Supplemental Material 1: Water Quality Laboratory
These activities were performed in the Klamath Connection Summer Immersion, Critical Thinking course and First-year Seminar.

Studying Algal Blooms of the Klamath River

Background

River ecosystems are like other aquatic and terrestrial ecosystems in that rivers also contain primary producers (i.e. plants and algae that use photosynthesis to convert light energy into chemical bond energy that is stored in carbon molecules), secondary and tertiary consumers (i.e. herbivores, predators, omnivores) and decomposers (i.e. non-photosynthetic bacteria, fungi). In systems like the Klamath River, most of the primary producers are algae growing either in the water column or attached to shallow bottoms where there is light. These algae include cyanobacteria (blue green algae) as well as other algal groups, particularly green algae and diatoms. Cyanobacteria and green algae can be uni-, multicellular or colonial whereas diatoms, which contain gold pigments within their silica quartz cell covering, are unicellular or colonial. Abundance of algae in these riverine habitats can be controlled by anything that affects their ability to photosynthesize, such as light, nutrients, temperature, pH and flow of water. Relative abundance of these resources is affected by both natural factors and human activities and can change dramatically. When conditions result in rapid growth without depletion, large algal blooms result.

Blooms that degrade water quality and negatively affect organisms are called harmful algal blooms (HABs). Some algal species that bloom also produce toxins like microcystins. There are several forms of this molecule that are produced by at least six species of cyanobacteria, the most well-known of which is *Microcystis aeruginosa*. Microcystins can be absorbed by fish, and they are known to have killed pets and livestock by destroying the liver. Humans ingesting large quantities of contaminated water are also affected, with problems ranging from skin irritation, gastrointestinal upset and death from liver or respiratory failure (Carmichael 1981, Chorus and Bartram 1999, Chorus 2001).

Microcystis aeruginosa in the Klamath River has been monitored since September 2004, when the first documented blooms appeared in Copco and Iron Gate reservoirs. The reported levels of microcystin toxin have routinely resulted in health advisories that limit the recreational activities in the reservoirs and some stretches of the Klamath River in late summer. These river closures are particularly impactful to the native people of the basin. Direct river contact is an integral part of Karuk, Yurok and Hoopa cultural practices such as fishing and gathering plants as traditional foods, basketry materials, and for ceremonial and regalia purposes. Over 85% of the fish and freshwater mussel tissues sampled during a 2007 microcysis bloom contained microcystin levels that exceeded the daily intake limits issued by the World Health Organization (Kann 2008), prohibiting consumption of these traditional foods. Throughout the year, bathing in the Klamath River is an important part of religious ceremonies, funeral services, subsistence practices, recreational swimming, courtship, and individual hygiene. Ceremonial leaders have begun to modify some traditional practices during late summer to avoid bathing in and drinking the water. (Bureau of Reclamation 2011).

These public health concerns have led scientists to compare the environmental conditions in the river (e.g. temperature, nutrients) to the abundance of cyanobacteria and toxin levels, and to

complete manipulative experiments designed to identify what is causing the blooms. These studies use a variety of methods for quantifying algal abundance, one of which is to measure the amount of light emitted by the photosynthetic pigments used by these organisms to convert light to energy: chlorophyll *a* (Chl a) and phycocyanin. This emitted light, called fluorescence, is an index of algal abundance: more fluorescence should result from more Chl a, which should result from more algal cells. The water quality monitoring data from these studies suggest that although the limiting nutrient is phosphorus (P) for many freshwater systems, it is likely nitrogen (N) in the Klamath Basin (Committee on Endangered and Threatened Fishes in the Klamath River Basin, National Research Council, 2004, Moissander et al. 2009, Kann and Corum 2008).

Project Summary

We are now approaching late summer, the time when dangerous levels of *Microcystis aeruginosa* have been documented in the Klamath River by water quality scientists since 2005. Through a series of activities that begin during your Klamath Connection Summer Immersion experience and continue through your fall semester courses, you will determine if *Microcystis aeruginosa* and other algal cells are currently present in the lower Klamath River.

On either Monday or Tuesday of the Summer Immersion, you will sample water from the lower Klamath River using the best practices of water quality scientists. When you return to campus, you will examine the water samples for presence of algal cells with a microscope and treat the water with a nitrogen solution, which will allow you to test the hypothesis that nitrogen is the limiting factor of algal growth in the Klamath River. During the first week of your classes, you will evaluate your cultures for presence of algal growth through visual observation. In addition, you will measure the relative fluorescence of your cultures to determine the relative abundance of Chl a and phyocyanin producing cells in the culture.

Through a series of assignments, presentations, and discussions that will occur in many of your first-semester courses, you will train in the scientific processes and procedures necessary to characterize and monitor algal species and study how harmful algal blooms impact the water quality, environmental, and cultural issues of the Klamath Basin.

