

***STRATEGIC INITIATIVE  
Undergraduate Research Funding for  
STEM Majors at the University of Idaho  
FINAL PROJECT REPORT***

*Submitted to:  
Higher Education Research Council  
Idaho State Board of Education  
P.O. Box 83720  
Boise, Idaho 83720-0037*

*Submitted by:  
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875 Perimeter Drive  
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November 18, 2022*

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## *Executive Summary*

Undergraduate research is recognized as a high-impact educational practice that increases the rates of student retention and engagement. At the University of Idaho, it is practiced throughout all units on campus, and it is centrally placed in the institution's strategic plan. The Office of Undergraduate Research is taking the lead in enabling research opportunities for undergraduates at UI. It manages various competitive student grant programs that directly support student research.

During AY 2021-22, generous funding from the State Board of Education permitted UI to continue its Summer Undergraduate Research Fellowship (SURF) Program. This intensive multi-week summer research experience actively engages undergraduates in faculty- mentored, independent research. Over the course of 10 weeks, students are mentored toward increased independence on their projects. Each student is provided with a \$4,000 stipend in the form of a fellowship which allows them to devote full time effort to their projects. Each student is also provided with up to \$1,000 to help offset materials and supplies and other project-related expenses based on budget requests and justifications. Selection of student participants is a competitive process in which students submit research proposals to the Office of Undergraduate Research. State Board of Education funding supported 13 SURF awards and our Gen Ed funding supported another 7 during the summer of 2022. Enclosed in this report are Titles, Abstracts, Accomplishments, and Budgets of 18 of the 20.

Funding provided by the State Board of Education also allowed the Office of Undergraduate Research to support several undergraduate researchers during the academic year. This was accomplished through competitive Undergraduate Research Grants awarded to students during the spring semester of 2022. These grants supported semester-long research projects under the guidance of faculty mentors. These grants were in the amount of \$1,000 each for materials and supplies and other project-related expenses. For Fall of 2022, five projects were awarded funding. Names and titles for those five are also included. Those students will be required to present their research accomplishments at the April 2023 OUR symposium.

UI students supported by State Board of Education funds attended and presented the results of their projects at the 2022 Virtual Idaho Conference on Undergraduate Research held in Boise in July of 2022. These students will also be required to present their results at the UI Undergraduate Research Symposium in April 2023. Most significant about these awards are the many colleges that they touch. More than just the STEM colleges (College of Science, COS; College of Engineering, COE), these awards went to deserving students in the College of Letters and Social Science (CLASS), the College of Agriculture and Life Sciences (CALS), the College of Natural Resources (CNR), and the College of Education and Health and Human Sciences (CEHHS). End-of-project feedback from students and their mentors was overwhelmingly positive. Significantly, none of the undergraduate research projects described here would have been possible without the support provided by the State Board of Education. We sincerely thank the Higher Education Research Council and the Idaho State Board of Education for making these experiences possible for our students.

As for my role, I took on the task of Acting Director in the Fall of 2022 after working with the OUR for six years serving as the one Faculty Associate. We have now grown to have Faculty Associates in all Colleges (that feature undergraduates) and we are increasing our visibility to students in anticipation of growing the undergraduate research community and the distinction that undergraduate research brings to students.

Kristopher V. Waynant  
Acting Director of the Office of Undergraduate Research  
Assistant Professor of Chemistry  
[kwaynant@uidaho.edu](mailto:kwaynant@uidaho.edu)

## ***Final Project Reports: Office of Undergraduate Research (OUR) Undergraduate Research Grants SURF 2022***

**Fellowship Recipient:** Taylor Booker

**Faculty Mentor:** Sarah Wu, Dept. of Biological and Chemical Engineering, COE

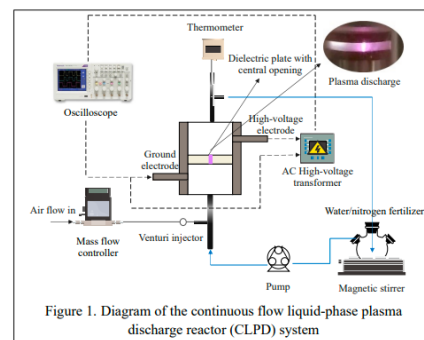
**Project Title:** Evaluating a green nitrogen fertilizer produced by plasma discharge of air and water

### **Abstract:**

Plasma activated water (PAW) has been identified as a green fertilizer and disinfectant. A new approach for improving crop yield and growth with an emerging PAW production method will be tested in this study. The objective of this study is to quantify seed germination and plant growth after repeated treatment with plasma activated water produced by a novel continuous flow liquid-phase plasma discharge (CLPD) reactor system. Optimal CLPD operating conditions for plant growth pertaining to nitrogen fixation with air and water will be determined. The findings from this study will help us to determine which plasma treatment time and power usage will be optimal for plant germination and growth and maximize yield, in order to develop CLPD produced PAW into a viable green fertilizer.

### **Project Accomplishments:**

- Germination rate did not change with the addition of plasma reacted water
- Root and Stem Length varied for each plant regardless of whether they received treatment or not.
- Plant quality was not altered with treatment
- Treatment did not alter *Medicago Sativa* growth



### **Summary of Budget Expenditures:**

<b>Description</b>	<b>Cost</b>
Lab supplies:	
4 boxes of Nitrate TNTplus Test Vials (0.2-13.5 mg/L NO <sub>3</sub> -N)	\$188
4 boxes of Nitrite TNTplus Test Vials (0.6-6.0 mg/L NO <sub>2</sub> -N)	\$162
4 boxes of Nitrate TNTplus Test Vials (5-35 mg/L NO <sub>3</sub> -N)	\$188
4 boxes of Nitrite TNTplus Test Vials (0.015-0.600 mg/L NO <sub>2</sub> -N)	\$162
Sprout seeds	\$100
Air filters, TiSO <sub>4</sub> reagent, H <sub>2</sub> O <sub>2</sub> standards, and other chemicals	\$110
Miscellaneous	\$15
UI Undergraduate Research Symposium Poster	\$75
<b>Total</b>	<b>\$1000</b>

**Acknowledgement:** This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.



Fellowship Recipient: Christina Briggs-Mathers

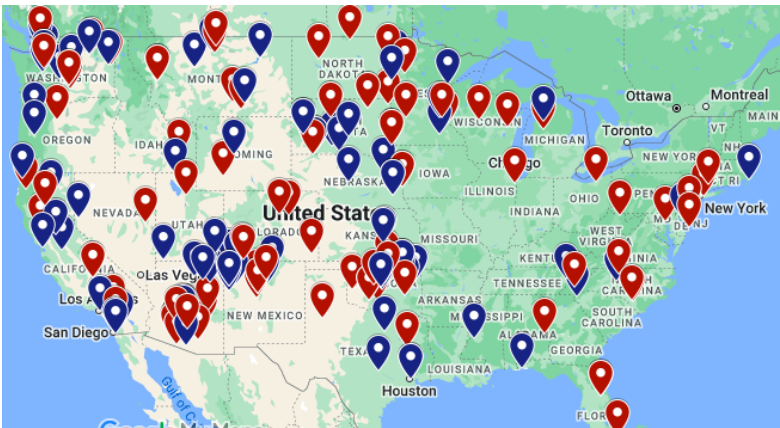
Faculty Mentor: Omi Hodwitz, Dept. of Culture, Society and Justice, CLASS

Project Title: Missing and murdered indigenous women, girls, and Two-Spirit (MMIWG2) database

Abstract:

There is an epidemic of missing and murdered Indigenous women, girls, and Two-Spirit people (MMIWG2).<sup>1</sup> In the United States, we know little about this phenomenon. Academics have largely overlooked this issue, leaving it in the hands of the Indigenous population to collect information about MMIWG2 (King & Hodwitz, 2020). The problem with this is that the information tends to be scattered across a lot of different databases managed by various organizations. Sovereign Bodies Institute or Justice for Native Women are just two of the organizations led by Indigenous community members that collect the data on MMIWG2. There needs to be a single database that is exhaustive and accurate to create a foundation upon which to work when looking into this very important problem. This need is addressed by the MMIWG2 database, a project scheduled to begin in the summer of 2022 at the University of Idaho.

<sup>1</sup>Two-Spirit is a term for individuals who do not identify by the gender binary used by Indigenous communities.



Project Accomplishments:

The growing set of coded cases suggests a few key trends. First, most of the cases originate on the west side of the country, illustrating the importance of location. Second, the police do not have high closure rates and the cases they do solve usually involve a perpetrator that is already known to the victim. Third, the victims are more likely to be female and the perpetrators are more likely to be male.

Summary of Budget Expenditures:

Purpose	Amount Requested
Supplies, Services, and Other Materials (UI Symposium Poster)	\$75
Total Amount:	\$75

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI’s Office of Undergraduate Research.

Fellowship Recipient: Bailey Briggs

Faculty Mentor: Mark Coleman, Dept. of Forestry, Rangeland, and Fire Sciences, CNR

Project Title: Nitrifier Abundance and Nitrification Rates in Reclaimed Water Irrigated Forest Soils

Abstract:

Pristine forests are nitrogen limited with conservative nutrient cycles, although anthropogenic nitrogen (N) inputs cause many ecosystems to saturate and lose N to the surrounding environment, causing an increased risk of leaching and eutrophication in freshwater ecosystems. The effects of nutrient additions are being studied in a time-series of five forest water reclamation facilities operating in Northern Idaho with treatment durations ranging from 9 to 44 years. This time-series presents an opportunity to compare microbially controlled nitrification processes. Insight into sustainable forest wastewater application has the potential to allow greater rates of carbon sequestration and increase water quality in surrounding freshwater systems. Nitrifying bacteria and archaea oxidize ammonium from wastewater to nitrate, which is highly mobile in soils. The rate-limiting step of nitrification is catalyzed by ammonia monooxygenase. Ammonia monooxygenase is encoded by the *amoA* gene, which can be used to detect presence and abundance of nitrifiers. Using qPCR amplification of *amoA*, the abundance of nitrifiers across the time series of forest water reclamation facilities can be compared to adjacent non-treated control plots. Comparing the respective nitrification rates and *amoA* abundance between treated and control plots can help determine critical nitrogen saturation and subsequent leaching risk.

Project Accomplishments:



Bailey's project was selected to be presented alongside her advisor in a "paired research presentation" at the ICUR 2022. She and her advisor, Mark Coleman each gave 10-12 slides each on the nitrification project.

Her conclusions for her project were:

- Forest Water Reclamation increases forest productivity
- Regulating wastewater application in spring and fall will decrease hydrologic losses during season
- Nitrate leaching occurs with age, indicating nitrogen saturation

A Final Focal Question was: Are there tools to measure nitrogen saturation?

Summary of Budget Expenditures:

<i>Undergraduate Research Symposium poster</i>	\$75
<i>Zymo soil DNA isolation kit, 100 extractions</i>	\$37*
<i>amoA primers</i>	\$52
<i>qPCR plates and covers</i>	\$396
<i>Microbe-Lift, nitrifying culture</i>	\$12
<i>SYBR Mastermix, 500 reactions</i>	\$428
<i>Total</i>	<i>\$1,000</i>

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.

Fellowship Recipient: Melinda Cross

Faculty Mentor: Kattlyn Wolf, Dept. of Agricultural Education, CALS

Project Title: Development of Effective Marketing Strategies based on Consumer Perspectives of Junior Livestock Shows and Sales

Abstract:

A buyer's perspective and reasoning for their decisions is constantly changing, and often hard to predict. In order to keep up with and try to cater to the buyers, it is crucial that inquiries are made regarding their thought processes. From scholarship opportunities to community involvement--Junior livestock shows offer numerous benefits to both the producer and consumer. Unfortunately, there is little research on the subject of buyer demographics and what motivates them. Recently, there has been a decline in consumer support of livestock shows and sales. In this study, surveys and interviews will be conducted to better understand the consumer's perspective as well as their knowledge on the livestock showing industry. This information will be put to use in an experimental marketing campaign for the Latah County Livestock Show. The finding of this study will be used to discover effective marketing practices for this particular industry.

Project Accomplishments:

From interviews of all current and past buyers the data suggests that a later sale time was motivating in addition to providing thank you cards and actual stories from children who discuss their projects. There was a lack of knowledge in the county on what exactly 4-H and FFA were promoting or doing and therefore a lack of community support. We expect to improve the Latah Market Animal sale in the future by moving the sale to a later time, offering a buyer's luncheon, and asking for buyers to sign-up ahead of time to make time in the livestock show and sale more efficient.



Summary of Budget Expenditures:

<u>Item (Qty)</u>	<u>Justification</u>	<u>Cost</u>
Incentives for survey respondents/interview participants	Incentives for survey completion (i.e. stickers, online coupons, etc.)	\$300
Exhibit at Latah County Fair	Prizes, Posters, Brochures, etc.	\$350
Meeting Information	Posters, Brochures, Photos, Possible Recruitment of Business owners to chat with the kids.	\$350
Total Budget		\$1000

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.

Fellowship Recipient: Alexis Dunham

Faculty Mentor: Kristopher Waynant, Dept. of Chemistry, COS

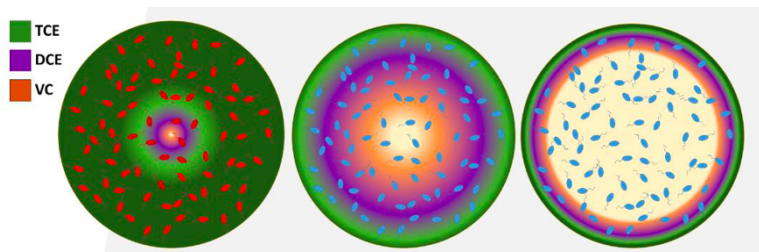
Project Title: Cryoprotectants for poly(vinyl alcohol) hydrogels

Abstract:

Bioremediation is an important process that treats pollutants using biological systems, and for this project, microorganisms. The microorganisms, a commercially purchased microbial consortia, are capable of metabolically eliminating the contaminants, specifically chlorinated aliphatic hydrocarbons (CAHs). The microbes, if added directly into these contamination sites will die due to the high concentrations, therefore a polymer hydrogel encapsulation process is utilized. Polyvinyl alcohol (PVA) hydrogels are known hydrogel polymeric systems for microbial encapsulation yet these systems are not optimized. Recent advances in our group have shown that a freeze/thaw gelation method is advantageous for optimal diffusion of CAHs in and byproducts out. The focus of this project is to add cryoprotectants to the gel formula to both keep the diffusion rates optimal and yet keep the microorganisms alive within the gels so they can do their job. A series of mixtures of PVA and cryoprotectants will be explored using trehalose, sucrose, dextran, glycine betaine, and glycerol as various cryoprotectants. The gels will be tested for both physical and chemical properties to make sure that their role plays little attention to the diffusion rates but helps sustain the cells to live. The desired outcome of this research is to find a cryoprotectant chemical that is compatible with PVA and will work well to keep the microorganisms that live within the gel alive.

