The Algae-in-a-Bottle Experiment:

A High-Impact Learning Activity

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Summary

The *Algae-in-a-Bottle Experiment* provides an engaging and flexible high-impact teaching tool for helping students to know, understand, and apply a number of concepts related to the biology and ecology of aquatic plants and their environments. It is also relevant to methods being developed for the use of algae as an alternative energy source, that is, biofuels. The protocols in this experiment can be adapted use as a demonstration activity in one or two class sessions, or as a nature of science, inquiry-based activity over a few to several weeks. The easy-to-obtain and inexpensive materials used in the experiment make it accessible to institutions where resources and space are limited, as long as sunlight or artificial light are available to carry out the experiment. Preliminary results with non-majors enrolled in introductory general education oceanography courses in a community college indicate increased engagement and a high level of enthusiasm for the experiments, and suggest a better knowledge and understanding of the effects of light and nutrients on photosynthesis, and greater appreciation for the nature of science. We believe that the *Algae-in-a-Bottle Experiment* offers an effective means for improving science literacy and for introducing scientific research to diverse learners from middle school to college.

Student Learning Outcome

Upon successful completion of this experiment, students will be able to write the equation of photosynthesis and explain how varying conditions of light and nutrients will affect the growth rates of microalgae and the productivity of food webs that depend on them.

Lesson Plan

The topics and steps outlined below represent a sequence of discussions and activities that introduce the process of photosynthesis and the factors that affect the growth of algae in aquatic environments. The lesson plan is applicable to students enrolled in introductory biology, botany, plant physiology, algology, marine biology, environmental biology, ecology, and oceanography where a general understanding of photosynthesis, primary production, the global carbon cycle, and aquatic food webs is desired.

The level of complexity of each step and activity can be tailored according to the goals of instruction, or background of the instructor. The sequence may be simplified, expanded, accelerated, or slowed down depending on the time and resources available to the instructor. The entire sequence may be completed as a demonstration activity in the timeframe of a single-class, or it may be carried out as an inquiry activity over a number of classes. In the longer timeframe, each step or activity may be interwoven with a lecture or other activity, as some steps can be completed in 10-15 minutes. The longer timeframe, a kind of *What About Bob?* baby-steps approach, offers the greatest opportunity to achieve the highest levels of content mastery and conceptual understanding. For example, the instructor may choose to give students bottles of water that already contain algae, and ask students to observe the changes in color that appear over the course of a few days or weeks. Students may even take the bottles home to make these observations. Alternatively, the instructor may choose to cover only 1-2 steps in the lesson plan each class, permitting greater time for introduction and discussion of concepts, and allowing students to carry out individual investigations in the form of an inquiry or research activity.

The main goal here is to provide instructors with a fairly simple activity (in practice) that is fun and engaging for students, and that allows students and instructors to explore and learn about fundamental processes of global importance. Students learn best by doing, and it's in that spirit that this activity is presented.

Why study algae?

Tell them they are going to create a home for a most marvelous organism, one that serves as the base of the food web, and, in doing so, provides fish and shellfish to much of the world; one that regulates carbon dioxide, and, as such, controls the temperature of our planet, and one that may one day provide fuel, what has become known as biofuels, for transportation. This organism belongs to a group of organisms known as the algae, or because they are tiny, the microalgae. In the ocean, we refer to microalgae as phytoplankton, the algae drifters.

Introduce some basic facts about microalgae.

Microalgae are single-celled, microscopic, photosynthetic organisms, related to seaweeds, that inhabit the lighted regions (i.e., the photic zone) of all aquatic environments, freshwater and saltwater, including your cat or dog's water dish, your Aunt Mary's bird bath, and your kid brother's goldfish bowl. The class will grow a species of marine microalgae. Distribute 8-oz bottled water to individual or teams of students. Ask the class to examine their bottles. What would be the ideal environment for their algae in a bottle? What are essential ingredients of their new home? Write their responses on the board.

Introduce photosynthesis.

Write on the board a basic equation for photosynthesis: $CO_2 + H_20 \rightarrow$ sugars + O_2. Discuss. What do the symbols in this equation mean? Simply put, plants and algae use carbon dioxide and water to produce sugars, a kind of energy molecule, and in the process, produce oxygen as a byproduct. What's missing?

Algae, like plants, need light to grow. Add light: CO_2 + H_20 + light → sugars + O_2. What else do plants need to grow? They also need fertilizers. In the algae business, we call them biologically important nutrients, or simply, nutrients. Examples of common lawn and garden fertilizers may be shown. On the front of the package are the letters, N, P, and K. What do these symbols stand for? These are the basic macronutrients required by all plants and algae. Add nutrients: CO_2 + H_20 + light + nutrients → sugars + O_2. (At this point, more advanced courses may develop further details of photosynthesis, such as the light and dark reactions, and the water-splitting reactions, which helps explain how solar energy is transformed into chemical energy which is then used as an energy source to manufacture cellular materials, and helps explain that the O_2 produced by plants and algae comes from the splitting of water, not the splitting of carbon dioxide.)

Introduce chlorophyll a.

The central player in photosynthesis is a molecule called chlorophyll *a*. This molecule is the primary light-absorbing molecule of all plants and algae and photosynthetic bacteria. Ask students if they have ever seen chlorophyll? If a window is near, ask them to look outside at a tree. Can they see chlorophyll? Explain that the green color of plants and algae comes from chlorophyll. This molecule absorbs sunlight and transfers the energy from sunlight into a system that transforms the solar energy into a form of chemical energy that can be used by the plant. The individual cells of algae are too small to see, but when the algae are abundant, their chlorophyll is visible. Ask students if they have ever noticed changes in the color of the ocean from winter to spring to summer to fall. In winter and summer, the ocean may be blue and mostly transparent. In the spring and fall, the ocean may be green and opaque. The difference in color and the change in color from blue to green comes from the presence of phytoplankton. When the ocean is blue, there are few phytoplankton in the water. When they ocean is green, phytoplankton are abundant. In the experiment, the color inside the bottle provides an indication of the amount of algae. Changes in the color inside the bottle over a period of days indicate how fast the algae are growing. It will be important to observe and document these changes.

Introduce respiration.

