

Department of Biology
College of Science
Undergraduate Research Symposium

Spring Undergraduate Poster Session



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Undergraduate Research

Exploring canid monogamy: Characterization of the distribution of vasopressin 1a receptors in the brain of coyotes (*Canis Latrans*)

Madeline Measom, Sophia Adamis, Dr. Sara Freeman

Arginine vasopressin (AVP) is a neuropeptide hormone that has been shown to be a factor in the biological basis of social attachment in monogamous species. It functions in the brain to modulate social memory, territoriality, and social attachment between pair-bonded adult mates of the same species. Research has mostly focused on the vasopressin 1a receptors (AVPR1a) in the brains of socially monogamous rodents and non-human primates. These studies have demonstrated a critical role of AVP and AVPR1a in the neurobiology of social attachment, especially influencing species-specific, pairbond-related behaviors. Coyotes are a unique species in the context of social research because they are socially monogamous and have been shown to also exhibit sexual and genetic monogamy, which is rare among mammals. The goal of this study is to establish the distribution of AVPR1a throughout the coyote forebrain in order to compare their receptor map to other known monogamous species. This comparative work will lay the neuroanatomical foundation for future studies of the AVP system of coyotes. In order to map coyote AVPR1a, we will use five brains that were opportunistically collected from captive-housed coyotes at the USDA Millville Predator Research Center. The samples were fresh frozen on dry ice within hours of death and sectioned at 20 microns using a cryostat. We will process them using AVPR1a autoradiography according to procedures that are well established in literature. Our areas of interest include regions previously shown to be important in social behavior in other monogamous mammals: the lateral septum, ventral pallidum, cingulate cortex, and hypothalamus. We expect to find a high density of AVPR1a in all of our regions of interest. To our knowledge, this study is the first to examine measures of the AVP system in coyotes and will serve as the basis for future research on the biological basis of social behavior in coyotes.

Maillard reaction impact on rodent and human health

Oran Wasserman, Dr. Robert Ward

Dr. Ward from the NDFS department and I are researching the effects of the Maillard reaction on the human gut microbiome health. The Maillard reaction, which is also known as non-enzymatic browning, is the result of the reaction between reducing sugar and a free amino group of a protein or nucleic acid. The Maillard reaction is significant in foods since it contributes to the formation of pleasant aromas, golden-brown color. And contribute to food product quality. However, it is a very complex chemical reaction that can produce some toxic by-products, and some of the products may be toxic. The goal of the experiment is to further our understanding of how isolated products of the Maillard reaction make mice healthier and how to perfect a method to make those products reproducible

Construction of vectors for correction of the intestinal CFTR gene expression in Cystic Fibrosis sheep fetal fibroblasts

Kaden Bunch, Irina A. Polejaeva

Cystic Fibrosis (CF) is a recessive human genetic disease that is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. This gene is responsible to transport Cl⁻ and HCO₃⁻ anions in epithelial cells. Previously, we generated CFTR^{-/-} lambs using CRISPR/Cas9 and SCNT techniques. The CFTR^{-/-} lambs display many features similar to human CF disease, including meconium ileus (MI), pancreatic fibrosis, portal fibrosis and biliary hyperplasia, small gallbladder, and absence of vas deferens. In CF patients, MI affects only 15-20% of human babies, whereas it was observed in 100% of newborn CFTR^{-/-} lambs and was the primary cause of death. We here hypothesized that the transgenic expression of the ovine CFTR cDNA under regulation of an intestinal-specific expression promoter would promote the correction of MI in CFTR^{-/-} sheep. In this study, we are constructing three potential vectors with different promoters to be evaluated prior to the generation of transgenic animals. Rat intestinal Fatty Acid Binding Protein (iFABP), rat liver Fatty Acid Binding Protein (LFABP), and Villin1 promoters have already been characterized and successfully used for intestinal-specific expression. After digestion and ligation cloning, the three constructs will be sequenced to confirm the presence of all segments (promoter, cDNA, and vector) in the correct orientation. Subsequently, we plan to evaluate the transient gene expression of the constructs in CaCo-2 cells to ensure they are fully functional. Therefore, we will construct the pcDNA3.1>promoter>CFTR expression vector in order to generate intestine-CFTR transgenic CFTR^{-/-} sheep.

300 Recombinant Inbred Lines Used to Identify Genes Involved in Cowpea Resistance to Seed Beetles

Alex Lish, Frank Messina

Cowpea is an important source of dietary protein throughout the world, but especially in sub-Saharan Africa and South America. A major pest of cowpeas in storage is the seed beetle *Callosobruchus maculatus*. Recently, eight cowpea cultivars were crossed to develop 300 recombinant inbred lines (F8) with agriculturally desirable traits. Previous assays of the eight parents revealed significant variation among each component of larval fitness, including development time, weight at adult emergence, and survival. Here, we assayed the RILs themselves by measuring oviposition, survival, development time, and weight at adult emergence. Similar to the parents, we found significant variation in each fitness metric. We also found negative correlations between development time vs. adult weight and development time vs. survival, indicating that some RILs may be simultaneously poor for multiple fitness components. Genome-wide association will eventually be used to map genomic regions and identify candidate genes associated with beetle resistance. Future studies will use a second beetle population from a different geographic region to determine if the level of plant resistance and its genomic basis vary among pest populations.

A pilot study comparing maternal immunity, egg yolk immunity, and offspring wound healing in Side-blotched lizards (*Uta stansburiana*) following an immune challenge

Kelsi Elkins, Susannah French

Maternal allocation of physiological factors before birth can drastically alter offspring phenotype and quality. In vertebrates, mothers can invest antibodies or immune factors via egg yolk or placenta to provide protection from pathogens. Furthermore, infection or sickness during reproduction can alter maternal investment of immune components which may reduce the physiological costs of an immunological challenge. In this study, we compared the maternal immunity, egg yolk immunity, and offspring wound healing ability in Side-blotched lizards (*Uta stansburiana*) following an immune challenge. We hypothesized that offspring of immune challenged females would have larger eggs and more yolk investment of bactericidal factors relative to females given a saline control or no injection at all. In addition, we hypothesized that offspring of immune challenged females would be larger and have quicker wound healing ability relative to offspring of control females. In a split clutch design, we extracted yolk samples from half of the eggs within a clutch to measure egg yolk bactericidal capacity, while the other half were incubated until hatching to measure offspring size and wound healing ability. Results of this experiment will be discussed in my poster presentation at the USU Undergraduate Research Symposium.