Project Accomplishments:

- The 12% Betaine PVA hydrogel has the highest average diffusivity when compared to the other cryoprotectant hydrogels.
- 12% Betaine, 20% Glycerol, and 16% Sucrose PVA hydrogels have a higher average diffusivity than the regular 10% PVA hydrogel.
- The 14% Trehalose PVA hydrogel has the lowest standard error however, it has a lower average diffusivity than the rest of the hydrogel mixtures.
- Based on the average diffusivity of the 12% Betaine PVA hydrogel, the 12% Betaine would make for a good cryoprotectant as it keeps up the integrity of the hydrogel without sacrificing the movement of the liquids through the gel.



Future work: Further research will go into testing the compatibility of the cryoprotectant PVA hydrogel mixture with the microorganisms. They will be tested to see if they hold up as a cryoprotectant in bead form and if they are able to keep the microorganisms alive in colder environments.

Summary of Budget Expenditures:

**Budget: Project Budget**

Common Reagents to Gel formation	Cost
Poly(vinyl alcohol) (1 kg)	\$ 110.00
Sodium alginate (500 g)	\$ 115.00
Chitosan (250 g)	\$ 231.00
CaCl <sub>2</sub>	\$ 35.00
Trehalose	\$ 100.00

Glucose	\$ 30.00
Glycine betaine	\$ 65.00
Characterization (GelipHish, Powder XRD)	\$ 150.00
General Lab supplies (gloves, glassware)	\$ 89.00
<b>Poster Printing</b>	\$ 75.00
<b>Total</b>	\$ 1,000.00

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.

Fellowship Recipient: Hailey Faith

Faculty Mentor: Nathan R. Schiele, Dept. of Chemical and Biological Engineering, COE

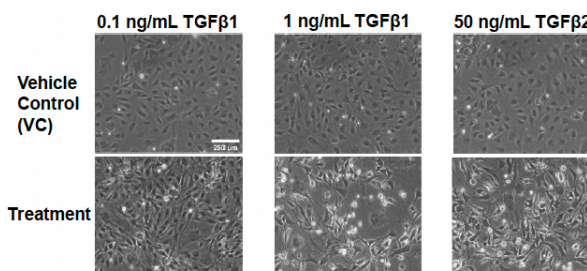
Project Title: Exploring regulators of collagen crosslinking enzyme production by stem cells

Abstract:

Tendon is a type of collagenous connective tissue that attaches muscle to bone, allowing mechanical force transfer in the body. Tendons are characterized by their low healing capacity, and current tendinopathy treatments are ineffective, motivating the need for an effective tendon regeneration technique. Mesenchymal stem cells (MSCs) are multipotent stem cells known for their self-renewal and differentiation potential. Tissue regenerative methods using MSCs have been explored, suggesting a promising regenerative approach to tendon healing. There remains a limited understanding in how MSCs can be used to control tenogenic mechanical function during tendon healing. To further reduce this gap in knowledge, I aim to investigate how collagen crosslinking enzymes are regulated by MSCs. This research proposal aims to explore how transforming growth factor (TGF) $\beta$ 1 impacts collagen crosslinking enzyme lysyl oxidase (LOX) production in MSCs through the Akt signaling pathway. The objectives of this project are to 1) determine how MSCs treated with different concentrations of TGF $\beta$ 1 impact LOX level production and 2) analyze how the Akt pathway regulates LOX production in MSCs. Results of this research will enhance the tendon tissue engineering field by determining how tendon mechanical function can be impacted while using MSCs as a tendon regenerative approach.

Project Accomplishments:

- TGF $\beta$ 1 and TGF $\beta$ 2 impact MSC morphology.
- TGF $\beta$ 2 is a driver of LOX production.
- TGF $\beta$ 1 was cytotoxic at high concentrations and did not upregulate LOX at low concentrations.
- Akt inhibition may decrease LOX production, but more work is needed to understand what role Akt is playing in LOX regulation.
- Findings improve understanding of the factors that impact tendon formation by MSCs.



**Fig. 4. TGF $\beta$ 1 and TGF $\beta$ 2 impact MSC morphology after 3 days.** Images of MSCs treated with 0.1 ng/mL TGF $\beta$ 1, 1 ng/mL TGF $\beta$ 1, and 50 ng/mL TGF $\beta$ 2 with their respective vehicle control.

Based on her work this summer as a SURF awardee, Hailey was the primary author of an abstract she submitted to the 2022 Biomedical Engineering Society (BMES) Annual Meeting in San Antonio, TX. BMES is one of the largest meetings of biomedical engineers and typically has an attendance of over 3,000. Her abstract was accepted, and she presented a research poster (citation below). Her poster was very well received, and several labs recommended that she apply to their programs for graduate school next year. Building on the research outcomes from over the summer, Hailey was also able to continue in my lab this fall semester to conduct additional experiments and work toward submitting a manuscript. Due to the opportunities that SURF provided, Hailey has a great start on her research career.



Faith HL, Schiele NR. Exploring TGFβs as regulators of collagen crosslinking enzymes for tendon tissue engineering. *Biomedical Engineering Society Annual Meeting*. October 12-15, 2022. San Antonio, TX. Poster Presentation. *The UI OUR supported her travel*.

Summary of Budget Expenditures:

**Consumable Laboratory Supplies**

Rat MSCs (cat # scr027, Sigma) + shipping \$730.00

TGFβ1 (cat# 100-21, PeptroTech) \$195.00

**SURF Required Costs**

Poster printing for UG Research Symposium \$75.00

**TOTAL \$1,000.00**

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.

Fellowship Recipient: Emily Hill

Faculty Mentor: Ann Brown, Dept. of Movement Sciences, CEHHS

Project Title: The Effect of a 4-week Tonal Strength Training Intervention on Body Composition, Muscular Strength & Emotional Well-Being Among Premenopausal Working Mothers

Abstract:

Premenopausal working women with children are often overlooked in the fitness world, dismissed as being too busy to prioritize strength training. However, the benefits of strength training on physical and emotional well-being can impact mothers and promote longitudinal health. The Tonal at home fitness program gives pre-menopausal women the opportunity to incorporate strength training without the stress and time of going to the gym and arranging childcare. Many physical changes occur following pregnancy and the needs of a child win over self-care. However, a premenopausal working mother population has not been studied in conjunction with strength training. This study aims to make not only fitness more accessible to premenopausal working mothers but encourage the incorporation of strength training into their workout routines. Over 4 weeks, working female mothers with at least 1 child older than 6 months and under the age of 18 will be recruited to participate in workouts 4 times a week of varying focuses and intensities. Physical and emotional measures will be taken before and after to assess the differences that the Tonal program can have. This pilot study can open future opportunities for this specific population to be studied with strength training and the Tonal equipment.



Project Accomplishments:

This study aimed to reframe the stigma around women strength training and to encourage and implement strength training into mother's routines. The stigma around hypertrophy has prevented women from considering ST and the Tonal system allows them to explore fitness in the comfort of their own home. The convenience eliminates the time consuming and nerve-wracking barriers that come with a gym membership. The system helps to train and educate users on proper form and routine. We are hoping to see an increase in enjoyment of working out and a positive increase in body perception and body composition. This study is one of the first studying this specific population and will open the doors for future expansion of strength training studies on women, specifically working mothers.

Summary of Budget Expenditures:

Dual-Energy X-Ray Absorptiometry (DEXA) Scans: in order to assess body composition DEXA scans will be conducted in the Human Performance Laboratory at the University of Idaho. Scan cost is \$20/participant and contributes toward maintenance of the equipment. 15 participants x \$18 x 2 scans = \$540	\$540
Participant Compensation: incentive to participate, compensation for travel to the HPL and time invested in the study. 15 participants x \$25 = \$375	\$375
Poster Printing For presentation at the Undergraduate Research Symposium 2023	\$75
Total Requested	\$990

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.



Fellowship Recipient: Klara Isbell

Faculty Mentor: Lisette Waits, Dept. of Fish and Wildlife Science, CNR

Project Title: Building a Species Inventory and Characterizing the Foraging Behavior of Bats in the UI Experimental Forest Using Acoustic Monitoring

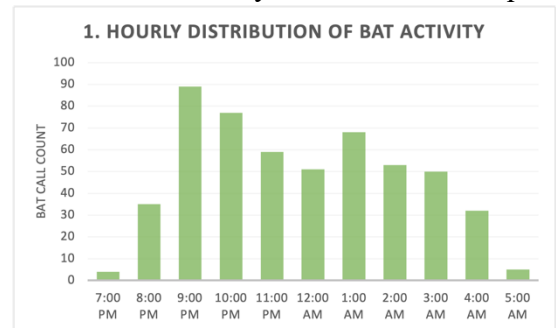
Abstract:

There is a deficit of information about bats world-wide, and this holds true for bat populations in Idaho. As bats become increasingly exposed to diseases, human disturbance, and climate change, research to fill these knowledge gaps is essential for effective management and conservation. Bats use echolocation for navigation, foraging, and communication, which allows the use of acoustic monitors to noninvasively study bat populations. Using bioacoustic data collected in the University of Idaho (UI) Experimental Forest with Audiomouth monitors, my research would compare foraging patterns between seasons and characterize peaks in foraging activity observed in different species. Analysis of the bioacoustic data will also allow for the development of a species inventory for the UI Experimental Forest, providing information about bat community composition in this region of Idaho. This research will be part of my senior thesis project in the Ecology and Conservation Biology degree program.

Project Accomplishments:

According to the Kaleidoscope automatic identification, 11 species of bats were detected in the UI Experimental Forest. There are 14 species of bats native to Idaho.

- Most of the recordings require manual identification, and the automatically identified calls require verification by comparison with recordings from bat call libraries.
- There is more activity in the evening than morning. The peak activity time occurs between 9 -10 pm followed by a gradual decline with another peak between 3 – 4 am.
- The objectives of this study are to create a species inventory and to analyze for potential seasonal changes. Patterns relative to weather will also be assessed.



Klara Isbell had an abstract accepted and presented a poster presentation to the National Wildlife Society meeting in Spokane Nov 6-10, 2022. *The UI OUR supported her travel.*

Summary of Budget Expenditures:

<b>Item</b>	<b>Quantity</b>	<b>Unit Cost</b>	<b>Cost</b>
Gas	10 weeks	\$46.8 per week	\$468
AA batteries (packs of 24)	14	\$21	\$294
64GB MicroSD cards (packs of 3)	7	\$23	\$161
Poster printing	1	\$75	\$75
<b>TOTAL</b>			<b>\$998</b>

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.



Fellowship Recipient: Dawson Mathes

Faculty Mentor: Christopher Marx, Dept. of Biological Sciences, COS

Project Title: Phenotypic Heterogeneity of PHB Production in *Methylobacterium Extorquens*

Abstract:

Lignin serves as an unexploited potential biofuel stock in order to produce butanol, due to the presence of methoxylated aromatics produced during the breaking down of lignin. Methoxylated aromatics have proven difficult to break down through microbial conversion due to their toxic nature. However, *Methylobacterium extorquens* has shown a promising ability to resist the toxicity from formaldehyde produced during the breaking down of such aromatics. The production of polyhydroxybutyrate (PHB) from methoxylated aromatics in *M. extorquens* is useful as a proxy for investigating potential 1-butanol production, as it follows the same carbon flow. This project aims to understand under which conditions *M. extorquens* yields the highest production of PHB while investigating the role of methoxylated aromatics as a carbon source. Diving further, this project goes past population level PHB quantification and aims to quantify single cell PHB production. Using a combination of flow cytometry and fluorescent microscopy this project will investigate the phenotypic heterogeneity of PHB production from methoxylated aromatics in *M. extorquens*.



Project Accomplishments:

- Growth of ancestral strains tend to lag behind evolved strains while the max growth rate remains equal
- Flow cytometry data suggests that there may be phenotypic heterogeneity in PHB production among cells of the same population

Dawson presented another poster at a recent UI College of Science Poster Session, Oct 28<sup>th</sup>, 2022.

Summary of Budget Expenditures:

<i>Item</i>	<i>Justification</i>	<i>Cost</i>
Poster	Printing cost associated with poster for Undergraduate Research Symposium.	\$75
Flow Cytometry 3-Month Pass	A 3-month pass is the most cost-effective route for using flow cytometry opposed to \$75/hr for normal use.	\$500
Lab Consumables (Described Below)	General lab materials needed in order to complete the proposed research with <i>M. extorquens</i> .	\$250
Growth Media	Media needed to culture <i>M. extorquens</i> .	\$25
PHB Extraction Chemicals	Chemicals needed for PHB extraction of aim 1.	\$50
Nile red (100mg Sigma #72485)	Staining used for single-cell PHB quantification.	\$100
Total:		\$1000

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.

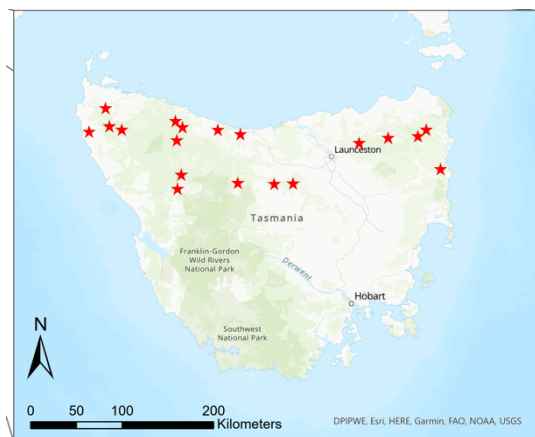
Fellowship Recipient: Cooper Moon

Faculty Mentor: Laurel Lynch, Dept. of Soil and Water Systems, CALS

Project Title: Exploring the role of scavenger declines on soil microbial function

Abstract:

Top scavengers are essential to food web structure, and their declines have cascading effects on trophic levels below them. Tasmanian devils are critical scavengers endemic to the island of Tasmania. A highly transmissible and extremely lethal cancer (devil facial tumor disease; DFTD) is threatening devil populations across roughly 95% of their habitat. The east to west spread of this disease since 1996 provides a rare opportunity to test how the decline of top scavengers affects the rate of nutrient cycling from carcasses to soils and whether these dynamics shift microbial community composition and ecosystem function. Soil samples from five locations spanning the devil-density gradient will be tested to evaluate (1) how scavenger density impacts soil biogeochemistry and nutrient cycling; and (2) whether scavenger decline alters microbial community structure and function. Working alongside Drs. Lynch and Osburn I will extract DNA from the soil to characterize the diversity and functional potential of bacterial and fungal communities. Additionally, I will analyze the soil to quantify how biogeochemical properties change across the DFTD disease gradient. I will use various statistical analyses to determine the effect that scavengers have on ecosystem structure and function which is an important area of research that has not been widely explored.



