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Prismatic Little Plankton. Subjects: Life Sciences, Physical Science/Chemistry; Marine/Ocean Science - Grades: 9-12

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PRISMATIC LITTLE PLANKTON

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Grade Level
High School

Subject area
Biology, Physics, Environmental Science

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Title Prismatic Little Plankton

Focus Pigments and Photosynthesis: Determining phytoplankton species present in a sample based on chromatography tests

Grade Level HS Physics, HS Biology, HS Environmental Science

VA Science Standards

- BIO.2 The student will investigate and understand the chemical and biochemical principles essential for life. Key concepts include the capture, storage, transformation, and flow of energy through the process of photosynthesis.
- PH.4 The student will investigate and understand how applications of physics affect the world. Key concepts include examples from the real world, and exploration of the roles and contributions of science and technology.
- PH.8 The student will investigate and understand wave phenomena. Key concepts include wave characteristics, fundamental wave processes, and light in terms of wave models.
- PH.9 The student will investigate and understand that different frequencies and wavelengths in the electromagnetic spectrum are phenomena ranging from radio waves through visible light to gamma radiation. Key concepts include the properties, behaviors, and relative size of radio waves, microwaves, infrared, visible light, ultraviolet, X-rays, and gamma rays; wave/particle dual nature of light; and current applications based on the respective wavelengths.

Learning Objectives

- Students will extract pigments from common plant-based foods
- Students will conduct paper chromatography tests
- Students will generate sketches showing presence of different pigments in an unknown "algae" sample based on the results of paper chromatography tests
- Students will infer the species composition of an unknown sample through performing experiments and creating graphs based on qualitative observations
- Students will explain how a variety of pigments found in plankton allow the organisms to absorb or reflect different wavelengths of light

Total length of time required for the lesson Two 90-minute class blocks

Key words, vocabulary

Photosynthesis; electromagnetic spectrum; photons; reaction centers; chloroplasts; chlorophyll; carbohydrates; primary producers; cyanobacteria; phytoplankton; mixotrophic; absorbance; reflectance; accessory pigments; carotenoids; phycobilins; phycoerythrin; phycocyanin; fucoxanthin; diatoms; rhodophytes; High Performance Liquid Chromatography; chlorophyll; accessory pigments; absorbance; reflectance; transmittance; cyanobacteria; phytoplankton;

Background Information

In terms of global biochemical processes, perhaps none is as significant and important as **photosynthesis**. Photosynthesis is the process whereby green plants and other select organisms convert light energy from the **electromagnetic spectrum** into organic chemical energy (food) using sunlight, water, and carbon dioxide. Oxygen is generally released as a byproduct of photosynthesis. The general equation for the photosynthetic process is included below:

$$\underset{\text{(carbon dioxide)}}{CO_2} + \underset{\text{(water)}}{H_2O} + \underset{\text{(light energy)}}{\text{photons}} \longrightarrow \underset{\text{(carbohydrate)}}{CH_2O} + \underset{\text{(oxygen)}}{O_2}$$

The process begins when **photons** of energy found in wavelengths of light in the visible light spectrum are absorbed by proteins called **reaction centers**, which are located in organelles called **chloroplasts** found in the membranes of most plant cells. Inside these reaction centers are special green pigments called **chlorophyll**. These pigments and reaction centers use the absorbed photon energy to excite the electrons in water (H_2O) to the point where the hydrogen and oxygen atoms are split apart, producing oxygen gas. The newly-freed hydrogen atoms fuse together with the carbon dioxide to create **carbohydrates**, which are very nutrient-rich compounds that can be stored and later released by the organisms to support their metabolic activities. Through this process, these organisms (called **primary producers**) effectively convert light energy into chemical energy and provide the base nutrition and energy that supports all of Earth's global food webs while also providing the oxygen needed for life to exist on the planet.

In the ocean, there are a variety of photoautotrophs responsible for this important process. Seagrasses, macroalgae (i.e. seaweeds and kelp), corals, and anemones are photosynthesizers in the ocean. In the case of corals and anemones, it is not the animals themselves but symbiotic **cyanobacteria** (blue-green algae) that live inside the animal's tissues and perform the photosynthetic process to provide energy for both organisms. Cyanobacteria are just one of many groups of organisms that make up the global network of **phytoplankton**. Phytoplankton are microscopic (most less than 0.1 millimeter in length) protists and bacteria that drift through the water column in the global ocean, estuaries, rivers, and freshwater lakes. All phytoplankton photosynthesize, however, there are also a large amount of species which are **mixotrophic** – meaning that they not only photosynthesize to convert light energy into organic compounds, but also consume other algae to get nutritional energy from them as well.

