SES: Methods in Microbial Ecology, Fall 2018
Problem Set 2 (*Due Thurs, 20 Sept 2018*)

To get partial credit, please show all your calculations. If you give only a number answer, and it's incorrect, you will get zero on that question.

1) Plate count lab: A) Report the number of colonies counted on ***each*** dilution plate (make a table). If a particular dilution was uncountable, then label it as such. From the **statistically meaningful** plates, calculate the concentration of bacteria in the original sample. Provide only **one answer** and state which plate count is being used for the calculation (i.e., report only one number as your final value); *also include the Winogradsky column number the sample was taken from*. B) Report the colonies YOU counted on the fecal coliform plates, the volume of sample water filtered, the location of where the sample was collected and calculate the number of fecal coliforms (i.e. CFU) per 100 mL of sample. Indicate if the water is safe to drink, safe to shellfish, or safe to swim in based on the number of CFUs.

2) DAPI count lab: Report the average number of bacteria per grid counted under the microscope using the DAPI stain. If you used the Zeiss Zen software to determine area, then report the area, number of bacteria counted and the average number of bacteria per mm^2 or μm^2. From your average number, calculate the bacterial concentration in the original sample. Specify the volume of *sample* (not PBS) you stained/filtered. Also specify which Winogradsky column the data are from. Note, the size of the grid in the microscopes not connected to the computers is approximately 100 μm x 100 μm with the 100x objective. For the microscopes without a grid, assume the diameter of the field of view is 150 μm.

3) A) What did we use to preserve the bacterial samples in Tuesday's lab for later counting? B) Why did we add a preservative (give two reasons)?

4) A) What are two disadvantages of using Acridine Orange for direct bacterial counts? B) What cellular macromolecule(s) does Acridine Orange bind to?

5) Why is bacterial abundance measured by plate counts lower than bacterial abundance measured by direct counts?

6) A) What organism caused the largest outbreak of a waterborne disease in US history? B) Does the fecal coliform count measure the concentration of pathogenic organisms in a water sample? Briefly explain your answer.

7) Assuming a bacterial concentration of 5.4×10^6 cells ml^{-1} (this notation is the same as cells/ml) and that a cell contains 20 fg C (f is femto), what is the carbon concentration associated with the bacterial biomass? Please give your answer in μmol C l^{-1}.

8) A) Mechanistically, it would be possible to stain bacteria with DAPI, then put a drop of the stained bacteria on a microscope slide and count them. So why do we filter the sample in the DAPI method? B) Why do we run a blank along with the sample in the DAPI counts? C) If the blank was not zero, how would you correct your counts?

9) A) Why do we use epi-fluorescence and not transmitted light to count bacteria in natural samples? B) If an objective has a numerical aperture of 1.25, what is the greatest magnification that can be achieved with the objective?

10) A) What benefit does immersion oil provide in light microscopy? B) What is the approximate size of the smallest object that can be resolved using light microscopy?