Ask students what happens to plants when they don't get enough light? Students will generally answer that the plants will die, but the instructor may counter by asking if plants die at night? Why don't plants die at night? Plants and algae store energy in the same manner as humans do. When light (or food) is not available, plants (and humans) use their stored energy. Plants, algae, and humans store energy as fatty acids, which we may think of as energy molecules, just like sugars. When plants, algae, and humans need energy, they break down these fatty acids in a process called cellular respiration. Here's the equation:

fatty acids + O_2 → CO_2 + H_20. Does this equation look familiar? In fact, respiration resembles photosynthesis in the opposite direction. Organisms use oxygen to break down energy molecules (sugars and fats) and, in doing so, produce carbon dioxide and water. Why is this important? Because plants and algae use some of the energy they produce, and they use oxygen to break down that energy. Simply put, plants and algae carry out respiration and require oxygen. Repeat: plants and algae require oxygen. Fortunately for us, plants and algae produce more energy molecules and more oxygen than they need. Otherwise, we might only find plants on Planet Earth!

Emphasize that photosynthesis plus respiration make algae grow.

Photosynthesis turns solar energy into chemical energy, and respiration takes that chemical energy and uses it to build all of the things that make up a plant or algal cell, including the molecular machinery that lets plants and algae photosynthesize. Photosynthesis and respiration work together. Growth in plants and algae occurs via cellular division: one cell divides into two cells, two cells divide into four cells, and so on and so forth. Because algae are unicellular, we can measure how fast they are growing by counting the increase in cell numbers over a period of time. Alternatively, we can measure changes in the concentration of chlorophyll, either visually as changes in color, or quantitatively, using scientific instruments designed to detect chlorophyll (e.g., colorimeters, spectrophotometers, fluorometers, etc.) If we want to conduct experiments on what makes algae grow, then tracking their growth rate will be important. The instructor may want to hold up two different bottles of algae with different concentrations of chlorophyll to emphasize this point, or project a microscopic image of microalgae. (Advanced classes may be introduced to growth curves and growth equations, if desired.)

Introduce limiting factors.

In nature, something always limits how fast plants or algae can grow. If it's not light, then it might be nutrients. If it's not light and nutrients, then it might be water (for plants) or some other factor (for algae). The idea that something always limits how fast plants or algae can grow proves to be an important one, especially in agriculture. If you want to grow more crops faster, you need to identify the factor that is limiting the growth of the crops. In the mid-1800s, Justin von Liebig, a useless chemistry student (in the eyes of his professors) came up with the idea of a limiting factor, the one factor required for the growth of a plant that is in the least supply. It might be light, it might be nutrients, or it might be something else. Thus was born Liebig's Law of the Minimum. An illustration of a wooden bucket with different lengths of planks serves as a useful analogy: the length of the shortest plank determines the amount of water held by the bucket. Analogously, the limiting factor determines the growth rate of the plant or algae. This "law" has important implications for understanding the growth of algae in a bottle, and in nature. In addition to agriculture, Liebig's Law of the Minimum has been applied to understanding the biology and ecology of phytoplankton growth in the ocean.

Ask scientific questions.

The *Algae-in-a-Bottle Experiment* may be conducted as a demonstration activity, where students optimize water levels, prepare saltwater, add nutrients, find suitable light, add algae, and watch the algae grow, or it may be presented as an inquiry activity, where students come up with (or are given) scientific questions to explore, formulate hypotheses and predictions, set up experimental conditions, carry out the experiments, and analyze and communicate the results. In either approach, it's useful to ask students to work in pairs or teams to come up with a few scientific questions concerning the growth of their algae at different light levels and different nutrient concentrations. What hypotheses might they test? What do they predict will happen? Alternatively, the instructor may write a few questions, hypotheses, and predictions on the board. To avoid confusion, start with light as a limiting factor first. For example, how fast will algae grow in high light? How fast will they grow under low light? How fast will algae at high light grow with short days and long nights, such as found in winter in temperate zones? How fast will they grow with long days and short nights, resembling summer in temperate zones? There are a number of variations here, but it can be useful to allow students to explore the possibilities, just as a scientist might. Similar question-generating can be carried out with nutrients, but with one important extension: the concentration of nutrients in the bottle will determine the maximum biomass of the algae in the bottle. Lower nutrient concentrations will produce lower biomass, and higher nutrient concentrations will produce higher biomass.

Discuss carbon dioxide.

Hold up a piece of wood. What kind of atoms does it contain? Carbon. From where does the carbon in the wood come? Carbon dioxide. How do plants obtain carbon dioxide? From the atmosphere. How do algae obtain carbon dioxide? From carbon dioxide dissolved in the water. (It's not necessary to introduce the forms of dissolved carbon dioxide in seawater, i.e., the carbonate buffering system, as this tends to overcomplicate the matter.) How does carbon dioxide get into the water? Across the air-water interface. Hold up a bottle and ask, if algae use the carbon dioxide in the water, how will they get more? It might take some prompting to get students to realize it, but the cap will have to be removed from the bottle to periodically refresh the air in the bottle. The bottle may need to be shaken. What else can be done besides removing the cap? (More prompting.) Create a headspace. At this point, students may drink or pour out a portion of the water so that the water level is even with the shoulder of the bottle, right where it begins to taper.

Discuss and add salts.

If the class is growing marine algae, what must they do to the water in the bottle? Add salts. (Depending on the level of complexity desired, the instructor may introduce the properties of water, the nature of dissolving, anions and cations, the major constituents of seawater, the Principle of Constant Proportions, salinity, and measuring salinity using conductivity, but these concepts are not essential for meeting the learning outcomes for the experiment.) What kinds of salts make up seawater? Mostly, we find sodium chloride in seawater, but we also find nine other major elements, the major constituents, and more than 80 trace elements, the minor constituents. Fortunately, we can buy salts for saltwater aquariums, and that's what we will use for our algae. (At this point, the instructor may instruct the students to add a teaspoon of sea salts to their bottle, or let students calculate the amount needed according to the directions, for example, a half cup of salts per gallon of water. The proportionality calculation is a good exercise for students. Students will need to measure the amount of freshwater in their bottle, now that they have reduced its volume to less than 8 ounces.)

Discuss and add nutrients.

