Nutrient Enrichment of Phytoplankton in the Chesapeake Estuary
A MWEE for 9th Grade Environmental Science Classes

Third Day of PLANS – Teacher-led classroom activity, supported by PLANS staff through online resources.

Overview

During this class students will be introduced to the model enrichment lab experiment and will set up their bioassay. At this point, students will take their first observations of their test tubes. Note: students will spend 6-8 days monitoring their experiment and making observations on the initial day, day on the 4th and 8th day of the experiment.

Objectives

- Introduction of model enrichment lab experiment
- Setting up experiment and initial observations
- **6-8 days of observations** *(supervised by teacher)*
- Students complete FieldScope activity either in class or as homework (provided by PLANS staff)

Background

Phytoplankton are a diverse group of aquatic autotrophs that have many of the same nutrient requirements as land plants. However, unlike terrestrial plants, which gain most of their essential elements from the soil, phytoplankton receive their nutrition from substances dissolved in the surrounding water. Natural precipitation contains little, if any, of the macronutrients necessary for the growth of plants. Air pollutants can impart some nutrient enrichment to the precipitation, namely in the form of nitrogen-containing compounds, but many elements are still lacking. By contrast, surface waters such as runoff, streams, and rivers, may contain high levels of all the nutrients needed to support plant growth. These additional nutrients originate from the interaction of newly fallen precipitation with components of the geosphere – primarily dust, sediments, bedrock, and soil.

Every surface water sample is enriched to some degree by these interactions and is, therefore, at least somewhat capable of supporting algal growth. The maximum algal biomass that can be produced in a natural water sample under standardized laboratory conditions is termed the water sample's "Algal Growth Potential". In most cases a sample's algal growth potential is a measurement of its degree of nutrient enrichment. The nutrient enrichment bioassays to be conducted by your students during PLANS are just such measurements. These bioassays of natural water samples can be used to predict the likelihood of excessive algal growth in aquatic environments.

Nutrient Enrichment Bioassays and Experimental Design

In order to design a robust experiment, it is important that students first understand the basic concepts and terminology of experimental design before applying it to the task at hand – designing a nutrient enrichment bioassay.

Experimental design is often approached by students as a mundane task that is done by rote. However, this approach is unproductive and leads students down the path of confusion and inaccuracy. Good experimental design is a very creative and thoughtful endeavor that must adhere to a set of guidelines in order to produce unambiguous and coherent results. Experimental design is an opportunity for scientists to be resourceful and inventive, as they acquire new information about the world around them.

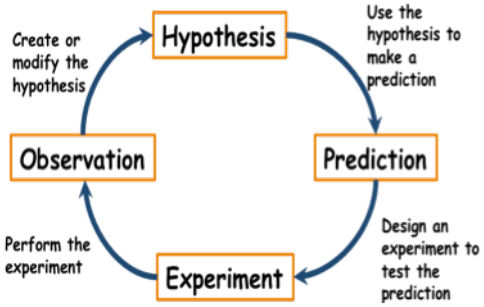

Figure 1. A schematic of the experimental process.

Students are often confused about what an experiment is, and what it is not. It is not unusual for teachers to use the term "experiment" when an activity might be better described as a "demonstration" or "analytical procedure". For example, demonstrating that saltwater is denser than freshwater, while instructive, is not an experiment per se. Likewise, determining the salinity of a water sample is not the same as conducting an experiment.

So what is an experiment? The dictionary defines the term *experiment*, in part, as "an operation or procedure carried out under controlled conditions in order to discover an unknown effect or law; to test a hypothesis". It is the hypothesis testing aspect of this definition that is especially critical. Making observations and forming a hypothesis leads to an iterative process by which the hypothesis can be confirmed or refined (Figure 1).

A *hypothesis* is a statement - not a question or objective - that describes some aspect of the natural world. It is usually based on initial observations. For example in the case of nutrient enrichment bioassays, the hypothesis could be stated as: "Algal growth will be enhanced by the addition of excess nutrients". Based on this hypothesis you could make the following *predictions*: 1) "The addition of nutrients will enhance algal growth in my water sample" or 2) "Withholding nutrients in my water sample will inhibit algal growth". The next step is to design an experiment to test these predictions.

Experiments are designed so that only one factor varies. This is the *manipulated (or independent) variable*. In our model example this would be the addition of nutrients

added to each test tube. The additions of different concentrations of nutrients are called *treatments*.

