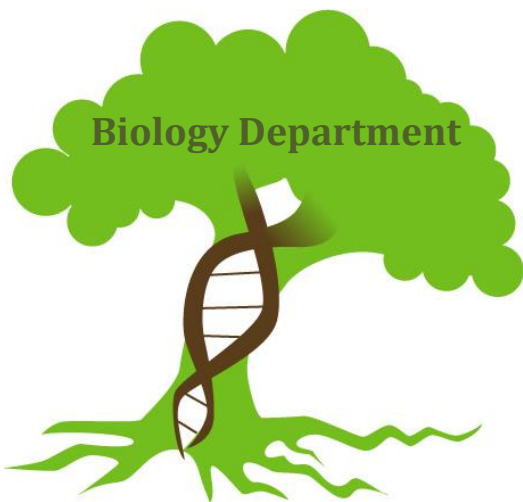


**22<sup>nd</sup> ANNUAL**

**JAMES MADISON UNIVERSITY**



**BIOSYMPOSIUM**

**2013**

**Keynote Presentation:**

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**Dr. Francis X Farrell**

Scientific Director, Drug Discovery  
Adjunct Professor, Microbiology and Immunology  
Drexel University School of Medicine

*"Biotherapeutics: Designing Novel Molecules to Treat Chronic Diseases"*

**Friday, April 19, 12:20-1:10, Bioscience 2007**

Lunch follows in second floor foyer

**The students and faculty in the Biology Department gratefully acknowledge support for their research from:**

- ❖ Jean D. Acton Scholarship
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*Student authors whose research has been supported by one of these gifts are noted with an asterisk.*

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## Schedule: Thursday April 18<sup>th</sup>

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SESSION 1	12:45pm-2:25pm	POSTERS	Bioscience 2009
P1	Emily Peterman, Andrew Loudon, Dr. Reid Harris, Dr. Eria Rebolgar, Dr. Alex Bannigan	Negative Chemotaxis of <i>Bd</i> in Response to Violacein and DAPG	
P2	Michael Partin, Kevin Libuit, Dr James Herrick	Identification and Characterization of Staphylococcus spp. in Stream Water and Sediment	
P3	Sinead Gilmore, Hailey Huebner, Dr. Terrie Rife, Dr. James Turner (VMI)	The Effect of Estrogen and Cardiovascular Health on Nitric Oxide Synthase Expression in a 'Listless' Model of Zebrafish ( <i>Danio rerio</i> )	
P4	Emily Thyroff, Dr. Heather Griscom	Experimental Greenhouse And Field Trials On American Ginseng <i>Panax Quinquefolium</i> : Implications For Reintroduction In Appalachia	
P5	Elena Balkanska, Seana Sears, Dr. James B. Herrick	Capture and Characterization of Stream Sediment Plasmids Conferring Resistance to Ceftazidime And Ciprofloxacin, Two Antibiotics Important In Human Clinical Therapy	
P6	Allyse Harris, Matt Wallace, Dr. Mark Gabriele	Development of Inputs from the Lateral Superior Olivary Nucleus to the Inferior Colliculus in EphA4 Mutant Mice Prior to Hearing Onset	
P7	Abdalla Sheikh, Dorottya Boisen, Amelia Tavangar , Chris Lantz, Ph.D, Ken Roth, Ph.D.	IL-3 Dependent Cytokine Regulation In <i>P. berghei</i> -infected BALB/c mice	
P8	Kimberly Okafor, Ian Brown, Dr. Steve Cresawn	Cytotoxicity Screening of Bacteriophage Genes in Mycobacteria	
P9	Kandyce Hamilton, Rebecca Smith, Dr. Terrie Rife	The Effect of High Fat Diet on Nitric Oxide Synthase Expression in the Kidney of Rat	
P10	Rebecca Dickey, Eria Rebolgar, Molly Bletz, Reid Harris	Persistence of Amphibian Probiotics in an Aquatic Environment	
P11	Sheyda Maghsoudi, Dr. Michael Renfroe	Antioxidant Analysis of Seven Spices from the Apiaceae Family	
P12	Jonathan Schmitz, Jonathan Monroe	Two plastidic $\beta$ -Amylases From Arabidopsis Are Involved In Osmotic Stress Resistance.	
P13	Alexandra Heil, Hannah Wines, Dr. Michael Renfroe	Quantitative Analysis of Antioxidants in Specialty Wines	
P14	Victoria Callahan, Dennis Churchin, Kristin McKenna, Andrew Sharp, Christopher Watson, Dr. Katrina Gobetz	Research Activities of the Trelawny Learning Community	





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## Schedule: Friday April 19<sup>th</sup>

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SESSION 4	FRI 2:00-4:00	POSTERS	BIOSCIENCE 2009
P15	Katie O'Donnell, Jessie Ward, Dr. Heather Griscom	Effects of Soil and Light on the Growth of <i>Castanea dentata</i> and Competing Species: Implications for Reintroduction	
P16	Jennifer Cahill, Dr. Chris Rose	Measuring the Effects of Exogenously Applied Thyroid Hormone on Cartilage Growth and Shape Change in <i>Xenopus</i> Tadpoles	
P17	Virginia Hoffman, Dr. Alex Bannigan	Effects of Oleic Acid on Arabidopsis Growth and Microtubule Rearrangement	
P18	Hayley Norian, Dr Steven Cresawn	A Bioinformatics Approach to Revealing the Genetic Basis for Bacteriophage Host Preference	
P19	Ian Richardson, Sina Kipry, Dr. Roshna Wunderlich	Plantar Pressure and Kinematics of Walking and Running in a Habitually Barefoot Population in Madagascar	
P20	Caitlyn Klotz, Matthew Wallace, Dr. Mark Gabriele	Countergradients and Modular Expression Patterns of Eph-Ephrin Signaling Proteins in the Developing Auditory Brainstem.	
P21	Brittany Hatchett, Lauren van Reesema, Dr. Terrie Rife	Nitric Oxide Synthase Expression in Skeletal Muscle of Prediabetic Rat Models	
P22	William Shoemaker, Dr. Eria Rebolgar, Dr. Reid Harris	A Study of Horizontal Probiotic Bacterial Transmission Rates in <i>Agalychnis callidryas</i> : the Effects of Population Density	
P23	McKenzie R. Quinn, Steven G. Cresawn	Analyzing Relationships of Adjacent Phamilies in Bacteriophage Genomes to Help Determine Gene Function Using A New Phamerator Tool	
P24	Kevin Tomlinson, Dr. Jon Kastendiek	The Effect of Additional Protection From Deer Browsing on Growth and Survival of Seedlings at the Smith Creek Restoration Site.	
P25	Lauren van Reesema, Keith Zirkle, Kassim Rahawi, Dr. Terrie Rife, Dr. Nusrat Jahan	The Effects of Metabolic Syndrome on Gene Expression in the Kidney: A Meta-Analysis of Microarray Studies	
P26	Morgan Donovan, Maria Wilkins, Dr. Janet Daniel	Investigating the Effect of Osmotic Stress in Root Growth in STP 1 k/o and WT Plants	
P27	Katie Garbarini, Ben Boward, Chelsea Cockburn, Michael Ferras*, Cy Lampugnale, Ashley Miller, Abby Pyper, Dr. Justin Brown	Exploration of the Role of Ambient Temperature in the Recovery From Major Surgical Stress.	
P28	Michelle Clower, Rebecca Turner, Dr. Grace Wyngaard	Genome Size: A Tool to Aid in Delineating Putative Species of Copepods (Crustacea: Copepoda)	
P29	Melissa Maloof, Rachel Stottlar, Laura Johansen, Pria Chang*, Katherine Sinclair, Maureen Filak, Fafa H. Koudoro, Rahul Warrior, Dr. Susan R. Halsell	In vivo Time Lapse Confocal Analysis of the RhoA Head Involution Defect and Molecular and Genetic Characterization of Five Extant RhoA Mutant Alleles	
P30	Nurlybek Mursaliyev, Chris Berndsen, Nathan Wright and Jonathan Monroe	Structural investigations of a Catalytically-inactive $\beta$ -amylase9 from <i>Arabidopsis</i>	

- P31 Jessie Doyle, Margaret King, Mycah McNett, Joseph Noel, Rebecca Sanders, Ty Steve  
Dr. Bruce Wiggins  
A Comparative Stream Water Quality Survey of Smith Creek Using Benthic  
Macroinvertebrates
- P32 Jacob Mesler, Emily Cate, Dr. Patrice Ludwig  
A Comparison of Developmental Thresholds for Horn Length in Male Dung Beetles.
- P33 Shelby Burns, Renzo Renteria, Dr. Alex Bannigan  
Creating a Digital Online Catalog of Microscope Slides in the Biology Department
- P34 Kyle Bonifer, Brandi Volkers, Dr. Kyle Seifert  
Antimicrobial Analysis of the *Meta*-Pyridine Series of Novel Amphiphiles
- P35 Nicholas Minahan, Jason Floyd, Dr. Kyle Seifert  
Antimicrobial Analysis of the Xylylene Series of Novel Amphiphiles
- P36 Lena Husnay, Dr. Wunderlich, Dr. Rittenhouse, Dr. Daniel  
Effects of Omega-3 Fatty Acids on Bone Mineral Density and Overuse Injuries in Female  
Athletes