What else do algae need in their seawater to grow? If students were paying attention earlier, they will answer nutrients. But the instructor may need to refer them back to the equation of photosynthesis written on the board. What would happen if no nutrients were added? Where is a place on land with low nutrients? Deserts. Where is a place in the ocean with low nutrients? The tropical ocean. There's very little plant growth in deserts because of water and low nutrients, and very little phytoplankton growth in the tropical ocean because of low nutrients. So if no nutrients were added, there would be little to no growth of algae. What if lots of nutrients were added? Do more nutrients necessarily mean higher growth? It can be useful to show a graph of growth rate versus nutrient concentration for limiting concentrations of nutrients. At some point, a saturating concentration of nutrients is reached, beyond which additional increases in the concentration do not produce faster growth. Instructors may also want to refer back to the discussion of limiting factors to explain that at high nutrient concentrations, nutrients may no longer be limiting, and, as a result, some other factor might limit their rate of growth. What happens when the nutrients run out? The algae stop growing, and the color inside the bottle remains constant. (Ultimately, the amount of nutrients inside the bottle determines the final biomass within the bottle. So one simple experiment simply involves comparing the color of two bottles—one with low nutrients and one with high nutrients—at the end of a given period of time. If nutrient concentrations are saturating, and the two bottles held at the same light intensities, they may both grow at the same rate, but the bottle with the higher nutrient concentration will permit greater growth and a higher final concentration of algae. This is a point that the instructor may choose to explain when the experiments have been completed to allow students to derive this conclusion on their own.) As with sea salts, algae nutrients are available for purchase, albeit in a highly concentrated form. The instructor will want to prepare a solution of nutrients ahead of time from which students can pipette a given amount. The amount dispensed to students will depend on the type of experiment that the instructor or students have chosen to carry out. In the simplest approach, some students will add a few drops and others will add double or triple the number of drops. In the absence of instrumentation to measure the concentrations of nutrients in the bottles, there's a bit of guesswork here. Experiment with it. That's science!

Discuss light and remove the label on the bottle.

Fortunately, discussions of light involve more familiar territory. Students generally understand high light and low light. They may be less familiar with light:dark (L:D) cycles, though some prompting and questioning about length of days in summer versus winter, time it gets dark at night, time it gets light in the morning, etc., helps students grasp the idea. Key to this step is a discussion of the light environment inside the bottle. Holding up a bottle, the instructor might ask what properties of the bottle itself may impede the quantity of light that the algae receive inside the bottle. Some students will immediately recognize that the cap blocks light. A discussion of the orientation of the bottle with respect to the light source can be helpful. Which is better, illumination from above or illumination from the side? What are the potential advantages and disadvantages of either approach? Savvy students will also recognize that the bottle's label will block light. The class should remove the labels from their bottle at this point. If the instructor has already chosen the light intensity or light intensities for the experiment, then he or she may want to ask students to work in pairs or teams to predict growth rates under that light intensity (or range of light intensities), assuming that nutrients are not limiting. If natural sunlight is to be used, the instructor might ask how growth rates will vary through the day, and at night (!) A discussion of L:D cycles, which offer another experimental variable, may be carried out among students or as a class. In the end, it will be important for students to know or to have decided the light intensity (low, medium, or high) and the light regime (12:12, 16:8, 8:16) for their particular experiments. (Adventurous instructors may want to introduce Beer's Law, the exponential diminishment of light as it passes through a liquid. This can be useful for discussions of self-shading of algae within the bottle, or as an explanation for differences in growth rates that may occur in shallower or deeper bottles. Opportunities also exist to cover electromagnetic radiation, the seasonal cycle, the absorption properties of water, the absorption spectra of photosynthetic pigments, why the ocean is blue, and satellite oceanography, among others.)

(Optional) Discuss light and nutrients and maximum biomass.

As mentioned earlier, the concentration of nutrients will determine the maximum biomass of algae that will grow within the bottle. However, the speed with which the maximum biomass is achieved will depend on light intensity. For a given nutrient concentration, a high light intensity will promote faster growth and a quicker use of nutrients than a low light intensity. As a result, under high light, the maximum biomass may be reached in a matter of a few days, whereas under low light, the maximum biomass may not be reached for several days. Instructors may want to prompt students to think about what is happening inside the bottle as the algae grow. For example, what is happening to the nutrient concentration of the seawater in the bottle? It's decreasing. What is happening to the biomass? It's increasing. What happens to the light intensity inside the bottle as the biomass of the algae increases? The light is diminished because the algae are absorbing it. Where time permits, discussion of these interactions can really help to solidify students understanding of the concepts. Students may be asked to create a matrix of possible outcomes, or they may be asked to create concept maps. Short answer and essay questions on the topics covered to this point will help instructors identify misconceptions, and will help ensure that students are making the link between the theoretical concepts and the actual experiment. Do students grasp what is going on within the bottle? Do they have the conceptual understanding to support and explain whatever results they achieve?

Don't forget to make notes: the scientific notebook.

Depending on the type of course and the experience level of the students, it may be necessary to introduce appropriate methods for documenting the experimental methods, observations, and data in a scientific notebook.

Take pictures! Learn good scientific housekeeping.

Once students have prepared their seawater and nutrient solution, and have a clear idea of the light protocol that they will observe, it's a good time for students to take pictures of their bottles (using their smartphones or other devices), and even selfies with their bottle. We highly recommend that instructors simply instruct students to take pictures without supplying any additional details. Students should be asked to share their photos with each other, even post them on Facebook or Instagram. If possible, students should ask students to email their photos to the instructor so that he or she can project them on the classroom projection screen. Two or three photos from different students works ideally to make the following points: 1) How will students be able to identify their bottles among a classroom of bottles? 2) How will they be able to tell from a picture if their bottles have turned greener? and 3) Why might it be important to reproduce exactly the way in which the photograph was taken? The first point may be addressed by assigning unique identifiers to each student. The instructor might ask if it matters where students place their labels. (The best approach is to use a permanent marker and label the plastic cap on the bottle.) The second point may be addressed by suggesting that students take their pictures against a white background. A whiteboard works great for picture taking. The third point refers to creating a time sequence of photographs that are identical in every respect: same distance from the camera, same lighting, same angle. Reproducing the photographic conditions as exactly as possible ensures that any changes observed are due to the growth of the algae, not the way the picture was taken.

Add algae. Observe. Take time zero measurements.

It's finally time to add algae. The instructor will provide a flask or container of stock culture from which students may pipette a small amount (~1 ml), or the instructor may add the algae. To the degree possible, identical amounts should be added to each bottle to ensure consistency and reproducibility in the results. Following the addition of algae, students should cap their bottle, and gently shake it. They may then take pictures (using the picture-taking criteria established in the previous step). If quantitative measurements are to be taken, the students should be instructed to take these measurements at this time. Students should make visual observations of their algae, and write those observations, along with the time and date, in their notebooks or on their worksheet. The instructor may want to remind students to be sure that they have properly labeled their bottles.

Let the algae incubate!