During the model experiment we would monitor algal growth in each tube. This is our *observed (or dependent) variable*. All other factors that could conceivably affect the outcome of the experiment must be the same among the treatments. These are called *constants.* In our example, constants would be such factors as the initial amount of water in the tubes; the temperature at which the tubes are incubated; the amount of light exposure provided each tube; and so forth. It is often advisable to monitor constants during an experiment to document that they did indeed remain unchanged.

Well designed experiments also contain controls. These act as internal tests of the validity of your design. *Positive controls* are designed to determine whether the experimental procedures are capable of observing the predicated effect (e.g., enhanced algal growth in the presence of excess nutrients). *Negative controls* confirm that the procedure is not observing an unrelated effect (e.g., enhanced algal growth unconnected to nutrient additions). In our example, additional test tubes that have increasing concentrations of nutrients are positive controls. Negative controls would be tubes containing the algae and water but no additional nutrients. The entire experimental design scheme is summarized in Figure 2.

Another important component of good experimental design is *replication*. Each control and treatment within an experiment should be replicated a number of times. Replication allows us to document and quantify the variability of our experimental system in response to the manipulation that we have just performed. How many replicates are necessary? There is no magic number of replicates. Generally, it's advisable to do as many as your time and resources will allow. Classroom time and resources are often at a premium; however, try to perform at least some replication.

Lastly, it is important to point out that our model experiment is trying to demonstrate nutrient enrichment in an algal sample, which will mimic an algal bloom in the Chesapeake Bay. In our case, the test tube is considered to be an *experimental model* of one of these much larger systems. Models are customarily used to provide replicates of larger, more complex natural systems. However, the model and the natural system will differ in many ways. For example, water in the river or bay:

- contains many more organisms than are found in your model
- receives rainfall and runoff continuously, unlike your model
- interacts with bottom sediments not found in your model
- is subject to different temperature and light conditions than your model

These differences may or may not be important to the hypothesis being tested. However, it is critical for your students to consider each of them (and possibly

others) when interpreting the findings of your classroom experiment and trying to infer their results to the larger ecosystem.

Measuring Algal Biomass

There are a variety of methods for measuring a change in the size of a phytoplankton population. Often the cell density in a water sample is determined by counting cells with the aid of a microscope. Alternately, the algal population size can be estimated by measuring the amount of chlorophyll that can be extracted from a water sample.

Another, simpler way of determining at least large differences in algal density is to filter a known amount of algal culture through a filter and catch all the algae on the surface of the filter. The color of the filter is proportional to the density of algae in the original culture - the darker the coloration, the greater the algal density.

We standardized these observations somewhat by filtering known numbers of cells on to filters and scanning the filters to obtain the coloration (see Figure 1). You can see that as greater numbers of cells are filtered, the coloration on the filter pad becomes markedly darker.

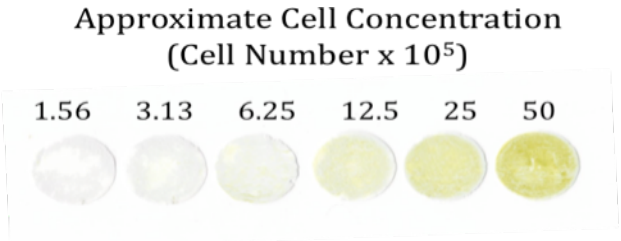

Figure 2. Coloration differences in filter residues from filtering 10 ml of cultures of different densities.

Note that the color differences are most easily seen in cultures that have higher cell densities; and that the sensitivity of this method declines at lower cell densities.

From these color differences we have developed a color comparator to use in estimating cell densities in classroom incubations (see Figure 3). The comparator will allow students to gauge the amount of color on their filter pads and convert that color to a relative value. These values can be used to compare results from group to group and from one experiment to another.

