Phytoplankton-Bacteria Competition

Phytoplankton obtain their C requirements from CO_2 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.

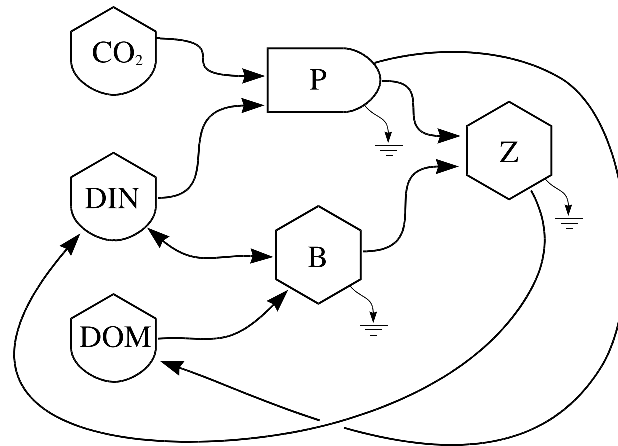

Fig. 1. Interaction of phytoplankton (P), bacteria (B), and higher trophic levels (Z).

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 and the low C:N ratio of bacteria.

Readings:

Bratbak, G. and Thingstad, T.F. (1985) Phytoplankton-bacteria interations: 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

(31 Oct 2019)

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:

	Final Concentration (μM)	
Nutrient Added (mg)	Treatment A	Treatment B
Glucose (270)	0	75 (450 μM C)
KNO_3 (73)	36	36
$Na_2SiO_3 \bullet 9H_2O$ (296)	52	52
KH_2PO_4 (6.3)	2.3	2.3

Microcosms 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 μM). If we assume a bacterial carbon yield of 40%, then 180 μM C will be incorporated into bacterial biomass, the other 270 μM being converted to CO_2. Assuming a bacterial C:N ratio of 5, the bacteria will consume 36 μM N, which is almost all the available N (Woods Hole seawater contains probably ~1 μM 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 7th*** (This means

your 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. You will also need to email me your raw and calculated data for the problem set.

<u>Dissolved Organic Carbon</u>

Collection: Filter sample through GF/F filter and fill DOC scintillation vials (25 ml, tall glass vials) with 20 mL of sample. Using a pipette add 100 uL 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.

<u>Bacteria counts</u>

Collection: Fill standard scintillation vial with 19 ml of sample and preserve with 1 ml formaldehyde (in fridge). Note that there is a 1 mL syringe that can be used to dispense the formaldehyde (but a pipette can be used too). Remember, formaldehyde is pretty nasty, so wear gloves, eye production, 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) of preserved sample and with 50 µL of DAPI working solution (refer to DAPI lab handout for protocol). DAPI and PBS wash are both in the fridge. 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).

<u>Nanoflagellates and Ciliates</u>

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

Analysis: Ciliate counts are conducted similarly to the bacterial DAPI counts (refer to DAPI lab handout for protocol), **except** that 10 ml of sample is filtered down to ~1 mL using a 0.8 µm (or 1.0 µm) Nuclepore filter (with GF/F backing filter) using the glass filter tower. With the ~1 mL of concentrated sample still in the filter tower, add 50 µL DAPI/mL and incubate in the filter tower (this method saves on DAPI, as we are staining only 1 mL, but the sample is 10 mL). After 5 min of staining, draw the sample through the filter and rinsed with PBS. Place the filter on a microscope slide as we have down with bacteria counting. 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 μl 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: You will be measuring chlorophyll using two different methods, described below.

Method #1

Collection: Each day, fill a 100x13 mm test tube with sample.

Analysis: Each day, for *in vivo* fluorescence, place test tube from above in Turner fluorometer marked for Chl and read fluorescence. The fluorometer has been calibrated for chlorophyll in μg Chl a/L.

Method #2

Collection: Each day, 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 place in a 50 mL centrifuge tube. If you are unable to filter the entire 300 mL (i.e., filter clogs), record the volume you filtered, and pore off excess water, remove filter. Label the centrifuge tube with time, date, treatment, and volume filtered and place the filter immediately into the freezer until analyses are run.

Analysis: To measure the chlorophyll on each of your filters, extract the Chl from each filter in 25 mL of acetone. Fill the 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 µl 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 µl of "P" MUF-phosphate substrate (this is located inside a foil wrapped scint vial. 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 and marked Enzymes. 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 (µM). The fluorometer should hold the calibration, but if your incubation doesn't seem to be working correctly, let the TA know.

Bacterial Production (2 students)

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 small refrigerator designated for ^{14}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.