

JOURNAL OF WINCHELL UNDERGRADUATE SYMPOSIUM ABSTRACTS

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Spring 2024

TABLE OF CONTENTS

Abstracts

<i>Biochemistry</i>	1
<i>Cellular and Molecular Biology</i>	10
<i>Chemistry</i>	19
<i>Ecology and Environmental Science</i>	21
<i>Epidemiology</i>	29
<i>Exercise Science</i>	30
<i>Neuroscience</i>	31
<i>Organismal and Physiological Sciences</i>	33
Symposium Sponsors	35

The abstracts included in this publication describe research presented at the Minnesota Academy of Science's Winchell Undergraduate Research Symposium, held on April 13, 2024, at Saint Mary's University of Minnesota, in Winona.

BIOCHEMISTRY

EFFECTS OF MUTATION P126E AND P126R IN WATERMELON GLYOXYSOMAL MALATE DEHYDROGENASE (WGMDH)

Lois J. Campbell, Mirnesa Delic, Finn J. Melchior, and Betsy Martinez-Vaz (Advisor)

Department of Biology and Biochemistry Program

Hamline University, St. Paul, MN

Malate Dehydrogenase, MDH, is a multimeric enzyme that is involved in the catalysis of malate to oxaloacetate, coupled with a cofactor reduction reaction of NAD⁺ to NADH. This reaction is reversible and is an integral part of many different cellular processes, such as the tricarboxylic acid (TCA) cycle, the malate-aspartate shuttle, gluconeogenesis and the glyoxylate bypass. Mutations in the mobile loop of MDH have been shown to affect the specific activity of the enzyme. Being able to target these mutations and control the activity of the enzyme could prove useful in treating parasitic infections that take over pathways like the TCA cycle. Additionally, MDH has been shown to be involved in treatment for ischemia by reducing oxidative stress in the brain, and regulating p53, a known tumor suppressor gene involved in the formation of cancer. In our research we have isolated two mutants at position P126 in the mobile loop. Based on previous research, proline could prove to be integral for the rigid turning in the conformational changes in the mobile loop, which are essential for catalysis. The mutations are from genetically altered MDH from 1SMK, a watermelon glyoxysomal MDH. We hypothesize that the specific activity of the mutation, P126E and P126R, would decrease compared to that of the wild type. We conducted analysis of specific enzyme activity and turnover rate using a UV-Vis spectrophotometer. Our hypothesis was supported as the specific enzyme of the WT was greater than either mutation.

IDENTIFYING CANDIDATE DRUGS TO TREAT RARE TYPES OF MUSCULAR DYSTROPHY

Brenna A. Powers¹, Nathaniel P. Mohar², Benjamin E. Hinz^{1,3}, Hailey McCoy-Munger³, and Lori L. Wallrath^{1,2} (Advisor)

¹*Department of Biochemistry and Molecular Biology/Carver College of Medicine*

²*Interdisciplinary Graduate Program in Genetics*

³*Department of Biomedical Engineering*

University of Iowa, Iowa City, IA

Mutations in the LMNA gene result in a group of diseases called laminopathies, the most common of which are the LMNA-associated muscular dystrophies (LMNA-MD). The disease mechanisms of LMNA-MD are poorly understood, and there are currently limited treatments available. Here, we investigate the disease mechanisms of a LMNA-MD causing mutation (c.746G>A p.R249Q) and identify potential treatments. The *Drosophila* orthologue of LMNA is Lamin C (LamC). Flies expressing LamC R264Q (analogous to human R249Q) in larval body wall muscles are 100% lethal at the pupal stage. We performed an unbiased drug screen using the Prestwick Chemical Library and assayed for adult viability. Each compound was mixed with fly food at a 5 μ M final concentration. Flies possessing the UAS-LamC R264Q transgene were crossed to flies possessing a larval body wall muscle-specific Gal4 driver, producing offspring that ingested the drug mixed with food throughout development. The parental generation was removed after four days, and adult viability was scored after 14 days. This screen identified 68 drugs that rescued adult viability, corresponding to a hit rate of 4.75%. Among these positive hits were multiple voltage-gated calcium channel blockers, drugs targeting serotonin signaling, and inhibitors of the cyclooxygenase (COX) enzymes. Taken together, these data provide insights on the molecular mechanisms of muscle pathology and identify potential novel therapeutic targets. Future research includes a secondary screen of the positive hit compounds involving a dose response, and a tertiary screen for broad efficacy across additional lamin mutants.

THE INFLUENCE OF MEDIA COMPOSITION ON PLASMID COPY NUMBER AND FOLD INDUCTION IN *Escherichia coli* DH5A

Catherine Sothy and Thomas C. Marsh (Advisor)

Department of Chemistry

University of St. Thomas, St. Paul, MN

This study focused on experimentally determining sustainable “food sources” (i.e. media formulations) to supply cell cultures used in the pharmaceutical industry. These bacterial cultures require nutrient rich media for growth and the industry standard is to use casein hydrolysate or beef extract as a protein source in media. However, bovine derived extracts are not the most efficient source of nutrients and increase the risk of bovine protein contamination in the final product. A more sustainable option would be to use plant derived peptone hydrolysates in media formulations. This project focused on comparing sustainable plant-based media formulations to animal derived medias for high yield plasmid production. Optimized growth conditions were determined by growing *E. coli* DH5a in LB media using four hydrolysates: tryptone, soy, pea, or a soy/pea blend. Cultures were tested for yield by isolating plasmid with a QIAGEN spin miniprep kit and comparing specific yield. Cultures were also analyzed by qPCR to compare plasmid copy number and fold induction. Initial testing suggests that tryptone and soy cultures performed the best in yield and plasmid copy number. However, qPCR has demonstrated that the soy and pea peptone hydrolysates may contain carbohydrates that cause uncontrolled induction of fosmids with induction systems to control copy number. These data are important for plasmid production in the pharmaceutical industry to understand when these peptones can be used effectively to decrease environmental impact of the industry.

VEGF AND NRP-1 NEURAL EXPRESSION IN CD-1 MICE EXPOSED IN UTERO TO ATRAZINE

Elle Petersen, Nina Langley, and Debra Martin (Advisor)

Department of Biology

St. Mary's University of Minnesota, Winona, MN

Atrazine is an herbicide used for removal of weeds that has been found in water supplies with quantities higher than the US EPA limit of 3 ppb. The known effects of atrazine include the disruption to circadian rhythm. Circadian rhythm is known to control the expression of vascular endothelial growth factor (VEGF) and Neuropilin-1 (NRP-1), which are responsible for angiogenesis during embryonic development. Since defective or excessive growth of vasculature during development has been shown to impact neural function, understanding the effects of atrazine on VEGF and NRP-1 protein expressions is crucial. Utilizing an ELISA assay, the effect of *in utero* atrazine exposure at 0 ppb, 3 ppb, and 30 ppb on circadian rhythm expression of the neural proteins VEGF and NRP-1 were investigated. Data indicated 3 ppb *in utero* atrazine exposure caused a significant increase in neural VEGF expression at 4 Zt ($p=0.004$), 8 Zt ($p=0.012$), and 20 Zt ($p=0.042$) with a shift in circadian expression occurring at 4 Zt compared to 0 Zt for 0 ppb and 30 ppb. A similar shift in circadian expression was observed for neural NRP-1 occurring at 4 Zt for 3 and 30 ppb atrazine treatments compared to 0 Zt for 0 ppb atrazine treatment. Significant increases in NRP-1 expression were detected at Zt 4 between 0 ppb and 3 ppb ($p=0.006$) and between 0 ppb and 30 ppb ($p=0.035$). At Zt 8, significant increases in NRP-1 expression were found between 30 ppb and 0 ppb ($p=0.007$) and 30 ppb and 3 ppb ($p=0.030$).

EFFECTS OF MUTATIONS D32R AND C171R ON THE CATALYTIC ACTIVITY OF GUANYLUREA HYDROLASE

Fran Zyla, Aubrey Chavarria, Katie Beaudette, and Betsy Martinez-Vaz (Advisor)

Department of Biology and Biochemistry Program

Hamline University, St. Paul, MN

Metformin is one of the most often prescribed medications in the world with over 80 million prescriptions per year in the U.S. alone, and its use is expected to increase as the global prevalence of diabetes rises. Guanylurea, metformin's transformation product, is said to be a "dead-end" metabolite that is accumulating in water. Recent studies found an enzyme, guanylurea hydrolase (GUH), in *Pseudomonas mendocina* strain that breaks down guanylurea into guanidine and ammonia. The purpose of this study was to determine the effects of mutations in amino acids D32 and C171 on the catalytic activity of guanylurea hydrolase. These amino acids are part of a catalytic triad necessary for enzyme activity; C171 is proposed to be a nucleophile while D32 acts as a general base to enhance its nucleophilic properties. We hypothesize that changing aspartate (D32) or cysteine (C171) to arginine will disrupt the catalytic triad and lead to lower enzyme activity. Site-directed mutagenesis was performed to construct enzyme variants and compare their catalytic activity to that of the wild type guanylurea hydrolase. Enzymes purification was done using His-tag- Nickel affinity chromatography. Enzyme activity was tested by measuring the amount of ammonia released using the Berthelot reaction. Activity assays showed the specific activity of the mutants was more than 90% lower compared to the wild type GUH. These results suggest that amino acids D32 and C171 are essential for catalysis. These experiments are key to understanding the breakdown of metformin and its transformation product, guanylurea.