Project Accomplishments:

- Measured pools of soil carbon, nitrogen, and phosphorus (solid- and dissolved-phase), pH, & moisture at 20 different sites from across Tasmania.
- Extracted bacterial and fungal DNA, quantified total microbial biomass
- Created linear mixed effects models and figures in R learning how to model along the way.

Cooper will be presenting his findings in December at a regional conference and will be second author on an upcoming manuscript. He will also be joining the project team on a 6-week trip to Tasmania.

Summary of Budget Expenditures:

DNeasy Power Soil Pro Kit	\$500
SURF poster printing	\$75

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.



Fellowship Recipient: Gabriel Nelson

Faculty Mentor: Mark Roll, Materials Science Engineering Program, COE

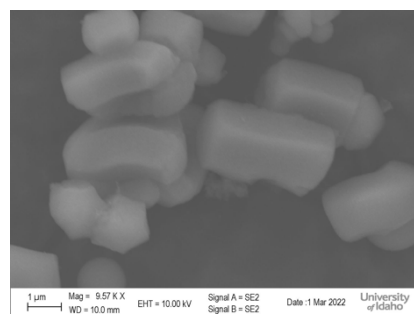
Project Title: Ordered mesoporous silica synthesis by self-catalyzing surfactants

Abstract:

Mesoporous silica nanochannels are an important material with uses in fields such as drug delivery, thermal energy storage, and filtration. Synthesis of these structures requires a surfactant template, a silica precursor, and a reaction catalyst. A technique has been identified to ionically bind a nitrate catalyst to a cetyltrimethylammonium bromide surfactant template; effectively eliminating the need for additional catalysts when later synthesizing mesoporous silica. Preliminary data shows that mesoporous silica synthesized in this way produces promising results. This project will further explore key chemical relationships in the process of attaching the nitrate catalyst to surfactants. Additionally, it will provide a better understanding of the method of using these modified surfactants to synthesize organized mesoporous silica structures. This project will result in x-ray diffraction patterns confirming the crystal structure of synthesized silica particles and electron microscopy data verifying the microstructure of the synthesized silica. This project will allow for exploration of a new mesoporous silica synthesis technique. The results will be compared with silica synthesized by traditional means to see if this new process is an effective technique for producing mesoporous silica.

Project Accomplishments:

- Qualitative observations from synthesis indicate successful CTAN production
- CTAB and CTAN templates produce very similar ASNC
- Calcination decreases d-spacing and increases peak breadth
- Methanol degrades structure at RT and in Soxhlet extraction
- Current self-catalyzing procedure produces disordered structure. May be due to methanol wash step degrading structure



Future Work: Obtain Infrared spectroscopy of synthesized CTAN as well as an SEM of CTAN templated ASNC. Develop a methanol free synthesis of CTAN-templated silica and an ethanol based chemical surfactant removal and acquire ASNC synthesis with CTAC and F127 surfactant templates.

Summary of Budget Expenditures:

Item	Quantity	Price
<i>Tetraethyl orthosilicate</i>	500 mL	\$35
<i>Cetyltrimethylammonium Bromide</i>	100 g	\$40
<i>Nitrile Gloves</i>	5 boxes at \$40/box	\$200
<i>X-ray diffraction</i>	12 hours at \$10 per hour	\$120
<i>Scanning Electron Microscopy</i>	5 hours at \$65 per hour	\$325
<i>Focused Ion Beam/Scanning Electron Microscopy</i>	1 hour at \$200 per hour	\$200
<i>Poster printing</i>		\$75
<b>TOTAL</b>		<b>\$995</b>

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.



Fellowship Recipient: Charis Peever

Faculty Mentor: Elizabeth Cassel, Department of Earth and Spatial Sciences, COS

Project Title: The Effects of Elevation and Evaporation on Soil Water Isotopic Composition Across the Cascades and Rocky Mountains

Abstract:

Stable isotope ratios of hydrogen and oxygen ( $\delta D$  and  $\delta^{18}O$ ) in surface water are widely used as tracers of modern climate, water vapor sources, and atmospheric circulation. Geologic materials, such as clay minerals and volcanic glass, preserve ancient hydration waters in their structure, so their  $\delta D$  and  $\delta^{18}O$  values can be used to understand past climate and circulation. Modern meteoric water distributions are needed to interpret geologic records, but there are currently a limited number of precipitation stations across the northwestern U.S., and almost no soil water data, which is a better representation of the geologic record than the more common river water data. My project will use hydrogen and oxygen isotope ratios collected from soil water to create an isoscape (the distribution of  $\delta D$  and  $\delta^{18}O$  values across a region). This isoscape will be used 1) as a modern baseline for active paleo-studies, 2) to compare to the existing river water data to identify where river water data varies from soil water, and 3) most importantly, to identify areas with high evaporation rates, which are likely experiencing variations due to climate change.

Project Accomplishments:

My project had me digging a soil pit near each of the locations where volcanic glass is sampled for the paleo-elevation data for the most accurate comparison. At each soil pit, I took a sample every 20 cm, up to 1 m depth, sealing the sample immediately to limit any evaporation due to air exposure. Sampling at multiple depths within the soil column will help in identification of evaporation trends (Breecker et al., 2009). Soil Water Extraction: For preparing the samples, I will be using the cryogenic vacuum extraction line in Dr. Cassel's lab to extract all water from the soil sample, following the methods of Orlowski et al. (2018). Isotope ratio analysis of the extracted waters will be done at the University of Texas – Austin, where the samples will be analyzed for both  $\delta D$  and  $\delta^{18}O$  values.



Summary of Budget Expenditures:

Budget Item	Cost	Justification
Travel	\$250	Rental vehicle and fuel to travel to each sampling site.
Lodging	\$75	Campground fees
Per Diem	\$150	Per Diem is budgeted at (\$50/trip x 3 trips)
Analysis for Deuterium	\$250	25 samples for deuterium isotope analysis @ cost of \$10 per sample.
Analysis for Oxygen	\$200	25 samples for Oxygen isotope analysis @ cost of \$8 per sample
Poster	\$75	ICUR Undergraduate Research Symposium.
Total Cost:	\$1,000	

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.

Fellowship Recipient: Zach Preston

Faculty Mentor: John Shovic, Department of Computer Science, COE

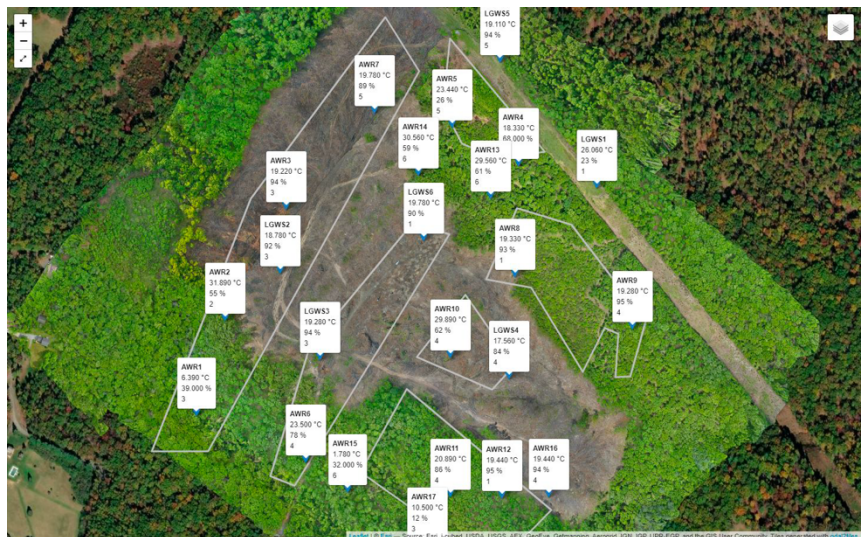
Project Title: Project VineHeart

Abstract:

To improve the efficiency of their vineyard, Laurel Grove Wine Farm is introducing a system of sensors that will read information about various elements of the vineyard and create aggregate data, to help the owners make decisions about the ways they use resources and tend to the vineyard. This “smart vineyard” system will improve the agricultural practices used in the vineyard and make its operation more environmentally friendly. The smart vineyard system will increase efficiency for Laurel Grove Wine Farm and enhance the care for the vineyard. Additionally, the plan is to create a modular and scalable system that can be applied to a variety of agricultural scenarios. Sensors could be added or removed to fit the needs of a wide variety of environments. The smart vineyard system will include a dashboard that will make it easy for Laurel Grove Wine Farm to review the data that’s collected, which will be the primary focus of my contribution to the project.

Project Accomplishments:

The Project VineHeart dashboard is a robust interface for analyzing the vineyard at Laurel Grove Wine Farm. The options that it provides give a multitude of ways to interpret the conditions of the vines and their environment. The benefit of the dashboard is more-informed decisions when taking care of the vines, and maintaining the land and air where they grow. This dashboard is just one step in the sequence for the utilities and services provided by Project VineHeart.



Summary of Budget Expenditures:

*Poster \$75*

*Laptop Capable of running Unity for Dashboard \$875*

*Total \$950*

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.



Fellowship Recipient: David Reetz

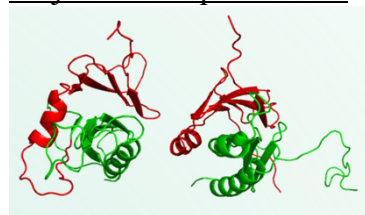
Faculty Mentor: Paul Rowley, Dept. of Biological Sciences, COS

Project Title: Elucidating the 3D structures of *Saccharomyces* killer toxins using the cutting-edge protein prediction algorithm AlphaFold2

Abstract:

Fungi cause millions of deaths every year and are responsible for a significant portion of food spoilage around the world. There is a great need to find new, more effective methods to combat harmful fungi. Killer yeasts, which produce antifungal ‘killer’ toxins, are a potential solution to this problem. This research is aimed at gaining structural understanding of known killer toxins from *Saccharomyces* yeasts to better understand their mechanism of action against pathogenic fungal species. I have already generated preliminary structural models of eleven *Saccharomyces* toxins using the neural network AlphaFold2. This has provided the first glimpse of the tertiary structure of proteins that have resisted attempts to determine their structure empirically for decades. To build more confidence in these *in silico* models, each will be energetically optimize using the molecular dynamics software GROMACS. The most confident model will undergo *in silico* mutagenesis using FoldX. Finally, the accuracy of the simulated mutations will be validated by testing their effect on toxin function against pathogenic yeasts using wet lab techniques from molecular and cell biology. This multidisciplinary approach will put researchers at the University of Idaho at the leading edge of antifungal protein research with the aim to develop new classes of therapeutics.

Project Accomplishments:



K1 is a killer toxin with 3 distinct domains. By using AlphaFold2 modeling software, we were able to build homologs of K1. These homologs indicated that the interaction between the alpha and gamma domains of the K1, K1L, and KKT proteins. Now exploring yeast 2 hybrid assays to test the interaction between the alpha and gamma domains of K1 and autoimmunity assays. David Reetz recently presented a poster at the American Society of Microbiology (ASM) and won a best poster prize.

Summary of Budget Expenditures:

Materials	Price/qt	Quantity	Total
Nitrile Gloves	\$ 140.00	1	\$ 140.00
Primer Synthesis	\$ 5.00	14	\$ 70.00
Phusion Master Mix	\$ 650.00	0.1	\$ 65.00
HyPure Water	\$ 12.00	1	\$ 12.00
T4 DNA ligase	\$ 64.00	1	\$ 64.00
Competent E. coli	\$ 233.00	1	\$ 233.00
S. Cerevisiae BY4741	\$ 90.00	1	\$ 90.00
Fisher Bioreagents Agar	\$ 230.00	0.4	\$ 92.00
Yeast Extract	\$ 58.00	0.25	\$ 14.50
Peptone	\$ 189.00	0.24	\$ 47.10
Dextrose	\$ 130.00	0.25	\$ 32.50
Methylene Blue	\$ 48.00	0.05	\$ 2.40
Galactose	\$ 25.00	1	\$ 25.00
Petri dishes 100mm x 15mm	\$ 75.00	0.5	\$ 37.50
<b>Poster Printing</b>			\$ 75.00
<b>Total</b>			\$ 1,000.00

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI’s Office of Undergraduate Research.

Fellowship Recipient: Julia Woods

Faculty Mentor: Brenda Murdoch, Dept. of Animal, Veterinary and Food Sciences, CALS

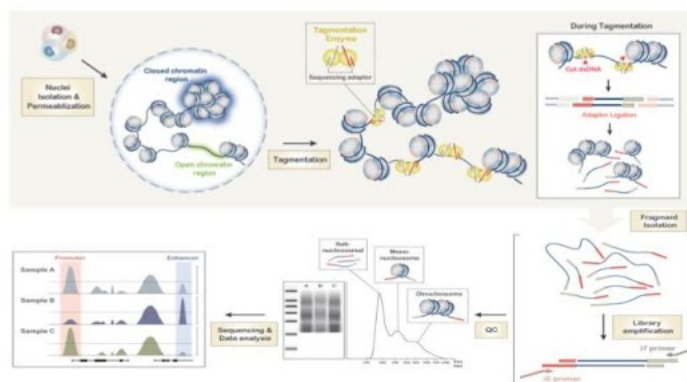
Project Title: Transcription levels in open chromatin regions and RNA expression in Angus cattle

Abstract:

The sustainability of the livestock industry hinges upon the improvement of production efficiency to the benefit of both consumers and producers. A large contribution to this betterment has been achieved through innovation in genetics, including the rapidly expanding field of epigenetics. Our goal for this research is to improve our understanding of the relationship of open chromatin regions and RNA expression in Black Angus cattle. Knowing what chromatin is open and where RNA is transcribed helps indicate the presence of other factors that may affect transcription. Their interactions will be analyzed using ATAC-seq and RNA-seq of four tissue types from four animals as biological replicates. Having a multitude of sample types from different specimens allows for better comparison to draw broader conclusions about open chromatin regions and RNA expression. Not only will the results from this project contribute to the completion of the overall bovine epigenome project, but they will also provide information to researchers that can in turn aid in the application of genomic tools and data toward production strategies.

Project Accomplishments:

Through characterizing the transposase-accessible and actively transcribed regions of the genome of 4 biological replicates and 3 tissue types, we expect to define tissue-specific signals as well as capture biological variation. The results of this study will contribute to the current understanding of chromatin accessibility profiles in economically important livestock breeds. It was discovered that proximity of regulatory enhancers decreases in tissue-specific open chromatin regions, indicating a long-range modulation of transcription. Ongoing work will provide significant verification of the epigenome in relevant species of livestock genetics.



