

SYLLABUS

BIOL 5480 – Comparative Physiology

Meeting Times: Tuesdays 9:30 – 10:50 AM & 1:00 – 2:50 PM; Thursday 9:30 – 10:50 AM

Instructor: Jim Harper, Ph.D.

Office Hours: MW 10 AM – 12 PM (LDB 100B)

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Course Overview

This is a hypothesis-driven experimental course meant to give students the opportunity to assess physiological function in animals using one of two model systems (**choose one**):

1. Mice. Students will generate hypotheses concerning the effect of psychological stress on the physiology of mice. Because working with vertebrates requires prior approval by the Institutional Animal Care and Use Committee (IACUC) there are limitations regarding what can be done with live animals. See below for the specific protocols that have been approved. There are no limitations on what can be done post euthanasia, however (the mice will be sacrificed at the conclusion of the experimental phase).

Note: ALL students MUST complete the following CITI training modules (<https://about.citiprogram.org/en/homepage/>):

Working with the IACUC

Working with Mice in Research.

Failure to do so will result in an 'F' in the course.

2. Invertebrates. Students may design their own experiment from top-to-bottom using any invertebrate model to examine the effect of a given treatment on a specific physiological response. Invertebrates are not covered by the Animal Welfare Act of 1966, Animal Welfare Act Regulations, and the Health Research Extension Act of 1985 (HREA); hence working with these animals does not require authorization by the IACUC for either research or teaching purposes. It also does not require specific CITI training.

There is no lecture component to this course. It is entirely laboratory-based and the onus is on you to gain the knowledge needed to generate specific hypotheses, as well as specific methodologies best suited to test your hypothesis given time, financial and equipment restraints. We have a spectrophotometer capable of performing colorimetric, luminescent and fluorescent measures, as well as DNA and protein gel apparatus and detection systems. There are also gas and temperature-controlled incubators, as well as a biosafety cabinet available to perform organ and/or cell culture measures, microscopes (dissecting and compound light) and most of the other equipment (such as water baths) needed for routine physiological experiments.

Due to the nature of the course, students will almost certainly have to work outside of the scheduled class times in order to conduct specific assays, *et cetera*. Many of the assays you are likely to need require > 4 or more hours to complete. Sometimes a given assay will require multiple days. ***If you feel this is going to be an issue then I would recommend dropping the course.*** In addition, because the lab space is used for other courses, you will need to be cognizant of who is in the lab when. Most faculty do not mind if you work quietly in the background, but it is better to double-check. You are also welcome to work in my lab space (Room 134) if you can somewhere to setup.

A significant portion of your time for this course, outside of data collection, should be devoted to the analysis and interpretation of these data. ***You will need to generate statistical models and publication grade figures for the final report (see below).***

Course objectives

- To learn fundamental principles of experimental design
- To learn specific techniques used to assess physiological function in animal models
- To learn how to gather and analyze data to refute the validity of specific hypotheses

Particulars (also see Sam Houston State University Student Handbook and Appendix)

- We do not have the resources to allow each student to work independently. Consequently, you are to work together in groups of no more than three (but preferably two) to complete the course objectives.
- **Attendance:** You are expected to attend activities that require group participation. Regardless of whether you choose to use mice for your specific study, some days will require the efforts of the entire class to ensure everything is completed in a timely manner. Once you have begun the experimental/data generating phase in earnest, you will have the choice of attending during set class times dependent on need. Note that I will be available during scheduled class times for guidance and troubleshooting.
- **Grading:** Final grades will be assigned based on ***your cumulative point total*** earned from each of the following:
 1. **Participation (200 points):** This is simple. You must be an active member in your group and attend organized class meeting times. There is no sliding scale; i.e. this is an all or none grade. If you lose 200 points due to a lack of participation, you will fail the course.
 2. **Benchmarks (100 points):** These are simple goals to ensure everyone is staying on track. Failure to do so on the specific date will result in the loss of points for that benchmark (all or none). ***More importantly, complete failure to meet a specific benchmark will automatically disallow you from completing all subsequent benchmarks.*** What this means is that failure to meet any of the benchmarks will almost certainly ensure that you fail the course (Note: although undergraduates can receive a 'D' for the course, graduate students cannot and would receive an 'F').
 - a. Form a group (10 points)
 - b. Submit your hypothesis for approval (25 points)
 - c. Submit experimental design for approval (30 points)
 - d. Submit a provisional analysis of your data (20 points)
 - e. Submit the final figures/tables for approval (15 points)
 3. **Presentations (100 points):** Each group will present their findings to the class. Presentations ***must include*** sufficient background material to provide the rationale for the specific hypotheses tested, as well as an overview of the methodologies used. Remember that each group will not have performed the same assays. In total, your presentation should last about 20 minutes (15 minutes + 5 minutes for questions).
 4. **Final report (100 points):** Each group will submit a paper that is consistent with a 'Short Communication' typical of many journals. By and large, they follow the format of a normal journal article, but are limited in terms of the number of figures and text allowed. For our purposes the format will be:
 - a. Title page
 - b. Abstract (limit 400 words)
 - c. Introduction
 - d. Methods
 - e. Results
 - f. Discussion
 - g. Conclusions
 - h. References
 - i. Figure captions
 - j. Figures
 - k. Table Captions
 - l. Tables

Figures and tables are not typically incorporated into the text when submitting for publication; please refrain from doing so. In the end, the text should be about 2000 – 2500 words and include no more than 2 figures and/or 2 tables (so up to 4 total). You should have at least 10 references in support of your hypothesis as well as to support your interpretation of the data. References concerning methodologies are not necessary unless you have performed a unique or uncommon assay or to provide the rationale for how/why you may have modified an existing protocol.

