Chlorophyll *a* Lab

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Overview: Measurements of the concentration of photosynthetic pigments of algae and cyanobacteria can be used to estimate the composite biomass of phytoplankton populations. Although pigment concentrations generally are correlated to the biomass of phytoplankton, pigment concentrations of algae can vary widely depending on metabolism, light, temperature, nutrient availability, and many other factors. Plant pigments of algae and cyanobacteria consist of the chlorophylls and carotenoids. The three major chlorophylls, *a*, *b*, and *c*, absorb light maximally at specific wavelengths when dissolved in organic solvents. From these absorption characteristics an estimate can be made of the concentrations of pigments. Chlorophyll a is by far the most dominant chlorophyll pigment and occurs in greatest abundance. Thus, often chlorophyll *a* alone is used to estimate algal biomass. A Fluorimeter can be used to estimate chlorophyll but requires fluorescence to be measured both prior to and following acidification in order to correct for degradation products of chlorophyll that may be present.

The mini-lecture (Chl_Lab_MiniLecture), available on Canvas, is also an informative reference to help you complete this lab.

Summary: Because the extraction process takes 24 hours, we have already placed the filters you collected in Horsetooth Reservoir in acetone the day before the lab. Briefly, you will need to find and measure your sample before and after the addition of HCl. Once you have taken your first measurements and are satisfied with it add 2-3 drop of HCL to your sample. Wait 30 minutes and then repeat your measurement. Then use the worksheet below to calculate Chla values for your Horsetooth sample.

Procedure:

1. Obtain one of your group's glass-fiber GFF filters from the Horsetooth Reservoir trip, that has been sitting in acetone for the past 24 hours.
2. Fill a cuvette with distilled water (a blank) and obtain a measurement for it from the fluorometer. Use the following procedure for using the fluorometer, for this step and subsequent steps:
    a. Read each cuvette twice, giving the cuvette a slight turn between readings.
    b. If difference between readings is more than 10 percent, repeat reading and keep two most similar readings.
3. Fill a cuvette with your group's chlorophyll sample and obtain measurements for it from the fluorometer. Enter them in the table below (#1). Repeat for your second filter.

4. After the first measurement, add 2-3 drops of HCl to both samples. Wait 30 minutes for the acidification to take effect.
5. Fill another cuvette with your group's sample and obtain measurements for it from the fluorometer. Enter them in the table below (#2)
6. Repeat steps 1 to 5 for your group's other GFF filter. Fill in the rest of the table below.

Results:

Your group number: 6

Chlorophyll *a* Calculations

#	Variable	Units	Results for your group's first filter	Results for your group's second filter
1	Before acidification value (H_B) from fluorometer	μgL^{-1}	9.90	
2	After acidification value (H_A) from fluorometer	μgL^{-1}	6.91	
3	Volume of acetone used in the extraction ($V_{Acetone}$):	ml	10	10
4	Volume of water filtered (V_{water}):	ml	100	
5	V_{water} / $V_{Acetone}$ (aka Concentration Factor)		100/10 10	
6	H_B / Concentration Factor *[This gives you combined phaeophytin and chl a]*	μgL^{-1}	9.90/10 .99	
7	(H_B - H_A)/ Concentration Factor *[This gives you just chl a]*	μgL^{-1}	9.90 – 6.91 = 2.99	

Calculate the following:
1. Mean of chlorophyll *a* of your two filters: ___NA_____
2. Standard deviation of chlorophyll *a* of your two filters: 0

Questions

1. **Was your standard deviation high or low? If it was high, why do you think your samples were dissimilar?**

We couldn't successfully calculate a standard deviation because we only have one filter to take measurements from.

2. **One of the hardest things about collecting samples in the field (especially for the first time) is understanding why specific protocols are necessary and which details matter and why. Now having measured chlorophyll a in the lab, is there anything you would have done differently when collecting your samples?**
   First- we would have taken more than just the singular filter sample, that way we could complete the analysis. Additionally, we could label our specimens with more information as to make our data noticeably clear for analysis. Additionally, taking more time and precaution through the entire process would allow us to create a more polished dataset with easier interpretation. We believe that the best way to accomplish these protocols for future sampling is with better preparation.

To Be Completed At Home

We have posted results from the Lake Yojoa nutrient enrichment bioassay from January on Canvas. Please use that data set to answer the following questions.

1. **What was the average chlorophyll content for the control bottles? Nitrogen addition? Phosphorus? Nitrogen and Phosphorus? What is the standard error of each? Results may be displayed on a table.**

