Environmental Microbiology Essentials Laboratory
EVPP306 – Fall 2019
(Updated 12 Aug 2019)

Lecturer:	Dr. Jennifer Salerno
Office:	David King Hall
Office Hours:	By appointment
Email:	jsalerno@gmu.edu
Lecture Time:	Tuesdays, 1:30 - 4:15 p.m.
Lab Location:	David King Hall 3021
Text:	Lab manual provided in a downloadable format on Blackboard.

Because we constantly evolve EVPP306 to take advantage of changing opportunities and improved methods, we update our lab manual each time we teach the lab. The manual will be available in the most updated version on Blackboard. Having a hard copy of the lab manual during the lab session is essential. It provides you with procedural directions that can be easily followed, data entry forms, and questions to be answered. You will be answering the questions and submitting the hard copy to your instructor. You can choose to print each section as needed and bring that to class, or print the entire manual. It is highly recommended that you read over the lab manual before class.

DATE	TOPIC
Aug 27	Introduction to Laboratory **Ex. 1- 4**; Lab and Microbiological Safety **Ex. 5 (start)**; Human microflora - can you wash to sterility? **Discuss Bee Gut Microbiome Project**
Sep 3	**Ex. 5 (cont'd)**; Human microflora - Examine plates from experiment and record observations from plates in lab notebook **Bee Gut Dissection,** prep bee gut samples for DNA extraction, inoculate on TSA plates, preserve bee guts for microscopy **Ex. 6 (start)**; Light Microscopy – microscope assignment, use, calibration, storage (be sure to record your individual microscope number)
Sep 10	**Lab Manual Questions Due (Ex. 4, 5)** **Ex. 6 (cont'd)**; Light Microscopy - Bacterial Observations – view prepared slides, illustrate shapes and sizes with colored pencils **Ex. 7**; Aseptic Technique, Streak Plate (using colonies from bee guts) **Ex. 8**; Simple Stain
Sep 17	**Quiz #1, Lab Manual Questions Due (Ex. 6, 7*, 8)** **Ex. 7 (cont'd)**; Check results of Streak Plate - *answer related questions in lab before turning in **Ex. 23;** Identifying Unknown Bacteria - Read intro paragraphs (no need to prepare slants at this point) **Ex. 24;** DNA extraction from whole bee guts (isolates extracted later)

Sep 24 **Bee Gut Microbiome Project – Introduction Due**
Ex. 9; Winogradsky Column Construction
Ex. 10; Practice Gram stain procedure on provided cultures
****Prepare two slants and one broth culture from your bee gut streak plate (Refer to Ex. 23 for slant preparation)**

Oct 1 **Quiz #2, Lab Manual Questions Due (Ex. 9, 10)**
****All tests performed on bee gut isolate**
Ex. 10; Perform Gram stain
Ex. 11; Oxidase Test
Ex. 12; Motility tests – hanging drop, stab MTM cultures*
Ex. 13; Oxygen Requirements – inoculate FTM*
***Come in the day after your lab to check and record the results of these cultures**!
RECORD RESULTS FROM ALL OF THESE TESTS IN SPREADSHEET!

Oct 8 **Bee Microbiome Project – Methods (culture dependent) Due**
Ex. 9; Check Winogradsky Columns
Ex. 12 (cont'd); Motility tests for bee gut isolates - flagella Ryu stain
Ex. 24 (cont'd); PCR amplification (prep for sequencing)

Oct 15 **NO LAB – FALL BREAK**

Oct 22 **Quiz #3, Lab Manual Questions Due (Ex. 11, 12, 13)**
Ex. 14; Period 1: Coliforms by membrane filter and MPN (water samples)
Ex. 15; Fungal culture (humidity chamber), budding in yeast

Oct 29 **Bee Microbiome Project – Methods (DNA extraction)**
Ex. 14 (cont'd); Period 2: Confirm MPN (BGLBB)
Ex. 15 (cont'd); Fungal humidity chamber observations, observe and draw fungi from slides **(google search and report out)**

Nov 5 **Quiz #4, Lab Manual Questions Due (Ex. 14, 15, 24)**
Ex. 14 (cont'd); Period 3: Isolate coliform EMB agar
Ex. 16, 17; Spore, acid fast stains known and unknowns
Ex. 24 (cont'd); Gel electrophoresis