**T1** Samantha Platt, Nurlybek Mursaliyev, Dr. Jonathan Monroe  
**Identification Of Binding Partners For A Catalytically Inactive *Arabidopsis thaliana* B-Amylase (BAM9) Using Phage Display**

Phage Display is a technique used in the study of protein-protein, protein-peptide, and protein-DNA interactions. Developed in 1985, it employs the use of bacteriophage to identify novel proteins that bind to a target of interest. This project is focused on BAM9, a  $\beta$ -amylase found in chloroplasts of *Arabidopsis thaliana*. BAM9 is catalytically inactive but mutants accumulate starch so we hypothesize that it plays a regulatory role in starch metabolism. We suspect that BAM9 is a maltose-sensing protein, which acts through an unknown protein-binding partner. In order to identify the binding partner we are using a phage library engineered to display millions of unique 12-mer peptides in their coat proteins. The phage library was incubated with BAM9 with and without maltose. Unbound phages were washed away and the bound phages were eluted from the target protein using an affinity resin and low pH. These phages were then amplified using *E. coli* strain ER2738 and are being put through an additional three rounds of selection to enrich for phages that had the highest affinity for BAM9. Additionally, controls using dry milk and BSA were used to account for phage affinity for blocking agents, which was seen in previous experiments. After four rounds of selection the individual clones are amplified using PCR and then sequenced to identify the 12-mer peptide sequences. These peptide sequences can then be analyzed and compared to known protein sequences encoded in the *Arabidopsis* genome to shed light on BAM9's true binding partner.

**T2** Elizabeth Badley, Samantha Platt, Jonathan Schmitz, Emily McGlone, Jonathan Monroe  
**Diurnal Plastidic B-Amylase Activity In *Arabidopsis* Leaves.**

*Arabidopsis* contains nine  $\beta$ -amylase genes, six of which encode plastid-targeted proteins. Of these six, four are catalytically active including BAM1, -2, -3, and -6. BAM3 is active at night and plays a role in mesophyll cell starch degradation, while BAM1 is active during the day and functions in guard cells. BAM1 is also induced in mesophyll cells by osmotic stress where it may contribute to daytime starch degradation in support of osmotic adjustment. Microarray data and the phenotypes of mutants lacking BAM1 and -3 suggest that there may be diurnal changes in BAM activity encoded by different BAMs, and reported measurements of total leaf amylase activity in the mutants showed moderate decreases due to the lack of BAM1 and -3. However, measurements of total leaf activity can be misleading due to the presence of BAM5, which encodes a cytosolic enzyme that can sometimes account for up to 80% of activity in leaves. We therefore constructed a set of double mutants lacking BAM5 and each of the plastidic BAMs (BAM1, -2, -3, and -6) and measured total leaf activity during the day and at night. All of the activity in *bam5* could be accounted for by BAM1 and -3 but surprisingly, there were no diurnal differences in activity in any of the double mutants. WT plants that were osmotically stressed with 200 mM mannitol showed some leaf damage after 5 days but damage to *bam1* plants was more severe. The only BAM protein affected by osmotic stress appears to be BAM1.

**T3** Ian Huang, Dr. Chris Rose  
**Estimating The Role Of The Cell Cycle To Cartilage Growth And Shape Change In Frogs**

The shape of skeleton determines its function. To understand how the shape of skeletal tissues is regulated throughout life, we need to understand how the cells that make up skeletal tissue contribute to their growth. Amphibians are good models for addressing this question because their pharyngeal arch cartilages pass through a larval period of growth without changing shape and a metamorphic period of shape change without growth. By measuring variation in the cell cycle in two cartilages of the frog *Xenopus laevis*, we will be to understand the contributions of the cell cycle to cartilage growth and shape change. We will use three techniques to measure variation in the cell cycle as a function of developmental stage. We will use antibodies to phosphohistone H3 and proliferating cell nuclear antigen to label cells in mitosis and the rest of the



cell cycle respectively. This technique should reveal differences in cell cycle length between cartilages and among developmental stages. We will treat animals with different BrdU exposure times to estimate the time it takes to go through the whole cell cycle. We will also treat animals with a BrdU pulse with varying chase times and label for both BrdU and phosphohistone H3 to estimate the times required to go from S phase to mitosis and to complete a second mitosis. These and other data will be used to develop a model for how cell behaviors impact the growth and shape change of cartilage skeleton.

**T4** A. Elizabeth Nichols, Jillian Myers, Dr. Reid Harris,  
**Rana muscosa Antimicrobial Peptides and the Bacterially-produced Metabolite 2,4-Diacetylphloroglucinol Have a Synergistic Effect on the Inhibition of the Zoospores of the Amphibian Fungal Pathogen Chytridiomycosis**

The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), which causes the disease chytridiomycosis in amphibians, is implicated in the significant population declines and extinctions of amphibians around the world. However, some species and populations coexist with the pathogen. Recent research has focused on how amphibian-produced antimicrobial peptides and bacterially-produced metabolites inhibit *Bd*. Synergy of antimicrobial peptides and the metabolite 2,4-diacetylphloroglucinol to kill *Bd* was explored in this study. It was found that significantly lesser amounts of both peptides and the metabolite were needed when used in combination compared to the amounts needed when used individually. The finding of synergy supports the general hypothesis that amphibians and their skin microbes are engaged in a mutualistic ecological relationship and may also explain why some amphibians are able to coexist with *Bd*.

**T5** Kathryn Ogborn, Dr. Chris Lantz  
**Increased Anemia Seen in *Plasmodium berghei*-Infected Interleukin-3 Deficient Mice is Not Due to Impaired Erythropoiesis**

Malaria is a mosquito-borne disease caused by a eukaryotic protozoan parasite, *Plasmodium*. Every year, approximately one million people die from malaria, with most deaths occurring in Africa alone. Common symptoms include fever, chills, sweats, headaches, nausea and vomiting, splenomegaly, and anemia. We have examined the role of the cytokine interleukin-3 (IL-3) in infections caused by *Plasmodium berghei*, a lethal rodent strain that induces symptoms similar to those observed in humans. Using *P. berghei*-infected wild-type (WT) and IL-3-deficient (IL-3<sup>-/-</sup>) mice, we show that infected IL-3<sup>-/-</sup> mice survive longer than similarly infected WT mice. IL-3<sup>-/-</sup> mice also exhibit greater splenomegaly and are more anemic. Because previous in vitro studies have suggested that IL-3 may act as an erythropoietic growth factor, we hypothesized that IL-3<sup>-/-</sup> mice may be more anemic due to impaired erythropoiesis. We examined erythropoiesis in WT and IL-3 mice by quantifying levels of colony forming unit-erythroid (CFU-e) and blast forming units-erythroid (BFU-e) present in the spleen and bone marrow. CFU-e and BFU-e represent two distinct types of erythroid progenitors that can be detected and quantified by culturing bone marrow or spleen cells in semi-solid media containing growth factors. Following proliferation and differentiation, colony-forming units of the erythroid lineage can be enumerated based on their distinct morphology. Surprisingly, we found that infected-IL-3<sup>-/-</sup> mice actually have greater numbers of erythroid progenitors than similarly infected WT mice. This suggests that greater anemia observed in infected-IL-3<sup>-/-</sup> mice may reflect excessive red blood cell destruction in these animals.

**T6** Christina Anderson, Dr Jon Kastendiek, Mr Mark Hudy, Ms Amanda Colton  
**Riparian Canopy Restoration of Smith Creek: a Cost Analysis of Three Different Riparian Tree Plantings**

The Smith Creek Restoration Project was assembled to restore riparian canopy cover to a tract of stream historically inhabited by eastern brook trout (*Salvelinus fontinalis*). A number of different restoration techniques were implemented in 2005 and 2011 to re-establish riparian forests along Smith Creek. The purpose of this study was to perform a cost benefit analysis of these different techniques. Other restoration plantings in the ridge and valley regions of Appalachia had average

success rates of less than 70%. Low survival rates decrease the return of planting investments making restoration ventures costly and a better understanding of cost effectiveness of different planting techniques is vital. Three different plantings conducted over a seven year period were compared to determine the most cost effective technique to establish riparian buffers. A CREP planting was undertaken in spring, 2005. The second; a monocrop of American sycamore (*Platanus occidentalis*) seedlings, and third; a variety of large root ball trees. A viability census of all plantings was conducted between 2011 and 2012. Cost effectiveness was determined by assessing the initial cost at planting compared to final costs after viability assessments in 2012.