Students can now place their bottles in the location chosen for the experiment (a windowsill, a tabletop with a light source, a plant growth cart, etc.). They will want to note the orientation of the bottle with respect to the light source as small perturbations in the bottle, bottle caps, label residue, may slightly change the light regime. Students will want to maintain the same orientation throughout the experiment.

Make observations or measurements periodically.

Following the initial addition of algae, the timetable for making further observations or measurements will depend on the class schedule. If students are taking pictures, observations may be completed in a short amount of time at the beginning of class over a period of a week or a few weeks. If they are making measurements of chlorophyll, it may take longer, but with enough resources and proper organization, these measurements shouldn't take long either. A natural endpoint for the experiment is the point where no further changes in color (or chlorophyll) are observed (or measured), the maximum biomass point, or a shorter time period, depending on the initial questions being investigated.

Frame those photos!

The availability of apps that permit users to create collages of photos proves very useful for illustrating before-and-after photos of the algae in the bottle. A time sequence of several photos may also be created. Students generally know these apps well, and will require little direction for creating a collage of their experiment.

Compile results and graph them.

In classrooms where iPads or computers are available, students may enter data into a spreadsheet as they collect it. Otherwise, they may create tables and graphs of data in a computer lab or on a computer at home. Bar charts illustrating changes in color (measured by a colorimeter or spectrophotometer) or chlorophyll (measured in a fluorometer) help illustrate changes and rates of change in algal growth.

Share and interpret the results.

We encourage instructors to allow students to share and interpret their results with the rest of the class. The online availability of free presentation software (e.g., Google Slides) makes it easy for students to prepare a few short slides of their results, and makes it easy for students to project their slides on a classroom projection system connected to the Internet. Alternatively, students may prepare PowerPoint presentations, and submit them on a USB drive, or they may write up their experiments in a laboratory report or scientific paper. We encourage instructors to focus on the presentation and interpretation of the results versus a proper accounting of methods, though this is important. Far too often students feel like the results of their experiments are not valid, that they did something wrong, or that some experimental error gave them the results that they achieved. On the contrary, it's important to come up with possible explanations for any results the students find. Algae may grow or not grow for a variety of reasons, including temperature fluctuations (unaccounted for in our experiments), bacterial contamination, differences in bottles that induce differences in the internal light intensity, accidental differences in the amount of salts, nutrients, or algae added. Students should be encouraged to compare their results with those of their team members. If the treatments were identical, shouldn't the results be identical? A lively discussion of results demonstrates that students were engaged in the process, that they were invested in the experiments, and that they understand the conceptual underpinnings of the experiments. This is the most rewarding part of the experiment for students and instructors.

Background and Explanation of Approach

The idea for a classroom experience in growing algae emerged from an undergraduate research project in algae biofuels at Fullerton College, a large community college in Southern California. Lacking space and resources for sophisticated bioreactors, students grew an "off-the-shelf," euryhaline (tolerant of a wide range of salinity), marine algal species, *Tetraselmis*, in a 120-gallon aquarium equipped with LED aquarium lights. Successful propagation of the algae (i.e., they didn't kill it) led to the idea of having a classroom of students grow the algae in 8-oz water bottles. (Smaller water bottles were used to reduce the expense, but 11- or 12-oz bottles should work equally as well.)

The Slow Approach

The Basics

In the simplest version of the experiment, students are introduced to the basics of photosynthesis, including the limiting effects of light and nutrients on marine algal growth. Students are then given an 8-oz bottle of water purchased at a grocery or superstore, and asked to discuss what are the necessary "conditions" to turn their bottled water into a suitable home for their marine algae. Students generally understand that light is necessary for algal growth, but they are less certain what will happen under low light conditions, or under conditions with varying light-dark cycles. Similarly, students generally understand that some kind of "fertilizers" (i.e., biologically important elements) are important to make algae grow, but lack the sophistication to predict what might happen at different concentrations of nutrients. Nonetheless, students are on the right track in knowing that their algae will require light and nutrients.

The Scientific Notebook

Following a general introduction to the experiment, and in preparation for carrying out the experiment, it's a good idea to introduce students to the importance of documenting every step along the way. If scientific notebooks and scientific notetaking have not been introduced to the class, then now would be a good time. The instructor may ask students to take notes individually in their own notebooks, or they may provide composition notebooks to the teams of students who will work together on the experiments. Introduction to the scientific method, posing questions, formulating hypotheses, making predictions, carefully documenting observations and measurements, and data recording can be introduced. Otherwise, if time is restricted, the instructor may wish to present a common question or questions to the class, and guide students through a pre-chosen set of hypotheses and predictions for a given set of experimental conditions.

Pre-Tests

Instructors may also wish to administer pre-tests developed for the algae-in-a-bottle experiment. (See Appendix.) Student attitudes towards science and student knowledge of photosynthesis and algal (or plant) growth may be assessed to gauge levels of enthusiasm and levels of knowledge prior to the activity. Following the experiment, a set of post-tests can be administered to determine improvements in attitudes and understanding, and as a tool for improving the way in which the experiment is carried out.

Dissolved Gases

Following the introduction, the instructor may provide each student with a bottle of water. The first discussion with students, bottle in hand, may concern the presence of dissolved gases, and specifically the deliver of carbon dioxide, to algae in the bottle. The instructor may want to introduce the concept of respiration, and even gas exchange across an air-water interface, if desired. Any student who has owned a goldfish should be familiar with the importance of aeration for oxygenation. Algae, too, need oxygen, except this is not usually a problem because they produce their own, a point that can be reinforced by referring to a simple equation for photosynthesis. However, algae also need dissolved carbon dioxide to grow. Students may be asked how their algae will obtain carbon dioxide in a bottle that is filled to the brim and capped. A number of solutions can be explored—removing the cap, adding an air pump, shaking the bottle regularly—all of which serve the purpose. The most elegant (and easiest to implement) solution is to simply pour water out of the bottle. We recommend reducing the water level to the shoulder of the bottle. Here the instructor may introduce the concept of head space in a bottle to allow for an appropriate exchange of gases. A demonstration of head space in a soda bottle for preventing explosions can be helpful. For advanced classes, the inverse relationship between the saturation concentration of a gas and water temperature may also be explored.

