

Lab Three

Carbon uptake by phytoplankton – exploring the iron hypothesis in the laboratory

Purpose: The purpose of this lab is to build on the experimental problem solving skills developed in the two prior labs and to introduce you to some important laboratory techniques.

Lab overview: In this lab you will design an experiment to test the hypothesis that iron concentrations in marine environments CAN limit primary productivity. This is not the same thing as proving that they do limit primary productivity. However, this lab will give us a chance to begin to explore the complex relationships between trace metals and global element cycles and will give you an opportunity to begin to think about how useful laboratory experiments can be for exploring facets of environmental science.

Background: Environmental science is characterized by its complexity—lots of variables interacting with each other and affecting each other in ways that are poorly understood. Judicious choices of reductive experiments can help to tease out connections between variables. Care needs to be taken in reassembling the knowledge gained from laboratory experiments. One needs to think diligently about the biases introduced by the laboratory environment. For example, only a fraction of all microorganisms have been successfully cultured in the laboratory. Therefore studying the biochemistry of a cultured microorganism may not yield important insights about natural communities of organisms.

Over the past two decades the idea has emerged that iron, and perhaps other trace metals, may be the limiting factor in phytoplankton growth in the oceans. This idea, known as “the iron hypothesis”, has been explored now in several large-scale experiments. These experiments have demonstrated a connection between the short-term iron fertilization of marine ecosystems and phytoplankton growth. This demonstrated connection has inspired some to suggest that large-scale fertilization of oceans with iron may be a potential way to ameliorate some of the effects of human carbon consumption. We will discuss this idea further in our discussion sections this week.

Plan of action: We will divide the lab sections into groups of two. Each group will conduct a simple experiment to test whether it is possible to limit the growth of phytoplankton by simply limiting the amount of iron available to the organisms.

Each group will set up cultures to grow over the winter break. In the lab period that follows the winter break, we will extract the chlorophyll from the phytoplankton in the experimental cultures and quantify it, using a fluorometer. Chlorophyll concentration is assumed to be proportional to the number of phytoplankton.

In addition, we have a related modeling question we would like you to tackle. We'll make some time available in each lab period to work on it

Experimental Details: This lab introduces a number of technical details.

First, it is technically difficult to limit the levels of metals in a system when one is working with what are known as “trace metals”. Trace metals are defined as metals that are present in ppm concentrations. There are plenty of ways to contaminate an experiment and inadvertently introduce metals. To minimize contamination of our experiment, we have washed all of the glassware you will use in an acid bath (10% trace metal grade HCl). We allow the glassware to soak in that bath for at least a few hours, and then rinse the glassware thoroughly with distilled deionized water (Milli Q). All solutions will be made up with this water as tap water contains unacceptably high levels of metals. Depending upon the levels of metals one is interested in, even more drastic measures are sometimes called for. Some scientists work in what are known as “clean rooms”. These are rooms that have been specially designed to minimize the use of metals and that are rigorously maintained to keep the environment free of spurious metals.

Second, there are technical difficulties associated with trying to grow microorganisms. One common concern is to prevent the growth of unwanted organisms. A common approach to limiting the growth of unwanted organisms is to sterilize everything so that only the living organism one wants to introduce into a culture is introduced. To work under sterile conditions, ALL instruments and solutions must be sterilized. Care must be taken to sterilize things at the appropriate times. For example, all pipet tips must be sterilized prior to being used. Media is sterilized after it is made up but before the organism of interest is introduced. It is easy to pick up an unsterilized implement and stick it into a sterile culture before you realize what you have done, so good work habits are essential. I do not know how important it is to keep our solutions of phytoplankton sterile because for phytoplankton we do not add carbon to our media and bacteria require carbon for growth. However, to the extent possible, we will follow sterile techniques in this lab.

Third, many of you may be unfamiliar with making up solutions of precise concentrations of chemicals. This lab will give you a chance to develop these skills.

First Lab Period:

During this first lab period, we will have you make the f/2 mediums for the next lab group and then inoculate the sterilized mediums that have already been prepared with the phytoplankton culture.

Below is the standard recipe for making media for growing marine phytoplankton. Our strain of phytoplankton is grown in a f/2 medium. It assumes a 1 L volume of stock solution as the final volume. You will only need to make 500 mL and the quantity of compounds will need to be adjusted accordingly.

f/2 Medium

To 950 mL filtered seawater add:

Quantity	Compound	Stock Solution
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1 mL	Na NO ₃	75 g/L dH ₂ O
1 mL	NaH ₂ PO ₄ H ₂ O	5 g/L dH ₂ O
1 mL	Na ₂ SiO ₃ 9H ₂ O	30 g/L dH ₂ O
1 mL	f/2 trace metal solution	See recipe below
0.5 mL	f/2 vitamin solution	See recipe below

Make final volume up to 1 L with filtered seawater and autoclave.

f/2 Trace Metal Solution (Already prepared for you.)

To 950 ml distilled H₂O add:

Quantity	Compound	Stock Solution
3.15 g	FeCl ₃ .6H ₂ O	
4.36 g	Na ₂ EDTA.2H ₂ O	
1.0 mL	CuSO ₄ .5H ₂ O	9.8 g/L dH ₂ O
1.0 mL	Na ₂ MoO ₄ .2H ₂ O	6.3 g/L dH ₂ O
1.0 mL	ZnSO ₄ .7H ₂ O	22.0 g/L dH ₂ O
1.0 mL	CoCl ₂ .6H ₂ O	10.0 g/L dH ₂ O
1.0 mL	MnCl ₂ .4H ₂ O	180.0 g/L dH ₂ O

Make final volume up to 1.0 L with dH₂O. Autoclave.

f/2 Vitamin Solution (Already prepared for you.)