**T7** Brian Schwenk, Dr. Dean Cocking

**Presence of Total Mercury (Hg) Within Invertebrates Collected in Rockingham Co. VA**

Distribution of Airborne Hg is known to occur at the landscape level. Passive air samplers consisting of inverted plastic Petri plates containing Tangle Trap (a sticky organic) have been used to examine its presence. The assumption is that this deposition serves as a relative index of Hg at the different locations in the landscape. The total Hg content when plates were exposed to the air over a fixed period was measured. Five “contaminated” locations, which previously had consistently above average Hg concentrations, and a low Hg control site, were selected for more extensive study in 2010-2012. In addition to dust, Hg vapor, and Hg dissolved in precipitation, the Tangle Trap captured various invertebrates and debris. All items greater than ~0.5 mm in diameter were removed for airborne determination. Insects were analyzed separately. During 2010-2012, separate fly, beetle, wasp, and spider traps were deployed with the intent of obtaining greater invertebrate biomass for analysis. A comparison was made of the relative Hg content of the air dried invertebrate tissue (mostly insects) to the relative airborne Hg measured by the plate samplers. Some insects from the “contaminated” locations had 2-5 times higher Hg concentrations than those at the low Hg control site. This supports the presence of a repeatable pattern of distribution of low level Hg in a rural, non-industrial landscape. Whether or not this is a result of existing background airborne contamination or is due to local sources remains to be determined.

**T8** Taylor Evans, Dr. Heather Griscom

**The Effect of Evergreen Understory on Forest Regeneration After Hemlock Removal**

The eastern hemlock (*Tsuga Canadensis*) is an evergreen conifer native to eastern North America; this tree is under threat from the hemlock woolly adelgid (*Adelges tsugae*), an invasive species of insect from Japan that has contributed to rapid decline in eastern hemlock populations in the United States, creating large gaps in once hemlock-rich forests. Research has shown that evergreen shrubs, such as *Rhododendron maximum*, have the potential to arrest succession and decrease overall forest productivity. The purpose of this study was to assess the effect of two evergreen shrub species, *Rhododendron maximum* and *Kalmia latifolia*, on forest regeneration in areas where eastern hemlock had been removed by the adelgid. The areas of study included two locations located in the George Washington National forest (Rockingham County, VA). A total of 109 forest inventory plots were created. Data on seedling and sapling density and diversity were collected as well as evergreen shrub coverage and soil moisture. At either site, *Acer rubrum* was found to compose a large percentage of both seedlings (>40%) and saplings (>8.0%). ANOVA analysis showed seedling and sapling diversity, as well as seedling density were shown to significantly ( $p < 0.05$ ) differ by site. Evergreen coverage was found to significantly affect seedling density. Seedling diversity and sapling density were affected most by interactions of the factors measured. It is expected that succession will progress towards a maple-birch (Fridley’s Gap) and maple-oak (Hone Quarry) forest structure in the future, with evergreen understory having little effect on forest density or diversity.

**T9** Emily Cate, Theresa Dalmut, Dr Patrice Ludwig, Dr Anthony Tongen, Dr Brian Walton

**Modeling Fitness of *Onthophagus taurus*: The Effects of Density on Mating Success**

Examples of sexual selection via male competition can be found throughout the animal kingdom. Dimorphic males can arise from this intraspecific competition where one morph adopts a guarding strategy and the other a sneaking strategy. Females of the dung beetle *Onthophagus taurus* dig tunnels beneath dung pats deposited by large herbivores. Males seek mating

opportunities within the created tunnel system and also exhibit a characteristic dimorphism that leads to differing reproductive strategies. Horned “major” males guard the females’ tunnel while hornless “minor” males dig accessory tunnels in order to sneak copulations. The success of these strategies depends on the number of other males with which an individual competes as well as the number of available females. Agent-based model simulations were created using MATLAB and NetLogo in order to explore the effects of density on male fitness in the form of varying sex ratios and varying the proportion of majors to minors. Minors were found to always have a higher paternal success given a 1:1 sex ratio and as the number of females was increased, major paternal success decreased while minor success increased. These findings support that more minors will be found in higher densities whereas majors thrive in lower density populations. Changing densities can also be a proposed hypothesis as to why the dimorphic threshold is different in geographically separate populations of *O. taurus*.

[ T10 Withdrawn]

**T11** Rania Sheikh, Dr. Chris Rose

### **How Does Stage Of Lung Inflation Affect the Developmental Anatomy of the Frog Lung**

Amphibian lungs differ from mammalian lungs in completing cellular differentiation and anatomical development and starting to function at postembryonic stages rather than embryonic stages or birth. Frog lungs are further distinguished by undergoing inflation at different stages in different species. *Xenopus*, *Rana*, and *Bufo* species inflate their lungs at early, mid, and late tadpole stages respectively. Stage of lung inflation in frogs is thought to be determined by natural selection based on water oxygen level, feeding requirements and predation risks associated with buoyancy and other ecological factors. Inflation represents a pivotal event in lung development because the onset of air pressure allows for blood flow in lung capillaries, exposes lung tissues to higher oxygen levels, and introduces new stretching forces, all of which might affect cell differentiation. Inflation also represents a pivotal event for the evolution of lung anatomy because it is the stage at which lung surface area becomes coupled to body size to meet oxygen demand. Since it determines when frogs can begin to develop the rudiments of septa that subdivide their lungs, timing of lung inflation might also pose a size constraint upon metamorphosis. We wish to know how lung development is affected by variation in stage of lung inflation. We will prepare histological sections for each species and compare development of the lung rudiment and internal anatomy as a function of developmental stage. The anatomical features to be examined include the elastic and smooth muscle fibers and blood vessels, and primary, secondary and tertiary septa.

**T12** Lauren van Reesema, Dr. Terrie Rife, Dr. Janet Daniel, Dr. Nusrat Jahan

### **The Effect of Metabolic Syndrome on Gene Expression in Skeletal Muscle: A Meta-Analysis of Microarray Studies**

Metabolic syndrome (MetS) is a clustering of risk factors that increase chances for developing various metabolic diseases, such as cardiovascular disease and type 2 diabetes. The pathology of MetS lies in a disruption of insulin signaling and fuel homeostasis. Skeletal muscle is the most abundant insulin-sensitive tissue, making up approximately 40 percent of body mass. Any changes in muscle fuel homeostasis drastically affects whole-body metabolism, so changes in gene expression within skeletal muscle related to MetS are of interest. This study uses two random effect size meta-analysis models to combine data of existing microarray studies of MetS in the gastrocnemius and soleus muscles to increase the statistical power and applicability of the results. Within the gastrocnemius, the expression of 650 genes was significantly altered in the MetS condition, with 99 up-regulated and 551 down-regulated. Genes were “novel” if expression had a fold change in the same direction for each study included, and was greater than/equal to +2.0 or less than equal to -2.0. Eight “novel” genes were found, including Pak1 and Cyr61, which were chosen for further analysis as well as a verification of the meta-analysis technique. Pak1 encodes a protein kinase involved in insulin signaling, while Cyr61 encodes a binding protein involved in cell adhesion and apoptosis. Neither gene has been associated with MetS, yet their related functions make them interesting for further study. Within the soleus, the expression of 66 genes was

significantly changed, with 21 up-regulated and 45 down-regulated. No novel genes were found within the soleus meta-analysis.

**T13** Bejan Rasoul, Dr Kimberly Slekar

**Investigating The Role Of The Saccharomyces Cerevisiae B-Nac In Stress Response**

When proteins fold incorrectly in cells there is a possibility of abnormal protein aggregation, which can lead to undesirable consequences for the organism. Protein aggregation is associated with diseases such as Alzheimer's and Parkinson's. To prevent misfolding, proteins are guided into correct conformations by chaperones. The chaperone-like protein NAC or Nascent Polypeptide Associated Complex binds to ribosomes near the exit site of the newly forming protein. The NAC is composed of  $\alpha$  and  $\beta$  subunits. It has been shown that a removal of  $\beta$ -NAC, the subunit that comes into contact with the ribosome during translation, hinders yeast cells in a noticeable way. Mutations of the NAC and the chaperone SSB (stress 70B) leads to protein aggregation. To protect itself from unfolded proteins, the cell initiates the unfolded protein response (UPR), which is a series of events that leads to the degradation or repair of misfolded proteins. In the eukaryotic model *Saccharomyces cerevisiae*, commonly known as baker's yeast, transmembrane proteins sense the increased levels of unfolded proteins in the ER lumen and initiate a process that leads to the upregulation of the transcription of specific genes. When the UPR is not sufficient to solve the problem, cells resort to autophagy, in which whole sections of the cytoplasm are degraded. To better understand these important cellular processes, we used yeast mutants deficient in  $\beta$ -NAC to investigate the role of this protein in cell stress response and longevity.

**T14** Kristie Prtorich\*, Marc Carpenter, Hilary Kurland, Dr. Joanna Mott

**Characterization of Enterococcus spp. from Water and Geese in an Urban Water Shed**

Newman Lake is a man-made lake on the campus of James Madison University formed through the damming of Sibert's Creek and serving as the collection point of storm-water drainage for the university. Monthly water monitoring has shown that the lake contains elevated levels of fecal indicator bacteria. Potential sources of contamination include geese (*Branta canadensis*) which aggregate around the lake. Water and geese feces samples were collected to isolate species of *Enterococcus* and compare the species present in Newman Lake to those found in the geese feces in order to determine any similarities between isolates from the two sources. Additionally, the antibiotic resistance profiles of the water and fecal isolates were compared. Some water and fecal isolates were found to exhibit multi-drug resistance including resistance to tetracycline. Tetracycline resistant isolates were studied to determine the transmissibility of the drug resistance. This data can be used to assess sources of contamination and potential risks to individuals exposed to these bacteria and to inform storm-water management planning to decrease impacts on downstream waters.

