3. Can both equations be made to create impossible values (e.g., negative light)?
4. What does light do if the lake is really, really deep?: (1) Does it reach a small, positive level and then not change further as depth continues to increase? (2) Does it reach a minimum and then begin to increase? (3) Does it go negative?
For each of the equations, check to see if you can force the equation to go counter to your intuition about how light changes.
It will help if you plug-in some possible values for constants and graph the two equations.)

9 The Beer-Lambert Law

From our previous work, we now have a candidate model for light extinction that has at least some of the minimal characteristics we need:

Beer-Lambert Law:

This is the **Beer-Lambert Law** of light extinction, where $I(z)$ is the light intensity at depth z , $I(0)$ is the incident light entering the column of water ($z = 0$), and a is an empirical constant that applies to a particular water column, called the *extinction coefficient*. a will differ from lake to lake if one lake is more [green]turbid than another; it must be estimated for each situation. (Forget what e is? Ask your instructor!.)

Mini-quiz: In a very turbid lake, will a be larger or smaller than the a of a clear lake? What is the relative magnitude of a for the Great Salt Lake and for Bear Lake?

9.1 Your Job

Given your light data, what is the value of a ?

If a is an empirical constant, then we must estimate it from our data. Your job is to find a method that uses our data to estimate a .

Try answering these questions by discussing them with your group members:

1. What do you want to know? Easy! We want to know a , but it’s stuck up there above that funny e constant.
2. In the Beer-Lambert equation, what parts do you know? What have you measured? List them here: these are **known**, so we can put in numbers for them later.
3. (The hard part) Can you transform the Beer-Lambert equation to make it easy to estimate a ?

(Have you already solved a similar problem? Yes! Recall how you estimated D in the cooling and osmosis exercises. What kind of an equation allowed us to easily estimate D ?)
(Don't give up; but if you're really stuck, ask your instructor for a hint.)

When the instructor re-convenes the class, help out the others by suggesting your bright idea.

10 The Equation to Estimate a

From the class discussion with the instructor, you now have a general, linear equation for the Beer-Lambert law. Copy it in the box below:

Linear Form of the Beer-Lambert Law

From this equation, you can see that a is the slope of the line. b is $\ln(I(0))$. In the box below, write the equation for the intercept. Below it, in the same box, write the equation that will give you the incident radiation ($I(0)$) from the intercept. Ask your instructor if you don't know how to do this last step.

Intercept =

$I(0)$ =

10.1 Your Job

1. In the datasheet above (Fig. 4), compute and record the necessary transformation of your aquaria data.
2. On supplied graph paper, plot the transformed data against distance from the light source.
3. Using your best guess draw a single "best fit" straight line through the points.
4. Estimate the slope and intercept of this line, which approximates your data.
5. Write this, using your values, as an equation of the form: $y = mx + b$.

Mini-quiz: What is the value of the incident radiation? What is the value of the extinction coefficient?

6. Once you have the slope and intercept, plug these values into the original Beer-Lambert equation to predict light levels for each depth (z) you measured. Graph the values and draw a smooth curve through them. On the same graph, re-plot the original data. Is the fit good? What do you mean by *good*?

Mini-quiz: What are the units of a ? How do the units change if we measured distance (z) with meters, not cm?