Water Chemistry

Further discussion can be elicited regarding the requirements for marine-versus-freshwater algae. Invariably, someone mentions saltwater, and a discussion ensues regarding the chemical composition of saltwater, and how to reproduce that formula in a bottle of water. A number of important water chemistry concepts, such as the nature of dissolving, cations and anions, the Principle of Constant Proportions, etc, can be introduced here, but the conversation can be shortened by telling students about “instant ocean” salts manufactured for the saltwater and reef aquarium hobbyist. One variant of the experiment asks students to calculate the amount of salts that should be added to bottle of water based on the manufacturer's directions for the amount of salts to produce a gallon of saltwater. Students must grapple with unit conversions and ratios to come up with the correct amount of salts. They also will need to measure the volume of water in their bottle after they have removed some water to allow for gas exchange (as mentioned above). Instructors may extend this part of the experiment to include lessons in measurement, methods protocols, and record keeping in a scientific notebook. Once students have prepared their saltwater solution, they may check the salinity inside their bottle using a refractometer or salinometer (if available). Otherwise, the instructor may simply provide an answer, allow students to add a pre-determined amount of salt (usually a teaspoon), and continue with the experiment.

Biologically Important Nutrients (aka fertilizers)

Once an understanding of ideal water conditions is established, and students have prepared their seawater solution, the next step is to introduce the need for biologically important nutrients, and the concept of a limiting factor. An expanded version of the equation of photosynthesis may be presented, or an introduction to "fertilizers" and the importance of nitrogen (N), phosphorus (P), and potassium (K) may be presented. The instructor may introduce students to Justin von Liebig, a worthless chemistry student, according to his professors, but the originator of the idea that one element (or factor) above all limits the growth of plants (or microalgae), that is, Liebig's Law of the Minimum. For marine algae, nitrogen in the form of nitrate is generally limiting in the ocean, but this fact may overcomplicate student understanding at this point. We generally ask what might happen if algae were simply introduced to their bottles at this stage. Some prompting of answers using household plants as examples may be appropriate. We generally ask how many students have grown plants, or helped to fertilize a lawn. For demonstration, we show different bags of fertilizer with their differing ratios of N, P, and K. Where's the soil in the aquatic algae home? What's the purpose of soil? Why do we fertilize plants? What happens if we don't? The standard nutrient enrichment medium for growing aquatic algae is Guillard's f/2, though other mixtures have begun to replace this one. A full description of these media is beyond the scope of this text, but links to useful resources on this topic can be found in the Appendix. For the purposes of the experiments here, we purchase liquid f/2 from an aquaculture vendor.

Limiting Factors-Nutrients:

The critical decision here is how much nutrient enrichment media to add. The instructor may wish to show students a graph of the relationship between nutrient concentration and growth rate. When concentrations are limiting (the linear part of the graph), algal growth will be regulated by the nutrient concentration. Where concentrations are saturating (the horizontal part of the graph), nutrient concentrations will be saturating and some other factor will limit the growth of the algae, or they will grow at their maximal rate. As an extension to the experiment, students may be asked to hypothesize what will happen under conditions of limiting and saturating nutrients. Which bottle will turn green faster? They may also be asked to consider the end result (i.e., the final concentration of algae in their bottle, i.e., the maximum biomass) under each of these conditions. Which bottle will turn greenest? If time is limited, or the complexity of this topic is one that instructors do not wish to introduce, students may be instructed to add a pre-set amount of saturating nutrients, or to simply give any amount a try. In our experience, students tend to want to add plentiful nutrients, so the instructor may want to give students a more narrow choice, 2, 4, or 6 drops. To those eager to add lots of nutrients, the instructor might ask if it's possible to add too much? (Yes.) What happens when you overfertilize a lawn? (It dies.) What happens to the salinity if you add too much nutrient media? (The salinity is lowered.)

Light Intensity

Once nutrients have been added, it's time to consider the light intensity under which the algae will be grown. Students should be asked to predict rates of growth versus different light intensities. The instructor may ask if there can be too much light, or too little. A graph of growth rate versus light intensity may be shown to students following discussion of their predictions. Were their prediction accurate? The instructor will want to point out key concepts such as the compensation light intensity, the minimum light intensity needed to meet the metabolic demands of the algae (or plant) itself. Below this light intensity, the algae will "starve." The saturation light intensity, the light intensity at which growth rate is maximal, should remind students of the saturating nutrient concentration. Above this light intensity, the growth rate of the algae will no longer increase; the growth rate is maximal. However, light intensity can become too high, and the concept of photoinhibition may be introduced. Students may be asked to name a few common plants that prefer the sun, and ones that prefer the shade. What happens to shade plants when you place them in bright light? (They wither and die.) Algae reduce their growth rate at extremely high light intensities.

The Light-Dark Cycle

In addition to light intensity, the light-dark (L:D) cycle plays an important role in the growth of the algae. It's instructive to probe student understanding of the L:D cycle and how it changes through the seasonal cycle. When are nights the longest and days the shortest? When are days the longest and nights the shortest? How long is daylight during the summer? How about during the winter? Common L:D cycles are 16:8 hours (summer), 12:12 hours (spring/fall), and 8:16 hours (winter). If the class is using natural light, students can look up the L:D cycle on the US Naval Observatory website (http://aa.usno.navy.mil/data/docs/Dur_OneYear.php). With artificial light, the L:D cycle can be controlled with a simple timer (such as those found in a hardware store). Students may be asked how the L:D cycle might affect their experiments. Will the algae grow faster under a longer L:D cycle, or will they simply grow for a longer period of time? Why? How will the L:D cycle affect the time needed to reach the maximum biomass in the bottle?

Chlorophyll a

At some point, instructors will want to mention the presence of chlorophyll *a* (hereafter, simply called chlorophyll). Chlorophyll is the major light absorbing pigment of all photosynthetic organisms (including cyanobacteria, algae, and higher plants) that produce oxygen. (This latter distinction may or may not be important to introduce: some bacteria carry out forms of non-oxygenic photosynthesis that do not use chlorophyll, and we'll leave it at that). The green that students observe in the leaves of a tree, or an algae-filled pond, comes from chlorophyll. While it's not necessary to introduce biochemical mechanisms by which plants capture and process light energy (i.e., the Z-scheme), it is important to point out that chlorophyll takes energy from the sun (or artificial lights) and turns it into chemical energy (i.e., ATP and NADPH) that the plant can use to grow. The term, chlorophyll antenna, is commonly used in the literature, but we prefer to think of chlorophyll as a kind of catcher's mitt that's used to capture baseballs (i.e., photons) of light. Ultimately, students will use changes in the concentration of chlorophyll as an approximation for growth rate. Using photography (smartphones or tablets work great), color chart comparison (e.g., Fore-ule scale), a colorimeter (homebuilt or purchased), or a fluorometer (e.g., Turner Designs Aquafluor), students will track changes in the growth of the algae in their bottles. Students may be asked to predict what will happen to chlorophyll concentrations in their bottles as the algae grow.

