SES: Methods in Microbial Ecology, Fall 2019
Problem Set 5, Microbial Grazers
(*Due 17 Oct 2019)*

1) From your incubation experiments, report in a table for each field of view the number of ciliates (including zeros) and the number of beads in each ciliate. From your table calculate A) the average number of ciliates counted per field of view and B) the average number of 0.75 μm beads counted in each ciliate. Calculate 1) the ciliate abundance (cells mL^{-1}), and 2) the specific ciliate feeding rate (prey hr^{-1} $ciliate^{-1}$); make sure to account for the fraction of prey that were beads (i.e., we could not see the bacteria that were also eaten). As stated in the methods handout, assume bead final concentration was $1.0x10^7$ beads mL^{-1} and the bacteria were at $7.1x10^6$ cells mL^{-1}. Also, the whole field of view (FOV) diameter is 200 μm with the 100x objective for the two older scopes. For the 3 new scopes, the diameter of the FOV is 225 μm (the monitor display is 190 μm × 100 μm if you used that.) (10 pts).

2) A) Several of the methods to measure bacterial grazing rate (size fractionation, dilution, inhibitors) have the disadvantage of requiring long incubation times (on order of days). Why are long incubations required? (5 pts) B) In the bacterial grazing experiment, why did we use 5 μm filters instead of 0.2 μm filters? (5 pts)

3) The mass balance equation for bacteria we discussed in class is given by,

$$\frac{dB}{dt} = \mu B - \varphi G$$

where B is bacterial concentration, μ is their true specific growth rate, G is the concentration of the grazers, and φ is their specific grazing rate on bacteria. If the bacterial concentration does not change rapidly with time (i.e.,$\frac{dB}{dt} \cong 0$), and the bacterial specific growth rate is of the order 5 d^{-1}, A) what does this equation tell you about the relative concentrations of bacteria versus grazers given the value you measured for φ for ciliates in part B) of question 1 (that is, calculate the value of B/G)? (8 pts). B) From all your data in Question 1, does it appear that $\frac{dB}{dt} \cong 0$? (2 pts).

4) A) Explain how measuring bacterial specific growth rates in unfiltered and 0.6 μm filtered water can be used to obtain bacterial grazing rates (provide the equation for φ and how the values are obtained)? (8 pts) B) Why is the dilution technique superior to the 0.6 μm filtration method (note, both have problems of cell rupturing during filtration, so that's not it)? (2 pts).

5) A) We did not run a control during our lab. How might you run a control to verify your results are not compromised by some strange artifact (like beads getting suck underneath ciliates)? (5 pts) B) Why is it important to add formaldehyde to a sample before the DAPI filtration if you want to count/examine eukaryotes? (5 pts)

Questions continued on back of page

6) A) What are the two main discoveries that lead to the microbial loop conceptualization? (5 pts) B) What evidence was used in the Azam *et al.* paper to support the hypothesis that nano flagellates graze bacteria? (5 pts)?

7) What is one possible hypothesis, discussed in class, for the increase in harmful algal blooms that is associated with eutrophication? (10 pts)

8) A) What is mixotrophy in the context of this week's lab? (5 pts) B) What does top-down and bottom-up control mean in food webs? (4 pts), C) Why don't I like the top-down, bottom-up concept (1 pt)?

9) A) What requirement must be met for the microbial loop to provide significant amounts of carbon to zooplankton and higher trophic levels? (5 pts). B) What other function might the microbial loop provide to higher trophic levels? (5 pts).

10) A) What two processes control the concentration of bacteria in nature? (5 pts). B) Why is organism size so often used to characterize microbial food webs? (5 pts).