# Microbial Grazing Lab

The microbial loop is responsible for the cycling of nutrients as well as potentially 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 today), we will introduce labeled pseudo-prey (fluorescent microspheres) at $4.6 \times 10^6$ beads/mL. The bacterial abundance in the sample is approximately $1.3 \times 10^7$ cells $mL^{-1}$. After a short incubation period, you will determine the specific grazing rate by counting the number of fluorescent beads within the DAPI stained ciliates, as well as the concentration of the 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 using the GFP filter set). The sample you will analyze comes from a composite of your Winogradsky columns (#2, #3 and #8). Grazing experiments can also be performed with labeled bacteria, but beads are much easier to differentiate and count. However, Sherr and Sherr (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 the Winogradsky composite sample with 500 µl of 0.75 µm bead (**vortex or swirl beads first**). Gently swirl sample to mix (do not vortex). Note time of addition. Final bead concentration will be 4.6 x $10^6$ 0.75 µm-beads $mL^{-1}$.

- At approximately 15-20 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 or swirl. 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, Al foil or in a drawer.

- Filter sample onto **5.0 µm** black polycarbonate filter. Rinse un-ingested beads, bacteria and excess DAPI stain twice with ~2-5 ml PBS from squirt bottle, but avoid allowing 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 can 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. On the microscope, you should use the DAPI filter set to count ciliates, them change the filter set to green fluorescent protein (GFP) to count beads within ciliates. Record 0 if no beads are found in a ciliate or if 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. Use the total field of view for ciliate counts (not just the grid of the reticle), we do not need the computer for this assay. Diameter of the field of view is 150 µm.

- If necessary, we can also filter the remaining 3 mL of your sample in the Falcon tube if the 20 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.

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