Also, please adhere to the following guidelines when preparing the text:

- a. **Font:** Tahoma, Arial or Calibri, no larger than 11 pt
- b. **Margins:** 1" margins on all sides
- c. **Line Spacing:** A maximum of 1.5

FINAL GRADE

Letter grades will be assigned based on **total** points earned as follows:

A, ≥450; B, 400 – 449; C, 350 – 399; D, 300 – 349; F, ≤300

Mouse Experimental Design

Two groups of mice (n = 12 per group divided equally among sexes) will be subjected to chronic stress and blood samples collected at specific time points after the stressor is applied (0, 30 and 90 minutes). Blood samples will be drawn via retro-orbital bleeding without anesthesia using standard methods. I have performed this procedure thousands of times over the past 20 years without ill effect and will be responsible for all blood samples collected in this manner; novices can cause significant damage.

Restraint is a classic rodent model to study the physiological effects of stress and is easy to perform with minimal impact on individual mice. To quantify the degree of stress faced by the animal, as well as for the quantification of various analytes of interest, an initial blood sample is drawn (T = 0 or baseline), then animals are confined to a small Sherman Trap for a total of 30 minutes. Immediately afterward, a second blood sample is collected (T = 30) and the mice are returned to their home cage for 60 minutes prior to the collection of a final blood sample (T = 90). About 25 µl of blood will be drawn each time; hence approximately 75 µl of blood is drawn over 90 minutes; mice can easily tolerate a single blood draw of up to 200 µl (personal observation). Chronic stress will be induced by performing this procedure over seven consecutive days. Individual graduate students will be responsible for performing this process restraint after training. Mice will also be weighed periodically using a standard benchtop digital balance.

Approved Procedures for Live Mice

1. Glucose Tolerance Testing: Intraperitoneal glucose tolerance testing (GTT) consists of challenging individual mice with a glucose load after an overnight fast (water will be available *ad libitum*). More specifically, individual mice are injected with a single bolus of D-glucose dissolved in sterile PBS at 2 µg/kg body weight (mice will have been weighed the previous night). Mice undergoing a GTT will experience 3 blood draws (immediately before T=0 and 15 and 30 minutes after injection) via retro-orbital bleeding. Immediately afterward, mice will be anesthetized using CO₂. Large blood samples (>500 µl) can be drawn via cardiac puncture under anesthesia at this time. Afterward mice will be euthanized via cervical dislocation to allow for the collection of tissue biopsies as needed.
2. Immune Challenge: Treating mice with a single i.p. injection of sheep red blood cells (SRBC) represents a simple immune challenge because the SRBCs are recognized as 'foreign' by the mouse's immune system. Here, mice undergo a blood draw (approximately 100 µl) immediately prior to injection via retro-orbital bleeding; 24 hours later, they are anesthetized for the collection of blood samples and tissue biopsies in accordance with the methods used for the GTT. Washed pooled SRBCs will be purchased from Lampire Biological Products and injected i.p. at 2.5×10^8 cells per mouse in accordance with published protocols (Kervleit and Ougthon 1993).

Please note that you are not required to perform either of these procedures. They will only be used if there is a group interested in quantifying the type of data these procedures would generate. Anything else involving live mice is disallowed without prior IACUC approval.

BIOL 4096/5480 – COMPARATIVE ANIMAL PHYSIOLOGY
SPRING 2018 COURSE SCHEDULE

Week	Dates	Tuesday (Lecture and Lab)	Thursday (Lecture & Lab)
1	1/15 – 1/19	No class	Introduction
2	1/22 – 1/26	General Q&A (Group Benchmark)	General Q&A
3	1/29 – 2/2	General Q&A (Hypothesis Benchmark)	Initial Prep (Labeling lots of Tubes)
4	2/5 – 2/9	Begin chronic stress (Experimental Design Benchmark)	Materials compilation (figuring out what to order)
5	2/12 – 2/16	End Chronic Stress/Sample Collection	OPEN TIME
6	2/19 – 2/23		
7	2/26 – 3/2		
8	3/5 – 3/9		
9	3/12 – 3/16	NO LAB SPRING RECESS	
10	3/19 – 3/23		
11	3/26 – 3/30		
12	4/2 – 4/6		
13	4/9 – 4/13		
14	4/16 – 4/20		Provisional Analysis Benchmark
15	4/23 – 4/27		Final Figures Benchmark
16	4/30 – 5/4	Presentations	
17	5/7 – 5/10	Finals Week – Final Report Due	

* The 3 ½ weeks prior, as well as the entire period after the spring recess, is open. This gives you over seven weeks to collect your data and begin analyzing what you have. During this time you may work at your own pace to perform specific assays as needed. Time management will be critical. Leave time for troubleshooting and repeating assays.

