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

Appendix 1: Lesson Script

** As students are walking into the classroom, direct them to the multiple small stations that you've set up prior to class. It may be helpful to use your discretion as to which students will work effectively and efficiently together. **

Class Block #1: Concept Introduction and Pigment Chromatography Tests

SLIDES 1 & 2: TITLE SLIDE & INTRODUCTION SLIDE

- Today we are going to be doing a lesson that was created by Kristen Sharpe, who is a Masters graduate student at the Virginia Institute of Marine Science. She studies zooplankton, which are all of the animals who live in the ocean and Bay that cannot swim against a current. An example of a zooplankton that you may be familiar with is Sheldon J. Plankton from Spongebob, who is a copepod! Kristen's research will likely be in Bermuda and the Chesapeake Bay, but her lab also does research in the North Atlantic and Pacific Oceans, the Amazon River, and even Antarctica! She led education programs at VIMS for five years and really loves teaching people about science, which is what led her to creating this lesson for us today! The lesson plan that she developed is going to focus on how light and energy are not only relevant in physics but also in marine biology.
- We will start by doing a quick experiment involving common food items. On your tables you have a variety of materials. I am going to come by to give you a copy of your worksheets which will include all of the instructions that you need to follow for this first part of the lesson. Please read the instructions carefully, and only touch the chromatography paper on the edges. Note that you will be using ethanol in this experiment, which is the clear liquid in the cups on your tables and is why you will be wearing safety goggles and gloves. Please be careful with the ethanol so that you do not spill it or unnecessarily inhale any fumes. I will be walking around and can help you if you need my assistance.

SLIDE 3: PHOTOSYNTHESIS

- Okay, now that we've completed the first step of our lesson, let's take a step back to what you learned way back in elementary school. *click*
- How do plants create energy to live? *click* [*Photosynthesis*] *click*
- Photosynthesis is the process in which plants use water, carbon dioxide, and sunlight to convert the light energy into organic chemical energy in the form of sugars. We call these organisms photoautotrophs because they use light to create their own food. The sugars that plants create become food for the animals who eat the plants, and therefore plants serve the critical role of providing the base nutrition that supports all the planet's food webs.
- In order to understand this process better, we will examine the electromagnetic spectrum.

SLIDE 4: LIGHT AND THE ELECTROMAGNETIC SPECTRUM

- Light is a form of electromagnetic radiation, a type of energy that travels in transverse waves. Other kinds of electromagnetic radiation that we encounter in our daily lives include radio waves, microwaves, and X-rays. Together, all the types of electromagnetic radiation make up the electromagnetic spectrum.
- Every electromagnetic wave has a specific wavelength, or distance from one crest (wave tip) to the next, and different types of radiation have different characteristic ranges of wavelengths. Types of radiation with long wavelengths, such as radio waves, carry less energy than types of radiation with short wavelengths, such as X-rays.
- The visible spectrum is the only part of the electromagnetic spectrum that can be detected by the human eye. It includes all electromagnetic radiation with wavelengths between about 400 nanometers (nm) and 700 nm.
- Although light and other forms of electromagnetic radiation act as waves under many conditions, they can behave as particles under others. Each particle of electromagnetic radiation, called a photon, has a certain amount of energy. Types of radiation with short wavelengths have high-energy photons, whereas types of radiation with long wavelengths have low-energy photons. This is very important when thinking about photosynthesis. *click*
- We call this visible light portion of the electromagnetic spectrum the "photosynthetically active radiation" region, or PAR, because these are the wavelengths of light that photosynthetic organisms can absorb in order to use the energy from the photons to create sugars.

SLIDE 5: ABSORPTION, REFLECTION, & TRANSMISSION

- When a photon of light from the sun hits an object, there are three possible fates of that photon: it can be absorbed by the item, can be passed through (transmitted), or can be reflected backward off the surface of object.
- Different photons of light have different fates depending on their wavelengths. In the case of sunlight hitting a leaf, some wavelengths of light are absorbed by the plant's tissues while others are transmitted (passed through) or reflected.