*\*Student research supported by Taliaferro Scholarship and Jean D. Acton Scholarship*

[T15 Withdrawn]

**T16** Joanna Gardiner, Brittany Wilhelm, Dr. Roshna Wunderlich, Dr. Anthony Tongen, Dr. Jon Kastendiek, Dr. Daniel Schmitt, Charlotte Miller

**Using Accelerometry to Study Leaping Performance in Free-Ranging Sifakas (*Propithecus verreauxi*)**

Measurements of performance are critical to analyses of fitness in wild populations and necessary in order to study locomotion as part of life history strategies. Sifakas (*Propithecus verreauxi*) are arboreal primates who travel using vertical clinging and leaping. Animals of all ages and all levels of physical ability travel along the same arboreal pathways and have to cross the same gaps. This presents potential challenges to smaller, younger animals, and less agile, older individuals. We used a novel 3-dimensional accelerometry system to identify locomotor behaviors and quantify leaping performance in one adult and one juvenile sifaka in both an enclosed experimental room with multiple substrates and a free-ranging outdoor enclosure at the Duke Lemur Center. Accelerations and simultaneous locomotor behavior data were collected in the experimental setting and then for a four-hour period in the free-ranging setting to clearly establish the relationship between movement

and accelerometer output. Data were filtered and waveforms and peaks were identified. Up to 94% of single and cyclic vertical leaps were distinguished from all other locomotor patterns using the vertical component of the acceleration signal. Cyclic leaping and bipedal galloping exhibit similar center of mass vertical acceleration patterns but are distinguishable from one another using the relative magnitude of the peaks and the horizontal component of acceleration. Resultant magnitudes of acceleration positively correlate with estimated leap distances. These data serve both to establish these methods for measuring leaping performance in the field and to reveal profound biomechanical similarities between leaping and bipedalism in sifakas.

**T17** Ben Stanley, Dr Christine May

**Explaining Localized Variation In *Cerion* Shell Morphology On San Salvador Island, The Bahamas**

*Cerion* is a remarkably diverse genus of terrestrial pulmonate snails that has attracted over 50 years of scientific investigation. The goal of this study was to investigate a possible behavioral mechanism behind the maintenance of distinct shell morphologies between populations of *Cerion* residing in localized areas on San Salvador Island, The Bahamas. Low dispersal rates and clumping behavior of this genus led to the hypothesis that individuals from high density areas would show a preference for low dispersal as a result of the relaxed selective pressure for finding a mate, and that this preference would ultimately result in less morphologic variability in high density areas. This hypothesis was tested with morphometric measurements made from photographic analysis combined with a dispersal study of marked individuals. Morphometric measurements including shell height, shell width, whorl count, rib density, and coloration were taken for all individuals residing on palm habitat found within a 40x10 meter transect (n=1,080). Coloration and rib density were the most variable of these measurements. Calculation of total dispersal for 333 individuals from varying population densities resulted in a mean dispersal distance of 3.5 m over seven months (ranging from 0 – 12.8 m). Dispersal was skewed, representing a sedentary majority and mobile minority. Average dispersal for individuals starting on high and low density trees was 3.4 and 3.6 meters, respectively. Although we do not see a significant difference in dispersal behavior with respect to initial population density, the results do show higher average dispersal rates than previously recorded for this genus.

**T18** Rebecca Turner, Dr. Grace Wyngaard

**Quantitative Behavior of DNA: Timing of Chromatin Diminution in *Mesocyclops edax* (Crustacea Copepoda)**

*Mesocyclops edax*, a freshwater zooplankton, possesses a novel DNA behavior known as chromatin diminution. During embryogenesis, 80% of the germline genome is excised from the somatic genome. The result is a germline genome that contains a diploid amount of 15 pg DNA per nucleus (~15 Gb) and a somatic genome that contains only 3 pg DNA per nucleus (~3 Gb). This excision of DNA during embryogenesis marks the timing of germline-soma differentiation, but the precise cleavage division in which chromatin diminution occurs is unknown in *M. edax*. The objective of this research is to ascertain the particular cleavage division at which diminution occurs. Image analysis densitometry was used to measure the amount of DNA per nucleus in embryonic nuclei in the 1C, 2C, 4C, 8C, 16C, 32C, and >32C cell stages. Preliminary results suggest that the diminution occurs in the 5th cleavage division. Additionally, the primordial germ cell that gives rise to the germline undergoes mitosis on a schedule that is asynchronous with that of the somatic line. This occurs before the diminution suggesting that the fate of individual cells is determined very early in development. No DNA was detectable in nuclei at the 1C stage, suggesting that the state of chromatin at this stage differs considerably from those at subsequent stages, and may have to be treated differently to quantitate DNA amounts.

**T19** Lina Chhuy-Hy, Michael Yeager, Ben Williamson, Heather Johnson, Christopher Watson, Tori Callahan, Dennis Churchin, Dr. Marta Bechtel

**Optimizing Isolation of Primary Rabbit Cornea Cells for Tissue Engineering Studies**

Cornea tissue engineering is an active area of research that seeks to understand the complex behavior of the cells that comprise cornea tissue, along with the tissue scaffold and signaling processes that contribute to a functional cornea. The cornea is comprised of three different tissue layers – the stromal, epithelial, and endothelial layers. The stromal layer represents about 90% of the cornea tissue. Keratocytes, the cells found in the stromal layer, play a major role in maintaining corneal transparency. When trauma or injury occurs to the stromal layer, the keratocytes convert to a wound healing phenotype that can lead to loss of transparency and corneal haze (National Eye Institute, 2004). Our lab employs a rabbit cornea model to study the corneal wound healing processes and to understand how to reconstruct a functional cornea. Recently, we have optimized our cell isolation protocols to obtain a high yield of healthy corneal cells of each of the three tissue layers. These optimized protocols will enhance our ability to characterize the roles of each cell type in regenerating a functional cornea.

**T20** Sara Bray Ischinger, Dr. Roshna Wunderlich

**Plantar Pressure During Vertical Climbing in Chimpanzees (*Pan troglodytes*)**

Metatarsal torsion and robusticity have frequently been used to infer locomotor function in extinct primates, including fossil hominins. Apes possess a robust, everted metatarsal 1 (lateral torsion), and inverted metatarsals 2-5 (medial torsion). It has been suggested that this distinct forefoot design allows the metatarsal heads of an inverted foot to lie flat on the substrate, and that the decreasing length and robusticity across the metatarsal row reflects load bearing during vertical climbing. We test this hypothesis by collecting the first quantitative data on plantar load distribution during vertical climbing. We trained two *Pan troglodytes* to climb a vertical pole instrumented with a flexible Pliance pressure mat (Novel GmbH). We quantified plantar pressure in 11 anatomical regions (phalanges; metatarsals 1, 2/3, 4/5; medial and lateral midfoot, heel) and described load distribution across the foot during stance phase of vertical climbing. When vertical climbing, *Pan troglodytes* transfer their weight from the midfoot to the lateral metatarsals and then to the medial metatarsals. Peak pressure was significantly greater under metatarsals 2/3 than under metatarsal 1 and 4/5 ( $p=0.0475$ ,  $p=0.0001$ ). Peak pressure under metatarsal 1 was significantly greater than under metatarsals 4/5 ( $p=0.0345$ ). *Pan troglodytes* use an inverted foot with an abducted hallux during vertical climbing. Torsional characteristics allow *Pan troglodytes* metatarsals to align plantar surfaces with the substrate facilitating lateral to medial weight transfer across the metatarsal row, while the robusticity of the *Pan troglodytes* medial forefoot is an adaptation for high push-off forces during climbing.

**T21** Jessica Holland, Andrew Loudon, Dr. Reid Harris

**The Interaction of the Lethal Fungal Pathogen *Batrachochytrium dendrobatidis* and Combinations of anti-*Batrachochytrium* Bacterial Species**

*Batrachochytrium dendrobatidis* (*Bd*) is the fungal pathogen responsible for the disease chytridiomycosis. *Bd* is the largest threat to amphibian biodiversity, with 41% of amphibian species threatened and over 200 species now extinct. To survive and contend against pathogens, amphibians display three main types of defense: innate, adaptive, and bacterial microbial. The latter refers to the symbiotic bacterial species from the skin of amphibians that serve as defenses. Several antifungal bacterial species have been identified as inhibitory against *Bd*. This study used four anti-*Bd* bacterial species both alone and in pairwise combinations with *Bd*. The combinations of bacterial species exhibited synergistic and additive relationships in terms of *Bd* inhibition and the lessening of facilitative effects of individual species, as well as relationships which had no effect on *Bd* growth. In addition, bacterial species combinations were co-cultured prior to exposure to *Bd* to compare the effects of co-culturing versus 'separate then combined' treatments on *Bd* growth inhibition. The co-cultured combinations inhibited *Bd* growth significantly greater than did the same combinations in 'separate then combined' treatments. These results provide further knowledge for the possible implementation of a probiotic approach (bioaugmentation) to amphibian survival.

