MONTANA ACADEMY OF SCIENCES

2018 ANNUAL MEETING

APRIL 6-7, 2018

Montana Tech of the University of Montana - Butte, Montana

Chrissie Carpenter, President, Montana Academy of Sciences
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INTRODUCTION

The Montana Academy of Sciences (MAS) was incorporated on the 20th day of March, 1961 as a non-profit, educational organization. The objectives of the Montana Academy of Sciences are to encourage interest and participation in the sciences and to promote public understanding of science and its contribution to society. The Academy accomplishes its objectives by conducting meetings of those interested in sciences and the education of scientists, by publishing contributions to scientific knowledge, by supporting research, by making awards to recognize accomplishments in science, by administering gifts and contributions to accomplish these aims, by assigning and cooperating with affiliated and other organizations with similar objectives and by engaging in such other activities as deemed necessary to accomplish its objectives.

We held our 2018 Annual Meeting at Montana Tech in Butte, MT. on April 6 and 7. Over 80 registrants participated, viewing 21 contributed oral presentations and 22 poster presentations over the day and a half meeting. We present the abstracts from our meeting here so that the readers of the Intermountain Journal of Sciences can see the quality and types of science supported by MAS. Please mark your calendars for our next meeting, April 5 and 6, 2019 in Butte. Finally, the Board of Directors of MAS would like to thank the sponsors of our 2018 Annual Meeting:

Dr. Doug Coe, Dean, College of Letters, Sciences and Professional Studies,
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Dr. Beverly Hartline, Vice Chancellor for Research, Montana Tech, Butte

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Dr. Jenny McNulty, Assoc. Dean, College of Humanities & Sciences,
Univ. of Montana, Missoula

PRESENTATION ABSTRACTS

Alphabetical by First Author's Last Name
*** Denotes Presenter**

ECOLOGICAL EVALUATION OF RECLAMATION SUCCESS OF MINE SITES OF THE SAPPHIRE MOUNTAINS

Jeremy Aal *, Biology, Montana Tech, Butte

Sapphires and gems have been mined from the Sapphire range of southwest Montana for more than 150-years. Across this region and elsewhere, an unknown count of pits and tunnels and tailing piles lay abandoned while the local ecology reclaims the disturbed earth. A historical practice of reclamation for decommissioned mines was to bulldoze the site flat, removing physical hazards from the landscape. This process mixes the soil strata and alters the composition of the topsoil. Soil compaction is also a consequence of this process. Altered composition and significant increases in soil compaction often cause native plant species to struggle or fail to thrive in an area, causing opportunistic weedy species to proliferate. Our research investigated the species richness and coverage of three sites: a disturbed mine without reclamation, a mine site with bulldoze reclamation, and a third site in the vicinity which was not mined. Our multivariate analyses confirmed that species composition was different among the three sites. The natural site had higher plant cover, however, it was not significantly different from the other two sites due to the higher coverage of exotic weeds in the reclaimed sites. A greenhouse-controlled species competition supported this as soil from the reclaimed site was significantly better for knapweed test plants. Soils from the natural sites proved to be significantly better for native bluebunch wheatgrass growth. These results show that reclamation success does depend on initial site preparation, on the presence of exotic plant species that can be further spread by inappropriate site management. Also reclamation sites need to be managed if exotic invasion could be a potential.

EXPLORATION OF THE EFFECT OF PLATELET YIELD ON 5-7 DAY STORAGE

Aja Anderson *, Biology, University of Providence, Great Falls, MT

Platelets are a blood component essential for normal blood clotting. Donated platelets are critical for the survival of many patients, but the currently accepted shelf life of platelets is only 5 days. Thus, maintaining an adequate supply of lifesaving platelets is an ongoing concern. Platelet supply could be increased if data supported an increased shelf life. Previous studies revealed that increasing numbers of platelets in the storage bag (platelet yield) is negatively correlated to shelf life. This study explores the effect of platelet yield on the longevity of the units and examines donor differences with respect to their platelet storage profiles. We studied 12 donor units, split into 36 experimental bags, loaded at four yield levels. Our goal was to ascertain the maximum platelet yield that maintained a bag pH above 6.2 at day 7. A platelet bag is considered "failed" when the pH drops below 6.2. We found that higher platelet yields correlated with decreasing bag pH, while donor differences were related to the magnitude of the bag failure. No bags loaded at nominal yields (the most commonly used yield) failed by day 7. Of note, none of the 18 bags loaded at high-nominal yields (a less common current practice) failed at day 5, but 3 failed before day 7. These results confirm previous studies and support manufacturer determined yield limits and shelf life. Yields at and above manufacturer limits were associated with high failure rates, and are not recommended.

ANALYSIS OF ANTIMICROBIAL PEPTIDE EFFICACY AGAINST CHYTRIDIOMYCOSIS FROM SKIN SECRETIONS OF COLUMBIA SPOTTED FROGS (*LITHOBATES LUTEIVENTRIS*)

Emma Esposito *, Biology, Carroll College, Helena

Amphibian populations have been declining in size in recent years. A major contributing factor to this decline is the fungal disease chytridiomycosis. Chytridiomycosis occurs when the zoospores of the fungus Batrachochytrium dendrobatidis (Bd) imbed into amphibian skin and disrupt the homeostatic functions the skin provides, leading to death in most amphibians. Amphibian skin can produce antimicrobial peptides (AMPs) that inhibit Bd infection. The goal of this experiment was to determine whether or not the amount and type of AMPs a frog produces affects its ability to defend against chytridiomycosis. Columbia spotted frogs were chosen as a model organism because they vary in susceptibility to the disease, yet their populations have not been drastically affected by the fungus. AMP samples were collected from ten Columbia spotted frogs at four different locations in western Montana (40 samples total). The AMP concentration for each sample was determined using a micro BCS assay. The minimal inhibitory concentrations (MICs) were determined using 96 well plate growth inhibition assays. In the assay a set of ten AMP dilutions from each frog (ranging from 100-1000 µg/mL) were used. The Bd was grown in each dilution and growth was measured after four days as change in absorbance measured at 492 nm. Infection load of frogs was determined using Quantitative PCR analysis. From the data collected, there was no statistically significant correlation between the MIC observed and infection load.

METAL-MICROBE INTERACTIONS IN SILVER BOW CREEK

Jordan Foster *, Environmental Chemistry, Montana Tech, Butte

This project studies the relationship between microbes and both metals and metalloids in Silver Bow Creek. Biotic manganese nodule formation in Silver Bow Creek was identified through previous studies. The formation of these nodules is of interest to Silver Bow Creek restoration since heavy metals can bind to the nodules and potentially lower contaminants of concern. The goals of this study were to identify the organism(s) involved in the formation of manganese nodules and to identify how the nodules were formed using proteomic analyses. In addition, water chemistry data was used to study relationships between several elements in the creek and the potential effect these factors have on the microbial life. A combination of microbial culturing and LC-MS was used in an attempt to isolate the microbes involved with nodule formation and identify the proteins produced by the microbes. Currently, this work is attempting to isolate a microbe in the creek and analyze metagenomic data to predict the proteins used in the formation of the nodules. Once the microbe has been isolated or the proteins predicted, proteomic analyses with LC-MS can proceed. This work, in its current phase, has implications upon the interactions of microbial communities with contaminants of concern in Silver Bow Creek.