Summary of Budget Expenditures:

<b>Item (Size, Amount)</b>	<b>Supplier</b>	<b>Cost Per Item (\$)</b>	<b>Number of Items</b>	<b>Total Cost</b>
RNA Extraction Kits	Qiagen	\$558.00	1 (50 spincolumns)	\$558.00
Misc. Lab supplies	Thermo Fisher	Various	Various	\$217.00
Shipping samples	FedEx Approx.	\$100	2 Shipments	\$150.00
Research Poster	U of I Printing Center	\$75	1	\$75.00
<b>Total Cost:</b>				<b>\$1,000</b>

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.

Fellowship Recipient: Elizabeth Worley

Faculty Mentor: Gwinyai Chibisa, Animal Veterinary and Food Science, CALS

Project Title: Determining stress response of dairy calves when transported at different ages

Abstract:

In past research studies from the U.S., it has been shown that the age at which a dairy calf is transported could impact their long-term health. Transport-related stress can contribute to a higher susceptibility of digestive and respiratory disease and can reduce production performance into adulthood. However, past studies have relied solely on the measurement of blood cortisol, a stress hormone, to determine the effects of transportation. This can be an issue since cortisol does not fully characterize the impact of transport-related stress, especially in young calves as the cortisol response is still muted. Therefore, this study will not only focus on cortisol, but will also evaluate other measures like white and red blood cell counts, body weight, and heart and respiration rates. This will hopefully provide a better picture of how calves at different ages respond to transportation.

By determining the ideal age to transport calves, dairy farms can ensure the welfare of each calf in the facility. Therefore, we can develop better management strategies to not only improve animal welfare, but to help reduce deaths and the use of antibiotics to treat sickness caused by transported-related stress. This will help increase productivity and efficiency in the dairy industry, while increasing profitably and the overall success of a dairy operation.

Project Accomplishments:

With this study, we will create new evidence-based recommendations for the ideal age to transport calves. Therefore, we can increase the sustainability of dairy operations, as well as increase the production efficiency of dairy cows in the future. Calves were transported over 8 hours. Body weights were measured both before and after transport. Blood work was done following transport.



Summary of Budget Expenditure:

<b>Budget: Item</b>	<b>Amount</b>
1. Blood Analysis	\$600
2. Lab Supplies	\$325
3. Poster Printing	\$75
<b>Total</b>	<b>\$1000</b>

Acknowledgement: This work was made possible by generous support from the Idaho State Board of Education which provided the funding for this work and the UI's Office of Undergraduate Research.

***Students, Project Titles, and Abstracts of UI Gen Ed SURF recipients not presenting at ICUR***

Fellowship Recipient: Madison Wolf

Faculty Mentor: Omi Hodwitz, Dept. of Culture, Society and Justice, CLASS

Project Title: Missing and Murdered Indigenous Women, Girls, and Two-Spirit (MMIWG2) database

Abstract:

In North America, Indigenous women, girls, and Two-Spirit (IWG2) are at an increased risk of victimization. The matter long predates the present-day movement for a resolution. As a result, there is a severe data deficit regarding Missing and Murdered Indigenous Women, Girls, and Two-Spirit (MMIWG2) in both the United States (U.S.) and Canada. On top of existing struggles within Indigenous communities, there is fear that they or a loved one will go missing at any moment and receive little to no aid in their recovery. Without adequate information, legislators cannot address the situation at hand. The MMIWG2 database provides this necessary information.

The MMIWG2 research consists of two phases. The first phase will involve gathering cases of MMIWG2 in the U.S. and Canada. The second phase will consist of verifying the authenticity of each missing person's case and collecting corresponding information. The corresponding information will include, among other things, the time between an individual being reported missing and law enforcement responses, and if the case received follow-up or resolution. This will provide the verified information needed to assess and understand MMIWG2 and the effectiveness of each country's response to the issue.

Fellowship Recipient: John Mansanarez

Faculty Mentor: Christine Berven, Dept. of Physics, COS

Project Title: Experimental Tests of Type-II Three-Dimensional Levitation and Energy Loss Quantization

Abstract:

This project will be a continuation of testing dynamic force models for Type-II Superconductor Permanent Magnet (SCPM) bearings. Experiments will continue to test horizontal restoring forces of the SCPM bearings. Further expanding trust into our three dimensional predictive model. We intended to use a variety of Halbach arrays and compare to predictive theory that requires no fitting parameters, while other popular models[EJ] require data fitting and bearing prototyping. The second objective is to gain a stronger understanding of the energy loss within our systems. There are currently a few flux flow and creep models are used to explain energy loss in superconductivity, but these require current measurements that can only be obtained through experimentation. Our goal is to construct a solid predictive theory that would not rely on experimental fitting. As well as design experimental procedures to rigorously test our hypothesis. The major goals to achieve by the end of the summer would be to finish the two sets of experiments mentioned before and follow up with finalizing two papers that would be submitted for publication.

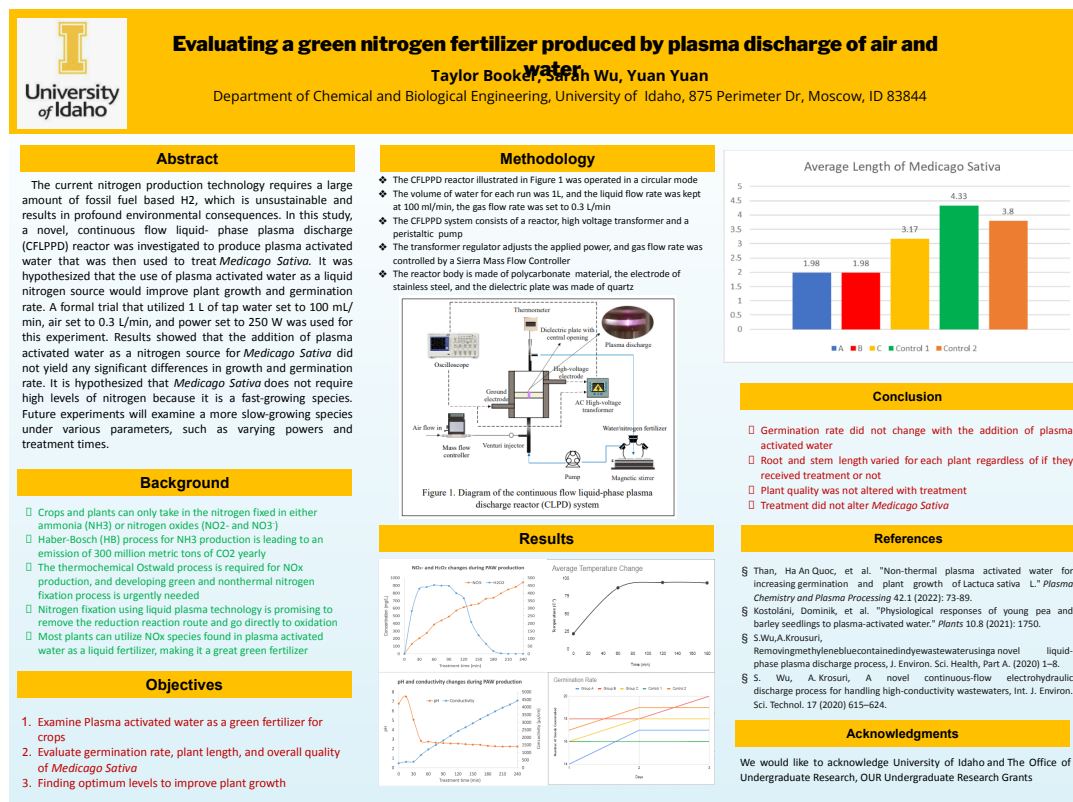
Acknowledgement: Acknowledgement: This work was made possible by generous support from UI's Office of Undergraduate Research.

*These Faculty members have been given a warning and will need to mention this warning in their letters of recommendation for future applicants of SURF funding.*

## Name, Mentor's Name and Titles of Fall 2022 Projects

Student Name	Faculty Mentor	Department	College	Project Title
Brazil-Geyshick, Asiah	Chelsey Byrd Lewallen	Family and Consumer Sciences	CALS	Kombucha Leather Research Project
Goebel, Peter	Eva Strand	Ecology and Conservation Biology	CNR	Sampling, Identifying, and Charring Experiments of Woody Taxa to Understand Past Palaeoecological Conditions and Early Human-Wood Relationships in the Great Basin
Hill, Katherine	Paul Rowley	Biological Sciences	CoS	An investigation into the role of the Krel membrane receptor in killer toxin immunity
LaVoie, Nathan	Paul Rowley	Biological Sciences	CoS	Site-Directed Mutagenesis of Conserved Cysteines found in an Aerolysin-like toxin
Major, Julia	Paul Rowley	Biological Sciences	CoS	The cell membrane protein Krel as a receptor for the K1 killer toxin in pathogenic yeasts
Reetz, David	Paul Rowley	Biological Sciences	CoS	Elucidating the 3D structures of Saccharomyces killer toxins using the cutting-edge protein prediction algorithm AlphaFold2

## Appendix of Posters:





## Trends of Missing and Murdered Indigenous Women, Girls and Two Spirit

Christina Briggs-Mathers (brig7281@vandals.uidaho.edu)

Dr. Omi Hodwitz (omi@uidaho.edu)

Department Of Culture, Society, and Justice



University of Idaho  
Department of Culture,  
Society and Justice

### Research Goals

There are numerous databases that record and report MMIWG2 in the United States. However, these are scattered over various sources and, as such, tend to be piecemeal at best, limiting our ability to understand this phenomenon. To address this issue, we are building a central database that brings these cases together so that the extent and nature of MMIWG2 can be better understood.

### Methods

Indigenous people are at higher risk of becoming victims of violence than any other racial or ethnic group in the United States. As such, the need to empirically assess this phenomenon is pressing. In order to do so, we have identified approximately 1000 cases of MMIWG2 in the United States between 1980 and 2020, of which 30% (and counting) have been verified and coded. Our primary sources include community databases, media articles, government reports, and government databases.

### Current Victim Demographics

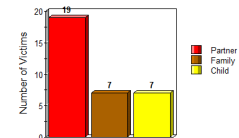
Men Murdered Women	55
Men Murdered Men	7
Men Murdered Young Girls	8
Women Murdered Men	1
Women Murdered Women	6
Women Murdered Young Girls	3

Indigenous Perpetrator	14
Non-indigenous Perpetrator	1

### Current Solve Rate

Police have arrested and charged someone in 67 cases out of 318. Which leaves a solved case rate of 21%

### Known Domestic Violence Murders



### Summary

Our growing set of coded cases suggests a few key trends. First, most of the cases originate on the west side of the country, illustrating the importance of location. Second, the police do not have a high closure rate and the cases that they do solve usually involve a perpetrator that is already known to the victim. Third, the victims are more likely to be female-presenting, while the perpetrators are more likely to be male-presenting.

### Funding

This research was funded by the University of Idaho's Office of Undergraduate Research.

### References

King, S., & Hodwitz, O. (2020). What's the plan? Broadening the MMIWG2 conversation in North America. *Comparative American Studies* an International Journal.  
<https://doi.org/10.1080/14775700.2021.1877082>

### Murdered and Missing Locations in USA

Murdered Missing



### Known Perpetrator Demographics

Sex	Age	Amount
Female	Under 18	38
Male	Under 18	12
Unknown	Under 18	7
Female	Adult	113
Male	Adult	83
Unknown	Adult	0
Female	Unknown	12
Male	Unknown	6
Unknown	Unknown	47

Sample Size: 318

## Development of Effective Marketing Strategies based on Potential Consumer Perspectives of Junior Livestock Shows and Sales



Melinda Cross

Latah Market Animal Sale, University of Idaho

University of Idaho

### Background

The number of buyers and supporters participating in Junior Livestock Shows has been decreasing across the country, especially after the COVID-19 pandemic. From financial responsibility to character development, Junior Livestock Shows help children build important life skills. There have been numerous studies done about the incentives behind junior livestock shows for youth. There are social skills learned, responsibility, attention to detail, time management, and the possibility of being connected with future employers. There is then the largest incentive: the financial potential. What is necessary for students to succeed is for there to be community support and involvement as buyers. While there is information on buyer concerns, there is little information published describing livestock buyer profiles. To completely understand the consumers and their motivations, research is needed to compile demographics and measure attendance.

### Methods

- Interviews were done with top buyers to gain an understanding of what buyers need, and what can be done to better accommodate them. They were given a mug with the LMAS logo and portable battery chargers as a thank you.
- A booth was set up at the Moscow Farmer's Market where 4-H/FFA members were educating people about the sale, and their projects. There were posters hung up with information about pricing for buying animals on the hoof at a sale like LMAS versus in the store. There was also a poster about freezer space, and how much you need for each animal. Lastly, there was a raffle to try to get people interested in signing up to receive more information about the sale.
- A social media page was started, and updated weekly. Educational posts were done often to show people why buying from the sale is beneficial for their wallet. 4-H and FFA members taking their animals through the sale were also given the opportunity to be featured.
- Local Latah county fair board and livestock leader's meetings were attended to gain an inside view on what is happening with the sale, and to relay the data from past buyers to those running the meetings.

### Data Collected

From interviews and research done all current and past buyers the suggested:

- A later sale time
- Thank you cards from kids with pictures
- More contact from kids talking about their own projects
- More appreciation for the buyers from the sale, and an easier way to sign up to be a buyer.

We also discovered there was simply a lack of knowledge in the county about what the sale and 4-H/FFA even were. This contributed to the lack of community support.

### Future Plans

We took all the words from the past buyers as well as from the community. We moved the sale to a later time and are offering a buyer's luncheon. We also have ways for buyers to sign up ahead of time to make it more time efficient. We are proud of the outreach and education we were able to provide in the hopes that we can improve the Latah Market Animal Sale.

# Cryoprotectants for PVA Hydrogels

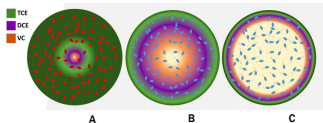
Alexis Dunham<sup>1,2</sup>, Carson Silsby<sup>2</sup>, Dr. Kristopher Waynant<sup>1</sup>, Dr. James Moberly<sup>2</sup>, and Dr. Mark Roll<sup>2</sup>  
Department of Chemistry<sup>1</sup>, Department of Chemical and Biological Engineering<sup>2</sup>; University of Idaho

## Objective

The purpose of this research is to optimize the PVA hydrogel solution with a cryoprotectant so microorganisms encapsulated in the hydrogel can survive freeze/thaw cycling. Glycerol, betaine, sucrose, and trehalose were tested as cryoprotectants in a PVA gel mixture to determine which is best by comparing the diffusivities of the cryoprotected gel mixture to the original PVA hydrogel.

## Introduction

- A Bioremediation process utilizing polymer encapsulated hydrogels allows for an efficient reduction of already existing CAH pollutants such as TCE and VC.
- TCE and VC are among the 15 commonly found CAH pollutants at all superfund sites.
- Microorganisms encapsulated in a PVA hydrogel proves to be an effective way to keep the microorganisms alive in contamination sites.