Development of *Bacillus subtilis* as a Platform for Research and Education in Low Resource Settings

Andrew Walters, Dr. Charles Miller

Synthetic biology is the application of engineering philosophies to traditional biological research. It focuses on building standardized, modular, and reproducible genetic/cellular tools to solve translational problems across several fields, including healthcare, agriculture, and commodities. Advancements in synthetic biology mirror the progress that was made in electrical engineering and computer science, which revolutionized the world as we know it. This field is characterized by off the shelf components like the Arduino that are affordable and easily programmable. Synthetic biology has that same potential. This project aims to build an analog of the Arduino for synthetic biology. However, current tools for synthetic biology research require significant infrastructure. In our work, we have shown that *B. subtilis* can be engineered comparably to *E. coli* by transforming it with GFP. After a rigorous literature review, a mechanism for engineering *B. subtilis* using only agar plates was discovered. We aim to combine this method with other studies showing *B. subtilis* can be grown at room temperature to create an engineering platform that will allow research and education to be done in low resource settings like classrooms and remote communities.

Role of novel receptor GPR171 in chemotherapy-induced neuropathic pain

Taylor Edwards, Erin Bobeck

First-line chemotherapies against solid tumors are highly efficacious in reducing the tumor burden, but have many adverse side-effects including nerve damage, leading to chronic pain. Non-addictive, efficacious pain relievers are an area of active interest, and we propose a novel target to address this pressing issue. GPR171 is a G-Protein Coupled Receptor that was recently deorphanized and was identified to be expressed in the brain in regions that regulate reward, anxiety, and pain. Within the pain circuit, it was shown previously that systemic administration of the GPR171 agonist enhances morphine antinociception in acute pain tests. Preliminary data from our lab has shown that GPR171 activation can also alleviate persistent inflammatory pain. However, the role of this receptor has not been investigated in other chronic pain models. Given these findings in acute and inflammatory pain, we hypothesize that GPR171 can reduce neuropathic pain. To test this hypothesis, we investigate the role of GPR171 in chronic neuropathic pain. We tested the efficacy of a GPR171 agonist in a chemotherapy-induced neuropathy mouse model. Neuropathic pain was induced by injecting paclitaxel (16 mg/kg) followed by assessment of the pain-relieving effects of activating GPR171 receptors. Mechanical pain thresholds were assessed using Von Frey filaments. We observed an increase in mechanical thresholds following GPR171 agonist treatment. Further, using immunofluorescence we observed that there is a decrease in GPR171 receptors in the periaqueductal gray (PAG) of these mice that have neuropathic pain, indicating that the agonist can bind to the available receptors to produce pain relief. Overall, this study proposes that GPR171 may be a novel target for the treatment of neuropathic pain.

Determining the Function and Structure of Cms1, A Type V CRISPR Endonuclease

Adam Tonks, Ryan Jackson

Cms1 is a Type V endonuclease that contains a novel domain, shares little sequence homology with other Type V endonucleases, and in some organisms, is found near genes coding for other single-subunit nucleases. Studies in rice (*Oryza sativa*) have shown Cms1 capable of RNA-directed DNA editing. However, the mechanism of DNA cleavage remains unknown. Here we present biochemical data that demonstrate Cms1 from *Sulfuricurvum* processes an RNA guide and binds/cleaves single- and double-stranded DNA through RuvC nuclease motifs. 2-D classification of structures obtained by negative staining electron microscopy show a major conformational change between SuCms1 bound and unbound to an RNA guide. The predicted global structure appears to be different than those reported for other Type V effectors. These data provide for a greater understanding of Type V endonucleases and may provide an alternative tool for genome editing applications.

Effects of Sex and Autism on Oxytocin Receptors in the Substantia Nigra of the Human Brain

Kip Dooley, Sara Freeman

Oxytocin, a hormone present in the mammalian brain, has been shown to be a vital component of social function in animals and may have a role in the social deficits associated with Autism Spectrum Disorder in humans. Based on

previous studies from our lab, there are oxytocin receptors in the human substantia nigra, a basal ganglia structure in the midbrain that is important in both movement and reward pathways. The substantia nigra contains two subsections that are defined by the neurotransmitters they contain: the pars compacta, which is dopaminergic, and the pars reticulata, which is GABAergic. By localizing oxytocin receptors in either the pars compacta or pars reticulata, we can infer the role of that region as it relates to social function. We used immunohistochemistry to stain human brain tissue and selectively identify the rate limiting enzyme in the synthesis of dopamine, highlighting the dopaminergic sub unit, which will be overlaid with oxytocin receptor autoradiography films to be quantified in a neuroanatomically informed way. The stained tissue was acquired from four distinct groups: eight typically developing males, seven typically developing females, eight males with Autism Spectrum Disorder, and seven females with Autism Spectrum Disorder. We will be using our determination of the subunits of the substantia nigra to anatomically accurately analyze the oxytocin receptor binding to determine the effect of sex and autism on oxytocin receptor density. Future directions of this research are aimed at defining the role of the oxytocin system in individuals with Autism Spectrum Disorder and how it relates to the social deficits present in those individuals.

Exploring Canid Monogamy: Characterization of the Distribution of Oxytocin Receptors in the Brain of the Coyote (*Canis latrans*)

Trevor Anderson, Nate Johnson, Sara Freeman

Oxytocin is a neuropeptide that has been shown to be a factor in species that display social monogamy. Its neural actions are necessary for social memory of familiar individuals of the same species. Much research has been done on oxytocin receptors (OXTR) in the brains of socially monogamous rodents and non-human primates, and these studies have demonstrated a critical role of oxytocin in the neurobiology of social attachment. Coyotes are a unique species in the context of social research because they are socially monogamous and have been shown to also exhibit sexual and genetic monogamy. The goal of the current study is to establish the distribution of OXTR throughout the coyote forebrain in order to compare their receptor map to other known monogamous species and to lay the neuroanatomical foundation for future studies of the oxytocin system of coyotes. In order to map coyote OXTR, we used five brains that were opportunistically collected from captive-housed coyotes at the USDA Millville Predator Research Center. The samples were fresh frozen on dry ice within hours of death and sectioned at 20 microns using a cryostat. We then processed these using OXTR autoradiography, according to procedures that are well established in the literature. Our area of interest includes regions previously shown to be important in social behavior in other monogamous mammals: the nucleus accumbens, striatum, lateral septum, cingulate cortex, and the hypothalamic regions where oxytocin is synthesized, the supraoptic nucleus and the paraventricular nucleus. We found a high density of OXTR receptors in all of our regions of interest. To our knowledge, this study is the first to examine measures of the oxytocin system in coyotes and will serve as the basis for future research on the biological basis of sociality in coyotes.

