Yara Alshwairikh, PhD student

Fine-scale Liver Transcriptome Profile of the Freeze-thaw Process in Wood Frogs

Research Highlights

- Wood frogs are one of few animals that have freeze tolerance abilities
- Currently, we do not have much information about the winter freezing conditions (*e.g.* hibernacula temperatures, snowpack) that wood frogs endure at Yale Myers Forest
- We also do not fully understand the molecular underpinnings of the freeze-thaw process in wood frogs
- This project explores the gene expression profile of the freeze-thaw process and relate it to the winter conditions that wood frogs experience at Yale Myers Forest

Research Summary

Many animals cope with environmental extremes in ways we do not understand very well. As the climate is changing, understanding those coping strategies and adaptations is more important than ever. A remarkable adaptation of the wood frog (*Rana sylvatica*) is its ability to freeze solid with up to 70% of its body water as ice. The wood frog is the most widely distributed amphibian in North America, occurring from Alabama to Alaska and Arctic Canada. There is high variation in its freeze endurance abilities across its range, such as minimum temperature tolerated and length of time spent frozen. To this day, we still lack data about its freeze endurance abilities and the winter conditions it can tolerate in many parts of its range.

In this project, I wanted to understand the freezing abilities of wood frog populations in Yale Myers Forest (YMF) in Tolland County, Connecticut, USA. My first objective was to record the hibernacula temperatures that wood frogs experience. In Tolland County, average snow fall is 6808 kg m^{-1} y^{-1}, and the county remains below freezing temperatures for an average of 90 days from late Fall through early Spring. Wood frogs hibernate at depths of 2-10 cm below the soil surface, and knowing the temperatures they experience in their hibernacula will allow me to estimate the number of freeze-thaw cycles they may undergo.

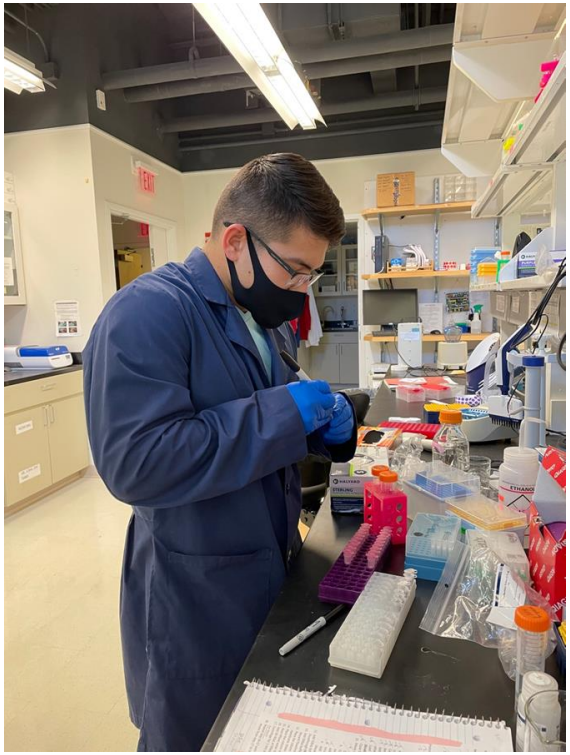
A wood frog (Rana sylvatica) that was frozen in the lab to a temperature of -2.5 °C

Research Summary cont.

My second objective was to examine the molecular regulation of the mechanisms involved in the freeze-thaw process of wood frogs. What genes and pathways are involved, and are they differentially expressed? Answering this question will shed insight on what genes and pathways may be important in this process and reveal new candidate genes for further investigation into their biochemical role in freezing and thawing.

During the fall of 2021, I installed microhabitat temperature loggers at three locations in YMF, each approximately 50m away from the edge of three ponds where wood frogs are known to breed every year. Each temperature station consisted of an approximately 1m-tall metal fence post with three temperature loggers associated with it. The first temperature logger was attached to the top of the fence post to record air temperature, the second logger was placed at the soil surface and covered lightly with leaf litter, and the third logger was placed 10cm below the soil surface. I will retrieve the loggers in early Spring 2022 to collect the data.

I also conducted a freezing experiment on adult wood frogs to explore their gene expression profiles. I collected 6 adult wood frogs from YMF and brought them to the laboratory to run the experiment. I collected tissue samples at different time points throughout their freeze-thaw process: at control levels (*i.e.*, cold, but not frozen frogs kept at 4 ^{o}C), when they were halfway frozen, fully frozen (at -2.5 ^{o}C), and completely thawed (back to 4 ^{o}C). With the help of an undergraduate student, Greg Jazwinski, we extracted RNA from the tissue samples and analyzed the quality of the RNA using a Bioanalyzer. To achieve better results and have more reliable data, I plan to collect more samples next year in 2022. I will repeat the same experiment and my next step will be to send the RNA samples for RNA-sequencing so I can begin data analysis.



Undergraduate student Greg Jazwinski performs RNA extractions from wood frog liver tissue