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
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Submitted by:

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Office of Undergraduate Research

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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 tasked with taking the lead in enabling research opportunities for undergraduates at the U of I. Among its roles, it manages various competitive student grant programs that directly support student research.

During FY2019, generous funding from the State Board of Education permitted the U of I to continue its Summer Undergraduate Research Fellowship (SURF) Program. This intensive 10-week summer research experience actively engages undergraduates in faculty-mentored, independent research. 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 $1,000 to help offset materials and supplies and other project-related expenses. 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 16 SURF awards during the summer of 2019.

Funding provided by the State Board of Education also allowed the Office of Undergraduate Research to support a number of undergraduate researchers during the academic year. This was accomplished through competitive Undergraduate Research Grants awarded to students during the spring semester of 2019. 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. State Board of Education funding supported 7 Undergraduate Research Grants during the spring semester of 2019.

Almost all of the U of I students supported by State Board of Education funds attended and presented the results of their projects at the Idaho Conference on Undergraduate Research (ICUR) held in Boise in July of 2019. Several of our students also presented posters of their work at the U of I Undergraduate Research Symposium in April 2019. Additionally, some presented their work at national conferences. A few of students were unable to attend this year's ICUR conference. In lieu of this, these students will be required to present their results at the U of I Undergraduate Research Symposium in April 2020.

The funding provided by the Idaho State Board of Education was in the form of two separate awards to the University of Idaho. These awards were managed by the U of I Office of Undergraduate Research and the awards were used together to help fund its competitive student grants program. This final project report combines all of the student project reports funded by both SBoE awards into a single document.

Collectively, the awards provided by the State Board of Education helped fund 23 student research projects. In a few cases, individual projects ended up coming in slightly under the $1,000 amount allotted for project-related expenses. Additionally, one student's summer project played out differently than planned and although he was able to complete the work, his project-related expenses will not come into effect until later in the fall of 2019. Consequently, he spent very little of the project money awarded to him. Taken together, these outcomes left

us with a small amount unexpended funds at the close of FY2019. These funds were returned to the State Board of Education.

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.

Final Project Report: Office of Undergraduate Research (OUR) Spring Undergraduate Research Grant - Spring 2019

Grant Recipient: Chloe Beall, Wildlife Resources, University of Idaho

Faculty Mentor: Dr. Lisette Waits, Professor and Chair, Department of Fish & Wildlife Sciences

Project Title: Columbia Basin Pygmy Rabbit Winter Field Sampling and Genetic Monitoring

Abstract: The pygmy rabbit (*Brachylagus idahoensis*) population in central Washington, United States, has declined significantly in response to habitat loss and fragmentation. The goal of my research was to assist the Washington Department of Fish and Wildlife (WDFW) in determining the number of active burrow sites in the sampling area, collecting fecal samples, and performing genetic analyses on the samples. This research is a continuation of the recovery program overseen by WDFW and will provide insight into population estimation methods using burrow numbers. My research seeks to answer the following questions: How many burrows are detected in the focal sampling area? How many pygmy rabbits are identified from fecal pellets collected in this area? What is the ratio between the number of rabbits detected and the number of active burrows detected? We performed transect searches to locate active burrows and collected fecal samples for genetic analysis across a 3.4 km2 region of the reintroduction area. Individual identification was determined using genotypes from 6-10 microsatellite loci. We collected samples from 103 new wild born individuals, representing 150 unique locations. Overall, we identified individuals from 150 active burrow sites. This resulted in an average of 0.687 rabbits per burrow in the ECRP site.

Project Accomplishments

1. *Determined the number of burrows in the sampling areas*
   Result: With the help of many volunteers, I was able to determine the number of burrows in my primary East Conservation Reserve Program Field (ECRP)study site in central Washington. We walked along belt transects for thorough burrow detection. We collected 271 fecal samples at burrows for genetic monitoring and positively identified 204 of those samples as pygmy rabbit samples. I was also able to compare these results with results from previous work in another study site in the Chester Butte area. The Chester Butte sample was smaller, with 14 burrows and 6 pygmy rabbit individuals detected, but it provided a potentially useful baseline for my study.

2. *Identified individual pygmy rabbits from the sampling areas using genetic analysis*
   Result: After collecting fecal samples from all the detected burrow sites and eliminating samples that were identified as other rabbit species using mitochondrial DNA, pellets underwent further genetic analysis utilizing 19 nuclear DNA microsatellite loci including one sex ID marker. Of the 204 pygmy rabbit fecal samples, 153 passed the M2 multiplex requirements. From those samples, we identified 103 new wild born individuals.

3. *Determined a possible ratio between number of burrows and number of pygmy rabbits in the sampling area*
   Result: Of the 103 individuals identified, 80 were identified at only a single location. The remaining 23 were detected at 70 locations collectively. Based on these data, it seems that there is an average ratio of 0.687 rabbits per burrow. Furthermore, the data from the Chester Butte site

resulted in an average of 0.429 rabbits per burrow. However, given the number of rabbits that were detected at only one location, this average likely has a large amount of variance.

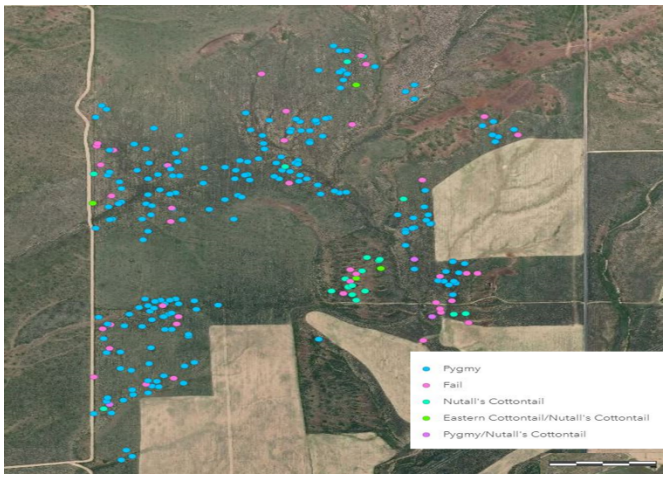


Figure 1. Map of sample locations from winter monitoring and species identification from CRP study area (204 Pygmy Rabbit samples, 20 Nutall's Cottontail, 4 Eastern/Nuttall's mixed, 3 Pygmy/Nutall's mixed, 42 failed).

Summary of Budget Expenditures

Item	Cost	Explanation
DNA extraction supplies	900	150 samples x $6 sample
Posters	100	For UI UG Research Symposium and ICUR
Total	**1000**	

Conference Presentations: I presented a poster of my work at both the Undergraduate Research Symposium at the University of Idaho in April 2019 as well as the Idaho Conference on Undergraduate Research (ICUR) in Boise in July 2019.

Acknowledgement: I greatly appreciate the generous support provided to me by the Idaho State Board of Education HERC in the form of an Undergraduate Research Grant from the U of I Office of Undergraduate Research. This grant funded the genetic analysis needed for the success of my project. I am also grateful to both my faculty mentor Lisette Waits and my graduate student mentor Stacey Nerkowski for all of their help along the way.

Final Project Report: Office of Undergraduate Research (OUR) Spring Undergraduate Research Grant - Spring 2019

Grant Recipient: Mallory Cullen, University of Idaho

Faculty Mentor: Dr. Joe Kuhl, Associate Professor, Department of Plant Sciences, University of Idaho

Project Title: Role of litchi tomato peroxidases in potato cyst nematode immunity

Abstract: Potato cyst nematodes (PCN) are obligate, biotrophic pathogens that are among the most damaging pests to potatoes. These nematodes can cause up to 80% yield loss and with soil fumigants becoming more restricted new strategies must be devised to deal with the infestation of this pest. Mechanical damage caused by pests resulting in a rapid oxidative burst and an upregulation of the peroxidase genes strongly implicate peroxidases in plant defense and immune response. Research conducted in *Solanum sisymbriifolium* (a plant resistant to PCN and a wild *Solanum* relative to potato) showed eleven peroxidase candidate genes are differentially expressed in infected versus uninfected roots, ten peroxidases were upregulated, and one was suppressed. In this project eight of these peroxidase gene candidates were amplified and the open reading frames for these genes cloned into a vector. The clones were submitted for sequencing and the resulting DNA sequences were analyzed. With the sequences for the open reading frames determined, the cloned peroxidases are ready to be placed into a suitable plant expression vector. Future research will be conducted to transform these peroxidase genes into susceptible potatoes to see if they confer resistance to PCN.

Project Summary and Accomplishments:
The goal of this project was to confirm peroxidase open reading frames, ORFs, that were differentially expressed in litchi tomato plants that were infected with potato cyst nematodes versus uninfected litchi tomato plants at the bench. RNA seq data showed 10 peroxidase candidate genes that could be linked with potato cyst nematode infection. Using these candidate open reading frames, primers were designed to amplify the fragments from cDNA. Once amplified, the fragments were cloned into the vector PENTR D-TOPO. From this vector, the plasmids were extracted and sent for sequencing. Using the sequencing results, the data was aligned to the reference sequences from the RNA seq data and analyzed for discrepancies. Due to some unforeseen complications, the confirmed sequence could not be directly integrated into PearleyGate 100 and introduced into agrobacterium. The construct was ligated into PearleyGate100 and transformed into JM109 cells. Using this construct, the plasmid was confirmed using sequencing. Once sequence conformation was complete, the plasmid was transformed into electrocompetent agrobacterium using electroporation.

A significant portion of this project was accomplished during the period of this grant. Starting with 10 predicted peroxidase open reading frames, 9 of them were able to be amplified using PCR and were of the correct size. One open reading frame, c10137/f1p4/96**,** did not match the predicted size of the fragment and did not proceed in the experiment. Next the amplified open reading frames were cloned into a PENTR vector. 8 of the ORFs were successfully cloned into the vector. One ORF, c90433/f240p109/1282, did not successfully clone and was therefore dropped at this stage. Once cloned, the plasmids were sent for sequencing. 6 of the ORFs had matching sequences to the reference sequence. c7557/f1p0/1301 and c6814/f2p4/1378 had 3 and 1 base pair changes respectively. All of these sequences are stored as glycerol stocks awaiting ligation into the PearleyGate 100 vector and then transformation into Agrobacterium. Some trouble was encountered with the ligation and transformation into agrobacterium process. The vector could not be confirmed in agrobacterium. To circumvent this process,

the construct is now confirmed in JM109 cells and then extracted, sequence confirmed and transformed into agrobacterium. Only one peroxidase open reading frame, c16456/f1p3/1223, has been successfully ligated into PearleyGate 100 and transformed into agrobacterium. The next step of this project will be to get the remaining 7 peroxidase ORFs into PearleyGate100 and transformed into Agrobacterium. After this, the construct will need to be introduced into potato plants and genotypic and phenotypic analysis conducted.

For the budget, the money was dispersed amongst portions of my project. The first item purchased was NotI-Hf enzyme for $72.00. This enzyme was purchased to use during restriction digestions for conformation in the pENTR vector. $327.00 went towards purchasing a 100bp and ultra-low DNA ladder for use in gel electrophoresis when running the PRC and digested products. A total of $354.64 was used for sequencing of samples by Genewiz. This amount can be broken down by two rounds of sequencing for $174.00 and $162.00 along with shipping which was $9.32 each time. $180.00 was spent on Timentin, a compound needed to grow the transformed agrobacterium. Finally, the poster for the poster presentation was purchased for $48.00. **TOTAL: $1,000.28**

Conference Presentation: This research was presented at the 2019 UI Undergraduate Research Symposium and at the 2019 Idaho Conference on Undergraduate Research in Boise, ID.

Acknowledgment: This research could not have been as successful as it has been without the generous support of the Idaho State Board of Education/HERC. I truly appreciate the support given to me in the form of a U of I Office of Undergraduate Research Grant.

Final Project Report: Office of Undergraduate Research (OUR) Spring Undergraduate Research Grant - Spring 2019

Grant Recipient: Ren Dimico, Biological Sciences, University of Idaho

Faculty Mentor: Dr. Peter Fuerst, Associate Professor, Department of Biological Sciences

Project Title: Unravelling Genetic Determinants of Synapse Formation in the Mammalian Visual System

Abstract:

Blinding diseases, such as age-macular degeneration and glaucoma, are common causes of vision loss and occur in 2-15% of the population. A detailed understanding of visual system organization is a limiting factor in developing treatments for such disorders. Genetic blinding diseases are studied to understand visual system organization and diseases. Stationary night blindness, is caused by mutation in the *Dscaml1* gene. *Dscaml1* encodes for the protein, Down Syndrome Cell Adhesion Molecule-like 1 (DSCAML1), which plays a role in organization of cells critical for night vision. In a previous study we used electron micrographs to visualize the cellular organization of rod bipolar cells (RBC) in the synaptic pathway within the mouse retina, an accessible model for human diseases. We found an increased number of dendrite terminals that do not contact rod photoreceptors in the absence of DSCAML1. This project focuses on using immunohistochemical (IHC) techniques to compare the development of the synaptic pathway at multiple post-natal time points in three genotypes. It is predicted that the loss of DSCAML1 results in termination of the pathway between the RBC and dendrites instead of a delay in formation. This study is intended to guide clinicians seeking interventions for people with similar disorders.

Project Description:
The aim of this project was to optimize and utilize an IHC staining protocol to stain cryostat sectioned retinas for RBCs, puncta, and rods. First optimal concentrations and staining times were determined with the antibodies used. After obtaining an optimized protocol the ratio of puncta to RBCs was calculated using florescent imaging to determine if there is a delay in synaptic formation as the mouse ages throughout different timepoints. Three genotypes are being used in this study: B6 (wild-type), DSCAML1$^{+/-}$, and DSCAML1$^{+/+}$.

Summary of Project Accomplishments:
Optimal staining concentrations, times, and temperatures were determined in order to derive an optimized protocol for this study. All age points that were taken for study, within the three different genotypes, were successfully stained, counted and analyzed. Limitations were presented at the end of the summer due to working with specific timepoints of mice, but all mice that were taken for this study were analyzed up until this point.

Conference Presentations: I presented a poster of my work at ICUR in Boise in July 2019 as well as at the UI Undergraduate Research Symposium in April 2019.

Summary of Budget Expenditures:
New antibodies to stain the RBCs and puncta were ordered ($685). Part of the budget was also used for care of the animals used in this study ($245) in addition to poster printing ($70) for the ICUR conference I attended at the end of July. TOTAL EXPENDITURES: $1,000.

*This project will continue into the Fall 2019 semester as more mice can be taken for study at the desired timepoints.

Acknowledgement: I am very thankful for the generous support provided to me by the Idaho State Board of Education/HERC in the form of an Undergraduate Research Grant. I am also grateful to the U of I Undergraduate Research for all of its support and for making this grant possible.

Final Project Report: Office of Undergraduate Research (OUR) Undergraduate Research Grant - Spring 2019

Grant Recipient: Jadzia Graves, Materials Science and Engineering, University of Idaho

Faculty Mentor: Dr. Indrajit Charit, Department of Chemical and Materials Engineering

Project Title: Microstructural and Mechanical Properties Evaluation of Friction Stir Welded High Entropy Alloys

Abstract: Traditional alloys that are commercially available have one base element and trace amounts of multiple other elements. That limits the capabilities of the alloys as they are largely dependent on the base element instead of utilizing the abilities of all the elements in the alloy. High entropy alloys (HEAs) are an advancement of traditional alloys as they have a larger percentage of multiple elements. In this proposal, we will work on the effect of friction stir welding (FSW) to determine how it affects the microstructures and the correlating mechanical properties.

Project Overview: In this project, the High Entropy Alloy (HEA), known as $Fe_{42}Mn_{18}Co_{10}Cr_{15}Si_5$, was looked at under various microscopes to see how Friction Stir Welding (FSW) affects the mechanical properties of the material. FSW is when a rotating tool heats and joins two side by side plates. It is important to know how the material is affected by this process to understand how the material changes as it undergoes this process.

Project Accomplishments:

1. Learned how to prepare samples for various forms of testing and microscopy

To prepare samples for the optical microscope, a small section was cut off of the main block. It was placed into a mold to create a cylindrical object with a 1in diameter and a thickness of .5in. The sample was then grinded with sandpaper from 120 grit to 1200 grit before it was polished with a diamond slurry. In order to see the microstructures it was lightly etched with a hydrochloric/nitric acid solution. After the samples were looked at with the optical microscope, they were also used on the Vickers Hardness machine.

For the scanning electron microscope (SEM), the sample was prepared in much of the same manner, but the alloy had to be extracted from the mold. This is to allow the electrons to properly bounce off of the alloy which is what provides the images.

Transmission electron microscope (TEM) samples are prepared slightly different. Rather than being placed in a mold, a thin sliver of the material is glued onto a metal disc. Once one side has been polished, the sample is removed and reattached on the other side. As the second side is being polished, it is also measured to ensure that the sample is less than 100 micrometers thick.

The Instron testing machine requires samples to be cylindrical, so the material was machined to the proper size.

2. Used the optical microscope, the scanning electron microscope (SEM), Vickers Hardness machine, and the Instron testing machine

The optical microscope is used to see basic microstructures. Typically, images are taken at magnifications from 50x to 100x, but can go as high as 500x. Multiple images were taken across the weld zone, so in the future a single image of the weld zone will be available.

SEM is used to look more in depth at the microstructures. The magnifications go up above 2000x. To use the SEM, the sample was loaded into a vacuum chamber. Cameras were used to position the sample under the electron beam.

The Vickers Hardness machine is used to determine the hardness of the material. This is done by pressing a diamond tip into the material and measuring the imprint. For this material, measurements were taken across the weld zone to see how the weld affects the hardness.

The Instron testing machine has many functions, for this project, compression tests were performed. From the compression test, mechanical properties such as Young's modulus and ultimate yield strength could be determined. Compression tests were chosen over tensile tests do the amount of material that was saved by doing compression, along with the fact that the microstructures can be analyzed after compression tests but not tensile tests.

Summary of Budget Expenditures:

Description	**Title**	**Cost**
0319 WSU PULLMAN 509-335-9651 WA	Analytical Services	**200.67**
Boise State University	Analytical Services	**187**
Supplies 04252019	Research Supplies	**612.33**
	Total	**1000**

Conference Presentations: I presented images taken from this project at the UI Materials Advantage Paper Night hosted by ASM International in April 2019. I presented a poster of my work at ICUR in Boise in July 2019.

Acknowledgements: I sincerely thank the State Board of Education for their support in the form of an Undergraduate Research Grant from the Office of Undergraduate Research at the University of Idaho. I would also like to acknowledge Franceshi Microscopy and Imaging Center at Washington State University for the use of their Transmission Electron Microscope.

Final Project Report: Office of Undergraduate Research (OUR) Spring Research Grant – Spring 2019

Grant Recipient: Reagan Haney, College of Agricultural and Life Sciences, University of Idaho

Faculty Mentor: Shirley Luckhart, Professor, Department of Entomology, Plant Pathology, and Nematology; Professor, Department of Biological Sciences

Project Title: Effects of Abscisic Acid (ABA) on *Anopheles stephensi* Reproduction

Abstract Hundreds of millions of malaria cases are reported every year despite significant global efforts focused on elimination. Accordingly, new vaccines, therapeutics, and strategies for vector control are needed to support these efforts. *Anopheles stephensi* is an aggressive malaria vector mosquito that has invaded and become established in Sri Lanka, Djibouti and Ethiopia, with significant risk for range expansion into Somalia, Eritrea and Sudan. In Djibouti, *A. stephensi* has been linked to a resurgence of severe infection with the human malaria parasite *Plasmodium falciparum*. Malaria control directed at reducing mosquito reproduction or fecundity is an important strategy, particularly for *A. stephensi* that is adapted to oviposition in artificial water sources in urban habitats. Mosquitoes consume blood to produce eggs. Following blood consumption, the protein Vitellogenin (Vg) is synthesized in the fat body of the female mosquito and transferred to developing eggs. Vg synthesis is stimulated following the blood meal by increasing titers of the hormone 20-hydroxyecdysone (20E). We have discovered that abscisic acid (ABA), a natural compound, can reduce mosquito fecundity. Based on studies in the flesh fly that showed that ABA can reduce Vg levels in this insect, we hypothesized that ABA reduces *A. stephensi* fecundity by reducing levels of Vg in the mosquito.

Project Description In 2016, there were 216 million reported cases of malaria worldwide and about half a million deaths occurred due to the devasting effects of the malaria parasite. Malaria is a vector-borne disease that is caused by a parasite, most commonly *Plasmodium falciparum*, transmitted through the bite of an *Anopheles* mosquito. There has been progression towards eliminating malaria in some areas of the world. However, there are still many challenges to overcome before eliminating malaria. Obstacles such as parasite resistance to antimalarial drugs and mosquito resistance to insecticides are just a few examples of obstacles that have yet to be tackled.

Project Accomplishments

1. Previous work in the Luckhart lab demonstrated that supplementation of adult female *A. stephensi* with ABA had no effect on egg production in the first reproductive or gonotrophic cycle. Given that female mosquitoes with access to blood can lay eggs approximately every 3-4 days and, thereby, complete multiple gonotrophic cycles during a complete lifespan, I determined whether supplementation of ABA to adult female mosquitoes could alter fecundity beyond the first gonotrophic cycle.

   Result: In the first set of experiments, adult female *A. stephensi* were supplemented with ABA and clutch size (number of eggs laid per female during one reproductive or gonotrophic cycle) was evaluated. I observed that ABA does not affect clutch size when supplemented to adult female mosquitoes.

2. Current research in the Luckhart lab has demonstrated that supplementation of ABA in water to mosquito larvae shortened time to pupation and body size of adult mosquitoes. Therefore, I tested

whether supplementation of ABA to mosquito larvae could alter reproduction of adult females derived from supplemented and control larvae.

Results: I observed that female *A. stephensi* derived from ABA treated larvae exhibited a significant reduction in clutch size relative to adult females derived from untreated larvae. These data indicate that ABA treatment of larvae results in physiological changes that persist into the adult stage and that are durable over time. It was also notable that ABA supplementation of *A. stephensi* larvae significantly reduced the lifespan of adult female mosquitoes derived from treated larvae relative to adult females derived from untreated larvae.

3. Based on our data, we hypothesized that ABA reduces *A. stephensi* fecundity by reducing levels of Vg in the mosquito.

Results: 20E and Vg levels were evaluated post blood meal. Adult female *A. stephensi* in the control group exhibited the expected pattern of increasing 20E titer following a blood meal. Female mosquitoes derived from larvae treated with 1μM ABA and 100μM ABA group did not show the typical peak of 20E at 24 hr, indicating they did not properly respond to the blood meal. Further, adult female *A. stephensi* derived from larvae treated with 1μM ABA and 100μM ABA had increased Vg mRNA levels at 12 hr post blood meal relative to control, but reduced levels in the following 36 hr. This early increase in Vg mRNA expression followed by reduced Vg expression could explain why egg production was reduced but not blocked in adult females derived from larvae treated with ABA.

Conference Presentation: This study was presented as a poster at the University of Idaho Undergraduate Research Symposium in April 2019 and ICUR in Boise, ID, in July 2019.

Summary of Budget Expenditures

Item Purchased	Cost	Balance Remaining	Receive date
Caisson ABA	$641.91	$358.09	2/7/2019
IBB Blood	$300.00	$58.09	2/10/2019
Fisher Cotton Balls	$58.09	- 0 -	3/18/2019

Acknowledgment: I greatly appreciate the generous support provided by the State Board of Education in the form of a Spring Undergraduate Research Grant from the UI Office of Undergraduate Research. This was an amazing experience for me. Without this support from the SBOE/HERC, I would not have been able to participate in this research.

Final Project Report: Office of Undergraduate Research Grant - Spring 2019

Grant Recipient: Jordan Howard, Food & Nutrition, University of Idaho

Faculty Mentor: Katie Brown Ph.D., formerly Associate Professor, Department of Family & Consumer Sciences (Dr. Brown now works at Utah State University)

Project Title: Low Energy Availability and Bone Density in Non-Athlete Males at the University of Idaho

Abstract: Low energy availability (LEA) is a condition resulting from an insufficient amount of energy required for normal function and metabolic processes after accounting for exercise. Collegiate athletes are prone to developing LEA due to insufficient energy intake, and LEA is associated with decreased bone mineral density (BMD) in competitive athletes. However, little is known about the occurrence of LEA or its relation to BMD in non-athlete populations. This study aimed to investigate the prevalence of LEA and its relation to BMD in non-athlete males at the University of Idaho. Non-athlete males (n=21) ages 18-26 took part in this study. EA was assessed using measurements of dietary energy intake (DI), exercise energy expenditure (ExEE), and body composition. BMD was assessed using dual-energy x-ray absorptiometry (DEXA). Five participants (23.8%) identified with LEA based on an EA of < 30 kcal/kg of LBM/day. There was no correlation between EA and total BMD ($p = 0.951$), spine BMD ($p = 0.641$), or hip BMD ($p = 0.786$). However, total BMD was significantly correlated with body weight ($p < 0.001$). These findings differ from previous research among competitive athletes which found associations between LEA and BMD.

Project Accomplishments:

1. Assess the prevalence of low energy availability among non-NCAA athlete male students at the University of Idaho.

I was successful in assessing the prevalence of low energy availability among this population. I was able to measure this prevalence by monitoring participants' physical activity and dietary intake over a period of three days. Participants wore accelerometers to monitor their physical activity and tracked their diet using ASA 24, on online dietary assessment tool. 21 non-NCAA athlete males at the University of Idaho successfully completed this study. Among these participants, 71% (n=20) were found to have reduced energy availability while 23% (n=5) were found to have low energy availability.

2. Study the relationship between low energy availability and bone density in non-NCAA athlete males.

By using dual-energy x-ray absorptiometry (DEXA) technology, I was able to assess bone mineral density in participants. A comparison between bone mineral density z-scores and energy availability using Spearman correlations revealed there was no correlation between low energy availability and bone density ($p = 0.951$). However, a strong correlation was found between bone mineral density and body weight ($p = 0.001$).

3. Study the relationship between low energy availability and testosterone levels in non-athlete males.

A goal of this study was to test the following hypothesis: Testosterone levels in non-NCAA athlete college males with low energy availability will be significantly lower than males without low energy availability. Salivary testosterone samples were successfully obtained from participants who completed the study. These samples were sent to Salimetrics® to be analyzed. However, I have not yet received these results back from the company. Once these are received, I will be able to study the relationship between energy availability and testosterone in non-NCAA athlete males and test this hypothesis.

Summary of Budget Expenditures:

Supplies	**Cost**
Salimetrics® Testosterone Sampling Kits	**$900**
Participant Incentive	**$100**
Total:	**$1000**

Additional Information:
This project was originally titled, "Low Energy Availability and its Relation to Testosterone Levels in Non-Athlete Males." However, because the results of the testosterone sampling were not ready in time to present at the University of Idaho Undergraduate Research Symposium in April, I included the study of bone density in my project. Due to delays in the processing of testosterone samples, I have not yet received these results. When these results are analyzed, I plan to update this report with my new findings.

Conference Presentation:
This study was presented as a poster at the University of Idaho Undergraduate Research Symposium in April 2019 and ICUR in Boise, ID, in July 2019.

Acknowledgement:
I truly appreciate the generous support provided by the State Board of Education in the form of this undergraduate research grant. This was an incredible experience which greatly enhanced my education at the University of Idaho. This grant enabled me to participate in research on a level that otherwise would not have been possible. I learned a tremendous amount about research design, protocols, and techniques related to nutrition, as well as how to properly analyze and disseminate results. Thank you again for your generosity.

Special thanks to my faculty mentor Dr. Katie Brown, Eric Vallin, Krista Story, Megan Follett, and Dr. Ann Brown for their tremendous support and help with this project.

Final Project Report: Office of Undergraduate Research, Spring 2019

Recipient: Paul Riebe, Materials/Mechanical Engineering, University of Idaho

Faculty Mentor: Dr. Mark Roll, Associate Professor, Department of Materials Engineering

Project Title: ABS Microstructures in Extrusion 3D Printed Samples

Abstract: ABS plastic exhibits an interesting set of properties, making it ideal for certain applications. It is a convenient material for use in consumer grade additive manufacturing, and its cost is relatively low. This makes it popular for use as a filament in 3D printers like the Makerbot. However, parts printed out of ABS do not behave the same way as parts produced using traditional methods, like injection molding. Preliminary research done previously on the project shows that the failure patterns of ASTM dogbone testing samples are radically different when comparing layered prints and injection moldings. During tensile tests, printed samples fail suddenly, with little to no necking at the failure point. Injection molded samples follow a typical stress/strain curve. It would seem that there is something happening at a microstructural level to explain this phenomenon. The purpose of this research was to pinpoint this microstructural difference by extruding custom filament from the exact same ABS stock that was used to injection mold the sister samples. By eliminating any material differences between stock suppliers, the microstructural differences, it was hoped, would become apparent.

Project Accomplishments

1. First, the old research had to be compiled and organized. The project had been worked on by two students previously, and the records were both old and slightly disorganized. Some samples had been tested, but test sample lengths after tensile testing were never recorded.
    1.1. I measured the remains of the tested samples from over the past several years, recording all of their lengths, and matching them with the conditions of their test environment in a large spreadsheet.
    1.2. After doing this it was realized that many of the old samples were the wrong size and shape and did not match any ASTM standard dogbone. Not only that, but some had been vapor annealed, and some simply had no notes on them. I compiled the lengths for all of them, but as it worked out there were never more than a few of any one distinct sample, so trying to compare them to the injection molds was going to be difficult.
2. After compiling the old tests, it was decided that new filament would be extruded, and some more tests should be conducted using standardized sample geometries. Upon requesting that some more filament be extruded, Dr. Armando McDonald politely declined, citing that he was already far too pressed for time to get the extruder running again.
    2.1. At this point I approached the individual in the Buchanan Engineering Lab who helped 3D print the previous samples. Upon discussing the project with him and asking whether there was any leftover filament from the custom stock that was made before I joined the project, he replied that it had never been used and that the samples had been printed with brand name filament. In other words, our non-standard samples were not made out of the same ABS plastic, probably due to a slight breakdown in communication several years ago.
3. It was at this juncture that it seemed prudent of us to pursue microtomy of the old 3D printed samples, simply to at least get an idea of where to go with the research.