DETECTION OF COLORADO TICK FEVER VIRUSIN DERMACENTOR ANDERSONI

Zach Hart *, Cell Molecular Biology, Montana Tech, Butte

Colorado tick fever virus is a double stranded RNA (dsRNA) virus transmitted by Rocky mountain wood ticks (Dermacentor andersoni), which can be found at high elevations in a number of western states including Montana. Although Colorado tick fever often presents as flu-like symptoms that are usually not life threatening, twenty percent of those infected are hospitalized. Little is known about the evolution of CTFV. The reference strain, Florio, has been sequenced, but this particular strain was isolated in Colorado in 1943. We hypothesize that isolates collected in Montana would have novel mutations because of the geographic distance and time between them and the reference strain. Ninety-seven ticks were collected in a previous SURF project this summer, mostly from Maud S. Canyon. RNA was extracted from crushed ticks and reverse transcribed. PCR successfully amplified CTFV cDNA from a positive control, but none have been detected in the tick samples to date. In contrast, using primers for a tick actin gene, PCR resulted in an amplicon of the expected size. This would suggest that the nucleic acids were collected from the tick. While no positive results for CTFV in the ticks have been obtained, there is reason to believe that the protocols developed are working. Our results are inconsistent with other studies that have found the virus in 21% of ticks sampled in Wyoming.

GROUNDWATER/SURFACE WATER INTERACTIONS ON THE CLARK FORK RIVER

Eva Hover *, Geological Engineering, Montana Tech, Butte

Grant-Kohr's Ranch is a National Historic Site located near Deer Lodge, MT. The ranch stretches along a two mile reach of the Clark Fork River, which has been contaminated by mine tailings. Plans for remediation are in progress, and information regarding where groundwater enters and leaves the river is important in this remediation process. A handheld temperature (T) and specific conductivity (SC) probe was used to look for spatial anomalies of these parameters that may indicate where groundwater enters the river. To do this, fifteen cross sections consisting of several T and SC readings were collected perpendicular to flow, and several readings were collected along the river banks (no cross sections) between each cross section. Significant spikes in specific conductivity values are consistent with groundwater entering the river. Zones of low high water temperatures in the winter is consistent with areas where groundwater warmer than the surface water is infiltrating the river. This idea holds true in warmer months as well, as areas where low temperatures are logged may show where cooler groundwater is infiltrating the warmer surface water. Radon-222 (222Rn) was collected along both the right and left banks at all fifteen monitoring locations to verify groundwater inputs inferred from T and SC measurements. 222Rn de-gasses quickly when a river flows from a gaining to a losing reach, so this is an ideal validation. Local lithology suggests a sandy gravelly aquifer that would interact with surface water. Both 222Rn combined with T and SC suggest intermittent zones of groundwater inflow along the river banks, often only occurring along one side of the river at a time.

INTEGRATION OF A FLUXGATE MAGNETOMETER AND GPS SYSTEM INTO AN UNMANNED AIRCRAFT SYSTEM

Sierra Luoma *, Geophysical Engineering, Montana Tech, Butte

Magnetic method has been widely used for exploration of magnetic minerals or rocks and the detection of artificial objects such as unexploded ordinances (UXOs). However, a ground-based magnetic survey is time-consuming. Aeromagnetic surveys will be faster and more efficient. In this project, we integrated a fluxgate magnetometer and GPS units with an unmanned aerial vehicle (UAV), i.e., Matrice 600 Pro, to measure magnetic anomalies for aero-geophysical exploration. To achieve this objective, we used an Arduino microprocessor to control a triaxial fluxgate magnetometer (FGM-301/310) and two GPS units (ANT-GPS-SH-SMA). Arduino IDE codes were written for simultaneous data collection from the magnetometer and the two GPS antennas and for storing the data to a microSD card. These components will be attached to the DJI Matrice 600 Pro UAV. The whole system will be tested using a UXO sample. We will fly the UAS with the magnetometer over a sample UXO. With the two GPS antennas, the location and orientation of the magnetometer is obtained so that the three components of the magnetic field can be calculated. The data collected will be processed to create a georeferenced map of magnetic fields and will be interpreted. The accuracy of the fluxgate magnetometer and UAV system will be evaluated.

CHARACTERIZING EXCITED STATE DIFFUSION IN PCDTBT USING TRANSIENT ABSORPTION MICROSCOPY

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Alexander R. Hathaway, Chemistry and Biochemistry, Montana State University, Bozeman
Erik M. Grumstrup, Department of Chemistry and Biochemistry and Montana Materials Science Program, Montana State University, Bozeman

Organic semiconducting polymers (OSPs) are an attractive alternative to traditional inorganic semiconductors for use in photovoltaic devices and other optoelectronic applications because they are cost effective and solution processable. Here we describe our efforts towards under-standing excited state transport in micron-scale domains of the OSP, poly [N-9’’-hepta-decanyl-2,7-carbazole-alt-5,5-(4’,7’-di-2-thienyl-2’,1’,3’-benzothiadiazole)] (PCDTBT) utilizing transient absorption microscopy (TAM). Using TAM, we directly image excited state diffusion across mi-cron scale domains of PCDTBT thin films, reducing the effects of morphological heterogeneity in these complex polymeric systems. To further understand exciton diffusion and dissociation dynamics we have begun the development of two individual experiments. This presentation will discuss the theoretical evaluation of both experiments as well as preliminary experimental development. The first experiment has been investigated by Monte Carlo simulation of exciton dissociation at microfabricated donor-acceptor interfaces. The second experiment relies on the fabrication of polymer devices so that carrier diffusion can be characterized under the influence of an electric field. Investigation and implementation of these experiments was made possible by support from the Montana Academy of Sciences and will provide a more thorough understanding of the excited state transport dynamics in micron scale regions of PCDTBT.

INVESTIGATING THE ROLE OF DNC-2 AND DLI-1 ON AMPA RECEPTOR MEDIATED BEHAVIORS IN C. ELEGANS

Natalie Oberding *, Biology, Carroll College, Helena
Stefanie Otto-Hitt, Biology, Carroll College, Helena

γ-amino-3-hydroxy-5methylisoxazole-4-proprionic acid (AMPA) receptors (AMPARs) are protein complexes involved in excitatory neurotransmission. AMPARs are tetrameric structures consisting of the pairings of GluA1, GluA2, GluA3, and GluA4 subunits. Dctn2 and Dync1li1 were found to interact with GluA2 in an immunoprecipitation screen that was performed on postnatal day 14 rat brains. DNC-2 and DLI-1 were identified as the C. elegans homologs of Dctn2 and Dync1li1, respectively, and are components of motor proteins that transport cargo throughout the cell. The goal of this project was to determine whether knocking down DNC-2 and DLI-1 by RNAi affects AMPA Receptor-mediated behaviors, including mechanosensation and chemosensation, in C. elegans.

HYALURONIC ACID CONJUGATED WITH ANTIOXIDANTS FOR TREATMENT OF CMV-INDUCED HEARING LOSS

Zoe Peach-Riley *, Hellgate High School, Missoula, MT

Cytomegalovirus (CMV) is the leading nongenetic cause of sensorineural hearing loss (SNHL) in children in the United States. Current antiviral treatments exist, however with only modest short-term improvements and uncertain long-term outcome as well as reports of complications. Because CMV causes SNHL through inflammation and oxidation of the inner ear, an anti-inflammatory compound such as hyaluronic acid (HA) can be modified and combined with an antioxidant compound like D-methionine as a potential treatment. Preliminary results indicate successful synthesis of the compound CMHA+Dmethionine as well as demonstrated cytocompatibility. Upcoming procedures will include testing the combined anti-inflammatory and antioxidant effects of the novel compound CMHA+D-methionine and investigating its potential to act as an effective treatment for CMV SNHL.