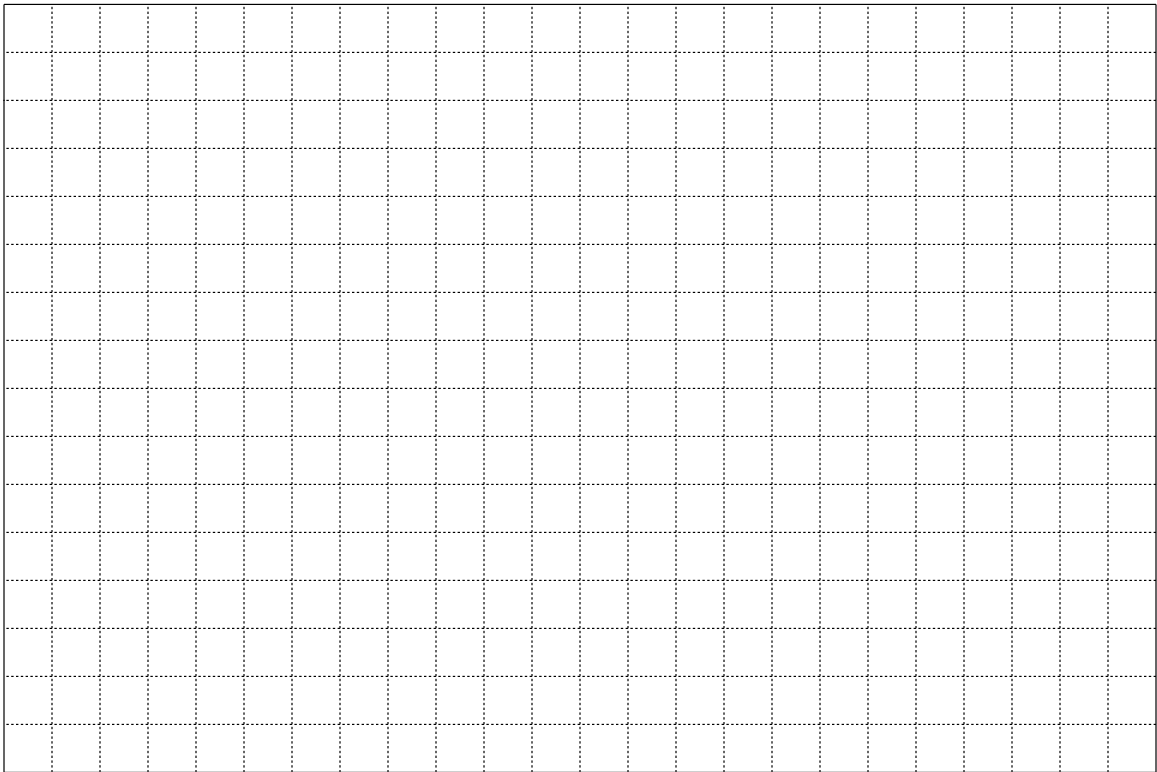
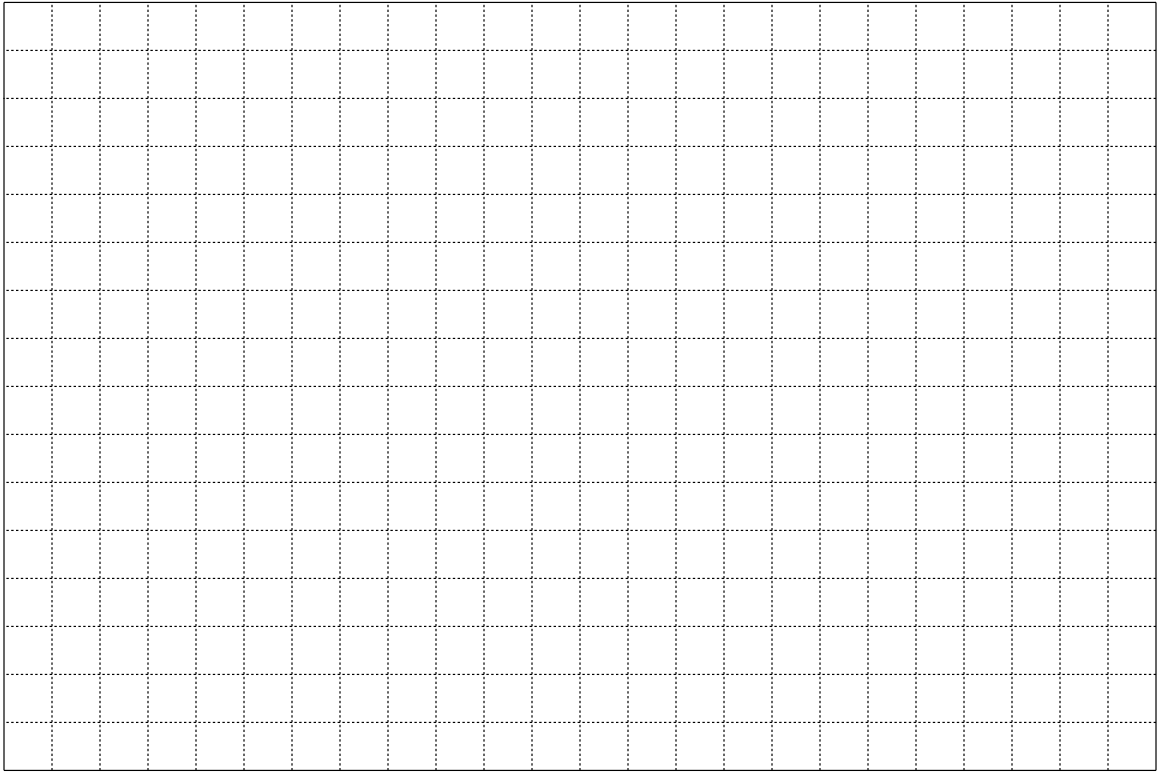