3.1. I began by asking around McClure, only to find that the microtome we thought was there had been surplussed several years ago. I reached out to the biology department, but turned attention toward WSU's Electron Microscopy Lab.

3.2. We knew that the ABS samples would need to be stained with Osmium Tetroxide, but when we tried to find anyone with a working knowledge of Osmium Tetroxide staining, we came up empty handed, including at WSU. It was proposed that I could go and pay for the training to use the SEM at WSU, but that would require a semester long course for which there simply was not time.

In the end, the conclusion of the Spring semester rolled around and Dr. Roll and I had hit nothing but dead ends in the course of the project. I express my sincerest apologies, as I had high hopes of being successful with this research. However, Dr. Roll has informed me that this is not unheard of.

Summary of Budget Expenditures:

Stock Material Costs: ABS $62.83 per 5 kg bag	$62.83
Stock Material Costs: Transparent ABS Filament $29.95 per roll	$29.95
Microscopy Supplies & Maintenance Costs	$225
Total*	**$317.78**

*Unspent funds were returned at end of funding period

Conference Presentations: Regretfully, I did not have any results in time to present at ICUR 2019 so I did not attend/present a poster at this conference. My plan is to continue research in Dr. Roll's laboratory this fall semester and present my results at the UI Undergraduate Research Symposium in Spring 2020.

Acknowledgement: I greatly appreciate the generous support provided to me by the Idaho State Board of Education/HERC in the form of an Undergraduate Research Grant from the U of I Office of Undergraduate Research. I truly appreciate this opportunity, and despite the misadventures along the way, I learned a lot and this experience meant quite a lot to me. This research, although occasionally troublesome, was of tangible benefit to my college career.

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Jacob Alderink, Computer Science, University of Idaho

Faculty Mentor: Terence Soule, Professor and Chair of Computer Science Department

Project Title: Examining the Behavior of Evolutionary Algorithms in the Starcraft II Environment

Abstract: Autonomous software has become a large part of everyday society. They drive our cars, deliver our packages, fly drones, and maintain our economy. These robots need to learn at both an individual scale but also learn how all the robots need to work together at a management scale. Evolutionary AI techniques could solve the problems that come with maintaining and teaching these robots. Starcraft provides a testbed for AI behavior analysis. Starcraft requires the player to create a military infrastructure, manage an army at both the micro and macro level, and collect and administer resources. Using Starcraft we examine the best method for evolving two algorithms a macro algorithm and a micro algorithm. For our results we examined: running the micro algorithm by itself, the macro algorithm by itself, running them both in parallel, running the macro algorithm for a short time then having the micro come in, and finally running them both separately and then combining them after a set amount of generations. Our results indicate that parallel evolutionary algorithms with interdependent goals learn best when infrastructure is learned solo, and then unit behavior is defined.

Project Design: The evolutionary algorithm is two-fold. One algorithm is in charge of developing the overall strategy that this bot takes (The Macro Algorithm). The second algorithm is in charge of developing strategy for the individual unit groups that the first algorithm produces (The Micro Algorithm). The Macro Algorithm is a Genetic Algorithm (GA) in which each individual is 100 integers long. Each integer ranging from 0-32 represents a building or unit that it wishes to produce. Once the game is started, the GA starts at the first gene in the selected individual and takes the number there and translates it to a corresponding unit/building. If it can build it, the game then produces that unit. If it can't (due to tech tree issues or possibly missing resources) then a fitness penalty is administered. Since the ultimate goal of the Macro Algorithm is to produce an army, every unit that is produced adds to the fitness of the individual.

The Micro Algorithm is also a GA in which each individual is (149*18) = 2682, float values long ranging between -1.0 and 1.0. These values are weights inputted in a neural network that are trained through evolutionary methods to determine the behavior of every type of unit in the game. Since the bot is protoss there are 18 unique army units that are in the game that need to have their behavior trained hence the 18. The neural network itself is 2 hidden layers with 11 inputs and 5 outputs. Information input into the neural network is:

Table 1: Neural Network Input Table

Input List	Format
Unit Position	(x,y)
Group Center Position	(x,y)
Enemy Start Location	(x,y)
Location Of Nearest Enemy	(x,y)
Percent of Health Remaining	Float(0.0-1.0)
Number of Adjacent Allies	Integer
Number of Adjacent Enemies	Integer

The output information is encoded as such:

Table 2: Neural Network Output Table

Input List	Format
Position to Go To	(x,y)
Execute Unit Ability	Float(0.0-1.0)
Attack or Move to Location	Float(0.0-1.0)
Attack Closest Enemy	Float(0.0-1.0)

Project Results: The method that produced the best results was when the Macro trained alone for 30 generations and then started training with the Micro algorithm. This indicates that autonomous AI programs that need to train with other algorithms will do best if the algorithm that creates infrastructure is trained alone first and the individual behavior algorithms are then attached.

Table 3: Results

Format	Generation .5 Ratio Was Reached On
Macro Alone Followed by Micro	Generation 55
Micro Alone Followed by Macro	Generation 99
Both Trained in Parallel	Generation 71
Both Trained Separate then Combined	Not Reached after 120 generations

It reached a 50% win loss ratio against the HARD bot at 55 generations which is 15 generations before the next method did. This potentially happens because the micro algorithm is more heavily dependent upon the macro algorithm to work. If there are no units produced by the macro algorithm, then for the first set of generations the micro algorithm might learn the wrong things. Thus, the macro-algorithm training by itself would create an environment so that when the micro does come it, it has units and behaviors to improve and train upon.

Conference Presentation: This study was presented as a poster at ICUR in July 2019.

Project Budget:

Item	Cost
SURF Fellowship	$4,000
ICUR Poster	$89.04

Acknowledgement: I truly appreciate the generous support provided by the State Board of Education/HERC in the form of a Summer Undergraduate Research Fellowship from the UI Office of Undergraduate Research. This was a tremendous experience for me. Without this support from the SBOE, I would not have been able to participate in this research.

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Jennavere Ball, Movement Sciences, University of Idaho

Faculty Member: Dr. Chantal Vella, Associate Professor, Department of Movement Sciences

Project Title: Associations of sedentary behavior and skeletal muscle mass in middle-aged to older adults

Abstract:
PURPOSE: To examine the associations of muscle mass and risk factors for sarcopenia with time spent in sedentary behaviors in middle-aged to older adults. **METHODS:** 12 adults (mean±SD: age: 56±9.5 y; 83% women) visited the laboratory on two occasions where anthropometrics, body composition, and blood glucose, hemoglobin A1c, and lipids were measured. Skeletal muscle index (SMI), total skeletal muscle, lean, and fat mass, as well as segmental lean and fat mass were estimated with a multi-frequency bioelectrical impedance analyzer. Sedentary behavior (SB) and moderate-to-vigorous physical activity (MVPA) were objectively measured for 7 days using a triaxial accelerometer worn on the right hip. Simple correlations were used to examine the associations between SB and variables related to lean body mass. **RESULTS:** On average, participants were classified as overweight with a body mass index of 29.1±5.1 kg/m^2 and body fat percentage of 36.6±8.4%. Participants had adequate skeletal muscle in the arms and legs indexed to height, as indicated by a SMI of 7.62±1.02 kg/m^2. On average, participants spent 494.6±75.5 min·d^{-1} in SB and 59.18±26.3 min·d^{-1} in MVPA. SB was positively correlated with skeletal muscle mass (r=0.35), SMI (r=0.51), and lean mass in the left leg (r=0.25), right leg (r=0.27), and trunk (r=0.40); however, these associations were not statistically significant (p>0.05 for all). **CONCLUSIONS:** Although our data show SB had low-to-moderate positive correlations with lean mass, these were not statistically significant and were in the opposite direction of our hypotheses. A larger sample size is necessary to draw conclusions regarding these preliminary findings.

Project description:

It is known that increased sedentary behavior has been positively related to many negative health outcomes. The effects of sedentary behavior have been shown to have negative outcomes on health even if an individual meets the recommended guidelines of 150 min per week of moderate-to-vigorous physical activity (MVPA). Although several studies have investigated the associations between sedentary behavior and body composition, a majority of these studies only examine body fat and fail to consider lean mass. Lean mass is composed of skeletal muscle, bone, and water. Sarcopenia, defined as gradual muscle loss with age, is related to the inability to perform activities of daily living with ease and loss of independence. Few studies have examined the relationship between sedentary behavior and risk factors for sarcopenia, and those that have are limited because of the use of self-reported levels of sedentary behavior. The purpose of this study was to determine the associations between total sedentary behavior and bouts of sedentary behavior with markers of muscle health, while controlling for risk factors, such as age, physical activity, diet, and self-reported diagnosis of chronic disease in middle-aged to older adults. The term "bouts" refers to the total number of times when a participant sits for an extended period of time. In this study we specify how many 10, 20, 30, 40, 50, and 60-minute sedentary bouts over the course of a week. Results of this study may help fill a gap in the literature by determining whether sedentary behavior and muscle mass are associated.

Summary of project accomplishments:
Although our study start date was delayed, we have been successful recruiting participants. To date we have recruited 18 participants total: 12 out of the proposed 30 participants have completed the study, and 6 additional participants are currently in the study. Based on the success level of recruiting we believe that we will be collecting data for a few more months. A preliminary analysis of the data using the 12 participants with complete data showed that sedentary behavior was positively correlated with skeletal muscle mass (r=0.35), skeletal muscle index (r=0.51), lean mass in the left and right leg (r=0.26 and r=0.27 respectively), and lean mass in the trunk (r=0.40). However, these correlations are not statistically significant, likely due to our small sample size. Additionally, we have not yet accounted for covariates in the model due to this small sample size. We hypothesize these correlations will decrease and possibly become negative as we gain more data. We will continue to collect data for this study until at least 35 participants are completed as that should be enough data for statistical analyses.
Through this project I've also had some personal accomplishments. I have gained valuable leadership experience conducting this study and have become very proficient in health-related data collection procedures and working with human subjects. I have learned how to effectively communicate with participants to explain consent forms and testing procedures, conduct body composition assessments using multi-frequency bioelectrical impedance analysis, obtain blood samples via finger stick for testing of blood sugar and fats, and collect and process activity monitor data. I have learned firsthand what the research process entails from the work that goes into developing a research question and methodology, organizing data, and analyzing and communicating results. I have also learned how much dedication working with a close-knit team takes and how to communicate with each other to have the study run smoothly. Although, I have been with Dr. Vella's lab for about a year now, I have learned the most during these last 2 months where I have had the opportunity to take the lead on every aspect of this study.

Summary of budget:
Of the $1,000 project budget, $700 of our budget went to participant gift cards as incentive to complete the study. The rest of the budget ($300) was used to pay for costs associated with the blood draws. The poster printing costs for the U of I Undergraduate Research Symposium will be paid out of my mentor's lab account as we don't anticipant printing the poster until spring semester. TOTAL EXPENDITURES: $1,000 project budget + $4,000 fellowship = $5,000.

Poster presentation at ICUR 2019:
Dr. Pfeiffer, Director of the Office of Undergraduate Research, gave me permission to miss the Idaho Undergraduate Research Conference in Boise for extenuating circumstances; however, I will present a poster at the UI Undergraduate Research Symposium in April of 2020 detailing the results of my study.

Acknowledgment:
I truly appreciate the generous support provided by the State Board of Education in the form of a SURF award from the UI Office of Undergraduate Research. Without this support from the SBOE/HERC, I would not have been able to participate in this research.

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Chanelle Brusseau, Animal Veterinary Science and Pre-Vet, University of Idaho

Faculty Member: Dr. Karen Launchbaugh, Professor, Rangeland Ecology

Project Title: Examining visual cues and cattle responses for virtual fences

ABSTRACT:
Virtual Fence is a new, innovative, and advanced technology designed to enclose grazing cattle with less labor and lower negative ecological effects than conventional wire fences. A student-professor team is developing an electronically advanced nose clip attached to livestock eliciting electrical stimulus when animals' cross virtual boundaries. My research advances our design by examining visual cues to facilitate animal learning. I conducted research to address two questions: 1) Will cows avoid visual barriers after receiving electrical shock to the nose? 2) Will animals recognize and stop at unconnected objects of varying distances as visual cues for a barrier? We found that after 3 days of training, where animals received a shocked after crossing a line on the ground, 89-91% of cows, depending on circumstances, would stop at that line. It was also discovered that in experiments with unconnected posts in various locations, animals were still able to avoid a visual barrier with 91% and higher success rate. Overall, we can conclude that cows are able to use differing visual cues to perceive a virtual barrier between unconnected objects. Results will be used by the UI Virtual Fence team to advance our design for ranchers to use on grazing lands. A manuscript is being prepared for a publication in a refereed journal and guidelines will be developed to help ranchers use visual cues to train animals for virtual fences.

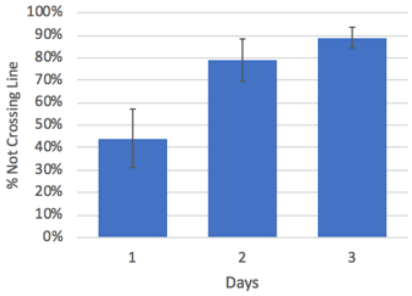
Project Description
Since livestock were domesticated 10,000 years ago, humans have engaged in animal husbandry to provide materials and food to humans. In the late 1800's, ranchers began using a new technology called, "barbed wire" to keep animals enclosed and graze in certain areas. Electrical fencing was introduced in the 1930 as an alternative to heavy wire fences. Fast forward to the 2000's, we see the agriculture industry using more electronically integrated technologies. Precision technology is also being developed for ranchers to manage livestock movement by using what is known as "virtual fence" which is designed to keep cattle out of or inside an area with a device worn by the animal that receives a signal when animal breaches a designated boundary and administers an electrical shock. My research examined if cattle can perceive a line on the ground, or unconnected points, as a barrier in a virtual fence.

Experimental Procedures and Accomplishments:
Animals used for this research were the University of Idaho's Charolais beef cows (n=29) whose age ranged from 2 to 10-years-old and weighed 650 to 850 kg. Protocols were approved by IACUC (Protocol #IACUC-2018-25) in February 2019. We used a Sport Dog YardTrainer 350 training dog collar connected to a nose clip by wire leads to emit an electrical shock at 4.7 kv, no resistance.

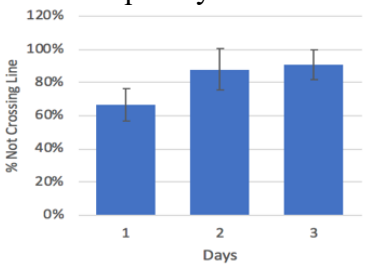
Before the experiments, we introduced cattle to molasses COB (i.e., corn, oats, barley) grain and observed and recorded cows' individual responses to distractions, nervousness, and motivation to eat grain. From these data we selected 18 animals most suited for the experiments. In all experiments, we haltered cattle and applied a nose clip to deliver an electrical stimulus. Cows were released from the working pen and moved to a treatment zone where they were observed and encouraged to cross a boundary line into an exclusion zone marked by a visual line or barrier with posts. In in experiments 1-4

there were grain pans in the exclusion zone to encourage cows to cross the barrier and enter the zone. In experiment 5, the grain pans were not present, but 10 other cows from the herd were held beyond the exclusion zone to draw cattle into the zone. Each day, cows were tested 4 times and places in a reset zone between runs. After trials, cows had halters and nose clip removed and were released to pasture.

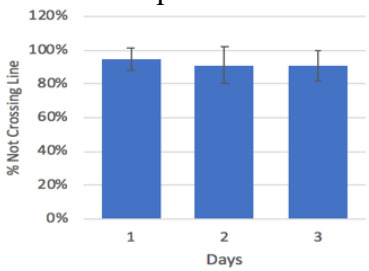
Experiment 1: We examined if cattle stop at a line in an alley after receiving an electrical shock and how long it would take them to learn this behavior. We tested 18 cows 4 times each day (30 sec/run) until >85% of them didn't cross the line. During the experiment we found that more cows stopped at the visual line on the ground each day ($P<0.05$) until day 3 when 89% of animals stopped (Figure 1). These results indicate that cows were able to learn to stop at a line in only a few days



Experiment 2: Like experiment 1, cows were encouraged to cross a line receiving a shock if they crossed that line. However, in this experiment we wanted to know if animals would still stop at a line in a larger area (corral) rather than a narrow alley. Each cow (n=16) was tested 4 times per day. We observed that cattle did quickly learn even in a larger area. By day 3 cattle stopped at the line >90% of the time. (Fig 2).

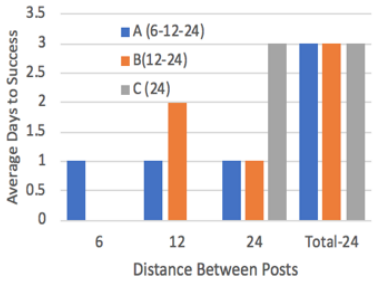


Experiment 3: In this experiment we examined if cattle would stop at unconnected posts instead of a line on the ground. Cattle were encouraged to cross a line posts spaced 1.5-meters apart in a corral. Each cow (n=16) had 4 runs/day (30 sec./run). We found that the cows throughout this trial stopped at the unconnected posts >90% of the time (Figure 3), indicating that cows perceive a virtual boundary.

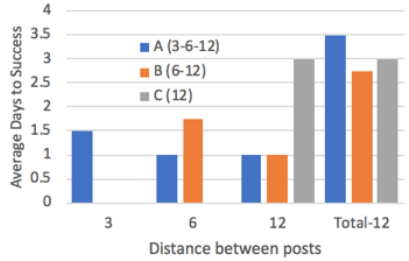


Experiment 4: In this experiment we increased the distance between posts to examine if animals perceive this as a boundary. Posts were spaced 6, 12 and 24 meters apart in a pasture. Cows started at a set distance and progressed a greater distance after not crossing the line 18 out of 20 times per group of five cows. Cattle were placed into 3 groups: 1) 6 → 12 → 24 meters, 2) 12 → 24 meters, and 3) 24 meters. Cows had 4 runs/day (2 min./run). We found that animals take about 3 days to learn to stop at a distance of 24

meters between posts (Figure 4). Previous experience at shorter distances doesn't decrease the time it took to perceive a barrier at 24 meters. There was concern that feed pans placed directly behind the line of posts indicated a virtual boundary to the cows.



Experiment 5: In the final trial, we removed feed pans from the exclusion zone to ensure they were not acting as visual cues for the boundary. Cows (n=15) were encouraged to cross posts spaced at 3, 6, and 12 meters apart in a pasture. To ensure cows in the trial cross into the exclusion zone we placed 10 other cows in the herd beyond the exclusion zone. As above, cows started at set distance and advanced to greater distance. Cows were in groups: 1) 3 → 6 → 12 meters, 2) 6 → 12 meters, and 3) 12 meters. Cows had 5 runs/day (2 min./run). Cows individually moved up to a greater distance when they didn't cross the line 4 or more times out of 5 runs. Animals took about 3 days to not cross the line at >91% (Figure 5). Previous experience with different distance smaller than 12-meters didn't decrease the days it took to stop at 12 meters.



In summary, I found that cattle learn very quickly with simple designs and they have the knowledge to remember what they learned to apply it to other situations. I will continue this research with my fellow teammates through the fall to discover more to help better the Virtual Fence Project.

Budget expenditures:

Type of Expense	Cost
Feed for experiments	$247.16
Field equipment	$ 238.68
Fuel for travel to site	$ 45.72
SportDog Collars-2	$299.98
Total	**$ 831.54**

Conference Presentation: I informed both Patricia Tilden and Dr. David Pfeiffer that I was not able to attend the ICUR 2019, however I'll present my findings in the Poster Symposium during the school year.

Acknowledgments: I appreciate this SURF grant that allowed me to explore science in a way I have never been exposed to, allow me to work with an interdisciplinary team, and spend time outside working with livestock to better the agriculture industry. Thank you to our professors on the project Dr. Karen Launchbaugh and Dr. Gordon Murdoch for all you help and support along with the UI Beef Manager Zane Garner for your cooperation.

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Courtney Carter, Animal Veterinary Science, University of Idaho

Faculty Mentor: Dr. Gordan Murdoch, Professor, Animal & Veterinary Science

Project Title: Audio Cues and its Application to Virtual Fence

Abstract: Virtual fence as defined in this project, is an animal-worn device that detects the animal's location relative to a virtual boundary and delivers an electric stimulus if crossed. This technology has the potential to improve management practices for grazing livestock. It could allow producers to use grazing areas that are otherwise unusable because they would be difficult or too expensive to fence. My research focused on audio cues applied before an electrical stimulus to signal a pending shock and facilitate avoidance behavior. The specific aims were to determine: (1) If sound, paired with an electric shock was an effective associative cue for cattle; (2) Does the tone and duration of the sound cue change its effectiveness; (3) Does the direction/location of sound affect associative learning in cattle. In experiment 1, 57% of cattle paused for at least two seconds after hearing a sound, 38% paused at the shock, and 5% didn't pause at all. In Experiment 2, 91% of cattle paused when they heard a 2,000 Hz beep, 78% paused to the sound of an air horn, 77% paused to a 300 Hz beep, and 66% paused a tolling bell. In experiment 3, 97% of animals paused if the sound came from in font vs 86% paused when the sound came from behind the animal. Animals were no more likely to pause if the sound came from near one ear (78% paused) compare to sound from near both ear (66% of animals paused).

Project Description and Accomplishments:

In early times, people used wood and stones to build fences to keep animals out of crop areas and contain them within pastures. Once westward expansion started, barbed wire fences became popular due to the lack of wood and stones in the plains. Fences are known to cause issues with wildlife by disrupting natural migration routes, inhibiting access to natural habitats, and entangling animals resulting in their death. These fence issues have contributed to population decreases in some wildlife species (Hanophy, 2009). Fences are also expensive to build and maintain. This has led researchers from across the globe to search for a way to manage livestock without physical fence. The idea of virtual fence gave is a potential solution though there are still concerns to be addressed. For example, several animal welfare concerns have prohibited the use of shock collars for training dogs in several countries and may limit use of virtual fences where electrical stimulus is used (Umstatter, 2011). Specific concerns include animals getting repeated electric stimulus due to faulty devices, inadequate training of animals, and the amount of distress animals may face when the electric stimuli are random (Umstatter, 2011).

1. Training animals to stop at a sound that is associated with a shock. Animals were enticed down an alley with grain and peers. Once they crossed a designated boundary, a sound was emitted. The distance to the boundary changed each time cattle walked down the alley. We recorded whether the animal paused at the sound, shock received after the sound, or didn't pause at all. Each animal repeated this, four times per day for four days. A Chi-Square analysis of pausing showed that day had no effect on whether the animals paused or didn't pause at the sound ($P=0.90$). This indicates that animals didn't learn to stop at a sound.

2. Will changing the tone or sound change the response to an associated sound and shock? As above, animals were enticed down an alley and a sound was emitted. We recorded whether or not the animal paused at the sound or shock. This was repeated four times each day for four days. A Chi-Square analysis of pausing vs not pausing showed a difference between sounds whether the animal paused or not

(P=0.013). When a 2,000 Hz beep was played, 70% of cattle paused, 63% paused to the sound of an air horn, 55% paused to a 300 Hz beep, and 46% paused a tolling bell.

3. Will the direction from where the animals hear a sound, change the response to an associated sound and shock? As above animals were enticed down an ally, and a sound was emitted. This was repeated for each animal four times per day for four days. In this experiment, sounds varied by the location from which they were emitted. When a sound was emitted from behind or in front of the animal, 97% of animals paused if the sound came from in front of versus 86% that paused when the sound came from behind the animal (P=0.03). It appears that animals were no more likely to pause if the sound came from near one ear (78% paused) compare to sound from near both ears (66% of animals pause: P=0.12) though based on the small sample size this may be prone to type 2 error.

Based on experiments conducted this summer, I believe future research can further evaluate effective use of sound in the most efficient way by using tones close to 2,000 Hz and have them emitted from the front of the animal. However, I also learned that cows don't inherently, nor do they quickly develop an association between the sound and the shock. This makes me question the types of virtual fence that are becoming commercially available that are based just on sound. Our team will look further into animal behavior and the best way to train them to make that association.

Conference Presentation: This study was presented as a poster at ICUR in July 2019.

Budget Expenditures:

Item	Price
Halters	$ 47.97
Training Collars	$458.93
Duct Tape	$ 12.93
Rolled Barley	$ 140.44
Batteries	$ 22.99
12-gallon Tote	$ 11.95
Poster Printing	$70.00
SURF Fellowship	$4,000
Total	$4,792.21

Acknowledgment: I greatly appreciate the generous support provided by the SBoE/HERC that made my SURF award this experience possible for me. It was a truly great experience.

References :

Hanophy, W. 2009. Fencing with Wildlife in Mind. Colorado Div. Wildlife, Denver, CO. 36 pp

Umstatter, C. 2011. The Evolution of Virtual Fences: A Review. *Computers and Electronics in Agriculture,* vol. 75(1);10-22

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Abby L. Davis, Animal and Veterinary Science, University of Idaho

Faculty Mentor: Dr. Brenda Murdoch, Dept. Animal & Veterinary Science

Project Title: Understanding how genetic variation in PRDM9 affects meiotic recombination

Project Description:
Meiotic recombination is an important process that contributes to genetic variation and produces viable gametes. Errors due to abnormal or improper recombination can result in reproductive consequences such as aneuploidy, developmental issues, fetal loss, and infertility (Baudat et al. 2013). The driving force behind this project is that very little is known about the influencing factors of meiotic recombination in mammals. Improving our knowledge regarding the effect of genetic variation on the meiotic recombination gene PR/SET domain 9 (PRDM9), the gene thought to be responsible for the positioning of recombination hotspots, can provide valuable insight regarding male infertility in both livestock and humans.

A recent study characterized and quantified the recombination protein mutL homologue 1 (MLH1), which is thought to be indicative of crossover (CO) events (Davenport et al. 2018). Davenport et al compared the number of COs per spermatocyte from three different breeds of sheep (Suffolk, Icelandic, and Targhee). The results of their study indicated that the number and location of MLH1 foci varied amongst the three different breeds. Suffolk rams exhibited the lowest number of MLH1 foci, followed by Icelandic rams, and lastly Targhee rams exhibited the highest number of MLH1 foci. The objective of this study is to expand on previous work by utilizing immunofluorescence to identify and characterize the histone mark, histone3 lysine4 trimethylation (H3K4me3), of PRDM9 using male meiotic prophase cells from Suffolk and Targhee breeds of sheep. H3K4me3 is thought to be the histone catalyzed by PRDM9 during meiotic prophase, (Davenport et al. 2018). We hypothesized that different breeds of sheep would express different H3K4me3 intensities, and that those breeds of sheep that exhibit higher MLH1 numbers also exhibit higher H3K4me3 intensities.

Testicular tissue samples of sexually mature Suffolk and Targhee rams were collected postmortem. The samples underwent surface spread preparation and were either frozen for later use or were immediately stained. Immunofluorescence staining was performed to identify three proteins: synaptonemal complex protein 3 (SYCP3), H3K4me3, and chromatin. Imaging of the meiotic prophase cells was done through the use of a Leica DM6 B fluorescence microscope and an Andor Zyla sCMOS camera. Throughout this study we imaged cells in the pachytene stage of prophase where MLH1 is thought to be initiating double strand break repairs through CO pathways (Baudat et al. 2013). The average intensity of the H3K4me3 signal was calculated per spermatocyte for each of four Suffolk and four Targhee rams using ImageJ version 1.51 software. Out of the four Suffolk and four Targhee rams utilized, approximately 50 spermatocytes per individual were examined, totaling 205 spermatocytes per breed. Using R Studio version 3.3.3, three statistical analysis were performed to determine if a significant ($p<0.05$) difference in H3K4me3 intensities were present. The first test was a Shapiro Wilk Normality test to determine if the data set was of a normal distribution. Following this test, a Kruskal-Wallis test and a post-hoc Tukey-Kramer test were performed to identify any significance within the data. This data was then compared to previously reported MLH1 data that underwent the same statistical analysis (Davenport et al. 2018). To

identify any correlation between MLH1 and H3K4me3 data, a Spearman's Rank Correlation was also performed.

The mean H3K4me3 intensity for Suffolk and Targhee spermatocytes were 17,552.15 and 17,678.44, respectively. The average difference of intensity measures for each breed were 15,593.19 for Suffolk and 18,362.32 for Targhee. The Shapiro Wilk Normality test indicated that the data did not show a normal distribution. The Kruskal-Wallis test and the post-hoc Tuckey- Kramer test indicated that a significant difference of H3K4me3 signal was present amongst individuals. Reference Figure 1 for a visual representation of the significant differences found. The Spearman's Rank Correlation resulted in a p-value of p=0.4198, showing no significant (p>0.05) correlation between previously reported MLH1 numbers and locations and H3K4me3 intensities.

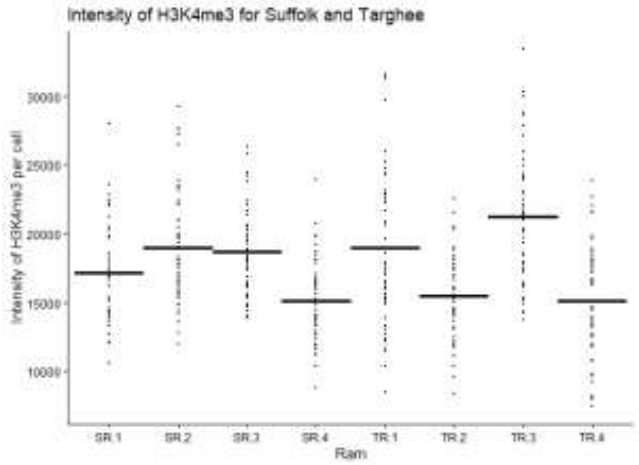


Figure 1. H3K4me3 intensity averages in individual rams. The dots represent the intensity average of each spermatocyte per individual. The black lines represent the mean intensity per individual. A, B, and C indicate significant (p<0.05) differences.

