

## Phytoplankton-Bacteria Competition

Phytoplankton obtain their C requirements from CO<sub>2</sub> via photosynthesis, while bacteria obtain their C requirements by assimilating dissolved organic matter (DOM) (Fig. 1). Bacteria can also obtain their N requirements from DOM, provided the C:N ratio of the assimilated DOM is low enough. Indeed, bacteria will excrete N (i.e., remineralize) if the C:N ratio of the DOM is below a certain threshold. If the C:N ratio of DOM is high however, bacteria will supplement their N demands via direct uptake of dissolved inorganic N (DIN). Under these situations, bacteria directly compete with phytoplankton for available DIN.

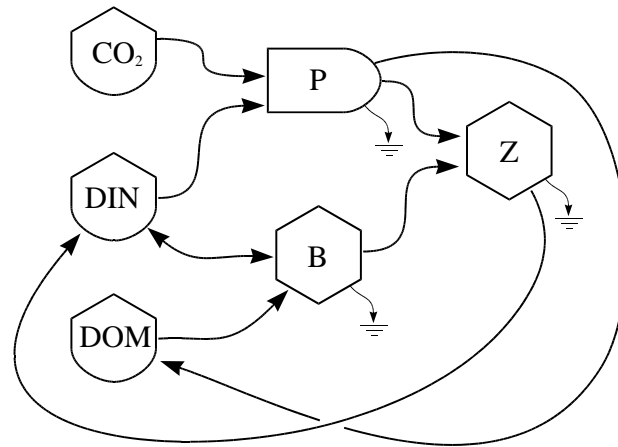


Fig. 1. Interaction of phytoplankton (P), bacteria (B), and higher trophic levels (Z).

Since bacteria are smaller than phytoplankton, bacteria have a greater surface area to volume ratio than phytoplankton. Consequently, bacteria can out compete phytoplankton for available dissolved inorganic nitrogen (DIN), provided there is enough dissolved organic carbon (DOC) to support bacterial growth.

Paradoxically, phytoplankton often excrete DOC while growing or during N limitation, thereby facilitating bacterial growth and possibly the competition for DIN. Whether phytoplankton excretion of DOC produces mutualism, commensalism, or competition between bacteria and phytoplankton in natural environments is still uncertain and a focus of research.

Even if bacteria do immobilize DIN and out compete phytoplankton, eventually the bacterial-immobilized N will be remineralized by organisms in the higher trophic levels due to the low efficiency C-transfer between trophic levels.

### Readings:

- Bratbak, G. and Thingstad, T.F. (1985) Phytoplankton-bacteria interactions: an apparent paradox? Analysis of a model system with both competition and commensalism. *Mar. Ecol. Prog. Ser.* **25**, 23-30.
- Caron, D.A., Goldman, J.C., and Dennett, M.R. (1988) Experimental demonstration of the roles of bacteria and bacterivorous protozoa in plankton nutrient cycles. *Hydrobiologia* **159**, 27-40.
- Wood, A.M. and Valen, L.M.v. (1990) Paradox lost? On the release of energy-rich compounds by phytoplankton. *Mar. Microbial Food Webs* **4**, 103-116.

## Microcosm Experiment: Bacteria-Phytoplankton Competition

(3 Nov 2016)

In this lab experiment we will use two microcosms to examine competition between bacteria and phytoplankton for inorganic nitrogen.

### Setup

The microcosm experiments will be conducted in 20 L carboys filled with Woods Hole seawater, unfiltered. Two treatments will be prepared as given by the following table:

Nutrient Added (mg)	Final Concentration ( $\mu\text{M}$ )	
	Treatment A	Treatment B
Glucose (270)	0	75 (450 $\mu\text{M}$ C)
$\text{KNO}_3$ (73)	36	36
$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ (296)	52	52
$\text{KH}_2\text{PO}_4$ (6.3)	2.3	2.3

Bioreactors will be placed near a window and additionally illuminated with 40 W fluorescent lights. All bioreactors will be monitored for, Temp, pH, PAR, Chl a and DO with Hydrolabs and you will take samples for later analysis (see Measurements below).