All the activities associated with this project are outlined in Table 1 and described on pages 5-6. The documents for the activities necessary for data collection in SCI 100 and ERE 280 are included in this document. Additional materials will be provided in your classes.

Table 1: Outline of Activities

Activity[1]	**Where**	**When**
Water Collection	Terwer River Bar, Klamath River	Summer Immersion Monday 8/12: ESM; FOR; ERE Tuesday 8/13: BIO, BOT, FISH, WLDF, ZOOL
Experiment	Botany Laboratories SCI D 141, 147, 151, 157	Summer Immersion (Wednesday 8/15) 9:00: BIO, BOT, FISH, WLDF, ZOOL 1:00: ESM; FOR; ERE
Data Collection	Experimental Greenhouse CNRS Core Facility	Assigned times outside of class 8/20-8/24
Data Analysis	FOR 100: Critical Thinking In Social and Environmental Responsibility ERE 280: Introductory Engineering BOT 105: Introduction to Botany MATH 101	See Syllabus for each course.
Context and Interpretation	FOR 100: Becoming a STEM Professional in the 21st Century , ERE 280: Introductory Engineering, CHEM 109: Introduction to Chemistry,	See Syllabus for each course.

Description of Activities

I. Water Collection – Monday or Tuesday of Summer Immersion (depending on your major)
Klamath Connection students will work in pairs to collect 45 mL of water at the Terwer River Bar on the Klamath River. *Please read page 7 of this document before you perform this activity.*

A. Students will receive an introduction to the experiment, sampling and labeling
B. Each group of students (2/group) will sample water at the Terwer River Bar
    a. Students will fill one labeled sterile 50 mL conical tube with approximately 45 mL of water. The tubes will be labeled with the location, date, and the group initials.
    b. The conical tubes will be stored in coolers and returned to HSU.

II. Experimental Set Up – Wednesday of Summer Immersion
The primary goal of this activity is to test the hypothesis that nitrogen (N) is the limiting factor in algal growth in the Klamath River. Additional objectives are to help students better understand some of the background information that informs this experiment and be introduced to the laboratory skills and procedures required to execute it. *Please read pages 8-11of this document in preparation*.

A. Each student group of 2-3 students will set up the experiment.
B. Klamath water samples will receive either nitrogen in the form of ammonium nitrate (NH_4NO_3) or Ultrapure water.
C. Cultures will incubate in the HSU Experimental Greenhouse for 6-7 days.

III. Data Collection – First week of classes

The primary goal of this activity is to collect data from the experiment begun on Wednesday, 8/15. Additional objectives include an introduction to relevant laboratory skills and techniques. *Please read pages 13-17 of this handout in preparation.*

A. Students will have a brief laboratory lecture in either SCI 100 or ERE 280.
B. Groups will meet Professor Sprowles and their SCI 100 break-out instructor at the HSU Experimental greenhouse at a predetermined time outside of your normal class sessions.
C. Students will observe cultures for color and clarity. Observations will be written in notebook.
D. Students will transfer 1 mL of each culture to a 48 well microplate and measure the amount of fluorescence in 1mL of each culture using the Spectramax© i6 spectrophotometer in the CNRS Core facility.

IV. Data Analysis

The goal of these activities is to analyze the data collected to see if the hypothesis that nitrogen is the limiting factor to algal growth is supported by the data collected. In addition, these activities will help students develop competency and comfort in working with numerical data. That analyses will be performed in different courses will illustrate that examination of scientific questions relies on integration of information from multiple individual disciplines.

Students will perform an initial analysis of the fluorescence data in their FOR 100 or ERE 280 courses. *The documents for this activity begin on page 18 of this handout.*

In addition, the cultures will be examined for presence of algal species in the BOT 105 'Introduction to Microscopy' lab week 1 of the semester and an expanded fluorescence data set will be used to calculate change in fluorescence over time in math courses. *The documents for these activities will be provided by your course instructors.*

V. Context, and Interpretation

The primary goal of these activities is to systematically analyze the overall issue of harmful algal blooms of the Klamath River through the collection and analysis of evidence and examples that results in informed conclusions or judgments. The hope is that participation in these activities will 1.) assist students in making simple connections among ideas and experiences that can be synthsized and transferred to new, complex situations and 2.) illustrate how scientists engage in activities of personal and public concern that are both individually life-enriching and socially beneficial to communities. The topic will be visited in many Klamath Connection courses. A few are highlighted below. *Documents for these activities will be provided by your course instructors.*

A. Guest lectures from Karuk Department of Natural Resources
B. Lectures by the instructors of the science, math and engineering courses participating in this activity.
C. Discussions of the importance of the water quality to the Yurok, Karuk, and Hoopa people of the Klamath Basin in Native American Studies courses

Materials for Summer Immersion Activities

Protocol I: Water Sampling
Monday or Tuesday of Summer Immersion

This activity will occur on the banks of the lower Klamath River. Your group leader will guide you through the sampling process outlined below. Note: it is extremely important that the water sample does not touch anything other than the sterile conical tube.

