Aquatic Microbiology Laboratory, MB 422/522

Spring

Instructor: Dr. Andrew Thurber

Contact Info: athurber@coas.oregonstate.edu, 737-4500, Burt 214

Office Hours: Tuesdays 9-10am and by appointment, Burt 214.

Overview: In this course we will be sampling from the headwaters of the rivers to offshore Oregon to look at the patterns of microbiological communities and how they relate to environmental factors. The goal of this course is field to lab – sample to synthesis where we will start by collecting samples, processing those samples, analyzing the resultant data, and presenting those results in both oral and written form. We will be focusing on the microbial ecology of freshwater and marine systems as a foundation for discovery and learning using techniques.

Meeting Time: Mondays 1-5 pm in addition to two (required) field sampling trips.

Mandatory Field Trips: This course requires two Saturday meeting times that will take the entire day both days.

Course Credits: 2

Course pre-reqs: MB 422: MB 303 (or equivalent) and MB 314 (either concurrent enrollment or previously taken) or approval by instructor
MB 522: Graduate Standing and approval by instructor.

Required Texts: None. Peer-reviewed literature and methods papers will be available on Canvas.

Lab Fees: Lab fees of $33 per student are required to cover reagents used in this course.

Grading 422:

<table>
<thead>
<tr>
<th>Field and lab notebook (8)</th>
<th>10 points per week</th>
<th>80 points</th>
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<tbody>
<tr>
<td>Final Paper</td>
<td>50 points</td>
<td>50 points</td>
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<tr>
<td>Paper summaries</td>
<td>5 points each (4 total)</td>
<td>20 points</td>
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<td>Assignments</td>
<td>10 point (2 total)</td>
<td>20 points</td>
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<tr>
<td>Project presentation</td>
<td>30 points</td>
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<td><strong>Class total</strong></td>
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<td><strong>250 points</strong></td>
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Grading 522:

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<tr>
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<td>50 points</td>
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Grades: A = 93-100%; A- = 90-92%; B+ = 87-89%; B = 83-86%; B- = 80-82%; C+ = 77-79%; C = 73-76%; C- = 70-72%; D+ = 67-69%; D = 63-66%; D- = 60-62%; < 60% = F. Final grades MAY BE curved at instructor discretion.

If you choose S/U grading, you need to get at least 70% (C-) to get an “S.”

While canvas will be used to convey grades, its accuracy is not guaranteed. However, please do convey any errors to the instructor if you spot them.

Overview of assignments:

Field and lab notebook: You are expected to take clear and concise notes on the activities performed in the lab and field throughout this course. This will require a specific hard bound (not spiral) notebook that must accompany you to every class and in the field. Copies of the notebook must be turned in the week following the lab and it will be inspected as you leave to make sure it has been filled out. You are encouraged to take notes in the notebook and keep track of data analysis as well. We will discuss exact expectations of this during the first class period.

Final Paper: A final synthesis paper will be required in which an aspect of the samples collected need to be analyzed and discussed. In this paper you will be required to have at least 10 citations to peer-reviewed papers published in the last 10 years. See schedule for paper topic identification deadlines. No more than 10 pages (double spaced) for students enrolled in 422 and 15 pages in total for students in 522.

Paper summaries: Throughout the course we will read specific peer-reviewed literature articles about the techniques we will use and or habitats that we are sampling. These are available on Canvas. A 1 paragraph, typed, half page summary of the papers on due on the day the papers are discussed. They must specifically state (1) the hypothesis of the paper, (2) the techniques used, and (3) the scientific advance that resulted. We will then discuss the paper at the beginning of class and when you participate in the discussion, you may turn in your summary to get credit.

Assignments: Throughout the course there will be 2 assignments which can be viewed as key points in the overall projects being undertaken. Please see the schedule for specifics.

Independent Data Project (522 only): The samples collected provide an opportunity to analyze a diversity of other components of the ecosystem that compliment the sequencing that we do. For this project an additional analysis will be decided on, in consultation with the instructor, and the analyses will be performed. Examples including Chlorophyll/ Pheopigment analyses, nutrient concentrations, or Org-C composition. The results will need to be provided in table form at the end of the course in included in the final project presentation and paper.

Project Presentation: You will prepare and present a 15 minute presentation on the topic that you have chosen to write your final paper on that uses the samples collected within the course. You are expected to follow the standard outline: Introduction, Hypothesis, Methods, Results, and Discussion format – most likely in PowerPoint or similar.
Statement Regarding Students with Disabilities: Accommodations for students with disabilities are determined and approved by Disability Access Services (DAS). If you, as a student, believe you are eligible for accommodations but have not obtained approval please contact DAS immediately at 541-737-4098 or at http://ds.oregonstate.edu. DAS notifies students and faculty members of approved academic accommodations and coordinates implementation of those accommodations. While not required, students and faculty members are encouraged to discuss details of the implementation of individual accommodations.