In addition to their role in being the base of the aquatic food web, phytoplankton create a significant amount of the oxygen that is diffused from the surface ocean into the atmosphere. It has been estimated by marine scientists that phytoplankton are responsible for creating up to ½ of the oxygen in the atmosphere at any one point. This means that phytoplankton are disproportionately responsible for the maintenance of Earth's oxygen budget and are incredibly important for all life processes occurring on the planet.

In order to perform photosynthesis, all phytoplankton species have a pigment called chlorophyll-a. This pigment **absorbs** certain wavelengths of light while it **reflects** the wavelengths which it does not absorb. That is why we perceive plants and these microalgae as being green – because chlorophyll-a absorbs heavily in the wavelengths of light that we perceive as red and blue but reflects green light.

In addition to chlorophyll-a, different species of phytoplankton have different **accessory pigments** to help them maximize their photosynthetic abilities. These accessory pigments absorb additional wavelengths of light and pass the energy to the chlorophyll molecules, expanding the range of wavelengths that the phytoplankton can use in photosynthesis. Examples of accessory pigments include **carotenoids** and **phycobilins** (**phycoerythrin** and **phycocyanin**).

Carotenoids are also found in some plant-based foods that we are more familiar with, including carrots and oranges. Carotenoids absorb additional wavelengths of blue and blue-green light that chlorophyll does not and reflects heavily in the orange/yellow wavelengths, which is why we see these food items as orange. One example of a carotenoid-based accessory pigment is **fucoxanthin**, a yellow-brown pigment which gives brown algae and **diatoms** their characteristic color.

Phycoerythrin absorbs heavily in the green wavelengths, reflecting red light – we see these pigments in the **rhodophytes**, or red algae. Phycocyanin absorbs heavily in the yellow and orange wavelengths, reflecting blue and green wavelengths. Appropriately, these pigments are found in the cyanobacteria (blue-green algae).

Since each different group of microalgae has a different combination of chlorophyll and accessory pigments, it is possible to identify the most common groups of phytoplankton present in a sample by analyzing the light that is absorbed and reflected by the sample. Scientists use a tool called **High Performance Liquid Chromatography** (HPLC) to do this analysis. The scientists will collect a sample of water from the field, filter the phytoplankton out onto a fine mesh filter, and then grind the plankton into a solution. This solution is placed into the HPLC chamber where a light is centered on it. A sensor in the machine measures the reflectance and absorbance of specific wavelengths of light and creates a graph of the results. The scientists can then use these results to estimate the abundance of different types of phytoplankton present in the sample.

Phycobilins are not only useful to the phytoplankton species which have them, but also have been very useful in scientific research. Each of the two pigments **fluoresces** at a particular wavelength. This means that when they are exposed to strong light, they absorb the energy from the light and then release it by emitting light in a specific wavelength. The light that is produced is so distinctive that it can be used as a chemical "tag." Medical scientists have chemically bound them to antibodies, which they have then added to a solution of cells. When the solution is sprayed past a laser and a computer sensor, a machine identifies whether the cells in the spray have been "tagged" by the antibodies. This has been very useful in tagging tumor cells in cancer research.

Student handouts and other materials needed

Lesson Script (Appendix 1), Student Worksheets (Appendix 2), Answer Key (Appendix3)
Supplementary Google Slides PowerPoint, Video resources included in the Google Slides PowerPoint

Materials & Supplies

For the lesson, the instructor will need a computer loaded with the supplementary Google slides presentation and an overhead projector.

For the activity, the instructor will need:

~ ½ L ethanol
8 strips chromatography paper (approx. 6" long x ¾" wide, or any combination of long and narrow)
8 large, plastic drinking glasses (12-16 ounces size)
8 small plastic or paper cups (Dixie-style)
12 popsicle sticks
4 50-mL measuring beakers/cups
1 roll of tape
1 pair scissors
4 small plastic strainers
10 leaves of spinach, kale, or other leafy green
1 orange, cut in half
16 red berries (e.g. strawberries or raspberries)
16 dark berries (e.g. blueberries or blackberries) OR a head of red cabbage
1 pipette
Copies of the student worksheet
Colored pencils (enough for each group to have a set of green, blue, red, orange, and yellow)

Classroom/Lab/Field Study Setup

Students will be working in small groups on this project, so a large table where they can work together is ideal. It may also be beneficial to place a protective covering of some sort (tablecloth, newspaper, etc.) down on the table to prevent any chemicals or dyes from staining furniture. Since students will be working with ethanol, it is also helpful to make sure there is enough ventilation or open space to minimize inhalation of fumes. The students will not be using enough ethanol to require a hood, however it may be beneficial for students to be wearing gloves and goggles for safety reasons.