Materials:
1 sterile 50 mL conical tube with screw cap/group of two students
Sharpie or other Laboratory marker
Cooler with ice
Rite in the Rain notebook
Pencil

Objective:
To see if addition of nitrogen to water sampled from the lower Klamath River at the Terwer River bar will increase algal growth.

Protocol

1. Listen to instructor's directions.
2. Begin an entry for this experiment in your Rite in the Rain notebook. The information should include:
    a. The date
    b. The experimental objective
3. Use the marker to clearly label your tubes with the following information:
    a. Location (Terwer River Bar)
    b. Date
    c. Initials of both Group Members
    d. FYS Abbreviation (ZOOL, BOT, BIO, FISH, ESM, FOR, WLDF or ERE)
    e. An identifying symbol that will allow you to quickly recognize your tube
4. Record the labeling information in your Rite in the Rain notebook.
5. Walk to the sampling area
6. Find a location that will allow you to fill the conical tubes with water from the middle of the water column—not the surface of the water, not the very bottom. If you feel safe, you may remove your shoes and wade out to an appropriate depth (not past your knees).
7. Observe the water. Do you notice any algae there? Other objects on or in the water? Is it clear? Is it turbid? Estimate the depth of the water. Record any observations in your notebook.
8. Working together, carefully remove the screw cap of the conical tube. One group member should hold the lid, taking care NOT to touch the inside.
9. Plunge the open conical tube down at least 4 inches from the surface and allow the tube to fill to the 45 mL mark.
10. Replace the lid and tightly secure.
11. Put the conical tubes in the cooler of ice.

Protocol II: Testing Klamath Water for the Presence of Algal species
Wednesday of Summer Immersion

The samples you collected will be evaluated for the presence of various algal species of the Klamath using two different measures, one visual and one chemical.

1. Visual identification of Primary Producer Species of the Klamath River

In this activity, you will examine water samples under a microscope in order to identify some of the primary producers (photosynthetic organisms) of the Klamath River. You will be walked through this exercise with a laboratory instructor. The images in Figure 1, below, are a guide to help you identify some of the prominent species.