SLIDE 6: PHOTOSYNTHETIC PIGMENTS

- The various wavelengths in sunlight are not all used equally in photosynthesis. Instead, photosynthetic organisms have specialized light-absorbing molecules called pigments that are found in their chloroplasts. These pigments, depending on their structure, absorb only specific wavelengths of light (while reflecting others).
- All photosynthetic organisms contain a pigment called chlorophyll. This pigment is greenish in color and contains a stable ring-shaped molecular structure that electrons freely migrate around. This means that chlorophyll molecules can gain or lose electrons very easily and can transfer those electrons to other molecules easily as well. This is the process in which the chlorophyll "captures" light energy from the sun and transfers it to the other organs in the plant to photosynthesize.

- The set of wavelengths absorbed by a pigment is called its absorption spectrum. In the diagram here, you can see the absorption spectra of three pigments that are important in photosynthesis: chlorophyll a, chlorophyll b, and beta-carotene. You'll see that the chlorophyll molecules absorb strongly in the blue and red portions of PAR. The set of wavelengths that the pigment doesn't absorb are reflected, and this reflected light is what our eyes perceive to be the color of an object. So, as far as plants go, they appear green to us because they contain pigments that are absorbing other wavelengths of energy while reflecting green and yellow wavelengths of energy.
- Phycologists (plant scientists) study things such as plant pigments to help classify different plant species. The differences in chlorophyll types found in different plants has helped scientists to determine that some plant species that they once thought were closely related were not as closely related as originally thought.

SLIDE 7: CHROMATOGRAPHY

- One way in which we can study plant pigments is through chromatography. Chromatography is a lab technique that is used to separate the different components of a mixture (in this situation, the different pigments found in the plants). The mixture is dissolved in a fluid called a solvent (in this case we used ethanol to create a pigment extraction that we will analyze). We use special chromatography paper that is designed to absorb this solvent along with the pigments dissolved in it. The solvent will carry the pigments as it moves up the paper. The pigments are carried along at different rates because they are not equally soluble in the solvent. Therefore, the less soluble pigments will move slower up the paper than the more soluble pigments. This causes a distinct layering of pigments along the chromatography paper, which we call a chromatogram.
- We will be doing a chromatography experiment to see the types of pigments that were contained in the plant produce that you all extracted at the beginning of class. You will once again be working with your partners at your table and will need to follow the instructions in part two of your worksheet to complete the steps of this next experiment. There are helpful diagrams on the side of the instructions portion to help you. Please ask for assistance if you need it.

Wait for students to perform this portion of the experiment, and once each group is finished and has set aside their chromatography cup, move on to explaining the phytoplankton portion of the lesson

SLIDE 8: WHAT TYPES OF THINGS PHOTOSYNTHESIZE IN THE OCEAN?

- While we are waiting for our chromatograms to develop, we are going to talk about some marine biology as promised. What photosynthesizes in the ocean? [allow students a couple of minutes to brainstorm – then run through the answers in the Google slide] *click*
- Kelps and seaweeds *click* – we call these macroalgae, or large plants *click*
- Seagrasses *click* – these plants are rooted at the bottom and grow in large rolling meadows, which provide a great habitat and a good source of food for some species such as turtles! *click*

- Coral reefs and anemones *click* – *click* but wait, coral and anemones are animals! How do they photosynthesize? *click*
- They have symbiotic organisms that live inside the polyps that can use energy from sunlight to create organic materials that the animals can then use for their own energy. *click* These symbiotic animals are a species of phytoplankton, or microscopic plant. *click* There are many different types of phytoplankton, or microalgae (small microscopic plant), species; and they have an amazing assortment of shapes and forms. We are going to be focusing on these organisms for the rest of this class!

