SPRING 2023

# BIOS 11174 Research Experience in Biology

*Research project summaries and registration information*

*This course provides students with an opportunity to work on a semester-long research project in biology. Different research projects are offered in specific sections of the course. Project descriptions and registration details (Table) are provided below.*

**Course prerequisite:** BIOS 11173 Biological Investigations or permission of instructor.

## Research project summaries (see pg. 5-6 for registration details)

### Evolutionary responses of salt marshes to global environmental change (Global Change)

*Laboratory professor: T. M. Olsen*
*Research Investigator: J. McLachlan*

*Sections available: T 2:00-5:00 PM; W 2:00-5:00 PM, Th 9:30 AM - 12:20 PM*

The abundance, distribution and, in many cases, survival of species in the next century will be influenced by an unprecedented combination of altered land-use, climate, and atmospheric chemistry. Anticipating these trends is difficult, but insight can come from how populations have responded to similar past environmental perturbations. The McLachlan lab gathers records of population change and connects them to environmental and biological processes using mathematical models linking the abundance of a foundational plant species inhabiting Atlantic salt marshes (*Schoenoplectus americanus)* to changing environmental variables (salinity, flooding, and atmospheric $CO_2$). Incredibly, seeds of *S. americanus* dating as far back as 150 years are still viable, and clones of these unique plants have been propagated in greenhouse experiments. Genetic experiments have identified and confirmed these “resurrected” plants as distinct genotypes from plants found currently in salt marshes. Consequently, the lab has a continuous record of evolutionary response to environmental change dating back over a century.

Student research teams in this project will examine the evolutionary responses of *Schoenoplectus americanus* to global environmental change using *S. americanus* genotypes “resurrected” from the seed bank and modern genotypes recently collected from the field, conducting greenhouse experiments to explore the effect of nitrogen, sea level and salinity on sedge growth, as well as incorporate molecular assays to identify *S. americanus* and its co-ocurringing conspecific sister species, *S. pungens* and hybrids via cpDNA RFLP and nDNA microsatellite markers.

Data generated by student's experiments are systematically collected each semester and managed by the McLachlan lab for use in their modeling efforts. This course project is part of the NSF grant "*Eco-evolutionary dynamics of coastal marsh response to rising $CO_2$"* awarded to Jason McLachlan and Michael Blum (University of Tennessee.)

This project requires collaboration and creativity in designing and structuring experiments, strong data management skills, solid analysis and writing skills for production of a project research paper and fortitude and attentiveness to successfully maintain living plants in the Jordan greenhouse throughout the semester-long experiment.

Project schedule:

*Project introduction, greenhouse growth experiment initiation and meeting with Dr. McLachlan*

1. Salt marsh ecosystems; *S. americanus* experimental system; Marsh Equilibrium Model
2. Experimental planning and potting of plant replicates
3. Growth experiment set-up in Jordan greenhouses and discussion with J. McLachlan

*Using molecular markers to identify sedge species and hybrids*

4. Isolation and PCR amplification of DNA from *S.americanus*, *S. pungens and* hybrids/Growth experiment maintenance and data collection
5. Restriction digest of cpDNA, electrophoresis of nDNA microsatellites and cpDNA restriction fragments/Growth experiment maintenance and data collection
6. Approaches for data visualization/Experimental maintenance and data collection
7. Statistical methods for data analysis/Growth experiment maintenance and data collection
8. Final week of Growth experiment data collection/start of plant dry biomass determination
9. Completion of plant dry biomass determination

*Project Synthesis Workshop with members of McLachlan lab*

Coastal communities, habitats, and sea-level rise: local to global perspectives and human impact

*Final data analysis and research paper writing*

10. Data analysis, graphing and paper writing clinic
11. Research paper submission

## Protein-DNA interactions in the ESX-1 secretion system of *M. tuberculosis* (Mycobacteria)

*Lab professors: B. Rudenga*
*Research Investigator: P. Champion*

*Sections available: M 2:00-5:00 PM; T 9:30 AM - 12:25 PM; Th 2:00-5:00 PM*

One in four people globally are infected with *Mycobacterium tuberculosis*, the causative agent of Tuberculosis (TB). Although only 5-10% of infected people develop active disease, there are still approximately 1.6 million deaths annually (WHO) making this the second leading infectious

disease killer (behind COVID-19). This burden of infection and disease is in part due to a lack of a viable vaccine that protects adults from pulmonary disease, the most common outcome of TB. To develop the vaccines and therapeutics needed to control the TB epidemic, we first need a better understanding of *M. tuberculosis* biology and the mechanisms that cause disease.

One key mechanism that bacteria use to promote their survival is the targeted transport of bacterial proteins, small molecules, or nucleic acids, directly into the host (secretion). The Champion lab is focused on identifying novel genes and mechanisms required for mycobacterial protein transport. We expect that this course of research will not only expand our understanding of *M. tuberculosis* biology, but may also lead to the identification of novel targets for anti-virulence based therapeutics against TB.

In this project, student research groups will explore the regulation of gene expression in the *ESX-1* secretion system of *M. tuberculosis*. This model examines the connection between an intergenic region of the TB genome and a protein known to negatively regulate gene expression. Each group will create a plasmid containing a specific mutation using a site-directed mutagenesis approach, which incorporates techniques of PCR, electrophoresis, and bacterial transformation. The mutated DNA will be amplified and tested to determine if the mutation affects the ability of the regulatory protein to bind to the intergenic region.