MECHANISTIC AND KINETIC INSIGHTS OF [CU(PDTC)L]

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Daniel Willems, Biological and Physical Science, Montana State University Billings, Billings
Alexander Fryett *, Biological and Physical Science, Montana State University Billings, Billings

Carbon tetrachloride (CT) is a known carcinogen. It is know that when pyridine-2,6-bis(thiocarboxylic acid) (PDTC) forms square planer coordinate complexes with copper(II) the combination can dechlorinate CT stoichiometrically. In this study three different complexes, where the position four ligands are varied Cu(PDTC)L (L= Cl-, CN-, and PPh3), were used to probe the mechanism of the dichlorination reaction. GC-MS kinetic assays were performed to assess the aqueous dechlorination kinetics while organic solvents were used in UV-Vis assays to test organic based solvents. Results on the UV-Vis show that Cu(PDTC)PPh3 shows the most significant reaction with CT in organic solvent, followed by Cu(PDTC)CN with very limited reactivity, and finally Cu(PDTC)Cl with no reaction. Water-based kinetics were done in phosphate buffer with Cu(PDTC)Cl, Cu(PDTC)CN, and Cu(PDTC)PPh3. Cu(PDTC)Cl showed the most significant loss in CT in air and under inert gas. In addition variable temperature kinetics assays were performed to elucidate activation energies for the reaction.

SINGLE PARENTS DESCRIBE BALANCING ROLES: PARENT AND STUDENT

Rayvn Scott *, Highlands College A. S., Montana Tech, Butte

The purpose of this qualitative study was to explore lived experiences described by single-parent students while balancing their roles: parent and student. A qualitative design included a hermeneutic approach with interpretation that consisted of a transcript coding process. Approximately 30 hours were spent reading through transcripts, while highlighting words which identified themes. Seven themes evolved: structure, stress, not enough time, guilt, isolation, self-care, perfectionism. For example, structure, isolation, and not enough time were themes reflected as Meg stated, “I know what a priority is and what isn’t. Friends are not a priority. It’s like this constant balancing act. Everything is going a million miles a minute, so fast - the teaching, homework, learning, my kids, the days, studying.” On-campus single-parent students’ academic support and family-friendly social activities were limited; inequities in social justice were evidenced. Following themes of structure, stress, and isolation, a homework-support group on campus was offered twice a week. There was scarce involvement by single-parent students. After the last six interviews, a different approach was used for support by following the themes of guilt and isolation. On campus, family-friendly social activities were offered and participation was successful. By offering family-friendly social activities on campus, single-parents received improved support while balancing their responsibilities of parent and student.

PROSOCIAL BEHAVIOR AND EMPATHY: ANALYSIS OF EMOTIONAL RESPONSES DURING THE USE OF TED TALK VIDEOS IN THE CLASSROOM

Robert Shelton *, Highlands College A. S., Montana Tech, Butte

Empathy may increase when observing prosocial behavior of others. The purpose of the study was to evaluate students’ emotional responses when observing prosocial behavior demonstrated in a Tedx Talk video through the lens of cognitive empathy and affective empathy. Tedx Talk videos are often shown in college classrooms to enhance learning outcomes. Student participants (N=157) completed the Interpersonal Reactivity Index pre/post assessment to examine if differences of empathy were measurable from before watching the video and after. Paired Samples t test Analyses were run to explore if there were differences between groups over time. Independent Samples t tests were used to understand differences between groups: age, gender and parental status. Results suggested statistically significant changes in empathy both over time and between groups.

REQUIREMENTS FOR AGGRESSION: ALTERING EAAT1 EXPRESSION TO MANIPULATE GLUTAMATE UPTAKE IN DROSOPHILA MELANOGASTER

Lucy Sirrs *, Hellgate High School, Missoula, MT

Aggression is a behavior required for survival and reproduction. Research has identified several neurons and neurotransmitters (signaling molecules) that are important for promoting aggression, but the neurotransmitter glutamate has only recently been identified, and its role is not fully understood. This research examines how manipulation of glutamate expression via altered expression of its transporter, EAAT1, alters aggression in the Drosophila model

organism. Glutamate function was reduced by increasing expression of the glutamate transporter, EAAT1 (Excitatory Amino Acid Transporter 1). EAAT1 recycles extracellular glutamate to regulate neuronal communication. By increasing the amount of EAAT1, more glutamate is recycled, reducing its ability to signal to the downstream neuron. EAAT1 expression was increased via controlled transgenic expression. Conversely, glutamate function was increased by reducing expression of EAAT1 via RNA interference. Using genetic tools and antibody labeling, it was verified that EAAT1 is widely expressed in glial cells throughout the brain. A reduction in glutamate produced male flies that were less aggressive compared to controls, similar to previous research that manipulated glutamate expression only in octopamine neurons. Specifically, experimental flies took longer to start fighting, lunged less, and produced fewer wing threats and wing extensions. Flies with increased glutamate levels were also less aggressive, though this may be due to limited function at the neuron or the neuromuscular junction, where a motor neuron and a muscle fiber meet. These results demonstrate glutamate function is required for sex-specific behavior and provide a foundation to determine the role of glutamate in aggression circuitry in any system.

CALIBRATION AND APPLICATION OF MONTANA TECH SHAKE TABLE APPARATUS

Brent Sordo *, Geological Engineering, Montana Tech, Butte

In the field of geotechnical engineering, a key task when designing earth structures is preparation for seismically induced loads. To directly study the impacts of these loads upon geologic structures, models or samples of the structures are tested with a seismic shake table, an apparatus that precisely accelerates back and forth according to a specific pattern. Montana Tech recently acquired a high quality, unique shake table, and this project was the first use of it. Initially, the table was installed and a booklet outlining its use and specifications created. With the table in use, the efficacy of its ability to model acceleration-induced loads was tested by subjecting a number of rock joint samples to seismic loads of varying intensity. The critical acceleration value, the value at which static friction is overcome, will then be identified and compared to other methods of frictional analysis such as tilt tests. The critical acceleration can be implemented into a calculation to find static coefficient of friction, as can a friction angle acquired from tilt tests of the same samples, allowing for direct comparison. Furthermore, samples subjected to sinusoidal wave patterns can also be compared to dynamically loaded discrete element models for further verification. This project itself is preliminary, buy with this relationship proven, the application of the shake table can proliferate to more complex simulations such as liquefaction, structural fundamental frequencies, and complex earthquake time histories.

COMPARISON & ANALYSIS OF LOCAL ENVIRONMENTAL METAGENOMICS AND DIVERSITY SEQUENCING DATA SETS

Lucas Stout *, Software Engineering, Montana Tech, Butte

Metagenomics is the rapidly advancing field that studies genetic material extracted directly from environmental samples. Recent advances in computational and sequencing methodologies now give an array of options to biologists who seek to analyze their samples; however, these new technologies are often run independently with little comparison of the results obtained between methods on the same sample. Prior to this study, field work by local biology instructors in conjunction with the labs of Drs. Marisa Pedulla and Alysia Cox made use of two such techniques to analyze the DNA of a locally-obtained soil sample. One technique, “shotgun,” or metagenomic sequencing, sequenced all of the DNA molecules in the sample; the second technique, “diversity” sequencing, only sequenced the molecules amplified from a single gene, the 16S ribosomal subunit rRNA gene, of the prokaryotic DNA in the sample. Because the 16S gene is highly recognizable and species-specific, the latter method also provided a count of the occurrences of each prokaryotic species. Due to this feature, the use of the 16S diversity sequencing approach is commonly utilized for studies aiming to understand prokaryotic species representation in samples. When metagenomics DNA sequences are known, represented species and their number of occurrences in a sample may also be derived computationally by comparison to procured databases of known sequences of organisms. This exploratory study compared the commercially obtained results of bacteria proportions in our sample by the diversity method, along with two experimental computational methods using these sequences compared to the public databases. Our hypothesis was that metagenomics data would provide the most accurate portrayal of bacteria in the sample at the phylum taxon. Results found significant disparities in results between each method, with implications in microbiome studies of the environment and human gut.

