Klamath Connection Background Reading

Learning Objectives: Gain background knowledge on algae affecting the Klamath River and how math modeling can help us test a hypothesis about algae growth.

Algae blooms in rivers have become an increasing concern. Human activity, like agriculture, in the watersheds affect algae blooms. Some algal species that bloom produce toxins like microcystins, which pose a threat to humans, pets, and fish. These public health concerns have led scientist to monitor the algal abundance and compare the environmental conditions in the river (e.g. temperature, nutrients) to the abundance of cyanobacteria and toxin levels.

The Klamath River is monitored for toxic algae. Dangerous levels of algae (specifically, a type of cyanobacterium called *Microcystis aeruginosa*) have been documented in the Klamath River by water quality scientists since 2005. Abundance of algae in riverine habitats can be controlled by anything that affects their ability to photosynthesize, such as light, nutrients, temperature, pH, and flow of water.

Klamath Connection students perform an experiment in summer immersion to address the question: **Is nitrogen a limiting factor of algae growth in the Klamath River**?

"Limiting nutrient" is a technical term that is used for a nutrient whose absence prevents organisms from growing or reproducing. Thus, introducing a "limiting nutrient" results in increased growth or proliferation. The limiting nutrient for many freshwater systems is phosphorous (P), but water quality monitoring data suggest nitrogen (N) is limiting in the Klamath (Committee on Endangered and Threatened Fishes in the Klamath River Basin, National Research Council, 2004, Moissander et al. 2009, Kann and Corum 2008). The Klamath Connection experiment specifically focuses on the growth of cyanobacteria, the toxic algae known to be present in the system.

Students sample water from the Klamath River at Terwer. Half of each water sample is treated with nitrogen, the other half is treated with sterile water. The samples are stored in the HSU experimental greenhouse for seven days, then analyzed for algal growth by comparing the "relative fluorescence units" (RFUs) of the samples before and after incubation. RFUs measure the relative amount of two compounds present in algae, Chlorophyll a and phycocyanin. The more algae present, the more light is going to reflect off the sample, and the higher the RFU. Chlorophyll a is a pigment found in nearly all photosynthetic life, therefore presence of Chorophyll a (Chl a) does not conclusively show presence of toxic algae. However, if there is a greater amount of algae present, we do expect a larger quantity of Chl a, and higher RFU measurements. Phycocyanin is a pigment found in cyanobacteria so it is a more accurate proxy of the amount of toxic algae present. Larger RFUs for phycocyanin indicate a greater number of cyanobacteria (our algae of interest).

The following hypotheses is tested.

Hypotheses

H_1: Addition of N will increase algal growth. *If N is limiting, then we predict that RFUs will increase more in flasks with added N than in control flasks.*

H_0: Addition of N will not result in increased algal growth. *If N is not limiting, then we predict that RFUs will be as high or higher in controls than in flasks with added N.*

In math class we test this hypotheses, by performing a simple linear curve fitting exercise with the average amounts of Chl a and phycocyanin for treated (nitrogen added) and untreated samples measured in RFUs. We expect that samples treated with nitrogen will have higher RFUs at day 7 then untreated samples and a higher rate of change of RFUs/time. Here is an example of a graph generated by past Math 101 students.

