SIO 126L
MARINE MICROBIOLOGY LABORATORY
Hubbs Hall 3300, Winter 2016

This laboratory course will employ current microbiological techniques to explore the diversity of marine microorganisms. Students will carry out experiments focusing on the enrichment, enumeration, identification, and metabolic and physiochemical adaptations of marine microbes. A large component of the course will consist of an independent research project conceived and carried out by the student. In the first part of the course, the students will learn a variety of experimental techniques by setting up and characterizing several microbial enrichments. Labs will also highlight microbial taxonomy, motility, pigment production, and protozoa-bacteria interactions. Students will also be coached during the beginning phase of the course in identifying and designing an independent project.

No textbook will be required, but it will be useful for students to read parts of "Brock, biology of microorganisms" by Michael T. Madigan, et. al (either the 2012 or the 2015 editions, which have been placed on reserve at the BioMed Library); as well as Marine Microbiology: ecology and applications, by Colin Munn (2011), also on reserve.

Additional reference materials:

The Prokaryotes : a handbook on the biology of bacteria : ecophysiology, isolation, identification, applications /, edited by Albert Balows et al., 2nd ed. New York : Springer-Verlag, c1992. 4 volumes. QR100 .P76 1992

The Prokaryotes:Prokaryotic Biology and Symbiotic Associations. Editors**:** Eugene Rosenberg, Edward F. DeLong, Stephen Lory, Erko Stackebrandt, Fabiano Thompson Electronic resource restricted to UCSD IP addresses.

Bergey's manual of determinative bacteriology. John G. Holt et al. (ed.). Baltimore: Williams and Wilkins. 1994. UCSD BML QW 4 B496 1994
Newer editions are also available as electronic resources restricted to UCSD IP addresses.

Manual of environmental microbiology (Editor in chief, C. J.Hurst) 2007. Electronic resource. Restricted to UCSD IP addresses. This is an excellent resource and provides background on the theory and methods of much of the work we will be doing in this lab. I strongly recommend consulting this as you are thinking about your independent project.

In addition, detailed protocols and references for each laboratory period will be provided by the instructor. The class will entail two four-hour lab periods per week, Tuesday and Thursday from 1–5 pm, and additional independent time outside of the scheduled lab time.

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Teaching Assistant: Raffaela Abbriano (rabbriano@ucsd.edu), 3100 Hubbs Hall

Lab Manager: Dr. Orna Cook (ocook@ucsd.edu), 3420 Hubbs Hall

Learning Objectives:
By the conclusion of the course, students will be able to:
-Use common microbiology lab equipment (pipettemen, spectrophotometers, counting chambers, phase contrast and fluorescence microscopes, PCR machines, gel electrophoresis apparatus)
-Be familiar with the theory and practice of: microbial enrichments, microbial enumeration, microbial isolation, microbial identification
-Find, read, and evaluate the primary literature
-Formulate a hypothesis and design and carry out experiments to test it
-Write a paper in ASM (American Society for Microbiology) style

Grading:

Taxonomy lab report:	25 points
Extremophile lab report:	25 points
Independent project proposal:	50 points
Independent project paper:	100 points
Independent project presentation:	25 points
Laboratory notebook:	25 points

You will be expected to keep a formal laboratory notebook for all of the experimental work you do in lab. Instructions on how to keep a lab notebook are included in the course manual. The notebook will be collected once during the course and will be graded.
Participation: 50 points
Students are expected to attend every class and attendance will be taken. If you are unable to attend lab for any reason, please email me or Raffi Abbriano ahead of time. You are expected to be on time, as class will start promptly at 1 and instructions for that day's activities will be given then. Although several of the exercises will be carried out with a lab partner, students are expected to perform and/or be familiar with all activities and procedures. Students are expected to clean up after themselves and to make sure their work areas are tidy before leaving.

Required materials:
-Bound laboratory notebook with carbon paper (bookstore)
-Safety glasses (bookstore)
-Lab coat (bookstore)

LAB SCHEDULE

Week 1:
Tuesday, January 5
A. Course overview
B. Lab safety
C. Check out lab equipment
D. Pipetting and dilutions
E. Sterile technique, plating and inoculation methods
F. Media preparation and autoclaving
G. Sign and turn in academic integrity agreement, lab safety agreement, equipment responsibility agreement

Thursday, January 7
A. Visualization of microbes (wet mounts, phase contrast microscopy)
B. Enumerating bacteria from seawater
1. Direct counts (SYBR green, and fluorescence microscopy)
2. Viable counts (Plating efficiency on different media (2216, SWT, TCBS))

Week 2:
Tuesday, January 12

A. Count plates and calculate plating efficiency on different types of media
B. Enrich for marine phytoplankton
C. Gram stain
D. How to use UCSD's library resources to research the primary literature
E. Students think about and discuss possible projects.

Thursday, January 14
A. Microbes in motion: swimming, swarming, and gliding
B. Enrich for marine actinomycetes
C. Discussion of assigned paper; how to write a paper in ASM style

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Week 3:
Tuesday, January 19

A. Adaptations of microorganisms to extreme environments
Halophiles/psychrophiles/thermophiles/piezophiles/alkaliphiles

Thursday, January 21
Preliminary proposal due (1 page)
Individual project considerations
Pier microbial community DNA extraction

Week 4:
Tuesday, January 26

Marine bacterial taxonomy
A. Phenotypic characters (growth temperature)
B. Use of Biolog in identification of bioluminescent bacteria
C. 16S rRNA gene amplification
D. Colony amplification

Thursday, January 28
A. Marine bacterial taxonomy
Run gel and prepare template DNA
Set up DNA and primer for sequencing reactions

Week 5:
Tuesday, February 2
A. Analyze sequence data
B. Extremophile report due

Thursday, February 4
Microbial source tracking methods in the evaluation of recreational water quality

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Week 6:
Tuesday, February 9
A. Analysis of phytoplankton enrichment
B. Flow cytometry
C. Extraction and analysis of pigments from various groups of phytoplankton

Thursday, February 11
A. Project proposal is due
B. Microbial source tracking

Week 7:
Tuesday, February 16
Independent project work

Thursday, February 18
A. Respiration and adaptations of microorganisms to alternative electron acceptors. Nitrate reducers/denitrifiers, b) Sulfate reducers, c) dissimilatory metal reducers
B. Microbes and metals, roles of siderophores in iron uptake
B. Independent project work
C. Taxonomy Report due

Week 8:
Tuesday, February 23
Open lab for individual project work

Thursday, February 25

Open lab for individual project work

Week 9:
Tuesday, March 1
Independent project work

Thursday, March 3
A. Bacteria-protozoa interactions
B. Independent project work

Week 10:
Tuesday, March 8
Open lab for individual project work

Thursday, March 10
Lab clean up
Hand in independent project report
Independent project presentations