IDENTIFICATION AND CHARACTERIZATION OF NOVEL SECONDARY METABOLITES FROM WASTEWATER BACTERIA USING COMPARATIVE GENOMICS

Alena C. Wadzinske, Justin J. Donato (Advisor), and Cassandra McCorison (Advisor)

Department of Biology

University of St. Thomas, St. Paul, MN

Applying comparative genomics, this research sought to identify novel secondary metabolites from wastewater bacteria. Within the wastewater treatment plant environment, bacteria grow in surroundings with carbon sources, electron acceptors, and relatively constant pH and temperature. Having a reliable food source and consistent environment encourages the creation of bacterial secondary metabolites. Bacterial secondary metabolites increase bacteria's fitness in their environment but are not required for survival. Previous secondary metabolite investigation contributed to antibiotic development and improved chemotherapy treatments. In this research, bacterial samples were extracted from three municipal wastewater treatment plants in the Upper Midwest. Using sequenced bacterial metagenomes, antiSMASH was applied to identify genetic regions—termed biosynthetic gene clusters (BGCs)—that encode enzymes responsible for secondary metabolite production. Research focused on Nonribosomal Peptide Synthetases (NRPS), one type of secondary metabolite. Of the genomic contigs analyzed in this research, approximately 19% identified BGCs were classified as containing NRPS regions. Within these NRPS-containing regions, 107 samples were deemed interesting to continue investigating. Factors considered for rating interest include NRPS region size, quantity of genes, quantity of identified and possible domains, and Norine similarity score. One secondary metabolite of interest and possible novelty was discovered, and cultivation attempts for this bacterium are ongoing.

PROTEIN ENGINEERING OF A FLOURESCENT WATERMELON MALATE DEHYDROGENASE

William Yu and Lisa Gentile (Advisor)

Department of Chemistry

College of St. Benedict & St. John's University, St. Joseph and Collegeville, MN

Malate dehydrogenase (MDH) is an enzyme that reversibly forms oxaloacetate and NADH from (S)-malate and NAD⁺. Tryptophan fluorescence would be ideal to monitor conformational changes, and watermelon glycosomal MDH does not contain any tryptophan. The research question investigates the possibility to mutate MDH to contain tryptophan while still retaining native-like specific activity. This hasn't been addressed as a literature search has shown nothing has been done to mutate Trp residues into wgMDH (watermelon). The mutant designed would be F353W. The selection factors would be shape similarity to Trp and being distant from the active site, both important factors towards maintenance of native-like activity. The forward and reverse primer was first designed for 353W. The criteria for successful mutagenesis would be to have C or G at the end of both primers, have 25-45 bases, and a melting temperature of over 78 degrees C using the QuickChange Site-Directed Mutagenesis Kit from Agilent Technologies. The reaction mixture was treated for DPN-1 and transformed into competent cells. Mutant colonies were picked, and mutant protein was synthesized overnight in LB AMP. Five replicas of the mutant were produced and plasmid was extracted. The five replicas were sequenced and used for further research, such as subsequent protein production and purification of the mutant protein. The impact of this research would help with further MDH research including substrate, enzyme, ligand and other research related to binding. Adding Trp helps track MDH and conformational changes as it controls diverse cellular processes, like signal transduction.

BIOCHEMICAL CHARACTERIZATION OF LINC00896, A NON-PROTEIN CODING RNA, WHICH, WHEN EXPRESSED, CONTRIBUTES TO THE DEVELOPMENT OF AUTISM
Micalyn Trihus, Trevor Rohder, Maycee Britz, Jacob Hanson, Andrew Rizea, Dylan Cleveland, Isabella Vannavong, Ashley Jensen, Emma Olson, Swedes Klogber, Ashley Stusse, Robert Mangiamele, Ridge Flatness, Donovan Floyd, and Rachel A. Hellmann Whitaker (Advisor)
Department of Chemistry
Bemidji State University, Bemidji, MN

Autism is a heterogeneous neurodevelopmental disorder that triggers early developmental difficulties that can be attributed to atypical cognitive issues such as impaired social precipitation, executive dysfunction, and perceptual and informational processing. Genetics is thought to impact environmental factors and embryonic development. Genetic analyses of patients with Autism indicate that the LINC00896 gene found in a 2016 Princeton University study may be linked to the development of Autism. It is thought to be one of the hundreds of genes associated with Autism. It is also known to play a role in other diseases, such as lung and colon cancer. The purpose of this research was to biochemically characterize the pathological role of LINC00896 in a developing brain and its potential link to Autism spectrum disorder. LINC00896 is a long non-protein coding RNA of which little is known. Structural analysis of LINC00896 RNA was done through a Thermostability Assay, which indicated when LINC00896 RNA was bound to magnesium, it formed a more stable tertiary structure when compared to apo-LINC00896 RNA. Further structural analysis was done using the Gel Mobility Shift Assay and Circular Dichroism Spectroscopy, indicating greater secondary structure elements such as double helices and stem-loop structures. These empirical structural analyses correlated with the computational secondary and tertiary structures rendered in silo. Currently, the biological function of LINC00896 is being determined through affinity column chromatography, mass spectrometry, and proteomics. Through this research, we hope to contribute to the biochemical understanding of the development of Autism Spectrum Disorder.

CATALOGING NOVEL QUORUM SENSING GENES FROM WASTEWATER SAMPLES

Alexander M. Drake, Justin J. Donato (Advisor), and Cassandra McCorison (Advisor)

Department of Biology

University of St. Thomas, St. Paul, MN

Antibiotic resistance genes rapidly emerge within bacterial communities following the introduction of antibiotics. New methods of microscopic warfare must be developed to have effective treatments, and one such method is targeting Quorum Sensing. Quorum Sensing is a phenomenon where bacteria release chemical signals known as autoinducers, which the bacteria use to gauge their concentration and coordinate collective actions. To study this phenomenon, samples were taken at various points in the wastewater treatment process in wastewater treatment facilities. Wastewater was chosen because the bacteria live in diverse communities in which signaling may impact functions. The DNA was extracted and sequenced using the Illumina HiSeq platform. Databases of DNA segments were made containing over 200,000 unique DNA sequences. Known I genes were matched against the sample databases to identify potentially novel I genes, and 298 potential I genes were found. The known I genes were luxI, r15, rhlI, bjaI, and lasI. The resulting sequences were matched against the NCBI nr database using BLASTp to determine novelty. Across all wastewater plants and timepoints, there were 35 potentially novel I genes. If a sequence had a potentially novel I gene, its conjugate R gene was also examined using BLASTp. One I gene was identified that was potentially novel and present in multiple stages of multiple wastewater treatment facilities. The DNA sequence of this I gene was ordered in a gene fragment. Subsequent research in the field of Quorum Sensing will enhance the identification of new Quorum Sensing genes.

COMPARATIVE ANALYSIS OF GLUTAMINASE 1 ISOTYPE EXPRESSION IN FIBROLAMELLAR HEPATOCELLULAR CARCINOMA TUMORS VERSUS NORMAL LIVER TISSUE SAMPLES

Joseph C. Tsukahara, Maya C. Lavendusky, Stephanie Yang, and Mong-Lin Yang (Advisor)

Department of Science

Concordia University - St. Paul, St. Paul, MN

Fibrolamellar Hepatocellular Carcinoma (FL-HCC) is a liver cancer that occurs in about 5% of all liver cancer cases. Like in most cancer types, FL-HCC hijacks different biosynthetic pathways to increase cell proliferation. In our project, we aimed to characterize the dysregulations of such pathways in FL-HCC samples in a way that can be compared to Hepatocellular Carcinoma (HCC) with a goal of cross-treatment. Using freshly frozen tumor samples given to us by our collaborator, the Mayo Clinic, western blot analysis, and a formulated membrane stripping technique was utilized to observe protein dysregulations. Based on data presented by the Mayo Clinic, one proteomic seat of interest is the proline pathway, as it is observed to be dysregulated by Fibrolamellar Hepatocellular Carcinoma and Hepatocellular Carcinoma. In previous groups, GLS1 and its isoforms have been identified as a protein of interest due to multiple unidentifiable bands. In our project, we used the above techniques to identify the unknown proteins. GLS1 antibodies were found to detect three separate proteins per sample. We identified the top band as the GLS1 isoform KGA. The identity of the other two bands as GAC is still unconfirmed. Analysis of tumor samples shows upregulation in KGA, and suggests upregulation in GAC. These results help to identify and promote future research on possible therapeutic targets for treating FL-HCC.

CELLULAR AND MOLECULAR BIOLOGY

A PUTATIVE YESMN TWO-COMPONENT SYSTEM ORTHOLOG IN *Streptococcus gordonii* IMPACTS BIOFILM FORMATION IN A ZINC-DEPENDENT MANNER

Georgi A. Aus¹, Bruno P. Lima², and Brittany L. Nairn¹ (Advisor)

¹Department of Biological Sciences, Bethel University, St. Paul, MN

²Department of Biological and Diagnostic Sciences, University of Minnesota, Minneapolis, MN

Streptococcus gordonii is an oral bacterium that forms plaque biofilms on the surface of the teeth. Two-component systems within bacteria allow for sensing of the external environment, the ability to respond to any external changes, and regulation of behaviors such as the formation of biofilms. We are interested in studying which two-component systems are involved in the regulation of biofilm formation by *S. gordonii* since it is currently poorly understood. A gene deletion mutant of an uncharacterized sensor kinase and response regulator SGO_1760/1761 was generated and evaluated for its role in biofilm formation. Interestingly, Δ SGO_1760/1761 had increased biofilm biomass compared to wild-type *S. gordonii* as measured by crystal violet staining. Evaluation of OD600 and ATP quantification suggested that this increase in biofilm formation was not due to an increase in bacterial growth. Sequence alignments showed that SGO_1760/1761 has significant homology to the two-component system YesMN in *Streptococcus pneumoniae*, which plays a role in zinc homeostasis and glycan metabolism. Therefore, wild-type *S. gordonii* and Δ SGO_1760/1761 biofilms were grown under a range of Zn-limiting conditions using the chelator TPEN. Zinc limitation restored Δ SGO_1760/61 biofilm biomass to wild-type levels. Together these data suggest that SGO_1760/61 plays a role in biofilm formation in a zinc-dependent manner, encodes a YesMN ortholog in *S. gordonii*, and provides insight into *S. gordonii* regulation of biofilm formation.