This course will include extended time on a research vessel within cramped spaces. In addition, field excursions will include 1-2 hour hikes on uneven trails and collection in mudflat areas that can only be reached by trails (and are muddy). The instructor is dedicated to be inclusive for all students. Please contact the instructor to discuss any mobility concerns and how to be successful in this course and accommodations that can be made.

Reach Out for Success: University students encounter setbacks from time to time. If you encounter difficulties and need assistance, it’s important to reach out. Consider discussing the situation with an instructor or academic advisor. Learn about resources that assist with wellness and academic success at oregonstate.edu/ReachOut. If you are in immediate crisis, please contact the Crisis Text Line by texting OREGON to 741-741 or call the National Suicide Prevention Lifeline at 1-800-273-TALK (8255).

MB 422/522 Course Policies

- Class information is posted on Canvas. Students must have an operational ONID account to access class material.
- Correspondence: email correspondence with the instructor must be done with a students’ ONID address. Please reference the course number in the subject line of your email (I get >100 emails a day, please make it easy for me to find yours). You may also directly contact me through Canvas.
- Grading: it is the student’s responsibility to look over all graded papers carefully upon return. Students have 2 weeks from the time that papers/exams are returned to contest a score. Any grade adjustments made after the 2 week period are at the instructor’s discretion.

The following information is summarized from the OSU Student Conduct Code:

http://oregonstate.edu/studentconduct/home/

Students are expected to be honest and ethical in their academic work. Academic dishonesty is defined as an intentional act of deception in one of the following areas:

- cheating - use/attempted use of unauthorized materials, information or study aids
- fabrication - falsification or invention of any information
- assisting - helping another commit an act of academic dishonesty
- tampering - altering or interfering with evaluation instruments and documents
- plagiarism - representing the words or ideas of another person as one's own
When evidence of academic dishonesty comes to the instructor’s attention, the instructor will document
the incident, present the evidence to the student so they may make a formal statement regarding the
allegation, and make a recommendation to the Dean as to the action that they recommend. **The
professor is under obligation to report all breaches of academic dishonesty to the College and University.** All final decisions about the penalties to the student are made by the Dean and University. These often range from mild (zero on assignment) to an F in the course, and in most cases are
accompanied by an indication on the student’s transcript that they breached the academic code of
conduct for dishonesty. **Please do not cheat.**

The goal of Oregon State University is to provide students with the knowledge, skill and wisdom they
need to contribute to society. Our rules are formulated to guarantee each student’s freedom to learn
and to protect the fundamental rights of others. People must treat each other with dignity and respect
in order for scholarship to thrive. Behaviors that are disruptive to teaching and learning will not be
tolerated, and will be referred to the Student Conduct Program for disciplinary action. **Behaviors that
create a hostile, offensive or intimidating environment based on gender, race, ethnicity, color,
religion, age, disability, marital status or sexual orientation will be referred to the Affirmative Action
Office.**

**Student Learning Outcomes for MB 499- Aquatic Microbiology Lab**

1. Understand the use of history and current microbiological methods used to quantify the distribution
   of bacteria and archaea
2. Demonstrate the ability to perform basic microbiological techniques used to quantify aquatic
   microbial communities.
3. Accurately report observations from both field sampling and laboratory analysis of microbial systems.
4. Acquire basic field sampling skills including freshwater, estuarine, and oceanographic approaches.
5. Demonstrate an appreciation of the diversity of microorganisms, using specific examples.
6. Familiarize yourself with primary literature and how to access it.
7. Analyze and compare the components of microbiological issues and communicate this analysis clearly
   and concisely in writing

**Student Learning Outcomes for MB 522 in addition to 422 Learning outcomes**

1. Demonstrate the ability to analyze samples independently for a state variable
2. Increase one’s knowledge about environmental data that is often used in the field of environmental
   microbiology (standard curves, assumptions of analysis, analytical techniques)
3. Acquire skills in comparing microbial and environmental data sets using multidimensional frameworks
4. Synthesize data and primary literature in written and oral format
Learner Expectations:

1. Attend lab (on time) and stay the entire lab period.
2. Read laboratory exercises in lab manual before they are to be performed.
3. Bring lab manual to class with days goals outlined.
4. Participate in learning activities and complete tasks on time.
5. Be a good team player and do not let other members of the team do all the work. There will be two very long days in the field, be prepared with a good attitude and enjoy the experience.
6. Communicate with the instructor and seek out help when needed.

Notes on drugs and alcohol use: This is a field course but as with all Oregon State University courses, drug and alcohol use is strictly forbidden. As we will be going on a UNOLS Oceanographic Research Vessel, we are also under the auspices of the US Coast Guard making the punishments to both individuals and the university severe (including impoundment of a 170 ft research vessel and jail time). This is not the time nor place for drugs and alcohol whether it is legal for you to take them in other scenarios. This includes substances that are legal in Oregon but controlled at the Federal level.