The Effects of Flywheel Squat Training on Water Polo Athletes' Jump Height Out of the Water

Coulton Hirschi, Dr. Earadic Bressel

This study investigated the effects of flywheel squat training on water polo athletes' jump height out of the water. This was a prospective cohort design with a control period that involved testing sessions at weeks 0 (Pre), 4 (Pre2) and 8 (Post) of the study. Each testing session assessed maximum jump height on land and in-water (WJH) and foot speed during the in-water jump. The first four-week block served as a control period with no intervention, and the second four-week block was the experimental period where the subjects performed flywheel squat training twice a week. There was no significant main effect for peak or average foot speed, with insignificance and small effect sizes between all trials. There was a significant main effect ($p = .001$) interaction for the in-water jump height variable (WJH), with significant interaction between Pre1 and Post ($p < .0001$) with a Cohen's d of .4, but between other phases there showed no significant differences and small effect sizes.

Vestibular Autonomic Interaction During Galvanic Vestibular Stimulation

Galen Huffcutt, Chris Warren

Galvanic Vestibular Stimulation (GVS) is a scientific method meant to improve the balance of an individual. A small current is placed behind their ears which, when fired, stimulates the subject's vestibular system and restores balance. This is useful especially for people with head injuries or debilitating diseases. What is unclear, however, is the range of effect of this stimulus. Branches of the sympathetic nervous system are also located in and around the ear and could be affected by this current. We know that Transcutaneous Vagus Nerve Stimulation produces salivary alpha amylase, which is a hormonal marker indicating that the sympathetic nervous system has been activated. Therefore, if GVS is producing an increase in salivary alpha amylase, then we know that it is also stimulating the sympathetic nervous system. We hypothesize that GVS also stimulates the sympathetic nervous system. We will recruit about 30 participants ages 18-30 recruited from the USU student population. They will be screened with a GVS questionnaire and a physical readiness questionnaire. These participants will receive SONA credits as compensation. Each participant will have 2 sessions, each with the same task. During one session, they will receive GVS, and in the other they will receive a sham stimulation. At various points before, during, and after the GVS, we will be collecting saliva samples that we will freeze, centrifuge, and then analyze. Participants will also complete a standing balance task on a force plate in each session to reinforce previous GVS data. We expect GVS to increase activity in the sympathetic nervous system. If it doesn't, however, then the data is still significant and important to know. If it doesn't affect the sympathetic nervous system, then it works as intended without these unintended consequences. If it does, then this increased stimulation needs to be accounted for.

Effectiveness of a YF17D Subunit Vaccine with Adjuvant

Parker Webber, Justin Julander

An effective Yellow Fever vaccine has been in use for several decades. The live-attenuated 17D vaccine is very effective, induces long-term immunity and is protective. The vaccine, however, has some limitations, including the need for a cold chain, adverse events after infection of immunocompromised individuals and a reduced stockpile of vaccine due to recent outbreaks and renovation of vaccine production facilities. There is therefore a need for additional options for YFV vaccines and antivirals. ViroVax has developed a subunit vaccine that is immunogenic and has shown protective efficacy. The vaccine is based on the effective 17D vaccine, but would not have the risks associated with a live-attenuated vaccine. The present study is designed to confirm this efficacy and to compare immunogenicity and protective efficacy with this vaccine and different adjuvants.

The Effects of Elevation and Solar Incidence on Nesting Behavior of Different Bee Species in Logan Canyon

Bailee Rasmussen, Will Pearse, Michael Stemkovski

There has been a decrease in bee habitats around the world, largely due to advancements in agriculture. A decrease in the bee populations will have negative side effects on our crop production. Monitoring the nesting behavior of different species of bees and determining what factors contribute to their selection of nest-site will help ecologists protect these species. The objective of this study is to understand how landscape determines where different species of bees are able to live and forage. Objectives of this study are to 1) determine if a higher abundance are present and low or high elevations, 2) determine if a higher abundance are present at low or high degrees of solar incidence, and 3) determine if insect abundance changes over time. These objectives were met by collecting bees at different locations in Logan Canyon and comparing data using statistical analyses. I hypothesize that as elevation and solar incidence decreases, bee nesting behavior will increase.

Antimicrobial Activity of Artemisia tridentate

Kayla Suisse, David Suisse, Amita Kaundal

Many plants secrete substances to create a more favorable environment, including chemicals that kill pathogenic microbes or competing plants. *Artemisia tridentata*, also known as "Big Sagebrush," is prevalent in the Rocky Mountain region of the United States and is known to have antimicrobial capabilities. We will study the potential antimicrobial activity of *Artemisia tridentata*. Studies report that chemicals released by the leaves and branches of A.

tridentata affect bacteria native to deer rumen. Another study showed that 27 actinomycetes (anaerobic bacteria that form colonies) strains found in the rhizosphere of *A. tridentata* demonstrated antibacterial activities when tested on *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus*. Native Americans traditionally used *A. tridentata* to relieve stomach pain, colds, coughs, sore eyes, snake bites and as an insect repellent. Researchers found several compounds, including flavonoids, that can affect antimicrobial activity. Articles regarding antimicrobial activities in *A. tridentata* were published between 1967 and 2004. With the chronological gaps and considering the progress that biological and molecular technology has made in recent years, knowledge of the chemicals released by *A. tridentata* lies largely untapped. In this study, we will investigate the antimicrobial activities of the leaves, stem, roots, and flowers of *A. tridentata* initially by the agar well diffusion method and followed by validating with the agar disk diffusion method. We will check the antimicrobial activity of the extract from different plant parts of *A. tridentata* on common bacteria such as *E. coli*, *Bacillus subtilis*, and some *Pseudomonas* spp. of plant pathogens. The knowledge obtained from this research will further help in the identification and characterization of the secondary metabolites or chemicals involved in antimicrobial activity of sagebrush. Medicinal plants provide a healthy, natural alternative to conventional medication, and may lead to new insights on antibiotics and pharmaceuticals. Besides, *Artemisia tridentata* is a plant native to Utah and Idaho. It grows everywhere in the surrounding area, making it inexpensive (free) to produce.