ANALYSIS OF sGLT-1 EXPRESSION IN DUODENUM TISSUES IN MICE EXPOSED CHRONICALLY TO ATRAZINE

Samuel J. Frick, Michelle Doering, and Debra Martin (Advisor)

Department of Biology

St. Mary's University of Minnesota, Winona, MN

Atrazine is a herbicide extensively used in agriculture that has been linked to various systemic dysfunctions, including alterations to the circadian rhythm, a mechanism essential for maintaining physiological homeostasis. This disruption is of particular concern as it may influence metabolic pathways, leading to an accumulation of fatty acids and the development of pre-diabetic conditions through compromised electron transport chain functionality. The regulation of glucose, critical in metabolic homeostasis, is mediated in the small intestine by the sodium-glucose cotransporter-1 (SGLT1). Given the importance of SGLT1 in glucose regulation, this study investigated the effect of atrazine exposure on the circadian expression of the SGLT1 gene expression in duodenum tissue. This study showed that SGLT-1 is regulated by circadian rhythm at both the protein and mRNA level. Furthermore, the circadian rhythm was influenced by the atrazine concentration.

REPEAT METHYLISOTHIAZOLINONE EXPOSURE ALTERS THE INFLAMMATORY POTENTIAL OF MURINE SKIN FIBROBLASTS

Oliver G. Lagasse and Elena Tonc (Advisor)

Department of Biology

Macalester College, St. Paul, MN

Vulvodynia is a chronic vulvar pain condition estimated to affect roughly 10% of woman-identifying individuals in the United States. Vulvodynia has been associated with a history of allergies and exposure to common household cleaning and personal care products. Methylisothiazolinone (MI), a preservative often found in these products, elicits inflammatory responses in allergic individuals. In our murine model of vulvodynia, dermal application of MI results in enduring anogenital hypersensitivity and other clinical findings. This suggests MI has the potential to lead to chronic pain. Vulvar fibroblasts from patients diagnosed with vulvodynia express higher levels of inflammatory cytokines upon activation in in vitro cultures. With this in mind, we investigate how the in vitro inflammatory potential of fibroblasts is altered in response to repeated in-vivo exposure to MI. We found heightened IL-6 and TNF- α but not IL-1 β production in response to in vitro activation of fibroblasts. This suggests that in vivo MI treatment increases the inflammatory potential of fibroblasts and could initiate a cascade of events that lead to chronic pain development, including inflammation in the tissue. We are currently further profiling the changes in the biology of fibroblasts from our animal model to help elucidate the hitherto obscure etiology of vulvodynia.

DYNAMIC RECIPROCITY BETWEEN FIBROBLASTS AND EXTRACELLULAR MATRIX IN CARDIOMYOPATHY

Eric M Puumala¹, Callie Knapp¹, Jackie Towner¹ (Advisor), Rachelle Crosbie² (Advisor), and Kirk Hansen³ (Advisor)

¹*Department of Biological Sciences, Bethel University, St. Paul, MN*

²*Department of Integrated Biology and Physiology, University of California Los Angeles, Los Angeles, CA*

³*Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO.*

Duchenne muscular dystrophy (DMD) is a degenerative muscular disease, resulting in chronic fibrosis that diminishes the effectiveness of contraction and repair in the heart and leads to heart failure and death. Fibrosis is an increased deposition of extracellular matrix (ECM) proteins that surround the cells in the heart. The ECM or matrix provides a microenvironment with dynamic reciprocity between the matrix and the cells interacting with it to influence cell behavior and proliferation. To study this interaction, we isolated the matrix from healthy three-month-old wild type mouse hearts and diseased hearts with DMD (mdx mouse model) and used the matrices as a scaffold for growing cells in vitro. One aspect of the dynamic reciprocity that occurs between the matrix and cells is the ability of cells to remodel the matrix by degrading or depositing matrix proteins. To measure this, we quantified the amount of laminin protein, a key matrix protein, by calculating the laminin remodeling index (LRI) using laminin immunofluorescence. We found a significant difference in the LRI of mdx matrices compared to wild type matrices, suggesting that a greater amount of cardiac remodeling occurs on mdx matrices than on wild type matrices.

EFFECT OF ARID3B ON INTRACELLULAR IRON AND ANTIOXIDANTS ASSOCIATED WITH FERROPTOSIS

Brendon N. Raabolle¹, Karen Cowden Dahl² (Advisor), and Matthew Rowley¹ (Advisor)

¹*Department of Biology, Saint Mary's University of Minnesota, Winona, MN*

²*Kabara Cancer Research Institute, Gundersen Health, La Crosse, WI*

Cancer is the second leading cause of death for Americans, and a poor prognosis is often associated with cancer due to high recurrence rates. High recurrence rates are common in cancers such as ovarian cancer and glioblastoma. Recurrence of the cancer is due to cancer stem cells (CSC) that are difficult to treat and can reform the tumor. More research is being conducted on targeting these CSCs and reducing the chance that the cancer recurs. Treating the CSC population is thought to lead to a better response. ARID3B is a transcription factor that can be overexpressed in ovarian cancer and glioblastoma. ARID3B affects the expression of PROM1, which is a common CSC marker and can lead to an increase in intracellular iron. Along with this, ARID3B regulates the expression of enzymes in the pentose phosphate pathway (PPP), which is involved in the production of NADPH. This study looked at the effects of ARID3B on intracellular iron and NADPH levels and applied it to ferroptosis in glioblastoma and ovarian cancer cell lines. We hypothesized that due to ARID3B's regulation of PROM1 and PPP, cells overexpressing ARID3B would have an increase in intracellular iron and NADPH levels. This study showed that in both glioblastoma and ovarian cancer overexpressing ARID3B, there are significant increases in intracellular iron levels but no significant differences in NADPH levels. This suggests that cells overexpressing ARID3B may be more susceptible to ferroptosis.

GENE EXPRESSION PROFILING OF EPIGENETIC REGULATED GENES IN FEMALE MONOZYGOTIC TWINS WITH DISCORDANT DIAGNOSIS OF BREAST CANCER

Haley M. Wangen and Claudia C. Preston (Advisor)

Department of Biology

St. Mary's University of Minnesota, Winona, MN

The incidence and death rates of female breast cancer have been increasing worldwide in the past 30 years. Epigenetic-related alterations have been known to play a key role in solid tumor pathogenesis. To investigate the effects of epigenetic differences associated with breast cancer, we used a discordant monozygotic twin approach to unveil potential molecular markers associated with regulation of transcription in breast cancer. A dataset (GSE37965) from the NCBI Gene Expression Omnibus website was used for analysis containing DNA-methylation sequencing of identical female twins with breast cancer discordant diagnosis (39-sets). Significance threshold was set at $p < 0.05$ and fold change (FC) > 1.1 or < -1.1 in breast cancer compared to controls. Functional enrichment analysis and gene prioritization was performed using Reactome Pathway Database Analysis tool and STRING interaction network database. Our analysis unveiled 28 genes that were significantly downregulated and 17 that were significantly upregulated (adj. $p < 0.05$). Further prioritization analysis identified four significant genes: DLG2, THBS4, NLGN4Y and COL4A2 (FC = -1.123, -1.105, 1.107, and 1.117 respectively). Functional enrichment analysis revealed direct impacts on pathways associated with neurexins and neuroligins, protein-protein interactions at synapses, and signaling by platelet-derived growth factor (PDGF). These preliminary results suggest that epigenetic variations of specific genes that have transcription regulation and tumor suppression functions might play a key role in the onset of breast cancer. The prioritized genes found to be significant are involved in key functions related to modulation of tumor growth and can serve as future pathways for breast cancer research.

INHIBITION OF COLIPHAGE T4rIIAB REPLICATION USING *Moringa oleifera* SEED EXTRACT

Megan Drayna and Jeanne Minnerath (Advisor)

Department of Biology

St. Mary's University of Minnesota, Winona, MN

Plants exhibit certain properties demonstrating their potential suitability as antimicrobial agents. Extracts from some plants, including seeds of *Moringa oleifera* (Drumstick Tree), contain compounds that are effective at inhibiting bacterial and viral growth. These results suggest that extracts from seeds of *M. oleifera* could be used to inhibit the growth of bacteria and viruses that cause human disease. The purpose of this study was to determine if *M. oleifera* seed extract was effective at inhibiting the replication of the bacteriophage, Coliphage T4rIIAB. Phage were exposed to *M. oleifera* seed extract or deionized water for 24 hours, mixed with the host cell, *Escherichia coli* B, spread on agar plates, and the plates were incubated at 37°C for 24 hours to allow for bacterial growth and phage plaque formation (plaque forming units). The number of plaque forming units were counted. Results indicated that the number of plaque forming units formed by phage incubated with *M. oleifera* seed extract was lower than the number of plaque forming units formed by phage incubated with deionized water. Overall, this difference in plaque forming units was statistically significant, indicating that *M. oleifera* seed extract was effective at inhibiting viral growth and could potentially be used as an agent to inhibit the growth of viruses that are of human concern.