SLIDE 9: WHAT ARE PHYTOPLANKTON?

- So, what exactly are phytoplankton? The root of the word plankton is “planktos,” which is Greek for drifter. Just like other plankton, phytoplankton cannot swim against a current, so they are transported around by them. We call these organisms “microalgae,” to distinguish them from the large photosynthesizing organisms such as seaweeds and kelps (which are usually referred to as macroalgae). Phytoplankton can be either eukaryotic or prokaryotic. As a reminder, eukaryotes are multi-cellular organisms with a complete membrane-bound nucleus and advanced cellular organelles; while prokaryotes are single-celled, smaller, and have a nucleoid rather than a nucleus. All phytoplankton photosynthesize, as we talked about. However, there are a large amount of species that are mixotrophic, which means that they not only photosynthesize to create their energy using sunlight but also consume other algae to get energy from them as well! [show video of dinoflagellate predating on other algae]
- [optional video script: This video is from the lab of VIMS Professor Kim Reece and shows cells of algae called *Dinophysis* swimming and feeding. The *Dinophysis* use their “peduncle” which is almost like a needle sticking off their body and insert it into prey algae and suck out all their nutrients which the *Dinophysis* can then use to grow in addition to all the energy harvested through photosynthesis.]
- Phytoplankton are very small, with many species being less than 100 micrometers, which is approximately 0.1 millimeter! Some species can be larger, up to 2 millimeters, but most you cannot see with the naked eye. Phytoplankton are important for a variety of reasons. Does anyone know of one?
- Phytoplankton are the link between inorganic nutrients and light energy and organic energy. For that reason, they are responsible for being the base of the oceanic food web. Zooplankton (animal plankton) and other animals such as whales, fishes, and invertebrates eat them. Without phytoplankton, there would be none of the large oceanic predators that we know and love! In addition, through photosynthesis, phytoplankton create a significant amount of the oxygen that is diffused from the surface waters 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! So, if you like breathing, you should thank phytoplankton!

SLIDE 10: PIGMENT ABSORBANCE SPECTRUM IMAGE

- In order to perform photosynthesis, all phytoplankton have the pigment we talked about earlier in class called chlorophyll-a, and many also have chlorophyll-b present in their tissues. However, different species of phytoplankton also have different accessory pigments to help them maximize their photosynthetic capabilities. Each accessory pigment absorbs and reflects light at different wavelengths along the electromagnetic spectrum. This graph shows the absorbance of each color of light by different accessory pigments. You can see that as we talked about before, chlorophyll a absorbs heavily in the purple and red regions and chlorophyll b absorbs in the blue and orange wavelengths. Therefore, we see the chlorophyll pigments as green, because they are reflecting the green light since they are not able to absorb it.
- Carotenoids are pigments that absorb in the blue wavelengths but reflect light in the orange and yellow wavelengths. These are commonly found in citrus fruits, carrots, pumpkins, and squashes. They are also found in a group of phytoplankton called diatoms, which makes them a golden orange-brown color.
- Phycobilins are a set of two separate pigments: phycoerythrin is a pigment that absorbs green light but reflects red light. These are found in strawberries, raspberries, tomatoes, watermelon, and red peppers. The pigment is also commonly found in a group of phytoplankton called the Rhodophytes, or red algae. Phycocyanin absorbs yellow light and reflects blue and purple light. This pigment is found in blueberries, blackberries, and red cabbage. It is also found in a group of phytoplankton called the cyanobacteria, or blue-green algae. The symbiotic algae found in corals and anemones that we talked about earlier are types of cyanobacteria.
- Now we will reflect back on the results from our chromatography tests. You were given four different produce types, and they all have different accessory pigments. You will sketch the results of your tests on your worksheet using the provided colored pencils, and then we will rotate clockwise to each group's station so that you may see the results of their tests as well before answering questions 2 and 3. I'd like you to use the graph here to try to determine which accessory pigment was in your produce.