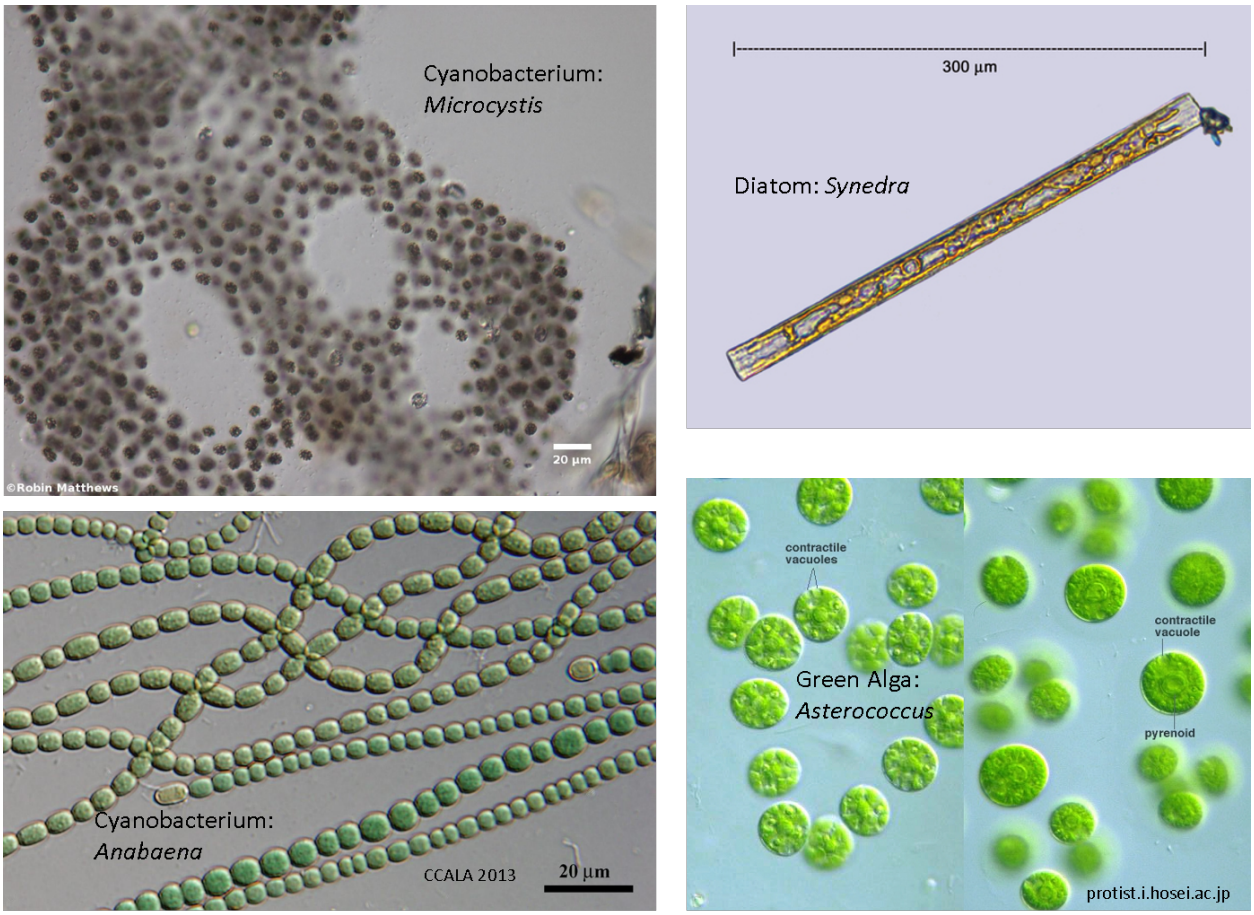

Figure 1: Primary producers common to the Klamath River

2. Analysis of the Role of Nitrogen in Algal Growth in the Klamath River

Scientists interested in understanding the environmental conditions that lead to algal blooms of the Klamath River have hypothesized that:

Hypothesis: Nitrogen is the limiting reagent for growth in the Klamath.

This means that although the algae will grow and survive under many conditions, there is normally not enough nitrogen to support the rapid growth necessary to produce an algal bloom.

Scientists use the hypothetico-deductive method (H-D method) to test hypotheses by deducing predictions that can be examined experimentally:
If N is limiting, then we predict that RFUs will increase more in samples with added N than in control samples.
If N is not limiting, then we predict that RFUs will be as high or higher in controls than in samples with added N.

Finally, to mathematically test the data in this experiment, we seek to confirm or falsify the statistical null (H_0) and alternate (H_1) hypotheses.

H_1: *RFUs are higher in samples with added N than in control samples*

H_0: *RFUs are as high or higher in controls than in samples with added N*

This exercise will allow you to test these hypotheses.

Materials

Laboratory Marker

Your Klamath water sample taken at the Terwer River Bar

1 25 mL graduated cylinder

2 mL serological pipettes

1 manual serological pipette controller

5 mM stock solution of ammonium nitrate (NH_4NO_3)

ultrapure water (H_2O).

Two clean, sterile, 50 mL conical Tubes

Trays to collect used serological pipettes

Styrofoam conical tube rack

Objective:

To see if the addition of nitrogen will increase algal growth in the water sampled from the Terwer River Bar on the Klamath River.

Both Conical Tubes will receive 19 mL of Klamath River Water

Conical Tube #1 will receive 1.0 mL of 5.0 mM ammonium nitrite (NH_4NO_3) for a final concentration of 250μM NH_4NO_3

Conical Tube #2 will receive 1.0 mL of ultrapure water. No additional nitrogen.

Safety Information:

Always wear close-toed shoes in the laboratory.

Do not touch a broken serological pipette. Inform your instructor so it can be disposed of properly.

Ammonium nitrate (NH_4NO_3) is a skin and eye irritant.

In case of skin contact, wash off with soap and plenty of water.

In case of eye contact, flush eyes with water as a precaution.

Protocol

1. Obtain two new, sterile 50 ml conical tubes
2. Label the tubes using the laboratory marker with the following information:
    a. Information for both labels should include
        ii. Conical Tube # (1 or 2)
        iii. Location of the Klamath River water sample (Terwer River Bar)
        iv. Date
        v. Initials of both Group Members
        vi. FYS Abbreviation (ZOOL, BIO, BOT, FISH, ESM, FOR, WDLF or ERE)
        vii. An identifying symbol that will allow you to quickly recognize your tube
    b. The water in Conical Tube #1 will receive nitrogen in the form of ammonium nitrate (NH_4NO_3). Therefore, include + NH_4NO_3 on this label.
    c. The water I Conical Tube #2 will receive an equal volume of ultra pure water. Therefore, include +H_20 on this label.

2. Use Table 1 to determine each solution you will add to the conical tube to create the appropriate experimental condition.

Table 1: Volumes of each component required for each conical tube.

Conical Tube #	Amt of Klamath Water (mls)	Amount of 5 mM NH_4NO_3 (mls)	Amount of ultrapure water (mls)	Final Volume (mls)
1	19	1	0	20
2	19	0	1	20

3. Remove the cap from Conical tube #1 and place it upside down on your laboratory bench, out of the immediate workspace.

4. Use your 2 mL serological pipette to transfer 1.0 mL of 5 mM NH_4NO_3 stock solution to conical tube #1.

When you are through with the serological pipette, put it directly into the dirty serological pipette holder.