Procedure

Advance preparation of lab materials – 45 minutes

Advance preparation of materials includes preparing the pigments for the "mystery" algal species tests. For this, you will need one half of all the fruits and vegetables that are listed above (5 spinach leaves, ½ orange, 8 red berries, and 8 dark berries).

1. Use the scissors to cut the spinach leaves into small pieces, and then separate each type of produce into one of 4 smaller plastic/paper cups. You will want to slice the half orange into thinner slices with a knife (leave the rind on).
2. Measure out 50 mL of ethanol and add to each cup with the produce.

3. Use the popsicle sticks to crush the produce/leaves slightly into the ethanol, and afterward let each sit for 30 minutes undisturbed.
4. Use a strainer to separate the produce pieces from the extracted pigments by pouring through the strainer into each of four large plastic glasses. Rinse the small cups so they can be used again.
5. Divide the spinach pigment equally between the four small cups.
6. To prepare the "mystery" samples, add enough of the following pigments to one of each of three of the four* cups of spinach pigment to double its volume:
    a. Cyanobacteria: add dark berry pigments into the spinach to double its volume
    b. Diatoms: add orange pigments into the spinach to double its volume
    c. Red algae: add red berry pigments into the spinach to double its volume
    d. * The green algae sample will be just the spinach pigment
7. It may be helpful for your reference to label each of the mystery cups as "Mystery sample A, B, C, or D" and keep track in your head of which is which so that you can help lead the students through their analysis later.

Lab Set-up – 15 minutes

1. Place one large plastic cup on each of four desks/working spaces, along with a 50-mL beaker of ethanol, 2 strips of chromatography paper, 1 small Dixie cup, 3 popsicle sticks, 1 small plastic strainer, and a pack of colored pencils.
2. Distribute the vegetables/produce so that each group has one type to test, placing them in the small Dixie cups.
3. Also, give a pair of scissors to the group who will be testing the spinach.

1. Project map of the monitoring stations by CBIBS. Right next to it is a map of one of the most severe fish kill event which is in Corrotoman River. Ask the students if we want to find out what has happened, data from which station should be used? Answer should be SR (Stingray Point).
2. Divide your students into groups of four. Give them Student Master.
3. Now have the student team examine the graphs on student master and complete the clues and questions. Team members should discuss. Walk around and assist if needed.

CLASS BLOCK #1: CONCEPT INTRODUCTION AND PIGMENT CHROMATOGRAPHY TESTS

Pigment Extraction & Google Slides Introduction – 30 minutes

1. Welcome students into the classroom and direct them to the working tables by dividing them into smaller groups of 4-6 students on the way in.
2. Tell students that they will be doing an activity that requires them to test compounds present in common food items, and that they will be using ethanol in this lab (which is a lab chemical) and that they should take care with the ethanol so that they do not inhale the fumes or spill on the table.
3. Give each student a copy of the student worksheet.
4. Tell the students that they should be pouring the ethanol from the beaker into the small Dixie cup that contains their produce. Tell the spinach group that they need to use the scissors to trim their spinach leaves into small pieces before pouring in the ethanol.

5. Have students use one of the popsicle sticks in their area to grind and mash the produce/leaves into the ethanol solution. Allow them to have a few minutes to accomplish this, and then tell them to set the cups aside so that we can wait for the chemical reaction to occur.
6. While the pigments are being extracted, explain to students what they are going to be doing using the supplemental Google Slides presentation and script (in the comments section of the slides, and in Appendix 1). You should stop after the "Chromatography" slide (slide 7).
7. Tell students that they will be using the worksheets to perform their paper chromatography tests of the pigments in the produce extractions that they made at the beginning of class. Tell them that all the instructions of what needs to be done are on the worksheets, and that you can answer any questions that they have as they do the activity.

Chromatography Activity – 30 minutes

1. Students will work together to create their chromatograms using the instructions on their worksheets (included in Appendix 2).
2. Make sure to walk around the room supervising what the students are doing, making sure that they are on task and doing what they are supposed to.
3. For quick reference, each student group should first be using the plastic strainers to filter out the pigments from the solid pieces of produce, so that the liquid is in the large plastic cup and the solid pieces are returned to the smaller cups. They will then secure one edge of the chromatography paper to the second popsicle stick and taping it so that it hangs below. They should then lay the popsicle stick sideways along the top of the plastic cup so that the end of the paper is in the pigment solution, making sure that the paper is not touching the sides of the container.
4. Have students leave their papers undisturbed for 15 minutes, while you return to the Google slide presentation to talk about photosynthesis in the ocean, phytoplankton, and pigments that phytoplankton have in order to aid in photosynthesis (slides 8-10).