METHYLISOTHIAZOLINONE INDUCES APOPTOTIC CELL DEATH IN PRIMARY SKIN FIBROBLASTS

Eunice Lim, Oliver Lagasse, Devavani Chatterjea (Advisor), and Elena Tonc (Advisor)

Department of Biology

Macalester College, St. Paul, MN

Methylisothiazolinone (MI) is a common chemical preservative used in many household and personal care products. Past studies have linked contact allergies and MI exposure to a chronic pain condition, vulvodynia. Studies have also shown that repeat dermal application of MI to murine labia leads to long-term tactile sensitivity and low-grade inflammation, mirroring clinical findings from vulvodynia. Clinical findings implicate fibroblasts in the pathophysiology of vulvodynia, so we investigated the effects of MI on fibroblasts in vitro. To determine the effects of MI on fibroblasts, we cultured primary fibroblasts derived from naive mice and treated them with varying concentrations of MI. Cell viability was assessed by Annexin V and propidium iodide (PI) staining using flow cytometry and the LDH assay measuring lactate dehydrogenase release. Metabolic activity of these cells was measured using a colorimetric MTT assay. Mitochondrial mass and membrane potential of MI-treated cells were assessed by MitoTracker Green and MitoTracker Red staining using flow cytometry. Our results indicate a decrease in cell viability after treatment with higher concentrations of MI, supporting our hypothesis that MI has a cytotoxic effect on fibroblasts. Currently, we are investigating the inflammatory responses of fibroblasts to MI. These results will help elucidate whether early fibroblast responses to MI are involved in initiating cellular events that contribute to the development of vulvodynia.

METHYLISOTHIAZOLINONE INDUCES CELL DEATH IN PRIMARY AND IMMORTALIZED MAST CELLS

Jo-Hsuan Chen, Ahlaam A. Abdulwali, Eunice Y. Lim, and Elena Tonc (Advisor)

Department of Biology

Macalester College, St. Paul, MN

Methylisothiazolinone (MI) is a common preservative in household and personal care products such as body washes and hand soaps. Repeated exposure to MI-containing products has been associated with an increased risk of developing the chronic pain condition vulvodynia. Characterized by chronic, debilitating pain in the vulvar region, this condition affects up to 10% of people with vulvas, but its etiology is still unknown. Vulvodynia has been epidemiologically associated with seasonal and contact allergies, and vulvodynia patient samples have also shown an accumulation of mast cells, which are skin-resident immune cells. Given the significant role of mast cells in allergies and their potential involvement in vulvodynia, we investigated the effects of MI on mast cells in an attempt to understand the early inflammatory responses leading to the development of chronic pain in vulvodynia. We treated primary and immortalized mast cell lines with increasing concentrations of MI for 24 hours and quantified cell viability using annexin V and propidium iodide staining. Using the same experimental protocol, we also conducted MTT and LDH assays, colorimetric assays that evaluate cell viability by assessing cell metabolism and plasma membrane damage respectively. Through these procedures, we found that MI treatment decreased mast cell viability in a dose-dependent manner. We also found that MI treatment reduced mast cell mitochondrial function, which was evaluated by concurrent MitoTracker Green and MitoTracker Red staining by flow cytometry. Collectively, these data show that MI is highly cytotoxic to both primary and immortalized mast cells, suggesting a need for its regulation.

OVEREXPRESSION OF TBX2 INDUCES INVASION OF MCF10A MAMMARY EPITHELIAL CELLS INDEPENDENTLY OF EPIREGULIN

Katherine M. Perkins and Matthew A. Rowley (Advisor)

Department of Biology

St. Mary's University of Minnesota, Winona, MN

Breast cancer is the second most diagnosed cancer in women. The majority of breast cancer originates in the breast ducts or lobules. Metastasis of breast cancer occurs when the primary tumor site loses its adhesion to the basement membrane and the cancerous cells can invade through the basement membrane entering the blood stream or lymphatic system. The overexpression of the transcription factor TBX2 has been found to downregulate E-cadherin which is important in maintaining adhesion between cells. An overexpression of TBX2 has also been found to upregulate two mesenchymal markers, N-cadherin and vimentin, which are involved in transitioning epithelial cells to mesenchymal cells. The transition to mesenchymal cells can lead to the release of proteases which can break down the basement membrane and induce invasion of cancerous cells. Epiregulin is a protein from the Epidermal Growth Factor (EGF) family. Studies have found that epiregulin is present in early breast cancer development. One student at Saint Mary's found that the expression TBX2 can induce expression of epiregulin. The purpose of this experiment was to examine if the expression of TBX2 in the MCF10A cell line impacts the invasion of cells using a Boyden chamber. The second goal was to examine if blocking epiregulin with an epiregulin neutralizing antibody influences the invasion of MCF10A cells expressing TBX2. The results demonstrated that more MCF10A cells were able to invade through the membrane than those expressing the control GFP plasmid. The results also indicate that invasion induced by TBX2 is not dependent on epiregulin.

STANDARDIZING THE EX OVO CAM PLATFORM: IMPROVING VIABILITY WITH NOVEL VESSEL DESIGN AND WATER SUPPLEMENTATION

Makenna Berger, Carson Krefft, Ryan Lee, Rachel Novak, and Mong-Lin Yang (Advisor)

Department of Science

Concordia University - St. Paul, St. Paul, MN

The chorioallantoic membrane (CAM) of chicken embryos has long been recognized as a valuable model system for investigating a variety of physiological and pathological processes. This study focuses on optimizing the design of a novel vessel crafted from 3D printed Truncated Hexagonal Pyramid (THP) layered with polymethylpentene (PMP) plastic wrap for robust and sustained ex ovo CAM experiments. This novel THP/PMP vessel offers enhanced convenience in manipulating and observing the CAM during its development, with a favorable CAM testing window of over 10 days (embryonic days 7-17). To further enhance chick embryo viability during this testing period, water-supplemented and non-supplemented THP/PMP vessels were examined. Our results indicate that higher viability is achieved with water-supplemented THP/PMP vessels, underscoring the importance of maintaining water availability and relative humidity in the design of ex ovo culturing vessels.

REIMAGINING A BETTER VESSEL FOR EX OVO CHORIOALLANTOIC MEMBRANE CHICK EMBRYO ASSAYS

Abby Lewerenz, Makenna Berger, Jarrid Pizel, Julian Yang, and Mong-Lin Yang (Advisor)

Department of Science

Concordia University - St. Paul, St. Paul, MN

The chorioallantoic membrane (CAM) is a network of blood vessels surrounding the chick embryo during development. This membrane has recently been of interest due to its ability to rapidly vascularize, making it an ideal model to study tumor development, stem cells, and tissue engineering. Normally, the chick embryo develops in ovo, inside the eggshell; however, access to the CAM is limited and visibility is greatly reduced. Researchers have been using the ex-ovo, outside the eggshell, model to combat these issues, yet survivability of the embryos is reduced. Many novel vessels have been created throughout the field, however, there has yet to be a vessel that both adequately supports the chick embryo and is able to be used for application purposes. The goal of this project is to design a novel ex-ovo CAM vessel that yields this dual function. The novel truncated hexagonal pyramid (THP)/polymethylpentene (PMP) vessel was created, tested, and compared to previously tested ex-ovo vessels. Our findings suggest that the novel THP/PMP vessel is able to both support a high embryonic survivability, comparable to that of a published chick embryo hatching vessel, in addition to being stable enough to begin implantation trial experiments. Testing was also performed to compare how certain design elements affected the survivability of the embryos, and it was found that the inclusion of water in the vessel promoted higher embryonic survivability.

CHARACTERIZING *Staphylococcus aureus* ON CONCORDIA'S CAMPUS

Eliot B. Sands, Nay H. Paw, Isabelle R. Boudreau, Amanda Brosnahan (Advisor), and Taylor Mach (Advisor)

Department of Science

Concordia University - St. Paul, St. Paul, MN

A team of over 120 students have been studying the Gram positive bacterium, *Staphylococcus aureus*, isolated from willing participants over the last seven years. Over 1,800 participants have been asked to complete a short survey and give a nasal swab. The swab is then deidentified and put through a series of culture tests including Gram staining, growth on blood agar and mannitol salt agar, and catalase, DNase, and coagulase enzymatic assays to look for the presence of *S. aureus*. Current carriage rates are around 25%, just slightly lower than the general observed rate of 30%. All *S. aureus* isolates undergo further characterization for methicillin resistance (MRSA) via Kirby-Bauer assay. Presence of virulence factors, including cytolytins and superantigens, were characterized through PCR and whole genome sequencing, in partnership with the Minnesota Department of Health. Analysis of 146 samples enabled the determination of virulence factor carriage rates. Among the identified genes, SEI-X was present in 79.45% of the samples, α -toxin in 79.72%, TSST-1 in 12.59%, and *mecA* in 3.42%. *S. aureus* poses a significant threat to humans due to its multifaceted ability to produce diverse toxins and developed antibiotic resistance. This versatility allows it to instigate a spectrum of human infections, rendering it inherently harmful.