IN VITRO SYNTHESIS OF APATITE COATINGS ON TITANIUM ALLOY SP-700 TO IMPROVE BIOCOMPATIBILITY

Ben Suslavich *, Metallurgical & Materials Engineering, Montana Tech, Butte

Titanium and its alloys are commonly used in the biomedical industry for their superior strength, corrosion resistance, machineability, fatigue resistance, elastic moduli, and biocompatibility; however, endosseous implants struggle with osteointegration due to poor adhesion between the implant and the receiver’s bone. It is known that by synthesizing a bone-like apatite surface coating, integration of the implant with surrounding bone is not only quicker, promoting shorter recovery times, but also reduces interface stress concentrators creating a more robust implant. This study focused on synthesizing an apatite coating via the sodium titanate method on the titanium alloy SP-700 which is a contender to be used as an implant material having a similar elastic modulus to bone. The synthesized coatings, which formed after treatment in simulated body fluid solution were characterized for topography and elemental composition using SEM/EDS analyses. The results of this study show that it is possible to synthesis thin apatite layers on the surface of SP-700 with a topography conducive to improving bone growth onto the surface of the implant.

CLONING GRNAS TO ENABLE CRISPR-MEDIATED HUMAN GENE KNOCKOUTS

Teal Taylor *, Organismal Biology, Montana Tech, Butte

The human genome encodes over 80 members of the tripartite motif (TRIM) protein family. Many of the TRIM proteins are upregulated in cells responding to interferon-beta (IFNβ). Interestingly, screens have shown that approximately half of the TRIM proteins act on interferon signal transduction pathways in positive and negative feedback loops. Cell lines are being created that can be used as tools for identifying the step of IFN signaling acted upon by TRIM proteins. To create tools for this goal, we have cloned guide RNAs targeting IFNB, IFNAR1, IRF3, and IRF7 genes into the pSpCas9(BB)-2A-EGFP vector using recombination techniques. Success of cloning was confirmed by restriction digests and DNA sequencing. Human cells (293T) were successfully transfected and are currently being cultured to attain monoclonal EGFP+ lines. Screening for loss of gene expression in clonal lines will assessed by immunoblotting as well as tested functionally using luciferase reporter assays.

ERGONOMIC EVALUATION OF A HIGH-PERFORMANCE GAMING INPUT DEVICE AND A COMPARISON WITH TRADITIONAL DEVICES

Jacob Van Dyke *, Occupational Safety and Health, Montana Tech, Butte

Objective: The goal of this study is to address the need for research on the ergonomic effects of computer gaming by providing an objective, ergonomic evaluation of a gaming input device using a simulated gaming task that compares ergonomic and performance measures with traditional computer input devices. The computer input devices included one traditional, a rollerball, and a gaming input device.

Methods: 20 participants wore sEMG sensors placed on the abductor pollicis brevis of the right hand. The subjects' maximum voluntary contraction (MVC) was recorded at the beginning of the study. Performance was measured by recording the number of correct sequences that subjects completed during their trail, while force was measured using sEMG. A Welch's one-way ANOVA (Analysis of Variance) was used to analyze statistical significance of the data; a Games-Howell Pairwise Comparison test was used for the post hoc analysis.

Results: The difference in mean force exerted by the abductor pollicis brevis for the input devices was significant ($p<0.001$). The number of correct sequences completed by each subject, was also significant ($p<0.001$). Two post-hoc comparisons were statistically significant for each data set. The %MVC for the gaming input device was significantly different from both the rollerball input device ($p= 0.021$) and the traditional input device ($p<0.001$). The number of correct sequences for the traditional input device was significantly different from both the gaming input device ($p<0.001$) and the rollerball input device ($p<0.001$).

Conclusion: The results of this study suggest that there is a possibility that gaming input devices increase force exertion while performing gaming tasks which may increase the risk of developing MSD's of the hand and thumb, without an apparent increase in performance within the constraints of this study. Further research is needed to determine if similar results exist during a gaming task performed by actual gamers.

ELONGATOR FUNCTION IN THE ANTERIOR PITUITARY AND ITS RELEVANCE TO FAMILIAL DYSAUTONOMIA

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Familial Dysautonomia (FD) is a devastating neurodegenerative childhood disease characterized by a diminished number of autonomic neurons. FD children suffer from a multitude of autonomic symptoms including cardiovascular instability, gastrointestinal incoordination, and respiratory dysfunction. FD patients also exhibit an abnormal autonomic stress response, show poor growth velocity, and have difficulty gaining and maintaining weight. Treatment with growth hormone (GH) has been shown to increase growth velocity in FD patients. FD results from a mutation in the IKBKAP gene and diminished levels of the corresponding protein IKAP, a scaffolding protein that assembles a multi-subunit complex called Elongator. Elongator functions in the modification of tRNAs that mediate translation of AA- and AG-ending codons including lysine, glutamine, and glutamic acid. In the absence of Elongator, small AG biased genes are upregulated and large AA-biased genes are downregulated. IKAP is expressed throughout the autonomic nervous system and historically

FD has been considered a strictly neurological disease. Here we show that IKAP is robustly expressed in the pituitary gland, indicating a strong dependence on Elongator. We hypothesize that compromised growth in FD may actually result from dysfunction of somatotrophs in the anterior pituitary, a non-neuronal cell type. To test this hypothesis, we generated a conditional knockout (CKO) mouse where Ikbkap is selectively ablated in anterior pituitary somatotrophs. These CKO mice exhibit decreased growth compared to control littermates. Surprisingly, quantitative immunohistochemistry indicates that GH1 levels may actually be increased in the CKO pituitary. CaBP7, a calcium binding protein that negatively regulates vesicle trafficking, is also found at elevated levels in the CKO, likely because of its strong AG-bias. In combination, these results suggest that upregulation of CaBP7 may inhibit GH1 exocytosis from pituitary cells, decreasing the amount of circulating GH1 and compromising growth in FD patients.

Mineralogy of a Uranium-Copper Breccia Pipe

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In northern Arizona, uranium-rich breccia pipes are common, however, these deposits are rarely found to contain 36% copper. Energy Fuels sent fifteen drill-core samples from their Canyon mine to Montana Tech. These samples contain varying amounts of copper and uranium from different areas of the deposit. The samples were cut, trimmed, placed into epoxy, and polished. A total of 36 polished “plugs” were made. The plugs were scanned using an X-Ray Fluorescence (XRF) device to determine rough estimates of element composition. Each plug was then carefully observed under a reflected-light microscope. The mineralogy and estimated amounts of minerals were noted. Plugs that were seen to have interesting features, high amounts of unusual elements, or unknown minerals were placed in a scanning electron microscope (SEM). The SEM uses electron dispersive spectroscopy (EDS) to obtain a chemical analysis of a specific point on the plugs which aids in mineral identification. Some specimens were also examined by Raman spectroscopy and X-ray diffraction (XRD). The most abundant mineral found in our samples was tennantite (Cu6[Cu4(Fe,Zn)2]As4S13) followed by chalcopyrite (CuFeS2). We also found the minerals uraninite (UO2), pyrite (FeS2), bornite (Cu5FeS4), galena (PbS), sphalerite (ZnS), chalcocite (Cu2S), covellite (CuS), and rammelsbergite (NiAs2). These minerals were deposited by hydrothermal fluids into a quartz grain matrix, in which they act as a cement holding the grains together. The discovery of rammelsbergite, a nickel mineral, was an unexpected find. The results of this project will be sent to Energy Fuels where they will use the data to help in milling and metallurgy. This mineralogy study may also help the mine understand potential sources of metal contamination for future mining wastes. One of our recommendations to Energy Fuels will be to assay their ore for recoverable nickel in addition to the other metals (Cu, U, Pb).