Accomplishments:

Through this research, we were able to conclude that significant differences of H3K4me3 intensities were observed amongst individuals of both Suffolk and Targhee sheep. However, no significant correlation to the previously reported MLH1 data was present. A better understanding of the relationship between H3K4me3 and MLH1 was developed, but it is still unclear how variations among these proteins control or influence PRDM9. This study contributes to the overall understanding of PRDM9 as well as sets a precedence for future work. As a student, I gained a more in-depth knowledge of the process of meiotic recombination and the many factors that contribute to it. I also had the opportunity to improve my bench work skills as well as obtain one on one training in advanced cytogenetic techniques and fluorescence microscopy. This was a summer of growth, and I am grateful to have had the opportunity to learn from such great mentors. I am thankful to have gotten the opportunity to participate in this research project. I sincerely thank the ID SBoE for providing the funding that made this possible!

Budget Expenditures

Item	Size/Amount	Cost per item	Total cost	Supplier
ProLong Gold Antifade Mountant with DAPI	1 bottle 10 ml	$211	$211	Thermo Fisher
Microscope slides	5 packs of 114 slides	$75	$375	Fisher Scientific
SCIENCEEARE Spindrive Orital Shaker	1	$381.52	$381.52	VWR
Poster printing	1	$70	$70	
SURF Fellowship			$4,000	
TOTAL			$5,037.52*	

*$5,000 covered by SURF award, remaining $37,52 covered by mentor.

References

Baudat, F., Imai, Y., & De Massy, B. (2013). Meiotic recombination in mammals: localization and regulation. Nature Reviews Genetics, 14(11), 794.

Davenport, K. M., et al., (2018). Meiotic recombination differences in rams from three breeds of sheep in the US. Cytogenetic and Genome Research. DOI:10.1159/000493175

Hassold, T., & Hunt, P. (2001). To err (meiotically) is human: the genesis of huma aneuploidy. Nature Reviews Genetics, 2(4), 280.

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Kathryne Day, Animal and Veterinary Science, University of Idaho

Faculty Mentor: Dr. Pedram Rezaman, Dept. Animal and Veterinary Science

Project Title: Nutritional characteristics of a modified lignin product

Abstract
The objective of this preliminary study was to evaluate feeding behavior of Holstein dairy heifers when offered a lignin product as a component of pelleted feed. Five feed pellets were prepared: a positive control containing molasses, a negative control containing neither molasses nor the product, and three pellets containing the product in varying levels – low, medium, high – plus molasses. Growing Holstein heifers (16 months of age, average BW = 399 ± 9 kg) were placed into individual pens (3 x 5 meters) with one pelleted feed offered at a time to test the acceptability of each feed. Feeding behaviors were recorded for each animal in a specified amount of time (60 min). A total of 129 observations were statistically analyzed. Preliminary data show the animals accepted the pelleted feed containing the high inclusion lignin product more than the other feeds: heifers consumed more feed within 60 minutes ($P < 0.0001$) and per approach ($P < 0.0001$). Heifers spent less time ruminating ($P < 0.0006$) and eating ($P < 0.0001$) when the negative control was offered compared with that of other feeds except the low inclusion. We are currently evaluating rumen fermentation characteristics of pelleted feeds containing the modified lignin product.

Project Description
All plant material is made of cellulose, lignin, and hemicellulose. Cellulose and hemicellulose are made of glucose molecules bound by β-linkages. Most mammals lack the enzymes to break the bonds between the glucose molecules. Cattle contain microbes in there stomach that ferment cellulose and hemicellulose and convert them to volatile fatty acids that the animal can convert to glucose and ATP. However, there are always losses as the microbes cannot remove all the cellulose from the lignin. These losses can be detrimental, especially in dairy cattle. High-producing dairy cows struggle to eat enough nutrients to supply their maintenance requirements and produce milk. The cows need as much readily available food as possible. As such, many companies have worked and developed products that remove the cellulose from the lignin. One company has created a process to thermally and chemically modify products high in lignin – such as wood chips and wheat straw – and make them more degradable. The process creates a liquid mixture that could be used as a binding agent to replace molasses in pelleted feed.

This project used the modified lignin from the above process to make a feed pellet, observing how well the pellet binds. An acceptability test was performed on the pellet; heifers were observed on how they respond to this new feed. The pellets will be analyzed in vitro for digestibility and fermentability. Finally, a palatability test will be performed: heifers will be observed on how they respond to a feed while the negative control is present.

Project Accomplishments
Five feed pellets were developed using the heifer ration. The negative control did not contain the product or molasses. The positive control contained molasses. Three test pellets containing various levels of the product – high, medium, and low – as well as molasses.

An acceptability trial was performed using eight growing Holstein heifers 16 months of age and 30 days pregnant. The animals were removed from feed at least 30 minutes prior to each feeding. Each animal was individually placed in a pen (3.4 x 3.7 meters) with a feed. The order of animals and feeds were randomized. The feeds were weighed before and after each trial and then converted to dry matter intake. The animals were left with the feed for 45-60 minutes and recorded via camcorder. The video was analyzed for the time the animals spent eating, ruminating, wandering, drinking, and the number of approaches to the feed. The preliminary data show that the animals accepted the new product. They ate more of the high inclusion lignin product than the others ($P < 0.0001$). The animals spent more time eating ($P < 0.0001$) when in the pen with the high inclusion lignin product. The animals approached and ate the high inclusion lignin product more than the other feeds ($P < 0.0001$).

Two cannulated beef heifers were fed a Holstein heifer ration for one week. Then rumen fluid was collected to perform *in vitro* analysis. The samples were placed in the rumen fluid for varying amounts of time – 3, 6, 9, 12, 18, 24, and 48 hours – and then analyzed for fiber degradation and organic matter degradation. They were compared to the original sample degradation values. *In vitro* data is still preliminary and has not been statistically analyzed. More replications are required and will be performed over the next few weeks. The volatile fatty acids have not been analyzed yet as the GC machine was being used for another project. The palatability trial will begin August 9, 2019. The feeding portion of the trial should be complete by August 30, 2019. All the videos should then be analyzed by the end of September. The project is still ongoing under the supervision of Dr. Rezamand and three of his students.

Conference Presentations:
I presented a poster of my work at the Idaho Conference on Undergraduate Research (ICUR) in Boise in July 2019.

Budget Expenditures

price	Unit	Test	amount	additional units	
$700.00	Pelleting Machine	Animal Trials			$700.00
$177.99	Camcorder	Animal trials	1	$10.68 (tax)	$188.67
$5.99	SD Card (16 GB)	Animal trials	1	$6.36 (tax)	$112.35
$100.00	Tripod	Animal trials	1		
$20.69	SD Card (128 GB)	Animal trials	1	$3.50 (shipping) $1.24 (tax)	$25.43
$80	ADS Concentrate	*in vitro*	2 bottles	$67.38 (shipping)	$397.38
$85	NDF Concentrate	*in vitro*	2 bottles		
$1.20	Filter bags	*in vitro*	400 bags	$13.69 (shipping)	493.69
$47	Poster	ICUR	1 poster	$3.60 (tube for travel)	$50.60
				Total	$1968.12

*Budget costs above the grant were covered by research gift in the AVS department.

Acknowledgements
I want to thank the Idaho Board of Education for their support of this project through the University of Idaho Summer Undergraduate Research Fellowship Grant. I learned the skills necessary to set up a project and explain the process to my coworkers. I also learned how to explain my project to those who have little to no experience in my field. Thank you again!

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Iqbal Ahmer, Biological Sciences, University of Idaho

Faculty Mentor: Dr. Craig McGowan, Associate Professor, Department of Biological Sciences

Project Title: Anatomic coupling of locomotor and auditory neurons in desert kangaroo rats

Abstract: Acoustic stimuli-induced startle response in mammals may be modulated by vigilance and an elevated arousal state to allow for a more rapid acoustic stimulus-induced response in locomotor systems. Environmental modulation of this reflexive response may underpin defensive maneuvers in prey species. Though this phenomenon is found in many mammals, the nature of anatomical connectivity between auditory and locomotor neurons remains unclear in desert kangaroo rats. Identifying the neuroanatomical nature of this auditory-locomotor pathway is a major step towards understanding how species-specific anatomical and functional properties of this pathway may underpin success rate of kangaroo rats in the wild. To evaluate the anatomical connectivity, a trans-synaptic retrograde pseudorabies virus was injected into the right gastrocnemius muscle and induces the expression of green fluorescent protein in all presynaptic neurons that are synaptically connected to the afferent motor neuron of the muscle, whether they be motor or not. Following 5-7 days of recovery after viral injection, kangaroo rats were euthanized, and their brains removed, frozen and sectioned coronally. Tissue sections containing all central auditory nuclei were then mounted directly onto slides or immunohistochemically labeled to amplify visibility of GFP expression. Sections were imaged through a microscope where GFP expression was observed in motor nuclei within the brainstem and midbrain along with the cochlear nucleus, a key site for mediating the acoustic startle response. Slices from the auditory pathway were also taken to observe if there is further involvement of higher order auditory brain regions that may contribute to the acoustic startle in k-rats.

Project Description and Accomplishments:

Desert kangaroo rats (*dipodomys deserti)* are a desert adapted species that thrive off their ability to escape predators through evasive techniques. Evolutionary changes suggest that the kangaroo rat developed strong bipedal hops in relation to heightened auditory response when startled by predators. In this project, we hypothesize that a direct connection between the auditory system and motor neurons controlling the escape jump is what enables kangaroo rats to react at a faster rate than other small mammals under similar conditions. The goal of this project is to identify the neuroanatomical nature of a potential connection between the auditory and locomotor systems.

Live desert kangaroo rats collected by Craig McGowan and his research team from the Mojave Desert for their experiments were used. Samples were injected with a PRV-152 virus strain that infected nerve terminals at the neuromuscular junction that were connected to distal regions of the spine. The virus traveled up into the cervical region of the spine, onto the brain stem, where it then infected presynaptic neurons and subsequently motor and auditory cortices. After viral incubation period of 5-7 days, brains were perfused in a 4% paraformaldehyde with 30% sucrose solution that cryoprotected brain samples. Serial sections of the brains were sliced using a cryostat and stained using an immunohistochemistry protocol that expressed Green Fluorescent Protein (GFP) in brain slice samples.

The main takeaways from this project showed that using PRV-152, a trans-synaptic pseudorabies virus, allows labeling of any neural circuit. In our experiment, this viral tracer can label auditory brain stem neurons suggesting they are linked to motor neurons. By injecting PRV-152 virus in proximal limb segment

of the gastrocnemius, a connection between the locomotor and auditory system was seen. Evidence from data supports anatomical connectivity that would underpin acoustic startle response in kangaroo rats.

Expression was found in KRAT 1 sample at specific locations throughout the brain. Strong expression was found in reticular motor nuclei in posterior components of the brain that initially connected the brainstem and spine. Expression associated with movement was found in Nucleus raphe ragnus (RM), Nucleus raphe padillus (RPA), Magnocellular reticular nucleus (MARN), Gigantocellular reticular nucleus (GRN), Intermediate reticular nucleus (IRN), Parvicellular reticular nucleus (PARN). Additionally, expression was also presented in other portions such as Facial motor nucleus (VII), Dorsal cochlear nucleus (DCN), Ventral cochlear nucleus (VCN). The dorsal and ventral cochlear nucleus were one of the main components associated with auditory neurons and were a main component of startle circuit response. Expression was found in periaqueductal gray (PAG) which plays a critical role in autonomic function, motivated behavior, and behavioral responses to threatening stimuli. Expression was also found in the substantia nigra (SNr) which is a structure located in the midbrain that plays a role in reward and movement.

Budget Expenditures:

- $65.78 – Sucrose Crystals
- $46.67 – Scissors
- $241.16 – Alexa Fluro GOAT Antibody
- $129.00 – GFP for staining
- $172.50 – Prolong GOLD for mounting slides.
- $47.48 – Biolite 24WELL plates
- $215.88 – IHC/ICC Block for IHC staining
- $7.50 – Dry Ice from Chemstores
- ~$74.00 – Poster
- SURF fellowship: $4,000
  TOTAL: $5,000

Conference Presentations:

I presented a poster of my work at the Idaho Conference on Undergraduate Research (ICUR) in Boise in July 2019.

Acknowledgements

I thank the Idaho Board of Education for their support of this project through the University of Idaho Summer Undergraduate Research Fellowship program. This has been a tremendous experience for me. Thank you!

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship - Summer 2019

Grant Recipient: Natalie M. Jaeger, Biological Sciences University of Idaho

Faculty Mentor: Douglas G. Cole, Professor, Department of Biological Sciences

Project Title: Optimizing Fatty Acid Production in Strains of *Euglena gracilis*

Abstract

A promising candidate for biofuel and nutritional supplements is the photosynthetic protist, *Euglena gracilis*. In addition to producing essential ω-3 polyunsaturated fatty acids *E. gracilis,* under certain conditions, produces high yield of waxy esters that can be used without modification as biofuel. *E. gracilis* also produces a range of fatty acids including those with methyl branching. Previous studies that focused on industrial lipid production examined the effect of autotrophic (photosynthesis only) and heterotrophic conditions. Our preliminary studies examined changes in fatty acid profile as a result of changing nutritional factors. In the current study, we compared these nutritional factors with different strains of *E. gracilis*, and the effects of environmental factors common to farming. *E. gracilis* can be grown under constant light, but to mimic outdoor farming they were grown in a 14:10 light:dark cycle. *E. gracilis* were also cultured at different temperatures to reflect different climes. Preliminary results show that artificial constant light negatively effects fatty acid production, and that temperature and strain choice critically effect growth rate.

Project Accomplishments

1. **Compared the relative amounts of various lipid groups in different strains of *E. gracilis***
   We harvested two strains of *E. gracilis* grown in identical conditions for comparison of the relative amounts of different lipid groups. This will be sent to Microbial ID for FAME analysis. The results will be used to determine the importance of strain choice on *E. gracilis* farming.
2. **Compared the effect of a light:dark cycle on *E. gracilis* relative lipid production**
   The relative amounts of different lipid groups were compared in cells that grew under 24 hr of light and cells that grew in a 14:10 light:dark cycle. The relative amounts were tested in duplicates. We found that the relative amounts of key fatty acid groups (odd-chain, essential, and methyl-branched) were lower in the *E. gracilis* grown in 24 hr of light than the amounts in the light:dark cycle.
3. **Compared the effect of temperature on *E. gracilis* relative lipid production**
   Two strains of *E. gracilis* were grown in 16.5°C and 26.5°C. They were harvested at stationary phase and will be sent of Microbial ID for FAME analysis and the results compared between the two strains. Another strain of *E. gracilis* from the Yukon is being isolated for comparison in the study.
4. **Compared the impact of nitrogen starvation on different strains of *E. gracilis***
   Two strains of *E. gracilis* were grown in four different nitrogen treatment groups: with nitrogen, without nitrogen, with only isoleucine, and with only alanine. These were harvested and will be sent to Microbial ID for FAME analysis. We will then compare the impact on different key lipid groups.

Conference Presentations

I presented a poster of my research at the 2019 Idaho Undergraduate Research Conference. I intend to present another poster of this project at the 2019 University of Idaho College of Science Research Symposium

Budget Expenditure Summary

0.20 µm syringe filters (35 in a partial pack)	$47.43
Autoclave bags, case of 200	$74.56
Autoclave tape, 10 rolls	$35.20
Transfer pipets, pack of 400	$58.48
Bottle-top 0.2 µm filter, 500 ml, case of 12	$127.84
0.2 µm Filtration units, 500 ml, case of 12	$106.97
0.2 µm Flitration units, 150 ml, case of 12	$115.97
Disposable beakers, 50 ml, pack of 100	$11.47
Acetone, 4 L	$23.23
Methanol, 4 L	$28.74
Sterile screwcap vials, 2 packs of 100	$100.00
Sharps container, 6	$26.34
Sterile microtiter plates, 96 well, case of 100	$224.44
Labeling Tape, 4 rolls	$18.93
Shipping	$9.10
SURF Stipend (before tax)	$4000.00

The grand total is $5,009.00; the extra $9.00 was paid by discretionary Cole lab funds.

Acknowledgements

I appreciate the generous support provided by the State Board of Education/HERC in the form of a Summer Undergraduate Research Fellowship. This has been a tremendous experience and without the support of the SBoE I would not have been able to participate. Thank you!

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Laura Nutter, Chemistry, University of Idaho

Faculty Mentor: Dr. Kristopher Waynant, Chemistry Department

Project Title: Encapsulation of *Lactobacillus acidophilus* and *Lactobacillus casei* to Determine Cell Viability in a Hydrogel Biobead Matrix

Abstract:
Trichloroethylene (TCE), a commonly used industrial solvent, is a widespread, persistent, and carcinogenic groundwater pollutant. An effective treatment strategy for TCE contamination is bioremediation using reductively dechlorinating bacteria. However, during bioremediation changing pH levels can harm these degrading microbes. By incorporating the microbes into a polymer matrix, pH is buffered, and the microbes are protected. This study assessed the viability of model microorganisms (*Lactobacillus casei* and *Lactobacillus acidophilus*) in various compositions and molecular weights of polyvinyl alcohol (PVA) and sodium alginate (SA) hydrogels. A method to measure viability of bacteria cells in biobeads was developed. Viability was characterized using plate counts and optical density measurements. Preliminary data indicates increased viability in beads composed of higher molecular weight PVA. A second goal of the project was to determine if polymer modifications impact diffusion rates. Similarly sized ionic (methylene blue, metanil yellow) and neutral (caffeine) model compounds were used to investigate the effect of charge on diffusion. Diffusion of caffeine through hydrogel membranes was determined to be 40% slower in hydrogels containing bacterial cells than without biomass. Determination of encapsulated microbe viability assists in optimization of polymer formulations to better protect microbial consortia and improve degradation of contaminants.

Project Accomplishments

1. One of my goals was to create an effective method for encapsulation of *Lactobacillus acidophilus* and *Lactobacillus casei*.

   I was successful in creating and refining a method for aseptically inoculating the PVA and SA polymers with bacteria and forming beads by chemically cross linking the inoculated polymer for 10 minutes in a crosslinker of 5.5% $CaCl_2$ for SA beads and 2% $CaCl_2$ and saturated boric acid for beads comprised of both SA and PVA. These beads were successfully stored in MRS broth and PBS buffer, with no short term impact on viability.
2. To determine comparative cellular viability of different polymer blends and molecular weights.
   I was able to determine that in both 10% PVA 2% SA and 5% PVA 2% SA hydrogel beads that higher molecular weight PVA produced increased viability after 2 days in MRS broth compared to lower weights. I noticed the highest viability and number of cells in 4% SA beads.

3. To explore alternative methods of crosslinking the hydrogels.
   I successfully made small batches of beads crosslinked by repeated freeze/thaw cycles. This was done with 5% PVA, 10% PVA, and 5% PVA 2% SA. 10% PVA beads form more quickly than the other two polymers, requiring fewer cycles. Forming beads is most successful on copper plates in a -20 °C freezer. The inclusion of polyoxometalates in low concentrations increases crosslinking ability of freeze/thaw cycles despite minimal dissolution.

Summary of Budget Expenditures

Supplies	Cost
Single-Channel Miniflex Pump	$699.99
External Hot Plate Temperature Controller	$160.00
Isopropyl-beta-D-thiogalactopyranoside	$80.50
Beta-D-Galactose pentaacetate	$13.50
Sodium tungstate dihydrate	$100.00
Metanil yellow	$17.00
Methylene blue hydrate	$29.00
Supplies Subtotal	**$999.99**
Stipend	$4000.00
Total	**$4999.99**

Conference Presentation: I presented a poster of my work at the 2019 Idaho Conference on Undergraduate Research (ICUR) in Boise. I will be presenting a poster at the UI Undergraduate Research Symposium in April 2020.

Acknowledgement: I truly appreciate the generous support provided by the State Board of Education/HERC in the form of a Summer Undergraduate Research Fellowship. This was a tremendous experience for me. Without this support from the SBOE, I would not have been able to participate in this research. I am also very thankful to the U of I Office of Undergraduate Research for helping make this possible.

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Kate Seegmiller, Mechanical Engineering, University of Idaho

Faculty Mentor: Dr. Daniel Robertson, Department of Mechanical Engineering

Project Title: A novel assessment of maize strength through puncture testing

Abstract
Corn (maize) is one of the most important crops in the world. However, 5-20% of the annual corn yield is lost to stalk lodging. Stalk lodging is a phenomenon in which forces from wind or rain break crops, and tall, top-heavy crops are especially susceptible. In the past, researchers and plant breeders have examined this problem from an agronomic or biological standpoint. However, little progress has been made. One of the main impediments to this problem is the lack of a quantitative breeding metric for stalk strength. My summer research proposed a solution to this problem by investigating a new way to approximate the strength of each stalk by first examining the morphology of each stalk, using basic engineering theories. I accomplished this by performing puncture tests on a large sample of 1000 naturally-dried corn stalks. Each puncture test generates a force displacement graph, which can be analyzed to retrieve information such as diameter and rind thickness. This data was then analyzed by customizing a MATLAB algorithm. In particular, this algorithm created several values which combined the morphological values derived from the puncture tests (i.e. the moment of inertia and the section modulus) with the puncture resistance forces measured during testing. The results of this experiment showed that the puncture resistance-weighted values had a very strong correlation with stalk strength, and further testing and investigation would be of value.

Project Description:
Stalk lodging is a problem that affects some of the most important crops in the world. Lodging occurs when forces from wind or rain irreparably damage a crop, causing financial and food losses. One of the most essential crops affected by this phenomenon is maize, with 5-20% of the annual yield being lost due to lodging. This has long since prompted researchers to investigate methods of strengthening corn stalks, but several impediments still exist that prevent much progress from being made. One major problem is the lack of a quantitative breeding metric for breeders to assess lodging resistance. My summer research involved developing a novel technique to determine stalk strength that I believe could provide a solution for this issue.

This research employs puncture tests to evaluate maize stalks from a morphological standpoint instead of a biological or agronomical one, as has been done in the past. To begin, I performed puncture tests on a sample of 1000 dried cornstalks. To perform the tests, each stalk was loaded into a universal testing machine which had been fitted with custom attachments, including one to support each stalk and one which acted as a puncture probe. This probe had a 2-mm diameter and a 45 degree chamfer at the tip. The stalks were loaded in the same manner each time, with the minor diameter axis parallel to the vertical. Next, a puncture test was performed on each internode of each stalk. After each puncture test, a force displacement graph was produced which gave information about the diameter and rind thickness of the stalk when examined properly. One such graph is displayed in figure 1. This information was used to calculate other valuable information about the morphology of each stalk, such as the moment of inertia and the section modulus, both of which give insight into the strength of a stalk.

These values (the moment of inertia and section modulus) were also weighted with the puncture resistance forces seen in the force displacement graph. This meant that the outside of each stalk, which has a strong, hard rind, would count more towards these values than the inside pith, which is soft and full of voids. After each value had been calculated using a customized MATLAB algorithm, correlations were made between the newly calculated values and the approximated strength of each stalk, which had been calculated in previous experiments.

Project Accomplishments:

This project accomplished many things. First, a table of data from the 1000 stalks was created, leaving a valuable wealth of information that can be examined for trends for years to come. Already, other ways of optimizing maize stalks are being examined using this data.

In addition to this, I was able to customize a MATLAB algorithm specifically for maize puncture tests. This should ensure that anyone wanting to process future data will be able to do so quickly and easily using the same setup. This is especially important because during my project, I had a chance to travel to the University of Kentucky and train other students in performing puncture tests. This collaboration will allow additional data to be collected and examined for further studies and confirmation of results.

The most significant result of this project was the strong correlation found between the force-weighted values that were calculated and the approximated strength values found in previous studies. Correlations were created for each value calculated from the MATLAB algorithm. These values are shown in table 1.

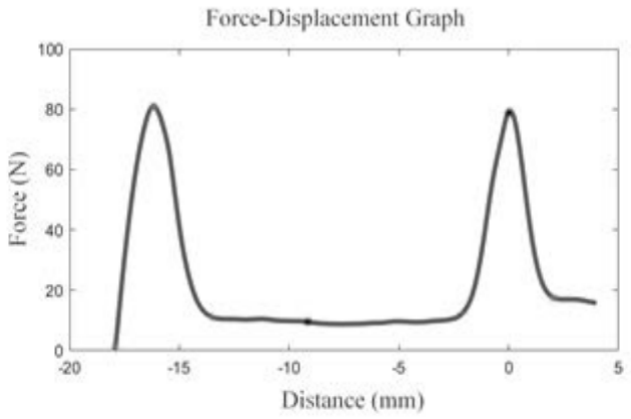


Figure 1: A force displacement graph from a puncture test. The peaks roughly indicate the entrance and exit of the probe, and the lower middle is the pith resistance.

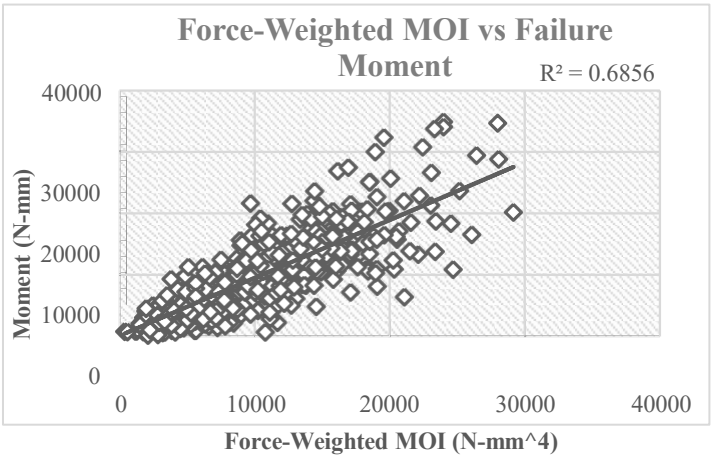


Figure 2: A graph of the puncture resistance weighted (force-weighted) moment of inertia vs the failure moment, which is an approximation of strength.

Table 1	Without Force-Weighting	With Force-Weighting
Section Modulus	0.2758	0.622
Moment of Inertia	0.258	0.686
Diameter	0.258	-

Table 1: A table of R-squared values for different correlations between stalk properties and stalk strength.

As can be seen in Table 1, the force-weighted values have a much better correlation with strength than the plain values. This information is extremely important. Puncture tests are much quicker and much less destructive to perform than other measurements of stalk strength, making them a more optimal option. Additionally, it might be possible to create a hand-held device to evaluate puncture resistance in the field instead of in a lab, which would make data collection quick, easy, and inexpensive.

Budget Expenditures

Round Trip to Lexington, KY	**558.00**
Hotel in Lexington	447.00
Total	**$1005.00**

This project required travel to Lexington, Kentucky, to complete a portion of the work. The entirety of the project budget was dedicated toward this travel. Dr. Robertson covered the project supplies and other project-associated costs through his own funding. The remaining funding for this SURF award covered the fellowship portion ($4,000) of my award.

Acknowledgment: I greatly appreciate the generous support provided by the State Board of Education in the form of a SURF award from the UI Office of Undergraduate Research. Without this support from the SBOE/HERC, I would not have been able to participate in this research. This has been a tremendous experience for me.

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Jennifer Smith, Animal Science, University of Idaho

Faculty Mentor: Dr. Jason Karl, Associate Professor, Dept. Forest, Rangeland, and Fire Sciences

Project Title: Wearable Technology for Cows: Applications for Virtual Fencing

Project Overview:
Managing the distribution of grazing animals is necessary for animal husbandry, effective land stewardship, and protecting sensitive and riparian lands. Virtual fencing is the idea where the negative reinforcement is worn by the animal rather than by restricting movement by wire and posts.

Virtual fencing pairs location-based communication technology with wireless fencing, such as that used in dog collars and invisible fencing, to control the distribution, location, and movement of livestock. Virtual fencing has the potential to significantly improve livestock management on open range and reduce the costs and impacts of physical fencing.

Virtual fencing can be deployed in an inclusion mode where animals are kept within a defined area for instance to graze residual crops or for rotational grazing within smaller pastures. Virtual fencing can also be used to exclude animals from riparian and other sensitive areas or achieve remote rotational grazing of pastures and ranges Virtual fencing will require a device that stays on the animal and does not negatively affect health and productivity.

Objectives:

1) Relationship between age, weight, and nose size of individual animals;
2) Best anatomical fit and least irritation to the animal by testing three shapes and sizes of nose pads;
3) Maximum weight to allow the device to remain in place on the animal for 1 month in a natural grazing setting.
This research facilitates designing technologies to study the application of virtual fencing and how it affects livestock which will ultimately contribute to a revolution in the way rangelands and riparian areas are managed and grazed.

Results:

Our results show that within the two age groups of cattle, primiparous yearling heifers and multiparous cows, there is little difference in nose size and shape among animals in the same age group. In addition, a spherical shape is a good starting point for a contact for the device and 40 grams of weight on the nose of a yearling heifer is too much for long term wear, while 110 grams appears to be less irritating to the mucosal tissues. Of the 20 yearling heifers that were used in the two (2) week device wearing study, seven (7) devices fell off; four (4) were 140 grams and three (3) were 110 grams. We know that we must create a finished working device of less than 110 grams.

Project Expenses:

Research Item	**Amount**
Hardware for nose clips *tubing, crimpers, plastic cement, tools, washers, etc.*	$ 242.84
3D Printer Filament	$ 168.99
Bluetooth locators for nose devices	$ 199.96
Poster Printing for ICUR	$ 75.00
Transportation	$ 313.00
SURF student fellowship	$4,000.00
Total spent	$ 4999.79

Acknowledgment:

I greatly appreciate the generous support provided by the State Board of Education/HERC in the form of a SURF award from the U of I Office of Undergraduate Research. This was an amazing experience for me. Without this support from the SBoE/HERC, I would not have been able to participate in this experience. Thank you.

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) – Summer 2019

Fellowship Recipient: Kael Stelck, Chemical Engineering, University of Idaho

Faculty Mentor: Dr. Mark Roll, Associate Professor, Dept of Materials Science

Project Title: Nanoreactors: Production of a Catalytic Membrane via Organo-Trialkoxy-Silanes

Abstract: Porous materials have many current uses that are already in place in some of the biggest industries today. The adsorption properties of high surface area materials are well known and used in gold mining to get higher yields. However, using porous materials as nanoreactors is a current source of discovery. This project seeks to make mesoporous materials from organo-trialkoxy-silanes (RTOS) in order to make highly structured nanoreactors that can aide in forming highly aligned polymer fibers. The making of highly aligned polymer fibers can be done, but the mesoporous materials used in previous experiments are hard to replicate. Using RTOS in enhancing the surfactants used to create the mesoporous material has not yet be done. By creating a highly structured nanoreactors that can be more easily replicated will greatly assist future catalyst research.