TBX2 SENSITIZATION EFFECTS OF ZR 75.1 CELLS IN RESPONSE TO CISPLATIN

Ella L. Wagner and Matthew A. Rowley (Advisor)

Department of Biology

St. Mary's University of Minnesota, Winona, MN

Breast cancer is ranked as the most diagnosed cancer in the world. Invasive ductal carcinoma is prevalent worldwide and shows the progression of cancer cells growing from the lining of milk ducts to surrounding tissues. Genetics can influence the growth of cancer and how well the cancer cells may respond to anticancer drugs. The TBX2 gene encodes for a transcription factor that impacts promoters that affect the cell cycle and apoptosis, leading to further cell proliferation. Expression of p21 and p53 is inhibited by TBX2. In addition, p21 and p53 are also targeted by a commonly used anticancer chemotherapy treatment called cisplatin. Cisplatin increases the production of the p21 protein. This research aimed to investigate the effect of TBX2 expression on cisplatin treatment of the Zr-75.1 ductal carcinoma cell line. Results show that cells with expression of TBX2 have reduced cell numbers, compared to cells expressing GFP when exposed to different concentrations of cisplatin over 4 days. This type of research is needed to understand cancer treatment resistance, as well as the impacts of genetic factors, such as TBX2, in response to treatment. Understanding the interactions of these impacts can influence and improve our responses to treating aggressive forms of cancer.

ISOLATION AND CHARACTERIZATION OF TETRACYCLINE RESISTANT BACTERIA FROM AQUATIC ENVIRONMENTS IN MINNESOTA

Eva Skipwith and Renu Kumar (Advisor)

Department of Biology

Minneapolis College, Minneapolis, MN

According to the Centers for Disease Control and Prevention (CDC), 266.1 million courses of antibiotics were prescribed in 2014 in an outpatient setting with an estimated 28% noted as redundant. This indiscriminate use of antibiotics has caused many bacteria, such as *Escherichia coli* and *Salmonella* species, to develop resistance mechanisms. Addressing bacterial-resistant crises requires a thorough understanding of antibiotics and their mode of action. This research was designed to characterize the antibiotic resistance bacteria isolated from aquatic environments in Minnesota. Sample selection consisted of targeting two different bodies of water, Minnehaha Creek, near the hospital (sample A), and Park Immersion Marsh, near dense areas of the population (sample B). We hypothesized that bacteria would be more resistant around the area where there is more exposure to antibiotics. Bacteria were isolated by using a serial dilution technique and plating on media with and without antibiotic tetracycline. After incubation, colonies on each plate were documented followed by the quantification of the total number of bacteria present in each of the original water samples. We also calculated the frequency of tetracycline-resistant bacteria present in each water sample. Preliminary results show that tetracycline-resistant bacteria are present in both samples. The frequency for tetracycline-resistant bacteria in MacConkey was higher (7-25%) in Sample A as compared to 12-18% in Sample B. The information acquired from this research will enhance our understanding of the prevalence of antibiotic-resistant bacteria in aquatic environments in Minnesota. An analysis will help us understand the relationship between outbreaks of antibiotic-resistant bacteria and the environmental reservoirs of resistant bacteria.

CHEMISTRY

OPTIMIZING LAB FOR BEST LAB PROCEDURE AND RESULTS FOR A LIBERAL ARTS SCIENCE COURSE

Sophie A. Hastings, Ikran I. Adan, and Heather Sklenicka (Advisor)

Department of Chemistry

Rochester Community and Technical College, Rochester, MN

For our Liberal Arts Chemistry course, students get a lab kit and do experiments at home. Analysis of their results showed a few labs that could be improved upon in this environment. The first lab to be examined was 'A Dash of Pepper and A Pinch of Salt,' which is a separation due to physical properties. The concern was that students had varied results in the ranges of below 50% and above 100% of the final recovered amounts of salt and pepper. Further experiments in the lab and at home were conducted, in which the salt mixture was boiled thoroughly resulting in the appearance of the salt being sand-like and set to dry on the filter paper with minimal excess water left. The process of drying the salt and pepper led to a more consistent percent recovery averaging 81.7% and 85.1%. The lab 'How Strong Is That Base?' is a small-scale titration which was having an issue with the titration volume overflowing the spot plate. Adjusting the amount of phenolphthalein allowed the titration to stay within the bounds of the container. This small adjustment allowed the concentration of the base to be determined consistently within 0.30 M for 0.5 M sodium hydroxide solution and within 0.08 M for 0.1 M sodium hydroxide solution. Changes to the lab procedures will be made for the upcoming semester to ensure students get scientifically accurate results and feel successful with science.

PROTEIN CONCENTRATION IN RICE PRODUCTS

Hoang Trung Doan, Ying Liu, and Heather Sklenicka (Advisor)

Department of Chemistry

Rochester Community and Technical College, Rochester, MN

Rice, a staple food, is increasingly popular due to its high macronutrient content. A single serving of rice contributes 15% of the recommended daily carbohydrates and 8% of the daily protein intake. A study was conducted to quantify rice protein content using colorimetry in an undergraduate laboratory setting. The methodology involved establishing a calibration curve using whey protein and a Coomassie Brilliant Blue G250 reagent. This reagent, upon interaction with protein, induces a distinctive blue color that can be quantified using a colorimeter. Rice samples were then subjected to the reagent, and their protein concentrations were determined by comparing the absorbance of light to that of the whey protein standards. The comparison of experimental protein quantities with the information provided on nutrition labels resulted in percent errors ranging from 10% to 57%. The relative protein quantities in the tested rice brands aligned with the proportions indicated on their nutrition labels. This approach demonstrates potential applicability in incorporating a nutrition-focused project into the General Chemistry curriculum, offering an innovative and hands-on educational experience for undergraduate students.

SYNTHESIS OF PHOSPHORAMIDE AS AN ORGANOCATALYST FOR THE CARBONYLOLEFIN METATHESIS REACTION

Alyssa K. Jones and Heidi A. Dahlmann (Advisor)

Department of Chemistry

St. Mary's University of Minnesota, Winona, MN

Carbonyl-olefin metatheses (COM) are carbon-carbon bond-forming reactions that can be used in synthesizing pharmaceutical compounds, organic electronics, and flavors or dyes. Traditionally, these reactions are catalyzed by Lewis acids, but we are investigating Bronsted-Lowry acids as an easier-to-use alternative catalyst. In this investigation, a novel phosphoramidate catalyst was formed from a commercially available phosphochloridate starting material. It was characterized by nuclear magnetic resonance (NMR) and by mass spectrometry. Meanwhile, previously known biaryl aldehyde and methyl ketone substrates were synthesized through Wittig reactions followed by Suzuki-Coupling reactions. Ultimately, we determined that our novel phosphoramidate catalyst was able to facilitate efficient conversion of the aldehyde substrate to the expected COM reaction product, with lower yields seen for the ketone substrate.

EXPLORING THE IMPACT OF MYCELIAL BINDING TYPE ON ABSORBENT CRYOGEL-LIKE FIBROUS MATERIALS

Grace M. Harper, Leyla F. Sengul, Katelynn R. Stariha, Phatee Yang, and William S. Boyle (Advisor)

Department of Chemistry

Anoka-Ramsey Community College, Coon Rapids, MN

Healthcare and personal hygiene products rely on single-use items, which are often petroleum-based. Replacing these items requires development of sustainable materials with shared physical and material properties. Mycelium (mushroom extracellular matrix) lends a cheaply produced and fast-developing medium. Our previous work demonstrated that base treatment and lyophilization generate a cryogel-like material with promising properties. Four species were evaluated, representing the three mycelium binding modes: monomitic, dimitic, and trimitic. These types are characterized by three mycelium tissues- generative, binding and skeletal- which are believed to influence the species' physical properties. This study investigates the effect of different binding types on the material properties of the resulting cryogel material. *Pleurotus ostreatus* (monomitic), *Ganoderma sp.* (trimitic), *Trametes versicolor* (trimitic), and *Grifola frondosa* (dimitic) were cultured on potato dextrose agar. Fluorescence microscopy, water absorption testing, and tensile testing were used to compare the qualities of the mycelium binding types. Microscopy revealed qualitative differences between species. ImageJ was used to quantify differences in diameter between the fibers. Monomitic fibers displayed a network of overlapping fibers. Similarly, dimitic fibers were curly, narrow and densely packed. Trimitic fibers were wider and straighter. Water absorption testing showed a correlation between thickness and amount of absorption, but not binding mode. *Ganoderma* and *Pleurotus ostreatus*, which generated thinner mats, exhibited the highest absorption rates. Tensile testing showed that trimitic fibers tolerated higher stresses before breaking. This study demonstrates that the binding mode of mycelium could be an important factor in the development of renewable bio-based alternatives for petrochemical absorbents.