Amphiphilic Neomycin Derivative

Taylor Shepherd, Tom Chang

Neomycin is an aminoglycoside antibiotic that is used to inhibit protein synthesis by binding to the bacterial ribosome. Bacteria have become more resistant to these treatments, so in previous years researchers have looked for other methods. Recently, researchers have reported amphiphilic neomycin which is selective toward bacteria. Our goal is to use amphiphilic neomycin and, through a series of reactions, attach a 12-carbon chain to amphiphilic neomycin. We will use this new compound on microbes to test for antimicrobial activity. We will then use the same methodology on 8 and 10 carbon chains. If found successful, this may lead to new medications for treating fungal and bacterial infections.

The Role of MicroRNA in Common Eastern Bumblebee Physiology and Behavior

Abby Tucker, Karen Kapheim

Eusociality is a social system involving division of labor found in insects, such as the common eastern bumblebee (*Bombus impatiens*), that results in a caste system with varied physiologies produced from the same genome. Division of labor in *Bombus impatiens* includes the queen that lays eggs and workers that cooperatively care for the brood. These two castes display different physiologies, especially ovary size and nutrient stores in the fat body, despite their shared genome, a phenomenon called polyphenism. We aim to investigate what genetic mechanisms (regulation of the expression of protein-coding genes) give rise to polyphenism, particularly the role of a microRNA sequence called miR-13b. MicroRNA (miRNA) regulates gene expression by binding to target messenger RNA molecules and inhibiting translation or causing degradation. In this study we plan to interfere with the expression of miR-13b by using an antagomir, a single-stranded synthetic inhibitor that is complementary to miR-13b, which will reduce the effect of miR-13b on gene expression. We are testing the hypothesis that altered gene expression will result in changes in the expressed phenotypes, especially in physiology and behavior, measured by ovarian and fat body development, which are physiological correlations of division of labor in *B. impatiens*.

Young ovarian dependent, germ cell-independent influence on aging immune health in mice.

Brendon Charlton, Dr. Jeffery Mason

While the lifespan of humans has increased in time, the health span of aging individuals has not turned away from deterioration. Medicine has been essential in extending the time of dying since as health is continuing to worsen, this is especially so in post-menopausal women. At a young age, reproductively cycling women hold a greater health advantage over similarly aged men. After menopause, the health advantage switches in favor of men as the ovarian cyclicity becomes senescent in women. Post-menopausal women are more at risk to immune deficiencies in naïve and memory cells. Previous work has demonstrated that young, cycling ovaries transplanted into aged, female mice

improves several of those health conditions mentioned previously. More recently, young ovarian somatic cells were isolated from ovaries and transplanted into aged female mice. Four different ages of control female mice whom had their original ovaries were compared to each other and surgery mice; 29 months, 20 months, 11 months, and 6 months. Treated mice consisted of; germ cell-containing mice of 20 months of age, germ cell depleted mice of 20 months of age, and ovarian somatic cell injected mice of 20 months of age. Changes in immune system cells were analyzed using a two-factor ANOVA. The data analyzed showed a significant increase of naïve and memory cells in all three mice groups that were tested compared to the controls. It is the hope of this research to improve the health of aging individuals.

Sex Differences in MAP Kinase Activation in the Periaqueductal Gray After Morphine Treatment

Ashley McCarty, Erin N. Bobeck

Morphine is a potent opioid analgesic, but its long term use can lead to negative side effects, including tolerance, which is a decrease in the effectiveness of the opioid. An area of active interest is looking into the molecular effects of chronic morphine treatment in the Periaqueductal gray (PAG), a brain region that controls descending pain modulation. One such molecular target within the PAG is extracellular-signal regulated kinase 1/2 (ERK). Previous studies have shown that pharmacological inhibition of ERK enhanced morphine tolerance, indicating that ERK activity is associated with better responsiveness to morphine. The PAG is known to contain a heterogeneous population of neurons including GABA and glutamate subtypes. However, which neurons ERK is activated in within the PAG following morphine tolerance is unknown. Further, there are known differences in PAG activity between male and female mice. These sex-differences have not been well studied after morphine tolerance using acute pain tests. The purpose of this research is to investigate differences in ERK activation following morphine tolerance in male and female mice. We treated wild-type male and female mice with morphine (10 mg/kg, i.p.) or saline for 5 days to induce morphine tolerance, following which both behavior and protein immunofluorescence were assessed. We observe sex-specific differences in ERK activation levels and morphine antinociceptive tolerance in mice. We also assessed co-localization of ERK with GABA and glutamate neurons after morphine tolerance. The study will help us understand the cell-type specificity of kinase activation following morphine tolerance. Further this will give us more information about the nature of neurons that are contributing to sex-differences in opioid functions within the PAG.

The Effects of Orchard Management Style on Pear Flower's Fungal Diversity --Impacting Resistance to Fire Blight

Chase Nelson, Robert Schaeffer

Pear Trees yield fruit that is a commercial staple across many countries and cultures. Pear is also the main victim of an infectious bacterium, *Erwinia amylovora*. *E. amylovora* takes advantage of damaged tissue and pollinator vectors to infect Pear trees, leading to a progressively fatal disease called Fire Blight. Fire Blight survives through winters and is highly contagious. Beginning in one flower or leaf, it can spread to sections of branches, entire trees, and even jump from tree to tree within an orchard. An orchard infected with Fire Blight can be devastated economically by reduced yield. Microbial competition through diversity may limit the establishment *E. amylovora* by occupying niches, direct cell to cell interaction, and other mechanisms. This study investigates how the management style of the orchard (the treatments the trees undergo) impacts the microbial diversity of the Pear Flowers, a key site of infection. The DNA of the microbes from flowers belonging to trees sampled from Organic, Conventional and Soft management types was sequenced and analyzed. Soft management showed statistically diverse microbes indicating a direct relationship between management type and microbial diversity. This, coupled with further research, can inform growers on best management techniques and bring us closer and provide quality data for further investigation into solutions for Fire Blight.