To 950 ml distilled H₂O add:

Quantity	Compound	Stock Solution
1 mL	Vitamin B ₁₂	1.0 g/L dH ₂ O
10 mL	Biotin	0.1 g/L dH ₂ O
200 mg	Thiamine HCl	-

I have prepared f/2 medium with and without iron for you to use in growing the phytoplankton since it requires autoclaving and sitting for 24 hours for pH stabilization before use. However, you need to prepare the f/2 medium (with and without iron) for others to use.

You will be broken into 4 groups. Two groups will make up 500 liter of f/2 medium using the standard recipe, utilizing the f/2 trace metal and vitamin solutions already prepared. Be sure to use acid washed glassware for your medium. We will use instant ocean for the filtered sea water. The other two groups will prepare f/2 medium using the standard recipe, but using the f/2 trace metal iron limited solution along with the vitamin solution. Be sure to label your mediums as either f/2 with Fe or f/2 without Fe!! I will autoclave them for you.

Following preparation of the f/2 medium, you will inoculate 200 ml of sterile f/2 medium with 50 ml of phytoplankton culture. You will do this twice: once with f/2 medium with iron and once with f/2 medium without iron. Be sure you use only sterile glassware for measuring and storing your culture and sterile caps for sealing your flasks. Once the cultures have been prepared and labeled, they will be placed in front of fluorescent lights in Carnegie B-21.

Second Lab Period:

Determining phytoplankton concentration by measuring the chlorophyll concentration using fluorescence is a common method used both in laboratory cultures and in field samples. We are grateful to Professor Will Ambrose who has agreed to allow us to use his fluorometer and many of his supplies in order to make these measurements. There are a number of steps required to prepare the phytoplankton cultures for fluorescence determination of chlorophyll. We will follow these steps carefully and be cautious in using his equipment.

You will need:

A porcelain filter funnel

42.5 mm glass filters

Graduated cylinder and/or volumetric flask- 50 ml and 250 ml

Forceps

50-60 ml plastic centrifuge tube

Acetone

Sonicator

Centrifuge

Transfer pipette

1N HCl solution

Fluorometer

Method for filtration/extraction and fluorometric analysis.

1. Place a glass filter in the porcelain filter funnel.
2. Gently shake your first phytoplankton culture flask and pour the contents into a graduated cylinder.
3. Record the exact volume.
4. Attach the filter funnel to the vacuum pump and turn on the pump.
5. Pour the contents into the filter funnel.
6. Turn off the vacuum pump. Using forceps fold the filter in half and lift it off the filter funnel.
7. Place the folded filter at the bottom of the centrifuge tube.
8. Label the centrifuge tube with tape with the group name and type of media.
9. Measure out exactly 35 ml of 90% acetone into the 50 ml graduated cylinder.
10. Pour the acetone into the centrifuge tube containing the filter.
11. Cap tightly and place in the sonicator for 2-3 minutes.

12. Remove and place in the dark.
13. Process next sample.
14. When all the samples have been extracted, clarify the extract by spinning on a centrifuge at 5 minutes at 1000g.
15. Take the samples to Will Ambrose's lab in Carnegie.
16. Using the transfer pipette, carefully withdraw 8-10 ml of extract to fill the fluorometer sample cuvette.
17. Zero the fluorometer against 90% acetone, then measure the fluorescence of the sample.
18. Add 2-3 drops of 1N HCl solution; mix very gently.
19. Allow the cuvette with the acid added to stand at least 90 seconds before measuring the fluorescence.

Data Analysis and Calculations:

The concentration of chlorophyll a must be "corrected" for the presence of phaeopigments, which are chlorophyll degradation products.

$$C_{E,c} = F_s(r/r-1)(R_b - R_a)$$

Where:

$C_{E,c}$ = corrected chlorophyll a concentration (ug/l) in the extract solution analyzed

F_s = response factor for the sensitivity setting S

r = the before-to-after acidification ratio of a pure chlorophyll a solution

R_b = fluorescence of sample extracted before acidification

R_a = fluorescence of sample after acidification.

Calculate the "corrected" concentration of chlorophyll a ($C_{S,c}$) in the whole water sample as follows:

$$C_{S,c} = \frac{C_{E,c} \text{ (Extract Volume (ml))}}{\text{Sample Volume (ml)}}$$

Lab Write up: Your lab write up should consist of a careful description of the experiment you designed, the observations you noted as you were working, and the data you collected. You should state what conclusions you can draw from your data. In addition, you will hand in a stella model with a description of what you observed for the question posed below.

Stella problem: Given the volume of oceans and concentrations of carbon in various compartments on earth, making some assumptions about mixing rates between deep ocean and surface waters, explore how rates of photosynthesis (i.e. phytoplankton growth) would affect the drawdown of all the ocean's nutrients and atmospheric concentrations of CO₂ if phytoplankton growth were increased due to iron fertilization. How much increased phytoplankton growth would there need to be in order to significantly lower atmospheric CO₂ concentrations?

Oceans cover ~70% of the earth's surface. Average depth of the world's oceans is ~4000m. Surface area of earth is $5 \times 10^{14} \text{m}^2$.

Week #1: Simply use Stella and your course pak to build a simple model of the carbon cycle

Week #2: Explore the effects of different phytoplankton growth trajectories.