**Figure 1.** The diffusion of TCE, DCE, and VC needs to be controlled so the microorganisms are able to complete a four-step reaction, in the case of TCE, that degrades the CAH to benign product. A) CAH diffuses too quickly and so the microorganisms become too overwhelmed and die. B) The CAH diffuses at the correct rate so remediation can be successful. C) The CAH is diffusing too slow so the microorganisms in the middle starve and die.

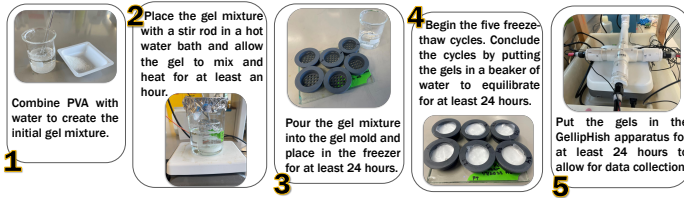
- Cryoprotecting the gels will allow for the incorporation of needed cell densities to optimize bioremediation.
- Glycerol, Sucrose, Betaine, and Trehalose are used in a PVA solution to serve as a cryoprotectant for the gels.

## Acknowledgements

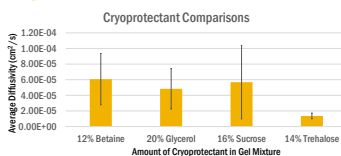
This research was funded in part by Summer Undergraduate Research Fellowships from the Office of Undergraduate Research at the University of Idaho

This research was funded in part by the National Science Foundation, Award Number: 1805358

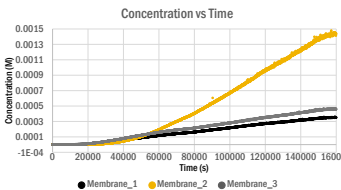
## PVA Hydrogel Preparation



## Cryoprotectant Results



**Figure 2.** A comparison of the average diffusion of the cryoprotectant PVA hydrogel mixture. They are compared using the average diffusivity.



**Figure 3.** The diffusion of hydrogen ions over time for a 20% glycerol 10% PVA hydrogel mixture.

## Results and Discussion

- The 12% Betaine PVA gel has the highest average diffusivity when compared to the other cryoprotectant gels.
- 12% Betaine, 20% Glycerol, and 16% Sucrose PVA gels have a higher average diffusivity than the regular 10% PVA gel.
- The 14% Trehalose PVA gel has the lowest standard error however, it has a lower average diffusivity than the rest of the gel mixtures.

- Based on the average diffusivity of the 12% Betaine PVA gel, the 12% Betaine would make for a good cryoprotectant as it keeps up the integrity of the gel without sacrificing the movement of the liquids through the gel.

## Future Work

Further research will go into testing the compatibility of the cryoprotectant PVA gel mixture with the microorganisms. They will be tested to see if they hold up as a cryoprotectant in bead form and if they are able to keep the microorganisms alive in colder environments.

University of Idaho  
Office of Undergraduate Research



## Exploring regulators of collagen crosslinking enzymes for tendon tissue engineering

University of Idaho  
Department of Chemical and Biological Engineering

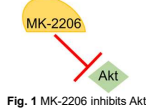
Hailey L. Faith, and Nathan R. Schiele

Department of Chemical & Biological Engineering, University of Idaho, Moscow, ID

University of Idaho  
Office of Undergraduate Research

## Background

- Tendon is a collagenous tissue that attaches muscle to bone, allowing mechanical force transfer and movement.
- Tendon injuries are common, heal poorly, and current treatments are ineffective.
- Transforming growth factor (TGFβ1) is a growth factor known to play a role in tendon injury and healing, while TGFβ2 promotes differentiation toward the tendon lineage in mesenchymal stem cells (MSCs).
- MSCs are multipotent stem cells known for their self-renewal and differentiation potential.
- MSCs have been explored as a regenerative therapy for tendon injury, but how MSCs are regulated to form mechanically functional tendon is unknown.
- Lysyl oxidase (LOX) is a collagen crosslinking enzyme that is crucial in forming a mechanically functional tendon, but how LOX production is regulated in MSCs remains unexplored.
- Akt cell signaling pathway may be activated by TGFβs and plays a role in tenogenesis, but impacts on LOX are unknown.
- MK-2206 is an inhibitor of Akt signaling.



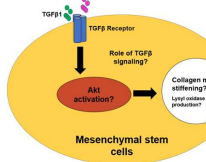
**Fig. 1** MK-2206 inhibits Akt

## Hypothesis & Objectives

**Hypothesis:** TGFβ1 and TGFβ2 increase LOX production through activation of Akt signaling in MSCs.

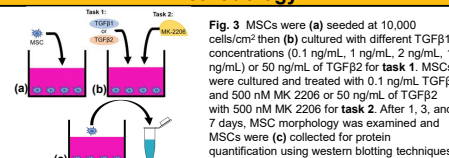
### Objectives:

- Determine how MSCs treated with TGFβ1 and TGFβ2 impact LOX production levels and Akt activation.
- Analyze how Akt signaling regulates LOX production in MSCs.



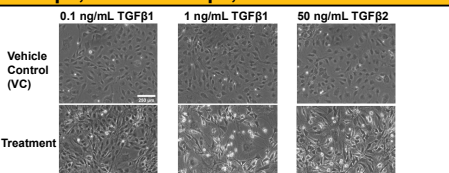
**Fig. 2** Overview of scientific questions

## Methodology

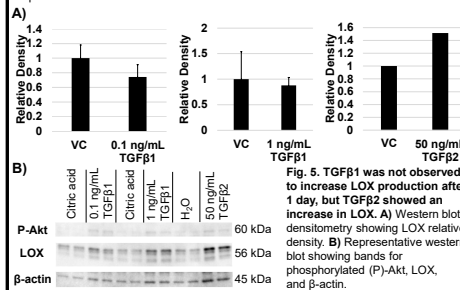


**Fig. 3** MSCs were (a) seeded at 10,000 cells/cm² then (b) cultured with different TGFβ1 concentrations (0.1 ng/mL, 1 ng/mL, 2 ng/mL, 10 ng/mL) or 50 ng/mL of TGFβ2 for task 1. MSCs were cultured and treated with 0.1 ng/mL TGFβ1 and 500 nM MK-2206 or 50 ng/mL of TGFβ2 with 500 nM MK-2206 for task 2. After 1, 3, and 7 days, MSC morphology was examined and MSCs were (c) collected for protein quantification using western blotting techniques.

## TGFβ2, but not TGFβ1, increases LOX levels

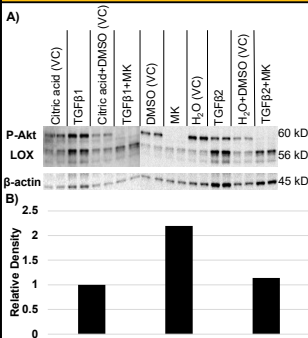


**Fig. 4** TGFβ1 and TGFβ2 impact MSC morphology after 3 days. Images of MSCs treated with 0.1 ng/mL TGFβ1, 1 ng/mL TGFβ1, and 50 ng/mL TGFβ2 with their respective vehicle control.



**Fig. 5** TGFβ1 was not observed to increase LOX production after 1 day, but TGFβ2 showed an increase in LOX. A) Western blot densitometry showing LOX relative density. B) Representative western blot showing bands for phosphorylated (P)-Akt, LOX, and β-actin.

## Akt inhibition reduces TGFβ2-enhanced LOX production



**Fig. 6** MK2206 inhibits Akt and reduces LOX production with TGFβ2 treatment after 1 day. A) Representative western blot showing bands for phosphorylated (P)-Akt, LOX, and β-actin. B) Western blot densitometry showing LOX relative density.

## Conclusion & Future Directions

- TGFβ1 and TGFβ2 impact MSC morphology.
- TGFβ2 is a driver of LOX production.
- TGFβ1 was cytotoxic at high concentrations and did not upregulate LOX at low concentrations.
- Akt inhibition may decrease LOX production, but more work is needed to understand what role Akt is playing in LOX regulation.
- Findings improve understanding of the factors that impact tendon formation by MSCs.

## Acknowledgements

This project was made possible by the University of Idaho Office of Undergraduate Research, Summer Undergraduate Research Fellowship (to HLF), the National Science Foundation # 2145004 (to NRS), and Colin R. Marchus for training in cell culture.



# The Effect of a 4-Week Tonal Strength Training Intervention on Body Composition, Muscular Strength, and Emotional Well-being on Premenopausal Working Mothers



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## ABSTRACT

**BACKGROUND:** Premenopausal working women with children are often overlooked in the fitness world, dismissed as being too busy to prioritize strength training. However, the benefits of strength training on physical and emotional well-being can impact mothers and promote longitudinal health. The Tonal at home fitness program gives pre-menopausal women the opportunity to incorporate strength training without the stress and time of going to the gym and arranging childcare. Many physical changes occur following pregnancy and the needs of a child with over self-care. However, a premenopausal working mother population has not been studied in conjunction with strength training. **PURPOSE:** This study aims to make not only fitness more accessible to premenopausal working mothers but encourage the incorporation of strength training into their workout routines. **METHODS:** Over 4 weeks, working female mothers with at least 1 child older than 6 months and under the age of 18 will be recruited to participate in workouts 4 times a week of varying focuses and intensities. Physical and emotional measures will be taken before and after to assess the differences that the Tonal program can have. **CONCLUSION:** This pilot study can open future opportunities for this specific population to be studied with strength training and the Tonal equipment.

## INTRODUCTION

The female body undergoes significant body composition changes following pregnancy and throughout the middle period, which can negatively impact health later in life. Because it is simple and inexpensive to assess, body mass index (BMI: body weight (kg)/height<sup>2</sup> (m<sup>2</sup>)) is typically used to identify changes in body composition however, BMI does not capture relative amounts of muscle vs. adipose tissue. Following pregnancy, many females experience loss of muscle and accumulation of adipose tissue. Infiltration of fat into muscle tissue hides unhealthy body composition from notice. Previous findings from our laboratory have demonstrated that normal weight obesity (NWO: normal BMI and >30% body fat) is prevalent in young females that are physically active but do not engage in strength training (ST). NWO is of distinct concern for females following pregnancy because of hormone changes that lead to heightened fat mass storage<sup>1</sup>. Recent literature demonstrates that 55% of working mothers do not meet physical activity guidelines<sup>2</sup> and ST is often avoided by females for fear of "bulking" based on previous ideals of a feminine body<sup>3</sup>. Although females could greatly benefit from ST, it is often an avoided gym activity because of fear of misuse and embarrassment<sup>4</sup>. Lack of confidence in ST abilities in combination with post-pregnancy body composition changes may contribute to dissatisfaction with both exercise experience and body image.

## PURPOSE

The goal of this study is to assess the physical and psychological impact that strength training has on premenopausal working mothers. **Specific Aim 1:** Compare physical attributes of body composition, muscular strength, and cellular strength in working mothers following ST (strength training) intervention. **Specific Aim 2:** Compare the psychological attributes that include physical activity enjoyment and body satisfaction in working mothers following ST intervention.

## METHODS

**PARTICIPANTS:** Participants between 18-50 years old will be recruited from the Moscow-Pullman area. Recruitment strategies include fliers posted throughout Moscow and advertisements on local Facebook groups. Participants must have at least 1 child between the ages of 6 months and 18 years old and be employed either full or part time. Exclusion criteria includes if participants are currently strength training (>20 min/day 2x/week), are currently pregnant, have medical conditions contraindicating to exercise, are currently smoking or have a diagnosed eating disorder.

**DATA COLLECTION:** Participants will visit the Human Performance Lab (HPL) hand Vandal Muscle Lab 1 time prior to beginning training on the Tonal. This visit will consist of a DXA scan, Body Parts Satisfaction Scale (BPSS), Physical Activity Enjoyment Scale (PACES), Dietary Assessment (ASA-24) and baseline max testing using Tonal equipment (Fig 1). The ASA-24 assessment will be taken 1x per week over the 4 weeks of the study to ensure no significant changes in intake are occurring. Participants will complete workouts of varying focus each week lasting 30-60 minutes per day (Fig 2). Following the intervention, participants will visit the HPL and Vandal Muscle Lab, performing the same assessments as pre-testing.

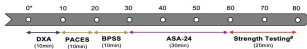


Figure 1. Human Performance Laboratory Pre-Post-Testing Timeline

\*minutes, \*includes familiarization at pre-testing, DXA = dual energy x-ray absorptiometry, PACES = Physical Activity Enjoyment Scale, BPSS = Body Parts Satisfaction Scale, ASA-24 = Automated Self-administered 24-hour dietary recall.

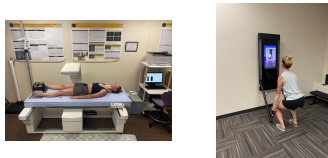


Figure 2. Vandal Muscle Laboratory Weekly Tonal Strength Training. Participants will complete 2 Whole Body Strength Training sessions, 1 Cardio session, and 1 Recovery session per week for 4 weeks. \*Vandal ASA-24 dietary recall day.

## METHODS CONT.

**STATISTICAL ANALYSIS:** Statistical analyses are completed using SPSS Statistics 24 and data were reported as means±SD. Significance was accepted at p<0.05. A repeated measures analysis of variance (ANOVA) was used to assess the effect of time and group on each variable.

## PRELIMINARY RESULTS

Table 1. Nutritional Information	(n=7)
KCALs	1670.6±636.8*
Protein (g/kg)	74.4±26.5170
Fat (g/kg)	82.5±36.9255
Carbs (g/kg)	140.1±59.7731

\*Means±SD, g/kg: grams per kilogram

Table 2. Body Composition Information	(n=7)
Weight (kg)	75.5944±15.6004*
LM (kg)	47.3936±4.5342
LM (%)	63.9176±7.9128
FM (kg)	25.4316±11.7804
FM (%)	32.5857±8.2743
VAT (g)	457.1428±287.6273
ASMI kg/m <sup>2</sup>	7.1485±0.8029
BMD	1.1338±0.073

\*Means±SD, kg: kilograms, %: percent, g: grams, kg/m<sup>2</sup>: kilograms over meters squared

## DISCUSSION

This study will aim to reframe the stigma around women strength training and to encourage and implement strength training into mother's routines. The stigma around hypertrophy has prevented women from considering ST and the Tonal system allows them to explore fitness in the comfort of their own home. The convenience eliminates the time consuming and nerve-wracking barriers that come with a gym membership. The system helps to train and educate users on proper form and routine. We are hoping to see an increase in enjoyment of working out and a positive increase in body perception and body composition. This study is one of the first studying this specific population and will open the doors for future expansion of strength training studies on women, specifically working mothers.

