The Effect of Eutrophication on the Health and Trophic Structure of an SAV Community

Background Information:

**Eutrophication** occurs when a body of water, like an *estuary*, receives an input of excess *nutrients*, especially nitrogen and phosphorous in forms that are easily taken up by plant life. **Phytoplankton** are microscopic plants that thrive in coastal waters. Because of their small size and corresponding high surface area/volume ratio, phytoplankton are able to absorb dissolved nutrients more rapidly than **submerged aquatic vegetation** (SAV). Nutrient additions result in rapid phytoplankton growth and reproduction, and have the potential to cause algal *blooms*.

There are many problems associated with these blooms. Initially increased photosynthetic activity spurred by the bloom results in increased oxygen production and ultimately higher levels of *dissolved oxygen* in the water column. However, as the phytoplankton die and sink toward the bottom, **heterotrophic** bacteria consume them. In the process of **cellular respiration**, these heterotrophic bacteria utilize the dissolved oxygen in the water column as they decompose the sinking phytoplankton cells. The resulting *hypoxic* (low $O_2$) or *anoxic* (no $O_2$) conditions make the environmental conditions near the bottom unsuitable for **aerobic** organisms.

Phytoplankton blooms also reduce water clarity. A reduction in water clarity reduces the amount of light that reaches the bottom, effectively limiting the amount of available habitat for SAV. In addition, some forms of algae, called *epiphytes*, actually grow on the surface of SAV leaves, further reducing the amount of light that reaches the plant. Reduction in light reaching SAV caused by phytoplankton blooms and epiphyte growth can destroy entire SAV beds. As the
SAV bed disappears, all the organisms associated with that habitat either emigrate or die, thus altering the **trophic structure** of the estuarine ecosystem.

**PART A- Design and Set-Up**

**Overview:**
In this experiment, students will be using aquaria (**microcosms**) to simulate SAV communities. Using the materials provided, students will manipulate nutrient addition and evaluate the effect of this addition on the SAV community health.

**Materials:**
- 5 small tanks
- Non-adhesive wrapping ribbons (artificial SAV) or actual plants
- Anchor (wire mesh, bolt, glass stirring rod, etc.)
- Pond water
- Masking tape and permanent marker
- Water soluble fertilizer (Miracle Gro)
- Air stone, pump, and tubing
- Ruler

**Pre-Lab Questions:**
1) What is eutrophication and what are some nutrient sources that might initiate this process?
2) What are some problems associated with algal blooms?
3) Why do hypoxic or anoxic conditions cause problems for organisms living in the benthic zone?
4) What are two direct causes for the reduction of light reaching the SAV?
5) What effect does a decrease in light have on the photosynthetic process of plants?
6) How might the disappearance of SAV impact the estuarine food web?
7) Using the “impacted vs un-impacted SAV habitat” diagram provided discuss with your
group the difference between the un-impacted and impacted food webs. Using a pencil
draw arrows to represent trophic linkages in both of the food webs. How does the
number and type of trophic linkages differ between the two food webs?
8) Think about your experimental design. What is are the dependent and independent
variables? Develop a hypothesis.
9) What is the control? What are the constants in your experiment?

Procedures:

1) Assign each group a tank and have them label their tank 15 cm from the bottom with
masking tape and permanent marker (Ex: control, pulse, continuous, pulse air,
continuous air).
2) Place all tanks near a window so they will all receive the same amount of light. Fill the
tanks with pond water up to the bottom of the label.
3) For each tank, cut 2 30-cm strips of ribbon. Place the anchor (wire mesh, bolt, glass
stirring rod, etc.) in the middle of your piece of ribbon and tie an overhand knot. You
should now have 2 15-cm ribbon strips (grass blades) attached to each anchor.
4) Place the artificial SAV in the tanks so that the blades of the SAV are not touching the
walls of the tank or each other.
5) Insert aerators into the appropriate tanks.
6) Determine the volume of water in your tanks in liters. Calculate the amount of fertilizer
(water-soluble Miracle Gro) needed for each fertilized tank using a 0.4 g/1 L ratio.
Weigh the calculated mass of fertilizer and dissolve this mass in 400 ml of water.
7) Pulse groups will add all of their fertilizer solution on Day 1. Continuous groups will add 80 ml per day of their fertilizer solution over the first 5 days of the experiment.

8) Be sure to take initial measurements for each parameter (dissolved oxygen, phytoplankton density, and font scale data) described in the following procedures (Part B- Data Collection).

**PART B- Data Collection**

**Overview:**

In this section, students will monitor their tanks over a period of 10 school days. Each day they will collect and record **quantitative** and **qualitative** measurements. The data will then be illustrated graphically so that trends can be analyzed. The students will draw conclusions and make inferences on how their microcosm simulates the natural environment.