**P1** Emily Peterman, Andrew Loudon, Dr. Reid Harris, Dr. Eria Rebollar, Dr. Alex Bannigan  
**The Negative Chemotaxis of *Bd* in Response to Violacein and DAPG**

In recent decades amphibian populations has declined due to a fungal infection caused by *Batrachochytrium dendrobatidis* (*Bd*). Some amphibian species have been able to resist *Bd* longer than other amphibian species due in part to metabolites produced by symbiotic bacteria present on the skin of these amphibians. Two different symbiotic bacteria produce the antifungal metabolites violacein and 2,4-Diacetylphloroglucinol (DAPG). Previous studies showed that DAPG has a negative chemotactic effect on *Bd*. However, the chemotactic effect of violacein is not known. The purpose of this study is to see the chemotactic effect of violacein and DAPG combined with violacein. If there is an additive or synergistic effect on the negative chemotaxis of *Bd* in the presence of both metabolites, then it would mean when both metabolites are present lower concentration of these metabolites would be needed to repel *Bd* from the amphibian. This could provide amphibians a better defense system against *Bd* when they have both of these bacterium and a fact to consider when bioaugmenting the environment.

**P2** Michael Partin, Kevin Libuit, Dr James Herrick  
**Identification and Characterization of *Staphylococcus* spp. in Stream Water and Sediment**

poultry and other meats with *Staphylococcus*. We hypothesized that intensive farming of poultry adjacent to streams would result in contaminated runoff, resulting in at least transient occurrence of *Staphylococcus* spp. in streams waters and sediments. This study intends to determine whether *Staphylococcus* occurs and persists within agriculturally impacted streams in the central Shenandoah Valley. In preliminary work, large numbers of *Staphylococcus* spp. were detected in water from Muddy Creek, which runs adjacent to a poultry processing plant. Vogel-Johnson agar, a reported medium for isolating *Staphylococcus*, was tested as a rapid isolation tool. However, the medium was not effective in isolating *Staphylococcus* from streams and sediments. Instead, Mannitol Salt Agar (MSA) was used to isolate two *Staphylococcus* spp. – one from the water and one from the sediment – from the Muddy Creek site. As the occurrence of *Staphylococcus* in Muddy Creek seems to be transient and highly variable, perhaps depending upon runoff and season, continuing efforts are underway to determine the temporal and spatial variability in its occurrence in both water and sediment. Future studies will include determining the susceptibility of *Staphylococcus* isolates to a range of relevant antibiotics – including oxacillin, which would identify methicillin resistance – and whether resistance is transmissible and/or plasmid borne. Overall, this study will provide insight into the occurrence of staphylococci in freshwater and a possible link between the microflora of agricultural animals and that of native streams.

**P3** Sinead Gilmore, Hailey Huebner, Dr. Terrie Rife, Dr. James Turner (VMI)  
**The Effect of Estrogen and Cardiovascular Health on Nitric Oxide Synthase Expression in a 'Listless' Model of Zebrafish (*Danio rerio*)**

Cardiovascular disease (CVD) is the leading cause of death among Americans. The hormone estrogen ( $E_2$ ) appears to influence cardiovascular health, as evidenced by the high incidence of CVD observed in postmenopausal women. Estrogen replacement therapy, considered a potential treatment for CVD, became controversial after a 2002 study that suggested it caused increased risk for heart problems and breast cancer. Due to conflicting evidence on estrogen's beneficial and harmful effects in postmenopausal women, its mechanistic roles must be further investigated to clarify estrogen's effect on cardiovascular health. The current study focuses on molecular changes that result from estrogen deprivation and replacement. This study utilizes differential gene expression in a model of zebrafish (*Danio rerio*) treated with an aromatase inhibitor (AI), which prevents the production of estrogen. This model, classified as 'listless,' results in congestive heart-failure and death due to tamponade followed by cardiac arrest. 'Listless' fish are compared to a control group in an embryo rearing solution, a rescue treatment group of AI+E<sub>2</sub>, and an E<sub>2</sub> treated positive control group. The genes myl7, NOS1, NOS2a and NOS2b, are likely regulated by estrogen

in the zebrafish model and may contribute to the rescue of the 'listless' phenotype. To examine the transcriptional changes of these genes, mRNA is isolated from the samples, reverse transcribed to cDNA, and subjected to real-time PCR for quantification. The housekeeping gene *ELF1-α* is used to normalize the data. Preliminary results suggest decreased expression of *NOS1* in the AI treatment group. Rescue with estrogen increases expression compared to AI.

**P4** Emily Thyroff, Dr. Heather Griscom

**Experimental Greenhouse and Field Trials on American Ginseng *Panax quinquefolium*: Implications for Reintroduction in Appalachia**

American ginseng *Panax quinquefolium*, a perennial herbaceous plant, is threatened in the Shenandoah and overall in Appalachia because of over-harvesting (North Carolina State University). Ginseng once was found everywhere in Appalachia, but now it is not abundant anywhere (McGraw, et al., 2003). Restoration work would benefit the environment as well as local economies because of ginseng's valuable market (Fournier and Gosselin, 2004). This study includes greenhouse and field trials in order to determine ginseng's optimal growing conditions. Knowing optimal growing conditions can help to naturally reintroduce ginseng in Virginia and West Virginia. In the greenhouse and field trials soil moisture (high, medium, low) and soil type (gilpin, dekalb, and limestone) will be manipulated resulting in nine different treatments. All other conditions will be controlled. The rationale for this study is the existence of conflicting material and discrepancies of ginseng's optimal growing conditions in the literature.

**P5** Elena Balkanska, Seana Sears, Dr. James B. Herrick

**Capture and Characterization of Stream Sediment Plasmids Conferring Resistance to Ceftazidime and Ciprofloxacin, two Antibiotics Important in Human Clinical Therapy**

Streams harbor large numbers of native and introduced bacteria, which can be both recipients and donors of antibiotic resistance genes on mobile elements. Transmissible, plasmid-borne resistance to expanded-spectrum cephalosporins and fluoroquinolones is of increasing concern in clinical settings, but is rare in natural environments such as streams and soils. Using a method developed in our laboratory, a rifampicin-resistant strain of *Escherichia coli* was used to capture, without culturing, two plasmids allowing growth on trypticase soy agar the beta lactam antibiotics ampicillin and the late-generation cephalosporin, ceftazidime. Plasmids were captured directly from stream sediment taken from Muddy Creek, a stream heavily impacted by agricultural runoff. Transconjugants were also tested – using a modified "Stokes" disk diffusion method – for resistance to tetracycline, gentamycin, ciprofloxacin and ceftriaxone. One transconjugant, originally isolated from a rifampicin/ceftazidime plate, was resistant to ciprofloxacin, ampicillin, and tetracycline, in addition to ceftazidime. A second transconjugant, originally isolated from a rifampicin/ampicillin plate, was also resistant to ciprofloxacin and tetracycline, in addition to ampicillin. To confirm that the transconjugants were indeed the rifampicin-resistant capture strain, they were fingerprinted using PCR amplification of BOX repetitive sequences. The presence of plasmids was confirmed via a plasmid preparation method specifically developed in our lab for large plasmids; the plasmids appear to be in the 50-100 kb range. Plasmids will be electroporated into an electrocompetent *E. coli* to further confirm that resistance is plasmid-borne. Recipient bacteria modeling bacteria native to streams – *Pseudomonas* and *Aeromonas* spp. – are also being developed as alternative recipients to *E. coli*. The recovery of transmissible plasmids encoding resistance to late-generation, human therapeutic antibiotics such as ceftazidime and ciprofloxacin from streams is surprising since such antibiotics are not approved for use in agriculture. It also suggests the presence of a reservoir – of unknown size and composition – of transmissible antibiotic resistance genes in stream sediment bacterial communities impacted by agricultural runoff.

**P6** Allyse Harris, Matt Wallace, Dr. Mark Gabriele

**Development of Inputs from the Lateral Superior Olivary Nucleus to the Inferior Colliculus in EphA4 Mutant Mice Prior to Hearing Onset**

Hearing loss affects many Americans in the United States. The organization of the auditory circuitry is complex and requires a better foundational knowledge in order to continue to improve



treatment strategies for hearing loss. We are interested in understanding how central auditory connections develop prior to experience (postnatal day 12, P12 in mouse). My project focuses on inputs from an auditory center in the brainstem, the lateral superior olivary nucleus (LSO) to the auditory midbrain or inferior colliculus (IC). Before hearing onset, LSO to IC projections are arranged tonotopically (i.e. frequency-mapped) and subsequently segregate into discrete layers. We are interested in the potential role a family of signaling molecules plays in establishing this early topography. The Eph family of receptor tyrosine kinases and the ephrin ligands are proteins that are involved in guiding the development of axonal targeting and topographic map formation in other systems (Flanagan, J.G. and Vanderhaeghen, P. 1998). Comparatively less is known about their involvement in the development of the auditory system, particularly their role in the development of patterned inputs to the auditory midbrain. Recent studies in our lab have shown members of the Eph-ephrin family are expressed in the developing IC when LSO axons are being shaped (EphA4, ephrin-B2, and ephrin-B3). The present study focuses specifically on EphA4 and its involvement in establishing ordered LSO-IC projection patterns utilizing fluorescent tract-tracing approaches in our EphA4 mutant model. We hypothesize that EphA4 is necessary for accurate topographic mapping of LSO inputs to the IC prior to experience.

