**Experiment: Understanding Nutrient Cycles and Algal Blooms**

(Please note, there are student adapted versions of this resource within the module lessons)

**Why study diatoms?**

In this experiment, you will be studying how diatoms grow in varying nutrient and light conditions. Diatoms are eukaryotic phytoplankton that play a large role in the global carbon cycle and ocean ecosystems and form the base of food webs in coastal and upwelling systems supporting important fisheries. Even though they only account for 1% of the world’s biomass (Falkowski, Raven, 1997), they account for 1/5th of total photosynthesis on Earth, and each year they “generate about as much organic carbon as all the terrestrial rainforests combined” (Armbrust, 2009).

One of the ways diatoms have come to dominate primary production in the oceans is through their unique cell cycle. With each cell division, diatoms get smaller and smaller, until they reach what is called a critical minimum size. When diatoms reach this size amidst certain environmental cues, instead of dividing and reproducing asexually, they reproduce sexually. After sexual reproduction, which increases genetic diversity, the diatoms revert back to their original, large size. Because they can only reproduce sexually when they reach a certain size, sex is “spaced optimally” throughout the species, such that no energy is wasted, but diatoms are able to maintain a high genetic diversity as well as great abundance (Lewis, 1983).

Each spring, when nutrient levels, light levels, and temperatures are high, diatom populations soar across the world. These bursts of production are referred to as blooms. The largest bloom, the North Atlantic Spring Bloom, is so big that you can see it from space! Below is a picture of an algal bloom viewed from a satellite.



During these blooms, increased photosynthesis leads diatoms to sequester huge amounts of CO2. CO2 levels have risen dramatically since the industrial revolution (from 280 ppm (parts per million) to 385), and are predicted to reach levels close to 800 ppm by the end of the 21st century. Right now, spring blooms play an important role in sequestering atmospheric CO2, but little is known about the specific mechanisms of these blooms, and how their ability to sequester CO2 will change as atmospheric levels continue to rise. Thus, it’s important for us to understand how diatoms bloom and what sort of conditions are required for their growth, in order for us to make accurate predictions about the future of the global carbon cycle and climate.

**What nutrients do they rely on?**

Diatoms have shells made of silica, and “control the biogenic cycling of silicon in the world’s oceans to such an extent that every atom of silicon entering the ocean is incorporated into a diatom cell wall on average 39 times before being buried on the sea floor” (Armbrust, 2009).

Diatoms also rely on Nitrogen and Phosphorus for their growth, and thus play a role in oceanic nitrogen and phosphorus cycles. Nitrogen is required to make amino acids – the building blocks of proteins. Phosphorus is required for DNA. Diatoms are able to extract nitrogen from the ocean in many forms. They can use NO3-(nitrate), NO2- (nitrite), NH3 (ammonia) and (NH2)2CO (urea). Their ability to use these different forms of nitrogen helps them in other ways too: the urea cycle provides important proteins for building their silica shells.

Samples of marine phytoplankton communities have shown that Nitrogen, Phosphorus and Silica exist in the ratio N:P:Si = 16:1:15.

**\*Observe sterile technique as much as possible so you don’t contaminate your cultures.**

**Day 1: Pouring media, inoculating cultures, taking first measurements, variations.**

In order to learn more about what nutrients diatoms are dependent on, and how their blooms develop, you will grow diatoms in four different types of media.

* All Nutrients: This media contains three necessary nutrients, nitrogen (N), phosphorus (P), and silica (Si) in excess, meaning the diatoms won’t run out.
* N-: This media is limiting with regard to nitrogen, meaning all the other nutrients (Si and P) will be present in excess, but N is present at a level similar to what is found in real ocean ecosystems.
* P -: This media is limiting with regard to phosphorus.
* Si-: This media is limiting with regard to silica.

You will also grow your diatoms in two different light conditions – high light and low light. High light cultures will be grown directly under two fluorescent cool lamps (with the top of the flasks approximately 5 inches below the edge of the lamp). Low light cultures will be grown three inches further from the lights than the high light cultures, and will be covered with one layer of mesh. Your lamps are set to turn on at 8 AM and turn off at 8 PM.

Diatoms and other photosynthesizing organisms adapt to different light conditions. When there is less light, they produce more chlorophyll, enabling them to capture as much light as possible. These photoadaptations can affect some of your measurements.