APPENDIX – ACADEMIC CALENDAR/CLASS POLICIES/STUDENT CONDUCT

ACADEMIC CALENDAR:

http://www.shsu.edu/~reg_web/academic_calendar/

RESIGNATION NOTE:

Students may resign with a "W" grade from the 13th class day through the last class day, but prior to any final exams being taken or course completion.

DROP NOTE:

Courses may be dropped without a grade of "F" through the last class day, but prior to any final exams being taken or course completion. Students wishing to drop courses after the term's online drop deadline must submit a drop request form, signed by the professor, to the Registrar's Office. To process a "Q" drop after the online drop closes, students must come to the Registrar's Office, Estill Building Rm. 331 between the hours of 8:00 a.m. and 5:00 p.m. No schedule changes may be made after the deadline specified in the Academic Calendar.

I take violations against the Sam Houston State University Code of Student Conduct very seriously and will pursue all available options in the event of a violation. Students are expected to engage in a manner that is above reproach and are expected to maintain complete honesty and integrity in the academic experiences both in and out of the classroom. Any student found guilty of dishonesty in any phase of academic work will be subject to disciplinary action **at my discretion**. The university and its official representatives may initiate disciplinary proceedings against a student accused of any form of academic dishonesty including, but not limited to, cheating on an examination or other academic work which is to be submitted, plagiarism, collusion and the abuse of resource materials. (<http://www.shsu.edu/students/guide/>).

Visitors in the classroom: Only registered students may attend class. Exceptions can be made on a case-by-case basis by the professor. In all cases, visitors must not present a disruption to the class by their attendance. Students wishing to audit a class must apply to do so through the Registrar's Office.

Students with Disabilities: It is the policy of the university that no otherwise qualified disabled student shall, solely by reason of his/her handicap, be excluded from participation in, or denied benefits of, or be subject to discrimination under any academic or Student Life program or activity. Disabled students may request help with academically related problems stemming from individual disabilities by contacting their instructor, school/department chair, or by contacting the university Chair of the Committee for Continuing Assistance for Disabled Students/ Director of Counseling Center. A student with a disability is encouraged to register with the university Counseling Center, as well as contacting their instructor about assistance needs. Accommodation cannot be made until the student has initiated the request with the Counseling Center. Every semester that the student desires accommodations, it is the student's responsibility to complete a Classroom Accommodation Request Form at the Counseling Center and follow the stated procedure in notifying faculty. Accommodations for disabled students are decided upon documentation and need on a case-by-case basis by the Counseling Center. All requests are handled with confidentiality according to university procedures.

Religious Holy Days Policy: Section 51.911(b) of the Texas Education Code requires that the university excuse a student from attending classes or other required activities, including examinations, for the observance of a religious holy day, including travel for that purpose. A student whose absence is excused under this subsection may not be penalized for that absence and shall be allowed to take an examination or complete an assignment from which the student is excused within a reasonable time after the absence. University Policy 861001 provides procedures to be followed by the student and instructor. A student desiring to absent himself/herself from a scheduled class in order to observe (a) religious holy day(s) shall present to each instructor involved a written statement concerning the religious holy day(s). This request must be made in the first 15 days of the semester or the first 7 days of a summer session in which the absence(s) will occur. The instructor will complete a form notifying the student of a reasonable timeframe in which the missed assignments and/or examinations are to be completed.

Disruptive Behaviors: You are expected to adhere to the Sam Houston University Code of Student Conduct. I take violations against this code very seriously and will pursue all available options if students violate this code. See below, or your handbook for details. Violations include but are not limited to:

- a. talking out of turn in lecture,
- b. obscene language,
- c. reading the newspaper,
- d. completing assignments other than Biology 134,
- e. cell phones ringing,

- f. answering cell phones,
- g. text messaging,
- h. cheating,
- i. and plagiarism.

Class Attendance

1. Regular and punctual class attendance is expected of each student at Sam Houston State University.
2. Each faculty member will keep a record of student attendance.
3. Each faculty member will announce the policies for accepting late work or providing makeup examinations. Students are expected to show appropriate cause for missing or delaying major assignments or examinations.
4. A student shall not be penalized for three or fewer hours of absences when examinations or other assigned class work has not been missed; however, at the discretion of the instructor, a student may be penalized for more than three hours of absences.
5. Each instructor is obligated to clarify in writing to each student enrolled in class at the beginning of the semester or summer session the instructor's classroom policy regarding absences.
6. Class absences will be recorded and counted only from the actual day of enrollment for the individual student in that specific class.

FINAL NOTE

The Sam Center (http://www.shsu.edu/~sam_www/) is an outstanding resource available to students to assist with a host of academic and personal issues.