POSTER ABSTRACTS

Alphabetical by First Author's Last Name
*** Denotes Presenter**

CREATING AN EFFECTIVE PUBLIC HEALTH CAMPAIGN REGARDING THE POTENTIAL HEALTH RISKS OF CHRONIC WASTING DISEASE

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Margaret (Mari) Eggers, Microbiology & Immunology, Montana State University, Bozeman

Creating an effective public health campaign regarding the potential health risks of Chronic Wasting Disease

Introduction: Chronic Wasting Disease (CWD) is a neurodegenerative disease in the family of Transmissible Spongiform Encephalopathies (TSE), also known as Prion diseases. CWD has recently been detected in cervids in Montana, posing a potential health risk to game meat consumers. Appropriate places to obtain information on CWD and test meat for infection are low profile. The purpose of this study is to identify and create an effective public health campaign about CWD.

Methods: Information regarding CWD is being obtained from the scientific literature and government websites. Additionally, presentations are being made to obtain ideas from the public. Both print and online educational materials will be made and disseminated to the MSU and Bozeman public.

Results: Recent experiments have shown that CWD can be transmitted to Macaques, genetically closely related to humans, via feeding infected muscle or brain tissue from elk and deer, including from asymptomatic cervids. Although there are no reported cases of animal to human transmission of CWD, people who have contact with and consume game meat are at possible risk of becoming infected. Prion diseases are 100% fatal. The State of Montana is offering the opportunity to get your game meat tested.

Conclusion: Despite all the risk factors, education regarding CWD in the community is limited to a couple State websites. More detailed information is needed, especially for hunters and butchers and must be disseminated to the local public.

ANALYSIS OF MODIFIED VACCINIA ANKARA TROPISM AND GROWTH CONDITIONS

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Vaccinia is a large enveloped virus belonging to the poxvirus family. It has linear, double stranded DNA, averaging about 190 kbp in length. Although vaccinia's origin is unknown, it is closely related to cowpox, smallpox and horsepox. It is used as a vaccine for smallpox. Recombinant vaccinia is also used a vaccine for multiple other diseases. We employed a mutant form of the virus, modified vaccinia Ankara (MVA), which has a limited host range and limited virulence compared to the wild-type vaccinia, which infects almost all cell types. MVA was established to infect the hamster cell line BHK-21. This cell line was used to test culture conditions and to determine the tissue culture infectious dose 50% (TCID50), which is the measure of the results. This work was undertaken to establish how different culture and infection conditions affect virus growth. This study reports several factors that influence growth and preliminary results for infection rates in several cell lines.

Effects of Dopamine Beta Hydroxylase Levels in a Mouse Model of FD

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Familial dysautonomia is a severe, recessive disease that devastates the peripheral nervous system, culminating in death of most patients by age 40. The most debilitating feature of familial dysautonomia is the severe autonomic crises that occur. These crises, which can sometimes last for days, cause extreme vomiting and nausea, among other symptoms. The crises have been shown to coincide with an increased level of circulating dopamine following stress. The current hypothesis suggests that elevated levels of tyrosine hydroxylase cause an overproduction of dopamine. The chromaffin cells cannot convert this dopamine into norepinephrine quickly enough; therefore, this dopamine is released into the blood stream. We propose an alternate hypothesis in which the levels of dopamine beta hydroxylase are instead reduced. Reduction of dopamine beta hydroxylase, the enzyme that converts dopamine to norepinephrine, would result in a larger amount of dopamine being released from chromaffin cells during the response to stress. This reduction in enzyme levels is also seen in dopamine beta hydroxylase deficiency, a disease that shares many of the same symptoms of familial dysautonomia. In support of this hypothesis, we have shown through quantitative RT-PCR that dopamine beta hydroxylase transcript levels are decreased in Wnt1-Cre; IkbkapLoxP/LoxP conditional knockout (CKO) embryos in which Ikbkap is ablated in the adrenal gland. Further analysis of the CKO using immunohistochemistry indicates that DBH protein levels may also be diminished as well as mis-localized within the cell.

Investigating the Binding of BH31-1 Derivatives to the Bcl-XL Protein

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In order to develop promising drugs for infectious disease, it is important to find molecules that specifically target the infectious agent without harming human cells. The Butler/Toenjes Lab has recently published an article showing that a small organic molecule called BH3I-1 and several of its derivatives have potent anti-fungal activity against Candida albicans, a common human pathogen. BH3I-1, however, is known to be toxic to human cells through binding to the Bcl-XL and Bcl-2 proteins. The goal of this project is to identify anti-fungal derivatives of BH3I-1 that do not bind the Bcl-XL and Bcl-2. Such BH3I-1 derivatives would have the potential for low human cell toxicity.

The Effects of Pulsed Electrical Stimulation on the Quadriceps Femoris Muscle Prior to Exercise

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Neuromuscular electrical stimulation (NMES) is a commonly employed modality for rehabilitation and is growing in utilization due to its physiological effect in muscular activation. Recent comparative studies have examined utilizing stimulation versus isometric exercise and differences between genders on the effects of administering NMES after physical activity for recuperative purposes, but very little information exists regarding the effects of pre-stimulation to specific muscle groups for strength enhancement. This study aimed to answer this question by pre-stimulating the quadriceps femoris muscle prior to resistance training to determine if there is greater improvement in overall strength performance of the lower body. Fifteen healthy, recreationally and/or competitively active college students (male and female, ages 21 + 3 years, weight 152.8 + 42.2 lbs, height 66.1 + 6 inches) with at least 2 years of resistance training experience performed a 8-week strength training program targeting the quadriceps femoris muscle and its synergists (gluteus maximus, adductor magnus, soleus, gastrocnemius) for three nonconsecutive days each week. Individuals were randomly selected for placement in either the experimental (n=7) or control group (n=8). The experimental group received pulsed NMES prior to the workout for 15 minutes. Training volume for each participant was collected weekly to measure participants' progress, and 1-repetition maximums were collected before and after the workout program to quantitatively determine any impact on strength from pulsed NMES. A Mann-Whitney U-test was used to statistically compare the improvements in strength performance between groups. The results indicated that pre-stimulation of the quadriceps muscle before exercise will result in enhanced strength performance. This study shows that administering NMES prior to resistance training is an efficient method of pre-activating muscle fibers, conditioning the muscle to tolerate longer and more forceful contractions during exercise, and allow for greater improvement in strength to be achieved more quickly.

XFEB, a Direct Target of ZIC1, is Involved in Neural Crest Development

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The peripheral nervous system, melanocytes and craniofacial cartilage and bone arise from neural crest cells that develop during early embryonic neural development. Transcription and signaling factors form a network to regulate this development. For example, it has been shown that Zic1 and Pax3 in conjunction are able to induce full neural crest cell development (Monsoro-Burq et al., 2005). Xfeb and Gbx2 also play roles during neural crest cell development as they are present in the same regions and developmental stages as the neural crest (Plouhinec et al., 2014; Li et al., 2009). A microarray identified Xfeb as a direct, downstream target of Zic1 (Li et al., 2006). An additional lab also identified Xfeb as a neural crest gene induced by Zic1 (Plouhinec et al., 2014). We hypothesize that Pax3,

Xfeb, Gbx2 and Zic1 are all part of the same gene regulatory network controlling neural crest development. To investigate the relationship between the Xfeb, Pax3, Gbx2, and Zic1 genes, we first upregulated Xfeb gene expression with sense RNA and down regulated Xfeb gene expression with morpholino oligonucleotides (MO). We used in situ hybridization to visualize neural crest induction by staining for Slug RNA expression, a known neural crest marker. Our results showed that embryos injected with Xfeb sense RNA expanded Slug expression while those injected with Xfeb MO diminished Slug expression. Given other labs' results suggesting that Zic1 plus Pax3 or Zic1 plus Gbx2 induced ectopic Slug expression, we will determine whether Xfeb plus Pax3 or Xfeb plus Gbx2 genes can induce ectopic Slug expression. These experiments will allow us to determine whether Xfeb acts in neural crest induction and will allow us to place Xfeb into the gene regulatory network that drives neural crest development.