Photoadaptation

Depending on time and the level of complexity that the instructor wishes to introduce, the concept of photoadaptation may be introduced. The physiology of algae (and plants) permits them to tune their photosynthetic machinery to available light conditions (nutrient conditions, too, but that's another topic!). While we may normally consider that the amount of chlorophyll per algal cell is constant, in reality, this ratio changes with light conditions. When light intensity is low, individual algal cells produce greater amounts of chlorophyll. Why? More catchers' mitts (i.e., more chlorophyll antennae) enable the algal cell to capture more photons of light. (Technically speaking, more chlorophyll increases the probability of light capture. Algae may increase the size of their antennae, or they may make more antennae, depending on the species.) Thus, under low light conditions, individual algal cells will become greener. At the other end of the spectrum, under high light conditions, algae will reduce their concentrations of chlorophyll per cell (to avoid photoinhibition and to conserve energy). As such, under high light intensities, individual algal cells will appear less green. The instructor may want to ask students to examine the leaves of shade and sun plants and compare their color. Typically, shade plants will appear a darker green because of their high chlorophyll per cell ratio, as compared to sun plants, with a lighter green color, that is, a lower chlorophyll per cell ratio. It's possible at this point to introduce students to other photosynthetic pigments, such other forms of chlorophyll (e.g., chlorophyll *b*, chlorophyll *c*) and accessory pigments (e.g., fucoxanthins) that help capture light (especially, wavelengths of light that chlorophyll a does not capture), or photoprotective pigments (e.g., carotenoids) that protect the photosynthetic machinery much in the same way that sunglasses protect our eyes. These other pigments become visible in climates where the leaves change color: absorption of chlorophyll during fall makes these pigments apparent as the yellows, oranges, and reds of fall leaves. Different species of plants (and algae) make different kinds of accessory and protective pigments, and, as such, are responsible for imparting visible color differences in the kinds of plants that we see in nature. Additional topics that may be introduced here include the characteristics of visible light (i.e., colors and their wavelengths), why we see color (pigments in materials absorb different colors and the color we see is the color not absorbed), the absorption properties of different algal pigments (chlorophyll primarily absorbs a narrow range of blue light, ~430 nm, and other chlorophylls and other pigments differ in the colors they absorb), algal and plant taxonomy (which, to some extent, is based on the presence of particular pigments), and the evolution of algae and higher plants (the presence of chlorophyll b in high plants suggests that they evolved from chlorophytes, a grouping of algae that contain chlorophyll *b*).

Light Sources

The decision of light intensities at which to grow the algae in a bottle depends mainly on the availability of artificial lights and space. Though some light sources are better suited to algal growth than others, just about any light source can be used, even a tungsten light bulb. High and low light intensities may be simulated by placing the bottles of algae closer to or further from the light source, respectively. Alternatively, household window screen may be cut and wrapped around individual bottles to reduce light intensity. If space and a light source are limited, the bottles of algae may be simply place in a windowsill for students to observe over the course of a few days or weeks. There are a number of different options here. See the Appendix for possible light sources and where to buy them.

Light in the Bottle

Following a discussion of light, students should be asked to look at their bottles, and determine if anything about the bottle might block the path of light from the light source into the bottle. Some students may suggest that the cap may block light, a correct assertion. But it's typical to get smiles and laughter when one or more students suggest that they remove the labels from their bottles. (The importance of this step cannot be overstated: the degree to which students understand the role of light in algal growth will be expressed in their confidence that removing the label is a no-brainer.) At this point, we let them remove the label and dispose of it properly. The instructor should now ask how the bottles should be oriented in relation to the light source. Should the bottles be lit from the top or the side? Would it be better to lay the bottles on their side for a light source above the bottles? If time permits, a discussion of Beer's Law can be introduced here, the exponential decrease in light intensity as light passes through water. Though it's not necessary to introduce absorption and scattering, the instructor can provide these processes as an explanation for the reduction in light intensity with depth in a column of water. A graph of this relationship can be useful, especially as Beer's Law helps students to understand how concentrations of chlorophyll can be determined using an instrument that measures reductions in light as it passes through a test tube of chlorophyll solution, i.e., a colorimeter or spectrophotometer. Beer's Law may also be applied to understanding why the ocean is blue, as blue light is the color least absorbed by seawater. Beer's Law has a number of applications in chemistry, and the interested instructor may find it useful to explore this literature, and introduce additional concepts, depending on the emphasis and complexity of the course. The key point here is that light intensity will be reduced from the lighted side of the bottle to the opposite side of the bottle as a result of absorption and scattering by water (a strong absorber and scatterer), by the algae suspended in the water (specifically, chlorophyll and other pigments), and, possibly, the plastic of the bottle

Scientific Questions & Predictions

After students have removed their labels and decided on the orientation and placement of their bottles in the light path, the instructor may ask students to predict the growth rate for the light intensity that they chose or that's available to them. Students may be asked how fast will their bottles turn green? How long will it take before the bottles stop turning green? What will cause growth to stop in the bottle, if anything? Will algae grown in high light reach their peak "greenness" faster or slower than algae grown in low light? What role do nutrients play in all of this? The manner in which these questions are explored and the time devoted to their discussion will depend on the degree to which this experiment serves as an inquiry-based activity or a demonstration activity. The algae in a bottle experiment is adaptable to a number of pedagogical approaches with simplifications or expanded discussions possible at any of the steps leading up to introduction of the algae into the bottle.

The Seasonal Approach

One way to connect the experiments with ecosystem processes is to take a seasonal approach whereby teams of students “duplicate” conditions of light and nutrients that are present during each of the four seasons in temperate zone waters. Typically, light intensity is lowest in winter, but dissolved nutrient concentrations are highest. Conversely, light intensity reaches its maximum in summer, but nutrients may be undetectable in surface waters. Spring and fall, times when algae blooms are common in lakes and in the ocean, provide sufficient light and plentiful nutrients. L:D cycles and water temperatures differ, too. The following combinations may be explored for exploring the seasonal cycle of productivity in aquatic ecosystems:

- Winter: low light high nutrients, 8: 16 L:D cycle, coldest temperatures
- Spring: medium light, high nutrients, 12:12 L:D cycle, warmer temperatures
- Summer: high light, low nutrients, 16:8 L:D cycle, warmest temperatures
- Fall: medium light, medium nutrients, 12:12 L:D cycle, colder temperatures

Students should be asked to predict growth rates and time to reach maximum biomass for each of the “seasons.” In presenting their results, students may be asked to compare what happened in their bottles to the typical seasonal response of algae “in the wild.” Were the results as expected? If not, why?