FIGURE 6: More Graph paper

11 Solved Problems

Here are some problems with worked answers. Be sure you understand how to do these, since similar questions will be assigned as homework and will appear on future exams.

1. Below are some data for light ($\mu\text{moles} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) as a function of lake depth (measured in meters). Calculate a .

z	Light
0	750.0
5	275.91
10	101.50
15	37.34
20	13.74
25	5.05
30	1.86
35	0.68
40	0.25

First of all, use all the digits shown. Then take the natural logarithm (\ln) of the light level at each depth

z	$\text{Ln}(\text{Light})$
0	6.62
5	5.62
10	4.62
15	3.62
20	2.62
25	1.62
30	0.62
35	-0.38
40	-1.38

Next plot the log of light on the y-axis and depth on the x-axis. Draw a single straight line through the data and calculate its slope. The intercept is the natural log of the incident radiation. The slope should be close to 0.2. The intercept should be the natural log of 750.0. Check that it is.

2. **Lake 1 has $a = 0.02$ and Lake 2 has $a = 0.1$. Which lake is more turbid?**
The constant a is the extinction coefficient, so large a means high extinction. I.e., lots of light is lost with depth. This is caused by suspended particles in the water column, so Lake 2 is more turbid.
3. **If a lake's light extinction coefficient is 0.5/meter and the incident radiation is $1,500 \mu\text{moles} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ what are the light levels at 0, 5, 10, 15, and 20 m?**

Use the Beer-Lambert Law:

$$I(z) = I(0)e^{-0.5z}$$

for each value of z in the problem. Light levels are: 1500.0; 123.1274979; 10.1069205; 0.829626555; and 0.068099895.

4. **If a is 0.2 measured on a sunny day when the incident radiation is $2,000 \mu\text{moles} \cdot$**

$m^{-2} \cdot s^{-1}$, what is a on a cloudy with half the incident light? Why?

$a = 0.2$, because the extinction coefficient does not depend on the amount of light that enters, only the fraction that is absorbed.

5. At the same time that photosynthesis is occurring in a plant cell, the cell is also using the energy and O_2 that the cell produces. This is called *cellular respiration*. As the light decreases with depth in a lake, the rate of photosynthesis decreases. (You'll prove that next week.) Eventually, the rate of production of O_2 will equal its rate of use for normal cell respiration. When this light level is reached it is called the *compensation point* (photosynthesis gains exactly compensate for respiration losses). This will occur at a particular depth in any given lake. Below this depth there will be no plants (not enough light). In most lakes, the light level of the compensation point is 1% of the incident light. In Lake Witchykoocha, $a = 0.25$ and $I(0) = 1250$. Below what depth would you expect to find no plants?