**P7** Abdalla Sheikh, Dorottya Boisen, Amelia Tavangar, Chris Lantz, Ph.D, Ken Roth, Ph.D.

#### **IL-3 Dependent Cytokine Regulation in *P. berghei*-infected BALB/c mice**

Mortality rates due to malaria have doubled in the past thirty years; 1.3 million deaths occur every year, mainly among children under the age of 5. Malaria is a parasitic infection caused by a protozoan of the Plasmodium genus, which is transmitted by an adult female Anopheles mosquito. The inflammatory response that results is characterized in part by increased levels of interferon-gamma (IFN- $\gamma$ ), tumor necrosis factor-alpha (TNF- $\alpha$ ), and other cytokines in the blood of the infected individual. The focus of our study is interleukin-3 (IL-3), a cytokine that has been shown to be involved in immune system functions. Using wild-type (WT) and IL-3-deficient "knockout" (KO) mice, we are monitoring the immune response to malaria by infecting mice with *Plasmodium berghei*, a rodent parasite that causes a disease with symptoms that mimic human *Plasmodium falciparum* malaria. To determine if IL-3 plays a role in the immune response, we are measuring levels of IFN- $\gamma$ , TNF- $\alpha$ , and other cytokines in plasma and in supernatants of splenocyte cultures at various time points post infection. Our preliminary data indicate that at four days post infection, WT mice have significantly higher levels of TNF- $\alpha$  and IFN- $\gamma$  than KO mice ( $p < 0.05$ ). By day eight, however, levels of TNF- $\alpha$  in both genotypes are lower and are not significantly different (day 8 levels of IFN- $\gamma$  are currently being evaluated). With our findings we hope to better understand the role of IL-3 in malaria infections. This information may be used in the future to help develop new treatment strategies.

**P8** Kimberly Okafor, Ian Brown, Dr. Steve Cresawn

#### **Cytotoxicity Screening of Bacteriophage Genes in Mycobacteria**

*Mycobacterium ulcerans* is responsible for the skin lesions experienced by people with Buruli Ulcers, a neglected tropical disease affecting more than 30 countries with no effective mode of treatment. Mycobacteriophages (viruses that infect mycobacteria) are being explored in this lab as a possible treatment because antibiotics have proven ineffective and phages have been seen to act as a growth control to mycobacteria in culture. In this project, we aim to identify cytotoxic genes from bacteriophages isolated by JMU students. Mycobacteriophages known to infect *Mycobacterium smegmatis*, LeBron, George and Rumpelstiltskin, were isolated. *M. smegmatis* was chosen for infection instead of *M. ulcerans* because of its close relation to *M. ulcerans*, its non-pathogenicity and quicker incubation time. The genomes were sequenced and annotated using Phamerator and BLAST by Viral Discovery students. From here, the specific gene region responsible for holin, endolysin A and endolysin B (viral proteins responsible for bacterial cell lysis) will be amplified via designing primers for PCR. This region was chosen to establish a positive control for known cytotoxic genes on plates induced with Anhydrotetracycline (used to evaluate cytotoxicity). Once the genes have been successfully amplified, we will clone the region into an inducible plasmid using the Gateway Cloning Method before introducing into *M. smegmatis*. Then, the cloned genes will be plated in the presence and absence of the inducer in order to evaluate the level of

cytotoxicity. This process will be repeated with phage genes of unknown functions to identify genes in the viral genome with cytotoxic properties.

**P9** Kandyce Hamilton, Rebecca Smith, Dr. Terrie Rife

#### **The Effect of High Fat Diet on Nitric Oxide Synthase Expression in the Kidney of Rat**

Diabetes has become a prominent issue in today's health care system as over 8% of all Americans have Diabetes. Much of the health care cost comes from the development of secondary symptoms for example; over 35% of all diabetics develop chronic kidney disease. Nitric Oxide Synthase (NOS) is an enzyme that plays a role in the development of this disease. There are three isoforms of NOS and if all three isoforms are knocked out of the kidney symptoms of kidney disease develop. This study aims to understand the effect of diet on the development of kidney disease. Rats fed either a high fructose or a high fat diet develop pre-diabetes and changes in expression of at least two of the NOS isoforms, NOS1 and NOS3. This study will verify previous findings about these two isoforms and examine the third NOS isoform, NOS2. For these studies RNA will be isolated from previously isolated rat kidneys. The RNA will be reverse transcribed into cDNA. Finally, using the real-time polymerase chain reaction a portion of the cDNA is enlarged multiple times to be able to evaluate the presence of NOS, also in duplicates. The expected result is the down regulation of NOS1 and a slight up regulation of NOS3 due to experiments seen by previous students and in literature and we expect a slight upregulation in NOS2.

**P10** Rebecca Dickey, Eria Rebollar, Molly Bletz, Reid Harris

#### **Persistence of Amphibian Probiotics in an Aquatic Environment**

Mitigation strategies for *Batrachochytrium dendrobatidis* involving probiotic applications to reservoirs within amphibian environments contain a number of variables that may impact effectiveness and practical applications in the field. Information concerning the persistence of beneficial bacteria in aquatic environments is critical if probiotic reservoirs are to be considered for implementation in areas in which *B. dendrobatidis* infections are a threat to amphibian species. Three strains of beneficial bacteria isolated from *Notophthalmus viridescens* were developed for rifampicin resistance and then tested for length and concentration of persistence in a laboratory setting using an artificial pond water media. We expect that results will aid in future research and may possibly direct conservationist efforts to select specific candidates for the creation of probiotic reservoirs in the wild.

**P11** Sheyda Maghsoudi, Dr. Michael Renfroe

#### **Antioxidant Analysis of Seven Spices from the Apiaceae Family**

Free radicals are organic molecules responsible for aging, tissue damage, and possibly some diseases. These molecules are very unstable, often bonding with other molecules and further continuing the damage process. Normally, the body can handle free radicals, but if antioxidants are unavailable, or if the free radical production becomes excessive, more severe damage can occur. Antioxidants present in many spices are molecules that prevent free radicals from harming healthy tissue. This study quantified the total antioxidant activity, a combination of hydrophilic and lipophilic antioxidant activity, of seven spices from Apiaceae family. Data were recorded in fresh and dry weight measurements using the ABTS/H<sub>2</sub>O<sub>2</sub>/HRP decoloration method. On a fresh weight basis, cumin was found to have the highest level of total antioxidants with a mean of 45.04 ± 0.40 μmol TE/g FW, followed by fennel, dill, celery, anise, caraway, and the lowest mean was found in coriander, with a mean of 9.54 ± 0.41 μmol TE/g FW. The dry weight data also gave similar results. The mean differences of the hydrophilic antioxidant activity, lipophilic antioxidant activity, and total antioxidant activity were analyzed using a one-way analysis of variance and Dunnett's T3 pairwise comparisons. The sample means were also placed into statistically significantly similar groups. Results found in this experiment shows that spices are a good source of antioxidants, and may contribute to a healthy diet.

**P12** Jonathan Schmitz, Jonathan Monroe

**Two Plastidic  $\beta$ -amylases from Arabidopsis are Involved in Osmotic Stress Resistance.**

Starch, a polymer of glucose, is a source of stored energy for plants. Starch accumulates in chloroplasts during the day and is broken down during the night, primarily by the  $\beta$ -amylase (BAM) family of proteins. The BAM family in Arabidopsis consists of nine proteins; six of which are plastid targeted. Two of these plastidic BAMs are BAM1, which is catalytically active and functions during the day, and BAM9, which is catalytically inactive. Because mutants lacking BAM9 accumulate starch, we suspect that it plays a role in regulating starch metabolism. When exposed to osmotic stress, plants close their stomata, slowing water loss, but this also prevents photosynthesis. To alleviate the loss of available photosynthate, BAM1 is expressed in mesophyll cells and is thought to hydrolyze starch to provide carbon skeletons for the production of osmolytes. Osmolytes help mesophyll cells maintain a water potential gradient, which allows roots to take up water. In an experiment to see if BAM1 and BAM9 play a role in long-term osmotic stress resistance, we exposed mutants lacking these  $\beta$ -amylase genes to drought stress and evaluated their health over time. Interestingly, both bam1 and bam9 plants experienced strong signs of wilting and chlorosis, whereas the wild-type plants did not experience as much chlorosis. Differences in response to stress between these genotypes suggest that BAM1 and -9 may both play roles in stress resistance, which will hopefully be brought to light in further experiments.

**P13** Alexandra Heil, Hannah Wines, Dr. Michael Renfroe

**Quantitative Analysis of Antioxidants in Specialty Wines**

Wines are sources of antioxidants such as procyanidins and resveratrol along with polyphenolics that provide important health benefits and help protect against hypertension, arteriosclerosis, cancer, and other diseases. Most commercial wines are made from the domesticated European grape (*Vitis vinifera*). However, there are specialty wines that are made from native American grapes (*Vitis labrusca*), and wines that are fruit based or blended with fruit extracts. Our research compared the antioxidant levels found in muscadine and scuppernon wines to those found in fruit-based or fruit-flavored wines. Antioxidants were measured using the trolox equivalent antioxidant capacity assay (TEAC) and results were reported in  $\mu\text{mol}$  trolox equivalents (TE) per mL. Muscadine and scuppernon wines from a single vineyard were compared and antioxidant concentrations ranged from 2.2-5.5  $\mu\text{mol}$  TE/mL wine. Four fruit wines were compared from a single vineyard and the raspberry and peach were low in antioxidants (2.0 and 1.7  $\mu\text{mol}$  TE/mL, respectively), whereas the blackberry and chocolate-flavored wines were very high in antioxidants (10.7 and 12.6  $\mu\text{mol}$  TE/mL, respectively). An apple wine from a different vineyard contained 2.6  $\mu\text{mol}$  TE/mL. These results indicate that wines can vary significantly in their antioxidant content, and that scuppernon and muscadine wines were comparable to some reported values of European grape-based wines. Additional wines are being evaluated for their antioxidant content.