LONG-TERM VEGETATION RESPONSE TO GRAZING ON A SOUTHWEST MONTANA FOOTHILLS RANGE

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Long-term vegetation data collected in area under known management provides an opportunity for understanding the potential implications of vegetation response to grazing management. Short-term studies (2-3 years) are unable to capture vegetation response due to the ecological inertia of the system and temporary fluctuations due to weather patterns. When Montana State University purchased Red Bluff Ranch in 1956, it was heavily stocked but the range was thought to be in acceptable condition. Stocking rates were lowered nonetheless. In 1958, 74 vegetation monitoring transects were established. In 2017 we attempted to relocate transects in two pastures currently used for winter grazing. Of the 28 transects in both pastures, we were able to relocate and read 11. Using original methodology, we collected basal cover data along five five-foot subplots randomly located around each transect. Data were analyzed using a paired Mann-Whitney U test. Cheatgrass (Bromus tectorum) exhibited the greatest change in cover, increasing from 0.4% in 1958 to 16.1% in 2017 ($P < 0.01$), and was present at 9 of the 11 monitoring sites. Perennial grass cover increased from 4.3% to 13.0% ($P < 0.01$). Litter cover increased from 49.2% to 58.4% ($P < 0.05$). Bare ground decreased from 35.2% to 3.0% ($P < 0.01$). The increase in cheatgrass cover explains a majority of the increase in litter and the extreme reduction in bare ground. The data indicate that the condition of the range may not have been as high as first thought, and, while cover of perennial species can increase under moderate stocking, rangeland may still be susceptible to invasion by cheatgrass. The invasion of cheatgrass may be attributable to the early spring flush of organic nitrogen associated with winter grazing.

HOST SPECIALIZATION OF THE PROKARYOTIC PREDATOR BDELLOVIBRIO BACTERIOVORUS

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Bdellovibrio bacteriovorus is a small gram-negative bacterium with the ability to parasitize other gram-negative bacteria via periplasmic invasion leading to host cell death. B. bacteriovorus is considered a generalist and as such has been touted as a potential "living antibiotic". To be useful in treating human/veterinary infections, antibiotics should have minimal impact on commensal microflora, yet the ability of B. bacteriovorus to specialize on specific hosts while ignoring others has not been documented. In this experiment, we

investigated the ability of B. bacteriovorus to specialize on one of two different hosts: Escherichia coli or Erwinia amylovora. Our results show that over approximately 875 generations of growth, some B. bacteriovorus populations grown only in the presence of E. coli demonstrate improved growth on E. coli and significantly diminished ability to infect and kill E. amylovora. B. bacteriovorus populations exposed only to E. amylovora show growth deficits on E. coli, but the magnitude of this effect is much smaller and perhaps reflects a longer association of the B. bacteriovorus strain used here with Erwinia spp. in nature. Future work examining the genetic basis of host preference in these populations will allow greater insight into mechanisms of host specialization and serve to inform the utility of B. bacteriovorus as a targeted rather than non-specific "living antibiotic".

Natural and Synthetic Drug Analysis in Blowfly Larvae and Pig Tissue

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Blowfly larvae (*Diptera: Calliphoridae*) has long been used in connection with human death. While most commonly used as a mechanism to determine time since death, blowfly larvae have come to be a part of drug analysis in human remains. This study investigates the presence of drugs, both natural and synthetic, in pig tissues which are consumed by blowfly larvae. The classes of drugs examined in this study include opiates, psychoactives, NSAIDs, and stimulants. These active ingredients will be extracted from natural and synthetic sources and injected into pig tissue. Blowfly larvae will then be exposed to the tissue, allowed to feed, and treated with chemical digestion to extract the active ingredients. The GC-MS will determine if there is any difference in compounds digested by the larvae when comparing the natural and synthetic sources. This study may allow forensic scientists to differentiate between natural and synthetic drug sources in remains based on blowfly larvae subjected to drug analyses.

Determining the Place of AQP-3B in the WNT/CA2+ Noncanonical Pathway

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During Xenopus laevis gastrulation, convergent extension is required for the mesoderm to extend into the embryo and shape the embryonic body plan. Recent results from our lab suggest that the inhibition of aquaporin3b (aqp3b) prevents convergent extension of the mesoderm and that aqp3b acts through noncanonical Wnt signaling. Wnt signaling is a key signal pathway for embryo and tissue development and is composed of the canonical and the noncanonical pathways. Our lab has shown that aqp3b acts through a specific noncanonical pathway, the Wnt/Ca2+ pathway, and acts upstream of the cytoplasmic Wnt signaling pathway member Disheveled (Dsh). Frizzled7 (Fz7) is a membrane receptor in the noncanonical Wnt/Ca2+ pathway which also acts upstream of Disheveled. Our question for this project is whether aqp3b acts upstream or downstream of Fz7? When Fz7 was present, protein kinase C fused to green fluorescent protein (PKC-GFP) attached to the cell membrane but when Fz7 was absent, PKC-GFP remained freely in the cytoplasm. This served as our control injections: PKC-GFP + fz7 or PKC-GFP alone, respectively. The experimental injections included a morpholino (MO), small oligonucleotide that inhibited aqp3b expression, or a control MO which did not inhibit aqp3b. We have shown that the MO has kept PKC-GFP localized to the cytoplasm despite the presence of Fz7, while the control MO is allowing PKC-GFP membrane attachment. These data demonstrate that aqp3b acts downstream of the Fz7 receptor in the noncanonical Wnt signal cascade.

INVESTIGATION OF ON-SITE WASTEWATER TREATMENT SYSTEM SUITABILITY FOR FLOODPLAINS (POSTER)

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Gallatin is the fastest growing county in Montana, hence land with high groundwater, including property within the floodplain, is under increasing pressure for development. Homes and businesses without access to municipal wastewater service are required to have a permitted on-site wastewater treatment system (OWTS) that can remove solids, nutrients and pathogens from waste before releasing the effluent into the environment. Failing OWTS can create public health risks. While drain fields for septic systems can be adapted to high groundwater by installing them in a "sand mound," limited literature indicates sand mounds cannot withstand flood events. The project goal is to determine whether sand mound systems or other OWTS have been proven to withstand flooding and hence would be appropriate technology for floodplain installation. Online and database literature searches were conducted regarding local health department regulations and policies regarding OWTS. Phone calls were also made to various health departments in the western part of the United States. There are articles from the EPA, the University of Wisconsin – Madison and the Nevada Division of Environmental Protection stating that sand mounds should not be placed in floodplains. There are some OWTS that have potential to safely work within the floodplain, which are being investigated further. A failing or inadequate system can cause a variety of environmental and public health risks. Gallatin County is quickly growing, and it is imperative that the septic systems in floodplains will not adversely affect public health because of flood events.