Students will have a chance to further their skills in molecular biology, gain additional experience working in the lab, and will be introduced to the relevant primary literature around mycobacterium secretion. This project requires strong attention to detail both in working at the bench and in keeping lab notebooks, and resilience with troubleshooting procedures when they do not generate the expected result.

<u>Project schedule:</u>

*Introduction*

1. ESX-1 secretion system

*Producing a plasmid construct*

2. Bioinformatics and designing PCR primers for cloning
3. Miniprep isolation
4. Site-directed mutagenesis PCR
5. Electrophoresis and *DPNI* treatments for site-directed mutagenesis
6. Transformation into DH5alpha *E. coli*
7. Miniprep isolation and mutant plasmid conformation

*Evaluating protein-DNA interaction.*

8. PCR amplification of promoter
9. Electrophoretic mobility shift assay for protein-DNA binding

*Data analysis and scientific communication*

10. Data analysis and paper writing clinic
11. Final research paper submission

**From phenotype to genotype: *Daphnia,* mutagenesis, and a whole lotta' gene expression**

*Lab professors: T. Agrelius*
*Research Investigator: M. Pfrender*

*Sections available: M 2:00-5:00 PM; T 9:30 AM - 12:25 PM; Th 9:30 AM - 12:25 PM*

Have you ever wondered what would happen if a gene no longer existed? How would that impact an organism and its perception of the environment? Would the loss of one gene change the expression of other genes?

The microcrustacean *Daphnia* is a keystone species in aquatic freshwater systems. *Daphnia* are used as models for environmental, ecological, evolutionary, and developmental studies due to their high levels of phenotypic plasticity, or rather their ability to respond to changes in their environment. Understanding how *Daphnia* are able to respond to environmental changes presents a fascinating line of investigation.

In this project, students will assemble and screen a gene editing system for *Daphnia* using CRISPR-Cas9 ribonucleoprotein complexes. Student designed behavior assays comparing mutated and wild type animals will address how the loss of a single gene alters light perception, either color or intensity. Finally, RNA will be extracted from both wild type and mutated animals for the assembly of whole transcriptomes. In case you're wondering, a transcript is a single mRNA molecule made by a single gene. Transcriptomics is essentially the study of every gene in an organism and how many mRNA molecules there are for each gene!

Students in the *Daphnia* mutagenesis CURE of BIOS 11174 will learn about *Daphnia*, an NIH-model system, and design experiments that investigate how the mutagenesis of a single gene impacts organismal behavior and gene expression. Students will conduct research in collaboration with Professor Michael Pfrender, Professor and Director of the Genomics and Bioinformatics CORE facility at Notre Dame, and Dr. Sheri Sanders. Students will have the opportunity to interact with Dr. Sanders and learn about the CORE, demystifying "big data" and bioinformatic analyses.

Students will have a chance to further their skills in molecular biology, gain additional experience working in the lab, and will be introduced to the field of bioinformatics. This project requires strong attention to detail both in working at the bench and in keeping lab notebooks, and resilience with troubleshooting procedures when they do not generate the expected result.

Project schedule:
*Introduction*

1. *Daphnia*
2. Bioinformatics and the Conserved Domain Database

*Testing Gene Function and CRISPR*

3. Designing sgRNAs
4. Testing sgRNAs and PCR amplification
5. CRISPR screening

*Behavioral Assays*

6. Light sensing ability in *Daphnia*

*RNA Extractions*

7. Extraction of RNA from both wild type and mutated animals
8. Quantification, purity, and integrity of RNA subunits

*Transcriptomics*

9. Differential gene expression
10. KEGG pathway analysis

*Data analysis and scientific communication*

11. Data analysis and paper writing clinic
12. Final paper presentations

# Registration Information

*Note: approximately ½ of seats in each section are reserved for 1st year students.*

| **MYCOBACTERIA** | | |
|---|---|---|
| Time period | Lab Faculty | Section # (CRN) |
| Monday<br>*2:00-5:00 PM* | Ben Rudenga | 1 (25782)<br>2 (25788) |
| Tuesday<br>*9:30 AM - 12:25 PM* | Ben Rudenga | 3 (25789)<br>4 (25790) |
| Thursday<br>*2:00 AM - 5:00 PM* | Ben Rudenga | 5 (25791)<br>6 (25783) |

| **GLOBAL CHANGE** | | |
|---|---|---|
| Time period | Lab Faculty | Section # (CRN) |
| Tuesday<br>*2:00-5:00 PM* | Mark Olsen | 7 (25784)<br>8 (25801) |
| Wednesday<br>*2:00-5:00 PM* | Mark Olsen | 9 (25802)<br>10 (25785) |

| | | |
|---|---|---|
| Thursday<br>*9:30 AM - 12:25 PM* | Mark Olsen | 11 (25794)<br>12 (25795) |

| **DAPHNIA** | | |
|---|---|---|
| Time period | Lab Faculty | Section # (CRN) |
| Monday<br>*2:00-5:00 PM* | Trenton Agrelius | 13 (25796)<br>14 (25797) |
| Tuesday<br>*9:30 AM - 12:25 PM* | Trenton Agrelius | 15 (25786)<br>16 (25800) |
| Thursday<br>*9:30 AM - 12:25 PM* | Trenton Agrelius | 17 (25787)<br>18 (25799) |