**P14** Victoria Callahan, Dennis Churchin, Kristin McKenna, Andrew Sharp, Christopher Watson  
Dr. Katrina Gobetz

**Research Activities of the Trelawny Learning Community**

Trelawny 2012-13 spans 6 research areas. While some students collaborate on posters with their labs, others will present a group poster detailing their experiences and plans for next year. Three students joined Dr. Bechtel's lab to isolate epithelial/endothelial cells from corneal tissue. They trained in aseptic cell culturing techniques as well as microscopy to ID apoptotic and necrotic cells. Next year's preparation includes dilutions for Real Time PCR on the GAPDH dengue protein and literature review on the dengue virus, to help students understand what they will achieve in the next few years. In the Mott lab, students learned to filter soil containing *Enterococcus muntii*. Filtration data was used to statistically analyze *E. muntii* viability under different temperature, strain, pH, and humidity treatments. Students practiced water sampling and used dilution and fluorescence to quantify bacteria. Work in Dr. Kastendiek's lab took place entirely at Smith Creek, where students learned to distinguish between species of young trees and determine evidence of deer browsing. Fieldwork consisted of measuring, tagging, and placing deer-proof devices on young trees. Lastly, the group compares invertebrate counts from the Blacks Run monitoring

project begun in 2011 to current data to assess stream health. Aside from lab/field skills, students gained perspective on challenges like balancing research hours with first-year courses and reconciling time spent collecting data for unsuccessful results. Each student will continue research in Fall 2013 based on training and preparation this year, and some will mentor next year's TLC.

**P15** Katie O'Donnell, Jessie Ward, Dr. Heather Griscom

**Effects of Soil and Light on the Growth of *Castanea dentata* and Competing Species: Implications for Reintroduction**

The American Chestnut, *Castanea dentata*, once dominated the canopy of Eastern United States forests until the chestnut blight, caused by a fungus, *Castanea parasitica*, began in 1904. By 1950, nearly the entire 9 million acre range of *C. dentata* was infected, removing an important hardwood product from the region's economy. *C. dentata* was found to successfully grow in a variety of soil and light conditions, as well as, develop as a shade-tolerant species. In order to determine the ideal growth conditions for *C. dentata*, four different soil and light treatment combinations were applied to 12 plots. In 2008, seedlings of pure and hybrid *C. dentata*, *Liriodendron tulipifera*, and *Quercus prinus* were planted randomly in each plot varying in treatment combination of light (large or small gap) and soil (xeric or mesic) conditions. Diameter (mm) and height (cm) were measured in October for five years. An ANOVA was carried out for the relative diameter growth,  $\alpha = 0.10$ , displaying that species was not significant,  $p = 0.277$ , however, treatment was found to be significant with a  $p < 0.000$ . Tukey comparisons showed that the large, mesic treatment resulted in significantly higher relative diameter growth than other treatments. An ANOVA for the relative height growth rate resulted in significance of species,  $p = 0.073$ , and treatment,  $p = 0.003$ . Tukey comparisons displayed large gaps resulted in higher relative height growth than the small gap treatments. This suggests that light conditions are more important than soil conditions for the height growth.

**P16** Jennifer Cahill, Dr. Chris Rose

**Measuring the Effects of Exogenously Applied Thyroid Hormone on Cartilage Growth and Shape Change in *Xenopus* Tadpoles**

Amphibians are creatures whose skulls grow without changing their shape during larval stages and then change shape without growing during their metamorphosis. The growth and shape changes of skull tissues have been well documented for a number of frog and salamander species. Amphibians regulate much of their postembryonic development with thyroid hormones (TH), which appear in the blood at mid-tadpole stages and increase steadily until the end of metamorphosis. Many experimental studies have applied thyroid hormones to induce metamorphic changes precociously, meaning in early and mid-tadpole-stage specimens. However, it is not known how the shape changes that are induced experimentally when TH is applied exogenously to immature tadpoles compare to those that occur naturally when mature tadpoles progress through metamorphosis. We also don't know the specific roles of the two kinds of TH, T3 and T4, in producing each kind of shape change and whether either hormone contributes to skeletal growth before metamorphosis. To answer these questions, we exposed *Xenopus* tadpoles at early and late tadpole and early metamorphic stages to either T4 or T3 and compared the effects on body size and size and shape of two cranial cartilages to the changes that occur in natural metamorphosis. TH-treated and control specimens were also treated with methimazole to block the production of endogenous T4 and with iopanoic acid to block the conversion of T4 and T3 to other forms of TH. Results will be discussed.

**P17** Virginia Hoffman, Dr Alex Bannigan

**Effects of Oleic Acid on Arabidopsis Growth and Microtubule Rearrangement**

Microtubules are small tubular structures found in all eukaryotic cells, including plants, that direct cell growth and help in cell division. Microtubules rearrange by polymerization and depolymerization, allowing them to be highly dynamic. In plants, organization of interphase microtubules is strongly correlated with cell elongation rate. The orientation of microtubules can be affected by a number of things, including pathogenic attacks, light and hormones. Phospholipase D is a plasma membrane associated enzyme that is known to interact with

microtubules (Gardiner et al., 2001). Oleic acid is among the stimuli known to affect Phospholipase D activity (Wang & Wang, 2001). Here, we show that activation of Phospholipase D by oleic acid is correlated with microtubule rearrangement and Arabidopsis growth rate. Increasing concentrations of oleic acid decreases the Arabidopsis growth rate and increases the degree of microtubule reorientation.

**P18** Hayley Norian, Dr Steven Cresawn

### **A Bioinformatics Approach to Revealing the Genetic Basis for Bacteriophage Host**

#### **Preference**

Mycobacteriophages are viruses that infect the genus *Mycobacterium*. The complete genome sequences of 220 mycobacteriophages have been determined. These have been grouped into 42 distinct clusters based on average nucleotide identity and protein family composition. While all 220 phages infect *Mycobacterium smegmatis* strain mc<sup>2</sup>155, the host on which they were isolated, a subset also infect other related mycobacterial hosts. These include human pathogens such as *Mycobacterium tuberculosis*. The extent to which these phages infect various mycobacterial hosts has been determined. The quantification of host range phenotypes was described as an efficiency of plating relative to mc<sup>2</sup>155. Phages with an efficiency of plating of one for a particular host have the same titer that they have on mc<sup>2</sup>155. Those with an efficiency of plating of zero for a particular host do not detectably infect that host. Efficiency values between zero and one can indicate a reduced replication rate or the emergence of viral mutants that can infect a host that is typically non-permissive for that virus. All three types of efficiency were observed, and efficiencies generally correlated with the phage genome clusters. Phages capable of infecting mycobacterial hosts other than mc<sup>2</sup>155 are of particular interest. We are developing software to find genotypes associated with the host range phenotypes. Genotypes that consistently correlate with the ability to infect a particular host will be validated experimentally. A more thorough understanding of the molecular basis for host range will contribute to the utility of phages as therapeutic and diagnostic tools.

**P19** Ian Richardson, Sina Kipry, Dr. Roshna Wunderlich

### **Plantar Pressure and Kinematics of Walking and Running in a Habitually Barefoot Population in Madagascar**

The hominin lineage is defined by our characteristic bipedal gait, but interpretation of fossil material, including footprints, is hindered by a lack of functional data on people whose foot shape and function are unhindered by the presence of footwear. Furthermore, recent debate regarding foot strike patterns (FSP) in habitually unshod groups has questioned whether FSP variation is a result of running speed, individual choice, or population-specific running styles. We asked whether footwear has an effect on plantar pressure distribution and FSP and whether there is variation among unshod populations. We compared plantar pressure distribution and FSP during walking and running in two habitually unshod groups from Madagascar and Kenya (Daasanach) and one habitually shod group from JMU. We quantified peak plantar pressures in 8 foot regions using an EMED- ST pressure mat (Novel GmbH). During walking habitually unshod groups have lower metatarsal, elevated toe 2-5 pressures (Malagasy), and elevated midfoot pressures (Daasanach). The Malagasy subjects placed more pressure on the medial heel, while the Daasanach exhibited higher lateral heel pressures. During running Daasanach rear foot strike (RFS) 72% of all steps, while the Malagasy RFS 79% of all steps but at slower speeds. However, our data shows no relationship between speed and FSP. Daasanach showed higher metatarsal 1 and heel pressures while running than Malagasy ( $P < 0.05$ ). Variations in plantar pressure distribution may result from the effects of footwear or foot soft tissue morphology. Habitually unshod populations may use footfall patterns that allow for greater load distribution across the forefoot.