INFLUENZA GROWTH VARIATIONS WITHIN MDCK CELLS

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Regardless of the preventative measures taken to keep the seasonal influenza virus under control, millions of people still get sick and tens of thousands die each year in the United States alone. This virus does not only affect the health of our nation but is also an economic burden. One study found that $16.3 billion is the average lost earnings due to flu-related illness or death each year. The total economic losses of annual influenza amounted to $87.1 billion in the US on average. This is merely the standard virus that comes around on a yearly basis causing the average "flu season." During the 1918 Spanish influenza pandemic; this virus affected every age group in every region, it quickly spread worldwide. The purpose of this research study is to better understand influenza growth within a laboratory setting. This becomes important when growing high titer virus for use in challenge studies and other procedures, where the most effective dose of virus is needed to test the proposed vaccine. To be able to grow the virus, there need to be cells available for infection and propagation. For this purpose, the MDCK cell line was used. There are many conditions that can affect influenza growth in MDCK cells. The assay of virus concentration is also cumbersome and somewhat variable. We show here an analysis of some of the variables affecting growth and some comparison of alternate quantification methods. The preliminary data shown here include effects of cell confluence ('thickness") upon initial infection; the amount of virus inoculum added for infection; when and how much TPCK – trypsin to be added to the media; when to harvest; and the growth medium type and volume. We are reporting the effect of these parameters on resulting virus titer.

Synthesis and Characterization of [PD(PDCT)L] Coordination Compounds

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Carbon Tertrachloride (CT), a popular industrial solvent, and potential environmental contaminant of water and soil. An approximate ten square Kilometer plume of soil at the department of energy’s site in Hanford, Washington has been polluted with CCl4. Our research is based on the study of potential CT environmental remediation technologies. Furthermore, we are interested in 2,6-pyridinedithiocarboxylic acid, or PDTC, and the dechlorination properties of its copper based coordination compounds, [Cu(PDTC)L] X. We seek to understand the link between coordination compound electronic structure and reactivity. To further understand electronic structure and reactivity of these copper coordination compounds, we synthesized several Palladium-based (Pd) coordination compounds. In this study we present IR, UV-Vis, and NMR evidence for the successful synthesis of a series of [Pd(PDTC)L]X coordination compounds. Here L-Br, CN, PPh3 as well as the dimer species [Cu(PDTC)2].

Synthesis and Characterization of [CU(PDTC)L] Coordination Compound Series

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Carbon Tertrachloride (CT) is a potential environmental contaminants in water, and soil. We are interested in studying potential CT environmental remediation technologies. Specially we are interested in 2,6-pyridinedithiocarboxylic (PDTC) and the dechlorination properties of its copper based coordination compounds, [Cu(PDTC)L]X. In the interest of creating better performing more soluble coordination compounds we seek to understand the link between coordination compound electronic structure and reactivity. With the ultimate goal in mind we present IR, UV-Vis, and NMR evidence for the successful synthesis of a series of [Cu(PDTC)yL]X coordination compounds. Here L-Br, CN, PPh3 as well as the dimer species [Cu(PDTC)2]. These experimental spectra are compared to computational data calculated using DFT optimized structures utilizing a wide range of exchange and correlation density functionals.

Examining the Correlation Between Coliform Bacteria and Human Wastewater in Home Well Water

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Nearly 15% of the U.S. population relies on home wells for drinking water, and approximately 34% of U.S. wells test positive for coliform bacteria. However, the presence of coliform bacteria alone does not confirm the presence of fecal matter, leaving the well users uncertain of their health risk and which mitigation measures to take. Therefore, understanding the correlation between human waste and the presence of coliform bacteria is vital to public health. A significant correlation would inform well owners and public health practitioners that mitigation must include addressing home septic system(s) (the well owner's system as well as neighbors' upgradient systems). The goal of this project is to analyze rural residential well water on the Crow Reservation to determine the degree of correlation between coliform presence, E coli presence and markers of human wastewater. The three primary analytes we are looking for are caffeine, cotinine and urobilin. All three chemicals are biomarkers of human waste. The methodology we are using to identify and quantify analytes within our water samples is solid phase extraction to concentrate the unknowns for further analysis using Gas Chromatography Mass Spectrometry. Subsequent analysis with colleagues will determine whether there are any significant correlations between the biomarkers of human waste and (1) the presence of coliform bacteria, (2) the presence of E. coli bacteria and/or (3) the absence of either coliform or E. coli. I will present my results to team members at a monthly meeting of the Crow Environmental Health Steering Committee, whereupon my colleagues on the Crow Reservation will use the data collected to inform and work with home well owners to properly mitigate home well contamination. After the completion of this project my colleagues and I plan on presenting this project at an additional conference and publishing in a peer review journal.

DOES AQUAPORIN 3B AFFECT THE NUMBER AND CHARACTERISTICS OF CALCIUM WAVES IN THE NEURAL PLATE OF XENOPUS LAEVIS EMBRYOS?

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Early in the development of the nervous system, vertebrate embryos undergo neural tube closure. This is a process where the cells in the dorsal part of an embryo, the neural plate, constrict on their outward facing side (apical constriction) to form a tube. The Merzdorf lab has found that inhibiting expression of the protein Aquaporin 3b (Aqp3b) in Xenopus laevis embryos prevents neural tube closure from happening. Given that aqp3b is only expressed in a well-defined line along the outer edge of each side of the neural plate, this action at a distance suggests some form of intercellular communication. In fact, calcium waves are required for neural tube closure. My hypothesis is that Aqp3b triggers the calcium waves that cause neural plate cells to apically constrict. To address this question, the number and characteristics of calcium waves will be compared between normal embryos and embryos that have been inhibited from expressing Aqp3b. My specific hypothesis is that the neural plate in embryos with inhibited aquaporin expression will have fewer calcium waves with different characteristics. Working towards the goal of answering this question I have developed and finetuned a method for injecting the embryos with a calcium indicator and imaging them. I have begun collecting time lapse images of calcium activity during neural tube closure and am designing methods for analyzing the time lapses I capture. This research is significant because, while calcium transients are known to be necessary to neural tube closure, no triggers of these calcium waves are known.

HOW AQP3B INFLUENCES CONVERGENT EXTENSION THROUGH NONCANONICAL WNT SIGNALING

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Aquaporin-3b, Aqp3b, is an aquaglyceroporin, a membrane water channel, that is present during gastrulation and various other stages of embryonic development. Gastrulation organizes cells into germ layers, which will later form different body tissues. During gastrulation, cells fold into the embryo, then merge by convergent extension to form the long body axis. These cell movements are regulated by noncanonical Wnt signaling, an intercellular signaling pathway that controls the migration and polarity of tissues. When Aqp3b is inhibited using a morpholino oligonucleotide (MO), convergent extension does not occur properly, suggesting a link between Aqp3b and noncanonical Wnt signaling. To assay these defects, we use the Keller tissue explanting method to observe convergent extension. Our goal is to determine which Wnt signaling pathway(s) are influenced by Aqp3b. We conducted rescue experiments by inhibiting Aqp3b with morpholino oligonucleotides and co-injecting an RNA or DNA construct of several proteins involved in Wnt signaling. Successful rescue with Dvl1ΔDix and Dvl2ΔDix constructs indicated that Aqp3b is involved in noncanonical Wnt signaling, since DvlΔDix acts in all noncanonical Wnt signaling. Further, Aqp3b acts through the Wnt/Ca2+ subpathway, indicated by rescue by PKC and PMA, and through a branch of the Wnt/PCP pathway, indicated by successful rescue with RhoA but not with Rac1. Aqp3b does not directly affect the Wnt/Ror2 pathway. In conclusion, I have demonstrated that the ability of Aqp3b to influence convergent extension is dependent on noncanonical Wnt signaling, specifically the Wnt/Ca2+ pathway and the RhoA branch of Wnt/PCP pathway.