Project Accomplishments

1. Determine method of easily synthesizing mesoporous silica material from tetraethyl orthosilicate (TEOS) with ionic surfactants.

   I used two different methods to create MCM-41 using ionic surfactants and TEOS. Both methods recommended cetyltrimethylammonium bromide (CTAB) as the ionic surfactant. The first method called for TEOS to be added to a basic solution of surfactant along with expanding agents. The total time for this method takes about 26 hours. The second method adds TEOS to an acidic solution of surfactant and takes a total time of about one hour.

   Results: The second method proved to be the quicker reaction and had a yield of 76%. The X-Ray Diffraction (XRD) also was shown to be more similar to MCM-41 when using the second method. Based upon both those factors I decided to move forward using the second method as a base for creating MCM-41.

2. Optimize surfactant removal via calcination and dissolution with ethanol.

   Using the method decided upon above I then attempted to remove the surfactant from the MCM-41 after the solid product had formed and been filtered out. Calcination at 550°C removes all the surfactant by oxidation. However, by oxidizing the surfactant, it all is destroyed. This would make any large-scale application potentially costly. Surfactant removal via dissolution with ethanol was used as the surfactant can then be recovered.

   Results: By using dissolution with ethanol to remove the surfactant 72% of the surfactant added to the reaction was recovered.

3. Analyze XRD and Thermogravimetric Analysis (TGA) of silica products.

XRD was used to determine the ordering of the mesoporous silica products. TGA was used to determine mass reduction during surfactant removal via calcination.

Results: XRD of MCM-41 products made by the method suggested in 1 proved that it was MCM-41 material. However, XRD of the MCM-41 after surfactant removal by either calcination or dissolution showed a breakdown in the MCM-41 structure. TGA data showed that the solid product before surfactant removal was about 58% silica oxide.

4. Explore hydrothermal treatment as a method to prevent MCM-41 structure decomposition during Surfactant removal.

   The breakdown of the MCM-41 structure during surfactant removal showed that the method being used to create the material was not adequate. By introducing hydrothermal treatment, we hope to prevent the breakdown. Currently, experiments with hydrothermal treatments with varying temperature and time are being conducted to determine which is best for the preservation of the MCM-41 structure after the removal of the surfactant.

5. Synthesize ionic surfactants for future use in Nano-ordering mesoporous silica.

   For future research it is important that many types of surfactants are experimented with. By combining and alkyl halide with an alkyl chain of 16 carbons to a tertiary amine a quaternary ammonium salt can be made. These types of surfactants like CTAB are ionic. Currently products are made, but still need to be purified and analyzed. More still need to be synthesized.

Summary of Expenditures:

Supplies	**Cost**
Chemstores: Consumables, Safety supplies	$104.42
Matrix Scientific: Cetyltrimethoxy silane	$46.41
Fisher Sci.: Silane and Surfactants/Ligands	$228.65
Sigma Aldrich: Silicon Tetrachloride	$63.05
AK Scientific: Silica and surfactant precursors	$240.45
Sigma Aldrich: Colloidal silica and surfactants	$269.12
Surplus optical polarizing filter	$47.90
SURF Stipend	$4000
Total	$5,000.00

Conference Presentations: I with be presenting a poster of my research in April 2020 at the UI Undergraduate Research Symposium, and I have already presented a poster at the Idaho Conference on Undergraduate Research (ICUR) in Boise during July 2019.

Acknowledgements: I truly appreciate the generous support of the State Board of Education in the form of a Summer Undergraduate Research Fellowship. The experience I gained during this was invaluable. With this support from the State Board of Education, I would not have been able to participate in this research.

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) – Summer 2019

Fellowship Recipient: Isabell Strawn, Department of Biological Engineering

Faculty Mentor: Dr. James Moberly, Department of Chemical and Materials Engineering

Project Title: Development of a Protocol to Measure Viability of Microorganisms Encapsulated Within Polymer Hydrogel

Abstract:

Trichloroethylene (TCE), a commonly used industrial solvent, is a widespread, persistent, and carcinogenic groundwater pollutant. An effective treatment strategy for TCE contamination is bioremediation using reductively dechlorinating bacteria. However, during bioremediation changing pH levels can harm these degrading microbes. By incorporating the microbes into a polymer matrix, local pH gradients can be controlled, and the microbes are protected. This study assessed the viability of model microorganisms (*Lactobacillus casei* and *Lactobacillus acidophilus*) in various compositions and molecular weights of polyvinyl alcohol (PVA) and sodium alginate (SA) hydrogels. A method to measure viability of bacteria cells in biobeads was developed and evaluated. Viability was characterized using plate counts and optical density measurements. Preliminary data indicates increased viability in beads composed of higher molecular weight PVA. A second goal of the project was to determine if polymer modifications influence diffusion rates. Similarly sized ionic (methylene blue, metanil yellow) and neutral (caffeine) model compounds were used to investigate the effect of charge on diffusion. Diffusion of caffeine through hydrogel membranes was determined to be 40% slower in hydrogels containing bacterial cells than without biomass. Determination of encapsulated microbe viability assists in optimization of polymer formulations to better protect microbial consortia and improve degradation of contaminants.

Project Accomplishments:

The initial goal of this project was to assess the viability of microorganisms within hydrogels of various polymer compositions and molecular weights. This project developed methodology for conducting viability tests with biobeads. Several procedures were proposed, tested, and modified to optimize bacteria survival rates and to most accurately depict the viability of the microorganisms during the polymer formation step. The best procedure developed (detailed in **Figure 1**) was used to characterize the viability of bacteria in 10% PVA (of three different molecular weights)/2% SA beads and the viability of the cells in 4% SA biobeads. The results indicate that the bead composition most

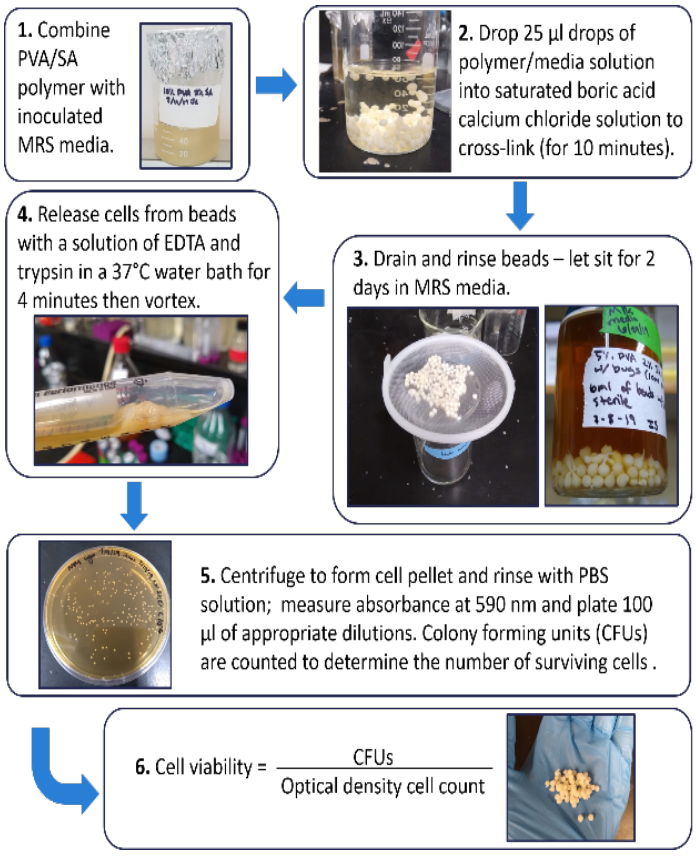


Figure 1. *The procedure to determine the viability of* L. casei *in polymer hydrogel biobeads.*

conducive to cell survival of those tested is 10% PVA (MW 146,000-186,000)/2% SA beads. Though the procedure worked sufficiently, future work will include further modifications to the viability testing process as well as testing of more polymer combinations. Characterizing the viability of microorganisms within the polymer hydrogels is a critical first step towards implementing this technology to improve bioremediation processes for TCE contaminated sites.

The second goal of this project was to investigate the diffusion properties of the polymer hydrogel, and this was accomplished by investigating the diffusion of three similarly sized neutral (caffeine) and ionic (metanil yellow and methylene blue) compounds through polymer pucks. These compounds were selected to quantify different electrostatic versus size filtering interactions with the polymers. Caffeine diffused the fastest, while diffusion of the charged compounds was substantially slowed, presumably due to electrostatic interactions with the polymers (**Figure 2**). Understanding reactions and interactions of molecules with the polymer hydrogels helps in optimization of the biobead size and polymer combinations to best accommodate the microorganisms and most efficiently degrade contaminants.

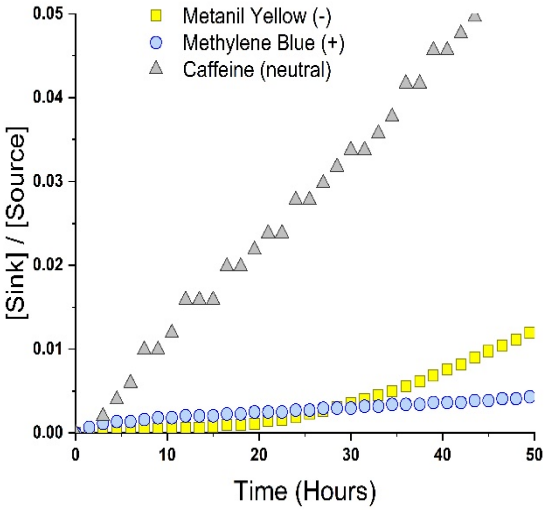


Figure 2. *Diffusion of metanil yellow, methylene blue, and caffeine in 10%PVA/2%SA hydrogel versus time.*

Summary of Budget Expenditures:

SURF Stipend	$4,000
Materials and Supplies IPTG ($299/5g), X-Gal ($199/100mg), lab coat ($24/each), consumables ~$400 (gloves, pipet tips, hydrogel polymer, plasticware)	$ 924
Other Expenses Poster printing for the UI Undergraduate Research Symposium ($75)	$75
Total	$ 4,999

Conference Presentation:
I presented a poster of my work at the 2019 Idaho Conference on Undergraduate Research (ICUR) in Boise, and I plan to present my research at the UI Undergraduate Research Symposium in April 2020.

Acknowledgement:
I truly appreciate the generous support provided by the State Board of Education in the form of a Summer Undergraduate Research Fellowship. This was a tremendous experience for me. Thank you for making this possible!

Final Project Report: Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship recipient: Silpa Subedi, Biological Engineering, University of Idaho

Faculty mentor: Dr. Ching-An Peng, Department of Biological Engineering

Project Title: Engineering Nano Carriers for effective gene delivery in T cells

Abstract: Immunotherapy is a therapy that uses the power of our body's own immune system to find and destroy cancer cells. With the rapid development of nanotechnology in the recent decade, novel gene delivery in T cells is being studied to replace the expensive viral vectors in immunotherapy. In this study, calcium-alginate nanoparticles were synthesized with water-in-oil emulsion method using a tip-sonicator. The obtained size and morphology of the nanoparticles were observed to be varied with volume and concentration of sodium alginate, and surfactant used. To examine the potency of Ca-alginate nanoparticles as carriers for gene delivery in human cells, GFP-encoding plasmids were encapsulated in these nanoparticles. The transfection rate was then investigated in A549 cells, Mesenchymal stem cells and Jurkat T cells. Our results showed that Ca-alginate nanoparticles with an average size of 200 nm in diameter were capable for delivering gene in A549 cells and Mesenchymal Stem cells. We have not observed any gene delivery in T cells.

Project Accomplishments

1. One of my goals was to synthesize the nanoparticle with the average size of 150 nm

Alginate nanoparticles in this study were developed with water in oil emulsion method using a tip sonicator. The nanoparticles were formed by calcium crosslinking of guluronic acid units of alginate polymer where dichloromethane was used as the oil phase. To get an accurate size of around 150 nm, the nanoparticles were synthesized using different volume and concentration of sodium alginate and surfactant. The alginate nanoparticles were collected by ultracentrifugation and then characterized by measuring its size and charge by a zeta potentiometer.

Result- The average size of nanoparticle characterized by zeta potentiometer was 200 nm. As expected, the size of Ca-alginate nanoparticles decreased with the decrease in the volume and concentration of sodium alginate and surfactant used.

2. To investigate the transfection rate of synthesized calcium alginate nanoparticles in A549 cells, mesenchymal stem cells and Jurkat T cells the nanoparticles were capsulated with GFP-encoding plasmids.

In a 2-well cell culture plates A549 cells, MSC and Jurkat T cells were allowed to adhere at 37 °C overnight. The alginate nanocarriers were suspended in DMEM and was added directly to the cells. Alginate nanoparticles were incubated with the cells for 12 hours at 37 °C. Transfection efficacy in these cells were evaluated by measuring the percentage of cells expressing the exogenously delivered GFP in fluorescence microscope.

Result-The gene delivery in A549 cells and MSC were more efficient than T cells, because MSC and A549 cells grow as a monolayer, attaching to the culture flask T cells are suspension cells; that grow by floating in the cell culture medium.

Future Work

A CAR-gene capsulated nanoparticle will be synthesized and test its ability for effective gene delivery in T cells. The CAR-T generated from the project will be further tested for its ability to recognize tumor cells among healthy cells *in-vitro*.

Summary of Budget Expenditure

Supplies	Cost
Jurkat T cell line	$385
Cell culture medium	$200
Materials for Ca-alginate nanoparticles	$200
T-flasks and 6-well plates	$140
Poster	$75
Sub Total Supplies:	$1000
Stipend	$4000
Total	$5000

Conference Presentations: I have already presented a poster at 2019 Idaho Conference on Undergraduate Research (ICUR) in Boise. I will be presenting a poster of my work at the Undergraduate Research Symposium 2020.

Acknowledgement: I appreciate the generous support provided by the State Board of Education in the form of a Summer Undergraduate Research Fellowship. It was a great experience to work on my own research and to get exposure in a research environment. I am very much thankful to SOBE for providing me with this opportunity.

Final Project report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Bishal Thapa, Biological Engineering, University of Idaho

Faculty Mentor: Dr. Xiao Wu, Professor, Department of Biological Engineering

Project Title: Fertilizer Production from Air and Water by An In-Liquid Electric Discharge Process

Abstract:
With the growing interest in sustainable farming, many eco-friendly alternative methods to produce plant accessible nitrogen is being studied. The aim of our research was to explore the feasibility of the fixation of nitrogen into NO3– and NO2– ions using a novel electrical discharge process (EDP) for producing a green fertilizer out of the air, water and electricity as input. At the discharge point in the EDP reactor, nitrogen and oxygen molecules dissociate into various reactive radicals and recombine into stable oxidative ions of NO3– and NO2– as the plasma discharge takes place. With a fixed air flow rate of 0.8 L/min, the concentration of NO3– and NO2– were profiled at different applied power levels for a batch of 300 ml water circulating through the EDP reactor for treatment. It was found that the concentration of NO3– increased with the increase in power and time of treatment. However, the NO2– concentration stably increased at lower power levels and decreased significantly at higher power. At 235 watts (W), the concentration of NO2– increased from 0.571 mg/l to 19.1 mg/l within 32 min. Similarly, the concentration of NO3– increased from 0.031mg/l to 84.6mg/l at the same operational conditions. When the power was increased to 358W, the concentration of NO2– increased from 0.005 mg/l to 17.8 mg/l in 16 minutes then it decreased to 0.019 mg/l by the end of 32 minutes, with NO3– increased from 0.114 mg/l to 266mg/l. At 417W, the concentration of NO2– fluctuated at a low level but the NO3– elevated from 0.354mg/l to 241 mg/l in the 32-minute period. This technology could be potentially developed for large scale production of nitrate fertilizer.

Project Accomplishments:

Objective 1: Study the effect of air flow rate on NOx production

In our previous preliminary experiments, we used 1-slpm as our input. So, one of the objectives of this research was to experiment with different air flow and understand its effect. For this experiment we added mass flow meter to our experimental setup to achieve consistent air flow.

Result: We found out that the production of NOx ions increases with the increase in air flow rate. But at high flow rate, if water circulation is not enough then burning occurs. The highest concentration we achieved at 100slpm water flow rate is 815gm/liter.

Objective 2: Study the effect of water flow rate on NOx production

To understand the effect of water circulation we are still conducting more experiment with multiple liquid flow rate. Since our system design did not withstand higher liquid pressure, necessary changes are recommended and are being made. As of now we are inconclusive of the effect of the liquid flow rate of NOx production.

Result: I will volunteer to complete experiments regarding effect of liquid flow rate during fall and I expect that with increasing flow rate the production increases.

Finally, after the completion of second objective, I will determine the optimum flow rate for production of NOx using plasma reactor.

Summary of Budget Expenditures

Description	**Cost**
4 boxes of Nitrate TNTplus Test Vials (0.2-13.5 mg/L NO3-N)	**$188**
4 boxes of Nitrite TNTplus Test Vials (0.6-6.0 mg/L NO2-N)	**$162**
4 boxes of Nitrate TNTplus Test Vials (5-35 mg/L NO3-N)	**$188**
4 boxes of Nitrite TNTplus Test Vials (0.015-0.600 mg/L NO2-N)	**$162**
6x customized quartz dielectric plates	**$225**
UI Symposium Poster	**$75**
SURF Stipend	**$4,000**
Total	**$5,000**

Conference Presentation: I had a time conflict which prevented me from attending and presenting my work at ICUR this year. However, I presented my research in Annual meeting of American Society for Agricultural and Biological Engineers on June 9th in Boston Massachusetts. I will also be presenting my research at UI undergraduate Research Symposium in Spring of 2020.

Acknowledgement: Summer Undergraduate Research Fellowship has helped me grow professionally and academically. With this experience, I feel confident on building a reactor for my future research, and now have the experience to plan the entire research project and execute it. I am very grateful for the generous support provided by Idaho State Board of Education through Summer Undergraduate Research Fellowship. Without the support of SBOE, I would not have been able to conduct such rigorous research project. I also thank Dr. Pfeiffer and the Office of Undergraduate Research for making this possible!

Final Project Report: Office of Undergraduate Research (OUR) Summer Undergraduate Research Fellowship (SURF) - Summer 2019

Fellowship Recipient: Eric Vallin, Biological Sciences, University of Idaho

Faculty member: Dr. Katy Brown, Associate Professor, Department of Human Sciences

Project Title: Low Energy Availability and Resting Metabolic Rate in Non-athlete College Males

Abstract:
Low energy availability (LEA) results in low bone mineral density, hormonal changes, and menstrual dysfunction in females. This has been extensively studied in female athletes and is known as the Female Athlete Triad. Studies have shown a link between low energy availability and a low resting metabolic rate. Low energy availability is scarcely studied among the male demographic and minutely studied in sedentary populations. The aim of this study was to assess the relation between LEA and resting metabolic rate in college-aged non-athlete males. 19 participants completed this portion of the study and we've found no correlation between resting metabolic rate and energy availability (r_s= .184, p=.450). However, we did find a correlation between lean body mass and RMR (r_s=.570, p<0.001).

Project Objectives and Accomplishments:

1. To establish and confirm a correlation between body mass and resting metabolic rate.

Upon doing our research we found a correlation between lean body mass and resting metabolic rate. Resting metabolic rate was taken with a "Body Gem" indirect calorimeter right after waking, with zero food or drink 8 hours prior to the test. This machine measures your resting metabolic rate, which is how many calories your body burns in a day completely at rest. It does this to obtain and continue equilibrium and regular physiological functioning. Muscle tissue requires a lot more energy to obtain and continue to have. Muscle cells are very active physiologically. So when we found that the males with more lean body mass (muscle), had a higher RMR- (r_s=.570, p<0.001), it confirmed my hypothesis.

2. Determine the relationship between Energy Availability and Resting Metabolic rate.
My hypothesis was that if an individual had low energy availability, it would mean their body would have to operate at a lower RMR (lower caloric intake= lower energy surplus). Although this was determined and confirmed true in female populations with the female athlete triad, it was not true for the males in our study. We found no correlation between the two - (r_s= .184, p=.450).

Summary for Budget Expenditures:

- RMR testing supplies: $11.96 per participant x 40 participants = $478.4
- DXA scan = $10.54 per participant x 40 participants = $421.6
- Incentive (drawing for 4, $25 Amazon gift cards) = $100
  SURF Stipend = $4,000
- **Total = $5,000**
- *Poster printing covered by FCS Department = $0

Acknowledgement: I am very grateful for the people on the State Board of Education that allowed me the funds to pursue research such as this. It was the first time I was able to take my scientific curiosity and scientific self to new heights. I am very happy to have done such amazing work with my fellow peers and professors. Thank you to the U of I Office of Undergraduate Research for facilitating this!

Examining the Behavior of Evolutionary Algorithms in the Starcraft Environment

Jacob Alderink, Dr. Terence Soule
Department of Computer Science, University of Idaho

Methods:
1. Two separate evolutionary algorithms manage the infrastructure(Macro) and unit behavior(Micro). Combined they create EvoBot.

2. Macro Algorithm represents Starcraft buildings and units through 100 encoded integers between 0-32.

3. Micro Algorithm runs a Neural Network though an encoding of 2682 floating point values.

4. EvoBot underwent 4 different training techniques:
- Training Macro by itself then in parallel
- Training Micro by itself then in parallel
- Training them in parallel
- Training the Macro and Micro separately then in parallel.

Results:
-The method that produced the best results was when the Macro trained alone for 30 generations and then started training with the Micro algorithm. Thus parallel evolutionary algorithms with interdependent goals learn best when infrastructure is learned solo and then unit behavior is defined.

Autonomous management AI learns faster by first learning an efficient infrastructure and then learning individual unit behavior

POLYMORPHIC GAMES

Table 1: Neural Network Input Table

Input List	Format
Unit Position	(x,y)
Group Center Position	(x,y)
Enemy Start Location	(x,y)
Location Of Nearest Enemy	(x,y)
Percent of Health Remaining	Float(0.0-1.0)
Number of Adjacent Allies	Integer
Number of Adjacent Enemies	Interger

Table 2: Neural Network Output Table

Input List	Format
Position to Go To	(x,y)
Execute Unit Ability	Float(0.0-1.0)
Attack or Move to Location	Float(0.0-1.0)
Attack Closest Enemy	Float(0.0-1.0)

Table 3: Macro Algorithm Parameters

Population Size	80
Mutation Rate	3%
Selection	Tournament, size 5
Crossover	Single Point Crossover

Table 4: Micro Algorithm Parameters

Population Size	80
Mutation Rate	.1%
Selection	Tournament, size 5
Crossover	Single Point Crossover

Columbia Basin Pygmy Rabbit Winter Field Sampling and Genetic Monitoring

Chloe Beall, Stacey A Nerkowski (Graduate Student Mentor), and Lisette Waits (Faculty Mentor)

Department of Fish and Wildlife Sciences, University of Idaho, Moscow, ID

Introduction

Figure 1a. Pygmy rabbit kit.

Figure 1b. Pygmy rabbit adult.

The pygmy rabbit (*Brachylagus idahoensis*) is:

- Smallest rabbit in North America (Figure 1).
- Dig their own burrows (Ecosystem engineer).
- Rely heavily on sagebrush for both diet and habitat.

The Columbia Basin Pygmy Rabbit:

- Present within the sagebrush-steppe community of the Columbia Basin in Washington State for over 100,000 years.
- Have been separated from the rest of the species' range (Figure 2) for ~10,000 years.
- Loss and fragmentation of native shrub-steppe habitats played a primary role in the initial decline of the Columbia Basin pygmy rabbit.
- Single remaining population in Sagebrush Flats, WA (2001).
- Emergency listed under the ESA as an endangered distinct population segment in 2001.

Conservation Efforts:

- Approximately 1900 rabbits have been bred and released through captive breeding program since 2012.
- Genetic monitoring critical for adaptive management.

Figure 2. Geographic distribution of the pygmy rabbit

Research Questions

- How many burrows are detected in the focal sampling area?
- How many pygmy rabbits are identified from fecal pellets collected in this area?
- What is the ratio between the number of rabbits detected and the number of active burrows detected?

Study Area

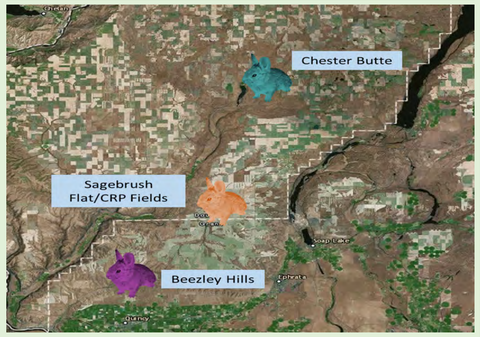


Figure 3. Locations of the three populations of Columbia Basin Pygmy Rabbits

Methods

- Field surveys conducted December through April.
- 50-m-wide belt transects to locate active burrows and collect fecal samples for genetic analysis.
- Species identification conducted using mitochondrial DNA cytochrome b.
- Pellets underwent genetic analysis utilizing 19 nuclear DNA microsatellite loci including one sex ID marker.
- Individual identity determined after using 2 multiplexes (12 microsatellites) to identify matching pellet samples.

Results: Chester Butte

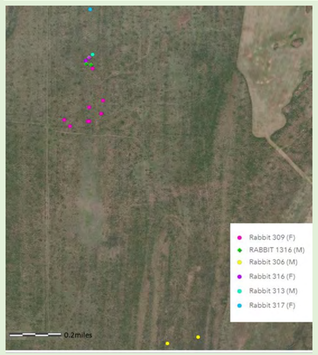


Figure 4. Locations of pellet samples and individual rabbits identified in Chester Butte study area (n=19). Circles represent burrow locations of individually identified rabbits. Diamonds indicate locations where rabbits but no burrows were detected.

Table 1. Results of winter field surveys and subsequent genetic analysis from Chester Butte study area

Field Data		Genetic Results	
Samples Extracted	20	Pygmy Rabbit Samples	19
Burrows Detected	14	Female Pygmy Rabbit Individuals	3
Samples at Burrow	14	Male Pygmy Rabbit Individuals	3
Samples not associated with Burrow	5	Average Rabbits per Burrow	0.4286

Results: CRP

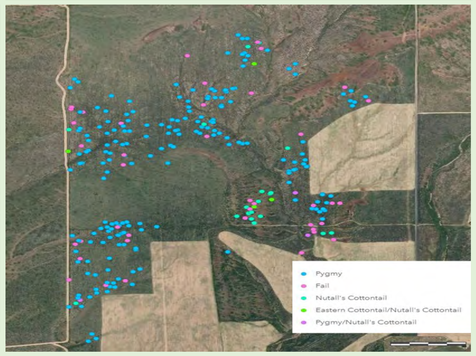


Figure 5. Locations of pellet samples collected during 2019 winter field sampling (n=271) in Sagebrush Flat/CRP study site.

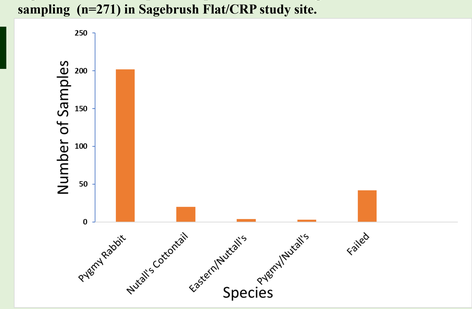


Figure 6. Bar graph of results from winter monitoring and species identification from CRP study area (202 Pygmy Rabbit samples, 20 Nutall's Cottontail, 4 Eastern/Nuttall's mixed, 3 Pygmy/Nutall's mixed, 42 failed).

Future Directions

- Individual identification for CRP ongoing
- Determine the relationship between number of rabbits to number of burrows more clearly.

Management Implications

- These preliminary results suggested that pygmy rabbits share burrows and a single rabbit tends to use more than one burrow.
- Continued individual identification and comparison between study sites will determine a possible ratio or relationship between population size and burrow use.
- If a ratio can be determined, it will decrease time and resources used for genetic analysis.

Acknowledgements

For funding and other support, we would like to thank the U.S. Fish and Wildlife Service, Washington Department of Fish and Wildlife, Oregon Zoo's Future for Wildlife Fund, UI Department of Fish and Wildlife Resources, UI Department of Biology, the UI Waits lab group, the UI Office of Undergraduate Research, and the students from the WLF 404 Winter Monitoring course.

University of Idaho
College of Natural Resources

U.S. Fish & Wildlife Service

Washington Department of Fish and Wildlife

Audio Cues and its Application to Virtual Fence

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College of Agricultural and Life Sciences

University of Idaho
College of Natural Resources

Introduction

Virtual fence is the idea that animal movement could be controlled by a device on the animal rather than wires and posts on the ground. This revolutionary idea has the ranchers to improve livestock management and land health. It will allow producers to access grazing areas otherwise unusable due to the lack of fencing and make rotational grazing easier to manage. A significant concern that arises with virtual fence is how it affects animal welfare. Previous research has shown that an associated cue paired with an electrical stimulus reduces stress to the animal. My research focused on using an audio cue immediately before electrical stimulus to determine if cattle were able to associate the sound with a shock and therefore avoid a shock. I also manipulated the tone and the direction from which the sound came to determine the most pronounced and effective response from the cattle.

Objectives

1. Examine if a sound associated with an electric shock to the nose will stop an animal's forward motion.
2. Determine whether the tone and type of sound influences the behavioral response and effectiveness of an auditory stimulus to stop movement.
3. Assess if the location from where the sound originates will affect the animal's behavioral response.

Methods

Research was conducted at the University of Idaho's Beef Center with 16 Charolaise-Lowline yearling heifer. The experiments were conducted in 2 alley ways set perpendicular in an L-shape with electrical shock delivered by a Sport Dog 350 collar mounted on halters with electrical leads connected to a nose clip delivering a shock to the cow's septum (**Figure 1 and 2**).

Experiment 1*: Will an animal pause or completely stop forward motion at a sound that is associated with a mild electrical shock?* Animals had three chances to pause or completely stop forward motion after hearing a sound administered randomly at 15, 20, or 25 meters down the alley way.

Experiment 2: *Will changing the tone or type of sound change the response to an associated sound and shock?* Sounds included a tolling bell, air horn, 300 Hz or 2000 Hz were played when a cow reached 20 meters down an alley way. Each animal received all four noises spaced across the four-day trial. Animal response to the sound or shock was recorded.