You will also have one, larger flask to demonstrate the power of mixing in developing algal blooms. Blooms occur in spring, after months of winter storms have mixed nutrients in the ocean. In the most intense storms in the Atlantic, waves can mix water at a depth to 200 meters!

**Pouring Media:**

1. Label sixteen 70 mL canted neck flasks as listed below:
	* All Nutrients, High Light (flasks 1 and 2)
	* All Nutrients, Low Light (flasks 3 and 4)
	* N-, High Light (flasks 5 and 6)
	* N-, Low Light (flasks 7 and 8)
	* P-, High Light (flasks 9 and 10)
	* P-, Low Light (flasks 11 and 12)
	* Si-, High Light (flasks 13 and 14)
	* Si-, Low Light (flasks 15 and 16)

Note: Make sure your flasks are clearly numbered. These numbers will help you keep track of the data you are collecting.

Label one 250 mL canted neck flask as “All Nutrients, Mixing.” Call this flask 17.

1. Into flasks 1-4, pour 40 mL of the media labeled “All Nutrients.” Your canted flasks have mL markings on the side. They can be difficult to read while pouring, so before you pour, use a sharpie to mark the 40 mL mark on the sides of your flasks.
2. Into flasks 5-8, pour 40 mL of the media labeled “N-.”
3. Into flasks 9-12, pour 40 mL of the media labeled “P-.”
4. Into flasks 13-16, pour 40 mL of the media labeled “Si-.”
5. Into flask 17, pour 40 mL of the media labeled “All Nutrients”

**Inoculating Cultures:**

1. Locate the flask entitled “stock culture.” Carefully pour about 40 mL of this culture into a clean falcon tube.
\*Diatoms (*Thalassiosira*) are available from Carolina Biological:

[**http://www.carolina.com/algae/thalassiosira-living/153110.pr?question=diatoms**](http://www.carolina.com/algae/thalassiosira-living/153110.pr?question=diatoms)

1. Pipette 1.5 mL of stock from the falcon tube into each one of your 17 flasks. When you unscrew the tops to your flasks, do not set them directly onto the lab bench. Try to keep them in between your fingers as you work in order to minimize the risk of contamination. Into flask 17, also drop the small stir bar you’ve been provided with.
2. Place all flasks labeled “High Light” directly under the lamps set up along the windows (with the top of the flasks approximately 5 inches below the edge of the lamp). Stand these flasks up so the bottom is flat and the top is where the cap is.
3. Place all flasks labeled “Low Light” three inches further from the lights than the high light cultures, and cover them with one layer of mesh. Stand these flasks up so the bottom is flat and the top is where the cap is. Use tape to make sure the mesh covers all sides of the flask.
4. Place flask 17 on its side on top of a stir plate. It should be oriented such that the logo of the bottle (which reads BD Falcon) is on top. Turn the stir plate onto medium.

**Variables:**

fish shake (fish emulsion)

Infant iron drops \*1mL trace minerals or vitamins per 1000mL seawater

Iron nail (non-galvanized)

Chalk or Rolaids (calcium carbonate)

Copper Wire

Magnesium Sulfate (Epsom salts)

**Taking Your First Measurement:**

Because you just inoculated your cultures, they should all have the same cell density. Therefore, you only need to take measurements from one culture today. These measurements will provide that starting values for all of your samples.

You never want to place pipette tips directly into your cultures, because they may be contaminated. In order to avoid contamination, wipe down your bench with ethanol and, **once your bench is completely dry**, start a flame with your Bunsen burner. Pour about 2 mL of your culture into a clean test tube. You will take all of your samples from this test tube.

1. Cell count using hemocytometer: A hemocytometer is a special microscope slide that has a grid on it. This grid allows you to count cells easily, and with a simple calculation, determine the density of your culture. You can use plastic disposable hemocytometers, a reusable glass hemocytometer, or a slide with a gridded sticker on it. When viewing diatoms, generally less microscopic light allows for optimal viewing. If you cannot see the cells, try dimming your light source.

Below is a picture of a hemocytometer grid. When you use it, you will count the cells in each of the four corners. Figure B shows one of those four corners. Notice that this corner has 16 small squares in it. When you’re using the microscope, you will focus on those small squares.



