**DIATOM POPULATION STUDY**

Diatoms are a crazy-small type of phytoplankton

that account for only 1% of the earth’s biomass, yet they account for 20% of the global photo-synthesis and play a huge role in carbon cycling, especially carbon sequestering. Each year they generate about as much organic carbon as all the terrestrial rain forests combined!! Most species are marine/ocean, but there are some freshwater species.

While most organisms are negatively affected by dropping pH (due to dissolved CO2 in the water) diatoms are expected to react positively. Diatoms absorb huge amounts of CO2 during photosynthesis, but unlike other marine organisms, they use SILICA to build their support structure rather than calcium carbonate--so a dropping pH will not cause their framework to dissolve.

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 small 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 so that not energy is wasted, but diatoms are able to maintain a high genetic diversity as well as great abundance.

Each spring when nutrient levels, light levels and temperatures are high, diatom populations soar across the world. These bursts of productions are referred to as “blooms” and can be seen from space!! During these blooms, increased photosynthesis leads diatoms to sequester huge amounts of CO2. CO2 levels have risen dramatically since the industrial revolution (from 280ppm to 385ppm) and are predicted to reach levels close to 800ppm 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. Diatoms 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 (Ambrust, 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 for 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)2CO2 (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.

**GENERAL INSTRUCTIONS FOR DIATOM POPULATION LAB:**

The species we will use is *Thalassiosira pseudonana*, or “Thaps” for short.

You and your lab partner(s) will do ONE of five class experiments. You will prepare a control and an experimental culture and monitor their growth rates over a week (3 data plus the initial). You will graph your data and share your findings with your class.

\*LABEL the sides of your two flasks with your initials, the NAME of the experiment and which flask is the control and which is the experimental.

\*Prepare your two flasks with media and Thaps (see instructions below)

\*Count the diatoms FOUR times: once to begin (Friday) and then three counts in one week (Mon, Wed, Fri).

\*Put 1 drop (ONLY ONE DROP) of your culture onto the gridded slide and top it with a coverslip.

\*Use medium to low light, then use the 10X objective to find the Thaps and count them.

**HOW TO ESTIMATE POPULATIONS GROWTH OF THAPS**

Use the following techniques and the coaching of your teacher to calculate the population of Thaps throughout the week. Record these calculations on the data sheet.

1) Count how many Thaps are in 4 squares (count quick--they float around!) and calculate an AVERAGE how many Thaps in those 4 squares.

 Example: \_\_ + \_\_ + \_\_ + \_\_ = \_\_\_\_\_÷ 4 = \_\_\_\_\_\_\_\_\_ Thaps per square on average.

2) Next, make an estimate for how many Thaps are in ONE DROP (34µl).

  **µl** means “micro” which is one-millionth of a liter

 \_\_\_\_Ave # Thaps per square x 400 squares on the slide =\_\_\_\_Thaps/34µl (one drop)

It takes 34µl to make one drop. It takes 30drops to make1ml of liquid (which is 1000µl). With that info, you can calculate how many Thaps are in one ml:

 \_\_\_\_\_\_\_\_\_Thaps/34µl (one drop) x 30drops/ml = \_\_\_\_\_\_\_\_\_\_\_Thaps/ml

3) And finally, calculate how many Thaps are in your 25ml culture flask:

 \_\_\_\_\_\_\_\_\_Thaps /ml x 25ml = \_\_\_\_\_\_\_\_\_\_\_Thaps/25ml

\*Keep a data table of your control and your experimental cultures.

\*Graph BOTH sets of data onto ONE graph.

\*Write a paragraph conclusion of your data.

\*Present findings to the class.

 **FIVE LAB EXPERIMENTS WITH DIATOM POPULATIONS**

**A: High vs Low light**

Measure 20ml ESAW and 5ml of Thaps to both flasks. Wrap one flask with a piece of white plastic and tape it so the plastic won’t slip off. This one will receive considerably lower light than the other flask. Stack your two flasks and put the wrapped one on the bottom. Put both flasks under the lamp. The lamp is set up to shine light through a water tank, which allows light to penetrate without heating up the diatoms. (If you choose to use compact fluorescent lights, you will not need to use a water tank since they give off much less heat than incandescent bulbs).

**B: CO2 Rich (acidic) vs CO2 normal**

Measure 20ml ESAW and 5ml of Thaps to both flasks. In one flask, blow bubbles (CO2) into the ESAW medium for exactly one minute. Use a serological pipet with a plug of cotton in it (this will trap most of the contaminants from your breath). This will drop the pH from 8.0 to 5.5. Then add 5ml Thaps and the lid. Stack the two flasks under the regular lights.

\*NOTE: EVERY DAY you will have to blow bubbles into the experimental flask for one minute to maintain a lower pH of about 5.5.

**C: Nitrogen Rich vs Nitrogen Poor**

Prepare the control flask with 20ml ESAW and 5ml Thaps. Prepare the experimental flask with 20ml ESAW -N (minus nitrogen) and 5ml Thaps. Stack the two flasks under the regular lights.

**D. Phosphorus Rich vs Phosphorus Poor**

Prepare the control flask with 20ml ESAW and 5ml Thaps. Prepare the experimental flask with 20ml ESAW -N (minus Phosphorus) and 5ml Thaps. Stack the two flasks under the regular lights.

**E. Silicon Rich vs Silicon Poor**

Prepare the control flask with 20ml ESAW and 5ml Thaps. Prepare the experimental flask with 20ml ESAW -Si (minus silicon) and 5ml Thaps. Stack the two flasks under the regular lights.

**DIATOM POPULATION STUDY DATA SHEET**

**GROUP MEMBERS** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Investigative Problem/Question:** How are diatom populations influenced by environmental changes such as lighting, CO2 and nutrients (N, P, Si)?

**Hypothesis:**

**DATA TABLE** for \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_(control).

Day Date T/25µl T/ml Total Pop. Clarity of culture (describe)

**DATA TABLE** for \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_(experimental)

Day Date T/25µl T/ml Total Pop. Clarity of culture (describe)

**GRAPH TITLE**:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**CONCLUSION:**

• Answer the experimental question.

• Include supporting data-- high and low data points.

• Explain how these data points support your conclusion.

• Provide a scientific explanation for the trend in the data.