1.6) CARBON CYCLING BETWEEN FISH AND PRODUCERS

Objective: In this laboratory exercise you learn to quantify the oxygen consumed or produced by a known biomass of consumers and producers. **Video instructions:** https://vimeo.com/839263337

Introduction: In 1991 nine volunteers entered into Biosphere II, a man-made self-contained ecosystem that was sealed off from the outside world for two years. The long-term goal of this controversial project was the colonization of space. Unfortunately, the original mission was discontinued after only two years due in large part to declining levels of oxygen. Today, this facility is used mainly for climate-change research.

Procedure A: Oxygen Consumption by Fish

1) Organize the feeder goldfish (between 1 and 2 grams each) into the following two groups: 2 and 4 fish per flask.
2) To weigh the fish, first tare the balance using a beaker with water (Fig. 1).
3) ...then add the fish and record the difference (Fig. 2).
4) Measure temperature and dissolved oxygen of about 2 liters of aerated aquarium water, then use this water to fill four 250-mL flasks about halfway.
5) Place each group of fish into its corresponding flask, and fill to the brim with the same water, and cover with a rubber stopper so there is no air space in the flask.
6) Place all flasks in front of the fluorescent lamp and measure dissolved oxygen and temperature after 30 and 60 minutes (Fig. 4).

Fig. 1

Fig. 2

Fig. 3

Procedure B: Oxygen Production by Plants

1) Organize the *Elodea* sprigs or *Spirogyra* portions into the following two groups that weigh 2-4 grams per group. Choose only healthy sprigs that do not have discolored leaves.
2) Weigh each sprig or group of sprigs by placing it directly on a clean platform such as a watch-glass (Fig. 3). Water tends to cling to *Elodea*. This can have a significant effect on the total mass, so make sure you shake off the water before you weigh each group of sprigs. If you are using *Spirogyra*, remove the algae from the water for 10 seconds to allow the water to drip out before weighing it (no squeezing!).
3) Fill one 250-mL flask near the brim with aerated aquarium water. Measure temperature and dissolved oxygen after pouring the water into the flasks.
4) Insert each group of *Elodea* or *Spirogyra* into its corresponding flask and fill to the brim with water.
5) Place each flask in front of the fluorescent lamp and measure dissolved oxygen and temperature after 30 and 60 minutes (Fig. 4).

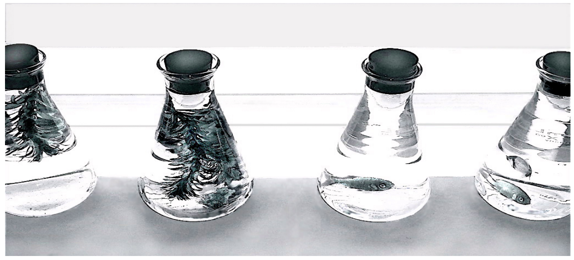
Fig. 4

Procedure C: Carbon Cycling Between Fish and Plants

1) Weigh out the following (approximate) mass ratios of fish and plant material; 1:2 and 1:1 if you are using *Elodea,* or 1:1 and 2:1 if you are using *Spirogyra* (because *Spirogyra* produce more oxygen per gram than *Elodea*).
2) Measure temperature and dissolved oxygen of about 1 liter of aerated aquarium water; then use this water to fill four 250-mL flasks about halfway.
3) Place each group of fish with *Elodea* or *Spirogyra* into separate flasks, fill to the brim with the same water, and cover with a rubber stopper so there is no air space in the flask.
4) Place each flask in front of the fluorescent lamp and measure dissolved oxygen and temperature after 30 and 60 minutes (Fig. 4).

Spreadsheet instructions for parts A and B:

Use a prepared spreadsheet to calculate the milligrams of oxygen production per kilogram of biomass per hour for both these time intervals. Keep in mind that ppm is the same as mg per liter. Since the biomass of the fish and plants is small we will assume that the entire volume of each flask is the water (a 250-mL Erlenmeyer flask typically holds 270 mL).

Below is a sample spreadsheet (minus oxygen readings) in which the total volume is 270 mL per flask and the biomass values in flasks x and y are 1.5 and 3.0 grams, respectively. Rows 3 and 6 correspond to readings that will be taken after 30 minutes. Rows 4 and 7 correspond to 60-minute readings:

Oxygen Consumption / Production by Fish / Plants									
◊	A	B	C	D	E	F	G	H	I
1	Flask	ppm O_2	Δ O_2 ppm	Vol. (liter)	Δ total mg O_2	Biomass (kg)	mg O_2 /kg	Δ Hours	mg O_2 /kg/hr
2	x		N/A	0.27	N/A	*0.0015*	N/A	0	N/A
3	x			0.27		*0.0015*		0.50	
4	x			0.27		*0.0015*		0.50	
5	y		N/A	0.27	N/A	*0.0030*	N/A	0	N/A
6	y			0.27		*0.0030*		0.50	
7	y			0.27		*0.0030*		0.50	

Questions:

1. Oxygen consumption by fish:
a. What is the average mg O_2 consumption per kg fish per hour at the end of the first half hour?

b. What are these values at the end of the second half hour?

c. Are these values more than 20% different from each other? If yes, why do you think they increased or decreased?

2. Oxygen production by plants:
a. What is the average mg O2 production per kg plant per hour at the end of the first half hour?

b. What are these values at the end of the second half hour?

c. Are these values more than 20% different from each other? If yes, why do you think they increased or decreased?

3. Use the data from parts A and B to predict the ratio of goldfish to plants that will give you no net change in oxygen and compare this with the actual data. Use the following formula to solve for the ratio (R):

$$\left[\frac{\text{overall average mg O2 / kg plant / hour}}{\text{overall average mg O2 / kg fish / hour}}\right] = R$$

R = mass ratio of fish to plants needed to get a 1:1 ratio of O2 consumption by plants vs. O_2 production by plants.

Show your work:

4. Based on your previous calculation, how many grams of plants do you need to provide enough oxygen for 20 grams of fish? (Show work):

5. Unlike plants, fish do not generate oxygen. Why did you place the fish in front of the lights?

6. Why is it necessary to completely fill and seal the flasks with plants or fish? How is the experiment affected if the flasks are not sealed or have too much airspace?

7. How does the prediction in your calculation compare with the actual ratios in the flasks where you combined the fish and plants? What do you think is responsible for the differences?

8. The title of this lab is "Carbon Cycling," but carbon dioxide is never measured in the experiment. How does dissolved oxygen relate to the carbon cycle?

9. Your experiment only measured oxygen production and consumption over a period of 1-2 hours. Do these ratios apply to a sealed environment on a 12-hour photoperiod designed to sustain fish for 365 days? What are two new things you need to take into account in order to keep these fish alive over this prolonged period of time?

10. Suppose you are doing this 365-day experiment with lab rats. Do you think the plant/animal biomass ratio will be different from that of the fish? To answer this question, think about how rats are different from fish:

11. Suppose you are doing this 365-day experiment with ferrets. Do you think the plant/animal biomass ratio will be different from that of the fish? To answer this question, think about how ferrets are different from rats:

Assignment Checklist:

1. Did you completely answer questions 1-11?
2. Did you fill out the blank spreadsheet form by hand?