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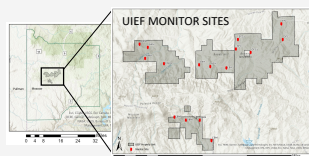
University of Idaho  
College of Natural Resources  
University of Idaho  
Office of Undergraduate Research

## Using Acoustic Monitoring to Detect Bat Species in the University of Idaho Experimental Forest

Klara Isbell, Elyce Gosselin, Dr. Robert Keefe, Dr. Lisette Waits

### INTRODUCTION & OBJECTIVES

Acoustic monitoring is an emerging non-invasive technique<sup>1</sup> for studying bats, birds, dolphins, and other echolocating animals<sup>2</sup>. This study will analyze echolocation recordings collected in the UI Experimental Forest (UIEF) to identify bat species in northern Idaho and characterize their foraging behaviors.



### STUDY AREA

- 8,300 acres
- Four units: West Hatter, East Hatter, Flat Creek, Big Meadow

### MONITOR PLACEMENT

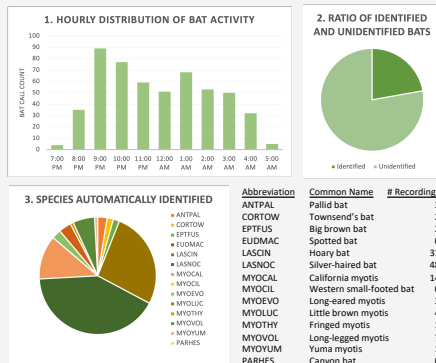
- Three habitat types: open, edge, narrow<sup>3</sup>
- 16 total monitors

### METHODS

- Data collection (May 27-August 10, 2022)
  - Audiomoth monitors for collecting recordings<sup>4</sup>
  - Kaleidoscope Pro software for processing recordings and conducting an automatic species identification<sup>5</sup>
  - Manual species identification and verification

### CURRENT RESULTS & SPECIES OBSERVED

- 523 bat calls recorded
- 11 species detected
- (1) The activity detected between May 27 – June 17th suggests a peak in activity between 9-10pm followed by a gradual decline. (2) Only 28.5% of bat calls received an automatic identification from Kaleidoscope. (3) The most frequently detected species was the silver-haired bat (LASNOC)<sup>6</sup>.



UI Summer Undergraduate Research Fellowship (SURF) Program  
Adele Berkland Undergraduate Research Scholar Award  
Kenneth Hungerford Research Award

### CONCLUSION

- According to the Kaleidoscope automatic identification, 11 species of bats were detected in the UI Experimental Forest. There are 14 species of bats native to Idaho<sup>7</sup>.
- Most of the recordings require manual identification, and the automatically identified calls require verification by comparison with recordings from bat call libraries.
- There is more activity in the evening than morning. The peak activity time occurs between 9-10pm followed by a gradual decline with another peak between 3-4am.

### FURTHER WORK TO BE COMPLETED

The data displayed here only represents recordings collected May 27<sup>th</sup>-June 17<sup>th</sup>, 2022. Data collection will continue through August 10<sup>th</sup>, 2022, after which detailed manual identification of recordings will be conducted. The objectives of this study are to create a species inventory and to analyze for potential seasonal changes. Patterns relative to weather will also be assessed.





# The Cell Membrane Kre1 as a Cell Receptor for K1 Killer Toxin in Pathogenic Yeasts

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University of Idaho, Department of Biological Sciences

## BACKGROUND

Yeasts of the Fungal kingdom are single-celled eukaryotes, with some possessing the ability to produce antifungal proteins named "killer" toxins. Killer toxins provide an evolutionary advantage over competing yeasts by inhibiting growth and causing cell death. These antifungals are primarily studied in biomedical research for their potential applications against fungal pathogens resistant to current front-line therapeutics. Antifungal resistance is a common problem, a prime example being the growing drug resistance of *Candida* yeasts. It is estimated that 1 in 3 women will suffer from a vaginal yeast infection at least once in their life, caused by a *Candida* yeast. Azoles, currently the most commonly prescribed class of antifungal drugs to treat vulvovaginal candidiasis, are as much as 32% ineffective [1]. Additionally, in patients prescribed the azole fluconazole, an estimated 10% experienced adverse side effects [2]. Azoles are considered the most effective class of antifungals for vaginal candidiasis; yet, they are only 60% effective, and 25% of these infections are caused by highly drug-resistant yeasts (*C. glabrata*). The other 75% are caused by other species of the *Candida* genus such as *Candida albicans* and *Candida auris*.

The first described killer toxin, named K1, was discovered when sterile filtered growth media derived from *Saccharomyces cerevisiae* (baker's/brewer's yeast) was shown to inhibit the growth of competing yeasts [3]. In yeast cells, K1 is processed into a mature toxin and secreted into the environment. Killer yeasts create zones of growth inhibition when inoculated onto a growth medium with a susceptible competing yeast. Recently, the Rowley lab has successfully inhibited the growth of 100% of *C. glabrata* strains and isolates from the human vagina using the K1 killer toxin [4].

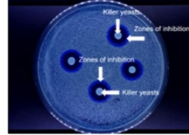


Figure 1: Killer yeasts inhibiting the growth of competing yeasts on a solid growth medium. After incubation for 48 hours, visible zones of growth inhibition can be observed as holes around each killer yeast strain.

After initially binding to the cell wall, K1 attaches to Kre1, what's thought to be its secondary receptor on the cell membrane. Kre1 is a GPI anchored protein found in the membrane covalently attached to the cell wall. K1 kills cells by disrupting cell membrane function by forming ion channels specifically for cations [5]. The formation of ion channels allows for the efflux of potassium and possibly hydrogen ions, subsequently leading to apoptosis and cell death. It was previously found that *kre1Δ* cells are completely resistant to K1, and expression of *KRE1* restored sensitivity [6]. To further test the relationship between *KRE1* and K1 intoxication, I will be taking the *KRE1* gene from a variety of different yeasts and inserting and integrating them into the UTR of *S. cerevisiae*. These modified yeasts will be exposed to K1 through killer toxin assays, and the effects observed. It is expected that the engineered yeasts will be susceptible to K1.

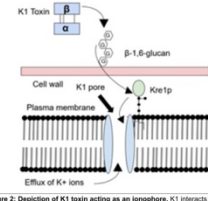


Figure 2: Deposition of K1 toxin acting as an ionophore. K1 interacts with β-1,6-glucan of *S. cerevisiae* yeast's cell wall. It is transported to the plasma membrane where it interacts with membrane receptor Kre1p, causing pore formation within the cell membrane.

## PRIMER DESIGN

Primers are short single stranded pieces of DNA (usually 18-25bp long) designed for use in PCR (Polymerase Chain Reaction). I designed 9 different primers to amplify the *KRE1* gene of each yeast I was working with. PCR works in three major steps, denaturation, annealing, and elongation. Initially, the template DNA is denatured, and new DNA strands are synthesized, using the old strands as a template. Next, the primers anneal to the ends of the DNA strands, bracketing the region of interest. These strands are then elongated, and the process repeated 25-35 times, ending with a multitude of copies of the targeted gene. Using the NIH protein blast, I collected the nucleotide sequences of each ORF of every *KRE1* in my 9 yeasts, plus ~1 kb of the UTR. Primer3 was used to design the forward and reverse primers for each yeast's *KRE1* using the UTRs. These yeasts were chosen due to the variance in size and species. Due to the pathogenic nature of yeasts in the *Candida* genus, they were not included in the initial rounds of PCR, but will be used later.



Figure 3: Domain diagram of *KRE1* from *S. cerevisiae* and *C. albicans*. Deposition of *KRE1* genes with noted areas of homology and high serine/threonine; light blue areas represent the hydrophobic domain of the C-terminal.

Strain	Yeast
19990	<i>Saccharomyces kudriavzevii</i>
555411	<i>Saccharomyces kudriavzevii</i>
NCTC_2729	<i>Kazachstania africana</i>
Y-17079	<i>Tetraspora phaffii</i>
33317	<i>Saccharomyces arboricolus</i>
CB5432	<i>Saccharomyces paradoxus</i>
CBS_12357	<i>Saccharomyces eubayanus</i>
Y_2379	<i>Torulaspora delbrueckii</i>
NCTC_777	<i>Neurospora dairensensis</i>
NCTC_2688	<i>Neurospora castellii</i>

Figure 4: 10 yeast with respective strains. Table detailing the full names and strains of each yeast chosen for this project.

## PCR AND gDNA EXTRACTION

Before PCR could be done, the genomic DNA needed to be extracted from each yeast. Each of the 10 yeasts were grown overnight in 10 ml cultures. gDNA extraction involved three main steps, lysis, precipitation, and purification. Lysis allows the cell membrane to be broken down so that the DNA can precipitate out of the solution and separate from the other cellular components in the second main step. Lastly, the purification step is necessary to get rid of any residual salts, proteins, and the like. After resuspension of the gDNA in TE buffer, I ran PCRs with my previously designed primers to amplify the *KRE1*s. To ensure it was successful, the expected product sizes were calculated in, adding the length of the gene and the primers together.

Yeast	Expected product size
<i>S. kudriavzevii</i>	1129 base pairs
<i>K. africana</i>	617 base pairs
<i>T. phaffii</i>	1202 base pairs
<i>S. arboricolus</i>	1151 base pairs
<i>S. paradoxus</i>	1197 base pairs
<i>S. eubayanus</i>	1184 base pairs
<i>T. delbrueckii</i>	1389 base pairs
<i>N. dairensensis</i>	1433 base pairs
<i>N. castellii</i>	1492 base pairs

Figure 5: Table of the 9 yeast species used in the project. Predicted product size based on length of the species's *KRE1* and primer length.

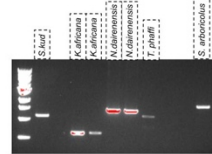


Figure 6: Amplification of *KRE1* in 5 different yeasts. Deposition of amplified *KRE1*s with the first of each yeast is a 1/100 DNA dilution, and the second is a 1/1000 DNA dilution.

## NEXT STEPS

Now that the *KRE1* of all 10 yeasts has been successfully amplified, the next step is to engineer a *KRE1* knockout strain of *S. cerevisiae* BY4741. Once this strain is made, new primers allowing for integration of orthologous *KRE1*s in *S. cerevisiae* will be made. These primers will target the *KRE1*s of the 10 respective yeasts, with homology to *S. cerevisiae*'s *KRE1*'s UTRs to allow for integration into the cell. After successful integration, killer assays will be performed where the engineered yeasts are exposed to K1. We expect the yeasts to be susceptible to K1, regardless of the orthologous *KRE1*s. The same experimental process will be repeated for yeasts of the *Candida* genome, after sufficient Biosafety Level 2 training, due to their pathogenic nature.

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## ACKNOWLEDGEMENTS

Thank you to the Office of Undergraduate Research for funding and the chance to participate in research as an undergraduate.

# Phenotypic Heterogeneity of Polyhydroxybutyrate Production in *Methylobacterium extorquens*

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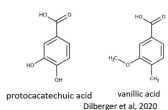
**Introduction:** Lignin is one of the most abundant organic materials on Earth; however, the resource is widely unused as a biofuel stock as it contains aromatics which are toxic in nature. Additionally, some aromatics offer a secondary source of toxicity in the form of methoxy groups that subsequently form formaldehyde when broken down. *Methylobacterium extorquens* was used for the microbial conversion of methoxylated aromatics into polyhydroxybutyrate (PHB). The production of PHB from methoxylated aromatics in *M. extorquens* is useful as a proxy for investigating potential 1-butanol production, as it follows the same carbon flow.<sup>1</sup> This project goes past population level PHB quantification and aims to quantify single cell PHB production using a combination of flow cytometry, fluorescent microscopy, and HPLC.



PHB granules (green) accumulate at different rates at the single-cell level. This can be viewed using a fluorescent microscope and Nile red staining.

## *M. extorquens* Formaldehyde Resistance

Due to the natural formaldehyde resistance of *Methylobacterium extorquens*, it serves as a promising candidate for the microbial conversion of lignin to butanol.<sup>2,3</sup> However, the growth of *M. extorquens* on aromatics needs to be investigated further in order to optimize the potential production process.

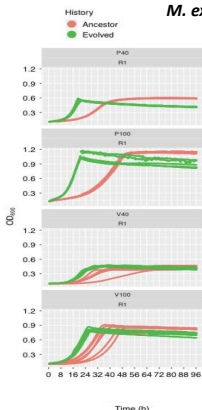


PCA (left) and VA (right). Note the methoxy group (CH<sub>3</sub>-O) on VA as a source of secondary toxicity.

## Methods

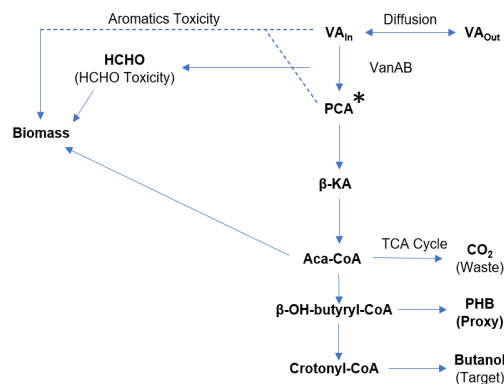
- Grow *M. extorquens* on respective evolved media
- There are many strains of *M. extorquens* and those used in the Marx lab have been evolved to grow on carbon sources such as vanillic acid, methanol, and succinate.
- Extract PHB
- Polyhydroxybutyrate (PHB) was used as a proxy for butanol as butanol is difficult to extract and quantify in relation to PHB.
- Quantify PHB production
  - Through HPLC
- Investigate for phenotypic heterogeneity of PHB production
- Using flow cytometry and microscopy

## *M. extorquens* Growth



Growth curves showing the pattern of *M. extorquens* growth on respective media at varying carbon concentrations. Red depicts the ancestral strain while green represents the evolved strains.

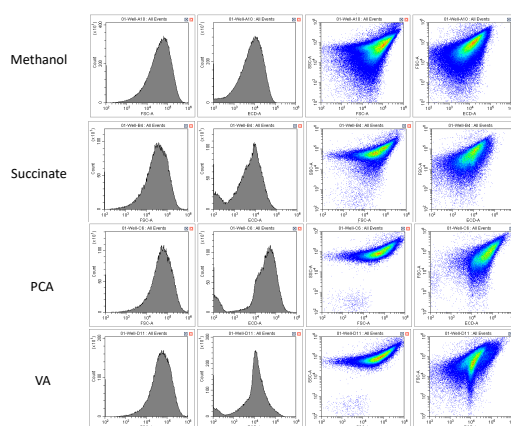
- Evolved strains have larger initial growth rates
- Max growth rates remain the same between ancestral and evolved strains



**VA catabolism in *M. extorquens*.** Formaldehyde (HCHO) is derived from the enzymatic cleavage of VA (Vanillic Acid). Additionally, note that butanol follows the same carbon as the proxy PHB.