To use the plastic hemocytometer, load 10 uL of your culture onto the slide. When using the glass slide, use ethanol and a kimwipe to clean off both the slide as well as the slide cover, before you load your sample (being very careful not to break them – these slides cost $150!). Both the plastic and glass hemocytometers have two counting grids, meaning they can hold two samples at once. Place the slide under the microscope on 40x magnification and focus until you can see the hemocytometer grid.

Find the top right corner of the grid. Now, find the bottom left square (of the 16 small squares) within that corner. Using a clicker, count the number of cells in that square. Using the microscope controls, move the slide so that you are now focused on the square to the right of the one you just counted. Continue using the clicker to count the cells in that square. Continue moving the slide so you can count the cells in all sixteen squares of that corner.

Once you have counted all sixteen squares, write down your total. Reset your clicker, move your field of view to the top left box and repeat your counting process. Write down this total. Repeat this process for the bottom left and right boxes. Below I have drawn the order in which I usually count the squares on the hemocytometer slide. Whichever order you decide to count them in, make sure you develop a system that is consistent between all of your countings. This will help you keep track of all the numbers without getting confused about which things have been counted and which haven’t. You need to count at least 100 cells for your results to be significant.



Average the cell counts you recorded for the four corners. Each corner is 1mm by 1mm in size, and has a depth of 0.1 mm. Cell counts are usually recorded in cells/mL. Remember that 1 cm3 is equal to 1 mL. The volume of the area you counted cells for is 1mm x 1mm x 0.1mm = 0.1mm3. In order to convert to cells/mL do the following calculation:

(average number of cells per corner) x (1000 mm3) = cells

 0.1 mm3 (1 cm3) mL

Each time you finish counting with a plastic hemocytometer, throw it away. Each time you finish counting with the glass hemocytomter, clean it again carefully with ethanol and a kimwipe. Make sure to dry it well.

1. OD600: Another way to determine cell density is to determine the optical density of your cultures at the wavelength equal to 600nm (The visible spectra goes from 400 to 700nm).

Prepare your blank by putting 200 uL of your “All nutrients” media into an appropriately sized cuvette. You never want to dip tips directly into media for diatoms because it can be contaminated. So, start up your flame again, and pour a small volume of your media into a test tube. From this test tube, pipette 200 uL of the media into your cuvette.

Next, pipette 200 uL of your sample into a clean cuvette. Bring both your blank and sample cuvette to the OD reader. Place your blank in the machine, ensuring that it is oriented properly. Press “blank” on the machine. Remove your blank and place your sample into the machine. Press “sample.” Remove the print-out of your results and record them.

Place a strip of parafilm over your blank cuvette so you can re-use it for your measurements every day. Wash out your sample cuvette and leave it to dry.

This first measurement will serve as the OD reading for all 16 of your cultures. Every other day, however, you will measure and record OD for each sample.

1. Pigment Observation: Though this won’t give you a cell count, writing down observations about the color of your cultures is a good way to spot differences between cultures. Your cells will usually settle to the bottom of your flasks. Note how dark the settled cells look. Now, swirl the media in your flask to spread your cells uniformly throughout the media. Write down the color of the homogenized media. Though it may look very clear at first, every day the color will change slightly, and you will begin to see differences between the cultures in different media.

**Days 2-5:**

Continue to collect all measurements outlined above, but on days 2-5 collect measurements for all 17 of your cultures. Once again, make sure to work near a flame, and pour out 2 mL from each of your cultures into clean, labeled test tubes. This way you will not contaminate your cultures.

Record and graph your results using Excel. You used multiple methods to determine cell growth. Compare the curves each measurement provided. Which one do you think was most precise, and why? Why did some methods give less precise curves?

Look for patterns among cells in the same media. What happens to their growth when they’re in limited media? What happened in the flask that was being mixed? If you had to graph a curve of the nutrient levels in the media, what patterns do you think you would find?

**Further Thoughts and Conclusions:**

As diatoms bloom and use up nutrients, what do you think happens to populations that feed on diatoms? What role do you think mixing plays in providing nutrients? (Note: nutrients often settle towards the bottom of the ocean, but diatoms can only grow in the presence of light).

It is predicted that with rising CO2 levels, diatoms will flourish. Given what you’ve learned about how diatoms use nutrients, what do you think will happen to nutrient cycles if diatom blooms are stronger and more productive? How do you think this will affect ocean ecosystems? How will it affect communities that feed on diatoms and the resources they provide?