# **Diurnal Cycling Experiments**

# **Background**

Eutrophic ponds and lakes experience large diurnal (daily) fluctuations in dissolved oxygen, carbon dioxide, pH, and water temperature.

• At night, dissolved oxygen is consumed by both plant and animal respiration, and carbon dioxide is produced. Carbon dioxide reacts with water and causes the water to become more acidic:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

- During the day, animal respiration continues to consume oxygen, but photosynthesis by plants consumes carbon dioxide and produces oxygen. The net effect may be a drop in CO<sub>2</sub>, an increase in pH, and an increase in O<sub>2</sub>.
- Complicating the relationship described above are the effects of temperature. During the day, water temperatures tend to climb, especially in shallow lakes and ponds. Both oxygen and carbon dioxide are less soluble in warm water than in cool water. A 10°C increase in temperature, for example, reduces the solubility of oxygen by about 20%.

Daytime water monitoring may not provide a complete picture of the chemical stresses that aquatic organisms face.<sup>1</sup> However, it may be logistically impossible for teachers to take students into the field at different times of the day or night. Hence, the experiments that follow!

# **Experimental Problem #1 (Standard Problem)**

Carry out an experiment to monitor diurnal fluctuations in dissolved oxygen, carbon dioxide, pH, and water temperature. This should be an experiment that can be carried out in the science classroom, during regular school hours.

# **Materials**

- Dissolved oxygen meter and supplies
- pH meter and calibration kit
- Pond water and replacement macrophytes (e.g., *Elodea*)

<sup>&</sup>lt;sup>1</sup> For example, in 1971 in Mulatto Bayou, Florida (an estuary on the Gulf of Mexico), a loss of oxygen in the water one night led to a fish kill of over 5.5 million fish.

- Thermometers
- Six aquaria with established plant and animals (artificially eutrophied)
- Three grow lights for household plants
- Three digital timers
- Dissolved oxygen test kits
- CO<sub>2</sub> kits
- Six bottles with established open-water organisms (artificially eutrophied)

#### **Suggested Steps**

Engagement	Set the stage for Experiment #1. We suggest that you do this
	in two ways:

- Some simple hands-on activities, and
- Some simple data analysis and interpretation.

These two steps can be accomplished in a single class period.

Before class, set up "water samples" of two types around the classroom, in sealed bottles. Label each sample with a "date" and a "time." Fill some of the bottles with well-aerated tap water (high oxygen, low CO<sub>2</sub>, neutral pH). Label these "2:00 p.m." Fill some of the bottles with a mixture of flat water and seltzer (low oxygen, high CO<sub>2</sub>, low pH). Label them "4:00 a.m." Plan on two bottles per group of students, one for each time. We suggest that you label the pairs of bottles with successive dates, e.g., "3/6/94 4:00 a.m," "3/6/94 2:00 p.m.," "3/7/94 4:00 a.m.," etc. You may also want to vary the amount of seltzer you add to the 4 am bottles. Obviously, the dates and times are fictitious--a fiction you should share with your students eventually!

Have the students determine the CO<sub>2</sub> and pH of the samples (and optionally, dissolved oxygen). It is up to you how you have them do this. You may wish to have everyone determine pH with indicator papers, or you may want to have different groups use different methods (e.g., LaMotte titrations or pH paper or pH meter readings). Be sure to have each method applied to both a 4 a.m. and a 2 p.m. sample, however.

After students have done their measurements, have them record their data on the board, on both a data table and a graph. You'll want to set up graphs of date & time (graph 1, X axis) vs. pH (graph 1, Y axis), and date & time (graph 2, X axis) vs. CO<sub>2</sub> (graph 2, Y axis). You'll want to be sure that students see that both pH and CO<sub>2</sub> are diurnally varying and cyclical. You should also be sure that students understand that their "simulated" results would be consistent with the findings from an aquatic system in which respiration at night drove

Environmental Inquiry

 $CO_2$  up (and hence pH down, because of  $CO_2 \leftrightarrow HCO_3$ -equilibrium) and photosynthesis in the daytime drove  $CO_2$  down and pH up.

Before you go on to the next step, you'll need to teach your students to measure dissolved oxygen, either via the Winkler method (test kits) or the dissolved oxygen meter.

ExplorationRun Experiment #1. We recommend that you walk students<br/>through Experiment #1. Although your eventual goal may be to<br/>have students design their own experiments, most students will find<br/>experimental design much easier if they first get solid experience with<br/>a protocol.