\*PCA is created from the enzymatic cleavage of CH<sub>3</sub> from VA

## Flow Cytometry



**Flow cytometry on varying media at the same carbon concentration (40 mM C).**

- Overall cell size (column one) reveals a normal distribution
- PHB production among cell size varies
  - While the general trend shows a positive correlation between cell size and PHB there is a sizable distribution of small cells with high PHB levels and large cells with low PHB levels

## Conclusion:

- Growth of ancestral strains tend to lag behind evolved strains while the max growth rate remains equal
- Flow cytometry data suggests that there may be phenotypic heterogeneity in PHB production among cells of the same population

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Funding: University of Idaho, Office of Undergraduate Research (SURF)

# EXPLORING THE ROLE OF SCAVENGER DECLINES ON SOIL MICROBIAL FUNCTION

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## Introduction

- Globally, top predators/scavengers are in decline but the effects of their loss on ecosystem function remain difficult to predict<sup>1</sup>.
- The emergence of a highly transmissible, lethal cancer (DFTD) is pushing Tasmanian devils to the brink of extinction but provides an ideal natural experiment<sup>2</sup>.
- Because devils are the top scavenger in Tasmania, their decline may induce a trophic cascade but the effect on soil and ecosystem function is unknown.
- My research investigates the link between scavenger loss, microbial function, and soil biogeochemistry.



Healthy Tasmanian devil versus an individual with DFTD.

## Hypotheses

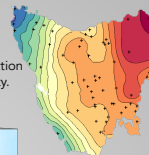
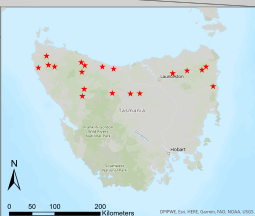
- Devils do not affect soil pH or cation exchange capacity (CEC).
- Lower devil density increases soil C:N by inhibiting rapid cycling of non-plant inputs.
- Lower devil density reduces microbial diversity but increases total biomass.



★ Sample Sites

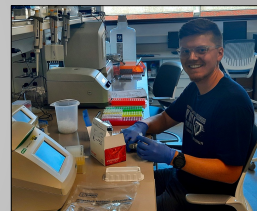
n = 19 sites with 3 replicates per site and 2 soil depths per replicate = 114 samples.

Sample sites spanning the East to West gradient of DFTD. Red = localized extinction while Blue = 95-100% of carrying capacity.



## Future Work

- DNA extractions and soil moisture analysis has been completed
- Remaining analytical work will be completed this summer
- Statistical analyses, figure development, and manuscript writing will be completed in the next two semesters.



## Literature cited

- <sup>1</sup>Ripple, et al. (2014). Status and ecological effects of the world's largest carnivores. *Science*, 343(6167).
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## Acknowledgments

This project was supported by a student grant from the UI Office of Undergraduate Research as well as NSF funding under NSF DEB 2054716.

## Driving Questions

Does scavenger presence affect soil biogeochemistry and nutrient cycling?

Does the decline of a dominant scavenger alter soil microbial communities?

## Methods

Moisture Content  
Soil pH  
Soil fractions (MAOM & POM)  
Dissolved C & N pools  
Cation exchange capacity

Microbial Biomass (C & N)  
DNA Extraction  
(bacterial 16S gene, fungal ITS1 region)



## Synthesis of substituted ATF ligands and their evaluation for metal dissolution and recovery from Waste electronic and electrical equipment (WEEE)

Natasha Muparutsa, Rabina Pradhan, Laxmi Tiwari, and Kristopher V. Waynant<sup>1\*</sup>, <sup>1</sup>Dept. of Chemistry, <sup>2</sup>Dept. of Chemical & Materials Engineering, University of Idaho, Moscow, ID 83844

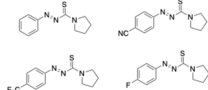
## Abstract

The dissolution and recovery of metals from waste electronic and electric equipment is important because the demand of precious metals in industrialization has increased and the quantity of natural raw metals has decreased therefore increasing the costs. Extraction of these metals is also important because it helps with environmental legislation thus making it easier to dispose WEEE. This is considered the secondary source of metals. Precious metals are used in a wide range of electronic appliances like, phones, modems, and computers. The types of precious metals that are usually found in waste electronic and electric equipment are gold, copper, silver, and palladium. In this study, there will be the synthesis of substituted ATF ligands and evaluation for metal dissolution of waste electronic and electrical equipment. The process utilizes a mild redox-active ligand series (azothioformamides or ATF) capable of dissolving metals and metal salts into ligand-metal coordinative complexes. These recovered complexes will undergo electrochemical processing to recover high purity metals and fresh ligand, providing a fully recyclable system.

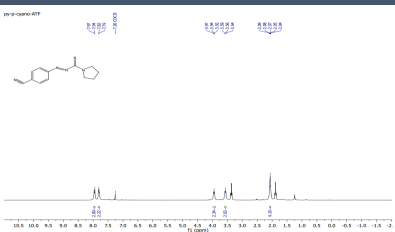
The recovery of metals from waste electronic and electric equipment has a positive impact on the environment because it reduces the toxic chemicals released by the WEEE into the atmosphere and reimagines how metals are sourced. The recovered metals can also be used for future industrial processes.

## Background

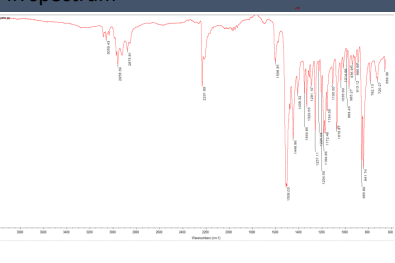
The ability of ATF ligands to chelate various copper species has been previously documented.<sup>1,2</sup> In addition to copper metal, 1:1  $\mu$ -X dimer crystals 3 form with copper(I)halides and 2:1 ligand to metal complexes are formed from copper(I) salts containing non-coordinative counterions (BF<sub>4</sub><sup>-</sup>, PF<sub>6</sub><sup>-</sup>) 4.<sup>2</sup>



## HNMR



## IR spectrum



## UV-Visible titration and Binding isotherm

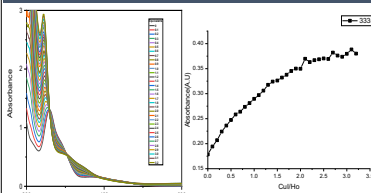


Fig. UV-Vis Titration spectra of CuI with Pyrrolidine-p-cyano-ATF Binding isotherm of CuI with pyrrolidine-p-cyano-ATF

## Conclusion

The Pyrrolidine-p-cyano-ATF has a melting point of 73 degrees with a yield of 40%. Electron-donating moieties resulted in more exothermic interactions, larger extinction coefficients, and an increased predicted binding association whereas electron-withdrawing moieties provided weaker binding association.

## Acknowledgements

University Of Idaho Office of Undergraduate Research

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## Introduction

- Arrays of silica nanochannels (ASNC) are a material that consists of an array of mesopores in a silica matrix
- ASNC are an important material with uses in fields such as:
  - drug delivery
  - thermal energy storage, and
  - filtration
- Surfactants are molecules used as a template for ASNC (Fig 1):
  - The most common surfactant is called CTAB
  - Surfactants self assemble into array of cylinders
  - TEOS is hydrolyzed by a catalyst to silicic acid, which condenses onto the surfactant array
  - The silica is stabilized and condensed by calcination, which removes the surfactant
- Structure is dictated by the surfactant template
- Surfactant removal technique can affect the final silica structure
- Nitrate catalyst can be attached to CTAB to make CTAN

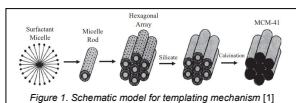


Figure 1. Schematic model for templating mechanism [1]

## Methodology

### CTAN synthesis

- Combine 1 g AgNO<sub>3</sub> and 2.2 g CTAB in 34 mL of water
- Precipitate AgBr byproduct in 90 mL methanol
- Let solvent evaporate to recover CTAN

### ASNC synthesis

- Dissolve 4 g surfactant in 60 mL of 37% HCl and 76 mL of water
- Added 2 mL of TEOS to solution at 0 °C
- Held at 0 °C for 4 hours to form silica nanochannels
- Vacuum filtered and washed with 50 mL of water
- Did low-angle XRD and indexed peaks by analytical methods

### Self-catalyzing CTAN templated silica

- Dissolved 2 g CTAN in 40 mL water
- Added 1 mL of TEOS to solution at room temperature
- Let stir for 16 days
- Isolate product with a methanol wash

### Surfactant Removal

- Heated at 330 °C for 2 h followed by calcination in air at 550 °C for 18 h
- Washed 400 mg ASNC in 40 mL methanol at RT for 24 hours
- Ran 50 mL methanol through 200 mg ASNC in Soxhlet extractor for 24 hours

## Results

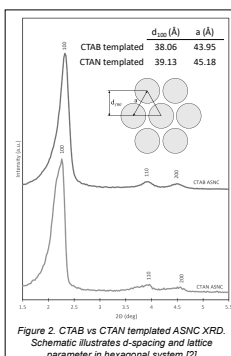


Figure 2. CTAB vs CTAN templated ASNC XRD. Schematic illustrates d-spacing and lattice parameter in hexagonal system [2]

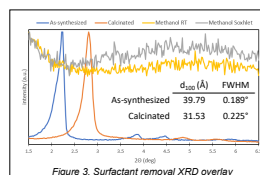


Figure 3. Surfactant removal XRD overlay

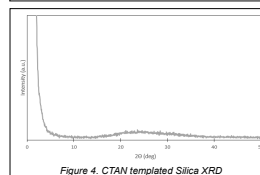


Figure 4. CTAN templated Silica XRD

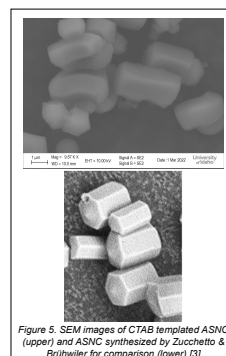


Figure 5. SEM images of CTAB templated ASNC (upper) and ASNC synthesized by Zucchetto & Brühwiler for comparison (lower) [3]

## Objectives

- Synthesize CTAN surfactant
- Compare CTAN templated silica to CTAB templated silica
- Compare methanol surfactant removal techniques to calcination
- Use CTAN as a self-catalyzing silica template

## Conclusions

- Qualitative observations from synthesis indicate successful CTAN production
- CTAB and CTAN templates produce very similar ASNC
- Calcination decreases d-spacing and increases peak breadth
- Methanol degrades structure at RT and in Soxhlet extraction
- Current self-catalyzing procedure produces disordered structure
  - May be due to methanol wash step degrading structure

## Acknowledgements

This research was funded by an Undergraduate Research Grant from the Office of Undergraduate Research at the University of Idaho.

## Future Work

- Infrared spectroscopy of synthesized CTAN
- SEM of CTAN templated ASNC
- Methanol free synthesis of CTAN-templated silica
- Ethanol based chemical surfactant removal
- ASNC synthesis with CTAC and F127 surfactant templates
- Gas adsorption analysis of all ASNC to determine:
  - Surface area
  - Pore size, and
  - Density

## References

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# The Effects of Elevation and Evaporation on Soil Water Isotopic Composition Across the Cascades and Rocky Mountains

Charis Peever; Advisor: Elizabeth Cassel, Earth & Spatial Sciences

## Background

The increasing effects of climate change are becoming apparent in yearly precipitation and evaporation rates, and this is affecting not only soil health, but also groundwater reservoirs (Konapala et al., 2020). An isotopic composition map constructed using soil water samples will be pivotal to understanding the rates of both precipitation and evaporation, and the distribution of areas that are and will continue to be affected by climate change. This isoscape will be useful as a baseline for current paleo-elevation studies, as it will be more accurate than isoscapes constructed with river water data, which is more prevalent and easier to collect, but skewed by water inputs from outside the local area. This isoscape will also be used to determine the amount of water being lost at various locations across the region due to evaporation. Soil water is a more accurate local sample than river water as soils tend to incorporate only local precipitation waters, much like the geologic proxy materials. It also averages rainfall over multiple years (Breecker et al., 2009).

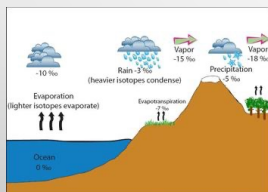


Fig. 1: Model of isotopes in precipitation showing a typical distribution of heavy mass isotopes (<sup>18</sup>O, D) in relation to elevation changes and vapor introductions due to evaporation. Based on Hoefs (2009) and Bruckner (2022).



Fig. 2: Study area with samples already taken marked with a white and black dot. More samples are being taken to fill in blank areas, specifically in Idaho, Oregon, and eastern Montana.

## Methods

**Sample Collection:** I will be digging a soil pit near each of the locations where volcanic glass is sampled for the paleo-elevation data for the most accurate comparison. At each soil pit, I will take a sample every 20 cm, up to 1 m depth, sealing the sample immediately to limit any evaporation due to air exposure. Sampling at multiple depths within the soil column will help in identification of evaporation trends (Breecker et al., 2009).

**Soil Water Extraction:** For preparing the samples, I will be using the cryogenic vacuum extraction line in Dr. Cassel's lab to extract all water from the soil sample, following the methods of Orłowski et al. (2018). Isotope ratio analysis of the extracted waters will be done at the University of Texas – Austin, where the samples will be analyzed for both δD and δ<sup>18</sup>O values.

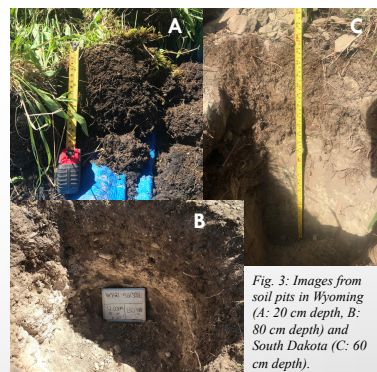


Fig. 3: Images from soil pits in Wyoming (A: 20 cm depth, B: 80 cm depth) and South Dakota (C: 60 cm depth).

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# Project VineHeart Dashboard

Zachariah Preston – College of Engineering – University of Idaho



## Abstract

Project VineHeart is an enterprise to install various sensors throughout the Laurel Grove Wine Farm in order to monitor the vines and their environment. To use the collected data, a dashboard is needed for displaying visuals such as graphs, maps, and tables that convert the raw data into meaningful representations. This dashboard, hosted on a website, allows the users to analyze the data from different perspectives, such as real-time data shown on a map or comparisons of readings for different sensors. It also has a modular software structure that makes it easy to expand the dashboard and use the data in more ways than just a website. The dashboard will be a powerful tool for tracking the growth and quality of the vineyard and maintaining it for the future.