ECOLOGY AND ENVIRONMENTAL SCIENCE

COMPARING BIODIVERSITY OF SMALL MAMMAL SPECIES IN REMNANT AND RESTORED PRAIRIE HABITATS

Katie P. Waugh and Joseph C. Whittaker (Advisor)

Department of Biology

Concordia College – Moorhead, Moorhead, MN

In Minnesota, few prairies have survived European colonization and widespread agricultural development. These remnant prairies are scattered and fragmented, interfering with the natural subsistence of the ecosystem. Restoration efforts by private and academic entities have been initiated to revive native prairies. However, the effectiveness of the efforts, in relation to small mammal populations, remains understudied. Using small mammals as an indicator, we hope to observe the efficacy of the current management techniques on prairie health and success of restoration. This research monitors small mammal populations, diversity, and species richness in restored prairies that have implemented management techniques compared to remnant prairies. Through comparison of these sites, we can observe the effectiveness of these techniques and hope to observe an increase in small mammal populations, richness, and diversity on restored prairies that approximates those seen on remnant prairies. Collecting data at remnant and restored prairies in northwestern Minnesota enabled us to observe the differences in mammal species richness, evenness, and diversity. Grids were established at each site with live-trapping stations (40-50 per grid), where captured small mammals were identified by species, sex, and mass before being marked and released. Since the project's initiation in 2012, we have seen an increase in small mammal populations in the restored prairies approaching the levels observed in remnant prairies. This suggests that current management techniques are successful, but specific species present may not represent the historical community. We hope to gather further information on small mammal populations to further understand their diversity and abundance dynamics.

ECOSYSTEM RESPONSE TO INCREASING HURRICANE DISTURBANCE: COMPARING NUTRIENT CYCLING DYNAMICS ASSOCIATED WITH EARLY- AND LATE-SUCCESSIONAL TREE SPECIES IN A WET TROPICAL FOREST

Miriam L. Kleit¹ and Christine S. O'Connell² (Advisor)

¹*Department of Biology*

²*Department of Environmental Studies
Macalester College, St. Paul, MN*

Puerto Rico has seen changes in the frequency and severity of disturbances in recent years as hurricanes have become more frequent and intense under climate change. In ecosystems experiencing increasing disturbances, we expect that species composition will shift as early successional trees become more common. These changes to species composition and community ecology are likely to impact terrestrial nutrient cycling both directly and indirectly, and it is still not well understood how shifting community composition may alter ecosystem functioning. To address this gap, we measured carbon and nitrogen variables in soils, microbial biomass, roots, leaves, and soil greenhouse gas fluxes within 1 m of individuals from three tree species across a topographic gradient in El Yunque National Forest in Puerto Rico. The three species of interest are likely to be differently impacted by changing hurricane regimes: an early successional tree species (*Cecropia schreberiana*), a secondary successional species (*Prestoea montana*), and a late successional species (*Guarea guidonia*). We hypothesized that the soil area surrounding early successional and late successional tree species would exhibit differences in carbon and nitrogen cycling and the resulting soil greenhouse gas emissions. We found that there were statistically significant species-related differences in leaf composition, soil nutrients, and soil gas fluxes. In combination, our results suggest that, as the successional state of the forest shifts to be dominated by early successional species for longer stretches of time due to increasing incidence of large-scale hurricane disturbance, the nutrient cycling of this forest may also be altered drastically.

QUANTITATIVE AND QUALITATIVE ANALYSIS OF DENTAL ANOMALIES AND PATHOLOGY IN WHITE-TAILED DEER (*Odocoileus virginianus*)

Tessa B. Danielson, Sophia R. Tobin, and Joseph C. Whittaker (Advisor)

Department of Biology

Concordia College – Moorhead, Moorhead, MN

An organism's teeth are essential for feeding, beginning the processing of food and for survival. Pathology and anomalies in teeth should be under intense natural selection as they could cause substantial decreases in fitness and impact survivorship. In this ongoing project we are documenting the number and types of dental anomalies and pathologies in white-tailed deer (*Odocoileus virginianus*) from Concordia College's Elsie Welter Museum of Natural History Museum. Deer were donated by hunters or salvaged from roadkill or individuals found in the field. Thus far we have documented 52 dentaries belonging to deer from Kentucky, Virginia, North Dakota, and Minnesota. Of the examined dentaries, 12 were single dentaries and 40 were paired. Sampled deer ranged in age from fawns (0.5 years) to well over 4.5 years. The majority of deer examined were 2.5 years (n = 13), followed by 1.5 years (n = 12), greater than 4.5 years (n = 11), 3.5 years (n = 7), 0.5 years (n = 6), and 4.5 years (n = 3). Eight jaws had pathology including damaged, cracked or broken teeth (15%). Two jaws had serious infections with osteomyelitis (4%). One jaw had hyperdontia or a supernumerary molar (2%). This study provides incidences of dental anomalies and pathology in free-ranging deer and in theory might serve to provide an estimate of incidences of such abnormalities in the wild.

SOUTHEASTERN MINNESOTA BAT RESPONSE TO AQUATIC INSECT EMERGENCES

Zachary T. Ryan and Benjamin Pauli (Advisor)

Department of Biology

St. Mary's University of Minnesota, Winona, MN

Riparian areas offer a unique environment to study energy transfers between aquatic and terrestrial habitats. Emergent aquatic macroinvertebrates are one way in which terrestrial and aquatic habitats can be linked. Bats are known to have high foraging activity in and around riparian habitats and to consume aquatic insects. Several studies have found correlations between aquatic insect emergences and bat foraging activity, however, conclusions as to the strength of such interactions vary. Limited research has been done on these interactions in the United States particularly in Minnesota. Emergent aquatic macroinvertebrates and ultrasonic bat calls were collected over twenty nights from eight riffle sites along Gilmore Creek on Saint Mary's University of Minnesota's campus. Multiple linear regression was used to determine if: 1) aquatic insect abundance were associated with bat activity levels, 2) aquatic insect richness and diversity were associated with bat activity levels, and 3) the emergence of any specific aquatic insect taxa was associated with bat activity levels. We found that local bat populations were not associated with emergent aquatic insect abundance, richness, diversity, or any particular insect taxa. The lack of relationships between aquatic insect emergence and bat activity may be due to several factors including: a lack of nocturnal and late season insect emergences, bats preferring terrestrial food sources, or minimal foraging over riffles by bats. While this study contributes to our understanding of the relationship between bats and emergent macroinvertebrates, due to limited data obtained here, we recommended that long-term, cross-seasonal studies be conducted.

THE EFFECT OF GARLIC MUSTARD CONDITIONED SOIL ON GARLIC MUSTARD, MYCORRHIZAL FUNGI, AND NATIVE PLANTS

Alfred Cobbinah, Olivia Gustafson, and Eric Baack (Advisor)

Department of Biology

Luther College, Decorah, IA

Garlic mustard (*Alliaria petiolata*) is an invasive species found throughout the United States, but particularly in the Northeast and Midwest. Garlic mustard can transform biological communities by outcompeting native species and releasing toxins that can disrupt the arbuscular mycorrhizal fungi associations between plants. In this study, we wanted to see how soils previously containing garlic mustard would affect native plant growth as well as the growth of garlic mustard. To do this, we grew garlic mustard and several species native to Northeast Iowa in soil previously beneath garlic mustard, soil where garlic mustard seedlings were observed but not adults, and soil never exposed to garlic mustard. We counted the number of germinated seeds, monitored survival, and calculated the average leaf area. We found that while some native species had higher germination rates in never-invaded soils, some species had significantly higher germination in soils previously invaded by garlic mustard. Additionally, some native species had significant differences between average leaf area and the soils they grew in. Garlic mustard seedlings had marginally lower survival in soils where garlic mustard adults were not seen. Future testing should be completed to better understand the changes in bacterial and fungal communities due to exposure to garlic mustard.

ASSESSING MICROPLASTIC CONCENTRATIONS IN THE GASTROINTESTINAL TRACTS AND FECAL MATTER OF TREE AND GROUND SQUIRRELS (*Sciuridae*) FOUND IN URBAN AND RURAL HABITATS

Yuden Dorji, Sharon Mac-George Nwabia, Favziya Rasulova, Fatima Mohammed, Mubina Rasul, and Joseph Whittaker (Advisor)

Department of Biology

Concordia College – Moorhead, Moorhead, MN

Microplastics, tiny plastic particles (MPs, < 5mm), are a threat to ecosystems, wildlife, and human health. Microplastics can result in gastrointestinal blockages, scrapes, and put and jeopardize animal's digestive health, behavior, and survival. Ingesting microplastics can disrupt an animal's feeding behavior, leading to reduced nutrient acquisition and malnutrition or starvation. Previous studies have observed MPs in mammalian liver, kidney, and gastrointestinal (GI) tracts. In our ongoing study we aim to investigate MP content in three different tree squirrel species, the American Red Squirrel, Eastern Gray Squirrel, and Eastern Fox Squirrels, and two ground squirrels, the Thirteen-lined Ground Squirrel and Franklin's Ground Squirrel. We hypothesized that urban squirrels will exhibit higher levels of MPs and that there will not be a difference between the number of MPs in ground versus tree squirrels. We examined gut content from feces of live-trapped squirrels and gastrointestinal tracts (GITs) from salvaged squirrels. Each sample was chemically digested using 10% KOH, filtered and examined using a dissection microscope. While there was high variability, there is a trend for more plastic in the guts of rural squirrels, but highest levels of plastic were collected from urban gray squirrels. Tree squirrels had significantly more microplastics than ground squirrels, thus far. In the future, we will obtain a small section of the gut to analyze its microbial content. Bacteria from each gut sample will be cultured and sent for further analysis. We hypothesize that higher MP levels in the gut may correspond to reduced diversity and fewer beneficial bacteria.