** Allow students ~ 25 minutes to perform these tasks. After they are finished, you may want to have a class discussion asking students which pigments were present in their samples and how they knew that.*

- During our next class, we will be investigating how knowing which pigments are present in each algae species can help marine biologists in their study of the ocean.

After you have finished reviewing major concepts with students to wrap up, have them help clean up all materials and help get their station ready for the next group

Class Block #2: Mystery Chromatography and Class Discussion/Review

**Begin by passing back the students' worksheets, and provide a quick discussion of major concepts and remind them of what they did the previous day.*

SLIDE 10: PIGMENT ABSORBANCE SPECTRUM IMAGE

- As we talked about last class, plants can have a combination of pigments within their tissues to maximize their ability to photosynthesize. Phytoplankton are no exception: different species have different combinations of these **accessory pigments** to absorb as more wavelengths of light to maximize their photosynthetic capabilities. So how can we use these pigments in marine biology studies?

SLIDE 11: PHYTOPLANKTON COMMUNITY SAMPLING

- How can we sample to determine which species of microalgae are present in a sample?
- Well, the first option is to look at a sample under the microscope to identify each individual plankton cell. As you can imagine, there are many millions of plankton per liter of sea water, so it would take a lot of time and a lot of energy (and eye strain!) to do this type of analysis.
- Another way is to test the concentration of chlorophyll a in a sample. If we can measure the amount of chlorophyll and compare it to a standard amount that we know each plankton cell produces, we can estimate the total biomass of phytoplankton in the sample. However, this only tells us how much phytoplankton is in there and doesn't necessarily tell us which species are present.
- As we talked about, phytoplankton are a hugely diverse group of organisms. There are about 5,000 species of phytoplankton that live in marine waters. So how can we get an idea of the major groups of phytoplankton that are in a sample? Well, we can use what we know about accessory pigments to help us find out!
- Different species will have different combinations of the accessory pigments that we talked about, so if you analyze a sample to determine which pigments are present you can get an idea of the types of plankton that are present in the sample as well.

SLIDE 12: BACK TO PIGMENT ABSORBANCE SPECTRA GRAPH

- We are going to attempt to do this in the classroom right now – we will separate pigments from an unknown mystery sample to determine the types of species of "plankton" present in the sample. You will work in the same groups as last class to run another chromatography experiment, this time with an unknown algae sample. Comparing your sketches of the original pigment chromatography tests and this mystery chromatography test, you will try to determine which of four algae species are present in your sample.
- Use the instructions on the second worksheet entitled "Mystery Algal Sample" to perform the next chromatography test. Once you are finished doing that, set it aside to develop for 15 minutes. While we are waiting for those results to develop, we will talk about how this process is used in marine biology labs around the country.

Give students plenty of time to complete these steps, then move on to the next slide

SLIDE 13: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

- Scientists around the country use a method called Performance Liquid Chromatography, or HPLC, to separate components of liquids.

- Scientists go out into the field to collect a sample of water that they'd like to test for phytoplankton. They use very fine filter paper to filter out all the phytoplankton from the water in the sample. They then grind the phytoplankton into a solution, to make the sample "homogeneous" (of the same appearance and composition throughout the solution). They inject this sample into the HPLC chamber, where the components/pigments are separated into bands. These bands run past a detector which is linked to a computer. The computer analyzes the different bands present in the sample and creates a chromatograph to show the amount of each pigment present in the sample. The scientists can then use the chromatograms created by the HPLC unit to determine which taxa (groups) of phytoplankton are present in the sample that they tested.

You may want to show students the video included in the slideshow for more clarification

- This is very similar to what you all just did, separating the pigments out so that you can now go back to attempt to determine which species were present in your mystery sample.

SLIDE 14: MYSTERY SAMPLE ANALYSIS

- Let's look back at the results of our mystery chromatography test. Take a few moments in your groups to sketch the results of your chromatogram, and attempt to answer the questions on your worksheet as a group (you will need the photos on the overhead to help you answer question #5).