Nov 12 **Bee Microbiome Project – Methods (PCR) Due**
***Check results of EMB agar plates from last lab**
Ex. 18; Bacterial Enumeration by direct microscopic method (bee guts)
Ex. 19; Bacterial Growth curve

Nov 19	**NO QUIZ, Lab Manual Questions Due (Ex. 16, 17, 19)** **Ex. 24**; Molecular identification of unknowns – BLAST and bioinformatics for community analysis **Discuss results of data table in Ex. 23**
Nov 26	**Ex. 24**; Molecular identification of unknowns – BLAST and bioinformatics for community analysis **Discuss results of Bee Microbiome Project - make graphs**
Dec 3	**Bee Microbiome Project – Results and Discussion Due** Work on Lab Reports and Review for Exam
Dec 17	**Lab Final Exam**: 1:30 – 4:15 p.m. (David King Hall 3021)

CLASS GOALS: EVPP 306 laboratory introduces the science and practice of microbiology for environmental science and other environmentally-focused students. In the study of the environment, microbiology often plays a central role. For example, secondary wastewater treatment is essentially a microbial reactor designed to metabolize organic matter. Our current standard for drinking water quality and shellfish sanitation is based on detection and quantification of coliform bacteria (*E. coli* in Virginia). Even "swimming in the Potomac River" as part of a triathlon is dependent on *E. coli* abundance in the river. Elimination of many xenobiotics from the environment is by microbial metabolism. A variety of human diseases are based on endemic bacterial species "re-inoculating" human populations on a repeated basis. Zoonoses, disease transmission between animals and humans, are quite common. Your understanding of environmental processes will be well supported by a broad understanding of the principles of general environmental microbiology.

The laboratory portion of the course emphasizes the methods by which various microbes can be safely handled, cultured, identified, and enumerated. This is likely your first practical experience with the laboratory study of microbes. The lab will introduce an entirely new class of organisms, a new vocabulary, and new techniques. At the completion of the laboratory, students should understand and be proficient in aseptic technique, light and epifluorescent microscopy, and in the basic techniques used to isolate, maintain, and identify microorganisms. Although you will not become practicing microbiologists from a one semester course, you will have the basic grounding to appreciate the importance of microorganisms and the techniques employed in their study, and to go on to more advanced courses in microbiology. For example, you could take our course in microbial ecology (EVPP643) – which would be especially valuable if you were interested in an accelerated BS/MS degree.

Our class will conduct an in-depth study of the bee gut microbiome. We will learn how to dissect bees, isolate and grow bacteria from their guts, extract genomic DNA, and identify the microorganisms that are present. This research study will be a collaborative effort among all students in EVPP305/306.

ATTENDANCE at the laboratory sessions is MANDATORY because, logistically, we cannot repeat a previous set of exercises and because each week's work will build upon previous exercises. Skills learned earlier will be required to successfully accomplish subsequent tasks. Because we will be working with living organisms, many of the exercises require that cultures be incubated for 24 to 48 hours after inoculation. This will require that **you make arrangements to be in the laboratory outside of scheduled hours**. Sometimes cultures can simply be refrigerated until the following lab meeting, but other exercises will require that tests be conducted immediately following the incubation period. If you are well prepared, this should not take an extraordinary amount of time. We will try to make arrangements within the class to reduce the inconvenience as much as possible.

LABORATORY RULES are listed in your lab manual. These rules are designed for your safety. Please make yourself familiar with these rules and follow them. Although we do not employ VIRULENT pathogens in this laboratory, any microbe is potentially hazardous when present in high concentrations. We will be growing bacterial cultures from natural environments and these must be treated as if they were pathogens. Perhaps the greatest acute hazard is the threat of fire in the laboratory. Be very careful in your use of open flames. If you have any questions about procedures in the laboratory, do not hesitate to discuss your concerns with your lab instructor(s).