GOOD GALL-Y! FIELD PREVALENCE OF GALL-FORMING INSECTS IN CARVER COUNTY, MINNESOTA

Joel W. Swanberg and Trevor Vannatta (Advisor)

Department of Biological & Health Sciences

Crown College, St. Bonifacius, MN

Goldenrod (*Solidago spp.*) is a common perennial plant inhabiting Minnesota that can host various gall-forming insects, making it an ideal system for studying plant-gall interactions. One common gall-former, *Eurosta solidaginis*, lays larvae within stems which stimulates the formation of spherical galls. In turn, multiple parasitoids, predatory beetles, or birds may attack these larvae. Other common gall-formers include *Asteromyia carbonifera* (blister-galls) and *Rhopalomyia solidaginis* (rosette-galls). This project aimed to determine if infection varies within a small geographic area. To study potential drivers of this variation, interactions between goldenrod symbionts, landscape features, and plant characteristics were examined. Field sampling was performed at five sites in Carver County, Minnesota: Crown College (CC), Lake Auburn (LA), Grimm Farm (GF), Steiger Lake (SL), and Monarch Singletrack (ST). No differences in *Eurosta* prevalence were found. However, rosette-gall prevalence was highly variable between sites. CC had fewer rosette-galls than GF ($p = 0.0420$) and ST ($p = 0.0007$). Additionally, SL ($p = 0.0054$) and LA ($p = 0.0381$) had a significantly lower prevalence of rosette-galls than ST. Blisters behaved oppositely than rosette-galls, with CC ($p = 0.0121$), GF ($p = 0.0011$), and LA ($p = 0.0886$) all having significantly (or near significantly) higher prevalence of blister-galls. Results may suggest an antagonistic interaction between rosette and blister-galls. This study contributes to the understanding of goldenrod-gall interactions which may have ramifications on larger ecological scales and across food webs. Further examination of interactions between gall-formers is warranted as model systems for studying pathogen-pathogen interactions are in high demand.

IDENTIFICATION OF MINNESOTA NATIVE HEMLOCK TREES FOR EX SITU CONSERVATION WORK

Jasmine M. Faue¹, David Remucal² (Advisor), and Aeisha Thomas¹ (Advisor)

¹*Department of Biological & Health Sciences, Crown College, St. Bonifacius, MN*

²*Department of Horticultural Science, University of Minnesota - Twin Cities, Minneapolis-St. Paul, MN*

The Eastern Hemlock is a coniferous tree native to North America experiencing population decline due to parasitic insects. Various groups of hemlock have become geographically isolated, resulting in inbreeding. In particular, the isolated Minnesota hemlock population has been declining. One conservation strategy being implemented by the University of Minnesota Landscape Arboretum is collecting seed and tissue samples from existing MN hemlock trees that can be preserved and later planted. This ex situ conservation strategy is contingent on confirmation that the sample is a MN native subgroup member. The goal of this research project is to determine whether sources of hemlock are MN natives. Polymerase chain reaction (PCR) was used to amplify 8 microsatellites previously determined to be useful in genotyping hemlock. Purified DNA from a positive control (known Minnesota native), negative control (e.g. WI, MI, etc.), and cohorts suspected of being Minnesota native underwent PCR to identify genotypic patterns of amplification. From the preliminary electrophoresis data, analysis indicated that the 8 identified primer sets showed stronger amplification with the Minnesota native genotype and that the experimental samples suspected to be Minnesota native showed similar genotypic amplification patterns as the known Minnesota native cohort. The DNA from these and additional samples is now being repurified with the goal of using a more conclusive approach, genotype sequencing. Results from this data will hopefully definitively differentiate between non-native and Minnesota native hemlock subgroup to support conservation efforts.

12 YEARS OF A PRAIRIE RESTORATION IN NORTHEAST IOWA: PLANT COMMUNITY SHIFTS, BURN REGIMES, AND MANAGEMENT RECOMMENDATIONS

Josephine Meyer¹ and Molly McNicoll² (Advisor)

¹*Environmental Studies Program, Luther College, Decorah, IA*

²*Department of Biology, Luther College, Decorah, IA*

Prairie reconstructions aim to create functionally diverse plant communities but have yet to fully replicate the structure of remnant prairies. Observing changes in the composition of reconstructions over time can help evaluate current seeding methods and other management techniques, informing recommendations for future projects and continued restoration of current projects. This study analyzed plant community structure of a 12- year-old tallgrass prairie reconstruction owned by Luther College in Northeast Iowa. Species diversity and abundance were assessed in 180 (1 x 0.5 m) permanently placed quadrats along nine transects during the summers of 2015 and 2023. Changes in community structure (species richness, coefficients of conservatism, and relative abundance of warm-season grasses) were assessed at four years of age (2015, unburned) and 12 years of age (2023, burned). One-third of plots were initially burned at five (2016), six (2017), and seven (2018) years of age. No differences in community structure were found amongst initial burn treatments, therefore sampling was not blocked accordingly. Species richness and coefficients of conservatism increased over time, reflecting a decrease in early successional species and emergence of more conservative species. Warm-season grasses increased in relative abundance over time, showing a more even distribution of major functional groups. Composition of this site indicates progression towards a functionally representative plant community, with supplementation being a potential tool to further increase floristic quality. Reconstructing habitats is a continual process moving towards a goal of remnant-like vegetation, with floristic assessments essential in monitoring this progression and making management decisions.

Epidemiology

CHARACTERIZING THE CORRELATION BETWEEN CANCER TREATMENTS AND CARDIOVASCULAR DISEASE IN ALL OF US DATABASE PARTICIPANTS

Christella Baluta and Aeisha Thomas (Advisor)

*Department of Biological & Health Sciences
Crown College, St. Bonifacius, MN*

In America, over 18 million people live with cancer, a disease characterized by uncontrolled cell division, leading to a higher and abnormal number of cells. In order to stop the growth of undesirable cells, patients take cancer drugs. However, several people who take cancer drugs end up with cardiovascular disease, while some do not, and this study aims to understand the difference between these two groups. The All of Us database (AoU) is a big research hub that stores health data from a diverse group of participants from across the United States and thus a potential source for characterizing the correlation between cancer treatment and cardiovascular disease. The first goal was to identify AoU participants who had taken four cancer drugs (capecitabine, trastuzumab, doxorubicin and fluorouracil) and had a history of four common cancers (breast, colon, prostate, or lung), and whether or not they had cardiovascular disease. The main limitation of this cross-sectional study approach is the uncertainty about when the participants had cardiovascular disease. To conduct this study different cohorts with appropriate criteria were built in AoU and we found that most of the people taking these drugs also had cardiovascular disease. Future directions are to analyze these AoU cohorts to find factors that might influence whether or not cancer drugs cause cardiovascular disease.

PREVALENCE OF NEWBORN SCREENING CONDITIONS IN THE ALL OF US DATABASE

Sierra E. Kicker and Aeisha Thomas (Advisor)

*Department of Biological & Health Sciences
Crown College, St. Bonifacius, MN*

The Recommended Uniform Screening Panel (RUSP) meets annually to revise the list of conditions for newborn screening in the United States. Currently, there are 37 core conditions and 26 secondary conditions. Each state determines which of the RUSP and possibly other conditions are included in its Newborn Screening Program. The National Institute of Health's All of Us Research Program (AoU) is a large database with geographic distribution, biomedical and other types of data. The goal of this project is to determine the prevalence of the RUSP conditions in AoU and collate available related medical and other data. This could inform state decisions regarding newborn screening conditions and be a resource for research in these often understudied rare diseases. This is a cross-sectional study where the electronic health record and other AoU data were mined to determine the prevalence of conditions listed in the 2023 RUSP, except for hearing loss. A separate cohort was created for each RUSP condition, but some cohorts include multiple types of the same condition due to a lack of distinction in the AoU database. Out of the 63 RUSP conditions studied, six had more than 20 persons in the cohort, 17 cohorts had fewer than or equal to 20 persons, and 33 of the RUSP conditions were not found in the AoU database. This prevalence data indicates that the All of Us database does have information regarding newborn screening conditions and can potentially be used as a resource for this area of rare disease research.

Exercise Science

DETERMINING THE IMPACT OF EXERCISE ON WOMEN'S BRAIN FOG

Natalie A. Mathews and Aaron Aslakson (Advisor)

Department of Biological & Health Sciences

Crown College, St. Bonifacius, MN

Brain fog, most commonly experienced by females, is a non-diagnosable medical condition characterized by reduced cognitive function. Symptoms are subjective and may last for weeks to years, depending on the severity and whether it occurs in conjunction with an external health condition. Due to the lack of biological markers and patterns of occurrence, existing research and understanding regarding diagnosis and treatment of brain fog is limited. The benefits of exercise are widespread, especially pertaining to inhibitory control and memory. Because brain fog impairs an individual's ability to concentrate or think cohesively, this research will primarily analyze exercise benefits in respect to cognition in the form of quantitative and qualitative data. The purpose of this study was to relate the impact of acute, aerobic exercise to brain fog, specifically short-term memory, for middle aged women. To determine the effectivity of exercise, fourteen women had their visual, episodic memory tested. Participants completed one subjective brain fog survey and memory examination without exercise, and both again following an aerobic exercise intervention. Accuracy, reaction time, and perception of brain fog were compared before and after exercise. Preliminary data indicates that participants experienced decreased symptoms of brain fog following exercise, with a statistically significant decrease in reaction time ($p < 0.05$). If an acute bout of aerobic exercise proves to effectively reduce brain fog and improve memory, it may serve as a low impact, easily accessible treatment.