A test of the applicability of Island Biogeography theory to Great Basin stream invertebrates

Allie Huish, Dr. Charles P. Hawkins

This project tests if Island Biogeography theory applies to aquatic invertebrate assemblages in isolated stream systems in the Great Basin of the United States. Island Biogeography theory predicts that island area and distance to sources of colonists will directly affect species richness via a dynamic equilibrium between immigration and

extinction rates. We defined islands as mountain ranges above 2000 meters in elevation. We hypothesize that macroinvertebrate richness in the Great Basin will be positively correlated with the area of mountain islands and negatively correlated with the distance to other mountain islands. We will test these hypotheses by assessing how taxonomic richness in previously collected stream benthic samples vary across different mountain ranges in the Great Basin. We will use ArcGIS to measure the area of each mountain range, their stream lengths (another measure of island size), and the proximity of mountain ranges to one another. High-mountain streams in an otherwise ocean of desert are important sources of aquatic biodiversity in arid regions. Understanding how habitat isolation and connectivity influence aquatic biodiversity will help inform policy regarding how to best protect and conserve aquatic biota in these regions.

Temperature and prey morphology influence attack rate and handling time in a predator-prey interaction.

Miles Robertson, Edd Hammill

Functional responses describe how the proportion of prey a predator consumes changes as prey density increases. For any predator feeding on a single prey species, its functional response is determined by two parameters: first, the rate at which it encounters its prey (i.e, attack rate), and the amount of time required to handle prey before it's ready to resume hunting. The flatworm *Stenostomum* is a predator of two species of *Paramecium*: *P. multimicronucleatum* and *P. aurelia*. *P. multimicronucleatum* are much larger than *P. aurelia*, making them poorer prey. Here we show that the speed of movement in both predator and prey changes significantly with temperature, leading to changes in the rate of attack. We also show how prey morphology affects the amount of time required to handle prey. We fit a mechanistic model to our data to quantitatively demonstrate how these changes in attack rate and handling time affect overall rates of predation. Our results therefore demonstrate how individual-level differences in movement and morphology can scale up to affect the dynamics of simple foodwebs and show how simple but mechanistic models can be used to describe ecological processes.

The Effects of Estrogen and Orexin on Latent Inhibition

Michael Herron, Mona Buhusi

The behaviors of humans are highly influenced by neurotransmitters and hormones that affect attention, alertness, and associative learning. These include the hormone estrogen (which plays an interesting role in cognition and attentional processes) and the neurotransmitter orexin (a heavy influencer of goal-related behavior, wakefulness, and arousal). We hypothesize that orexin and estrogen will have synergistic effects on latent inhibition (LI), a phenomenon that shows whether an organism possesses the ability to ignore irrelevant stimuli, thus serving as an excellent measure of associative learning and attention. Our experimental design compares LI between groups, demonstrating how estrogen and orexin may affect the paradigm. Female mice, aged 3-4 months, were tested in a 2x2x2 design comparing estrogen against placebo and wild-type mice against those with inhibited orexin reception (knock-out mice). To regulate estrogen levels, we performed ovariectomy surgeries and administered regular estrogen or placebo injections. In a 5-phase experiment, about half the mice were pre-exposed to a stimulus and then conditioned with a foot shock, while the others did not receive pre-exposure. Freezing time was recorded upon subsequent exposure as a measure of LI. Our preliminary results indicate that wild-type animals, given placebo, will likely have unimpaired LI while animals that are lacking part of their orexin system show impaired LI, regardless of whether they received estrogen or placebo. Because our results are somewhat preliminary, our sample sizes are too small to draw conclusive results. However, the results found thus far are promising and will hopefully lead us to further elucidate on the roles that orexin and estrogen are playing in this paradigm.

Effects of Temperature and Relatedness on Plant Phenology

Jeffrey S. Adams, Amanda Gallinat, William Pearse

Phenology is the study of seasonal cycles in organisms. This includes events such as leaf and flower budding, fruit ripening, and senescence. Previous research has shown that the timing of phenological events is very important for an ecosystem and that this timing can be affected by environmental conditions. However, it is unknown whether spring and autumn events respond similarly to environmental conditions, and whether closely related species are

similar in their timing. Using historical data from 1883 to 1912, we developed a method of digitizing the data. We then asked how average seasonal temperature affects the timing of spring and autumn events and looked at if closely related species had similar responses to each other. We found that most of the species in the same genus had similar timing in phenological events. Higher spring temperatures are correlated with earlier budding. Autumn events did not have a consistent relationship with autumn temperatures. This suggests that spring and autumn events do not respond similarly to seasonal temperatures and that autumn events are affected more by conditions other than temperature. This also suggests that there are evolutionary constraints on phenology in plants.

Transformation of *L. kunkeei*

Rylee Dalton, Dr. Dennis Welker

Lactobacillus kunkeei is a bacterial associate of honeybees and was identified as the most frequent isolate recovered from both the crop and hindgut of the bee. It was also found in the midgut, honey, floral nectar, and beebread. This study analyzed the frequencies at which two strains of *L. kunkeei* were transformed with different plasmids that confer erythromycin and/or chloramphenicol resistance. Transforming *L. kunkeei* cells is essential to allow genetic manipulations to study the roles of specific genes in *L. kunkeei*'s potential probiotic functions in honeybees. *L. kunkeei* cells from strains 3L and YH15 were electroporated and then incubated for several hours to allow the expression of the erythromycin or chloramphenicol resistance gene carried on the vectors (6 different vectors were used). The cells were then plated on MRS agar with erythromycin or chloramphenicol and incubated for 2-4 days to allow colonies to appear. The colonies were counted and transformation efficiencies calculated for each vector. All vectors transformed the *L. kunkeei* strain 3L. The vector with the highest transformation efficiency for strain 3L was vector pTRKH2. For strain YH15, two vectors (pGK13 and pNZ8048) did not yield any transformants. The vector with the highest transformation efficiency for strain YH15 was vector pGH9. The vectors that were used varied in their ability to transform *L. kunkeei* strains 3L and YH15. All six vectors were able to transform strain 3L but vectors pGK13 and pNZ8048 were not able to transform strain YH15. This is not due to an inability of the DNA to enter cells but must relate either to a restriction system that targets pGK13 and pNZ8048 DNA or an inability of pGK13 and pNZ8048 vectors to establish and replicate in YH15.