5. Measure 19 mL of the Klamath water sample from your sample collection conical tube with the 25 mL graduated cylinder.

6. Add the 19 mL of water into Conical Tube #1. Do your best not to let the graduated cylinder touch the conical tube. Also make certain the water does not splash onto the neck. This will reduce the possibility of contamination to your culture.

7. Return the cap to Conical Tube #1.

8. Remove the cap from Conical tube #2 and place it upside down on your laboratory bench, out of the immediate workspace.

9. Use your 2 mL serological pipette to transfer 1 mL of ultrapure H_2O to Conical Tube #2.

   When you are through with the serological pipette, put it directly into the dirty serological pipette holder.

10. Measure 19 mL of the Klamath water sample from your sample collection conical tube with the 25 ml graduated cylinder.

11. Add the 19 mL of water into Conical Tube #2. Do your best not to let the graduated cylinder touch the conical tube. Also make certain the water does not splash onto the neck. This will reduce the possibility of contamination to your culture.

12. Place the cap back onto Conical Tube #2. Secure the lid but keep it loose to allow for airflow.

13. Gently swirl the contents of each conical tube for about 1 minute so they are mixed.

14. Place your tubes in the Styrofoam rack at the front of the classroom. It should be labeled with your major abbreviation.

15. Inform your laboratory instructor you are done.

16. When instructed, use two hands to carry the conical tubes out of the classroom and to the experimental greenhouse. Place where instructed.

17. Your group will be assigned a time to meet back at the Experimental Greenhouse to collect your data before this laboratory session ends. This time should be entered into your Google Calendar

18. Prepare for your next laboratory session by answering the questions below and reading the Data Collection materials that begin on pg. 14 of this packet before arriving at the HSU Experimental Greenhouse at the time assigned for ERE 280 or your Wednesday Sci 100 class.

Answer the Following Questions:

1. Why do we call this a eutrophication experiment?

2. Why do you think the addition of nitrogen increases algal growth?

3. Why is it important to include a control sample with water only in this experiment?

4. Why is it important to add the same volume of water as the ammonium nitrate?

III. Fluorescent Data Collection

Background

As described in the background section of this document, public health concerns surrounding algal blooms of the Klamath River have led scientists to complete manipulative experiments designed to identify what is causing the blooms. These studies use a variety of methods for quantifying algal abundance. When scientists are interested in understanding the abundance of algal cells in vivo (in a natural setting or living system) they use fluorescence to determine the relative amount of pigments associated with the algal cells of interest. The absorption spectra for some of these pigments is shown in Figure 2. One of the most common measurements involves the pigment chlorophyll *a* (Chl a), which is found in nearly all photosynthetic life. Cyanobacteria also use three additional pigments, phycocyanin, allophycocyanin and phycoerythrin. Scientists trying to estimate relative cyanobacteria concentration in river systems typically have a meter able to measure fluorescence values for Chl a, which is taken after excitation (between 430-470 nanometers) at an emission wavelength in the red spectra (650-700 nm). They also can measure phycocyanin fluorescence at the emission wavelength of 670 nm after excitation at 590 nm (Hambrook et al. 2007). The amount of fluorescence is an approximation of algal abundance; more fluorescence should result from more Chl a and phycocyanin, which should result from more algal cells.

For our experiment, we will use a different instrument that is able to activate fluorescence in living cells. The Spectramax© i6 (Molecular Devices, Sunnyvale CA) can record fluorescence from a microplate (Fig. 3). These are flat plates with multiple wells that allow you to assay multiple samples at a time in very small volumes. This is a state-of-the-art instrument that has only been at HSU since 2015. It is housed in the CNRS Core Facility, which supports research projects for students, faculty and staff.

If you are interested in learning more about how absorption of light leads to fluorescence, visit the optical mocrosopy primer hosted by Molecular Expressions at: http://micro.magnet.fsu.edu/primer/java/fluorescence/exciteemit.

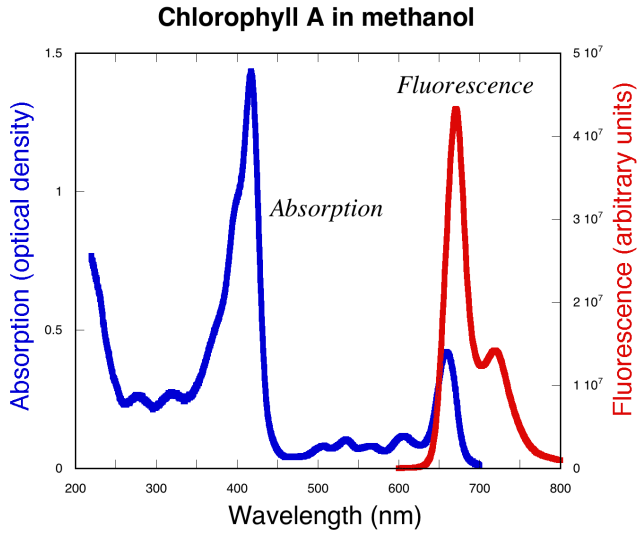

Figure 2: Absorption and Flourescence Spectra for Chlorophyll A. Note the same principle applies for phycocyanin, but that pigment has different absorption and fluorescence wavelengths. http://www.lakescientist.com/wp-content/uploads/2012/03/chlorophyll-292x300.jpg