The experiment is designed so that bacteria in treatment B will immobilize all nitrate, thereby preventing a phytoplankton bloom (or so we expect). The concentration of glucose is limited by oxygen availability, since we don't want the microcosms to go anaerobic (oxygen concentration in seawater at 32 ppt and 25°C in equilibrium with air at 1 atm is 215  $\mu\text{M}$ ). If we assume a bacterial carbon yield of 40%, then 180  $\mu\text{M}$  C will be incorporated into bacterial biomass, the other 270  $\mu\text{M}$  being converted to  $\text{CO}_2$ . Assuming a bacterial C:N ratio of 5, the bacteria will consume 36  $\mu\text{M}$  N, which is almost all the available N (Woods Hole seawater contains probably  $\sim 1$   $\mu\text{M}$  N). Silicon (required for diatoms) is added in excess, while phosphate is added at the Redfield ratio based on N.

### Measurements.

***Measurements will be taken every day during the seven-day incubation period.*** Each person (or persons) will be responsible for a particular measurement. However, if you cannot take a sample on a particular day, then arrange with someone to sample in your place. You need to accurately record time when a sample is taken. If you are about to run out of anything that is needed for your sample collection/analysis, let the TA know so they can restock. Try to give the TA a little advanced notice, especially make sure that you have everything that you need for the weekend. ***HAVE DATA READY FOR DISCUSSION ON Thursday November 10<sup>th</sup>*** (This means you will probably run/count your samples on Tues or Wed). **Please have a PowerPoint**

**slide or two to show your data, even if they are just preliminary results.** You can email me the slide beforehand, or have some way to access it from class, such as on a USB drive.

### Dissolved Organic Carbon

Collection: Filter sample through GF/F filter and fill DOC scintillation vials (25 ml, tall vials with black cap) with 20 mL of sample. Using a pipette add 100  $\mu$ L of 42% phosphoric acid (located in hood). This is a strong acid; wear gloves, eye protection and lab coat when working with the acid, and be gentle with the pipette. Make sure acid does not splash up inside the pipettor. Store samples in the refrigerator.

Analysis: Samples will be analyzed by persulfate oxidation (talk to TA or Rich). It is probably best to run all samples at end of experiment. TA will make arrangements for the run.

### Bacteria counts

Collection: Fill standard scintillation vial with 19 ml of sample and preserve with 1 ml formaldehyde (or glutaraldehyde) (in fridge). Note that there is a 1 mL syringe that can be used to dispense the formaldehyde. Remember, formaldehyde is pretty nasty, so wear gloves, eye protection, and work in the hood when adding the formaldehyde to your sample. Store samples in refrigerator prior to counting.

Analysis: Standard DAPI method, use 1.0 mL (may need to adjust volume) preserved sample and 1.0 mL of PBS and stain with 100  $\mu$ L of DAPI working solution (refer to DAPI lab handout for protocol). DAPI and PBS wash are both in the fridge. **BE SURE TO USE WORKING SOLUTION OF DAPI.** Remember, DAPI is nasty, and liquid waste generated from the DAPI counts needs to be discarded in the waste container. Counts can be done all together after all samples have been taken, or periodically throughout the incubation. You may also try to look for and count cyanobacteria (they are the same size as bacteria, but autofluoresce red, talk to Joe for more info on this).

### Nanoflagellates and Ciliates

Collection: Fill standard scintillation vial with 19 ml samples, preserve with 1 ml formaldehyde (or glutaraldehyde) (in fridge), and refrigerate for later analysis.

Analysis: Ciliate counts are conducted identically to the bacterial DAPI counts (refer to DAPI lab handout for protocol), except that 4 ml of sample are stained with 200  $\mu$ L DAPI (no PBS, and this is four times as much final volume compared to bacterial). This is then filtered onto black 0.8  $\mu$ m (or 1.0  $\mu$ m) Nucleopore filters. DAPI and PBS wash are in the fridge. **BE SURE TO USE WORKING SOLUTION OF DAPI.** Remember, DAPI is nasty, and liquid waste generated from the DAPI counts needs to be discarded in the waste container). Counts can be done all together after all samples have been taken, or periodically throughout the incubation. You may also try to look for and count phytoplankton (they autofluoresce red).