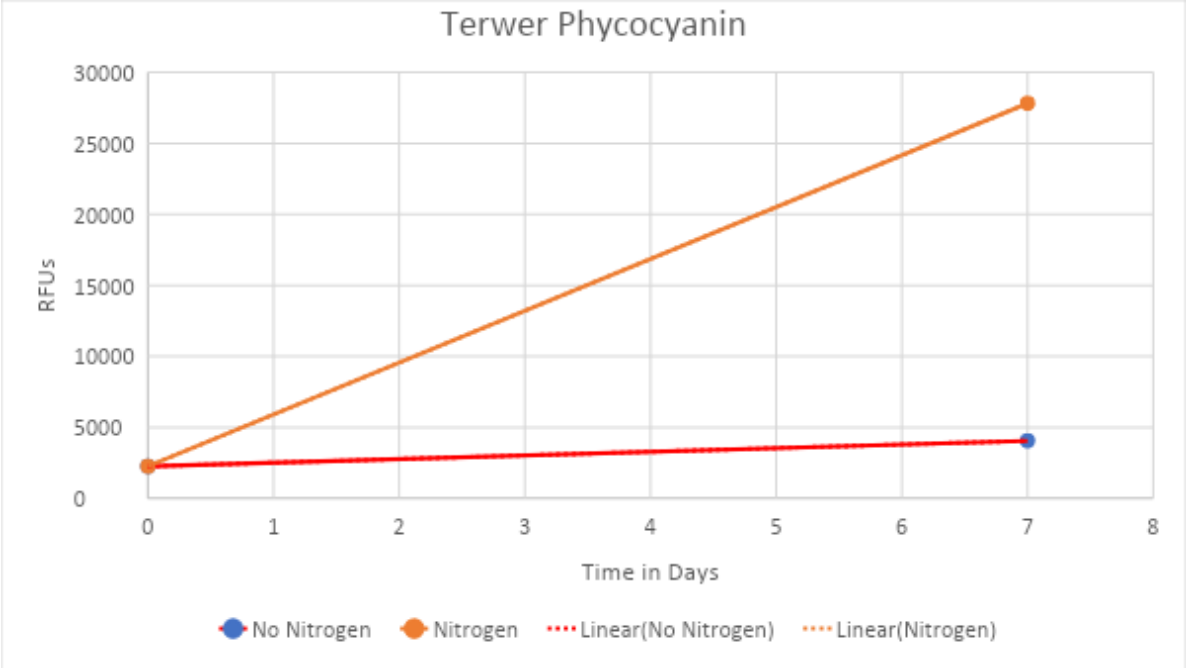


Figure 1: Amount of phycocyanin (pigment found in toxic algae) measured in RFUs found at day 0 and day 7 for samples treated with nitrogen and not treated with nitrogen. Water samples were collect at Terwer along the Klamath River.

Goals of the Lab: This lab will (1) introduce you to Excel; (2) give you quantitative tools to analyze data collected by Klamath Connection students; (3) connect mathematical concepts about linear models to scientific experiments.

Directions:

You will work **alone** to complete the following lab. You will need to create a typed and professional lab report in Microsoft Word (the requirements are described below), saved as a pdf and upload it to Canvas. You must clearly label each question in your lab, clearly put titles on the different lab sections, and answer all questions in complete sentences. All graphs should have titles, axes labeled, and equations.

Comparing florescence data for water samples treated with Nitrogen and not treated with Nitrogen

Clearly label this part of your lab report. Comparing Klamath florescence data.

READ THE BACKGROUND READING BEFORE ATTEMPTING THIS LAB.

Open the file "Klamath Data-linear.xlsx." (It is posted in Canvas). Once you open the data file in MS Excel, save it to your google drive as: **your.name.labKC.part2** for later use (You can also save it to a thumb drive, or email it to yourself).

This file contains the mean florescence data set from time zero and a subsequent mean florescence data set that was collected 7 days later measured in RFUs. This data was collected by Klamath Connection students at HSU. Recall, that Chl a is a pigment found in all algae and phycocyanin is found in toxic algae.

Step 1 – First, look at the data and calculate some numbers by hand.

*Answer the following questions (**in complete sentences**) and type your answers in your lab report:*

Question 1: You are going to use math to help answer a scientific question. What scientific question are you trying to address by analyzing the Klamath numbers?

Question 2: What is your hypothesis for this experiment?

Question 3: Refer to the Excel spread sheet containing the florescence data and calculate by hand the average rate of change of Chl A and phycocyanin measured in RFUs for samples treated with nitrogen and those not treated with nitrogen at Terwer. Type your answer in your lab write up using correct units.