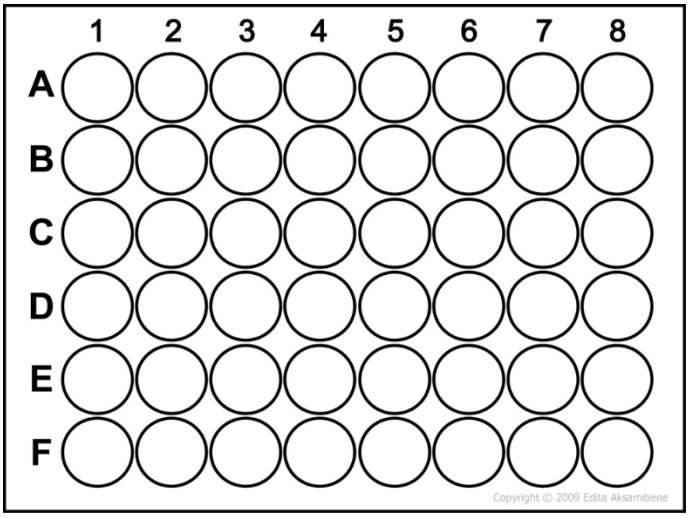

Figure 3: Diagram of a 48 well Microplate. You will use one of these to collect your data. The microplate has rows labeled with letters and columns labeled with numbers. http://www.cellsignet.com/media/plates/48.jpg

Materials

Rite in the Rain notebook

Two serological pipettes

1 manual serological pipette controller

1 48 well clear bottom microplate

1 map of a 48 well clear bottom microplate

Spectramax© i6

Objective: To collect data, the eutrophication experiment is designed to test proposed hypothesis H_1: addition of N will increase algal growth. In this experiment, you will examine the explanation that N is limiting algal growth in the Klamath. Algal growth will be estimated through observation and by relative abundance of Chl a and phycocyanin producing cells, measured as relative fluorescence units (RFU).

Procedure:

A. Students will meet Professor Sprowles at the HSU Experimental Greenhouse at a predetermined time.

B. Students will observe the solutions in their conical tubes and record observations in their Rite in the Rain notebook.

C. Students will transfer 1 mL of culture from each conical tube into a well of the 48 well microplate.

D. Students will travel to the CNRS Core Facility to measure the relative Chlorophyll *a* and phycocyanin fluorescence of their sample using the Spectramax© i6.

E. Data will be exported in a Microsoft EXCEL workbook and a plate map .pdf. It will be available to the students on the SCI 100 or ENGR 280 Canvas page.

F. Students will compare the average RFUs of Conical Tube #1 to Conical Tube #2. This information will be used to determine if the data supports the proposed hypothesis or the null hypothesis.

G. Students will formulate a conclusion to be written in the Rite in the Rain notebook.

Safety Information:

Always wear close-toed shoes in the laboratory.

Wear safety goggles when pipetting cultures of unknown organisms.

Wear gloves when handling the cultures of potentially toxic organisms.

If the culture splashes onto you or your clothing, inform your instructor. You will be instructed to rinse with running water for 20 minutes.

Do not touch a broken serological pipette. Inform your instructor so it can be disposed of properly.

Protocol

1. Find the pair of conical tubes containing the water collected by you and your partner during your field trip to the Klamath River.

   If you do not remember how your tubes were labeled, refer to your lab notebook.

2. Visually inspect the contents of each tube. What do you observe? Record all observations in your Rite in the Rain Notebook:

3. Locate a microplate station assigned to the location of your water sample.

   a. There are four microplate stations in the greenhouse. Each group of students should take their samples and go to one.

   b. At each station there are multiple 48 well microplates. Each microplate has a name and a plate map located next to each one. You will be **sharing a microplate** with up to 7 groups.

   c. You will also find manual pipette controllers, serological pipettes, and safety glasses.

4. Select a 48 well microplate.

   a. Column one of each microplate contains controls.

      i. Well A contains a positive control culture of photosynthetic algae

      ii. Well B contains water. Any RFUs reported are emitted from the plastic microplate and water alone.

5. Each group will select two wells for their samples.
   a. Remember each well has a column (number) and row (letter) ID

6. Fill out the table below for each of your samples. Copy this table in your Rite in the Rain notebook.

Write the last name of each group member and conical tube # in the microplate map such that rows A say conical tube #1 and B say conical tube #2. For example, if I claimed column #6, I would write my last name ("Sprowles") and "Conical Tube #1" in A6, and my last name ("Sprowles") and

"Conical Tube #2" in B6, as shown below. These will then serve as the "well IDs" for the microplate.

