Microbial Grazing Lab

October 20, 2011

The microbial loop is responsible for the cycling of nutrients as well as the support of higher trophic levels. The predator-prey dynamics of microplankton (eg. Ciliate 20-200 μm) and nanoplankton (eg. Flagellates 2-20 μm) grazing of picoplankton (eg. Bacteria 0.2-2 μm) provide the flow of energy and mass to higher trophic levels via the postulated microbial loop. In order to determine the grazing rates of ciliates (we will not count nanoflagellates), we will introduce labeled pseudo-prey (fluorescent microspheres) at $5x10^6$ beads/mL. After a short incubation period, you will determine the specific grazing rate by counting the number of fluorescent beads within the DAPI stained ciliates. In this experiment, we use small fluorescent beads (0.75 μm) that are excited at 488 nm and emit light at 515 nm as the prey (they will appear bright green). The sample you will analyze will come from your Winogradsky column. Grazing experiments can also be performed with labeled bacteria, but beads are much easier to differentiate and count. However, Sheer and Sheer (1987) found grazing discrimination with the beads such that the uptake ratios of bacteria to beads were 10:1 for ciliates and 6:1 for flagellates. Although such discrimination should be accounted for, we will assume no discrimination occurs in our calculations.

Lab Procedure:

- In a 15 ml Falcon tube, incubate 5 ml from your Winogradsky column with 100 μl of 0.75 μm bead (vortex beads first). Gently invert sample to mix (do not vortex). Note time of addition. Final bead concentration will be 5×10^6 0.75 μm-beads mL^{-1}.

- At approximately 10 minutes (record true time), remove 2 mL from the incubation and combine with 2 mL of **ice-cold** 4% glutaraldehyde in a 13 mm borosilicate tube and vortex. This both stops the grazing and fixes the cells. *Fixing the cells keeps them from rupturing during filtration*, which is critical to getting a good slide preparation.

- Stain the culture in the borosilicate tube for 5 minutes using 200 μl of 200 μg/mL DAPI. For better staining, keep samples in the dark by covering them with a box.

- Filter onto **5.0 μm** black polycarbonate filter. Rinse un-ingested beads, bacteria and excess DAPI stain twice with ~2 ml PBS, BUT DON'T ALLOW THE FILTER TO DRY BEFORE ADDING THE PBS WASH (this allows better distribution of the cells onto the filter). Also, avoid excessive vacuum, as this will cause cell lysis.

- Mount filter on a labeled slide using immersion oil on either side of filter as we did with the DAPI counts for bacteria. Ask us if you don't remember how.

- Under 100x objective, randomly choose a field of view and record the total number of ciliates (all sizes) in the entire view AND the number of beads you count inside each ciliate. Record 0 if no beads are found in a ciliate or no ciliates are present in a field of view. We are interested in estimating 1) the average number of ciliates per field of view (to get ciliate density) and 2) the average number of beads in each ciliate to get grazing rate.

- If necessary, we can also filter the remaining 3 mL of your sample in the Falcon tube if the 10 min incubation was not sufficient, but you will need to record how long that sample has been incubating. We will only do this if we have problems with the first sample, and I will let you know if you need to do it.

- Finally, if time permits (it probably won't), it would be good to get a DAPI count of the bacterial density in your column. If time does not permit, you will use the DAPI count we did in week two. The bacterial density will certainly have changed, but you will need a bacterial density number for your calculations.

For further info see: E.B. Sherr and B.F. Sherr (1987) Protistan grazing rates via uptake of fluorescently labeled prey. In Handbook of Mehods in Aquatic Microbial Ecology, P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole, Eds, Lewis Publishers, Boca Raton, 695-701