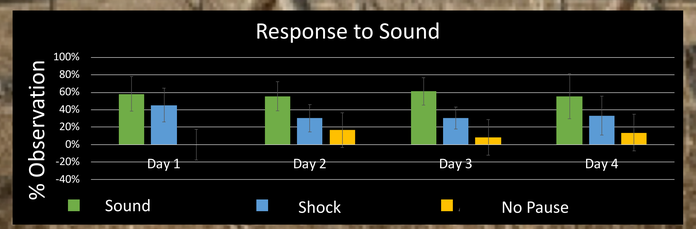
Experiment 3: *Will changing the direction from which the animals hears the sound change the response to an associated sound and shock?* The sound came from either the left ear, both ears, nose, or behind the ears. Whether an animal paused to the sound, required a subsequent shock to stop, or continued forward motion regardless of sound or shock was recorded

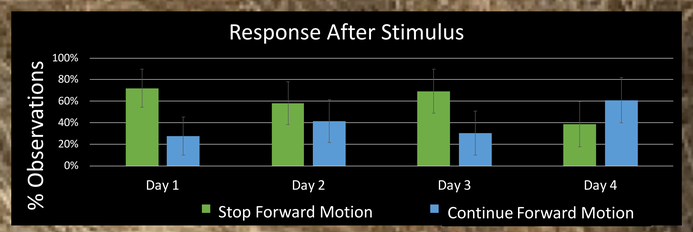
Figure1: Device used to administer the electrical stimulus

Figure2: Rivet on nose clip

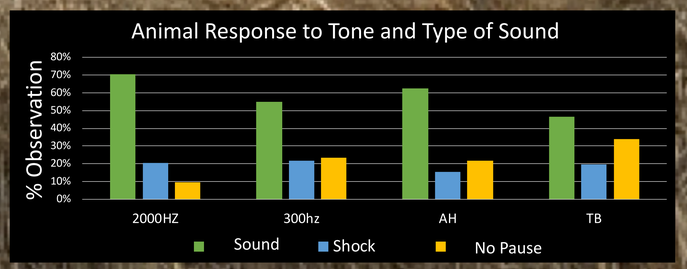
Results

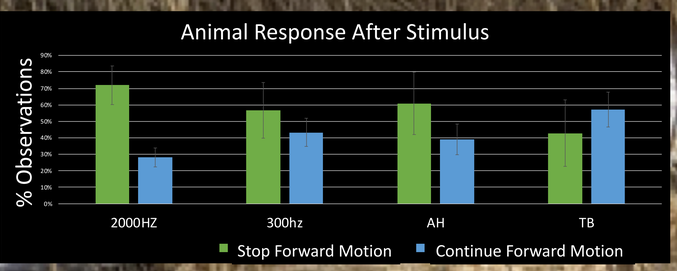
Experiment 1: **Inherent association between sound and shock**



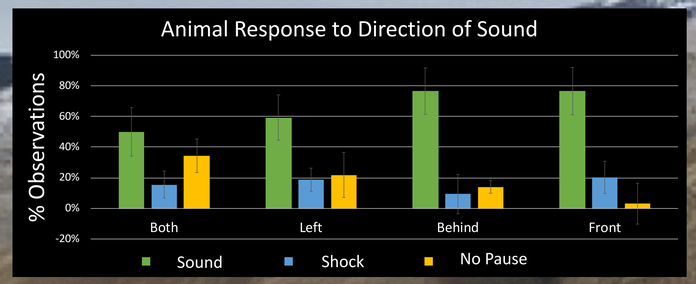


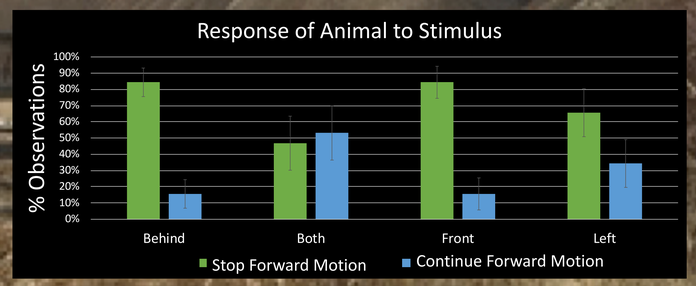
Experiment 2: Response to Different Sounds/Tones





Experiment 3: Response to Different Direction





Conclusion

-Animals did not initially learn to stop at a sound that was followed by electric shock

-Cows were apparently more responsive to some sounds (2,000 Hz) than others. Type of sound and the tone of sound influenced how likely animals were to pause and if they would subsequently continue forward motion

-Animals response was similar if sound came from one or both ears. However, animals were more responsive to sounds from in front of them rather than behind

This research will inform how specific sounds can be used in the development of virtual fence technologies.

Acknowledgments:

Research methods were approved by Institutional Animal Care and Use Committee (IACUC) 2018-74

The Role of Litchi Tomato Peroxidases in Potato Cyst Nematode Immunity

University of Idaho
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Introduction

Cyst nematodes are obligate, biotrophic pathogens of numerous plant species that present major threats to crop production worldwide. Two species of potato cyst nematode (PCN) are found in the United States; *Globodera rostochiensis* and *Globodera pallida* (Evens and Brodie 1980; Hafez *et al.* 2007). PCN is among the most damaging pests known to potato causing up to 80% yield loss. Eradication efforts have relied on fumigation primarily with the soil fumigant, methyl bromide. However, because of unexpected inorganic bromide residues and regulatory concerns, methyl bromide has not been applied to disinfest fields in Idaho since 2014. New strategies must be identified to deal with present and future infestations. A plausible alternative to control nematodes lies with the development of commercial crops expressing genes for resistance or immunity.

Figure 1: Cysts formed on roots by potato cyst nematodes in Peru. *(Photo provided courtesy of Rocio Silveste Casas, MS)*

Peroxidases are a subcategory of the larger family of enzymes called oxidoreductases. Peroxidases catalyze the oxidation of compounds in the presence of peroxides, R-O-O-R structure. Mechanical damage to plants will trigger a rapid oxidative burst of reactive oxygen species (ROS), followed by upregulation of peroxidase genes (Minibayeva *et al.* 2015; Masuta *et al.* 1991). These observations strongly indicate peroxidases are involved in plant defense and immune response.

Solanum sisymbriifolium or litchi tomato, is a distant relative of potato and tomato that has been shown to be nearly as effective as potato at inducing potato cyst nematodes to emerge from their eggs and cysts. Unlike a true host like potato, it fails to support their development into mature adults and cysts, leading to the death of the parasite. Litchi tomato is immune to *G. pallida* and *G. rostochiensis* infection by an unknown mechanism.

Figure 2: *Solanum sisymbriifolium* kept in tissue culture.

Characterization of the litchi tomato transcriptome using RNAseq (Wixom *et al.* 2018), has revealed expression changes in 277 defense-related genes 3 days post-infection with *Globodera pallida* compared to uninfected plants. Of these 277 genes, seventy have not been identified in other plant species and eleven genes out of the 277 appear to be putative peroxidases.

Sequence Name	Class of Genes (POX - peroxidases)	Length of Coding Region (nt)
c38565/f1p0/1276	anionic POX	978
c16456/f1p3/1223	POX-47	987
c90433/f240p109/1282	POX-72	1002
c6814/f2p4/1378	POX-7- like	522
c80297/f2p0/1141	Lignin forming POX	405
c15191/f1p3/1151	Lignin-forming POX	960
c25143/f1p1/1243	lignin-forming anionic POX	960
c7557/f1p0/1301	POX-27	975
c71321/f1p50/1658	Pox 45/72	822
c42476/f2p39/1402	Pox N1	987
c10137/f1p4/960	Pox 12	567

Table 1: The selected differentially expressed peroxidase genes from litchi tomato.

Objectives

1. Amplify selected peroxidase open reading frames, (ORFs), and screen to confirm predicted size amplification
2. Clone the amplified open reading frames into a pENTR vector.
3. Select bacterial colonies, grow bacterial cultures and extract the plasmid.
4. Screen the plasmids for the correct insert and conduct sequence confirmation.
5. Analyze and align the DNA sequences to reference sequences.

Amplifying the ORFs of Peroxidase Genes

A polymerase chain reaction, PCR, on cDNA using primers targeting each of the peroxidase genes was completed. Gel electrophoresis was run to screen for the correct amplified fragment size.

Results

All of the 10 selected peroxidase open reading frames were amplified. c10137/f1p4/960 was not the correct size of interest and therefore did not move on. As can be seen in Figure 2, c10137/f1p4/960 amplified at around 950 base pairs in length. The predicted size of c10137/f1p4/960 is 567 base pairs. The remaining PCR products moved on to cloning.

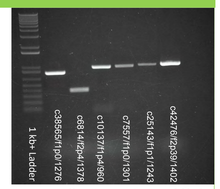

Figure 3: Amplified PCR products run on gel electrophoresis.

Cloning the Peroxidase ORFs

Figure 4: c7557/f1p0/1301 plated colonies.

The amplified fragments from the PCR reactions were cloned into a vector. The pENTR/D-TOPO cloning kit was used to clone these peroxidase open reading frames. These blunt-end PCR products were added to the pENTR vector which underwent topoisomerase directional ligation. Ligated product were chemically transformed into One Shot TOP10 chemically competent E. coli and the cells plated onto Luria-Bertani, LB, media with kanamycin. (Invitrogen, Carlsbad, CA)

Extracting and Screening the Plasmid

Transformed bacterial colonies were screened. An LB broth with kanamycin was made. A colony was selected from the LB plate, a master plate was made and the bacteria was added to the LB broth. The bacterial cultures grew overnight at 37 ℃. Once the bacteria grew in culture, the plasmid was extracted from the cells using a ZR plasmid mini prep kit (Zymo, Irvine, CA). After the plasmid was extracted, the quality and quantity of the plasmid sample was evaluated using a NanoDrop spectrophotometer. A restriction enzyme digestion was set up for each sample. 500ng of the plasmid was added into a reaction with *AscI* and *NotI* enzymes. Restriction patterns were used to verify the size of the insert present in the plasmid vector.

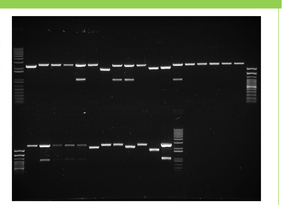

Figure 5: An example of the digested plasmids. The first band in the well is the vector. The second is the insert.

Results

All nine peroxidases; c15191/f1p3/1151, c38565/f1p0/1276, c42476/f2p39/1402, c7557/f1p0/1301, c25143/f1p1/1243, c6814/f2p4/1378, c71321/f1p50/1658 and c16456/f1p3/1223 had plasmids with the DNA fragment size of interest. c90433/f240p109/1282 was eliminated due to failure of the ORF to transform into the pENTR vector. This brought the final peroxidase count to 8 peroxidases for sequencing.

Sequencing the Plasmid and Data Analysis

Purified pENTR clones positive for an expected insert size of the peroxidase open reading frame fragment were submitted for sequencing. The samples were sequenced by GeneWiz (South Plainfield, NJ) and analyzed using SeqMan Pro (DNASTAR, Madison, WI).

Results

In analyzing the sequencing results, inconsistencies between the reference sequence and sequence data were identified. c25143/f1p1/1243, c16456/f1p3/1223, c38565/f1p0/1276, c42476/f2p39/1402, c15191/f1p3/1151 and c71321/f1p50/1658 had at least one clone that had an exact match to the reference sequence. c6814/f2p4/1378 consistently had three base pair differences from the reference which resulted in three different amino acids when the DNA was translated into a protein. c7557/f1p0/1301 had a single base pair change which results in a single amino acid change in the resulting protein. The samples that had 100% matching sequences have been stored as glycerol stocks. c7557/f1p0/1301 and c6814/f2p4/1378 with their base pair changes noted have been stored as glycerol stocks.

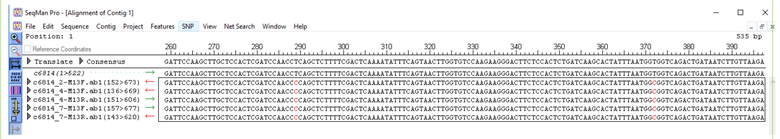

Figure 6: Peroxidase sequence alignment. The red base pairs are where there are inconsistencies between the sequencing data and the reference sequence.

Future Work

This project will allow for future research to be conducted on these peroxidase genes. Continued research will involve transfer of cloned peroxidase open reading frames into pEarleyGate 100. The pEarleyGate vector will then transform the cloned peroxidase open reading frame into the potato cultivar, Desiree. These genes will be over expressed in the potato plant to see if they confer resistance in potato to *G. pallida*. Conducting this initial work on peroxidases as I have done could help to create PCN resistant potato cultivars in the future.

Selected References

Dandurand, L., & Knudsen, G. (2016). Effect of the trap crop *Solanum sisymbriifolium* and two biocontrol fungi on reproduction of the potato cyst nematode, *Globodera pallida*. *Annals of Applied Biology*, 169: 180-189. doi:10.1111/aab.12295

Evans, K. & Brodie, B.B. (1980). The origin and distribution of the golden nematode and its potential in the U.S.A. *American Potato Jouranl*, 57: 79-89.

Hafez, S. L., Sundararaj, P., Handoo, Z. A., Skantar, A. M., Carta, L. K., & Chitwood, D. J. (2007). First report of the pale cyst nematode, *Globodera pallida*, in the United States. *Plant Disease*, 91: 325-325.

Ito H., Kimizuka F., Ohbayashi A., & Matsui H. (1994). Molecular cloning and characterization of two complementary DNAs encoding putative peroxidases from rice (*Oryza sativa* L.) shoots. *Plant Cell Reports*, 13: 361-366

Masuta C., Buicke M., Bauw G., Montagu M., & Caplan A. (1991). Differential effects of elicitors on the viability of rice suspension cells. *Plant Physiology*, 97: 619-629

Minibayeva, F., Beckett, R. P., & Kranner, I. (2015). Roles of apoplastic peroxidases in plant response to wounding. *Phytochemistry*, 112: 122-129. doi:10.1016/j.phytochem.2014.06.008

Wixom, A. Q., Casavant, C. N., Kuhl, J. C., Xiao, F., Dandurand, L., & Caplan, A. B. (2018). Assessment of an organ-specific de novo transcriptome of the nematode trap-crop, *Solanum sisymbriifolium*. *G3; Genes|Genomes|Genetics*, 8: 2135-2143. doi:10.1534/g3.118.200327

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Understanding how genetic variation in *PRDM9* affects meiotic recombination

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Introduction

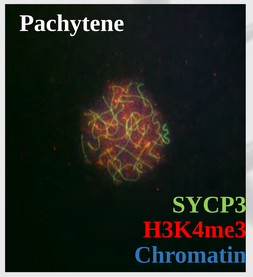
Homologous recombination plays an important role in gametogenesis through its contribution to genetic variation and aid in proper chromosome segregation. Errors due to abnormal levels or improper positioning of meiotic recombination events significantly contribute to aneuploidy, developmental disabilities, and infertility.[3] Developing a better understanding of the effect of genetic variation on the meiotic recombination gene *PR/SET domain 9* (*PRDM9*), the gene thought to be responsible for the positioning of recombination hotspots, can improve our knowledge regarding infertility in livestock and humans. A recent study characterized and quantified crossover (CO) events through the examination of the recombination protein mutL homolog 1 (MLH1), which resolves DNA double strand breaks into CO events. Through this study, they found that the number and location of COs (MLH1) differed amongst breeds of sheep. Specifically their study determined that the number of MLH1 foci was lowest in Suffolk rams followed by Icelandic rams, while Targhee rams had the highest number of MLH1 Foci.[2] Throughout this study, we characterized and quantified the histone mark histone3 lysine4 trimethylation (H3K4me3), the histone catalyzed by *PRDM9* during meiotic prophase, in male meiotic prophase cells from Targhee and Suffolk breeds of sheep. These observations were then compared to the previously reported data regarding recombination protein MLH1.[1] We hypothesized that different breeds of sheep would exhibit different intensities of H3K4me3, and that those breeds of sheep that exhibit higher MLH1 numbers also exhibit higher H3K4me3 intensities. This research contributes valuable information towards developing a better understanding of the effects of genetic variation on recombination and its impact on male infertility.

Objectives

To determine the relationship between the recombination protein MLH1 and H3K4me3 in male meiotic prophase cells from two different breeds of sheep, Suffolk and Targhee.

Methods

Testicular tissue samples were collected from sexually mature Targhee and Suffolk rams for surface spread preparation. Immunofluorescence staining was then performed to identify SYCP3 and H3K4me3 proteins and chromatin. This was performed four times, staining four slides each time. A Leica DM6 B fluorescence microscope and an Andor Zyla sCMOS camera were used for imaging of pachytene stage cells. Approximately 50 spermatocytes per individual were examined, totaling 205 spermatocytes per breed. The average intensity of each spermatocyte of four Suffolk and four Targhee were calculated using the ImageJ version 1.51 software to determine H3K4me3 signal. To determine if a significant ($p<0.05$) difference in H3K4me3 intensities were present, three statistical analysis were performed: the Shapiro Wilk Normality test, the Kruskal-Wallis test, and the post-hoc Tukey-Kramer test. To identify any correlation between MLH1 and H3K4me3 data, a Spearman's Rank Correlation was performed.



Acknowledgements

This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, award numbers USDA-NIFA-IDA1566 and USDA-NIFA-2016-09952.

Results

Table 1. H3K4me3 Average Intensity Calculations for both Targhee and Suffolk breeds.

	Animal ID	Mean	Min	Max	Range	# Cells
Targhee	**TR.1**	18973.59	8481.543	31615.48	23,133.94	52
	TR.2	15488.71	8360.01	22534.93	14,174.92	51
	TR.3	21257.33	13705.6	33422.29	19,716.69	50
	TR.4	15089.64949	7449.299	23873.01	16,423.71	52
Breed Average		**17678.44**	**9499.11**	**27861.4**	**18,362.32**	**51.25**
Suffolk	**SR.1**	17155.56	10593.09	28012.19	17,419.11	44
	SR.2	18968.38	11945.31	29266.98	17,321.67	56
	SR.3	18700.02	13866.12	26338.64	12,472.52	54
	SR.4	15123.85	8743.94	23903.4	15,159.46	51
Breed Average		**17552.15**	**11287.1**	**26880.3**	**15,593.19**	**51.25**

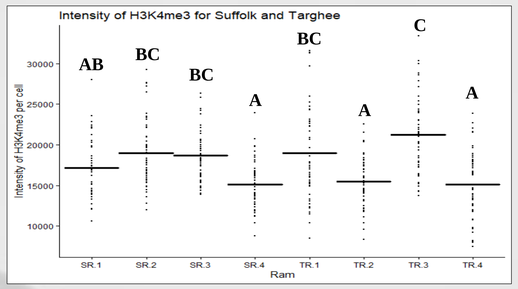


Figure 1. H3K4me3 intensity averages of each individual. The dot represents the intensity average of each spermatocyte per individual. The black lines represent the mean intensity per individual. A, B, and C indicate significant ($p<0.05$) differences.

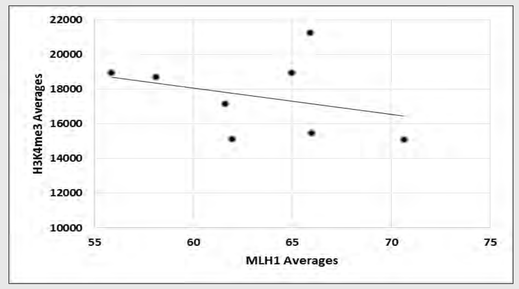


Figure 2. Relationship between the number of MLH1 foci averages and the H3K4me3 intensity averages. No significant correlation was observed.

Conclusion

- Significant differences of H3K4me3 signal were observed amongst individuals but differences did not significantly correlate to previously obtained MLH1 data.[1]
- A better understanding of the relationship between H3K4me 3 was developed, but further research in this area has the potential to understand their influence on meiotic recombination.

References

1. Baudat, F., Imai, Y., & De Massy, B. (2013). Meiotic recombination in mammals: localization and regulation. *Nature Reviews Genetics*, *14*(11), 794.
2. Davenport, K. M., et al., (2018). Meiotic recombination differences in rams from three breeds of sheep in the US. *Cytogenetic and Genome Research*. DOI:10.1159/000493175
3. Hassold, T., & Hunt, P. (2001). To err (meiotically) is human: the genesis of human aneuploidy. *Nature Reviews Genetics*, *2*(4), 280.

Feeding Behavior of Growing Holstein Dairy Heifers in Response to a Modified Lignin Product as a Feed Ingredient

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Abstract

The objective of this preliminary study was to evaluate feeding behavior of Holstein dairy heifers when offered a lignin product as a component of pelleted feed. Five feed pellets were prepared: a positive control containing molasses, a negative control containing neither molasses nor the product, and three pellets containing the product in varying levels – low, medium, high – plus molasses. Growing Holstein heifers (16 months of age, average BW = 399 ± 9 kg) were placed into individual pens (3.4 x 3.7 meters) with one pelleted feed offered at a time to test the acceptability of each feed. Feeding behaviors were recorded for each animal in a specified amount of time (60 min). A total of 129 observations were statistically analyzed. Preliminary data show the animals accepted the pelleted feed containing the high inclusion lignin product more than the other feeds: heifers consumed more feed within 60 minutes ($P < 0.0001$) and per approach ($P < 0.0001$). Heifers spent less time ruminating ($P < 0.0006$) and eating ($P < 0.0001$) when the negative control was offered compared with that of other feeds except the low inclusion lignin product. We are currently evaluating rumen fermentation characteristics of pelleted feeds containing the modified lignin product.

Introduction

- Forage contains fiber (cellulose, hemicellulose, and lignin).
- NDF (all three parts) correlates negatively with dry matter intake (DMI)
- ADF (cellulose and lignin) correlates negatively with degradability and digestibility
- Lignin is undegradable (Porter et. al., 1971).
- Research is exploring the use of treated high lignin products as feeds for ruminants.
- Thermally / chemically modified lignin products may be a substitute to molasses in pelleted feeds, as a binder.

Objectives

The objectives of this study were to: 1) make pelleted feed using a thermally/chemically modified lignin product, 2) study how cows respond to this product, and 3) analyze the degradability of the product.

Materials & Methods

- Five pelleted feeds were developed: a positive control containing molasses, a negative control containing neither molasses nor the modified lignin product, and three pellets containing the product in varying levels – low, medium, high – plus molasses.
- Eight growing Holstein heifers, 16 months of age, 60 days pregnant, and BW 399 ± 9 kg were used.
- Heifers were removed from feed 30 minutes prior to trial. Test feeds were weighed before and after each round.
- Three heifers at a time were placed in individual pens (3.4 x 3.7 meters) with one of five feeds offered. The order of feeds and heifers were randomized.
- The animals were recorded for 45 – 60 minutes.
- The observations were analyzed for the number of approaches, time spent eating, ruminating, and wandering. Dry matter intake (DMI) was calculated.
- The feeds were analyzed via *in vitro* fermentation for neutral detergent fiber, acid detergent fiber, organic matter degradability rates. They were cultured with ruminal fluid at 8 timepoints – 0, 3, 6, 9, 12, 18, 24, and 48 h.
- Responses were analyzed using GLIMMIX procedure of SAS (v. 9.4) with animals as the random effect.

Results

Figure 1. The number of approaches to the feed and the number of eating approaches by treatment.

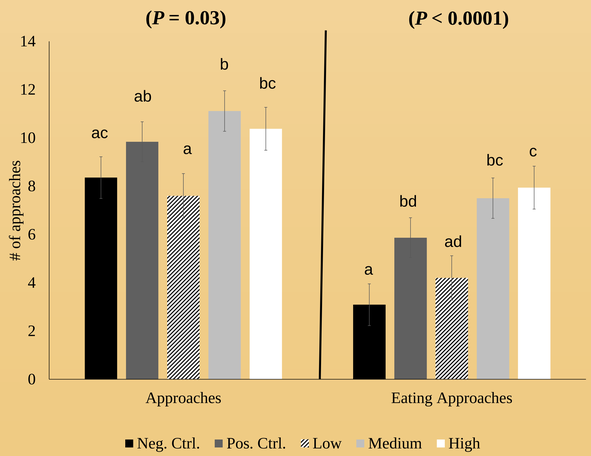


Figure 2. The dry matter intake (grams) by treatment over a fifty-five minute period.

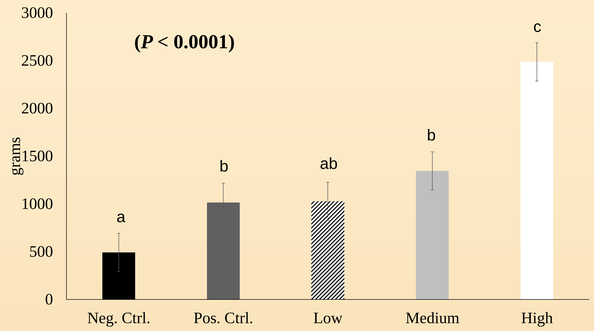


Figure 3. The dry matter intake (grams) per eating approach by treatment.

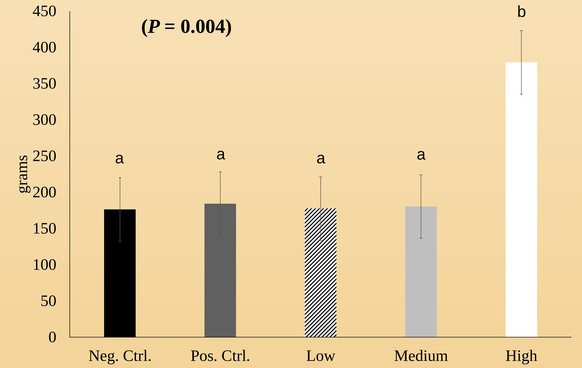


Figure 4. The time (minutes) spent eating and ruminating by treatment.

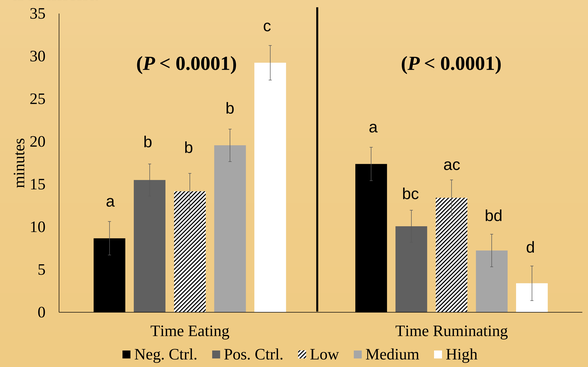
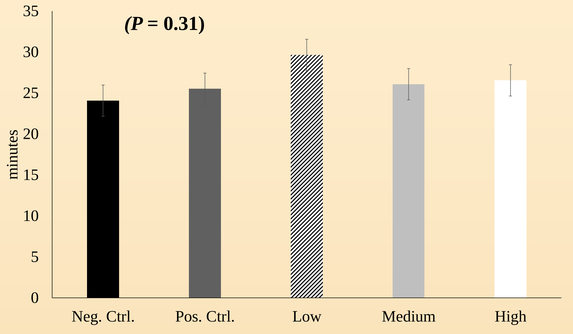


Figure 5. The time (minutes) spent restless by treatment.



Summary

- The heifers spent more time eating ($P < 0.0001$) when placed in a pen with the high inclusion lignin product feed.
- The animals spent less time ruminating ($P < 0.0001$) when placed in a pen with the high inclusion lignin product feed than the other feeds except the medium inclusion lignin product feed ($P = 0.17$).
- The heifers ate more high inclusion lignin product than the other ($P < 0.0001$)
- The heifers ate less of the negative control than the other feeds ($P < 0.0001$)
- There was no significant difference between heifers exposed to the feeds in the time spent drinking water nor wandering .
- Overall, animals accepted the high inclusion lignin product feed more than the other feeds.

References

Porter, P., & Singleton, A. G. (1971). The degradation of lignin and quantitative aspects of ruminant digestion. British Journal of Nutrition, 25(1), 3-14. doi:10.1079/bjn19710061

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Unravelling Genetic Determinants of Synaptic Formation in the Mammalian Visual System

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University of Idaho University Honors Program
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ABSTRACT

Blinding diseases, such as age-macular degeneration and glaucoma, are common causes of vision loss and occur in 2-15% of the population. A detailed understanding of visual system organization is a limiting factor in developing treatments for such disorders. Genetic blinding diseases are studied to understand visual system organization and diseases. Stationary night blindness, is caused by mutation in the *Dscaml1* gene. *Dscaml1* encodes for the protein, Down Syndrome Cell Adhesion Molecule-like 1 (DSCAML1), which plays a role in organization of cells critical for night vision. In a previous study we used electron micrographs to visualize the cellular organization of rod bipolar cells (RBC) in the synaptic pathway within the mouse retina, an accessible model for human diseases. We found an increased number of dendrite terminals that do not contact rod photoreceptors in the absence of DSCAML1. This project focuses on using immunohistochemical (IHC) techniques to compare the development of the synaptic pathway at multiple post-natal time points in three genotypes. It is predicted that the loss of DSCAML1 results in termination of the pathway between the RBC and dendrites instead of a delay in formation. This study is intended to guide clinicians seeking interventions for people with similar disorders.

BACKGROUND

Organization of the rod pathway depends on several genetic factors in humans. We utilize mice with similar mutations to understand why humans with these mutations develop blinding diseases. We will test the hypothesis that early failures in neural development result in later defects in visual acuity.

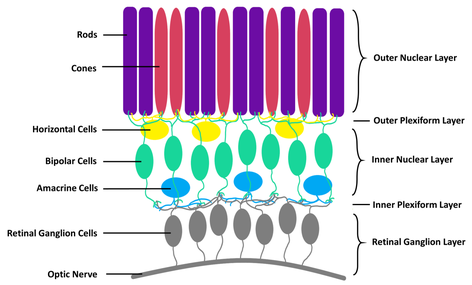
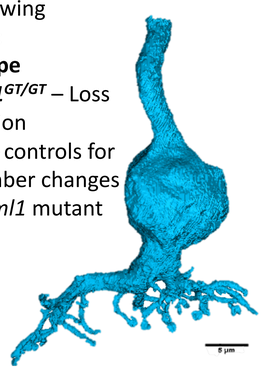


Fig 1: Layout of retina's cellular structure. The focus of these studies is on the rod bipolar cells that transmit signals from photoreceptors to ganglion cells.