Microplate Name:		
Row Letter	**Column #**	**Sample**
A	6	Sprowles Conical Tube #1
B	6	Sprowles Conical Tube #2

6. Each group will transfer 1 mL of sample into the appropriate well. Since you have three wells labeled to receive culture from conical tube #1, each of those three cells will get a separate milliliter of water.

a. Put on safety glasses and gloves before beginning the procedure.
b. Let groups go in order of the columns. The group in column 2 goes first, column three second, etc. This will not only help keep things moving efficiently, it provides a nice check to ensure the plate is being loaded correctly.
c. Swirl/flick your conical tube to evenly distribute the contents throughout the solution. After each swirl remove ONLY ONE mL of sample, transfer to the appropriate well and repeat. Because cells are dense and clumpy, they settle out of solution very quickly. By mixing and pipetting the liquid for only one well at a time, you will reduce variability in cell concentration.
d. Replace the lid on the conical tube. Put them back into in the rack provided.
e. When you are done with each sample, place the used serological pipette into the designated waste container.

7. When the microplate has been loaded, notify your instructor. You will then wait to be escorted to the CNRS Core Facility.

8. In the CNRS Core Facility, we will use the Spectramax© i6 microplate reader to measure the fluorescence of the cells at two different excitation/emission parameters: 470/700 for Chlorophyll a and 590/670 for phycocyanin.

The data will be taken under the following conditions:

- 20 flashes/read
- No lid

8. The resulting data will be in Relative Fluorescent Units (RFU).

a. You will receive the data in an EXCEL spreadsheet.

b. Students will discuss the data and how to analyze it in either SCI 100 or ENGR 280.

IV. Analyze & interpret Your Experimental results

You have been performing an experiment to test the hypothesis that nitrogen will increase algae growth in water samples from the Klamath River. This hypothesis is based on a proposed explanation derived from water quality monitoring data suggesting that nitrogen, not phosphorus, is the limiting nutrient for algae growth in the Klamath River (Committee on Endangered and Threatened Fishes in the Klamath River Basin, National Research Council, 2004, Moissander et al. 2009, Kann and Corum 2008).

Our experimental design makes the prediction that if nitrogen is limiting, relative fluorescence units (RFUs) will increase more in flasks with added nitrogen than in control flasks. You and your partner used the Spectramax© i6 microplate reader in the CNRS core facility to measure RFUs at two different excitation/emission wavelengths: 470/700 for Chlorophyll *a* and 590/670 for phycocyanin. The purpose of this portion of the experiment is to compare the average culture RFUs at both of these wavelengths. For the most accurate comparison, you will first adjust these values so they do not include fluorescence emitted from the plastic microplate. At the end of this analysis, you will be asked to make some conclusions drawn from you and your partner's experimental samples. Later in the semester in your math course, you'll examine and analyze data from the whole Klamath Connection cohort.

For Biology, Botany, Environmental Science and Management, Fisheries, Forestry, Wildlife and Zoology students, refer to your Science 100 Syllabus for details on assignment grades and due dates.

For ERE students, refer to your Engineering 280 syllabus for details on assignment grades and due dates.

Materials

Canvas page for FOR 100 or ERE 280.

.xls data files from the Spectramax© i6 microplate reader

These files will posted on your course Canvas pages. Pencil and Paper, a Calculator, and/or EXCEL.

Procedure

1. Go to the FOR 100 Canvas page or the ERE 280 Canvas page.
2. Go to the Week 1 module and locate the "data" file for your group's microplate. Download and open it.
3. Find the data for the wells containing your two samples (Conical Tube #1 and Conical Tube #2).
4. Identify the RFU values reported for your samples at excitation/emission wavelengths 450/700 and 590/670. These RFUs are generated by the plastic well and its contents. We will refer to this number as *total RFUs*.

To compare the amount of fluorescence generated in response to N addition, the total RFU values must be adjusted so that only the fluorescence generated from the water sample is evaluated. Therefore, you must calculate the *average culture* RFUs for conical tube 1 and conical tube 2.

5. Determine the Chl a Culture RFUs
    a. Copy the RFUs reported at excitation/emission wavelengths 450/700 for each of your two samples (values reported in the well containing 0.5mls of culture from Conical Tube #1 well and the values reported in the well containing 0.5mls of culture from Conical Tube #2) into Data Table 1, below.
    b. Find the RFUs reported for the Plastic well of your microplate. The number is highlighted in red. The RFUs in this well are emitted from the plastic microplate. Copy this value into column five, "Plastic RFUs @ 400/700".
    c. To determine the Chl a RFUs emitted from your Conical Tube #1 sample, the plastic RFUs must be subtracted from the total RFUs. Using either a pencil and paper, a calculator or a computational program like EXCEL, perform this calculation and insert the value into column six of Data Table 1, "Culture RFUs @ 450/700 (Total RFU- Plastic RFU)".
    d. To determine the Chl RFUs emitted from your Conical Tube #2 sample, the plastic RFUs must be subtracted from the total RFUs. Using either a pencil and paper, a calculator or a computational program like EXCEL, perform this calculation and insert the value into column six of Data Table 1, "Culture RFUs @ 450/700 (Total RFU- Plastic RFU)".

