Nutrient Limitation of Phytoplankton in the Chesapeake Estuary
Nutrient Limitation Bioassay Procedure – Patuxent River Sample
Honors & AP Environmental Science Classes

The following procedure outlines the steps in setting up a nutrient limitation bioassay using a Patuxent River water sample that you will obtain while on your field trip to the Morgan State University Estuarine Research Center (ERC). Your objective is to determine what nutrient, nitrogen or phosphorus, is limiting the growth of natural phytoplankton in the water sample taken from the river.

Procedure

Preparing your water sample (this will be done while you are on your field trip to the ERC):

- Obtain a screen mesh (53 µm mesh) and a 1L beaker to catch the screen water.

- To remove zooplankton, sieve your Patuxent River water through the screen mesh into a clean 1L beaker.

- Set a portion (approximately 5 ml) of the water sample aside for examination under the microscope. Make a wet mount of the water sample. Determine the relative amounts of different types of phytoplankton. Record this information. PLANS staff will help you to identify different groups of phytoplankton and record images of them using the digital cameras at the ERC.
- Return the remainder of the screened sample to your classroom and use it to set up the nutrient bioassay during your next class period. **Note: Store your water sample in a covered container in the light box until the experiment has begun.**
- You will also receive a Patuxent River water sample that has been recently collected and sterilized for you to take back to your classroom.

Preparing your treatments (back in your classroom)

- Your teacher will assign each team of students a letter designation (A, B, C, D …) and instruct you on the number of test tubes your team will be using for the limitation assay. Fill in the first column in Table 1 below with the number of tubes your team will set up for each treatment. Using label tape and a waterproof marker, label your tubes with your team letter and by treatment as indicated in the second column of Table 1.

  I am on team _____.

The following treatments will be:

No. of tubes (ask your teacher)	Label	Treatments for the Limitation Bioassay of the Patuxent River Water Sample
	Blank	Sterilized Patuxent water sample
	Control	Patuxent water sample
	+ N	Patuxent water sample + N
	+ P	Patuxent water sample + P
	+ NP	Patuxent water sample + N+P

Table 1. Treatment set up

- A portion of the water sample has been sterilized via boiling to kill all phytoplankton. This water will be used as your **blank**. A blank is not actually a treatment in the experiment, but is used in the analysis of your samples with the fluorometer. Why do you need a blank?

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- Add the following amounts to each of the labeled tubes (See Table 2 and Figure 1) Use a 100ml graduated cylinder to measure out the water samples and transfer them to the test tubes. Use 1ml pipets and a pipet-pump to transfer the nitrogen and phosphorus stock solutions. **Note: Be sure to use different pipets to transfer the two nutrients and do not mix them up!**

Label	Screened Water Sample (ml)	Nitrogen Stock (ml)	Phosphorous Stock (ml)
Blank	40 (sterilized)	-	-
Control	40	-	-
+N	40	0.4	-
+P	40	-	0.4
NP	40	0.4	0.4

Table 2. The volumes of materials to be added to each test tube.

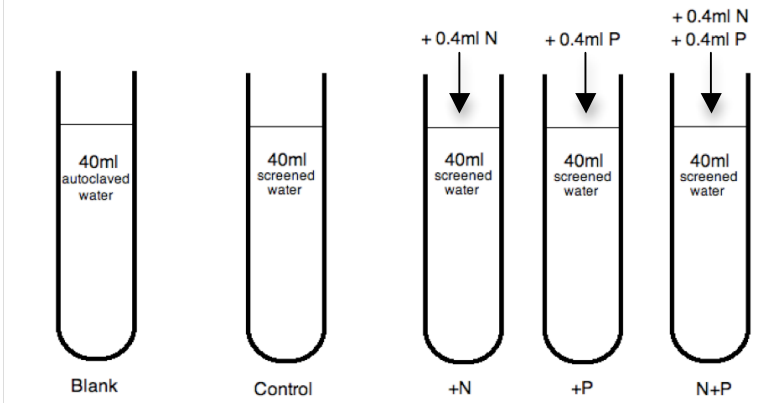

Figure 1. A diagram of the basic experimental design for this nutrient limitation assay.

- Make initial *in vivo* fluorescence for each tube as follows:

  - Cap and mix the test tubes gently by inverting them several times.
  - Remove 2 ml from each tube using a cleaned pipet and pipet-pump (at right). **Note: If the same pipet is used to obtain a sample from more than one tube, be sure to rinse it thoroughly and empty it of any rinse water**
  - Place the 2ml sample in a clean, dry plastic cuvette
  - Obtain an initial *in vivo* fluorescence reading using the hand-held Aquafluor fluorometer. Your teacher will demonstrate how to use the fluorometer correctly
  - Record your team's initial readings on the class data sheet
  - Replace the test tube cap loosely on the tube and place in a test tube rack
  - Place the test tube rack in the light box

- At least one member of each team should make daily fluorescence readings of each test tube using the procedure outlined above. Briefly, this entails:

  - Tighten the test tube caps and gently mix tubes
  - Remove 2 ml from each tube and place this volume in a cuvette
  - Obtain an *in vivo* fluorescence reading using the hand-held Aquafluor fluorometer
  - Record your team's readings in the proper location on the class data sheet
  - Replace cap loosely and place each tube back in the test tube rack
  - Place the rack in the light box

  Continue making daily readings until your teacher instructs the class to end the experiment.

- At the conclusion of the experiment, examine a sample of the control, +N and +P and +NP treatments under the microscope. What are the approximate proportions of each phytoplankton type identified in your sample when it was first collected during your field trip? How have these proportions changed from the beginning to the end of the bioassay?