The Fast Approach

Foregoing most of the detailed discussion outlined above, all of the steps to this point can easily be accomplished in a single class session provided the instructor has the materials ready and has prepared a place for the bottles to be placed ahead of time. Students can easily remove some of the water in the bottle, add a teaspoon of sea salts, add one to several squirts of nutrients, and remove the label on the bottle in the space of less than an hour. However, instructors will want to gather the class and slow down the pace to address a couple questions of methodology that will maximize the educational benefits of the experiment, and that will ensure the most reliable results.

Scientific Questions & Predictions

First, instructors will want to ask students what is the starting point for the experiment. Is it the bottle of prepared water without algae, or is it the bottle of water plus the algae? What are the differences between the bottle without algae and the bottle with algae? How can we observe and measure those differences? The most appropriate starting point for observing or measuring the growth of algae, the time zero, as it were, could be the observation or measurement that occurs once the initial aliquot of algae has been added. However, if quantitative measures are going to be used, such as fluorometry, it will be important to obtain a blank. What does the instrument read without any algae? We think it can be useful and important to introduce the concept of the instrument or observational blank. Students should be made aware of the state of the bottle and its water prior to introduction of the algae (the blank) as well as the time zero, the state of the bottle once the algae have been introduced. Quantitative determination of the concentration of algae in the bottle requires a blank, the measurement obtained prior to introduction of the algae to account for instrument effects on the measurement. The degree to which this is emphasized depends on the methods that will be used to document changes in algal growth. But, in any case, we think it's important to make observations or measurements prior to introduction of the algae to their new homes.

Pictures and Notebooks

Second, students may be asked how they are going to document the start of the experiment. Will they take pictures? Will they make observations on the color inside the bottle once the algae have been introduced? Will they pick a color on a color chart? Regardless of the methods chosen, we strongly recommend that students take pictures, but there's a lesson here. Does it make a difference how they take the picture? At the outset, we let students take pictures without any discussion of how they do it. Following the picture taking, including time for students to take selfies with their bottles of algae, we ask students to share their photos with each other. Do they notice anything? Instructors may ask a few students to email their photos to the instructor's email address so that the instructor can project them on a classroom LCD projector, if available. Typically, students place their bottles on their desks or a table and take pictures from any angle and any distance without any thought of whether they can see color inside their bottles, or take the same shot at a future date, once the algae have grown. At this point, instructors should emphasize the importance of reproducibility in making observations or measurements. Ask students to comment on each other's photos with regard to the visibility and color of the contents inside the bottle. Can they improve on the picture? Does it matter? Most students will realize that their pictures are not ideal. A discussion of the ideal photo should follow. In the end, instructors will want to emphasize that it is important to take the picture with the same camera in the same position (with respect to the bottle), and it will be important that the bottle is in a place with the same lighting and against a background where the color inside the bottle can be easily discerned. We have found that placing the bottles on the ledge of a white board works very well, as long as students take their pictures so that the entire bottle is captured, so that the bottle fills the frame of the camera, and so that the camera is held perpendicular to the bottle.

Labeling Bottles

Third, and this is a point that instructors can wait to introduce, how will students be able to identify their bottle of algae? How will they tell one bottle from the other? This can be a fun lesson if students are left to their own devices at the outset. If the instructor has asked for a few photos via email, he or she can show the photo and ask the class to whom it belongs. In most cases, students will have neglected to label the bottles or identify ownership of the bottle in a way that's visible in their pictures. Once students realize this shortcoming, they get it! We find that it's useful to discuss and come up with a set of identifiers unique to the class (if different sections are carrying out the experiment), the team (if students are working in teams), and the individual (the person responsible for a particular bottle). We also find that it's useful to ask students where they should place their labels. If students have learned the lessons of light penetration, they will suggest that the bottle cap is the appropriate place because placing a label or writing on the bottle will reduce light inside the bottle. At this point, we ask students to proceed with labeling of their bottles, and to retake pictures using a now-well-defined protocol.

Conduct the Experiment

Finally, it's time to add algae. We recommend a consistent and pre-determined number of drops for all students; typically 1-3 drops are ideal, more if the stock culture density is low. Obviously, the starting concentration will influence the number of days until peak algal density is reached. Instructors may wish to run the experiment ahead of time with a number of different starting concentrations to find the concentration that best fits the timetable devoted to the experiment. The important consideration is to allow a change to be observable or detectable for the conditions of light and nutrients under which the experiment is conducted. Bottles with high light intensities and high nutrient concentrations will show visible changes in a shorter period of time than bottles grown at limiting nutrient concentrations under low light. In truth, without quantitative measurements of light intensities and nutrient concentrations, it's going to be difficult to predict with any certainty the daily rate of change in growth. But that's the fun of it! Instructors may want to let some students start with more drops. It's an experiment. As long as all of the variables are carefully documented, then there's knowledge to be gained in any approach.

Keep Taking Pictures and Making Observations

Following addition of algae, students will want to take another round of pictures. In classrooms where quantitative measurements are being carried out, the first set of measurements should be taken at this point to establish the initial concentration of algae in each bottle. Very likely, despite careful attention to water bottle volumes and the amount of nutrients and algae added, there will be slight differences among students. Any students whose measurements differ widely from other students should be queried as to the procedures they followed. We typically require students to work in teams of 3 or 4 and carry out identical treatments. Thus, a given team of students performs the experiment in triplicate or quadruplicate, and results within each team should be similar. Depending on the degree to which their initial measurements differ from the expected, and time availability, instructors may choose to make the student or students to start over, or they may allow the team to proceed with their experiments as is.

A Simple Observation Activity

For some instructors, the algae-in-a-bottle experiment may consist simply of an activity where students prepare a bottle of algae, and watch it grow, either in the classroom or at home. For others, class sessions following the time zero measurements will require time for continuing to document growth using photograph and other measures. Data may be compiled for the class as a whole as the experiment proceeds, or at the end of the experiment. Instructors may ask students to create a picture collage of their bottles, prepare a table of observations, create bar or line charts of any quantitative measures, and/or write up the results of their experiment using a laboratory report format. Where sufficient quantitative measures are available, students may calculate the rate of change in chlorophyll concentration by subtracting previous values from subsequent ones, or by simply subtracting the time zero concentration from the final one. Results can be presented orally in front of the class, or the instructor may choose to compile and present select results to illustrate key findings. Students may be asked to explain any differences in their results from the outcomes they expected, or how they might improve upon their experiments. There are numerous possibilities for exploring and discussing the physical, chemical, and biological processes that occur in their algae homes.