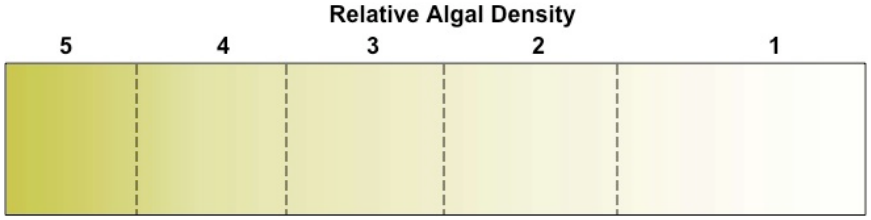

Figure 3. Color comparator

Setting Up the Model Enrichment Experiment

Materials Needed

- Enrichment Bioassay Kit(s) – provided by PLANS staff
- Constructed Light Box(es) – materials provided by PLANS staff
- Digital Camera (optional)
- Student Handout for *Nutrient Enrichment Bioassay Procedure* - downloadable from the PLANS website, under "Teacher Resources"
- Group Data Sheet (for recording periodic observations during the Bioassay) - downloadable from the PLANS website, under "Teacher Resources"
- FieldScope activity - downloadable from the PLANS website, under "Teacher Resources"

Preparation

- Construct the light box using the materials provided by PLANS staff and the instruction sheet on the PLANS website under "Teacher Resources". **Note:** You may want to enlist students in this task; however, this will require additional time.
- Make copies of the student handout: *Class Period 3*.
- Make a copy of the class data sheet
- Optional: Make copies of the FieldScope activity

Procedure

Set up the Bioassay:

Each team of students should set up one replicate of the control and each treatment in the Bioassay (5 tubes in total). Provide copies of the student handout of the Nutrient Enrichment Bioassay Procedure to be used as a precise guide in setting up the experiment.

Label 5 test tubes using tape and a marker. Label with the group letter (A, B, C, D or E) and the appropriate labels from Table 1 below. For example, the control tube for group A should be labeled "A-Control", the 12.5% tube should be labeled A-12.5%, etc.

Then fill each tube with the following:

Tube 1: Control
Using a graduated cylinder measure 20ml of spring water and pour into the test tube labeled "control"

Tube 2: 12.5%
Using a graduated cylinder measure 20ml of 12.5% fertilizer solution and pour into the test tube labeled "12.5%"

Tube 3: 25%
Using a graduated cylinder measure 20ml of 25% fertilizer solution and pour into the test tube labeled "25%"

Tube 4: 50%
Using a graduated cylinder measure 20ml of 50% fertilizer solution and pour into the test tube labeled "50%"

Tube 5%: 100%
Using a graduated cylinder measure 20ml of 100% fertilizer solution and pour into the test tube labeled "100%"

Now add 1 ml of the algae stock to each test tube.

Tube	Test Tube Label	20 ml of the following solutions	Algae
1	Control	Spring water	1ml
2	12.5%	12.5% fertilizer	1ml
3	25%	25% fertilizer	1ml
4	50%	50% fertilizer	1ml
5	100%	100% fertilizer solution	1ml

Table 1: Lab enrichment set up

Cap each tube and invert it several times to mix the algae and fertilizer solutions. Then return each tube to the rack and loosen the cap to allow for some exchange of air.

This bioassay will incubate for approximately 10 days.

Monitoring the Bioassay:

1. Have each team of students make their initial observations and record their findings on the group data sheet. Optionally, digital images can be taken of the tubes to record the initial coloration of the solutions.

2. Algae will settle to the bottom of the tubes, so students should invert tube daily or every other day to re-suspend them. Remind them to tighten caps before inverting and then loosen them again after algal re-suspension.

3. Students should observe the test tubes periodically (every 3rd or 4th day) during the experiment to note changes in coloration that might be developing. Encourage student to compare the colorations among the tubes. Can they discern any trends? Which is lightest? Which is darkest? Have them record their observations. Additionally, optional digital images may be taken at these times.

At the conclusion of the incubation, each algal suspension will be filtered and the color of the filters among the treatments will be compared using a color comparator. These methods will be described in detail in the Period 5 Teacher's Guide.

Check for Understanding – Teacher Guide

Which of the following scenarios would you expect a phytoplankton bloom to occur in the Chesapeake Bay?

1. During the spring after many days of rainfall
2. During the summer after a drought
3. During the winter

Answer: 1. Excess nutrients will run off into the bay during periods of high rainfall or snow melt. The addition of nutrients can create an algal bloom much like the simulation of a bloom in the lab enrichment experiment.

Make a list of factors you think would affect the growth of an algal population.
Answer: Students may identify one or more of the following:
Day length – increase hours of sunlight allow the algae to grow faster.
Rainfall – provides the addition of nutrients from soils via run off.
Carbon dioxide – plants need carbon dioxide to photosynthesize, therefore in short supply their growth will be inhibited and vice versa.
Salinity – depending on the species of algae salinity, increases may inhibit their growth.
Soil contaminants – pesticides, heavy metals and other toxins could all inhibit the growth of algae