1. Summarize this paper (10 points):

Reysenbach AL, Wickham GS & Pace NR 1994 *Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park*. Appl. Env. Microbiol. 60:2133-2199

... with respect to the following points:

A) What question or problem does this paper address? In other words, what was the purpose of doing this work?
B) What system do they work with? Think field sites, organisms, locations, this sort of thing.
C) What approaches did the authors use? Don't recite the Materials & Methods, just outline the methods and how they might have fit together, and any crucial details such as primers, probes, &c.
D) What were the main conclusion(s) of the paper?
E) What evidence did they present to support these conclusions?
2. Summarize this paper (10 points):

Moreno Am, Matz C, Kjelleberg S & Manefield M 2010 Identification of ciliate grazers of autotrophic Bacteria in ammonia-oxidizing activated sludge by RNA stable isotope probing. AEM 76:2203-2211

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A) What question or problem does this paper address? In other words, what was the purpose of doing this work?
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C) What approaches did the authors use? Don't recite the Materials & Methods, just outline the methods and how they might have fit together, and any crucial details such as primers, probes, &c.
D) What were the main conclusion(s) of the paper?
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3. In MB 452 Microbial Diversity lab, imagine you have isolated colonies on your agar degrader plate that contain both long rods and large cocci. No matter how many times you re-streak from well-isolated single colonies, you get the same mixture of cell morphologies. Your TA tells you to keep trying until you get the two different organisms separated, but it doesn’t seem to work and it occurs to you that they could be symbionts, two species can’t grow separately. On the other hand, Dr. Brown tells you it might be a single pleomorphic species, that the cocci are stationary-phase cells and the rods are growing cells.

You decide to figure it out once and for all in a research lab the following semester. How would you go about this? What would the results look like either way? Be sure to provide important particulars such as probes, labels, primers, &c. (10 points)
“Chlorochromatium aggregatum” is not a single organism, but consortia of two symbiotic organisms. Each of the colorless rod-shaped bacteria is surrounded by about 20 green cells. The rRNA sequences of both kinds of organisms have been determined; the colorless central rod-shaped cells are beta-proteobacteria that are not close relatives of any cultivated organism. The green surrounding cells are species of Chlorobium. These consortia cannot be grown in pure culture, but they predominate the microbial communities at the chemocline of meromictic lakes, and can be highly enriched from these samples by repeatedly shining a light on a sample and collecting the organisms that swim to the light. Any enriched sample of “Chlorochromatium aggregatum” contains what look like several kinds of related sets of organisms, and it has been confirmed in the phylogenetic analyses that not all consortia contain the same kinds of Chlorobi or beta-proteobacteria.

It is presumed that the Chlorobium is fixing carbon for consortia, since all known Chlorobi are obligate photoautotrophs. But what about nitrogen? These consortia can grow nicely with only atmospheric nitrogen present (no measurable nitrite, nitrate, ammonium or organic nitrogen) and so must fix nitrogen. How would you go about determining which of these two kinds of organisms fixes nitrogen for the consortia? How would the fact that you’d be isolating a mixture of related consortia (not a “pure” consortium) impact your results? (10 points)

NOTE: Stable-isotope probing won’t work in this experiment. For 5 extra credit points, explain why not.
5. In the paper “Changes in oral microbial profiles after periodontal treatment as determined by molecular analysis of 16S rRNA genes” by Sakamoto, et al, the authors primarily use t-RFLP, with some help from realtime PCR and traditional ssu-rRNA clone-and-sequence approaches, to see how periodontal microflora in periodontal disease patients changed after treatment. This paper is now 7 years old, and the experiments were probably designed about 10 years ago. How would you do this experiment today? What would be the advantages of the more modern approach? (10 points).
Final Exam : In-class questions  |  MB 451 Microbial Diversity

Honor pledge: “I have neither given nor received unauthorized aid on this test.”