GRADING:	Quizzes	25% (4x10 pts/quiz)
	Lab Manual Questions	25%
	Project Reports	25%
	Final Exam	25% (half practical, half written)

Grading Scale:

A+	96-100%
A	93-96%
A-	90-92%
B+	87-89%
B	83-86%
B-	80-82%
C+	77-79%
C	73-76%
C-	70-72%
D	60-69%
F	<60%

There will be four quizzes during the semester. Each will cover materials from the previous (two or three) laboratory periods (see syllabus for scheduled quizzes). The schedule allows a week for review and clarification. They will cover both conceptual and applied aspects of the exercises. The laboratory final exam will be cumulative and will include materials from the last three laboratory sessions. Additionally, in the final exam you will need to demonstrate your ability to successfully accomplish specific tasks (e.g.

streak plating for microbial isolation, gram stain, use of microscopes) in the laboratory. There will not be formal laboratory reports for each exercise. However, you will be required to keep a written record containing data, observations, analyses and drawings and answers to questions. You can do this using the pages in the laboratory manual. These pages will be collected and graded - specific due dates are in the syllabus. Lab manual questions will cover material from the previous two or three weeks of lab work.

This semester, we will be investigating the bee gut microbiome. In teams of two or three, you will develop a data-focused lab report of this investigation. The due dates for sections of the report are listed in the syllabus. We will guide you on data collection and analysis and will discuss results in lab. Your reports should contain the standard sections found in a formal lab report: Abstract, Introduction, Methods, Results and Discussion. At the end of the semester there will be an opportunity in lab to discuss what we have discovered. Grading will focus on the approach to the investigation, the results obtained, and analysis of those results.

Academic Integrity:

The main concern here is that you act as professionally as possible and that you not mistakenly act in manner that would be taken badly. One main issue comes up, of course – that is plagiarism. We all know it is easy in most cases to cut and paste others' words and put them in a document with our names on it. Clearly that is unacceptable. I have had some experience with cases in which student did not know the boundaries of plagiarism and so blundered into problems – sometimes severe ones. So if you have any question about what is acceptable and what is not, chat with your instructor. Especially, do not wait to work on your reports and any presentations so that you feel pressured. Get started early and work consistently on your reports and the class project. For various lab reports we will ask that you develop your own analyses. Discussions on approaches within the class are appropriate, but your report needs to be your own work. More discussion on that later.

Below are some statements from Mason.

MASON ACADEMIC INTEGRITY STATEMENT:

GMU is an Honor Code university; please see the University Catalog for a full description of the code and the honor committee process. The principle of academic integrity is taken very seriously and violations are treated gravely. What does academic integrity mean in this course? Essentially this: when you are responsible for a task, you will perform that task. When you rely on someone else's work in an aspect of the performance of that task, you will give full credit in the proper, accepted form. Another aspect of academic integrity is the free play of ideas. Vigorous discussion and debate are encouraged in this course, with the firm expectation that all aspects of the class will be conducted with civility and respect for differing ideas, perspectives, and traditions. When in doubt (of any kind) please ask for guidance and clarification.

GMU EMAIL ACCOUNTS
Students must activate their GMU email accounts to receive important University information, including messages related to this class. Your instructors are required to use your Mason email account.

OFFICE OF DISABILITY SERVICES
If you are a student with a disability and you need academic accommodations, please see me and contact the Office of Disability Services (ODS) at 993-2474. All academic accommodations must be arranged through the ODS. http://ods.gmu.edu

OTHER USEFUL CAMPUS RESOURCES:
WRITING CENTER: A114 Robinson Hall; (703) 993-1200;
http://writingcenter.gmu.edu
UNIVERSITY LIBRARIES "Ask a Librarian"
http://library.gmu.edu/mudge/IM/IMRef.html
COUNSELING AND PSYCHOLOGICAL SERVICES (CAPS): (703) 993-2380;
http://caps.gmu.edu
UNIVERSITY POLICIES
The University Catalog, http://catalog.gmu.edu, is the central resource for university policies affecting student, faculty, and staff conduct in university affairs.
OFFICE OF THE OMBUDSMAN: (703) 993-3306;
http://ombudsman.gmu.edu
The Office of the Ombudsman is a confidential, impartial, informal and independent problem-solving and conflict resolution resource for all students of the George Mason University community.