MATERIALS AND METHODS

3D RECONSTRUCTION

- Stacks of between 300 and 600 transmission electron micrographs of the following genotypes**:**
  - **Wild-Type**
  - ***Dscaml1***$^{GT/GT}$ – Loss of function
  - ***Bax***^{LOF} – controls for cell number changes in *Dscaml1* mutant retina



NEURAL SYNAPTIC DEVELOPMENT

- Stacks between 10-15 µm of coronal sections
- Genotypes: **B6, *Dscaml1***$^{GT/W}$**, *Dscaml1***$^{GT/GT}$
- IHC Staining:
  - 1° Antibodies: mouse PKC, mandag, ribeye
  - 2° Antibodies: DαR, DαGIgG2A, DαGIgG1
  - DAPI Stain

ImageJ Software from NIH
Nikon Confocal Microscope
Mice at following timepoints:

- P14, P21, P35, 3M, 5M, 8M, 18M

3D RECONSTRUCTION

The rod pathway transforms light stimulus into neural signals that are passed through rod bipolar cells (RBC), to retinal ganglion cells, continuing to the brain.

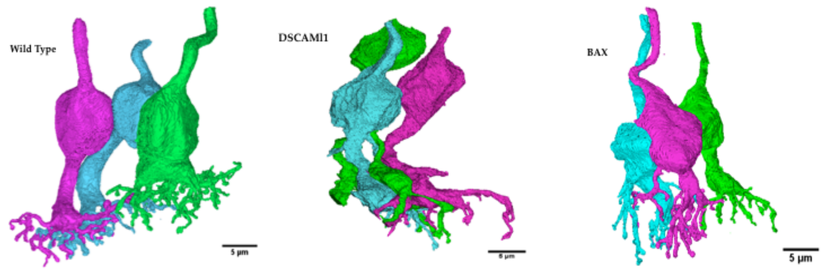
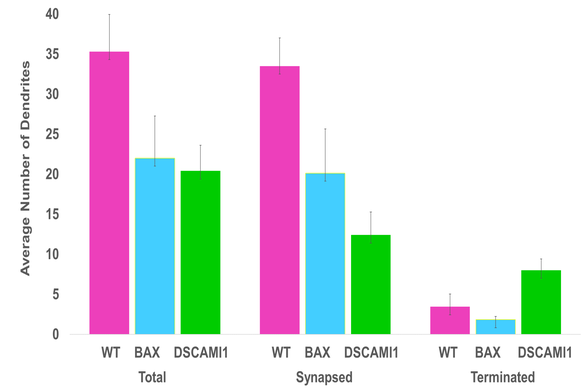


Fig 2: 3D reconstructions of RBCs showing the cell morphology and spatial arrangements of adjacent cells in Wild-Type (WT), *Dscaml1*, and *Bax* retinas.

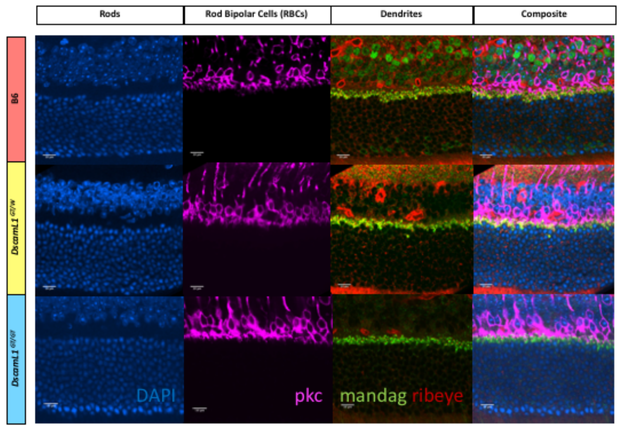


Changes in dendritic density occur in both the *Dscaml1* and *Bax* mutant retinas, suggesting cell density is a critical factor. Decreases in the number of dendrite terminals with synapses is specific to *Dscaml1* and suggests this protein plays a role in establishing connections between rods and bipolar cells.

Fig 3: DSCAMl1 is required for rod bipolar cell synapses. WT RBC have an increased number of total dendrites per cell, while *Dscaml1* and *Bax* have significantly fewer ($p<0.001$). *Dscaml1* RBC have significantly more dendrites that fail to synapse when compared to WT and *Bax* ($p<0.001$).

NEURAL SYNAPTIC DEVELOPMENT – IHC STAINING

Fig 4: Images from IHC staining results. Z stack images were taken using a Nikon Confocal Fluorescent Microscope at 600x total magnification. ImageJ was used to complete the counts and generate composite images.



TERMINATION COMPARISONS

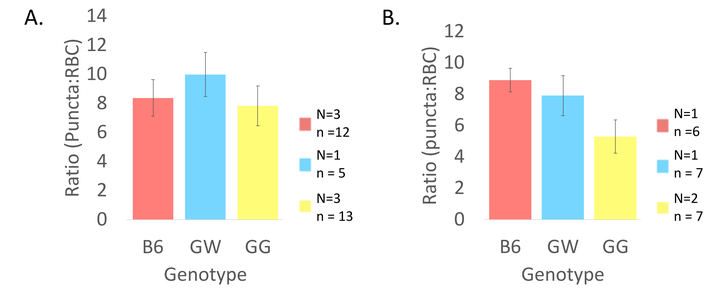


Fig 5: The ratio of puncta to RBCs changes between each genotype. A) The ratios of p14 mice with a p-value of 0.01657. B) The ratios of 3-month old mice with a p-value of 0.00003.

		p14		3 month	
B6	GW	Accept null	✓	Accept null	✓
GW	GG	Reject null	✓	Reject null	✓
GG	B6	Accept null	×	Reject null	✓

Table 1: Statistical results of ratio comparison. There is a difference in ratios between GW and GG mice and B6 to GG at p14 and 3 month with a critical value of 3.9.

Table 2: Comparison of ages within genotypes. For GW there is no difference in age starting at 3 month with a critical value of 3.79. Any possible effects of mutant allele is repaired by 3 months. Within the GG mice there is a difference starting at 3 months. The comparison of B6 mice also indicated that there is no difference as the mouse ages from p14 to p21 (p=0.28695).

GW	P21	P35	3M
P14	Reject null	Reject null	*Accept null*
P21		Reject null	*Accept null*
P35			*Accept null*

GG	P21	P35	3M
P14	Accept null	Accept null	*Reject null*
P21		Reject null	*Reject null*
P35			*Reject null*

B6	P21
P14	*Accept null*

DISCUSSION

- Functional connectivity in the rod pathway and functional synapses of rod to rod bipolar cells requires DSCAML1
- The decrease in successful synapses is due to DSCAML1 mutation, not overcrowding or changes in cell volume
- Confirmation that the mutation does lead to termination of synapses
- Preliminary results indicate possible synaptic pruning of less successful synapses over time and not a failure to develop dendrites

FUTURE WORK

- Increase sample size: three mice (N=3)/age/genotype n=15

ACKNOWLEDGEMENTS

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REFERENCES

Fuerst, P.G., et al., "DSCAM and DSCAML1 Function in Self-Avoidance in Multiple Cell Types in the Developing Mouse Retina," *Neuron* 64, no. 4 (November 25, 2009): 484–97

Fuerst, P. G., Koizumi, A., Masland, R. H., & Burgess, R. W. (2008). Neurite arborization and mosaic spacing in the mouse retina require DSCAM. *Nature*, *451*(7177), 470–474.

Ying, G., Maguire, M., Liu, C., & Antoszyk, A. (2008). Night Vision Symptoms and Progression of Age-related Macular Degeneration (AMD) in the Complications of AMD Prevention Trial. *Ophthalmology*, *115*(11), 1876–1882.e4.

Microstructure and Mechanical Properties of $Al_{0.4}CoCrFeNi$ High Entropy Alloy

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INTRODUCTION

High Entropy Alloys (HEAs) are an emerging class of materials having higher percentages of multiple elements, which allow enhanced properties for various potential structural applications.

One of the most studied HEAs is $Al_xCoCrFeNi$ where x = 0.1 to 2 [1]. The composition of $Al_xCoCrFeNi$ with x = 0.4 has not yet been studied. This alloy system is generally studied for their excellent mechanical properties, which are thought to be caused by both the lattice distortion effect and the cocktail effect [2]. It has bee found that FCC $Al_xCoCrFeNi$, has plastically deformed by twinning induced plasticity (TWIP).

The exceptional mechanical behavior of this alloy is contributed to the solid solution strengthening, where the alloy comprises of multiple solute elements, and twinning deformation during the deformation process [3]. Deformation by twinning contributes to plastic strain raising the strain hardening exponent value as the microstructure becomes finer.

The objective of this work is to study the microstructure and mechanical properties of $Al_{0.4}CoCrFeNi$ alloy and understand the unique deformation mechanism in play for this alloy composition.

PROCEDURE

The material used in this study is a dual phase High Entropy Alloy, $Al_{0.4}CoCrFeNi$. The nominal composition of the alloy is shown in Table 1. The alloy was made by vacuum induction cast melting.

Table 1: As cast material composition

Element	Al	Co	Cr	Fe	Ni
Atomic %	10.3	23.0	24.2	21.1	21.4

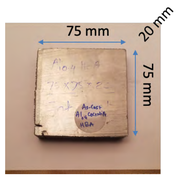

Figure 1: Specimen cast block

Compression tests were performed at room temperature and 600ºC using Instron 5982 Universal Tester Machine. Cylindrical specimens with height to diameter ratio of 1.5 were tested to prevent buckling after loading. After compression testing, specimens were characterized to observe any changes in microstructure.

Characterization instruments used are listed below.

- Optical Microscope Olympus PGM-3
- Zeiss Supra 35 FEG SEM equipped with EDS detector
- FEI Technai G2 20 Twin TEM operated at 200KV.
- Siemens D500 X-Ray Diffractometer Machine
- Vickers Microhardness LECO LM100

RESULTS

As Cast Microstructure

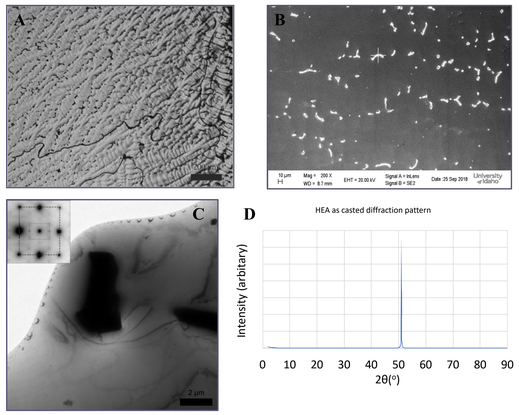

Figure 2: As cast microstructure analyzed with **A**. Optical Microscopy, **B**. SEM, **C**. TEM, and **D**. X-Ray Diffraction.

The as received specimen has typical cast dendritic structure with FCC phase in the dendritic region and B2 (BCC) phase with Al-rich and Ni-rich precipitates in the interdendritic region. The grain size is greater than 200 µm. The phase fraction of the second phase is estimated around 12%. From selected area diffraction analysis in TEM, crystallographic relation between BCC and FCC phase is found to be $[01\bar{1}]_{BCC} // [\bar{2}33]_{FCC}$. The lattice constant of the alloy is 3.587 nm as obtained from X-Ray Diffraction.

Compression Testing

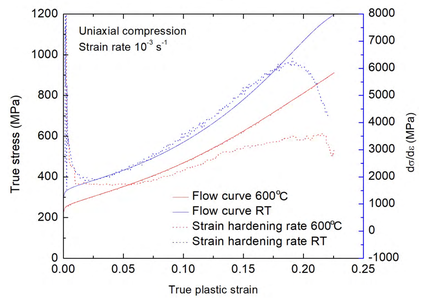

Figure 3: True stress vs. true plastic strain curve at a strain rate of 10^{-3} s^{-1}

Yield strength in compression is measured to be 300 MPa at room temperature and 250 MPa at 600ºC. After room temperature compression, Vickers microhardness changed from 174±4 $HV_{0.5}$ to 310±18 $HV_{0.5}$ (~78% increase). High temperature compression testing gives the Vickers microhardness to be 297±7 $HV_{0.5}$. Note the strain hardening behavior at room temperature and 600ºC is quite different.

Room Temperature Compressed Specimen

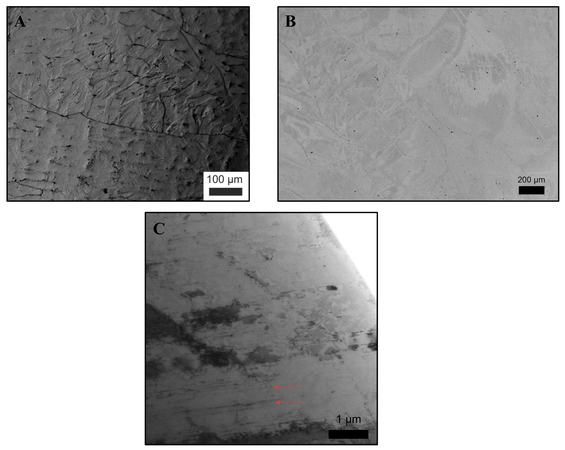

Figure 4: Room temperature compressed specimen analyzed with **A**. Optical Microscopy, **B**. SEM, and **C**. TEM.

Microscopic examination of the room temperature compressed specimen revealed formation of several fine twins. The SEM backscatter electron image, along with the TEM bright field image, agrees with the optical metallography results.

High Temperature Compression

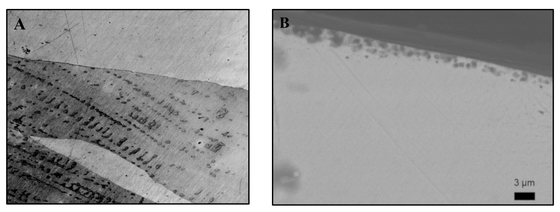

Figure 5: Compression at 600ºC analyzed using **A**. Optical Microscopy and **B**. SEM.

At 600ºC, compression testing does not show such twin boundaries as observed by optical microscopy or SEM.

DISCUSSION

Al addition to CoCeFeNi HEA helps form the B2 phase. This phase can be Al-Ni intermetallic. Although this alloy is heavily alloyed, the yield strength is at the similar level as conventional TWIP steel i.e. high manganese steel (low carbon) [4, 5] which have yield strengths around 300 MPa at room temperature.

During compression tests, at the same strain, twins form at room temperature whilst absent at high temperatures. This is likely from the effect temperature has on the mobility of dislocations. Twinning is promoted at lower temperatures whereas the thermal effect at higher temperature suppresses twinning and promotes slip.

SUMMARY

From this work, following conclusions can be drawn.

1. $Al_{0.4}CoCrFeNi$ is a dual phase HEA (FCC+12%BCC) due to the effect of Al.
2. The alloy shows non-monotonic hardening behavior at room temperature, while absent at high temperature.
3. Fine deformation twins were observed in the room temperature compression specimen whereas no twinning was noted in the high temperature compression specimen.

FUTURE WORK

Micropillar testing and nanoindentation testing will be conducted to study the intrinsic plasticity behavior of the alloy, *i.e.* crystallographic information of twinning. Mini-tensile testing can be done to evaluate ductility and fracture of the alloy which is not reflected in this work. Further compression tests will be conducted to study the effects of strain rate and temperature on twin formation.

REFERENCES

[1] Y. Kao, *et al.*: *J. Alloys Compds.* ***488*****(1)** (2009), 57-64.
[2] P. Rivera-Díaz-del-Castillo: *J. Mater. Res.*, ***33*****(19)** (2018), 2970-2982.
[3] X. Feng, *et al.*: *Scr. Mater.*, ***139*** (2017), 71-76.
[4] O. Bouaziz, *et al.*: *Curr. Opin. Solid St. Met.*., ***15*****(4)** (2011), 141-168.
[5] W. S. Choi, et al.: *Acta Mater.*, ***132*** (2017), 162-173.

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Effects of abscisic acid on *Anopheles stephensi* reproduction

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Abstract

Hundreds of millions of malaria cases are reported every year despite significant global efforts focused on elimination. Accordingly, new vaccines, therapeutics, and strategies for vector control are needed to support these efforts. *Anopheles stephensi* is an aggressive malaria vector mosquito that has recently invaded Sri Lanka and Djibouti (Africa), where it has been linked to a resurgence of severe infection with the human malaria parasite *Plasmodium falciparum* [1,2].

Mosquitoes consume blood to produce eggs. Following blood consumption, the protein Vitellogenin (Vg) is synthesized in the fat body (a liver-like tissue) of the female mosquito and transferred to developing eggs. Vg synthesis is stimulated following the blood meal by increasing titers of the hormone 20-hydroxyecdysone (20E).

Malaria control efforts directed at reducing mosquito reproduction (fecundity) is an important strategy. We have discovered that abscisic acid (ABA) can reduce mosquito fecundity. Previous studies in the flesh fly showed that ABA can reduce Vg levels in this insect[3]. Based on this work, we hypothesized that ABA reduces *A. stephensi* fecundity by reducing levels of Vg in the mosquito.

Background

Methoprene, pyriproxyfen, diflubenzuron are a few examples of insecticides that work by mimicking hormones that regulate insect growth and development. These insecticides prevent normal molting, egg laying, egg hatching, and development from the immature stages to the adult stage, thereby preventing insects from reproducing. Extensive use of residual insecticides for malaria vector control has resulted in *A. stephensi* resistance to DDT, dieldrin, malathion, other organophosphates and also pyrethroids [4]. Understanding the complex mechanisms behind hormonal control of mosquito reproduction and potential effects of ABA could help to develop novel strategies that can prevent vector-borne diseases like malaria.

Methods

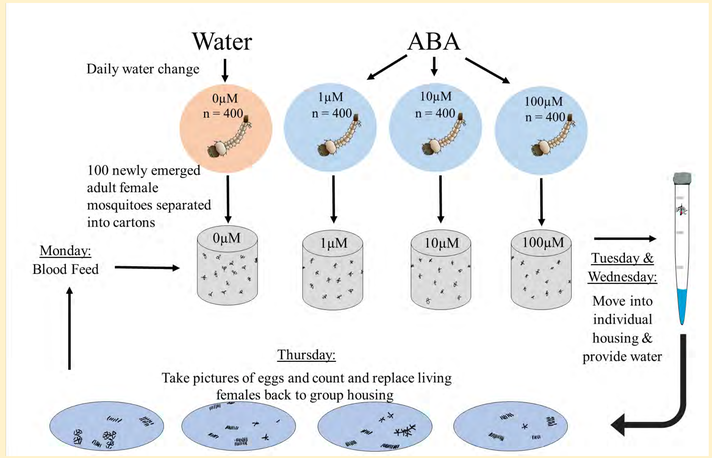

Figure 1: Experimental setup for ABA supplementation of *A. stephensi* larvae and for blood feeding and egg clutch collections of resulting adult female mosquitoes.

Quantification of 20E and Vitellogenin mRNA levels in adult female *A. stephensi*

- A total of 50 adult female mosquitoes were collected following pupation and adult emergence from each larval treatment group (0µM ABA or control, 1µM ABA, 10µM ABA, 100µM ABA).
- Each group of 3-5 day old adult female mosquitoes was provided a blood meal of washed human erythrocytes and serum.
- At 0 hr (immediately before feeding), 12 hr, 24 hr, 48 hr, and 72 hr post-feeding, a total of 10 mosquitoes derived from control and ABA-treated larvae groups were sampled for quantification of 20E (n=5 mosquitoes per timepoint) and Vg mRNA levels (n=5 mosquitoes per timepoint).
- Total RNA was prepared from whole mosquitoes sonicated in TRIzol® reagent (ThermoFisher) and 20E was extracted from whole mosquitoes in methanol. Total RNA was used for quantitative real-time PCR analysis of Vg mRNA transcript levels, while 20E was quantified using an enzyme immunoassay (Arbor Assays).

Results

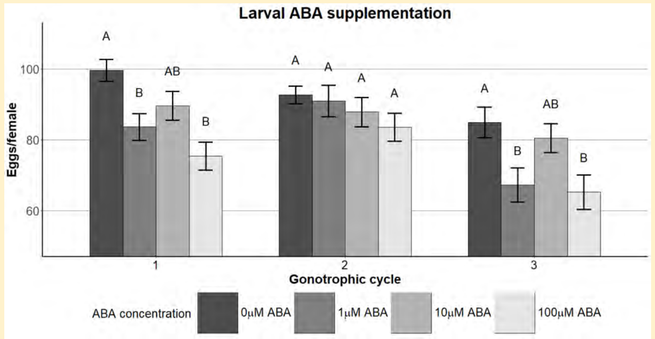

Figure 2: Adult female *A. stephensi* derived from larvae supplemented daily with ABA produced significantly fewer eggs per female in the 1st and 3rd gonotrophic cycles ($p<0.001$, $p<0.005$). These data indicate that ABA treatment of larvae results in physiological changes that persist into the adult stage and that are durable over time.

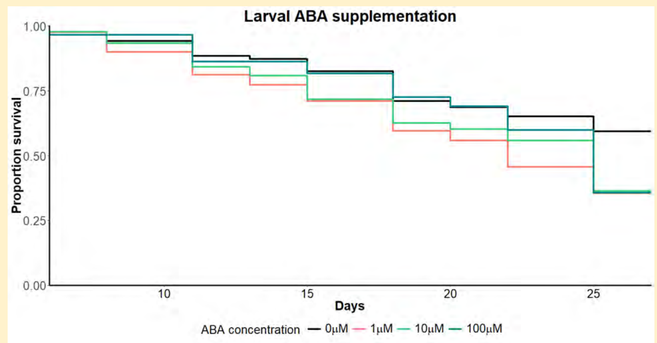

Figure 3: Adult female *A. stephensi* derived from larvae supplemented daily with ABA showed reduced survivorship compared to control mosquitoes over a three week time period. Data were analyzed by log rank test (1µM, p=0.0039; 10µM, p=0.0091; 100µM, p=0.0220).

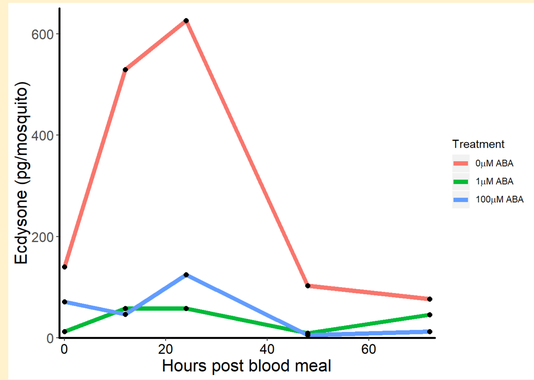

Figure 4: Adult female *A. stephensi* in the control group exhibited the expected pattern of increasing 20E titer following a blood meal. Female mosquitoes derived from larvae treated with 1µM ABA and 100µM ABA group did not show the typical peak of 20E at 24 hr, indicating they are not properly responding to the blood meal.

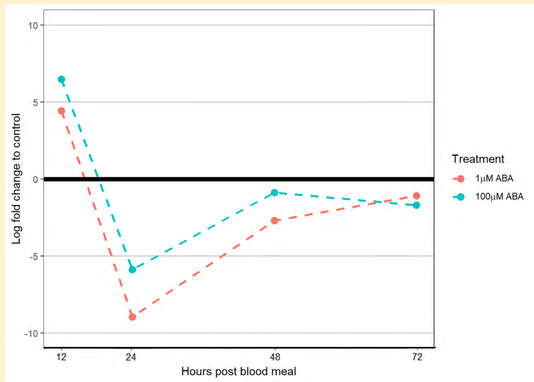

Figure 5: Adult female *A. stephensi* derived from larvae treated with 1µM ABA and 100µM ABA had increased Vg mRNA levels at 12 hr post blood meal relative to control (black line), but reduced levels in the following 36 hr. This early increase in Vg mRNA expression followed by reduced Vg expression could explain why egg production was reduced but not blocked in adult females derived from larvae treated with ABA.

Conclusions

- Relative to controls, ABA treatment of *A. stephensi* larvae significantly reduced fecundity of adult female mosquitoes derived from these larvae.
- ABA supplementation of *A. stephensi* larvae significantly reduced the lifespan of adult female mosquitoes relative to controls.
- Adult female *A. stephensi* derived from ABA-treated larvae exhibited reduced titers of regulating steroid hormone 20E and reduced post-blood meal Vg mRNA levels relative to controls.
- Innovative delivery of ABA to mosquito breeding areas could provide an environmentally friendly method to reduce mosquito reproduction.

References

[1] Faulde MK, Rueda LM, Khaireh BA. First record of the Asian malaria vector *Anopheles stephensi* and its possible role in the resurgence of malaria in Djibouti, Horn of Africa. Acta Trop. 2014;139:39-43.
[2] Gayan Dharmasiri AG, Perera AY, Harishchandra J, Herath H, Aravindan K, Jayasooriya HTR, et al. First record of *Anopheles stephensi* in Sri Lanka: a potential challenge for prevention of malaria reintroduction. Malar J. 2017;16(1):326.
[3] Man, W., Loof, A., Briers, T., & Huybrechts, R. (1981). Effect of Abscisic Acid on Vitellogenesis in *Sarcophaga bullata*. Entomologia Experimentalis Et Applicata., 29(3), 259-267.
[4] Enayati A. A., Vatandoost H, Ladonni H, Townson H, Hemingway J. Molecular evidence for a kdr-like pyrethroid resistance mechanism in the malaria vector mosquito *Anopheles stephensi*. Med. Vet. Entomol., 17 (2003), pp. 138-144.

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Low Energy Availability and Bone Density in Non-NCAA Athlete Males

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INTRODUCTION

Low energy availability (LEA) is a condition resulting from an insufficient amount of energy required for normal function and metabolic processes after accounting for exercise[1]. Collegiate male athletes are prone to developing LEA due to high levels of physical activity coupled with insufficient energy intake[1]. LEA adversely affects health and athletic performance in competitive athletes and is associated with decreased bone mineral density (BMD)[2]. Little is known, however, about the occurrence of LEA, or its relation to BMD, in non-athlete populations.

PURPOSE

This study aimed to investigate the prevalence of LEA and its relation to BMD in non-NCAA athlete males at the University of Idaho.

METHODS

Participants: This study recruited 21 non-NCAA athlete males ages 18-26 currently attending the University of Idaho.
Data Collection: EA was determined by the following equation[3]:

$$\text{EA kcal/kg of LBM/day} = \frac{\text{DI kcal} - \text{ExEE kcal}}{\text{LBM kg}}$$

Participants wore accelerometers (ActiGraphGT3X+) to measure their exercise energy expenditure (ExEE) and tracked their dietary intake (DI) using ASA24, an online dietary assessment tool. DI and ExEE were measured over a period of three days (two week days and one weekend day). BMD and body composition (fat mass (FM) and lean body mass (LBM)) were then assessed using dual-energy x-ray absorptiometry (DXA; Hologic Horizon™; Marlborough, MA). Criteria for LEA was defined as less than 30 kcal/kg of LBM/day[3].
Statistical Analysis: Descriptive statistics of participant characteristics, BMD, and EA were performed. Spearman correlations were performed to assess the relationship between EA and BMD.

RESULTS

Table 1. Descriptive Statistics of Non-Athlete Males[1]

Total (n)	21
Height (m)	1.79 ± 0.061
Weight (kg)	78.3 ± 10.6
BMI (kg/m²)	24.5 ± 3.33
[1]Mean ± SD (all such values)	

Figure 1. Energy Availability vs. Bone Density

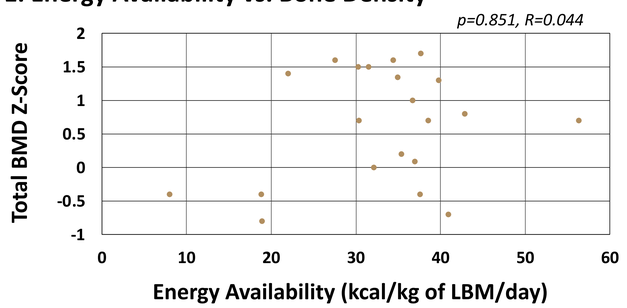

Figure 2. Weight vs. Bone Density

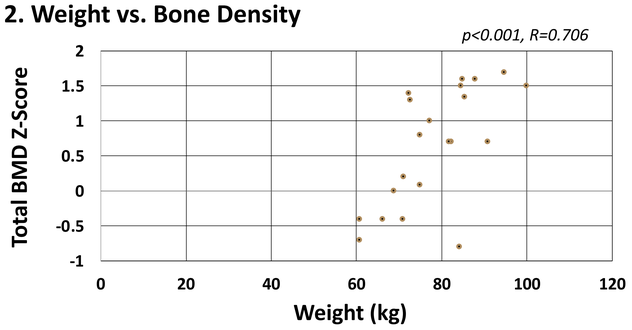

Figure 3. Prevalence of Low Energy Availability

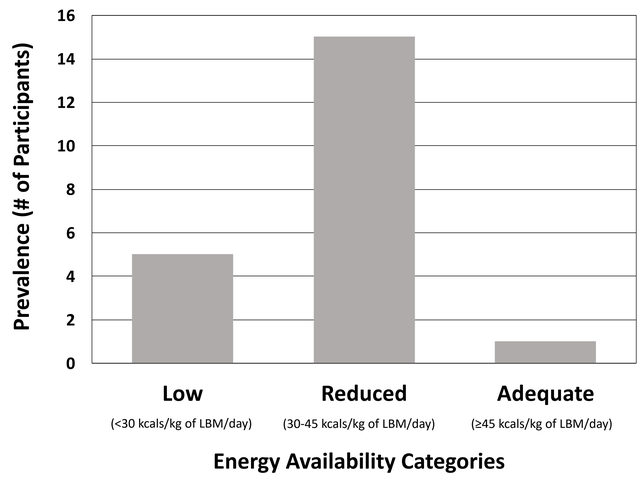

Table 2. Descriptive Characteristics of Body Composition and Energy Availability[1]

Total (n)	21
Body Composition	
FM (%)	20.5 ± 4.60
LBM (%)	79.5 ± 4.60
BMD	0.64 ± 0.84
Energy Availability	
EA (kcal/kg of LBM/day)	33.0 ± 10.0
[1]Mean ± SD (all such values)	

DISCUSSION

The prevalence of LEA in non-NCCA athlete males and its relation to BMD has not been extensively studied[4,5,6]. No correlation was found between EA and total BMD (p = 0.851), spine BMD (p = 0.641), or hip BMD (p = 0.786). However, total BMD was significantly correlated with body weight (p < 0.001). These findings differ from previous research among competitive cyclists which found associations between EA and BMD[7]. 23.8% of participants indicated having low EA (< 30 kcal/kg of LBM/day), and 71.4% of participants indicated having reduced EA (30-40 kcal/kg of LBM/day). Additional research is needed to fully understand the impact that LEA has on non-athlete populations.