SMALL MOLECULE INHIBITION OF GRAM-POSITIVE BACTERIA S. AUREUS AND C. DIFFICILE

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With the advent of antimicrobial molecules in the mid nineteenth century countless people were saved from life threatening infections. With decades of use, antimicrobials put enormous selective pressure on targeted pathogens resulting in resistances forming in many common microbes. These resistant strains of pathogens result in over two million illnesses, 23,000 deaths every year, and billions of dollars in medical expenses. This represents a need to research new molecules that have antimicrobial properties to combat the rise of resistance and prevent undue suffering to those afflicted. A small molecule is under investigation now that displays promising antimicrobial properties in preliminary studies against Candida species and many pathogenic gram-positive bacteria. The gram-positive bacteria that are inhibited by this small molecule at include Staphylococcus aureus, Streptococcus pneumonia, Listeria monocytogenes, Staphylococcus epidermidis, Streptococcus pyogenes, Bacillus cereus and Clostridium difficile. Research is currently focused on S. aureus and C. difficile with disk assays performed on both and plate assays performed on S. aureus to narrow down the minimum inhibitory concentration. C. difficile has a zone of inhibition of 9mm, while S. aureus has zone of inhibition at 10mm. The plate assay for S. aureus has shown a minimum inhibitory concentration to be between 150-100 uM of the small molecule, though further studies are needed. Plans are being made to find the mechanism of action of the small molecule by using mutant strains of S. aureus.

MATERNAL EFFECTS OF ASEPTIC AND SEPTIC INJURY ON EMBRYONIC LARVAL GENE EXPRESSION IN THE TOBACCO HORNWORM, MANDUCA SEXTA

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Cross-generational effects of physical and pathogenic stress have been demonstrated in several insect groups, including our model insect Manduca sexta. Prior studies in our laboratory have shown that maternal exposure to the soil-dwelling gram-negative bacteria, Serratia marcescens, just prior to adult eclosion alters egg morphology and larval immunity. Our goal is to identify mechanisms underlaying pathogen-associated parental effects on offspring. The current study advances this goal through measurement of embryonic size, embryonic histone modification, and both embryonic and larval gene expression. Two days prior to eclosion, parents were injected with saline, heat killed S. marcescens, or live S. marcescens. Embryos were collected at 24 (+/- 2) h or permitted to hatch for clearance assays (first instar) or measurement of fat body gene expression (fourth instar). We find that maternal, but not paternal, pathogen exposure significantly increases egg volume variability, and that maternal pathogen exposure may delay hatching. Furthermore, maternal injection with bacteria conferred on their offspring an enhanced ability to clear infection when compared to their saline injected peers. Histone analysis revealed that maternal treatment does not globally alter embryonic histones, however, several immune-related genes demonstrated altered expression in both embryos and fourth instar larvae.

COXIELLA BURNETII'S INFECTION-SPECIFIC SMALL RNA 12 (CBSR12) TARGETS CARA AND METK TRANSCRIPTS

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Coxiella burnetii is an obligate intracellular bacterial pathogen and the etiological agent of Q fever. Previous transcriptome analysis of C. burnetii by our group revealed several novel small RNAs (sRNAs) of varying sizes and expression patterns. Sequence alignments of these sRNA's across all strains of C. burnetii show strong conservation, indicating a functional role for these RNA's in C. burnetii's intracellular lifestyle. Furthermore, during C.

burnetii's biphasic life cycle of metabolically active (LCV) and inactive (SCV) states, several of the sRNA's have shown differential expression in SCV and LCV cells via RNA-Seq and Northern blot analyses. One such sRNA, termed CbsR12, showed a marked upregulation in infected Vero host cells when compared to bacteria grown in axenic media. Additionally, RNA-Seq data and qRT-PCR analyses show a marked upregulation of CbsR12 in LCV cells compared to SCV cells. Here, we show that C. burnetii RNase III cleaves CbsR12 into two fragments, an observation supported by both in silico and 5' RACE analyses. in silico sRNA target prediction programs were used to determine possible mRNA targets of Cbsr12. We subsequently determined through in vitro electrophoretic mobility shift assays (EMSAs) and in vivo luciferase reporter assays, that CbsR12 binds carA transcripts, which codes for carbamoyl-phosphate synthase subunit A, and metK transcripts, which codes for S-adenosyl methionine synthetase. These genes code for essential enzymes involved in pyrimidine biosynthesis and the methionine cycle, respectively.

GAP JUNCTION EXPRESSION AND FUNCTION IN XENOPUS LAEVIS EMBRYOS

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Connexins are intermembrane proteins that form protein complexes called connexons. These complexes bind together to form intercellular pores, called gap junctions, by binding to connexons of adjacent cells. These pores allow for passive transport of small molecules and ions involved in intercellular communication. Because gap junctions are required to transport of these ions, it is hypothesized that gap junctions are involved in developmental processes such as convergent extension during the processes of gastrulation and neurulation. In order to determine the role these genes play in development we must first determine the stages where they are expressed. To do this, cDNA (DNA reverse transcribed from RNA to only contain expressed genes) was made from developmental stages of Xenopus laevis embryos. The stages used were stage 6 (prior to activation of embryonic transcription), stage 8 (blastula), stages 10 and 12 (gastrula), stages 14, 16, 18 (neurula) and stage 20 (tailbud). This cDNA was then standardized by PCR using primers specific to ef1 alpha. This series of cDNA was then used as a template for PCR reactions of Xenopus connexin genes. The PCR reactions were then analyzed via gel electrophoresis in order to determine at what stages of development each gene is expressed. Gap junction gene alpha 3 was found to be expressed throughout neurulation and Gap junction gene alpha 7 was found to be expressed at all stages that were tested. Gap junction gene beta 1 and beta 2 were found to be expressed throughout gastrulation and neurulation. Gap junction gene alpha 2 was found to be maternally expressed as well as expressed in early gastrula, while Gap junction gene alpha 5 and alpha 4 are only expressed though early gastrulation. The next step of this project will be to use In Situ Hybridization to detect localized gene expression in embryotic tissue.

ASSESSMENT OF THE OCCURRENCE AND DISTRIBUTION OF UNPERMITTED WASTEWATER TREATMENT SYSTEMS ALONG A SECTION OF BOZEMAN CREEK AND IDENTIFICATION OF RECOMMENDATIONS TO ADDRESS FECAL CONTAMINATION

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Introduction - Bozeman Creek is considered “impaired” because sediment, nutrients and E. coli are at levels that impair the use of water for beneficial purposes such as irrigation or recreation. These E. coli can come from wildlife, livestock, pets or leaking septic tanks. Previous monitoring points to wastewater from septic systems as a major source of contamination to this stream. The research goal is to assess the impact of unpermitted wastewater systems on Bozeman Creek and to identify recommendations to address aging septic systems.

Methods - Online Health Department records are being reviewed to research the following questions: 1) What percentage of septic systems along a defined area of Bozeman Creek are unpermitted and what are their locations? 2) What percentage of permitted systems along a defined area of Bozeman Creek are >25 years old, and what are their locations? Additionally, a literature search is being conducted to identify best practices for the department and partners to address the status of onsite wastewater treatment along Bozeman Creek. GIS model is being created to assess which properties are most at risk of contaminating the creek.

Results - 34 properties were identified along the portion of Bozeman creek; 47 % of these properties lack septic system permits. Of those with permits, 56% are systems 25 years or older. Development of a GIS risk assessment model is underway.

Conclusions - The results of this project will be useful to health department staff to address wastewater sources contaminating Bozeman creek and serve as a model for other waterways.