The Phylogenetics of the Cleptoparasitic Genus *Evagetes*

James Richardson, James Pitts

In this study, we furthered previous research on the taxonomy of pompilids (spider wasps) in the genus *Evagetes*. We extracted and sequenced the DNA from 18 specimens of *Evagetes* and performed phylogenetic analyses in order to answer lingering questions from the original research. Previous studies were more limited with available technology, and, therefore, no molecular studies were conducted. Using the knowledge we now have regarding DNA, and the technology available today, we were able to extract, shear, and analyze the pompilid DNA to answer three major questions. 1) Are the known species of *Evagetes* valid?, 2) How many species are in *Evagetes* (are there any unknown species)?, and 3) What are the relationships between species in this genus? Our study found that our understanding of *Evagetes* is just beginning. Although the described species appear valid, there are many additional species that need to be described, and the relationships of species within this group need to be re-evaluated.

Elucidating larval host preference in the endemic Hayden's ringlet butterfly (*Coenonympha haydenii*)

Camden Treat, Amy Springer

The Hayden's ringlet, *Coenonympha haydenii*, is an endemic species of butterfly that has recently undergone a notable decline in abundance. Despite this decline, the basic life history of this species, including what host plant they feed upon, remains unknown. If their host is rare or patchy in distribution, habitat fragmentation and subsequent loss of gene flow may be placing the Hayden's ringlet at risk for further population decline. Determining *C. haydenii*'s host plant, and the degree to which they are specialized on a particular host, is thus of crucial importance to the understanding the conservation risk faced by the Hayden's ringlet. In this study, we seek to elucidate the larval host preference through a herbivory preference assay. Wyoming. Specimens were placed in oviposition cups and eggs were acquired from each female. Neonate larvae were placed in a petri dish equidistant from excised cuttings of three potential host species (*Carex*, *Harebell*, and *Kentucky Bluegrass*). Images of the host samples were taken before and

after herbivory. ImageJ software was used to quantify the area of each leaf, and the difference between the before and after images were calculated. ImageJ methodology for quantifying leaf area was centered around the use of the 'Threshold' tool. Using this feature, novel methodologies were designed to account for debris, leaf discoloration, and other abnormalities across the images. Following analysis of before and after images, percentage eaten was calculated for each sample. Our research goal was to quantify the degree of larval preference for a specific species. We also intended to assess the herbivory variation seen across the larval population sites. This research design also explored the limitations of the ImageJ software when quantifying minute differences in digital image areas.

Qualification of Diagnostic Assays for the Detection of Bovine Respiratory Disease Viruses

Bryan Daines, Rebecca Strong, Bart Tarbet

Our goal is to fill the need for diagnostic tests for viruses involved in bovine respiratory disease (BRD) or shipping fever. BRD is a significant cause of respiratory illnesses leading to death in cattle. BRD is a combination of viral infection, environmental factors, and bacterial infection. Viral infection is primarily caused by five respiratory viruses, including: bovine respiratory syncytial virus, bovine parainfluenza-3 virus, infectious bovine rhinotracheitis (bovine herpesvirus type-1), and bovine viral diarrhoea virus types 1 and 2. My research is focused on qualification of a neutralization assay for Bovine Respiratory Syncytial Virus (BRSV). BRSV has a higher potential to cause primary lethal disease. The history of epidemics related to BRSV include an initial outbreak in the mid-80s, the disease is still likely to be found in isolated herds. As the disease becomes more endemic, viral-bacterial synergism in BRD has taken the focus. Synergy occurs in part due to bronchial epithelial cell necrosis and inhibition of pulmonary alveolar macrophage functions. Proper treatment and control of BRSV rely upon recognition, diagnosis, and treatment of cattle when initially infected with one of the viruses mentioned above. We have been working to qualify and validate the virus neutralization assay protocol for diagnostic use for BRSV. As we qualify the protocols, we attempt to optimize the assay to decrease variability in test results. We evaluate factors such as cell line, virus concentration, and incubation time. The qualified assays will allow for identification of cattle that have been exposed to BRSV. I have specifically worked with Bovine Herpes Virus, after triplicating our tests we have been receiving inconsistent results. These serums would require more testing to decrease potential variables and identify if additional serums would be required.

Mapping a novel G-protein coupled receptor to understand its role in morphine reward

Nick Beus, Dr. Erin Bobeck, Max McDermott

Due to the addictive nature of opioid drugs, those exposed to opioid treatments over long periods of time are at high risk of abusing their medication and possibly overdosing. Clearly, alternative pain therapeutics are needed. Research in our lab has shown that a new G-protein coupled receptor enhances the pain-relieving properties of morphine (McDermott, et al. 2019). This suggests its possibility in enhancing morphine while necessitating a lower dose of opioid. In order to understand this novel GPCR's mode of action and its actions in vivo, molecular and behavioral actions must be evaluated. Behavioral research in our lab has shown that an agonist for GPR171 is not rewarding. (Figure 1) In this experiment we map GPR171's throughout the reward pathway to further our understanding of this novel receptor's role in reward. IHC was used to mark GPR171's presence in the brain. Expression distribution in the prefrontal cortex, amygdala, hippocampus, and nucleus accumbens were visualized using a Keyence microscope. Locations of mu opioid receptors GPR171 in the prefrontal cortex, amygdala, hippocampus, and Nucleus Accumbens may imply modulatory action between GPR171 and Mu Opioid Receptors in reward structures of the brain (DiFeliceantio & Berridge, 2012; Hioki, et al., 2020; van Steenberg, Elkemo, & Leknes, 2019; Steffenson, et al., 2006).

Bovine Respiratory Disease Viruses using Viral Neutralization Assays

Adam Allen, Dr. Bart Tarbet

In preparation for a joint analysis between the Institution for Antiviral Research and Utah State University Veterinary Diagnostics Lab testing for various bovine respiratory disease-causing agents, we have been focused on developing a reliable model to test cell susceptibility to infection. The production of Madin-Darby Bovine Kidney (MDBK) and Madin-Darby Canine Kidney (MDCK) cells is important; they are well-established culture systems that may be used

to grow viruses for vaccine production. MDBK and MDCK cell lines provide manageable and robust cellular models for studying the methodology that viruses employ to infiltrate and replicate by using host cell machinery. Using Madin-Darby Bovine Kidney cells as a viral hosting cell we specifically want to look at five viral agents which can cause bovine respiratory disease are the targets of our study: bovine respiratory syncytial virus, bovine parainfluenza-3 virus, infectious bovine rhinotracheitis (bovine herpesvirus type-1), and bovine viral diarrhea virus types 1 and 2. Currently validated diagnostic tests for the viruses associated with bovine respiratory disease are not available at the Utah Veterinary Diagnostic Laboratory. Our work will qualify and validate virus neutralization assay protocols for diagnostic purposes. As the protocols reach a qualification state, our group will optimize the assays to decrease variability in the expected test results. Factors such as cell line, virus concentration, and incubation time will be part of the minimization process. Qualified assays will allow for identification of cattle that have been exposed to bovine respiratory viruses. In my particular subset in the experimental series focuses on the development of qualitative assay aspects related to bovine parainfluenza-3 virus.

