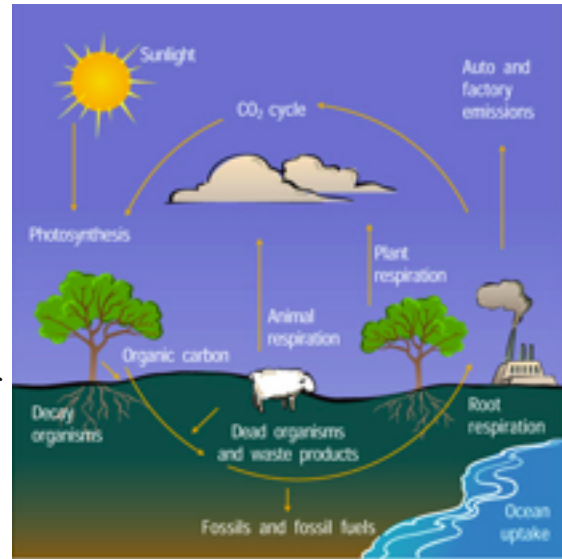
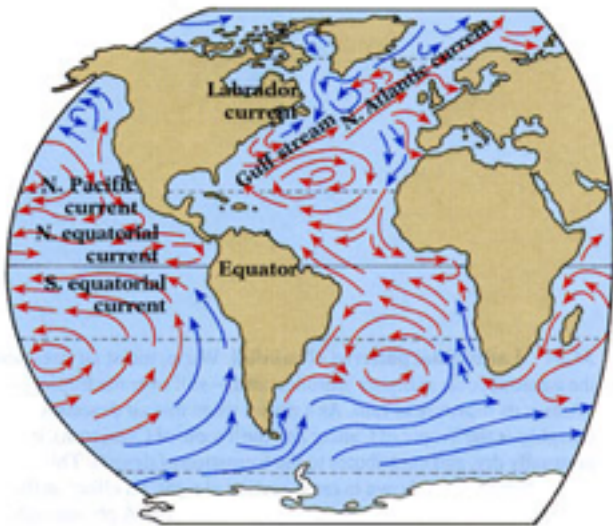


Phytoplankton Growth and Nutrient Cycling

Background Information: Ocean Carbon Cycle

The Earth's Carbon Cycle can be split into 3 major parts: *terrestrial carbon cycle, ocean carbon cycle and the atmosphere carbon cycle.*

The Carbon Cycle in the ocean has both organic and inorganic components. The inorganic part acts like as a pump- *bringing carbon dioxide (CO₂) in and out of the ocean depending on the temperature of the water.*



Carbon dioxide

is more soluble (*absorbed*) in cold water than in warm water. So in areas of cold water, *like the polar oceans*, CO₂ is more easily absorbed from the atmosphere into the ocean. So, the colder oceans tend to hold more carbon dioxide than the warmer ocean regions, where CO₂ is often expelled from the ocean back into the atmosphere as a gas. *Did you know that the equatorial Pacific is the biggest single natural source of CO₂ to the atmosphere?* This is because deep, cold water which is CO₂ rich is brought to the surface in the eastern part of the equatorial Pacific ("upwelling") and then warmed by the sun, causing the CO₂ to be released into the atmosphere.

The organic part of the carbon cycle also acts as a pump of carbon to and from the atmosphere. The carbon in the water first takes the form of dissolved *carbon dioxide gas (CO₂)*, *bicarbonate (HCO₃⁻)*, and *carbonate (CO₃²⁻)*. Many shelled organisms in the ocean use the carbonate ion to build their shells.

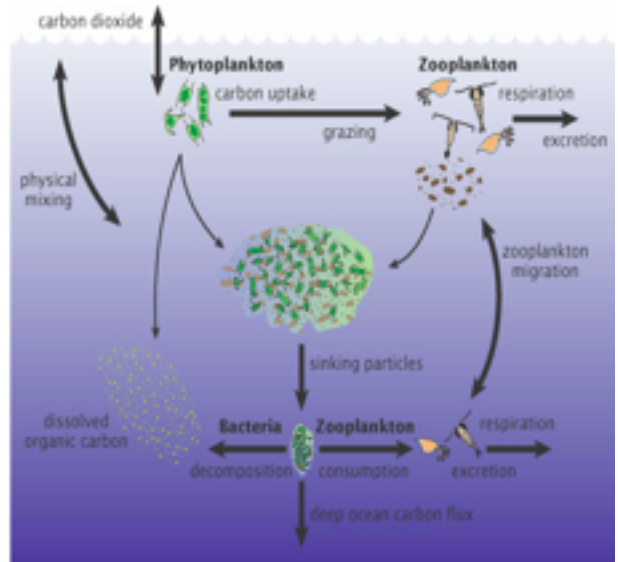
Plants, *such as phytoplankton (algae)*, use the carbon dioxide and bicarbonate to make food and energy through a process called ***photosynthesis***. Carbon is used during photosynthesis and then released during respiration. Because a large portion of the carbon in the ocean is in the form of biomass (*plants like phytoplankton*) after taking up CO₂ during photosynthesis and these phytoplankton produce oxygen, scientists are interested in studying various aspects of how phytoplankton grow. Global warming is a concern because it may change the CO₂ pump and thus may affect the amount of biomass (phytoplankton) that grows in the ocean. Phytoplankton is an integral part of the oceanic food web. If global warming affects the quantity of phytoplankton that are able to grow, it may alter, harm or destroy the entire oceanic ecosystem.