**Materials:**

- Dissolved oxygen kits
- Filter paper or weighing boats
- Razor blades
- Light microscope
- Slides and cover slips
- Dropper or micropipet
- Electronic scale (or triple-beam balance)
  Spectrophotometer (optional)

**Pre-Lab Questions:**

1) How can you measure epiphytic growth on the SAV? Is that measurement quantitative or qualitative?

2) How can you measure water clarity in your tank? Is that measurement quantitative or qualitative?

3) Can you think of a way to quantify the amount of phytoplankton in the water?
4) How will you measure the amount of dissolved oxygen in the water?

Data Collection Procedures:

Every other day, students will measure dissolved oxygen and estimate phytoplankton density in their tank. On the last day of the experiment, students will harvest the artificial substrates and estimate epiphytic biomass.

Dissolved Oxygen:

Follow the instructions on the DO kit.

Phytoplankton Density:

Cell Count-

1) Use a pipet or a graduated cylinder to pull a 5 ml sample of water from the middle of the water column. Agitate the sample to ensure equal distribution of suspended material.

2) Take a drop (~0.1 ml) from your sample and place it on a slide. Place a cover slip on top. Pour the remainder of the 5 ml sample back into the tank.

3) Using a light microscope at 100X magnification, count the number of algal cells in the field of view. Record this number as “algal cells in field of view” on the data sheet under the “Cell Count” section.

4) Repeat steps 1-3 two more times.

5) Calculate the mean of the three samples and record this value on the data sheet.

Water Clarity-

1) Obtain a Font-Size Scale of Water Clarity from your teacher.

2) Have one student hold the scale against the rear glass of the tank so that it can be viewed through the water column.

3) Read the smallest letter possible. Record the font size on the data table.
4) Repeat steps 2-3 two more times.

5) Calculate the mean font size and record this value on the data table.

**Epiphytic Biomass:**

1) Place four pieces of filter paper into an incubator or a dessicator (if available) for 24 hours prior to measuring the epiphytic biomass.

2) On the day of measurement, number each piece of filter paper with a pencil 1-4 respectively.

3) Weigh each piece of filter paper and record its weight in the data table as “filter weight” next to the appropriate number.

4) With forceps grasp the top of one of the ribbons in your tank and cut it at the base with scissors.

5) Carefully remove the blade from the water and immediately place it on the filter paper.

6) Using a razor blade or a straight edge, carefully scrape all of the epiphytic material from the artificial SAV leaf onto the filter paper.

7) Measure the length and width of the ribbon to determine the area of one side and then multiply this value by 2 [(L x W) x 2] to determine the surface area of the entire ribbon. Record this information in the “surface area” column of your data table.

8) Repeat steps 4-7 for each blade in the tank.

9) Place all pieces of filter paper in the incubator or dessicator for 24 hours.

10) When the filters are dry (~24 hours), weigh them to determine “filter weight + sample weight” and record this information on your data table.

11) Subtract the “filter weight” from the “filter weight + sample” to determine the “sample weight”. Record this value in your data table.
12) Divide the “sample weight” by “surface area” to calculate grams/cm$^2$ of epiphytic material. Record this value in the “epiphytic biomass/cm$^2$” column.

13) Determine the mean “epiphytic biomass/cm$^2$” for the tank. Record this value in the data table.

**PART C- Analysis**

**Overview:**

Students will report their findings to the class in order to provide all students with a complete data set. In the space provided, students will graphically analyze the data for the experiment.

**Materials:**

- Graph paper
- Calculator (graphing calculator optional)
- Ruler

**Graphing Guidelines:**

*Remember a complete graph always includes the following: title, labeled axes with units, equal intervals, and legend. Place the independent variable on the x-axis and the dependent variable on the y-axis.*

**Procedures:**

1) Develop a line graph for the dissolved oxygen data set.

2) Repeat this step for the three remaining data sets.

**Part D- Conclusions and Applications**

**Overview:**
In this section, students will draw conclusions about the experiment based on their analysis. These conclusions will then be used to infer how their results might apply to the problem of eutrophication in Chesapeake Bay. Students will compile their findings and conclusions in a post-lab report.

**Report Guidelines:**

* Title

* Hypothesis

* Results Section:
  1. Pre-lab questions and answers
  2. Data Tables (Cell Count, Font size, Epiphytic biomass, and Dissolved oxygen)
  3. Graphs (one line graph for each data set)

* Conclusions and Application:
  1. Restate your hypothesis.
  2. Discuss and interpret your results. For each graph, include a description of the relationship among the different treatments over time.
  3. Explain how your data did or did not support your hypothesis.
  4. Refer back to the diagram of the un-impacted and impacted SAV ecosystem. Explain how your results do or do not support the change in trophic structure represented in the diagram.
  5. Why is it important to decrease the amount of eutrophication occurring in Chesapeake Bay and give some examples of how we can reduce the amount of eutrophication occurring in Chesapeake bay.