Signed: __________________________ Date: __________________________

Name: __________________________

6. What are the 3 primary branches of life? (5 points)

Multiple-choice questions (2 points each)

7. ____ The pink filaments of Yellowstone National park have been cultivated and named...
   A. Thermus aquaticus
   B. Thermoleophilum album
   C. Thermophilum pendens
   D. Thermocrinus ruber
   E. none of the above

8. ____ The carbon-fixing symbionts in the scaly snail reside...
   A. in the esophageal gland
   B. in the muscular foot
   C. on the scales
   D. in the gills
   E. none of the above

9. ____ In Denaturing Gradient Gel Electrophoresis (DGGE), DNA molecules are separated by...
   A. G+C content
   B. denaturation point
   C. length
   D. sequence complexity
   E. none of the above

10. ____ “Unifrac” is ...
    A. a measure of the similarity of microbial populations
    B. a 3D plot of principle-component analysis
    C. a tree-generation algorithm
    D. a giant computer from the 1960’s
    E. none of the above

11. ____ A “phylogenetic probe” is ...
    A. A fluorescently-labeled oligonucleotide complementary to a region of rRNA used in FISH experiments
    B. a heavy-isotope-labeled growth substrate used to label rRNA in SIP experiments
    C. an ion-specific probe coated in specific oligonucleotides that can measure organisms in situ
    D. a program that scans metagenomic sequences for phylogenetic signal to identify the source organism
    E. none of the above

12. ____ The biggest advantage of using terminal RFLP to identify organisms in an environment is ...
    A. it’s well-established and reliable
    B. it’s quantitative
    C. it avoids PCR and the associated artifacts
    D. you don’t have to sequence anything
    E. none of the above
13. If you want to determine in a Stable Isotope Probing (SIP) experiment who in an environment is fixing nitrogen, the stable-isotope probe would be …
   A. $^{13}$CO$_2$
   B. a $^{32}$P-labeled rRNA primer
   C. $^{15}$N$_2$
   D. $^{133}$Cs-tetrafluoroacetate
   E. none of the above

14. Proteorhodopsin is a light-driven …
   A. Cl$^-$ pump
   B. H$^+$ pump
   C. Ca$^{++}$ channel
   D. ATPase
   E. none of the above

15. Which of the following can be used to argue that genes were acquired by horizontal transfer?
   A. clusters of genes with unusual (for the organism under examination) codon bias
   B. some regions have G+C contents that are different than that of the genome as a whole
   C. Many genes are more related to their archaeal homologs than to those of Bacteria
   D. all of the above
   E. none of the above

16. A “metagenome” is …
   A. the complete genetic complement of an environment
   B. the complete genomes of all organisms
   C. the complete genetic complement of an organism
   D. the complete genomic content of a metazoan
   E. none of the above

Short-answer questions (5 points each)

17. Why is cloning necessary in a traditional ssu-rRNA survey of a microbial population?
18. What is proteorhodopsin?

22. Describe the most interesting thing you learned from any of the papers we reviewed in class. Make sure to include why you think this is interesting - what does it mean to you?
Essay questions (10 points each)

18. Describe *in detail* how *one* of the following microbial communities was surveyed:
   - The human skin microbiome
   - Chloroflexi in wastewater sludge
   - Bacteria in different temperature zones of a Tibetan hot spring
   - Oral microflora before and after treatment for periodontal disease
19. Describe *in detail* any paper we’ve discussed in this course. You *are* allowed to use papers we reviewed in the Discussion session if you wish. **YOU ARE NOT ALLOWED TO USE ANY PAPER YOU’VE ALREADY DESCRIBED IN THIS EXAM!**
Papers reviewed in class:

- Moreno Am, Matz C, Kjelleberg S & Manefield M 2010 Identification of ciliate grazers of autotrophic Bacteria in ammonia-oxidizing activated sludge by RNA stable isotope probing. AEM 76:2203-2211

Papers reviewed in the Discussion sessions (you can use these if you wish):

- Somerville RA 2002 TSE agent strains and PrP: Reconciling structure and function. TIBS 27:606-612