Student Gallery Walk and Group Discussion – 25 minutes

1. Have students refer back to the results of their chromatogram. Give them five minutes to sketch out the results of their test on their worksheet using the colored pencils provided.
2. Allow students to do a "gallery walk" to rotate to the other groups so that they may look at the results of their tests as well. Students should provide a sketch of each group's results on their worksheets as well (~5 minutes at each station).
3. Have students go back to their original seats once done with this task to answer the "analysis" questions as a group.

Breakdown and Clean-up – 5 minutes

1. Have students pour all liquid solution down the drain and dispose of all fruit and vegetable solids in the trash can along with the popsicle sticks.
2. Students can also help collect and return the rest of the materials to the teacher.
3. Students may throw away the small paper cups but should rinse the large plastic cups so that they can be used again.
4. Student worksheets should be given to the teacher for use in the second class block.

CLASS BLOCK #2: MYSTERY CHROMATOGRAPHY TESTS AND CLASS DISCUSSION/REVIEW

Review of Previous Class and Introduction to Activity – 15 minutes

1. Hand back students' worksheets from the previous class block.
2. Google slides presentation (slides 8-10): Remind students that they had performed chromatography tests to examine pigments in common produce items, as well as reiterating what phytoplankton are and why they are important.
3. Explain that scientists use chromatography in the lab to examine and infer phytoplankton community structure (slide 11).

Mystery Chromatography – 45 minutes

1. Tell students that they will be replicating this research by analyzing a "mystery" plankton sample.
2. Hand out one of each of the four "mystery" samples that you had prepared in advance to each group along with an additional large plastic cup. Tell them to repeat the steps from the previous test, and to set these tests aside.
3. Leave tests undisturbed for ~10-15 minutes, while you use the Google slides presentation to explain to students the use of HPLC in marine science (slide 12). Show the video which explains how HPLC is used in the lab. Explain how what they just did is similar to HPLC because their chromatography results should show a breakdown of the different pigments present in the sample, as well as the relative amount of each one present.
4. Have students look at the results of their "mystery" analysis. Have them sketch the results on their worksheet in the appropriate place. Then, direct their attention to the presentation slide (#13) and have them guess which of the four algal species were present in their "samples." This should be done in their small groups.

Discussion – 20 minutes

1. Ask each of the four groups to explain to the class which mystery sample they analyzed and which species they thought were present in the sample and why. This can be done in a presentation format if desired.
2. Explain that this analysis is commonly used in phytoplankton analysis in the lab because it is very reliable as to a measure of the different types of algal species present in a sample and is much more time efficient and less labor-intensive than having a scientist sit at a microscope to identify each of the thousands of individual algal specimens in the sample.
3. Review why the lesson is important: why should they care about phytoplankton? Remind them that algae are very important in oxygen production (phytoplankton are responsible for nearly half of the oxygen in our atmosphere!) and that phytoplankton form the foundation for all aquatic food webs. All of the organisms they know and love rely on phytoplankton either directly or indirectly!
4. Explain that phytoplankton are important for human health as well. Use Google slide 15 to explain what fluorescence is (show included video), how phycobilins are used as chemical

fluorescent “tags” to aid in cancer research, and how many people use phytoplankton as a nutritional supplement given that they are rich in fatty acids which are good for your heart (“fish oil” and “krill oil” fatty acids actually come from the phytoplankton that they eat!) and that they also have a large amount of vitamin B-12 which helps keep the body’s nerve and blood cells healthy and also boosts energy levels!

Breakdown and Clean-up – 10 minutes

1. Have students pour all liquid solution down the drain.
2. Students can also help collect and return the rest of the materials to the teacher.
3. Students may throw away the small paper cups but should rinse the large plastic cups so that they can be used again.
4. Student worksheets should be given to the teacher for use in assessment of topic knowledge.

Assessment:

The instructor will primarily base assessment of the effectiveness of the activity on analyzing the questions and graphs created from the data worksheet that students will hand in at the end of class. The instructor may also assess the effectiveness of the students’ presentations on the results of their mystery sample analysis, if applicable.

References:

More information on photosynthetic pigments: http://www.ucmp.berkeley.edu/glossary/gloss3/pigments.html

Electromagnetic Spectrum video: https://vimeo.com/132634240

NOAA National Ocean Service Phytoplankton Fact Sheet: https://oceanservice.noaa.gov/facts/phyto.html

How Does High Performance Liquid Chromatography Work?: http://www.waters.com/waters/en_US/How-Does-High-Performance-Liquid-Chromatography-Work%3F/nav.htm?cid=10049055&locale=en_US