In this problem, what do we know? We know a and $I(0)$. BUT! we also know $I(z)$ because at the compensation point $I(z) = 0.01I(0)$. Putting these into the Beer-Lambert Law:

$$0.01I(0) = I(0)e^{-0.25z}$$

What do we want to know? The only thing left is z , the depth of the compensation point. How do we get z ? We isolate it on one side of the equation. How do we do that? The same way we solved for a , we take the natural logs of both sides and re-arrange.

The details are left to you, but to check, the answer is: $z = 18.4206m$

6. A spectrophotometer is an instrument that measures the concentration of a sample contained in a small glass chamber (e.g., 1 cm in length) by shining a light through the chamber and measuring the amount of light that emerges on the other end. Based on a known linear relationship between the light extinction coefficient and the concentration of the substance, if we know the amount of light that is absorbed by the sample, we can calculate the concentration of the sample. Suppose that the extinction coefficient varies with concentration by this equation:

$$a = 0.04C$$

where C is the concentration measured in millimoles per cubic meter ($\text{mmole} \cdot m^{-3}$). (We assume, for simplicity, that over 1 cm pure water has an extinction coefficient of 0.0.)

Also suppose that the light source emits $100 \mu\text{molephotons} \cdot m^{-2} \cdot s^{-1}$ and the sample absorbs 40% of the light ($60 \mu\text{molephotons} \cdot m^{-2} \cdot s^{-1}$ emerges from the sample chamber). What is the concentration of the sample?

Whew! Long question! What do we know? We know the amount of light that enters and emerges from the sample. We know the distance over which light is attenuated. We also know that if we can calculate a , then we can calculate C from the equation above. So now the problem is to find a . But you know how to do that from an earlier problem: Use the Beer-Lambert Law, and solve for a .

$$a = \frac{\ln(60) - \ln(100)}{-0.01}$$

or

$$a = 51.08$$

(Why use $z = 0.01$ and not $z = 1.0$?)

And so, $C = 51.08/0.04 = 1277.064 \text{mmoles} \cdot \text{m}^{-3}$

12 Homework Problems

Homework to be graded will be distributed in class.