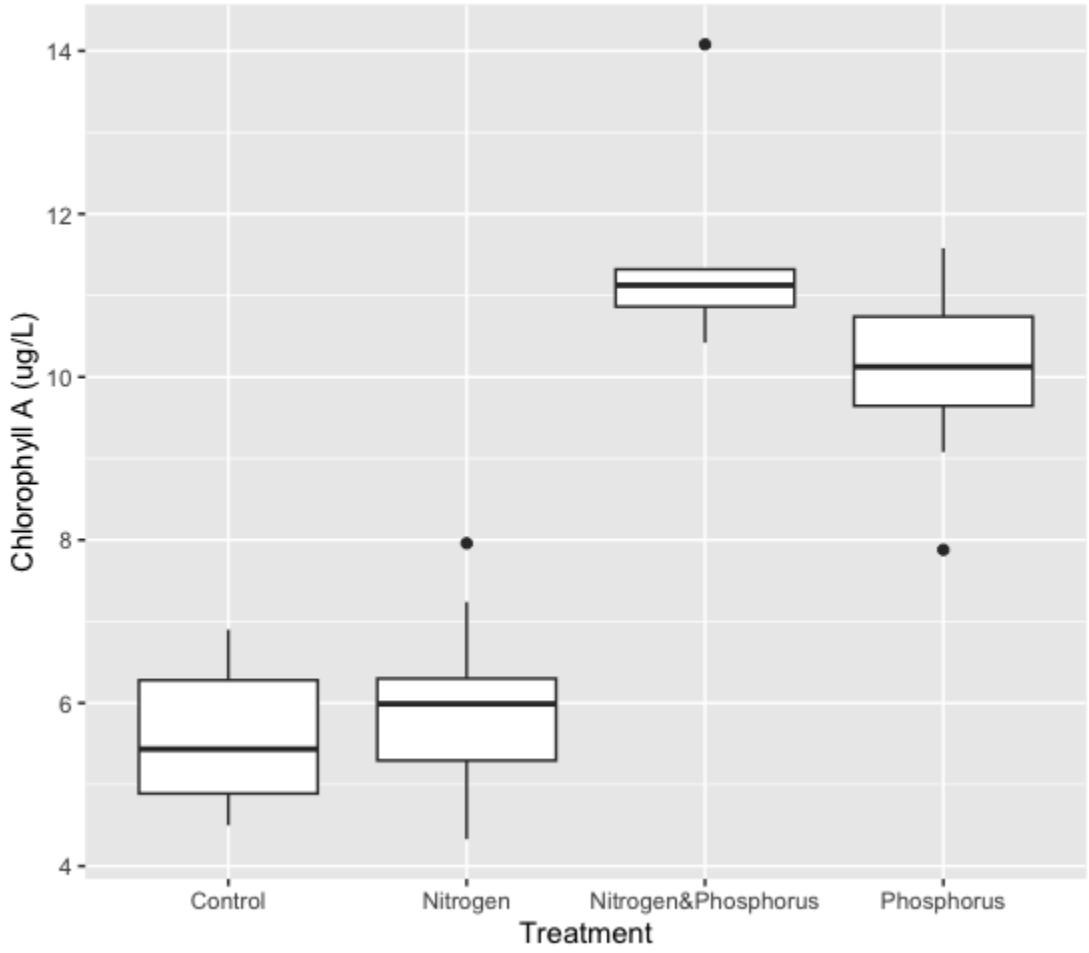
ug/L	Control	Nitrogen (N)	Phosphorus (P)	N & P
Mean	5.59	5.96	10.1	11.5
Std. Dev.	0.947	1.08	1	1.32
Std. Error	0.387	0.342	0.29	0.537
95% UCL	6.35	6.63	10.6	12.5
95% LCL	4.83	5.29	9.5	10.4

2. **Based on your calculations, what appears to be the primary source of limitation in Lake Yojoa in January: Nitrogen, Phosphorus or Co-limitation?**
   The primary limiting reagent in Lake Yojoa appears to be Phosphorus. While nitrogen contributes towards an increase in Chlorophyll-a concentration, it is minimal, with a mean concentration of 5.96 ug/L compared to the control test's 5.59 ug/L ($p=0.489$). Phosphorus has a mean concentration of 10.07 ug/L, an increase of 4.47 ug/L to the control ($p=2.057*10^{-6}$), and rejecting the null hypothesis that the relationship is unrelated. While Nitrogen and Phosphorus do show a considerable increase in Chlorophyll-a concentration (11.5 ug/L), it can be inferred that Phosphorus is the more

limiting reagent of the two because it is the more limiting reagent to chlorophyll-a concentration when examined on an individual basis.

3. **What are other potential sources of limitation not accounted for in this experiment?** Temperature, light availability/time of day, carbon dioxide concentration

4. **Produce one figure or statistical test of your choice that you feel best supports your conclusions in question 2 (i.e. bar chat with error bars, box plots, ANOVA etc.)**



N vs. P

t.test(NChl_a_ugL,PChl_a_ugL)

Welch Two Sample t-test

data: N$Chl_a_ugL and P$Chl_a_ugL

t = -9.1856, df = 18.658, **p-value = 2.379e-08**

N vs. Control

t.test(N$Chl_a_ugL,Ctrl$Chl_a_ugL)

Welch Two Sample t-test

data: N$Chl_a_ugL and Ctrl$Chl_a_ugL

t = 0.71417, df = 11.829, **p-value = 0.489**

P vs. Control

t.test(P$Chl_a_ugL,Ctrl$Chl_a_ugL)

Welch Two Sample t-test

data: P$Chl_a_ugL and Ctrl$Chl_a_ugL

t = 9.2684, df = 10.61, **p-value = 2.057e-06**