REFERENCES

1. M. Mountjoy et al., "The IOC consensus statement: Beyond the Female Athlete Triad Relative Energy Deficiency in Sport (RED-S)," Br. J. Sports Med., vol. 48, no. 7, pp. 491–7, 2014.
2. R. T. Viner, M. Harris, J. R. Berning, and N. L. Meyer, "Energy availability and dietary patterns of adult male and female competitive cyclists with lower than expected bone mineral density," Int. J. Sport Nutr. Exerc. Metab., vol. 25, no. 6, pp. 594–602, 2015.
3. A. B. Loucks and J. R. Thuma, "Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women," J. Clin. Endocrinol. Metab., vol. 88, no. 1, pp. 297–311, 2003.
4. K. Koehler et al., "Low energy availability in exercising men is associated with reduced leptin and insulin but not with changes in other metabolic hormones," J. Sports Sci., vol. 34, no. 20, pp. 1921–1929, 2016.
5. R. T. Viner, M. Harris, J. R. Berning, and N. L. Meyer, "Energy availability and dietary patterns of adult male and female competitive cyclists with lower than expected bone mineral density," Int. J. Sport Nutr. Exerc. Metab., vol. 25, no. 6, pp. 594–602, 2015.
6. E. Dolan et al., "An altered hormonal profile and elevated rate of bone loss are associated with low bone mass in professional horse-racing jockeys," J. Bone Miner. Metab., vol. 30, no. 5, pp. 534–542, 2012.
7. Keay, N., Franics, G., and Hind, K. (2018). Low energy availability assessed by a sport-specific questionnaire and clinical interview indicative of bone health, endocrine profile and cycling performance in competitive male cyclists. BMJ Open Sport and Exercise Medicine, 4;4(1):e000424.

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Anatomical coupling of locomotor and auditory neurons in desert kangaroo rats (Dipodomys deserti)

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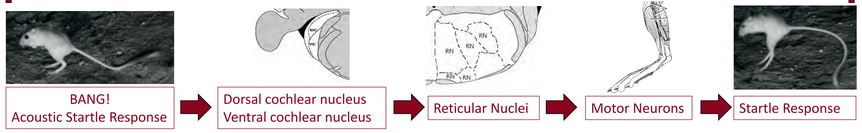
ABSTRACT

Acoustic stimuli-induced startle response in mammals may be modulated by vigilance and an elevated arousal state to allow for a more rapid acoustic stimulus-induced response in locomotor systems.[1] Environmental modulation of this reflexive response may underpin defensive maneuvers in prey species. Though this phenomenon is found in many mammals, the nature of anatomical connectivity between auditory and locomotor neurons remains unclear in desert kangaroo rats. Identifying the neuroanatomical nature of this auditory-locomotor pathway is a major step towards understanding how species-specific anatomical and functional properties of this pathway may underpin success rate of kangaroo rats in the wild. To evaluate the anatomical connectivity, a trans-synaptic retrograde pseudorabies virus was injected into the right gastrocnemius muscle and induces the expression of green fluorescent protein in all presynaptic neurons that are synaptically connected to the afferent motor neuron of the muscle, whether they be motor or not. Following 5-7 days of recovery after viral injection, kangaroo rats were euthanized, and their brains removed, frozen and sectioned coronally. Tissue sections containing all central auditory nuclei were then mounted directly onto slides or immunohistochemically labeled to amplify visibility of GFP expression. Sections were imaged through a microscope where GFP expression was observed in motor nuclei within the brainstem and midbrain along with the cochlear nucleus, a key site for mediating the acoustic startle response. Slices from the auditory pathway were also taken to observe if there is further involvement of higher order auditory brain regions that may contribute to the acoustic startle in k-rats.

INTRODUCTION

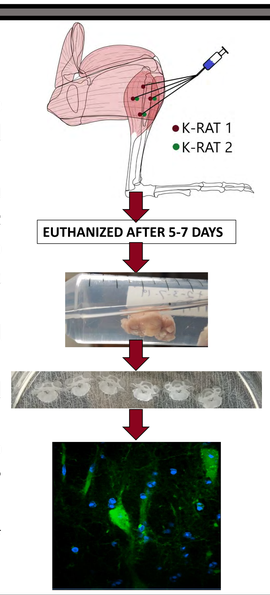
- Acoustic stimuli-induced startle response in mammals may be modulated for a rapid response in locomotor systems
  - Environmental modulation of this reflexive response may underpin defensive maneuvers found in prey species.
- Nature of anatomical connectivity between auditory and locomotor neurons remain unclear in kangaroo rats.
- Identifying neuroanatomical structures that are linked allows understanding of pathway
  - **May underpin the high success rate of k-rats in the wild**

~0.7 Seconds



MATERIALS AND METHODS

- Pseudorabies virus (PRV 152), a retrograde trans-synaptic viral tracer[2], was injected into right gastrocnemius muscle of subject
- PRV 152 induces expression of green fluorescence protein (GFP) in presynaptic neurons that are synaptically connected to the initially-infected motor neuron upon injection.
- Following 5-7 days of recovery from viral injection, subjects (n=2) were euthanized
- Brains were removed, frozen, and sectioned coronally using a cryostat.
- Tissue sections were directly mounted onto slides or immunohistochemically labeled for GFP chicken anti-GFP (1:500; NB100-1614).
- Images were taken by confocal microscopy at a wavelength of 488nm.



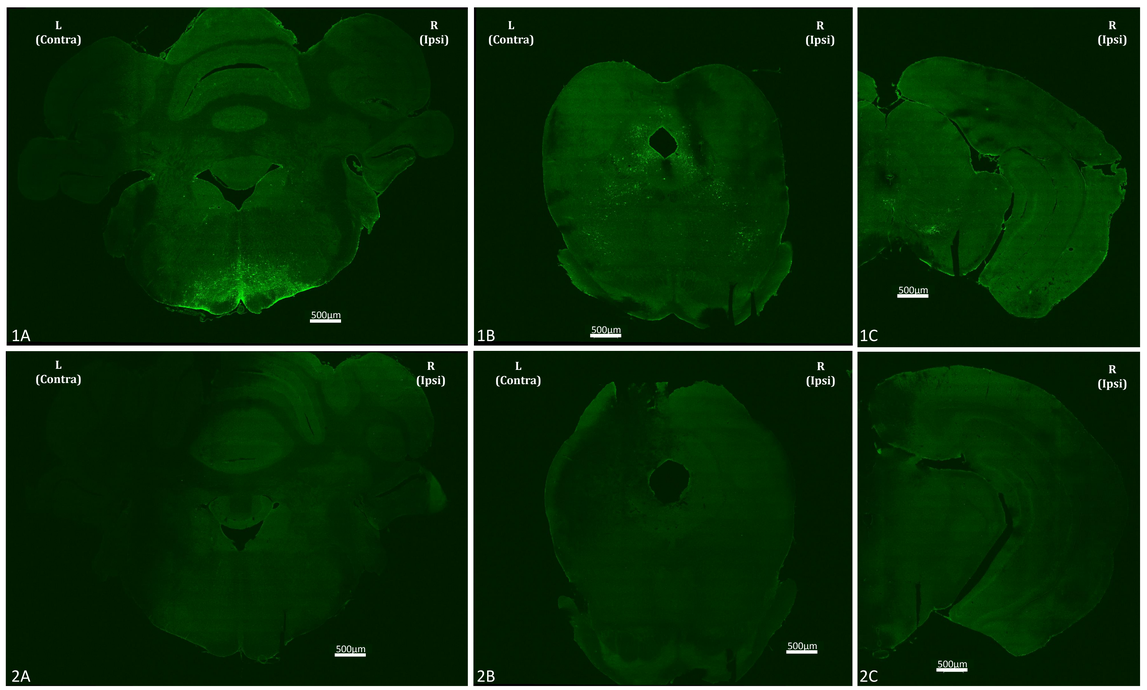
RESULTS

- **K-RAT 1**
  - 500nL/injection site, Total=2µL
  - Incubation Time: 5 Days
  - Settings:
    - 15% Intensity
    - 500ms exposure

Figure 1: Stained sections of Kangaroo Rat 1 (K-Rat 1) stitched with montage imaging.

- **K-RAT 2**
  - 300nL/injection site, Total=0.9µL
  - Incubation Time: 7 Days
  - Settings:
    - 15% Intensity
    - 500ms exposure

Figure 2: Stained sections of Kangaroo Rat 2 (K-Rat 2) stitched with montage imaging



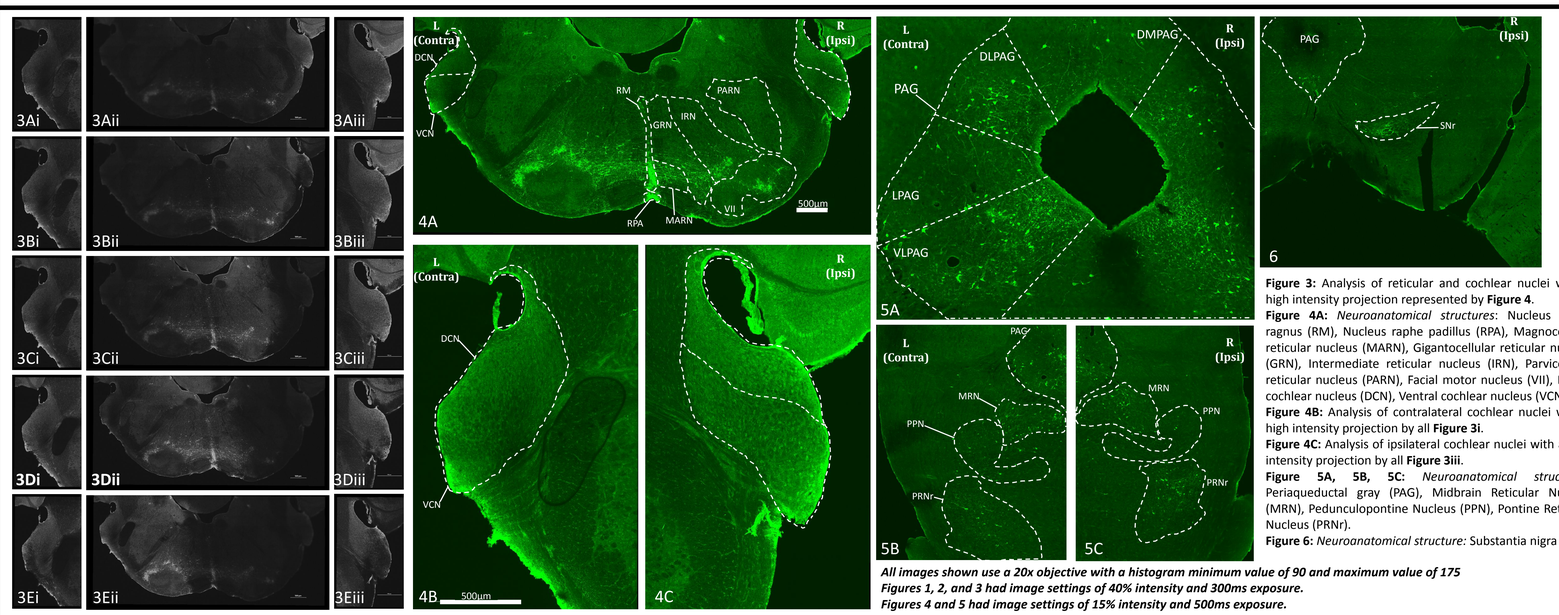


Figure 3: Analysis of reticular and cochlear nuclei with a high intensity projection represented by **Figure 4**.
Figure 4A: *Neuroanatomical structures*: Nucleus raphe ragnus (RM), Nucleus raphe padillus (RPA), Magnocellular reticular nucleus (MARN), Gigantocellular reticular nucleus (GRN), Intermediate reticular nucleus (IRN), Parvicellular reticular nucleus (PARN), Facial motor nucleus (VII), Dorsal cochlear nucleus (DCN), Ventral cochlear nucleus (VCN)
Figure 4B: Analysis of contralateral cochlear nuclei with a high intensity projection by all **Figure 3i**.
Figure 4C: Analysis of ipsilateral cochlear nuclei with a high intensity projection by all **Figure 3iii**.
Figure 5A, 5B, 5C: *Neuroanatomical structures*: Periaqueductal gray (PAG), Midbrain Reticular Nucleus (MRN), Pedunculopontine Nucleus (PPN), Pontine Reticular Nucleus (PRNr).
Figure 6: *Neuroanatomical structure:* Substantia nigra (SNr)

All images shown use a 20x objective with a histogram minimum value of 90 and maximum value of 175
Figures 1, 2, and 3 had image settings of 40% intensity and 300ms exposure.
Figures 4 and 5 had image settings of 15% intensity and 500ms exposure.

DISCUSSION

- Using PRV 152 virus with this method allows labeling of any neural circuit
- Viral tracer can label auditory brain stem neurons suggesting they are linked to motor neurons
- Efficient viral uptake seems to take more effect in proximal segment of limb rather than distal segment
- Together these data support anatomical connectivity that would underpin acoustic startle reflex in kangaroo rats

ACKNOWLDGEMENTS

Comparative Neuromuscular Biomechanics Laboratory
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University of Idaho College of Science
The McGowan Lab
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Literature

[1]Freymiller, et al. (2017)
[2] Card, P.J. (2014)

Using Environmental Cues to Modify Fatty Acid Production

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Department of Biological Sciences, University of Idaho, Moscow, ID

Abstract

A promising candidate for biofuel and nutritional supplements is the photosynthetic protist *Euglena gracilis*. In addition to producing essential ω-3 polyunsaturated fatty acids *E. gracilis,* under certain conditions, produces high yield of waxy esters that can be used without modification as biofuel. *E. gracilis* also produces a range of fatty acids including those with methyl branching. Previous studies that focused on industrial lipid production examined the effect of autotrophic (photosynthesis only) and heterotrophic conditions. Our preliminary studies examined changes in fatty acid profile as a result of changing nutritional factors. In the current study, we compared these nutritional factors with different strains of *E. gracilis*, and the effects of environmental factors common to farming. *E. gracilis* can be grown under constant light, but to mimic outdoor farming they were grown in a 14:10 light:dark cycle. *E. gracilis* were also cultured at different temperatures to reflect different climes. Different strains of *E. gracilis* will be grown at a range of pH values to assess lipid production, and growth under acidic conditions (pH 3-4) as a passive approach to reduce contamination. Preliminary results show that artificial constant light negatively effects fatty acid production, and that temperature and strain choice critically effect growth rate.

Background

Euglena gracilis

- Photosynthetic unicellular mixotroph
- Found in freshwater environments
- Produce a wide range of fatty acids

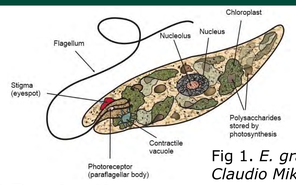


Fig 1. *E. gracilis* *Claudio Miklos*

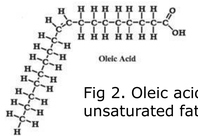


Fig 2. Oleic acid, unsaturated fatty acid

Fig 3. *E. gracilis* supplement sold by Euglena Co. Ltd

Fatty Acid Production

- Candidate for biofuel and nutritional supplements
- Produce short chain saturated waxy esters; Waxy esters can be used directly as biofuel
- Produces ω-3 and ω-6 essential fatty acids
- Produces methyl branched and odd-chain fatty acids
- Odd-chain fatty acids may reduce risk of type II diabetes (animal models)

Objectives

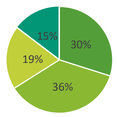
Compare factors involved in farming *E. gracilis* for their impact on fatty acid profile

- Nitrogen starvation (commonly used to boost algal fatty acid production)
  - Transferred at stationary phase to nitrogen-deficient(-N) medium
- Supplementation with branched aliphatic amino acids (Ile, Ala, Val)
  - 2 mM concentration
- Light:dark cycle (mimics outdoor farming)
  - 14 hr light:10 hr dark
- Temperature (reduced temperature mimics northern climate)
  - 26.5°C (~80°F) and 16.5°C (~62°F)
- Comparison of strains (geographically distinct)
  - Use strains isolated from different continents

Approach

Cultured in minimal acid medium (MAM pH 3.5)

Cell density counts to determine growth rate



Harvest to determine relative abundance of fatty acids through Microbial ID, Inc.

Results

Nitrogen Starvation

- Cells transferred to nitrogen-deficient medium after exponential growth phase
- Growth in nitrogen-deficient medium increased fatty acid percentage of dry weight

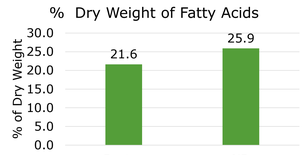
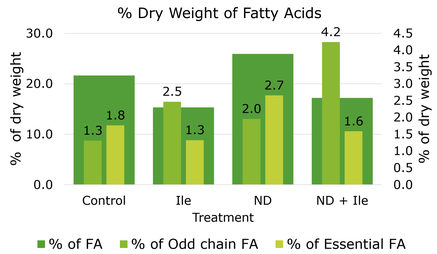


Fig 4. The percent fatty acid of dry weight in *E. gracilis*

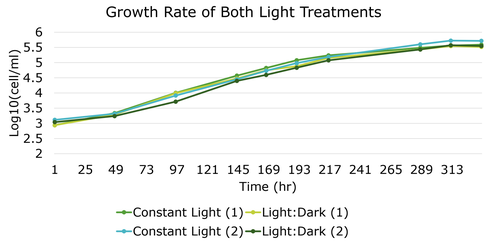
Branched Aliphatic Amino Acid



- Degradation of branched aliphatic amino acids produce propionyl-CoA, which result in odd-chain fatty acids
- When Ile is the only source of nitrogen the yield of odd-chain fatty acid tripled even though total fatty acid production dropped

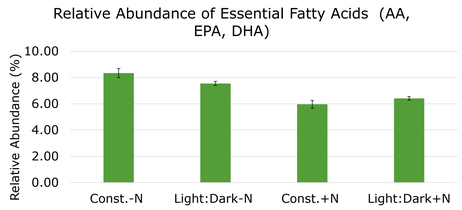
Fig 5. The percent fatty acid of dry weight compared to specific fatty acid groups grown in various treatments

Light:Dark Cycle



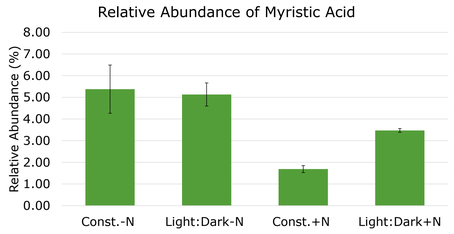
- Minimal difference between the growth rates under constant light and light:dark cycle based on cell density; bio-mass was not determined
- Each curve represents the average of four replicates

Fig 6. (Left) Growth curve of *E. gracilis* in constant light compared to 14:10 hr light:dark cycle



- Constant light with the removal of nitrogen at stationary phase yielded the highest relative abundance of essential fatty acids (AA, EPA, DHA)

Fig 7. Relative abundance of essential fatty acids in *E. gracilis* grown in 4 treatments: variable light and variable nitrogen(-N/+N).

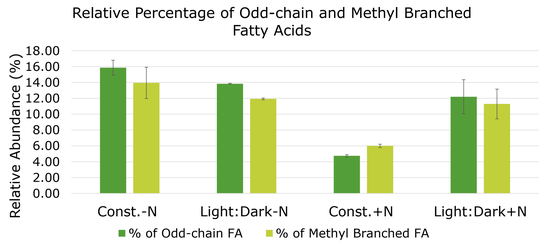


- Myristic acid is an indicator of myristyl myristate, a waxy ester
- When nitrogen is removed, relative abundance of myristic acid was elevated, and equivalent independent of a dark cycle

Fig 8. Relative abundance of myristic acid *in E. gracilis* grown in 4 treatments

- Artificial condition of constant light depresses the yield of both odd-chain and methyl branched fatty acids

Fig 9. Relative abundance of odd-chain and methyl branched fatty acids *in E. gracilis* grown in 4 treatments



Ongoing Research

Temperature

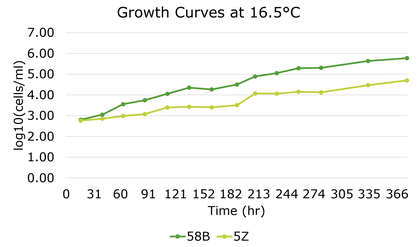


Fig 10. Growth curve of two strains of *E. gracilis* at 16.5°C.

- Two strains of *E. gracilis* grown at 16.5°C (~62°F)
- Grown in 14:10 light:dark cycle
- One strain (5Z) significantly slower doubling time at this temperature

Doubling Rate	58B	5Z
Hours	38.27	58.70

Fig 11. Doubling rates for two strains of *E. gracilis* grown at 16.5°C.

Branched Aliphatic Amino Acids

- Two strains of *E gracilis* grown at 26.5°C (~80°F)
- Grown in constant light, as in preliminary study
- Compare those grown with Ile as only nitrogen source to those transferred to Ile at stationary phase

58B

Doubling Time	+N	+Ile	+Ala
Hours	24.4	26.04	24.48

Fig 12. Doubling rates for one strain of *E. gracilis* grown in constant light at 26.5°C in differing media.

5Z

Doubling Time	+N	+Ile	+Ala
Hours	29.3	28.37	25.31

Fig 13. Doubling rates for one strain of *E. gracilis* grown in constant light at 26.5°C in differing media.

Conclusions

- Transfer to nitrogen-deficient medium resulted in highest yield of fatty acids (fig. 4)
- Temperature control and strain choice is critical to *E. gracilis* cultivation (fig. 10 and fig. 11)
- Artificial condition of constant light depresses the yield of key fatty acids (fig. 7 and fig. 8)
  - This is may prove beneficial because continuous light is not consistent with outdoor farming

Fig 15. Outdoor algae farming *Frank Fields, UC San Diego*

Acknowledgments

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References

Gissibl A, Sun A, Care A, Nevalainen H, Sunna A. Bioproducts From Euglena gracilis: Synthesis and Applications. Frontiers in Bioengineering and Biotechnology. 2019;7.
Nakazawa M. Alteration of Wax Ester Content and Composition in Euglena gracilis with Gene Silencing of 3-ketoacyl-CoA Thiolase Isozymes. Lipids. 2015;50(5):483–492.
Nettleton JA, Katz R. n-3 long-chain polyunsaturated fatty acids in type 2 diabetes: A review. Journal of the American Dietetic Association. 2005;105(3):428–440.
Wang Y, Seppänen-Laakso T, Rischer H, Wiebe MG. Euglena gracilis growth and cell composition under different temperature, light and trophic conditions. Plos One. 2018;13(4).
Zeng M. Fatty acid and metabolomic profiling approaches differentiate heterotrophic and mixotrophic culture conditions in a microalgal food supplement Euglena. BMC Biotechnology. 2016;16(1).

Encapsulation of *Lactobacillus acidophilus* and *Lactobacillus casei* to Determine Cell Viability in a Hydrogel Biobead Matrix

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Departments of [1]Biological Sciences, [2]Biological Engineering, [3]Chemical Engineering & Material Sciences, and [4]Chemistry; University of Idaho

Motivation

Trichloroethylene (TCE), a commonly used industrial solvent, is a widespread, persistent, and carcinogenic groundwater pollutant. An effective treatment strategy for TCE contamination is bioremediation using reductively dechlorinating bacteria. However, during bioremediation changing pH levels can harm these degrading microbes. By incorporating the microbes into a polymer matrix, pH is buffered and the microbes are protected. Determination of encapsulated microbe viability assists in optimization of polymer formulations to better protect microbial consortia and improve degradation of contaminants.

Objective

This study assessed the viability of model microorganisms (*Lactobacillus casei* and *Lactobacillus acidophilus*) in various compositions and molecular weights of polyvinyl alcohol (PVA) and sodium alginate (SA) hydrogels. A second goal of the project was determining the impact of polymer modifications on diffusion rates.

Introduction

- Chlorinated aliphatic hydrocarbons, including TCE, are some of the most common carcinogenic groundwater contaminants.
- TCE was widely used in many industrial processes and products over the last century (Industrial degreaser, extraction solvent, paints, pesticides, etc.).
- 60% of National Priority List sites have observed TCE (Doherty, 2000).
- Current bioremediation techniques involve pumping bacteria directly into groundwater.

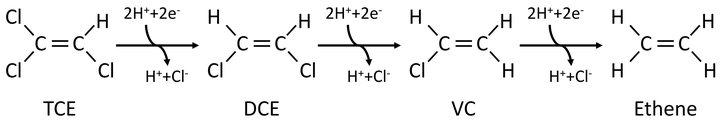

Figure 1. The dechlorination of TCE results in ethene and hydrochloric acid. This acidifies the environment, killing bacteria or inhibiting further degradation.

- To increase bacteria survival, they are put into a hydrogel bead (mimics a biofilm floc).
- Hydrogel biobeads are biocompatible in terms of toxicity and employ 'gentle' polymerization processes to optimize viability.

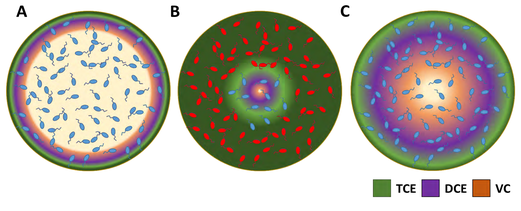

Figure 2. Protecting microorganisms from acids in hydrogels is a challenge. A) Hydrogel that restricts diffusion of nutrients and acids, starving/killing microorganisms within, B) hydrogel with too much diffusion can also be lethal to microbes, C) hydrogel with the proper balance of nutrient flux and wastes with protection from protons.

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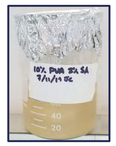
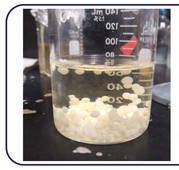
Biobead Production

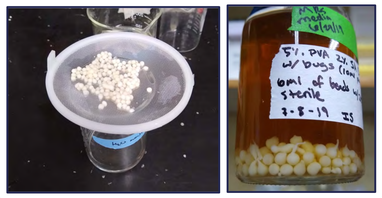
1. Combine PVA/SA polymer with inoculated MRS media.



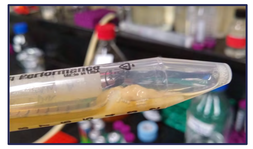


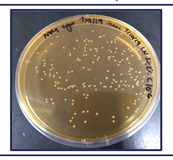
2. Drop 25 µl drops of polymer/media solution into saturated boric acid calcium chloride solution to cross-link (for 10 minutes).

3. Drain and rinse beads – let sit for 2 days in MRS media.



4. Release cells from beads with a solution of EDTA and trypsin in a 37°C water bath for 4 minutes then vortex.





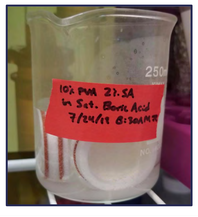
5. Centrifuge to form cell pellet and rinse with PBS solution; measure absorbance at 590 nm and plate 100 µl of appropriate dilutions. Colony forming units (CFUs) are counted to determine the number of surviving cells .

6. Cell viability = $\frac{\text{CFUs}}{\text{Optical density cell count}}$

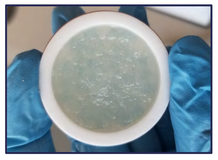
Diffusion Measurements

1. Heat PVA/SA polymer until pourable; pour into Teflon mold (pictured) and freeze.

2. Once frozen, cross-link in saturated boric acid and calcium chloride solution in fridge for 4 hours.



3. Equilibrate in water 1 hour. A completed puck (pictured).



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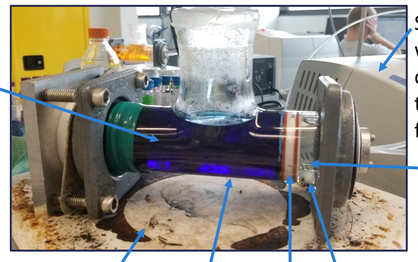
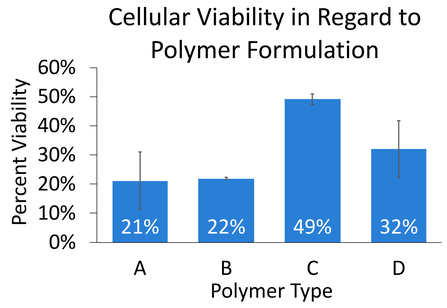


Spectrophotometer with flow through cuvette: a reading is taken every 5 min for 48 hours.

Sink: DI water

Biobead Results



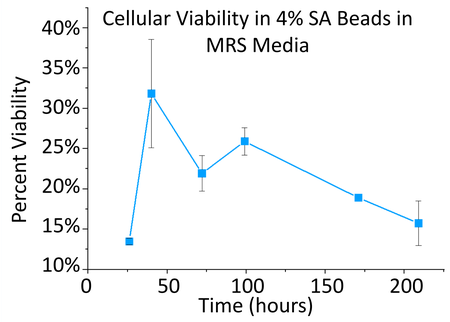


Figure 3.
A 10% PVA (MW 50-85 k) 2% SA
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Results and Discussion:

- Polymer C produces the highest cell viability.
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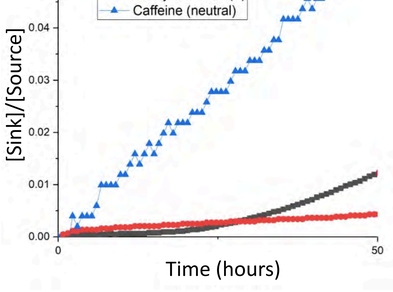


Figure 5. Diffusion of metanil yellow, methylene blue, and caffeine through a 10% PVA 2% SA puck.