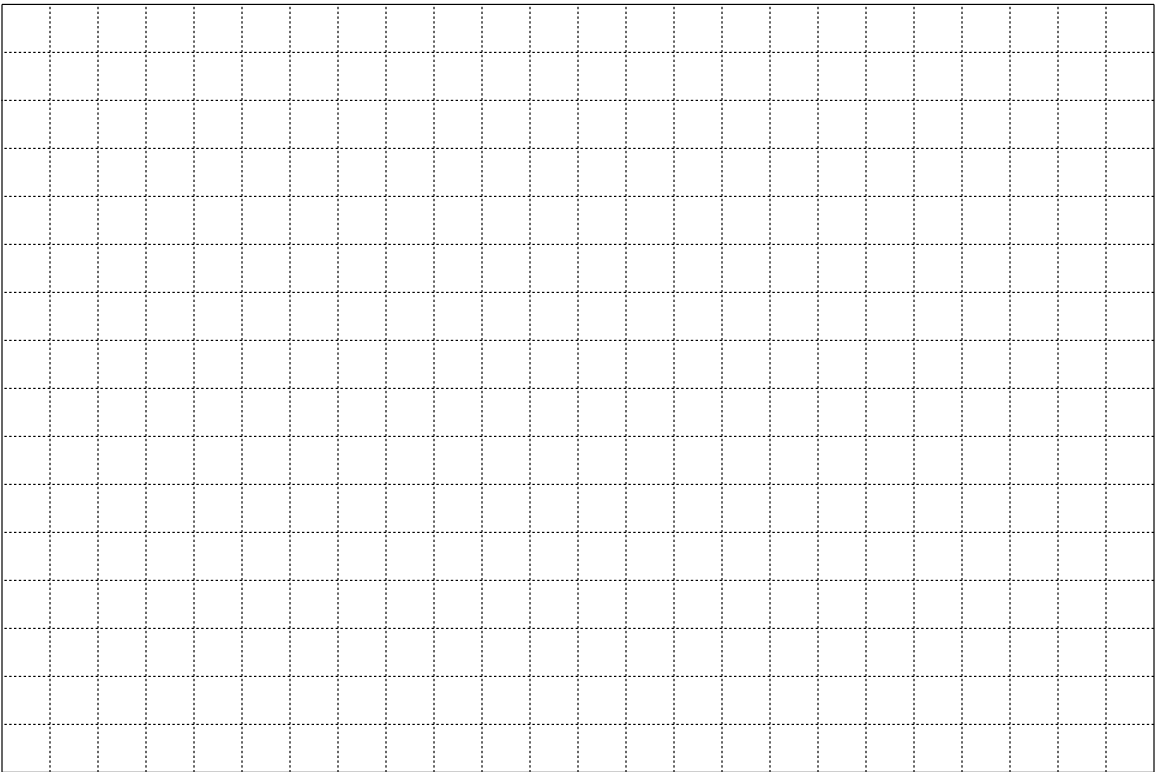
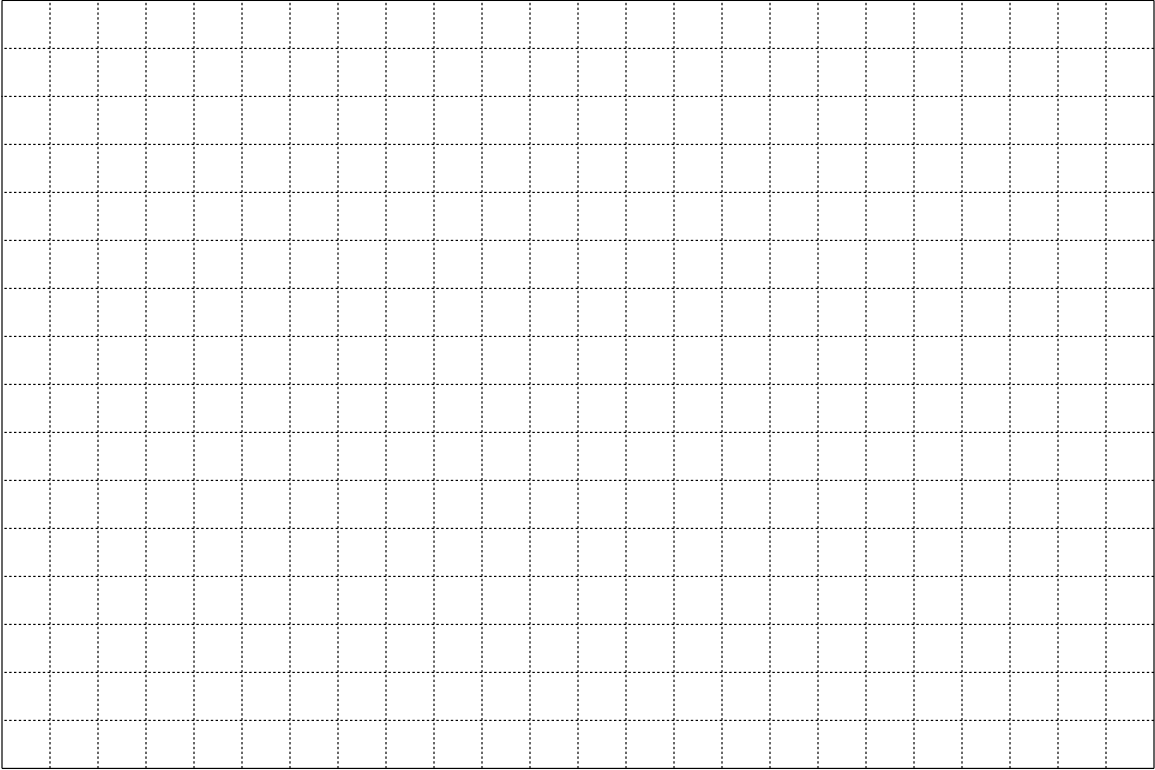


FIGURE 7: Still More Graph paper