



# 2018 Annual Meeting

## Montana Academy of Sciences

Student Union, Montana Tech, April 6<sup>th</sup> & 7<sup>th</sup>

### Abstracts for Poster Presentations

(alphabetical by title)

#### ANALYSIS OF MODIFIED VACCINIA ANKARA TROPISM AND GROWTH CONDITIONS (POSTER)

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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.

Key Words: Vaccinia, poxvirus, TCID50

#### 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 (POSTER)

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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. 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. Thirty-four 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. 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.

Key words: septic system, wastewater, Bozeman Creek, GIS, risk assessments

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

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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 (EMSA) 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.

Key words: small RNA, *Coxiella burnetii*, *metK*, *carA*, CbsR12

## CREATING AN EFFECTIVE PUBLIC HEALTH CAMPAIGN REGARDING THE POTENTIAL HEALTH RISKS OF CHRONIC WASTING DISEASE (POSTER)

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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. 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. 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. 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.

Key words: awareness, cervids, hunters, prion, zoonotic diseases

## DETERMINING THE PLACE OF AQP-3B IN THE WNT/CA<sup>2+</sup> NONCANONICAL PATHWAY (POSTER)

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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/Ca<sup>2+</sup> pathway, and acts upstream of the cytoplasmic Wnt signaling pathway member Disheveled (Dsh). Frizzled7 (Fz7) is a membrane receptor in the noncanonical Wnt/Ca<sup>2+</sup> 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.

Key words: aquaporin3b, noncanonical Wnt signaling, Frizzled7, protein kinase C, membrane attachment

## DOES AQUAPORIN 3B AFFECT THE NUMBER AND CHARACTERISTICS OF CALCIUM WAVES IN THE NEURAL PLATE OF XENOPUS LAEVIS EMBRYOS (POSTER)

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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 fine-tuned 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.

Key words: developmental biology, neural tube closure, calcium waves, *Xenopus laevis*, live imaging

## EFFECTS OF DOPAMINE BETA HYDROXYLASE LEVELS IN A MOUSE MODEL OF FD (POSTER)

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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.

Key words: familial dysautonomia, neurology, adrenal gland

## EXAMINING THE CORRELATION BETWEEN COLIFORM BACTERIA AND HUMAN WASTEWATER IN HOME WELL WATER (POSTER)

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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.

Key words: home wells, coliform bacteria, wastewater biomarkers, drinking water, mass spectroscopy

## GAP JUNCTION EXPRESSION AND FUNCTION IN XENOPUS LAEVIS EMBRYOS (POSTER)

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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 efl 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.

Key words: gap junctions, connexins, neural development, *Xenopus laevis*

## HOST SPECIALIZATION OF THE PROKARYOTIC PREDATOR BDELLOVIBRIO BACTERIOVORUS (POSTER)

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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".

Key words: *bdellovibrio bacteriovorus*, parasitize, host specialization, experimental evolution, living antibiotic

## HOW AQP3B INFLUENCES CONVERGENT EXTENSION THROUGH NONCANONICAL WNT SIGNALING (POSTER)

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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 Dvl1 $\Delta$ Dix and Dvl2 $\Delta$ Dix constructs indicated that Aqp3b is involved in noncanonical Wnt signaling, since Dvl $\Delta$ Dix acts in all noncanonical Wnt signaling. Further, Aqp3b acts through the Wnt/Ca<sup>2+</sup> 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/Ca<sup>2+</sup> pathway and the RhoA branch of Wnt/PCP pathway.

Key words: *Xenopus*, gastrulation

## INFLUENZA GROWTH VARIATIONS WITHIN MDCK CELLS (POSTER)

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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.

Key words: influenza, virus titer, MDCK cells

## INVESTIGATING THE BINDING OF BH3I-1 DERIVATIVES TO THE BCL-XL PROTEIN (POSTER)

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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.

Key words: small molecules, yeast, Bcl-2

## 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.

Key words: wastewater treatment, floodplain, sand mound, public health

## LONG-TERM VEGETATION RESPONSE TO GRAZING ON A SOUTHWEST MONTANA FOOTHILLS RANGE (POSTER)

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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.

Key words: long term monitoring, livestock grazing, perennial grass cover, annual brome grass

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

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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 underlying 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.

Key words: gene expression, septic injury, aseptic injury, maternal effects, *Manduca sexta*

## NATURAL AND SYNTHETIC DRUG ANALYSIS IN BLOWFLY LARVAE AND PIG TISSUE (POSTER)

Haley Fallang \*, Chemistry, University of Providence, Great Falls

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.

Key words: Diptera: Calliphoridae, opiates, psychoactives, NSAIDs, stimulants

## SMALL MOLECULE INHIBITION OF GRAM-POSITIVE BACTERIA *S. AUREUS* AND *C. DIFFICILE* (POSTER)

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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 pneumoniae*, *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  $\mu\text{M}$  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*.

Key words: antibiotic, resistance, *C. difficile*, *S. aureus*

## SYNTHESIS AND CHARACTERIZATION OF [CU(PDTC)L] COORDINATION COMPOUND SERIES(A) (POSTER)

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Carbon tetrachloride (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, PPh<sub>3</sub> as well as the dimer species [Cu(PDTC)<sub>2</sub>]. These experimental spectra are compared to computational data calculated using DFT optimized structures utilizing a wide range of exchange and correlation density functionals.

Key words: PDTC, [Cu(PDTC)L], synthesis, dechlorination, remediation

## SYNTHESIS AND CHARACTERIZATION OF [PD(PDTC)L] COORDINATION COMPOUNDS(B) (POSTER)

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Carbon Tetrachloride (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 CCl<sub>4</sub>. 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, PPh<sub>3</sub> as well as the dimer species [Cu(PDTC)<sub>2</sub>].

Key words:

## THE EFFECTS OF PULSED ELECTRICAL STIMULATION ON THE QUADRICEPS FEMORIS MUSCLE PRIOR TO EXERCISE (POSTER)

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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.

Key words: neuromuscular electrical stimulation (NMES), 1-repetition maximum, resistance, strength, training

### XFEB, A DIRECT TARGET OF ZIC1, IS INVOLVED IN NEURAL CREST DEVELOPMENT (POSTER)

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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.

Key words: *Gbx2*, *Pax3*, *slug*, *Xenopus*