Data Table 1: Determining Chl Culture RFUs

Sample	**Microplate Row Letter**	**Microplate Column #**	**Total RFUs @ 450/700**	**Plastic RFUs @ 450/700**	**Culture RFUs @ 450/700 (Total RFU- Plastic RFU)**
Conical Tube #1					
Conical Tube #2					

6. **Determine phycocyanin Culture RFUs**
    a. Copy the RFUs reported at excitation/emission wavelengths 590/670 f each of your two samples (values reported in the well containing 0.5mls of culture from Conical Tube #1 well and the values reported in the well containing 0.5mls of culture from Conical Tube #2) into Data Table 2, below.

7.
    a. Find the 590/670 RFUs reported for the plastic well of your microplate. The RFUs in this well are colored in Red. Copy this value into column five of Data Table 1, "Plastic RFUs @ 590/670".
    b. To determine the phycocyanin RFUs emitted from your Conical Tube #1 sample, the plastic RFUs must be subtracted from the total. Using either paper and pencil, a calculator or a computational program like EXCEL, perform this calculation and insert the value into column six of Data Table 2, "Culture RFUs @ 590/670 (Total RFU- Plastic RFU)".
    c. To determine the phycocyanin RFUs emitted from your Conical Tube #2 sample, the plastic RFUs must be subtracted from the total. Using either paper and pencil, a calculator or a computational program like EXCEL, perform this calculation and insert the value into column six of Data Table 2, "Culture RFUs @ 590/670 (Total RFU- Plastic RFU)".

Data Table 2: Determining Phycocyanin Culture RFUs

Sample	**Microplat Row Lett**	**Micropla Column #**	**Total RFU @ 590/670**	**Plastic RFU @ 590/670**	**Culture RFUs @ 590/670 (Total RFU- Plastic RFU)**
Conical Tube #1	A				
Conical Tube #2	B				

Conclusion:

Now it is time to use your data to determine if your results support the proposed hypothesis. Answer the following four questions and submit them into the Canvas Assignment Portal for either Science 100 or ERE 280 by the due date listed in your course syllabus.

1. Compare the Culture RFUs generated for Conical Tube #1 vs. Conical Tube #2. Does this data support the hypothesis that N is the limiting factor in algal growth in the Klamath River? Explain why or why not.

2. What is the purpose of including a positive control sample in Well 1A? How does a positive control help you have confidence in the results of your experiment?

3. You were asked to record your visual observations of the Klamath River water multiple times during this experiment. In the space below, type the observations you made at the Klamath River when sampling the water and the observations you made on the day the RFU data was collected. Do your visual observations support the RFU data that was generated? Why or why not?

4. Based on your results, do you believe *Microcystis aeruginosa* are in the water you sampled? Explain why or why not.

Literature Cited

Bureau of Reclamation. 2011. Karuk Tribe Sociocultural/Scocioeconomics Effects Analysis Technical Report for the Scretarial Determination on Whether to Remove Four Dams on the Klamath River in California and Oregon. Denver, CO.

Carmichael W.W. 1981. Freshwater Blue-Green Algae (Cyanobacteria) Toxins — A Review. The Water Environment. Algal Toxins and Health. Plenum Press, New York. pp 1-13.

Chorus I. and Bartram J. (eds.). 1999. Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management. World Health Organization, Routledge, London, England.

Chorus I, editor. 2001. Cyanotoxins: occurrence, causes, consequences. Springer-Verlag: Berlin

Committee on Endangered and Threatened Fishes in the Klamath River Basin, National Research Council, 2004. Endangered and Threatened Fishes in the Klamath River Basin: Causes of Decline and Strategies for Recovery. The National Academies Press, Washington, D.C..

Hambrook Berkman JA and Canova MG. 2007. Algal Biomass Indicators (ver. 1.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, section 7.4, August, available online only from http://pubs.water.usgs.gov/twri9A/.

Kann and Corum 2008. Summary of 2005 Toxic Microcystis aeruginosa Trends in Copco and Iron Gate Reservoirs on the Klamath River CA. Prepared for the Karuk Tribe.

Moisander PH, Ochiai M, Lincoff A. 2009. Nutrient Limitation of MIcrocystis aeruginosa in northern California Klamath River reservoirs. Harmful Algae (8) 889-897.