Schedule:

<table>
<thead>
<tr>
<th>Date</th>
<th>Plan</th>
<th>Due</th>
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<tr>
<td>March 30</td>
<td>Week 1: Background and Planning</td>
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<tr>
<td>April 4/TBD</td>
<td>Field Trip 1 – Oceanus or Andrews Forest</td>
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<tr>
<td>April 6</td>
<td>Week 2: Estuary Trip</td>
<td>Lab notebook 1.</td>
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<tr>
<td>April 11/TBD</td>
<td>Field Trip 2 - Oceanus or Andrews Forest</td>
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<td>April 13</td>
<td>Week 3: DNA extraction</td>
<td>Lab notebook 2 &amp; 3; Paper discussion 1</td>
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<td>April 20</td>
<td>Week 4: Finalize DNA extraction, PCR, state variables</td>
<td>Lab Notebook 4; Assignment 1; Paper discussion 2</td>
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<td>April 27</td>
<td>Week 5: DNA quantification, gels, and clean-up</td>
<td>Lab Notebook 5</td>
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<td>May 4</td>
<td>Week 6: Data Processing</td>
<td>Lab Notebook 6; Assignment 2; Paper discussion 3</td>
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<td>May 11</td>
<td>Week 7: Community analysis of biological communities</td>
<td>Lab Notebook 7; Paper discussion 4</td>
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<tr>
<td>May 18</td>
<td>Week 8: Data Processing / Figure generation and discussion.</td>
<td>Lab Notebook 8</td>
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<tr>
<td>June 1</td>
<td>Week 9: Final Paper due</td>
<td>Final Paper</td>
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**Schedule:** This course requires significant resources to be in place to follow the schedule exactly, including sequencer and ship availability. In certain cases this may require changing of the schedule after the start of the quarter, however Canvas will always have an updated field and lab plan.

**Week 1: Background and Planning:** This week will largely be a discussion of:

1. expectations in the course and notebooks
2. Lab, Field, and Ship safety.
3. Overview of methods employed
4. Introduction to Qiime and Statistics

Assignment 1 assigned: Complete the Qiime tutorial on mock data. *Due Week 5.*

**Sampling trip 1:** Scheduling dependent either Andrews LTER or R/V Oceanus. For both, please bring weather appropriate clothes including 1 layer more than you expect to need. **Plan on getting wet, cold, and tired so bring a disposition and clothes to allow you to enjoy the experience in light of these challenges.**

- R/V Oceanus: Depart OSU at 5am with estimated return at 10pm.
- Andrews LTER Forest: Depart at 9am with estimated return time at 5pm.

**Week 2: Estuary Trip.** We will depart and head back to the coast for a short but intense sampling of the Yaguina bay focusing on water column samples and sediment samples. Plan on getting muddy.

*Due:* Field notebook 1.

**Sampling trip 2:** Either Andrews or RV Oceanus – whichever was not carried out in the first term.

**Week 3: DNA extraction:** This week we will use DNA extraction kits (Qiagen powersoil kits) to extract DNA from samples from across the different sampling locations.

*Paper discussion 1:* Microbial biogeography: putting microorganisms on the map. Hughes Martiny et al. 2006

*Due:* Field notebook 2 and 3.

**Week 4: Finalize DNA extraction, PCR, state variables:** This week we will finalize any final DNA extraction steps needed, setup the PCR, and discuss state variables including chlorophyll and organic carbon quantification.

*Due:* Lab Notebook 4 and Assignment 1

*Paper discussion 2:* Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. Ramirez et al. 2014

**Week 5: DNA quantification, gels, and clean-up:** This is the week where we do the final steps getting the samples ready for sequencing. This includes cleaning up the DNA, making sure that there is no contamination brought about by the PCR by running the samples on a gel, and then cleaning up the DNA. Finally we will quantify the DNA using a spectrophotometric technique (Qbit) and calculate the
amount of DNA from each sample that is needed to have equal concentrations across all of the samples. At the end of this the samples will be submitted for sequencing.

Due: Lab Notebook 5

Week 6: Data Processing: This week we will (hopefully) have data from the sequencer to process and work through and begin processing. We will analyze the chlorophyll and organic carbon samples that we previously prepared for analysis. We will also discuss peer review literature, what it is and is not and ways at OSU to access.

Due: Lab Notebook 6


Assignment 2 due: Write a 1 page description contrasting the three environments sampled and expected differences in microbiota (taxa that may be present or not; diversity patterns, etc). This will form the basis for your final research paper.

Week 7: Community analysis and statistics for biological communities. In this week, we will have a discussion of how we compare communities across environments, methods to do it and what the different methods try and answer. We will then break into groups and work on the various challenges that each of us have on our projects.

Due: Lab Notebook 7

Paper discussion 4: Free choice. Bring in a paper from the peer-reviewed literature and be prepared to share it with the class. Take one figure from the paper and use that as a mechanism to convey the take home points of the paper. No more than 5 minutes please.

Week 8: Data Processing / Figure generation and discussion. For this week, we will discuss different mechanisms to generate figures that help convey information. The main point of this week is a short presentation by everyone (10 minutes max) on their topic.

Project presentation: Present the topic of your final paper and those data that you are going to use within your paper.

Due: Lab Notebook 8

Week 9: Final paper due.