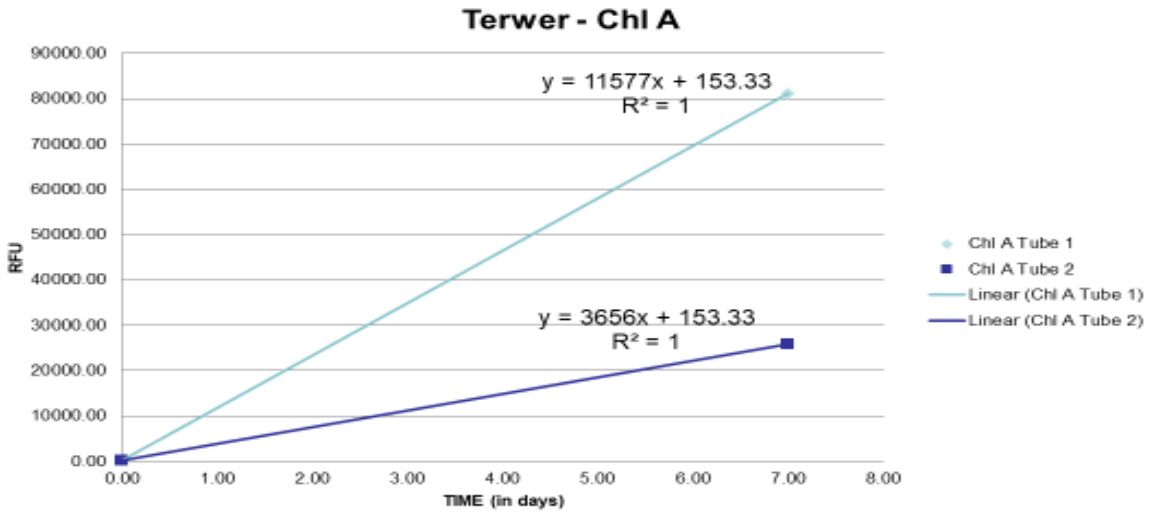
Question 4: How does the average rate of change of chl a and phycocyanin compare for samples treated with nitrogen versus samples not treated with nitrogen (Answer to question 3)? Why do you think this is true?

Step 2- Now turn to the computer! Make a graph of the data from Terwer only. You should have two graphs one for **Chl A** and one for **phycocyanin** (you should have both treated and untreated samples on the same graph for comparison. Include a chart title on your graphs and label both axes. Time in days on the x-axis and RFUs on the y-axis.

Step 3-Fit a linear regression line through the points on each graph**.** Click on add chart elements>trendline> more trendline options. Make sure to check "linear" and to check "display equation on chart." Also display the R^2-value on your graph. An example from last year for Chl A is provided on the next page. **Paste both of your graphs** in your lab report. Your graphs must be large enough to read.

The graph below is an example of what your graph should look like. **Your graph will be different**. Use your own graph to answer the questions.

Terwer-Chl A



Question 5: Type the linear equation you found in Excel which predicts the amount of phycocyanin from the treated (nitrogen added) sample as a function of time (t). Write your equation using function notation, p(*t*), phycocyanin as a function of time. What is the independent (input) variable and what is the dependent (output) variable in this model?

Question 6: What are the units of the slope in this equation? What does slope represent in practical terms? Use your numbers from question 5 to describe the change. Answer in a complete sentence.

Question 7: How much phycocyanin measured in RFUs would you predict from the sample using your model from question 5 at time 10 days? 100 days?

Question 8: Do you have any concerns about the predictions from question 7? Is it realistic? Explain your reasoning in a complete sentence.

Question 9: Consider again the slope for the Chl A and phycocyanin data and graphs. Compare the slope of the lines for the treated and untreated samples. Which is larger? Why?

Question 10: After looking at all of your graphs and linear regression lines, which line has the steepest slope? Why? What conclusion can you draw from this information?

Question 11: How could this experiment be improved? What limitations do you see?

Step 4- To better address the scientific question an expanded data set should be considered. Open sheet 2 of the Excel file containing the Klamath data. This data contains mean florescence from 4 points in time. Make a graph of the data from Terwer for phycocyanin for both treated and untreated samples. Fit a linear regression line to both treated and untreated samples. Paste your labeled graph in your lab write up. Include both equations and R^2 values.

Question 12: How well does a linear model fit this data?

Question 13: Looking at all the average rates of change and graphs that you have created do you have evidence to support your hypothesis? Do you think your results are valid? With this experiment can you conclude that nitrogen is a limiting nutrient for algae growth in the Klamath River? Recall, introducing a "limiting nutrient" results in increased growth or proliferation.