### Nitrate

Collection: Filter sample through GF/F filter into scintillation vial 4/5 full with sample and place in freezer for later analysis.

Analysis: Run all samples at end of experiment on Lachat autoanalyzer. Schedule a run time with the TAs.

### Ammonium

Collection: Filter sample through GF/F filter into scintillation vial, almost fill vial. Add 10  $\mu$ l of 5.5 N HCl (located in hood) and refrigerate for later analysis.

Analysis: It is probably best to run all samples at once at end of experiment. These will be analyzed by hand. Meet with TAs for scheduling.

### *In vivo* Fluorescence and Chl a extraction

Collection: A) Fill a 100x13 mm test tube with sample. B) Filter 300 mL of sample onto a 47 mm GF/F filter for determination of Chl a. This is done using the filter cup that is attached to the vacuum pump, and the 47 mm GF/F filters. Use the graduated cylinder that is provided to measure out 100 mL at a time. Pour sample into filter flask and turn on vacuum pump. Once the 300 mL sample has been filtered fold the filter in half (so that the chl. is on the inside) and wrap the filter in aluminum foil. If you are unable to filter the entire 300 mL (i.e., filter clogs), record the volume you filtered. Label the foil with time, date, treatment, and volume filtered and place the filter immediately into the freezer until analyses are run.

Analysis: A) For *in vivo* fluorescence, place test tube from above in Turner fluorometer and read fluorescence. The fluorometer has been calibrated for chlorophyll in  $\mu$ g Chl a/L. B) To measure the chlorophyll, extract the Chl from each filter in 25 mL of acetone. Fill 50 mL centrifuge tube with 25 mL acetone, located in the refrigerator. Extraction should be run for 12 hours. You can extract the chlorophyll overnight on Tues, and we can analyze them on the spec on Wed. You will need to see the TA to get the acetone and to arrange a time to meet to run the spec.

### Phosphate

Collection: Filter sample through GF/F filter and fill acid washed 15 ml Falcon tubes with 10 mL of sample. Add 10  $\mu$ l of 5.5 N HCl (located in hood) and refrigerate for later analysis.

Analysis: Standard spectrophotometric method: It is probably best to run all samples at once at end of experiment. These will be analyzed by hand. Meet with the appropriate TA for scheduling.

### Phosphatase

Collection: Remove 4 ml from each microcosm and place in 15 mL centrifuge tubes, incubate with 400  $\mu$ l of "P" MUF-phosphate substrate (this is located inside a foil wrapped 10 mL volumetric flask. It must remain cold and in the dark). Note time of

addition. At 0 min. and 60 min. remove 1 mL of enzyme-sample solution to a borosilicate tube. Add 4 mL of 200 mM glycine buffer (50 ml falcon tube in fridge) to the borosilicate tube to stop the reaction.

Analysis: Measure fluorescence at 445 nm (the fluorometer is preset). NOTE: You need to use the fluorometer that has been calibrated for MUF. The fluorometer needs to warm up for a few minutes, so turn it on when you start your incubation. Fluorometer has been calibrated, and should read output in concentration units ( $\mu\text{M}$ ). The fluorometer should hold the calibration, but if your incubation doesn't seem to be working correctly, let the TA know.

### Bacterial Production (we are not doing this this year)

Bacterial production must be measured when you collect a sample (it can't be preserved and analyzed later, why?). Use the same technique as we used earlier in class; however, you do **not** need to run a working solution (i.e., W sample), nor do you need to run more than one incubation time. You should have two microcentrifuge tubes for each microcosm; one for the blank (killed with TCA at time zero) and the other in which you conduct a 30 min incubation.

One other modification: You do not need to conduct the TCA and ethanol rinses each day. Instead, run the assay as usual, but after you add the concentrated TCA to kill the 30 min incubation, you can store the 4 microcentrifuge tubes in the refrigerator OKed for  $14\text{C}$ . After all the samples have been run, you can then spin down and rinse with TCA and ethanol all the samples at once as the procedure calls for.

Note, the  $^{14}\text{C}$  incubations need to be conducted in the dedicated Rad room in Lillie if our lab has been decommissioned for rad use.