Isolation, Purification and Analyses of Mice Gut Microbiome

Marielle Larsen, Dr. Abby Benninghoff

Colorectal cancer (CAC) is the second most common cancer-related death in the world. Approximately 1.4 million people suffer from inflammatory bowel disease, which is an etiological factor in development of colitis-associated colorectal cancer. Dietary interventions with the goal to reduce colon inflammation and encourage gut homeostasis may be a strategy to reduce the risk of CAC. The objective of this study was to determine whether the efficacy of dietary intervention with black raspberries (BRB) on colitis, tumorigenesis and the composition of gut microbiome of mice fed either a standard diet or a western type diet. Mice were fed one of four diets for 112 days. Mice were divided into one of two basal diet groups, a standard control diet that promotes health (AIN93G) or the Total Western Diet (TWD) which emulates the typical US nutrient intake. Mice were then further divided within their diet groups to be supplemented with 10% BRB (w/w) or not. At 21 days, these mice were injected with Azoxymethane (AOM), a carcinogen that specifically targets the colon. In conjunction with this injection, the mice were administered a 2% dextran sodium sulfate (DSS) via water for ten days which produced the necessary inflammation for tumorigenesis. Mice fecal pellets were collected throughout the project and are currently being processed with a series of protocols in order to separate the bacterial DNA in the fecal matter. Using various techniques, we isolate, purify and analyze the mice microbiome composition, giving us an insight to the changes of the microbiome throughout the project. For this presentation, I will be explaining the protocol used to analyze the dynamic mice microbiome.

To eat or not to eat? Feeding patterns in two groups of captive snakes

Jonah Fronk, Al Savitzky

One of the initially worrisome behaviors that captive snakes demonstrate is sudden rejection of food without seemingly any reason. Here we compare food rejection in wild-caught, temperate snakes (*Thamnophis elegans*), and captive-bred, tropical snakes (*Lamprophis fuliginos*) kept in identical conditions. By comparing the fraction of food consumed per feeding over a period of more than a year we were able to track changes in appetite among individuals and in the two species collectively. Though conditions were the same for both species and consistent year round, the *Thamnophis* colony showed a collective decrease in appetite during an extended period of the year and showed an increase afterwards. The *Lamprophis* colony showed more variation in appetite from feeding to feeding, but collectively showed very little change in appetite over time.

Internships

Teaching Secondary Science Education at Mount Logan Middle School

Nicole Tillmann, Dennis Welker

Mount Logan Middle School, located in Logan Utah, strives to live up to its district motto: "Ensure all students leave our schools ready to create a positive future for themselves and their community." I spent my internship living up to this motto by first observing science teaching by Mr. Bingham. I then helped out with student activities and learning by assisting students questions and concerns. I graded individual projects and essays. I also had the opportunity to independently teach students over a few class periods. I faced realistic challenges including guiding students to understand difficult concepts, supporting behavioral issues in the classroom, and pushing students with special challenges and individual education plans to work at a higher level. It was a great opportunity to help me learn if teaching is a career I wish to pursue by giving the actual experience of what happens in a classroom.

Farr West Animal Hospital

Brendon Charlton, Dennis Welker

Veterinary Medicine a very intriguing field for an individual to intern in. Prior to this opportunity I had very minimal experience in this field. At this hospital it was a quick learning curve as they let me get hands on the very first day. I mostly helped with taking notes during a physical examination or prepping patients for surgery. The hardest part of the internship for me was drawing blood from a jugular vein. With the help of the techs and guidance from the veterinary techs I have been able to consistently hit the vein. As I shadow the note taking process to learn how to do it before I did it by myself, I learned the importance of taking detailed notes to look back at for future exams. The details are important to be able to look back at to see if there were any prior issues or concerns that have gotten worse or resolved on their own. Learning basic operations of a veterinary clinic has made me excited for my future. This was huge because I am prepping to attend veterinary school.

Learn Valuable Job Skills While Making a Difference at Cache Valley Humane Society

Autumn Rees, Bri Smitm

The purpose of the project was to gain job experience related to my major while still being a full-time student and working part time. Research showed that volunteering was a beneficial way to learn job skills and often learn a broader variety of said skills since you are not assigned to one certain job but are able to travel around and diversify your skills in one workplace. I chose this workplace to be Cache Valley Humane Society because of my love of animals and my willingness to help them in any way I could. Methodology used while volunteering to improve my knowledge was assistance in veterinary work, animal behavioral assessments, clean up and care of sick or injured animals, working with the public for adoptions. The results were that after a few short months I already had a much greater understanding of how a non-profit was run, how to care for injured animals, and the basics of the administration of a simple veterinary clinic.

Epiphyte Abundance in Palms in Costa Rica

Katie King, Elleke Kofford, Jessica Murray

Epiphytes are plants that grow on another plant, but are not harmful to the host plant. One study found that epiphytes were more abundant on large branches and on branches that had an incline of 30° or less (Ingram and Nadkarni, 1993). Another study found that epiphytes are more abundant on larger trees which could be due to the additional growth of the tree, additional time for colonization, or increased genetic diversity of the microhabitat of the tree (Woods et al., 2015). Studies on epiphytes generally agree that epiphytes are more abundant on larger and older trees. Our research, conducted at La Selva Biological Station in Costa Rica, aimed to find if there was a difference in epiphyte significance between palms with stilt roots and palms without stilt roots. Epiphyte abundance in stilted palms was compared with epiphyte abundance in non-stilted palms. We found stilted and non-stilted palms on various old forest trails at La Selva. Data was collected from 20 trees of each root type. We counted and identified epiphytes up to 2.5 meters above the ground on a single tree. Statistical analyses were performed using a p-value of .05 to determine whether or not the difference in epiphyte abundance between the two root types is significant. The results of this study reflect no significant difference in total epiphyte abundance between palms with and without stilt roots, although the family Piperaceae, specifically *Polybotrya* sp., was significantly more present on palms without stilt roots. Based on our data, neither type of root in palms is more conducive to total epiphyte

abundance. This leads us to conclude that conservation efforts for both stilted and non-stilted palms are equally important to biodiversity in tropical rainforests.