Assess Attitudes and Understanding

Following the experiments, instructors may want to assess changes in attitudes towards the experiments (i.e., the affective domain) and changes in conceptual understanding (i.e., the effective domain) using the post-tests provided in the Appendix. The results of the pre-post assessments can provide valuable information on the effectiveness of each step of the activity, and point out ways that delivery of the experiment, i.e., the lesson plan, can be modified and improved. Above all, we hope that implementation of the experiment reaps benefits for helping students to understand the nature of science, and for bringing to students a deeper conceptual understanding of the factors that govern the growth of plants and algae in the natural world.

Materials and Optional Equipment

The Algae-in-a-Bottle Experiment relies primarily on easy-to-find, inexpensive-to-purchase materials available in local grocery or box stores, or online. Instructors wishing to adopt more rigorous, research-based protocols may, too, find materials easily available, albeit at greater expense.

Bottled Water

Bottles of 8-ounce, spring water work best and are the least expensive.

Saltwater

Morton or other brands of sea salts.

Professional grade sea salts: $75, Oceanic 81050 Natural Sea Salt Mix, makes 200 gallons
http://www.amazon.com/Oceanic-81050-Natural-200-Gallon-Bucket/dp/B000256EWG

Instant Ocean, Coralife, and others may be suitable.

Sources of Algae

Liquid microalgae cultures: $10-$30, Florida Aqua Farms
http://florida-aqua-farms.com/shop/liquid-microalgae-cultures/

Research-grade microalgae cultures, $175, National Center for Marine Algae and Microbiota
https://ncma.bigelow.org

Sources of Nutrient Media

Miracle Gro, $9, Amazon
http://www.amazon.com/Miracle-Gro-1001233-Purpose-Plant-Food/dp/B000P6QYJK

Microalgae Grow Mass Pack, $20+, Florida Aqua Farms
http://florida-aqua-farms.com/product-category/store/micro-macro-nutrients/

Research grade f/2 media, $85, National Center for Marine Algae and Microbiota
https://ncma.bigelow.org

Light Sources

An infinite variety of light fixtures are possible, ranging from simple bulb and fluorescent light fixtures to LED aquarium fixtures. The type you choose will depend on budget and space considerations. For best growth, choose bulbs designed specifically for plants (that is, bulbs that emit as much blue light as possible, the color most absorbed by chlorophyll a)

Bulb Fixtures and Bulbs

Bulb fixtures can generate lots of heat, but they are inexpensive, and a number of bulbs designed specifically for plants, including compact fluorescent bulbs and LED bulbs, are now available. Type "grow bulb" in Amazon or click below.

http://www.amazon.com/s/ref=nb_sb_noss_1?url=search-alias%3Daps&field-keywords=grow+bulb

Fluorescent Lights

Fluorescent fixtures are cooler, and offer the advantage that you can stand them on edge and place a lot of bottles in front of them. Choose full spectrum or plant grow bulbs for the best results. Type "grow bulbs fluorescent" in Amazon or click below:

http://www.amazon.com/s/ref=nb_sb_noss?url=search-alias%3Daps&field-keywords=grow+bulbs+fluorescent&rh=i%3Aaps%2Ck%3Agrow+bulbs+fluorescent

Aquarium LED Fixtures

Designed for true (hard or stony) corals, which contain algal symbionts, aquarium LED fixtures offer the most advanced lighting systems for growing algae. They are not inexpensive, but their wavelengths of emission are ideal for algae, and most LED fixtures permit you to vary the light intensity. We use the TaoTronics 165W Dimmable LED Aquarium Lights, but they are not available at the time of this writing. Type "LED reef aquarium lighting" in Amazon. We suggest that you read the reviews and make your own decision based on your budget and space considerations.

http://www.amazon.com/s/ref=nb_sb_noss_1?url=search-alias%3Daps&field-keywords=led+reef+aquarium+lighting&rh=i%3Aaps%2Ck%3Aled+reef+aquarium+lighting

Mobile Lighting Systems

A mobile system is ideal for use in multi-use classrooms (e.g., most college classrooms) where the experimental setup cannot be left in place. Mobile light carts can be wheeled in and out of a classroom, and stored safely in a closet or other location where electrical outlets are available. Prices range from the few to several hundreds of dollars. We use mobile, 4-shelf, plant grow carts with fluorescent lamps, purchased for $782.50 at Carolina Biological Supply. Type "plant grow cart" in Google for other options.

Scientific Equipment

Measuring cups, teaspoons, and tablespoons, available at your local grocery or box store

Plastic transfer pipettes, $4.39, pack of 100
http://www.amazon.com/Plastic-Transfer-Pipettes-Gradulated-Pack/dp/B005IQTSE0/ref=sr_1_1?ie=UTF8&qid=1428780519&sr=8-1&keywords=pipettes%27

Refractometer for measuring salinity, $49.99, Marine Depot
http://www.marinedepot.com/ps_viewitem.aspx?idproduct=MD2101&child=MD2101&utm_source=adwordsfroogle&utm_medium=cse&utm_campaign=adwordsfroogle&utm_content=MD2101&gclid=CjwKEAjwjKOpBRChjsTyicbFy3QSJADP1gTNYxtayxnYdL87VtARFk9DOuffxNO8mH_wVOCOelk-wBoCLGfw_wcB

Educational Colorimeter Kit, for measuring color changes, $145.00, IORodeo
http://www.iorodeo.com

AquaFluor Handheld fluorometer for measuring chlorophyll, ~$3000, Turner Designs
http://www.amazon.com/Plastic-Transfer-Pipettes-Gradulated-Pack/dp/B005IQTSE0/ref=sr_1_1?ie=UTF8&qid=1428780519&sr=8-1&keywords=pipettes%27

Helpful Resources

Culturing Methods and Information, National Center for Marine Algae and Microbiota
https://ncma.bigelow.org/culturing-methods
How to Grow Algae, Wiki-How
http://www.wikihow.com/Grow-Algae
Measuring Salinity with a Refractometer
http://reefkeeping.com/issues/2006-12/rhf/
The Basics of Photosynthesis, Simple English Wikipedia

http://simple.wikipedia.org/wiki/Photosynthesis

The Basics of Chlorophyll Measurement

http://www.ysi.com/media/pdfs/T606-The-Basics-of-Chlorophyll-Measurement.pdf