

## Interpreting Nutrient Limitation Data

You have completed a nutrient limitation bioassay. Now how should you interpret the results? First let's summarize and present the data; then we can determine its meaning with regard to limitation.

### Data Analysis:

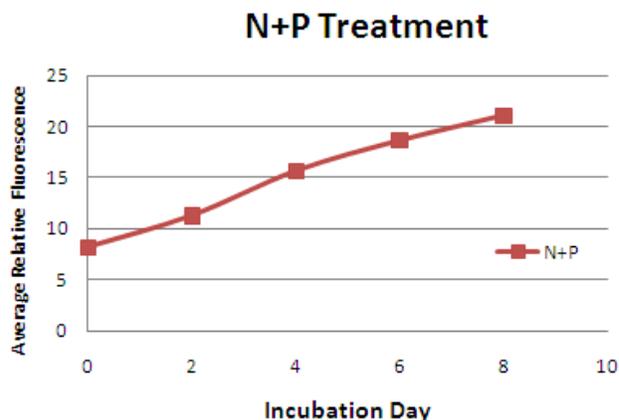
Recall that the class data contains replicates of five treatments: a Blank (sterilized water), a Control (live algae but no N or P added); a nitrogen treatment (only nitrogen added); a phosphorus treatment (only phosphorus added); and an N+P treatment (both nitrogen and phosphorus added). Each of these replicates was incubated for a number of days and *in vivo* fluorescence (IVF) measurements were made during most days of the incubation period. These readings are a good indicator of the algal population growth that is occurring in each test tube. Your data for one treatment might look like this:

#### N+P Treatment – IVF Readings

Replicate	Incubation Day				
	0	2	4	6	8
1	8.2	11.9	14.2	19.2	23
2	6.8	11.8	16.4	17.9	22.6
3	8.5	10.9	15.5	18.4	20.4
4	9.1	11.2	14.9	17.7	19.5
5	7.2	10.4	17.1	19.8	19.8
Average	8.15	11.24	15.62	18.6	21.06

#### YOUR JOB:

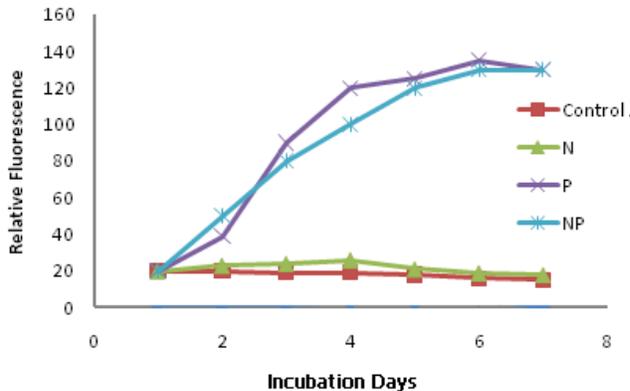
1. Compute the average IVF reading for each day of the incubation.
2. Create a scatter graph with the average IVF values on the Y-axis and the Incubation Days on the X-axis, as seen below.



Note that the graph to the left depicts continuous algal growth throughout the 8-day incubation. Your data may be similar or it may show inconsistent daily growth. If your incubation is sufficiently long, the line might also reach a plateau during which algal growth is equal to algal death.

3. Add lines to your graph that represent all of the additional treatment average IVF values (with the exception of the Blank). A completed graph might look like the one at the top of the next page:

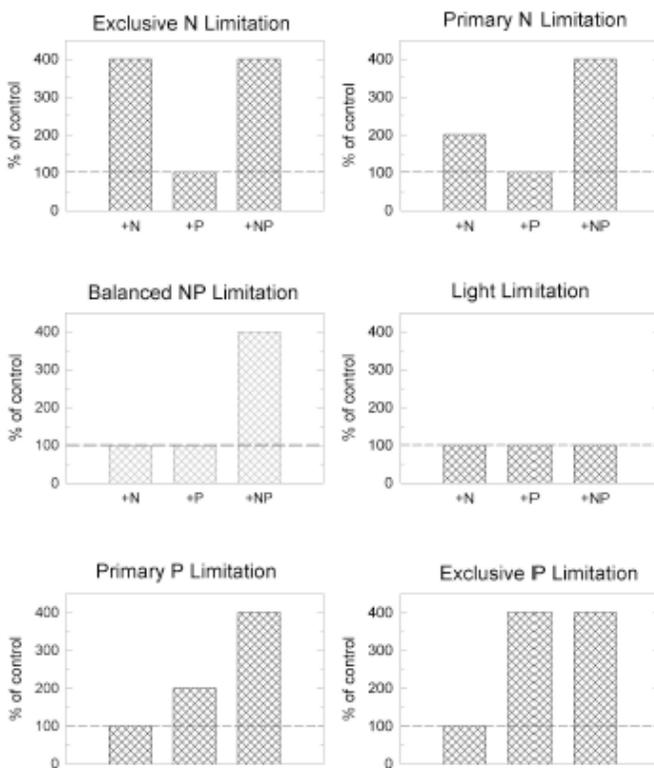
**Nutrient Limitation Bioassay Results**



This type of graph will summarize how your experiment proceeded from start to finish. Generally, your Control should show little increase in algal biomass during the incubation. Your N+P treatment will usually exhibit the greatest increase in algae. But what nutrient is limiting (controlling) the algal growth? Responses to your N and P treatments will allow you to make that determination.

**Interpreting Nutrient Limitation Results:**

Researchers studying nutrient limitation in the Chesapeake Bay have devised a series of model outcomes for nutrient limitation experiments that can be used to interpret your results. In each of these models, the average IVF values of N, P and N+P treatments for the final day of the incubation are compared to that of the Control. In each case, **the average IVF value is expressed as a percentage of the final average Control IVF value by dividing the final average treatment IVF by the average control IVF and multiplying the result by 100.**



The six graphs to the left show the range of results that you might observe. Note that your control should have the lowest final IVF average. Therefore, all the other treatment averages should be approximately 100% of the Control or greater. It's also important to note that in this scheme there are more outcomes than only simple N or P limitation. Here are descriptions of the six possible outcomes:

1. **Exclusive N Limitation** – The N treatment and the N+P treatment are nearly identical and both are much

greater than the Control, while the P treatment is similar to the control. Nitrogen is the only limiting factor for this outcome.

2. **Primary N Limitation** – The N treatment is somewhat larger than the Control but not as great as the N+P treatment. Nitrogen is the primary limiting factor for this outcome but other factors may also play a role in controlling algal growth.
3. **Balanced NP Limitation** – Both the N or P treatments are similar to the Control; however, the N+P treatment exhibits much greater growth than the control. In this case, both N and P are simultaneously playing a role in limiting algal growth.
4. **Light Limitation** – The final average IVF values of all treatments are similar to the control. Evidently some factor other than N or P is limiting algal growth. In the Bay that factor is most likely to be light.
5. **Primary P Limitation** – The P treatment is somewhat larger than the Control but not as great as the N+P treatment. Phosphorus is the primary limiting factor for this outcome but other factors may also play a role in controlling algal growth.
6. **Exclusive P Limitation** – The P treatment and the N+P treatment are nearly identical and both are much greater than the Control, while the N treatment is similar to the Control. Phosphorus is the only limiting factor for this outcome.

**YOUR JOB:**

1. **Express each of your final average treatment IVF values as a percentage of the final average Control IVF value.** (Refer back to the first paragraph in this “Interpreting Nutrient Limitation Results” section for how to do calculations.)
2. **Compare your percentages to the model outcomes just described and determine which model best fits your data.**
3. **From this comparison, state the nutrient or factor limiting algal growth in your bioassay experiment.**

Citations:

Fisher, T.R. and A.B. Gustafson. 2002. Nutrient-Addition Bioassays in Chesapeake Bay to Assess Resources Limiting Algal Growth. Maryland DNR 2002 Report. Available at: [http://www.dnr.state.md.us/bay/monitoring/limit/2002\\_level1\\_report.pdf](http://www.dnr.state.md.us/bay/monitoring/limit/2002_level1_report.pdf)