NEUROSCIENCE

ASSESSMENT OF GENE EXPRESSION PROFILING AND FUNCTIONAL PRIORITIZATION OF NUCLEAR ENVELOPE GENES IN ALZHEIMER'S DISEASE

Emma S. Schuster and Claudia C. Preston (Advisor)

Department of Biology

St. Mary's University of Minnesota, Winona, MN

Alzheimer's disease affects millions of people each year, however the pathological development of this disease is not yet fully understood. Current literature hints at involvement of the nuclear envelope in the molecular mechanisms behind multiple neurodegenerative diseases. This study focused on unveiling transcriptomic changes of genes coding for nuclear envelope-associated proteins in brains from patients with Alzheimer's. A dataset (GSE173955), from NCBI-GEO (Gene Expression Omnibus) website, was utilized for transcriptomic analysis, containing RNA sequencing on hippocampal samples from Alzheimer's and control patients (n=8/group). Threshold significance was set at adjusted p-value of < 0.05 . Functional enrichment and gene prioritization was performed using Reactome and the Database for Annotation, Visualization, and Integrated Discovery (DAVID) analysis tools. Additionally, protein-protein interactions were predicted using K-means clustering within the STRING database. Gene expression analysis yielded 1,165 significantly upregulated and 1,299 significantly downregulated genes (adj. p < 0.05). Of these, 37 upregulated and 63 downregulated genes were shown to be associated with the nuclear envelope. The most enriched functions were linked to genes coding for nuclear pore complex proteins (nucleoporins). Upregulated nucleoporin (Nup) genes were NUP98, NUP160, and NUP188, with fold changes (FC) of 1.22, 1.57, and 1.37, respectively. RAE1 and SEH1L were downregulated (FC of -1.35 and -1.56, respectively). These results suggest nucleoporins and other nuclear envelope-related proteins play an important role within the molecular mechanisms of Alzheimer's disease. Future directions will include protein expression visualization of these prioritized genes in diseased brain tissue.

CORRELATES BETWEEN SEASONAL HORMONES, SOCIALLY APPROPRIATE BEHAVIOR AND ANDROGEN/OPIOID/DOPAMINE GENE EXPRESSION IN EUROPEAN STARLINGS, *Sturnus vulgaris*

Misgana D. Mamo, Emily Schmidt, Elizabeth Johnson, Joanna Trinkle, and Melissa Cordes (Advisor)

*Department of Biological Sciences
Bethel University, St. Paul, MN*

Previous research has identified links between steroid hormones and reward pathways in the brain (specifically opioids and dopamine (DA)) that are known to support context-appropriate communication (Schroeder and Ritters, 2006). It has been proposed that androgens, such as testosterone have their effects on behavior and communication by interacting with downstream neuromodulators such as opioids and dopamine which in turn motivate and reward appropriate behaviors. But more research on animals whose vocal communication mimics humans in its social context specificity. During procedure, brains from birds in spring condition were sliced on a cryostat at 200um thick and then specific areas were removed by micropunch for processing. The brain punches were then processed to extract the RNA. This process is time consuming as only 6 samples can be processed at a time and we had 38 birds and 4 nuclei to complete. Once the RNA was extracted, it was converted by reverse transcription to cDNA which is more stable and can be used for quantitative real time analysis. We are also still gathering more data for more brain areas. We found in males that sang in spring, TH in HVC was correlated with the number of songs. There was also correlation between TH (which is our approximation for dopamine) and Dopamine Receptor 2. Once all the datasets are complete, we hope to continue collaboration with colleagues at the University of Wisconsin to work with these datasets.

ORGANISMAL AND PHYSIOLOGICAL SCIENCES

ADHESIVE PERFORMANCE OF THE PELVIC SUCKER DURING CLIMBING WATERFALLS IN A JAPANESE STREAM GOBY, *Rhinogobius fluviatilis*

Dylan Pakizegi, Erin Benson, and Takashi Maie (Advisor)

Department of Biology

St. Olaf College, Northfield, MN

The family *Gobiidae* is a speciose teleost family with over 2,000 species in more than 200 genera. A number of goby species in oceanic islands have evolved the ability of scaling waterfalls using pelvic fins that are fused into a suction disc (pelvic sucker). Positioned on the ventral side of the body, the pelvic sucker is used to gain traction as they migrate into upstream regions of torrential streams and rivers to reach unoccupied habitats for growth and spawning. These climbing gobiids must exert adhesive suction strong enough to support their body weight against gravity during climbing on the rock surface. A pressure transducer was used to measure adhesive suction pressure produced by the pelvic sucker in *Rhinogobius fluviatilis* while the fish were challenged to climb on an artificial climbing surface. With a wide range of body size (0.53-9.56 g BM; 3.86-10.77 cm TBL), *R. fluviatilis* generated a mean safety factor of $3.46 \pm 0.17 \times \text{BM}$ ($0.17 \pm 0.01 \text{ N}$). Consistent with other climbing gobiids previously studied, the suction force generated by the pelvic sucker of *R. fluviatilis* exhibited a strong positive allometry (OLS slope = 1.263) compared with the expected isometric slope despite the contact area of the sucker scaled in negative allometry. This preliminary result suggests a maintenance of the climbing ability throughout ontogeny.

ATRAZINE AND THE CIRCADIAN RHYTHM HAVE NO EFFECT ON INSULIN LEVELS IN MICE

Hanna C. Widdes and Debra Martin (Advisor)

Department of Biology

St. Mary's University of Minnesota, Winona, MN

Atrazine is one of the most commonly used herbicides in the world. Previous research suggests that chronic atrazine exposure may influence blood glucose levels. It is unknown if the change in glucose levels is due to less glucose being taken up by the gut or if changes in insulin levels influence glucose uptake by the cells since insulin and blood glucose levels are directly related. Circadian rhythm is also known to affect blood glucose and insulin levels. This study aimed to determine if chronic atrazine exposure and the circadian rhythm affect mouse serum insulin levels. The mice were exposed to 0 ppb, 3 ppb, 30 ppb, and 300 ppb atrazine via their water for 15 weeks and housed in 12-hour light and dark cycles. Serum was harvested at 6 different Zeitgeber time points to account for the circadian rhythm. Insulin levels were measured for each atrazine treatment at each time point using an Ultra Sensitive Mouse ELISA Kit (Crystal Chem). A two-factor ANOVA was run to determine the differences in insulin levels based on the two treatments. No significant difference was found between insulin levels with different concentrations of atrazine ($p=0.0867$). Insulin levels did not exhibit a significant difference based on the circadian rhythm ($p=0.591$). Circadian rhythm and atrazine interact to affect insulin levels together ($p=0.040$), but not when considered as separate factors.

TOWARDS PHYSIOLOGICALLY RELEVANT VASCULARIZATION TESTING: INNOVATIVE HEK293 CELL IMPLANTATION ON THE CHORIOALLANTOIC MEMBRANE

Makenna Berger, Carson Krefft, Ryan Lee, Rachel Novak, and Mong-Lin Yang (Advisor)

Department of Science

Concordia University - St. Paul, St. Paul, MN

The ex ovo culturing of chicken embryos offers a versatile platform leveraging the highly vascularized chorioallantoic membrane (CAM) for various experimental purposes. This study explored the use of a novel ex ovo culture vessel design to develop a more physiologically relevant method for implanting ortholog cells to assess vascularization. Traditionally, cell suspension in media followed by implantation on top of the CAM has been the norm for vascularization testing. However, this approach has limitations, as it allows post-implantation nourishment by media and unrestricted oxygenation through ambient air access, potentially affecting the vascularization process. To address these concerns, we devised a method to suspend HEK293 cells within a media-less agarose gel disc. Subsequently, we developed a technique to implant the HEK293 cell disc into the side of our novel vessel, where the CAM is accessible but ambient air oxygenation is limited. This approach aims to create a more physiologically relevant environment for vascularization testing by mimicking the natural conditions of tissue growth and development. Preliminary results suggest that this method holds promise as a more physiologically relevant implantation approach for vascularization testing utilizing the CAM. Further studies are warranted to fully elucidate its potential and optimize its application in experimental settings.

CORRELATION BETWEEN MORPHOLOGY AND ENVIRONMENT IN EURASIAN PERCH, *Perca fluviatilis*, AND YELLOW PERCH, *Perca flavescens* (Pisces: Percidae)

Briella Schmidt, Alyssa Snider, James Le, Sophie Higgs, and Takashi Maie (Advisor)

Department of Biology

St. Olaf College, Northfield, MN

Morphology of functional designs in animals often provides a reliable inference regarding how the design optimizes functional performance of particular behavior and how it changes to become better suited to meet specific functional demands from environments. Many teleosts exhibit intra- and inter-specific morphological variation that often correlates with differences in bioavailable resources and ecological characteristics in natural habitat. For example, difference in visibility of lake water may indicate the predator-prey interaction between species operates at different proximity when reacting to each other. With ontogenetic scaling analysis, we evaluated both intra- and inter-specific variation in body and fin morphology in Eurasian perch, *Perca flavescens* from two lakes from Minnesota and *P. fluviatilis*, from two lakes in Norway (Store Le and Østensjø vann). Although most of the morphological variables (e.g., pectoral fin base, anal fin base, caudal peduncle, caudal fin height, and second dorsal fin base) indicated a maintenance of shape in *P. flavescens* during ontogeny, these variables in *P. fluviatilis* exhibited negative allometry with respect to body size. However, thickness of specific body segments in both of these percids scaled in either isometry or positive allometry. These preliminary results may indicate correlations between morphology and environmental conditions, to which these fish are exposed, as well as potential genetic differences between species.

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