Future Work

There is ongoing research into buffering the internal environment of the hydrogels with polyoxometalates and PVA modifications (acetylation, phosphorylation, etc.). Other modifications include forming beads using repeated freeze/thaw cycles (**Figure 6**) instead of a chemical crosslinker and testing the viability of different bead sizes. We are conducting further research to improve our viability measurements by the use of alternative bacterial strains and stains. Additionally, we are still testing viability in various PVA/SA formulations.

Figure 6. Experimental cross-linking via freeze-thaw cycles.

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 National Science Foundation award number 1805358.

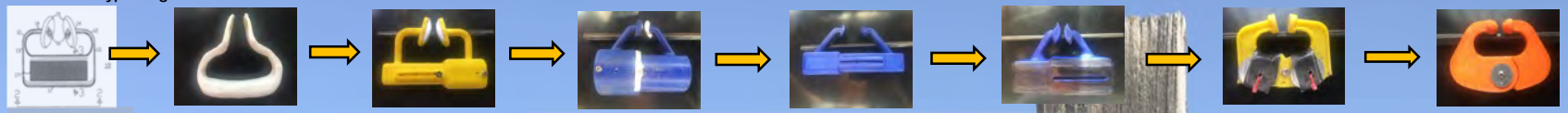
Wearable Technology for Cows: Applications for Virtual Fencing

Jenn Smith[1], Jason W. Karl[2], Karen Launchbaugh[2], and Gordon Murdoch[1]
1. College of Agriculture and Life and Sciences, University of Idaho, 2. College of Natural Resources, University of Idaho

University of Idaho College of Natural Resources

University of Idaho College of Agricultural and Life Sciences

Nose Prototype Progression



Inspired by patent from Andy Rose Wendel, 1984 Agricultural Engineering

Introduction:

Managing the distribution of grazing animals is necessary for animal husbandry, effective land stewardship, and protecting sensitive and riparian lands. Virtual fencing is the idea where the negative reinforcement is worn by the animal rather than by restricting movement by wire and posts.

Virtual Fencing for Livestock Management:

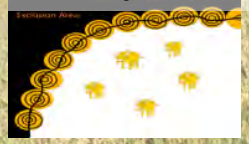
Virtual fencing pairs location-based communication technology with wireless fencing, such as that used in dog collars and invisible fencing, to control the distribution, location, and movement of livestock. Virtual fencing has the potential to significantly improve livestock management on open range and reduce the costs and impacts of physical fencing.

Virtual fencing can be deployed in an inclusion mode where animals are kept within a defined area for instance to graze residual crops or for rotational grazing within smaller pastures. Virtual fencing can also be used to exclude animals from riparian and other sensitive areas or achieve remote rotational grazing of pastures and ranges

Virtual fencing will require a device that stays on the animal and does not negatively affect health and productivity.

Virtual Fencing - Inclusion Mode

Virtual Fencing - Exclusion Mode



Objectives:

1) Relationship between age, weight, and nose size of individual animals;

2) Best anatomical fit and least irritation to the animal by testing three shapes and sizes of nose pads; and

3) Maximum weight to allow the device to remain in place on the animal for 1 month in a natural grazing setting.

This research facilitates designing technologies to study the application of virtual fencing and how it affects livestock which will ultimately contribute to a revolution in the way rangelands and riparian areas are managed and grazed.

Methods:

Five measurements of the bovine nose were taken on 16 heifers and 26 multiparous cows to determine correlation between nose size, age, and weight of the animal and best fit for a device. It was determined that weight was not a significant factor in nose size.

Shape, size, weight limit and duration of wear of the device was challenged on the University of Idaho herd of 20 Charolaise and Charolaise cross yearling heifers. Devices were printed on a 3-D printer using PLA filament.

Shape:
Horizontal
Vertical Oval-
Spherical/Round
Teardrop

Weights challenged:
110g (n=10)
140g (n=10)

Size of prototype device:
Depth: 3cm
Height: 3.5cm
Length: 10cm

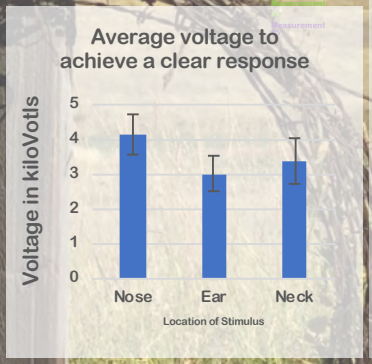
Length of time:
July 12- July 26

Results of the challenge:

Dimensions of animals noses were different by age group but were not significantly different within the same age group. The spherical/round contact shape device was superior with less irritation and moved more freely during grazing. The teardrop, horizontal and vertical ovals did not allow for free rotation of the device and proved to interfere with grazing on shorter forage. Device depth of 3 centimeters and weights of 110 and 140 grams did not adversely affect the animal's ability to graze or drink. Of the 10 devices of each weight that were initially deployed, six 110g and eight 140g devices remained in place for two weeks while the animals were turned out in a natural pasture grazing setting. There did not appear to be a significant difference in the staying ability between the weighted devices. The devices that did come off (and were found) showed weaknesses in design and material, and broke at the central pivot (see photos below).

1,2: Close up of the nose clip prototype
3: Heifer eating barley from a slick bunk
4: Resting in the pasture
5: Resistance testing on the nose
6: Resistance testing on the neck
7: Resistance testing on the ear

Measurement	Yearling Heifers (cm)	Multiparous Cows (cm)
1	7.69 (0.54)	10.5 (0.75)
2	7.25 (0.75)	9.13 (1.13)
3	3.06 (0.17)	4.06 (0.41)
4	4.18 (0.31)	5.46 (0.55)
5	2.38 (0.22)	4.00 (0.45)



Prior measurements indicated the nose was more sensitive to electrical stimulation than the neck. Our results, however, indicated the ear and neck required less electrical stimulation. However, this may be confounded by the cows' previous exposure to electrical stimulation. The nose is still considered the prime location for an electrical stimulation as nasal tissue is constantly moist and poses less risk than neck collars for injury to the cow or for getting caught on external objects (e.g., fence posts, trees).

Conclusion:

As virtual fence technology advances, our study has demonstrated that a device that is 3cm deep and 140g will have little effect on animal grazing and produce no noticeable discomfort to the animal. An efficient design is critical for successful implementation of virtual fencing. Our results have shown that training animals to remain within a designated area using audio or visual indicators with electrical stimuli is possible, we believe there is a way to make virtual fences a reality.

Furthermore, while other devices are currently under development for the neck, we feel our research shows devices attaching to the nose can be a successful alternative design approach.

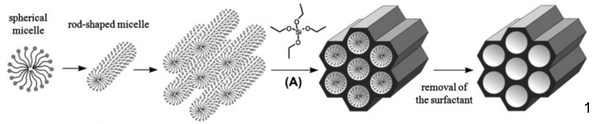
Acknowledgements:
A special thanks to Andrew Rose for his willingness to share his previous data and results, team members and cohorts Austin Grieve for prototype design and production assistance, Courtney Carter and Chanelle Brusseau for helping collect data and work the cattle. All research methods were approved by the Institutional Animal Care and Use Committee (IACUC 2018-74).

Impact of Surfactants on Mesoporous Silica Formation

Kael Stelck, Dr. Mark F. Roll
Department of Chemical and Materials Engineering
The University of Idaho

Introduction

- Ordered high surface materials applications
  - Adsorbents used in gold mining
  - "Nano"-reactors for polymers
- Two key physical phenomena
  - Surfactants form liquid crystals in water
  - Alkoxysilanes hydrolyze to give silica
- Goal: Create mesoporous silica structures with ordering from liquid crystals



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Experimental

Reference:	HCl	H_2O	Surfactant	TEOS	Time
Stuckey MCM-41	9.14 mol (pH= -0.59)	129 mol	Quaternary Ammonium Salts	1.0 mol	30 min
ChemEd SBA-15	4.24 mol (pH= -0.11)	178 mol	Alkylated Ethylene Glycol Oligomers	1.0 mol	48 hr

Steps:

1. Create acidic solution of surfactant
2. Add tetraethyl orthosilicate (TEOS)
3. Filter and dry solid product
4. Remove surfactant via calcination or dissolution.
5. Analyze using X-Ray diffraction

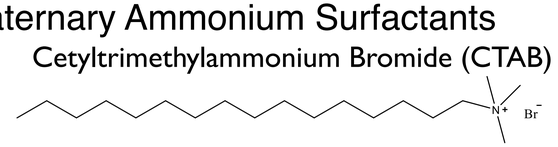
Conclusions

- Obtained MCM-41 material
  - Acid catalyzed synthesis
  - Surfactant still within structure
- Surfactant removal destroys MCM-41
  - Dissolution of surfactant using ethanol
  - Calcination at 550-650°C
- Hydrothermal treatment necessary
- Ambient conditions not ideal for CTAB
  - CTAB crystalize out at below 30°C
  - Average room temperature is below 30°C

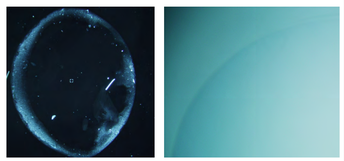
Surfactants

- Quaternary Ammonium Surfactants

Cetyltrimethylammonium Bromide (CTAB)



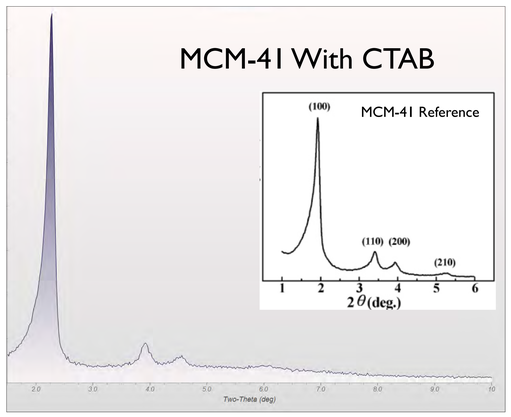
- CTAB liquid crystal under cross-polarized microscope



- Alkylated ethylene glycol oligomers also used as non-ionic surfactants

Results

- X-Ray Diffraction shows hexagonal order



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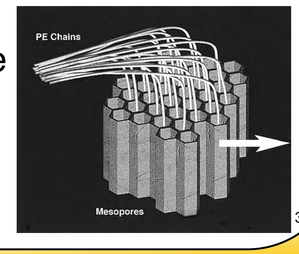
Future Work

- Low angle XRD for SBA-15 materials
- Thermogravimetric Analysis
  - To discern surfactant to silica ratio
  - Determine surfactant waste
- Hydrothermal treatment
- Ultimate Goal: Creation of "Nano"-extruder for the creation of crystalline polymers



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7. Acknowledgments

- University of Idaho College of Engineering and the Department of Chemical and Materials Engineering
- Dr. Tom Williams and the Electron Microscopy Lab for XRD and Cross-Polarizing Microscope
- Waynant Research Group and the Roll Research Group
- University of Idaho OUR program for Funding

References

1. Tiwari, A. (2013). *Nanomaterials in Drug Delivery, Imaging, and Tissue Engineering*. Somerset: John Wiley & Sons, Incorporated.
2. J L Shen. (2003). Bluegreen photoluminescence in MCM-41 mesoporous nanotubes. *Journal of Physics: Condensed Matter, 15*(20), L297-L304.
3. Kageyama, Tamazawa, & Aida. (1999). Extrusion polymerization: Catalyzed synthesis of crystalline linear polyethylene nanofibers within a mesoporous silica. *Science (New York, N.Y.), 285*(5436), 2113-2115.

Encapsulation of *Lactobacillus acidophilus* and *Lactobacillus casei* to Determine Cell Viability in a Hydrogel Biobead Matrix

Laura A Nutter[1], Isabell K Strawn[2], Addie E White[2], Jonathan R Counts[3], Connor Hill[3], Dr. Mark F Roll[3],Dr. Kristopher V Waynant[4], Dr. James G Moberly[3]
Departments of [1]Biological Sciences, [2]Biological Engineering, [3]Chemical Engineering & Material Sciences, and [4]Chemistry; University of Idaho

Motivation

Trichloroethylene (TCE), a commonly used industrial solvent, is a widespread, persistent, and carcinogenic groundwater pollutant. An effective treatment strategy for TCE contamination is bioremediation using reductively dechlorinating bacteria. However, during bioremediation changing pH levels can harm these degrading microbes. By incorporating the microbes into a polymer matrix, pH is buffered and the microbes are protected. Determination of encapsulated microbe viability assists in optimization of polymer formulations to better protect microbial consortia and improve degradation of contaminants.

Objective

This study assessed the viability of model microorganisms (*Lactobacillus casei* and *Lactobacillus acidophilus*) in various compositions and molecular weights of polyvinyl alcohol (PVA) and sodium alginate (SA) hydrogels. A second goal of the project was determining the impact of polymer modifications on diffusion rates.

Introduction

- Chlorinated aliphatic hydrocarbons, including TCE, are some of the most common carcinogenic groundwater contaminants.
- TCE was widely used in many industrial processes and products over the last century (Industrial degreaser, extraction solvent, paints, pesticides, etc.).
- 60% of National Priority List sites have observed TCE (Doherty, 2000).
- Current bioremediation techniques involve pumping bacteria directly into groundwater.

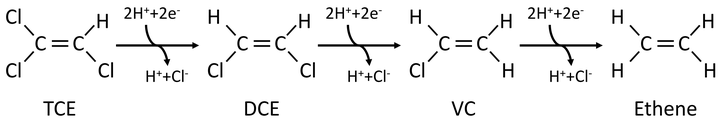

Figure 1. The dechlorination of TCE results in ethene and hydrochloric acid. This acidifies the environment, killing bacteria or inhibiting further degradation.

- To increase bacteria survival, they are put into a hydrogel bead (mimics a biofilm floc).
- Hydrogel biobeads are biocompatible in terms of toxicity and employ 'gentle' polymerization processes to optimize viability.

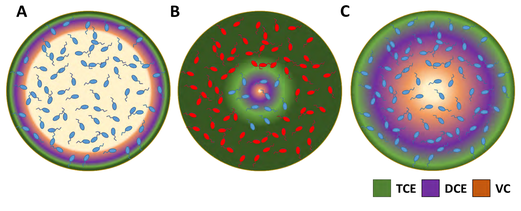

Figure 2. Protecting microorganisms from acids in hydrogels is a challenge. A) Hydrogel that restricts diffusion of nutrients and acids, starving/killing microorganisms within, B) hydrogel with too much diffusion can also be lethal to microbes, C) hydrogel with the proper balance of nutrient flux and wastes with protection from protons.

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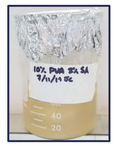
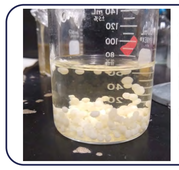
Biobead Production

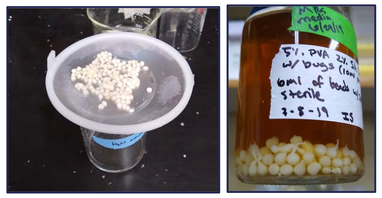
1. Combine PVA/SA polymer with inoculated MRS media.



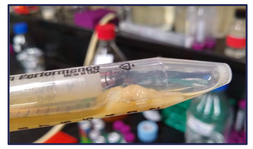


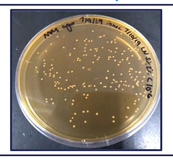
2. Drop 25 µl drops of polymer/media solution into saturated boric acid calcium chloride solution to cross-link (for 10 minutes).

3. Drain and rinse beads – let sit for 2 days in MRS media.



4. Release cells from beads with a solution of EDTA and trypsin in a 37°C water bath for 4 minutes then vortex.





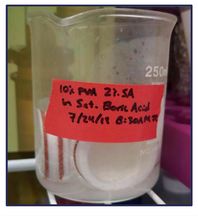
5. Centrifuge to form cell pellet and rinse with PBS solution; measure absorbance at 590 nm and plate 100 µl of appropriate dilutions. Colony forming units (CFUs) are counted to determine the number of surviving cells .

6. Cell viability = $\frac{\text{CFUs}}{\text{Optical density cell count}}$

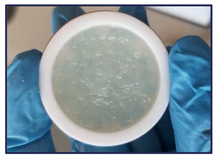
Diffusion Measurements

1. Heat PVA/SA polymer until pourable; pour into Teflon mold (pictured) and freeze.

2. Once frozen, cross-link in saturated boric acid and calcium chloride solution in fridge for 4 hours.



3. Equilibrate in water 1 hour. A completed puck (pictured).



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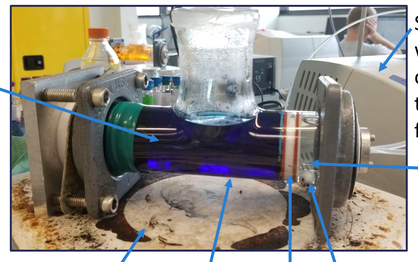

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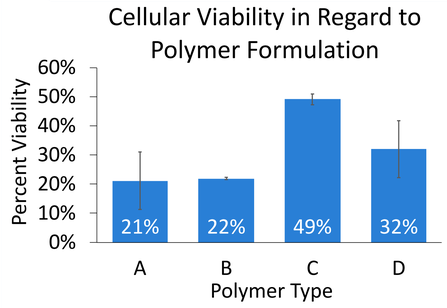

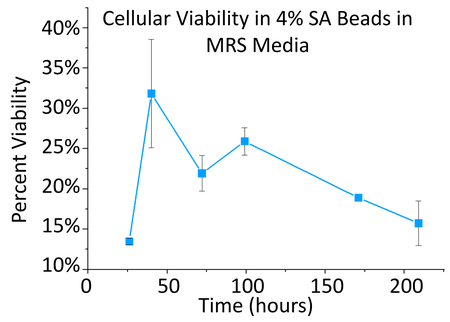

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A 10% PVA (MW 50-85 k) 2% SA
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- Growth in beads is evidenced by color change.
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L. Casei is most viable after two days in MRS media.

Diffusion Results

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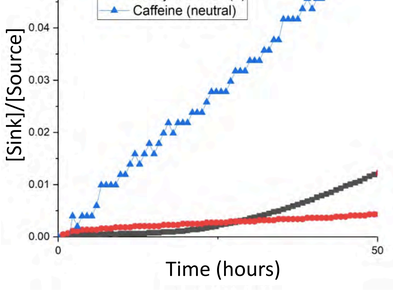

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Figure 6. Experimental cross-linking via freeze-thaw cycles.

Acknowledgements

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Engineering Nanocarriers for Effective Gene Delivery in T cells

Silpa Subedi, Xutu Wang and Dr. Ching-An Peng
Department of Biological Engineering, University of Idaho

University of Idaho

Overview:

The objective of this project is to design a GFP capsulated calcium alginate nanoparticle and test its ability as a gene carrier in T cells. The nanocarriers designed in this study are inexpensive and easy to manufacture, so may therefore, provide a broadly applicable non-viral gene delivery in T cells.

Background:

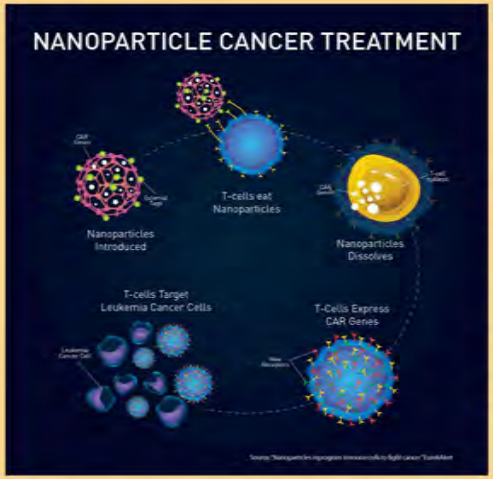

FIGURE 1. Nanoparticle mediated CAR-T cell therapy

The immunotherapy works by genetically engineering patient-derived T cells using a disarmed virus that could recognize cancer cells. The application of this therapy has produced impressive results, but the viral way to generate many tumor-specific T cells in-vitro is labor intensive and an expensive process. Recently developed approaches for using nanoparticles in cancer immunotherapy have enormous potential for improving cancer treatments. This new method can eliminate expensive and time-consuming steps that lagged in the previous T cell cancer therapies. The current protocol is that the T cells are removed from the patient, genetically altered, regrown, and infused back into the patient. The biodegradable nanoparticles will eliminate the removal, regrowth, and infusion steps by accomplishing the reprogramming step over a time span of 24 to 48 hours while the T cells are in the body.

Methodology:

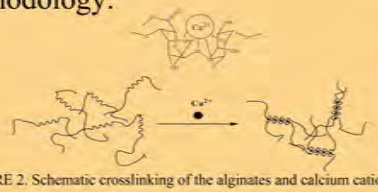

FIGURE 2. Schematic crosslinking of the alginates and calcium cations

Synthesizing alginate nanocarriers:

FIGURE 3.A. Tip sonication of sodium alginate in dichloromethane
B. White pellet obtained after ultracentrifugation

- Alginate nanoparticles were developed with water in oil emulsion method using a tip sonicator.
- The alginate nanoparticles were collected by ultracentrifugation and then characterized by measuring its size and charge by a zeta potentiometer.
- To get an accurate size of around 200 nm, the nanoparticles were synthesized using different volume and concentration of sodium alginate and surfactant.

GFP encapsulation:

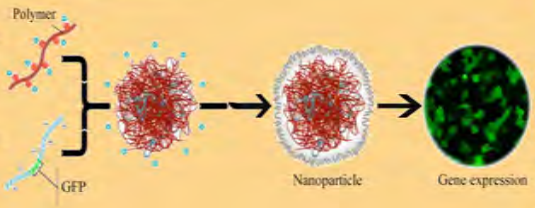

FIGURE 4. Non viral gene delivery of Green Fluorescent Protein(GFP) gene plasmid

- The alginate nanocarriers were suspended in DMEM and were added directly to the cells. Alginate nanoparticles were incubated with the cells for 12 hour at 37 °C.
- Transfection efficacy was evaluated by measuring the percentage of cells expressing the exogenously delivered GFP in fluorescence microscope.
- The transfection rate of the nanocarriers were investigated in A549 cells, Mesenchymal stem cells and Jurkat T cells.

Results:

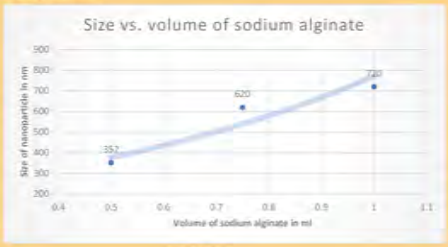

FIGURE 5.

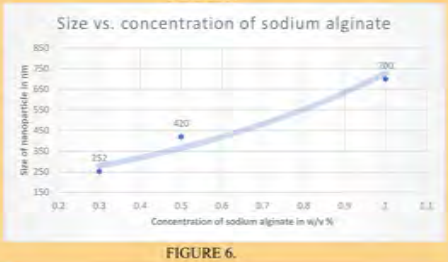

FIGURE 6.

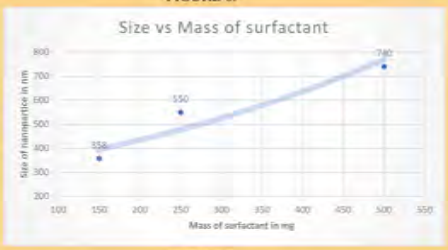

FIGURE 7.

Size and zeta potential analysis:

- Graphical representation for the size of Ca-alginate nanoparticles are shown in the figures 5,6 and 7. As expected, the graph indicates that the size of Ca-alginate nanoparticles decreased with the decrease in the volume and concentration of sodium alginate and surfactant used.

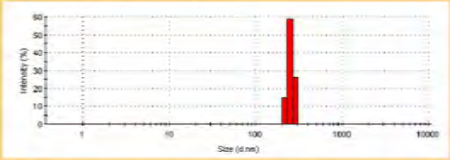

FIGURE 8. Dynamic Light Scattering results for the size of calcium alginate nanoparticles

- The average size of the nanoparticle used in this study was observed to be 200 nm and the zeta potential of calcium alginate was -38 mv.

FIGURE 9. Calcium Alginate Nanoparticles imaged by TEM

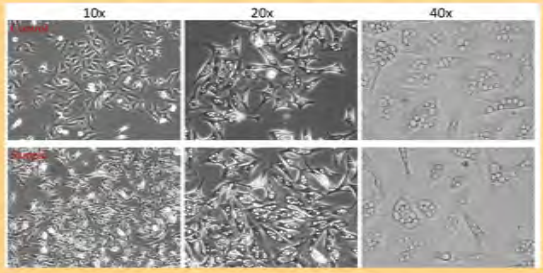

FIGURE 10. Nanocarriers in A549 cells bright field images

FIGURE 11. Mesenchymal stem cells transfected with green fluorescence protein bright field and fluorescent images.

Conclusion:

- The size of the nanoparticles were significantly modulated by the concentration and volume of sodium alginate solution and surfactant.
- Ca-alginate nanoparticles with an average size of 200 nm were able to encapsulate plasmid DNA and deliver gene in A549 cells.
- The gene delivery in A549 cells is more efficient than T cells because A549 cells grow as a monolayer, attaching to the culture flask.

References:

1. Peng, Ching-An. "Phagocytosis-mediated Retroviral Transduction: Co-internalization of Deactivated Retrovirus and Calcium-alginate Microspheres by Macrophages. " The Journal of Gene Medicine. December 23, 2004.
2. C-A, Peng. "Calcium-Alginate Nanoparticles for Nonviral Gene Delivery." TechConnect Briefs. May 08, 2005.
3. Smith, Tyrel T., Sirkka B. Stephan, Howell F. Moffett, Laura E. McKnight, and Matthias T. Stephan. "In Situ Programming of Leukaemia-specific T Cells Using Synthetic DNA Nanocarriers." Nature Nanotechnology. August 2017.

An engineering investigation of stalk lodging

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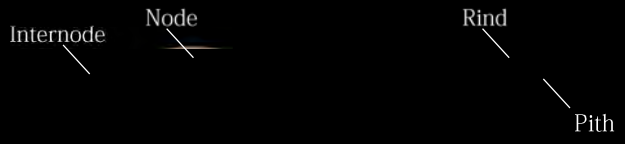
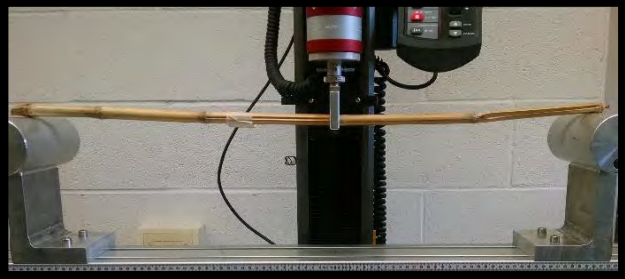
Hypothesis: Maize stalk morphology can be proven to be correlated with lodging resistance using puncture resistance and the thin beam assumption.

Thin Beam Assumption: $\sigma = \frac{-My}{I}$



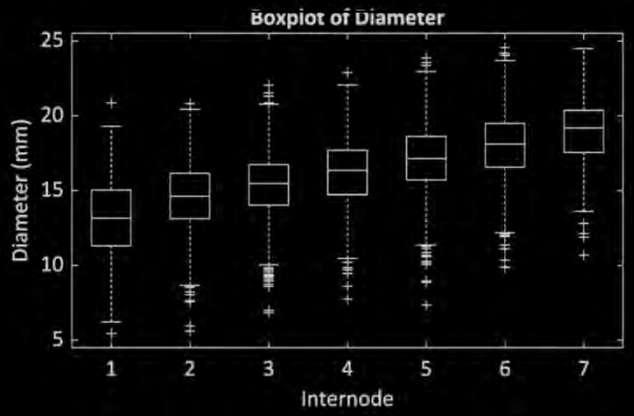
Stress Concentrators: Node-to-internode transitions, pith voids, and uneven stalk surfaces at the node are stress concentrators, which cause premature failure under a load.



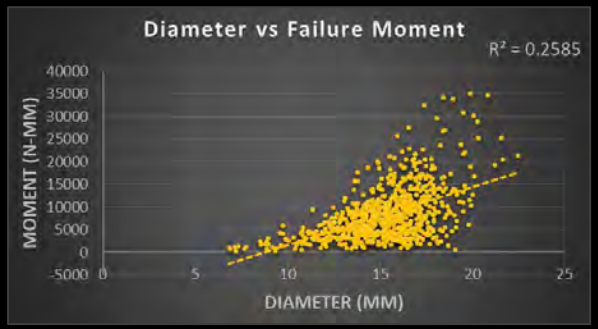
Tests: A universal testing machine was used to perform puncture and 3-point bending tests.

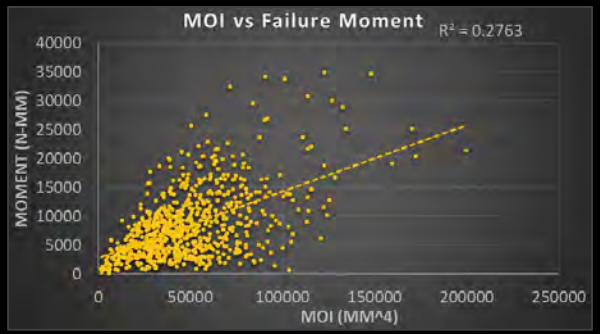
Corn is one of the most important crops in the world, but 5-20% of it lodges annually.

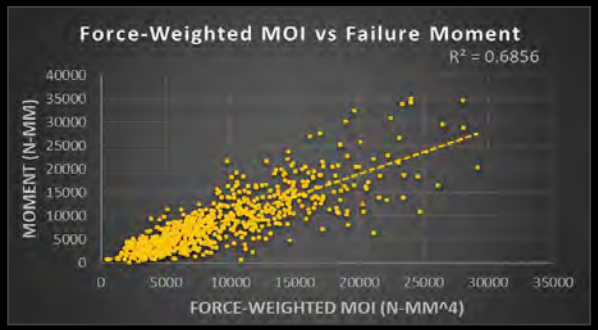
Morphology: Several population trends for maize were evaluated to understand more about the genetic morphology across different hybrids.



Results: The puncture resistance-weighted MOI has a much better correlation than both the MOI without puncture resistance-weighting and the diameter which MOI is derived.







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