- Set up several aquaria on staggered photoperiods, using plant grow lights and timers. Use pond or lake water if possible, and have lots of extra water on hand to replace water that evaporates (large soda bottles are good for storage). The photoperiods you use will depend on your class schedule. We set up three 10-hour light regimes: (Tanks 1 & 2) 5 am - 3 pm; (3 & 4) 8 am - 6 pm; (5 & 6) 12 noon - 10 pm. You're shooting to get "nighttime," "morning," and "midday" readings, at least. Ideally, students should be able to compare readings within their class, even if they will be pooling data with other classes.
- 2. Stock the aquaria with lots of macrophytes (e.g., *Elodea*) and give them plenty of nutrients. We fertilized our tanks with 80 drops apiece of Liquid Miracle-Gro.
- 3. Give the systems at least two or three days to adjust to the photoperiods.
- 4. On the day you are doing the analytical work, begin by writing one or more predictions on the board. Ask students to record them in their notebooks. Your predictions should say something about the relationship between time of day and oxygen, carbon dioxide, and pH. (This step is important. How you get it is less important. You may wish to dictate predictions to some classes and elicit predictions from others. But be sure everyone has the same predictions recorded).

Now have students determine dissolved oxygen, dissolved carbon dioxide, pH, and water temperature using the methods you prefer. If you wish, we can loan you extra pH titration kits, a pH meter, extra dissolved oxygen and carbon dioxide kits, and a dissolved oxygen meter. Environmental Inquiry

	How you distribute the work among students is up to you. With older students who are familiar with the LaMotte tests, you may want to have each lab group run all the tests on one tank from each two-tank set. With younger students who are not familiar with the LaMotte kits, you may choose to give students a more limited task. Make sure that, regardless of which test(s) a group runs, they run it on at least one tank from each photoperiod, however. That will ensure that each student sees cross-treatment variation. (It will also distribute procedural errors across treatments!)
Explanation & Elaboration	This is where your students analyze and interpret their data. We encourage you to try something different than the usual lab writeup, but obviously that's up to you. The format we suggest:
	I. Prediction Statement
	<u>II. Lab Log</u> What you did and when, problems that arose and how you addressed them.
	III. Interpretation A summary of your data, preferably in graphs.
	<u>IV. Tips for Future Experimenters</u> Recommendations for improving upon or extending the research, addressed to students taking this class next year.

**Evaluation** Some form of assessment (see sample assessment items at the end of this packet).

# **Experimental Problem #2 (Original Problem)**

These experiments should be original. Some possible ideas:

Design an experiment to monitor dissolved gases in aquaria exposed to different colors of light. {Background: If dissolved gas changes are a function of photosynthesis, variations should be seen in aquaria exposed to different wavelengths of light.}

Design an experiment to determine the light regime that best supports the growth of a particular aquatic organism. (Background: Plants such as duckweed may grow best under constant illumination. Other organisms, like some invertebrates, may need periods of darkness in order to feed, because they are adapted to hide during the day from visual predators)

Design an experiment to determine whether biotic activity will follow a light regimen that is not diurnal. (Background: Biological oxygen production in outer space may be optimum on a light/dark cycle different than day/night.)

# **Assessment Items**

#### **Traditional Items**

- 1. An aquarium in a dark room contains plants, invertebrates, and water. Susan measures the dissolved oxygen in the aquarium and finds that it is 10 ppm. She moves the aquarium into the sunlight for 8 hours, then measures the oxygen again. Which of the following is most likely to have occurred?
  - (a) The dissolved oxygen level has risen because warm water can hold more oxygen.
  - (b) The dissolved oxygen level has risen because the plants are photosynthesizing.
  - (c) The dissolved oxygen level has dropped because animals in the water are breathing.
  - (d) The dissolved oxygen level has dropped because the pH of the water has changed.
- 2. As carbon dioxide is added to distilled water, which of the following is most likely to result?
  - (a) The pH of the water rises.
  - (b) The pH of the water drops.
  - (c) The concentration of  $CO_2$  drops.
  - (d) The temperature of the water decreases.
- 3. Three plastic bottles full of water are sealed and placed in the sunlight. Bottle #1 contains filtered pond water. Bottle #2 contains filtered pond water and a number of *Elodea* plants. Bottle #3 contains filtered pond water and a goldfish. After three hours in the sunlight, the bottles are opened and the dissolved gases measured. Which bottle is likely to contain the most oxygen?
  - (a) Bottle #1.
  - (b) Bottle #2.
  - (c) Bottle #3.
  - (d) None of the bottles is likely to contain any oxygen after three hours in the sunlight.

#### **Authentic Assessment Item**

**Part I.** Design an experiment to test the prediction that diurnal variations in dissolved gases in freshwater aquaria are a result of changes in water temperature, nothing more. Write a paragraph clearly describing your experiment. Be sure to include in your design at least three replications of each treatment.

**Part II.** Swap papers with another student. Write a short paragraph explaining why your experiment is a better or worse experiment than the other student's. It is possible that you may find that your experiment is better in some ways and worse in other ways. Feel free to say this, but provide details.

# **Credits**

This exercise was written by William Carlsen (Pennsylvania State University) under NSF Award #9454428.