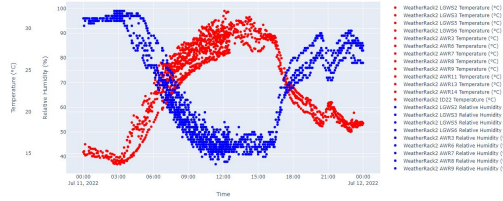
## Introduction

Laurel Grove Wine Farm is deploying sensors in their vineyard that record various qualities in the environment, such as temperature, humidity, soil moisture, rainfall, and more. The data collected by these sensors is transmitted via radio and aggregated into a MongoDB database. From here, the data needs to be transformed into representations that are informative for the users, and give them insight on the overall status of the vineyard as well as details about each sensor's area. So, a dashboard was implemented that queries raw data from the database and uses it to construct visualizations. It's accessible from a website so that the vineyard data can be checked at any time. A variety of filters and customization options are provided as well, which makes it easy to find details about any aspect of the vineyard.

## Structure

The software structure for the dashboard has a modular design to allow the program to be expanded upon in the future. The website has a driver that gets its data by sending a request to a central controller. Then, the controller forwards this request to the corresponding database for the data (MongoDB in this case). The database driver converts the request into a database query and searches for the data. Once it has the data, it's sent back to the controller, which passes it on to the website driver. With this setup, new databases can be added to the project by simply creating an additional driver. It also gives the option of using the databases for creating interfaces in addition to the dashboard.

Custom Graph



## Tech Stack

The dashboard was built using the Dash web framework developed by Plotly. Dash makes it simple to set up a website and start adding features. All of the HTML and JS for the site is implemented by Python objects and callback functions, so the code for each web page is all in one place and easy to maintain. For styling, Dash Bootstrap provides components that all match a selected theme, and has a simple grid system for arranging the content on the page. Dash also includes Plotly's graphing interface, so it's easy to generate and display graphs on the page. The interactive maps are made using Dash Leaflet, a wrapper for the React-Leaflet JS library. Images are rendered on these maps by converting them to tile maps with gdal2tiles. The website is hosted on Amazon Web Services so that it can be accessed at any time with a URL.

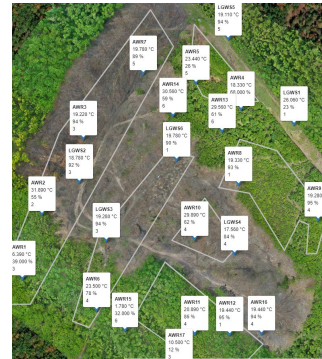
## Layout

Each page on the website gives a different view of the data. The "Home" page has quick access to graphs that are frequently needed. The "Real-Time Data" page uses Leaflet to show the location of each sensor and their most-recent data. It also generates alerts for the sensors to signal potential problems, such as a low battery or no data received in the past day. The "Sensor Data" page gives detailed information and graphs about individual sensors. This information includes tables with real-time data for that sensor, graphs of the data over time, and aggregations of the sensor's data such as averages and maximums. Finally, the "Custom Graphs" page has a wide range of parameters and settings for comparing any combinations of sensors and data fields. These graphs can be customized and saved on the website to view at a later time.

## Conclusion

The Project VineHeart dashboard is a robust interface for analyzing the vineyard at Laurel Grove Wine Farm. The options that it provides give a multitude of ways to interpret the conditions of the vines and their environment. The benefit of the dashboard is more-informed decisions when taking care of the vines, and maintaining the land and air where they grow. This dashboard is just one step in the sequence for the utilities and services provided by Project VineHeart.

Area ID	Value ID	Units ID	Area Type ID
Weather Station 1	1.7	°C	Weather
Leaflet Weather Station	1.7	°C	Weather
Weather Station 2	1.7	°C	Weather
Weather Station 3	1.7	°C	Weather
Weather Station 4	1.7	°C	Weather
Weather Station 5	1.7	°C	Weather
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Weather Station 99	1.7	°C	Weather
Weather Station 100	1.7	°C	Weather



# Exploring the structure-function relationship of killer toxin immunity

University of Idaho  
Department of Biological Sciences

David C. Reetz, Marty Ytreberg, Jack Creagh, and Paul A. Rowley

## Background: Killer yeasts, K1, and modeling approaches

- Harmful fungi affect millions of people every year
- Responsible for a significant portion of global food spoilage<sup>[1]</sup>
- Antifungal agents are challenging to develop

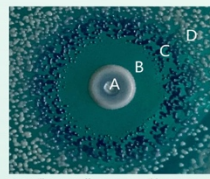


Fig 1. Killer yeast effect on susceptible yeast

- Killer yeasts secrete killer toxins (KTs)
- Kill other yeasts, create an inhibition zone
- Common Brewer's yeast
- Killer Assay (Left)

- little structural or mechanistic data on KTs
- Protein prediction tool AlphaFold2 - reliable structures in minutes<sup>[2]</sup>
- processed using GROMACS
- molecular dynamics simulator and the results can be tested in the lab.
- 22 KTs were modeled and optimized

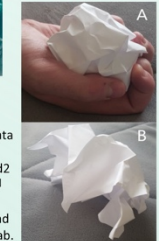
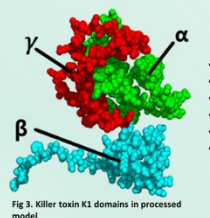


Figure 3: Representation of energy minimization  
A. crumpled paper representing unoptimized AlphaFold model.  
B. Relaxed paper representing energy optimized model.



- K1 is a killer toxin
- Effective on *C. glabrata*<sup>[3]</sup>
- Best studied killer toxin
- Three distinct domains
- Toxin, Immunity, Targeting.
- Gamma Cleavage

## K1, K1L, and KKT toxin modeling

- Alpha and gamma domains interact – Chaperone immunity.<sup>[5]</sup>
- Flexible glycine chain model for docking predictions.<sup>[6]</sup>
- Interactions between the two chains shown below

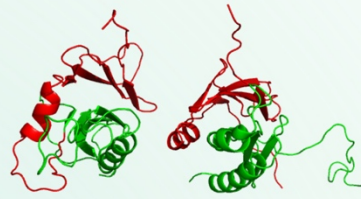


Fig 4. K1's alpha and gamma domains are predicted to interact closely. Processed AlphaFold2 model of K1 alpha and gamma domains directly connected, (Left) Processed AlphaFold2 model of K1 alpha and gamma domains modeled with 19 Gly residues, (Right)<sup>[6]</sup>

- Homologs can build confidence
- K1L – K1 like killer toxin – 18% sequence identity
- KKT family - 16-28% sequence identity<sup>[7]</sup>
- Similar interaction between gamma and alpha of related proteins – builds confidence in interaction

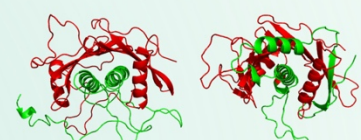


Fig 5. High structural similarity and immunity motif preserved between K1L and the KKT genes. Processed model of K1L (*S. paradoxus*) alpha and gamma domains (right) Processed model of KKT (*K. Africana*) alpha and gamma domains (left). Alpha and gamma domains for both toxins have been selected based upon likely cleavage sites and similarity in length to K1's domains.

## Testing the interaction between the alpha and gamma domains of K1

- Yeast 2 hybrid system selected for testing interaction
- Yeast grows on selective media if interaction is present

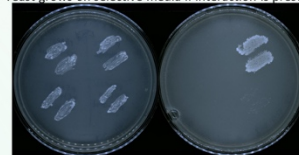


Fig 6. Yeast 2 Hybrid controls  
Strong interaction present

- Two plasmids must be constructed
- Gateway Cloning System
- Alpha and gamma each connected to proteins that induce expression when in proximity
- Expression of HIS3, URA2, and B-galactosidase proteins.



Fig 7. An agarose gel with PCR of the alpha domain and restriction digests of the gamma domain inside of the pCR8 Gateway donor vector. Wells 5-9, 11, and 12 have insert present.

## Project Direction:

- Finish Yeast 2 Hybrid assays
- Repeat with K1L and KKTs
- In the event of failure:
  - X-Gal, URA3, HIS2 (weak)
  - Autoimmunity Assay
- New plasmids to construct

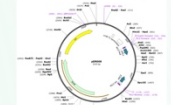


Fig 8. A vector map of the alpha domain

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# Open Chromatin Regions and RNA Expression in Multiple Tissues from Angus Cattle

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**AFFILIATIONS**



## INTRODUCTION

Our understanding of the epigenetic landscape in livestock animals is considerably less than within model species<sup>1</sup>. Epigenomic data is more notably important in agricultural animals due to the beneficial effect that the understanding of the pathway between genome and phenotype can have on quality production<sup>2</sup>. To address this need for epigenomic data, organizations such as the FAANG Consortium have put forth efforts in filling informational gaps<sup>1</sup>. To aid in these findings, this project will provide necessary information on the relationship between open chromatin regions and RNA expression found in three tissue types of Black Angus cattle. To do so, two complementary sequencing assays will be used: ATAC-seq and RNA-seq. The findings from these assays will be analyzed and the results will be overlaid to help us better describe the epigenetic landscape in cattle.

**OBJECTIVE** Aid in the characterization of the bovine epigenomic landscape to contribute to the betterment of livestock production

## METHODOLOGY

### Animal and Tissue Collection:

- Three target tissues: liver, muscle, and the frontal cortex
- Four biological replicates
- 2½ month old Angus calves
- Flash frozen and stored in -80°C until analysis

### RNA Extraction:

- Samples homogenized utilizing a chilled mortar and pestle
- Purified using the Quick RNA MiniPrep Plus kit from Zymo Research
- Nanodrop for quantification & quality measurements

### RNA Sequencing:

- RNA-seq → LC Sciences
- mRNA Poly-A total library prep and sequencing
- NovaSeq reads 150bp paired-ends for a 40 M sequencing depth

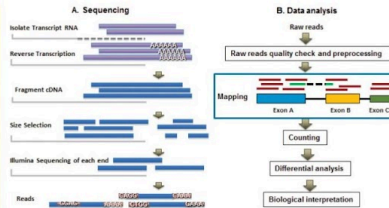


Figure 1. Depicted above is the general process RNA samples undergo in RNA-seq and subsequent analysis. Figure from "Bioinformatics for RNA-Seq Data Analysis" open source article.

### ATAC Sequencing:

- ATAC-seq → Center for Epigenetics at UC San Diego
- 100 mg aliquots of tissue
- Utilize the Tn5 transposase enzyme to cleave DNA at accessible regions
- Insertion of a primer sequence
- Primed DNA is sequenced
- Reads can be analyzed to identify regions of increased accessibility and possible transcription factor binding regions

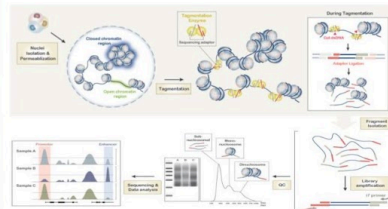


Figure 2. ATAC-seq procedure at UCSD School of Medicine Center for Epigenetics conducted to identify open chromatin regions and inference of possible regulatory element locations. Figure adapted from <https://medschool.ucsd.edu/om/cem/research/epigenomics/Pages/High-Throughput-Epigenomics.aspx>.

### Methods for Data Analysis:

- ATAC and RNA sequences:
  - Trimmed
  - Mapped to the bovine reference genome ARS-UCD1.3
- Analyses
  - Sequence peaks will be called using MACS2 for ATAC
  - Signal proximity will be examined
  - Analyzed and visualized in R

## EXPECTATIONS

Through characterizing the transposase-accessible and actively transcribed regions of the genome of four biological replicates and three tissue types, we expect to define tissue-specific signal as well as capture biological variation. The results of this study will contribute to the current understanding of chromatin accessibility profiles in economically important livestock breeds. A previous study examined the liver, muscle, and hypothalamus in *Bos indicus* cattle. Their findings showed increased open chromatin regions in the hypothalamus, covering 2.41% of the genome<sup>3</sup>. Liver and muscle covered 0.52% and 0.98% respectively<sup>4</sup>. It was discovered that proximity of regulatory enhancers decreases in tissue-specific open chromatin regions, indicating a long range modulation of transcription. In this ongoing work, we expect to provide significant impacts and verification of the epigenome in relevant species of the livestock genetics community<sup>5</sup>.

## ACKNOWLEDGEMENTS

I would like to thank my mentor Dr. Brenda Murdoch for all her generosity and guidance throughout my time working with her. Thank you to the kind graduate students within my lab who have supplied me support and encouragement. Finally, I would like to thank the University of Idaho's Office of Undergraduate Research for presenting me with the Summer Undergraduate Research Fellowship grant that allowed me to make this research and presentation possible.

## RELATED LITERATURE

- The FAANG Consortium, L. Anderson, A.L. Archibald et al. Coordinated international action to accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal Genomes project. *Genome Biol* 16, 57 (2015). <https://doi.org/10.1186/s13059-015-0922-4>
- Alexandre, P.A., M. Naval-Sanchez, M. Menzies et al. Chromatin accessibility and regulatory vocabulary across porcine cattle tissues. *Genome Biol* 22, 273 (2021). <https://doi.org/10.1186/s13059-021-02489-7>

# Determining the stress response of dairy calves when transported at different ages

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## Introduction

- Under current production practices in the United States, transportation is one of the most devastating events that dairy calves face (Hulbert and Moisi, 2016).
- This not only affects the calves in the moment, but it can also impact their future growth and performance, and reduce profits.

## Why?

- Currently, there are no regulations on when calves can be transported in the United States.
- However, there are indications that the negative impact of transport-related stress is more severe for younger than older calves.
- Without sound recommendations on the ideal age to transport calves, we will continue to have a high rate of diseases and deaths related to transport stress.
- Therefore, the objective of this study is to determine the impact of transporting Holstein bull calves that are 2, 4, 8 and 14 days old on growth performance, heart and respiration rate and blood cortisol concentration.

## Literature Cited

Hulbert, L. E., and S. J. Moisi, 2016. Stress, immunity, and the management of calves. *J. Dairy Sci.* 99:3199-3216. doi: 10.3168/jds.2015-10198

## Materials and Methods

- We will measure the impact of transporting Holstein bull calves of four distinct age groups: 2, 4, 8 and 14 days old on:
  - Body weight
  - Heart and respiratory rate
  - Blood cortisol concentration
- For each age group, there will be 9 calves, making the total number of calves being collected 36.
- Calves will be transported for eight hours. Body weight will be measured upon arrival at the university facilities. Heart and respiratory rates will also be measured on arrival. A blood sample will also be collected at arrival for cortisol analysis.

## Significance of the Project

With this study, we will create new evidence-based recommendations for the ideal age to transport calves. Therefore, we can increase the sustainability of dairy operations, as well as increase the production efficiency of dairy cows in the future.

## Acknowledgments

This project was supported by the SURF program  
Special thanks to the University of Idaho Animal Science Department, Dr. Chibisa and Lauren Gilbertson