Industrial Hemp Pests of Utah

Austin Taylor, Ryan Davis

Since legalizing the growth of non-potent (under 0.3% THC concentration) Cannabis in 2018, Utah farmers of industrial hemp are in need of state-specific hemp-pest resources. This project aims to create an easy-to-navigate print and online guide of the pests that affect industrial hemp grown in Utah. Little is known about which pests of industrial hemp are actually present in Utah due to the plant's recent legalization, thus the information contained in this guide will consist of the known hemp-pests to exist in Utah and surrounding states. In addition, this guide will contain potential and highly threatening pests to industrial hemp that are not known to occur in Utah but should be identifiable by all growers of industrial hemp. This guide will inform the reader of each pest's biology and management that are important to know when cultivating industrial hemp in Utah. Pest information was acquired from primary, peer-reviewed resources through various research databases and search engines. A list of pests found to plague industrial hemp was created. Each pest on this list was given a page within the guide where key biology and management information was stated. Photos of each pest and the damage they cause is also included. In addition, beneficial insects for industrial hemp are also included to ensure their survival rather than removal. The expected outcome of this project is that Utah farmers of industrial hemp may use this guide to help eliminate any pest problems or concerns they have whilst cultivating their crop.

Determining benthic macroinvertebrate abundance and diversity in two arctic lakes as part of an experimental lake warming

Allie Huish, Dr. Phaedra Budy

Global climate change threatens the integrity of freshwater ecosystems across the globe. At higher latitudes these ecosystems are experiencing the highest rates of temperature increase related to climate change. Freshwater invertebrate communities are important sources of food for fishes and bioindicators helping us understand the health of entire ecosystems. Our research is part of a larger experiment which aims to artificially warm an arctic lake by 2-4 °C, to try and determine the impact of rising temperatures on lake food webs and ecosystem function. Our objectives were to calculate benthic macroinvertebrate densities in two arctic lakes, Fog1 and Fog3 (one control and one reference), compare between these lakes as a function of abiotic and biotic differences, and assess whether these macroinvertebrate densities have responded to increases in lake warming. Using standard protocols, we analyzed benthic samples from 2016-2019 taken using an Ekman dredge from lake depths between 0-4 meters. We classified each taxon into four main groups: Diptera, Mollusca, zooplankton, and other aquatic macroinvertebrates and calculated the densities of each group (number/m²). Densities varied greatly from year to year, ranging from 609 organisms/meter² to 20,391 organisms/ meter². We observed a very large increase in "other aquatic macroinvertebrates" group in Fog1 (warmed lake) in 2018, comprised mostly of Hydracarina (mites) and including some unique Trichoptera and Annelids. While the densities of benthic macroinvertebrates ranged greatly across lakes and years, we did not observe a definitive trend in response to experimental warming (some groups increased one year but decreased the next and vice versa). In future work, we will explore the metabolic preferences of those taxonomic groups that may have responded to warming, as well as potential top down (e.g., fish predation) pressures on benthic macroinvertebrates.

Podiatry

Sam Tenhoeve, Dennis Welker

I work as a medical assistant in a podiatrist office here in Logan. I remove sutures, dress wounds, take x-rays, etc. The majority of our patients are in need of surgical intervention. Both podiatrists at the office are certified surgeons in foot reconstruction. They allow me to shadow surgery and are both great teachers. I have a great opportunity to learn everyday. I have thousands of hours of work experience along with hundreds of hours of surgical experience.

Students Come and Go, but Bacteria is Here to Stay

Madisen Pyper, Erin Jensen

For my internship, I researched how an analyzer used at Logan Regional Laboratory has impacted healthcare today. I explored how the BioFire analyzer has improved patient care in relation to testing times and costs. In order to make sure I understood the impacts this analyzer has had, I researched the involvement our lab has with this machine—from the cleaning processes to how the machine actually analyzes samples. My methods for researching the BioFire involved reading user manuals, reading through product descriptions on the BioFire website, reading peer reviewed articles on PCR testing, and consulting with medical technicians in the lab. With this information and the help of my mentor, we brainstormed ideas on how Logan Regional Laboratory's procedures involving the BioFire could be enhanced. Polymerase chain reaction (PCR) testing has, and continues to provide, cost effective options and rapid testing to the healthcare community. At Logan Regional Laboratory, the BioFire analyzer plays a big role in microbiology testing. The BioFire uses PCR testing to complete testing on various samples—blood cultures, respiratory, and gastrointestinal samples. The BioFire has provided many benefits to healthcare by decreasing the time it takes for sample testing to be done. Currently, using this process, test results can be received in less than an hour; whereas, in the past, results could take days to receive. This has allowed healthcare to treat patients faster with more targeted treatments and cut patient costs by reducing testing expenses and length of stays. Although there has been a lot of improvements due to the BioFire and PCR testing, there is still the potential of healthcare being improved with the BioFire and PCR testing in the future.

Teton Medical Group

Daniel Jones, Dr. Welker

I was able to participate in an internship with a Family Practice/Obstetric and Gynecology Medical Clinic in Rexburg, Idaho and St. Anthony, Idaho. The medical clinic's name is Teton Medical Group and there are two of them. I was able to intern at both sites and with many different medical professionals. My role in this internship was to observe to physicians, learn from the physicians, participate in paperwork, learn about the business and to learn about how a medical professional's personal life is. I was able to participate in a wide range of medical I am wanting to become a doctor so this internship has provided me an insight of what life would be like. The medical professionals I worked with gave me great advice and what separates good from bad doctors!

The Emergency Department

Taylor Burke, Liz Pedersen RN

This is an overview of my internship at Logan Regional Hospital's Emergency Department (LRH ED). My title while interning was known as Critical Care Technician. I learned many things about the emergency department and on this poster were a select few of the experiences of my internship. The overall experience was supervised by Liz Pedersen RN, who is the Emergency Department Manager for LRH. My coworkers as well helped in my learning and increase in my critical care skills. The objectives are not listed as to what I was working on specifically with my supervisor, but I do explain in the poster the new or improved skills I had acquired.