Allow students plenty of time to perform these tasks and to make their guesses, and then lead a class discussion about what species each group thinks that they have and why

- So why do we care to know which groups of plankton are present?
- Some species of plankton are "harmful algal species," which means that if they are present in high concentrations they can cause adverse ecological or human health effects.
- Different species of phytoplankton have different nutritional value for the organisms that rely on them, so knowing which species are present lets you know how likely the food web is going to support an organism of concern
- Some species grow better than others depending on environmental conditions - if you know which species is dominating, you can get a sense of the environmental conditions that had been occurring before the sample was collected

SLIDE 15: HUMAN USE OF PHYTOPLANKTON PIGMENTS

- In addition to their importance in phytoplankton, these pigments can be used by humans as well. Phycobilins (the red and blue pigments) are useful in scientific and particularly medical research. Each of the two pigments fluoresces at a particular wavelength. This means that when they are exposed to a 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 these tags to antibodies, which they then add 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.

- This fluorescence technology can also be used in the field or in the lab to determine the types of phytoplankton present in a water sample!

**Show students the video of the plankton fluorescing if time allows*

- In addition to medical uses, many of the health supplements like fish and krill oil is harvested from animals, but ultimately comes from the phytoplankton that they eat. Phytoplankton are rich in omega-based fatty acids which are great for heart health.
- Phytoplankton also have high levels of Vitamin B-12, which helps keep nerve and blood cells healthy and supports and boosts energy levels!

After you have finished reviewing major concepts with students to wrap up, have them help clean up all materials and their work stations

Appendix 2: Student Worksheets

The following three pages should be printed in advance and distributed to each student in the class prior to them doing the experiments. If desired, you may give one copy to each group of students and have them collectively turn in their worksheets with all group members' names listed in order to cut down on need for copies and materials.

Student Name ________________________________ Date __________
Group Member Names ____

* In the following experiment, you will be using ethanol. It is the clear liquid in the container on your table. Please be careful not to spill it or unnecessarily inhale any fumes. *

PART ONE: PIGMENT EXTRACTION

Which type of food item is your group using in your experiment? _________________________

INSTRUCTIONS:

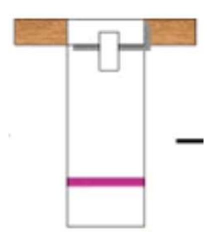
1. Place your produce into the small Dixie cup on your table. If you have been assigned the spinach leaves, make sure to snip the leaves into small (~ ½ inch) pieces before putting into the cup.
2. Pour the cup of ethanol into the small Dixie cup holding your produce.
3. Take a few minutes to use the popsicle stick to grind and mash your produce into the ethanol solution.
4. Set the cup aside so that we can wait for the chemical reactions to occur that we will use in the experiments for the rest of class.

PART TWO: PIGMENT CHROMATOGRAPHY

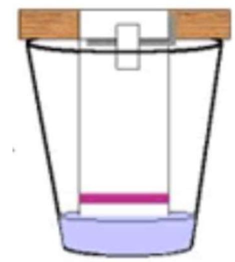
* You will be working together in your small group to perform a chromatography test on the produce extraction that you made at the beginning of class. This will help you to visualize the different pigments that different plants have to take advantage of light for photosynthesis. *

INSTRUCTIONS:

1. Set the large plastic cup standing upright in the center of the table and place the plastic strainer inside the mouth of the cup.
2. Pour your produce/ethanol mixture into the strainer so that the liquid runs into the plastic cup, but all large pieces/leaves are left behind on the strainer. Return these large pieces/leaves to the small Dixie cup and set aside.
3. Secure one end of one sheet of the chromatography paper (long skinny sheet of paper) on your table to the second popsicle stick in your station. Be sure to handle the paper so that your fingers are only touching the edges – oils from your hands will impact your chromatography results if you touch the front of the paper strip. It may be helpful to wrap the edge around the popsicle stick and then secure with tape so that you have a "T" shape with the paper hanging down from the popsicle stick. Refer to the drawing on the right for help.