In order to understand how these effects may manifest themselves, scientists need to understand what limits phytoplankton growth.

Iron and How it Limits Phytoplankton Growth

Phytoplankton is a single cell plant and grows in the upper layers of the world's oceans because of its need for light. It uses light through a process called **photosynthesis** to create food and energy for itself. *Recently, scientists have found that phytoplankton do not grow as well in ocean regions that are low in iron.*

Why do phytoplankton need iron? Well, all living things need macronutrients (major nutrients like nitrate and phosphate) and micronutrients (minor nutrients like iron and zinc) in order to survive. Iron is a micronutrient that helps our blood carry oxygen. Iron is important to phytoplankton because it acts as an electron carrier and a catalyst during photosynthesis. Phytoplankton need iron just like we do. *When there isn't enough iron around, then the phytoplankton can't grow.*



Experimental Overview:

You will be testing the effects of various nutrients on algal growth. You will be focusing on the nutrients nitrate, phosphate and iron, to see which conditions lead to the most optimal growth for the phytoplankton. You will be keeping track of the algal density by using microscope counts and also readings from a spectrophotometer for the percent transmission. You will be monitoring the growth of the phytoplankton for at least 5 days, and at the end of the experiment will make graphs of the different nutrient treatments. As a group, you will test one control and two different treatments of phytoplankton (Control, + Nutrients, and + Nutrients and Iron) to see which grew best.

Materials:

- 5 mL of phytoplankton culture
- 50 mL of filtered seawater
- 50-250mL Erlenmeyer flask
- Cotton ball
- Available light source (*sunny window is fine*)



- 3-300 µl pipettes
- Nitrate, Phosphate and Iron nutrient solutions
- Wax pencils
- Microscopes and microscope slides
- Spectrophotometer (if possible)

Procedures:

1. Each lab pair should have one flask. Each group should have three flasks between the three groups. *(The replication of the treatments is across the entire class- class data will be shared)*

Student pairs must label their flask with the appropriate label with the wax pencil:

- Control
- + Nutrients (Nitrate and Phosphate)
- + Nutrients and Iron (Nitrate, Phosphate and Iron)

3. Add an equal amount of pure filtered seawater (50ml) to each flask.

4. Cover the top of the container labeled “Control” with a cotton ball.

5. Add an equal amount of nitrate and phosphate to each “+Nutrients” container using the table below as a guide. Add iron to only one of the flasks and label it as “Nutrients + Iron.” Calculate how much you would have to add to each flask to get the final concentrations listed in the table.

*Hint: Use the following equation (Molarity final * Volume final = Molarity Initial (Stock Solution) * Volume Initial (Amount added of the stock solution)). You are solving for the “Volume initial” which is the amount of the nutrient stock solution you should add to each flask. Beware of units!*

Nutrient	Concentration of Stock Nutrient Solution (M)	Amount added (µl) to 50 mL	Final Concentration (µM) in 50 mL
Nitrate	0.008		48
Phosphate	0.004		24
Iron	0.00025		1.5

6. After you have added 300µl of each nutrient to your treatments, cover the tops of the flasks labeled “+Nutrients” and “Nutrients + Iron” with a cotton ball.

7. Add an equal amount of the stock algal culture (5 mL) to each flask.

8. Place all flasks in a sunny window or under grow lights where they can receive equal amounts of light and make sure they can be exposed to the same temperatures *(don't place some on a heater and others near the open window)*.

9. Over the course of 5 days, you will make observations regarding the algae using microscopes and the spectrophotometer.

Measuring the Phytoplankton:

1. To measure the phytoplankton using the **microscopes**, you should swirl the phytoplankton solution for 1 minute to mix it well and then take 3 drops of solution and place onto a microscope slide with a counting grid. Focus the microscope and count the amount of cells you see on the slide (without moving the slide) using a light microscope.

2. You will also monitor the phytoplankton concentrations using the **spectrophotometer** in the classroom. See the additional sheet on using the spectrophotometer. Microscope measurements and spectrophotometer measurements will be compared in the lab write-up.

Lab Write-Up:

Graphs:

1. Using the data you have collected in your lab notebook (share the data from each treatment with your group) you should make a graph of the growth of each treatment. Place the day on the x-axis and the growth on the y-axis (% transmission) and plot all three treatments on the graph (Control, + Nutrients, + Nutrients and Iron).

Questions to Consider:

1. Under what conditions did the algae grow?

2. Under what conditions did the algae grow best?

3. Under what conditions was there no growth?

4. What do the results of this experiment tell us about the needs of phytoplankton in the ocean? Explain.

5. What did you learn about chemical cycling and the importance of maintaining specific nutrient concentrations?

6. Why do you think it is important to know how algae grow best? Explain.