4. Lay your “T” shaped popsicle stick/paper sideways along the top of the plastic cup so that the end of the chromatography paper opposite the popsicle stick is in the pigment solution. Make sure that the paper is not touching the sides of the cup, but rather hanging down the center into the solution. Refer to the drawing on the right for help.
5. Set your chromatography experiment aside and do not disturb for the next 15 minutes. You will come back to this test later to make observations and draw conclusions.



OBSERVATIONS:

1. Go back to your original produce chromatography test. Make observations and sketch what you see on your strip of paper below using a pencil and colored pencils.

ANALYSIS:

2. Given what we’ve talked about as far as absorbance and reflectance, what colors are being reflected by the pigments in the produce?

3. What colors are being absorbed by the pigments in the produce? Which wavelengths of light do these colors correspond to?

Student Name __________________________________ Date _________
Group Member Names ____

PART THREE: MYSTERY PLANKTON SAMPLE

You will be working with your partners to analyze the different pigments found in a mystery algal plankton (phytoplankton) sample. This is similar to what scientists do in the lab with High Performance Liquid Chromatography (HPLC).

Which mystery sample is your group analyzing? ______________

INSTRUCTIONS:

1. Pour the mystery sample given to you by your teacher into the second empty plastic cup on your table. Be sure to record which sample your group is analyzing in the space above.
2. Repeat the procedure used in the previous chromatography experiment to secure the second blank piece of chromatography paper to the third popsicle stick on your table and secure with tape.
3. Once again, lay the popsicle stick across the top of the plastic cup containing your mystery sample, allowing the end of the chromatography strip to be sitting in your mystery sample while being careful not to let the paper touch the sides of the cup.
4. Once finished, set the experiment aside while you go back to your original pigment chromatography experiment and record your results.

OBSERVATIONS:

1. Create a sketch of your mystery sample's results in the space below.

ANALYSIS:

2. Which pigments do you think are present in your mystery sample?

3. Which wavelengths of light do your plankton reflect?

4. Which wavelengths of light do your plankton absorb?

5. Take a guess at which species of plankton was in your sample (using the photos on the overhead) and write the answer below.

Appendix 3: Answer Key

The chromatogram graphs that students create will be personalized to what they observe when they perform their chromatography experiment.

For the produce pigment chromatography, the answers to questions 2 and 3 will depend on what produce the group was testing.

Question 2 answers should be along the lines of:
- Spinach: green/yellow is reflected; may also have orange in their answer
- Orange: orange/yellow is reflected
- Red berry: red is reflected
- Dark berry: blue/purple is reflected; may also have red in their answer

Question 3 answers will differ because they rely on inference. However, as long as the students list the colors that are not reflected, they can be considered correct (all of the colors EXCEPT those above).

For the mystery algal sample chromatography, you will need to refer back to your notes on which "algal sample" each letter corresponds to in order to check correctness of answers. The answers to question 2 should be:
- Green algae: chlorophyll (may also say carotenoids, since they are present in spinach leaves)
- Red algae: chlorophyll, phycoerythrin
- Diatom: chlorophyll, carotenoids
- Cyanobacteria: chlorophyll, phycocyanin

The answers to question 3 should be along the lines of:
- Green algae: green, yellow
- Red algae: green, red
- Diatom: green, yellow
- Cyanobacteria: green, blue

The answers to question 4 should be along the lines of:
- Green algae: purple, blue, orange
- Red algae: purple, blue, green, orange
- Diatom: purple, blue, orange
- Cyanobacteria: purple, yellow, orange

A successful answer to question 5 will in part be a correct analysis of the plankton present in their samples, along with a comprehendible explanation of why they chose this answer.