**P20**

Caitlyn Klotz Matthew Wallace, Dr. Mark Gabriele

**Countergradients and Modular Expression Patterns of Eph-Ephrin Signaling Proteins in the Developing Auditory Brainstem.**

We are interested in gaining a better understanding of a family of receptor tyrosine kinase proteins called the Eph-ephrins, and their role in establishing the orderly arrangement of converging inputs to the inferior colliculus (IC) prior to hearing onset. Previously, we described the development of layered projections to the central nucleus of the IC (CNIC), as well as a modular input to the lateral cortex of the IC (LCIC) arising from the lateral superior olive (LSO). Recently, we showed in ephrin-B2 mutant mice that LSO inputs to the IC lack a clear topography, despite maintaining their characteristic patterns. These results led to the present studies that build upon preliminary expression data from immunohistochemistry and X-Gal staining experiments utilizing lacZ mutants. Quantitative methods were used to confirm discernible CNIC gradients and LCIC expression modules, as well as to explore the possibility of similar gradients in the LSO. Sampling methods reveal gradients of EphA4 and ephrin-B2 across the tonotopic axis of the CNIC, most concentrated in high-frequency regions. In addition, expression data reveals prominent modules for each throughout the LCIC. In contrast, the CNIC is devoid of ephrin-B3, although present in the LCIC and exhibiting an extramodular expression. Ephrin-B2 and EphA4 in the LSO are expressed in a complementary gradient, with protein most concentrated in low-frequency regions, while ephrin-B3 is absent. The described countergradient between ephrin-B2 (LSO) and EphA4 (CNIC) that have known binding affinities for one another may provide insights for the establishment of topographic connections between these auditory brainstem nuclei.

**P21**

Brittany Hatchett, Lauren van Reesema, Dr. Terrie Rife

**Nitric Oxide Synthase Expression in Skeletal Muscle of Prediabetic Rat Models**

In recent years, the metabolic health of the U.S. population has reached an all time low, and is steadily decreasing. High fat and high fructose diets are contributing to this epidemic, becoming precursors to conditions such as cardiovascular disease and diabetes. To study this, Sprague Dawley rats were fed either a 60% fat diet or a 66% fructose diet for 10 or 12 weeks respectively. Rats that were affected by this diet develop insulin resistance, making them a useful prediabetic model. Nitric oxide synthase (NOS) is predicted to be influential in the pathology of diabetes, as NOS triple knockout mice exhibit symptoms of diabetes such as insulin resistance. Changes in NOS expression will be examined in skeletal muscle, which is one of the largest insulin-sensitive organs in the body, and all three isoforms of NOS have been detected in this tissue. We expect to see differential NOS expression between skeletal muscle samples from prediabetic rats and healthy rats that may differ based on diet because of different signaling cascades. This project will examine the expression of NOS in skeletal muscle of prediabetic rats by extracting RNA from this tissue, reverse transcribing the RNA into cDNA, and PCR amplifying this cDNA to quantify NOS expression. Future work includes investigating other genes that could be involved in metabolic syndrome as well as the various NOS isoforms.

**P22**

William Shoemaker, Dr. Eria Rebollar, Dr. Reid Harris

**A Study of Horizontal Probiotic Bacterial Transmission Rates in *Agalychnis callidryas*: the Effects of Population Density**

Currently amphibians are threatened with population declines worldwide, a major cause being the fungal disease chytridiomycosis. To thwart the epidemic bioaugmentation via probiotic inoculation has been proposed as an option, though of the three modes of probiotic bacterial transmission there has not been any published research on horizontal transmission. The proposed experiment revolves around the hypothesis that probiotic bacteria are transmitted horizontally throughout a tadpole population. The main objective of this experiment is to determine whether phasing out environmental transmission would allow a rate of horizontal transmission within a tadpole population to be obtained. Environmental transmission occurs with horizontal transmission, so by having an environmental control the level of horizontal transmission can be obtained. In addition the experiment addresses two secondary objectives: whether population plays a role in the rate of horizontal transmission and whether the cutaneous probiotic bacterium

can be maintained throughout the metamorphosis of the tadpole. The experimental design will have two main treatments: one where environmental and horizontal transmission can occur and one where only environmental is possible. The treatments will be further differentiated by tadpole population and swabbed multiple times over two months. Each treatment will have a single *Agalychnis callidryas* tadpole which has been inoculated with a rifampicin resistant strain of a cutaneously native probiotic bacterium, allowing for quantifiable data to be readily obtained. Preliminary trials are currently being done to test the methods before moving on to a large scale study.

**P23** McKenzie R. Quinn, Steven G. Cresawn

**Analyzing Relationships of Adjacent Phamilies in Bacteriophage Genomes to Help Determine Gene Function Using A New Phamerator Tool**

The majority of bacteriophage genomes have genes of unknown function and with no conserved domains. This poses a problem when trying to annotate and compare these genomes. The bioinformatic tool Phamerator helps the user visualize conserved protein-coding regions grouped into “phamilies” of a relatedness greater than 32.5%. A large number of these phage genomes have been primarily isolated by the HHMI Science Education Alliance program. Although users can effectively visualize the similarities between genomes, there is currently no tool to visualize the conservation in the organization of the genome. “Pham Friends” is a new tool added to the Phamerator program that detects neighboring phamilies when a phamily is selected. By scanning the entire database of phage genomes, neighbors of this selected phamily are displayed and the frequency in which they occur. This display allows the user to visualize if specific genes appear together in a conserved operon structure and if synteny is occurring with these genes. Results may suggest a similarity in function if the neighbors are always paired together. Information gathered by this program can help elucidate the issues concerning the annotation and comparison of the genomics of bacteriophage genomes.

**P24** Kevin Tomlinson, Dr. Jon Kastendiek

**The Effect of Additional Protection From Deer Browsing on Growth and Survival of Seedlings at the Smith Creek Restoration Site.**

This study will determine if additional protection from deer browsing will increase growth and survival of tree seedlings planted at the Smith Creek Restoration site. In 2005 9,033 tree seedlings were planted in four-foot seedling protection tubes. In 2012 it was found that only 656 seedlings had grown above the deer browse line of at least 50 cm above the protective tubing. Of these only 8 were oak seedlings. Oaks are of particular concern because of the high ecological significance of their acorns, shading, and the habitats they provide wildlife. We will add plastic mesh tubing toppers to the protective tubes in order to protect the growing tip of the seedlings an additional foot and allow them to grow above the deer browse line. We will test the effect of the treatment by comparing growth and survival of seedlings with the extra protective tubing to those without it.

**P25** Lauren van Reesema, Keith Zirkle, Kassim Rahawi, Dr. Terrie Rife, Dr. Nusrat Jahan

**The Effects of Metabolic Syndrome on Gene Expression in the Kidney: A Meta-Analysis of Microarray Studies**

Metabolic syndrome (MetS) is a clustering of risk factors that occur together to increase the chance for developing conditions such as cardiovascular disease, type 2 diabetes, and kidney disease. Nitric oxide synthase (NOS) knockout rats develop kidney dysfunction similar to that associated with metabolic diseases, making the kidney an interesting organ for investigation. This study used a random effect size meta-analysis model to combine the results of three existing microarray studies of MetS in the rat kidney and aimed to find significant patterns in gene expression related to MetS, as well as the role of NOS within the etiology of this syndrome. By combining the studies, the statistical power and applicability of the results were increased since several subsets of the populations were investigated. Within the kidney, the expression of 126 genes was significantly altered in the MetS condition, with 110 up-regulated and 16 down-regulated. “Novel” genes were identified as genes that had an expression fold change of 1.5 or

greater in the same direction, for each study included. Using this method, we identified one novel gene (Mlc1), which has been associated with megalencephalic leukoencephalopathy with subcortical cysts (MLC), a rare demyelinating disease of the brain. Another gene (hnRNP L), identified as novel in previous analyses, has been shown to stimulate the splicing of eNOS pre-mRNA and promote degradation of iNOS mRNA, and thus may serve as a regulator of vascular inflammation. The expression of the NOS isoforms themselves remained unchanged, further emphasizing the intricately varied physiological functions of NOS.

**P26** Morgan Donovan, Maria Wilkins, Dr. Janet Daniel

**Investigating the Effect of Osmotic Stress in Root Growth in STP 1 k/o and WT Plants**

We are investigating the role of STP-1 in salt resistant phenotype observed in the STP1 k/o plant. We have shown that STP1 k/o plants grown in soil and on agar plants with 50mM NaCl are significantly longer than WT plants grown under the same conditions (Lee et al, 2012). In order to obtain increased plant tissue to initiate gene expression studies, we have adapted a hydroponics system to grow plants in larger quantities and higher efficiency. HYDROPONICS RESULTS: WT and STP1 K/O mutants were planted in 0.65% agar filled microcentrifuge tubes, and were suspended in a solutions of 0 and 50 mM NaCl Gibeau's solution. After four weeks the specimens were harvested, and measured. Average root lengths of STP1 k/o and WT plants grown in 50 mM NaCl were 9.69 mm and 5.91mm, respectively ( $p=0.08$ , unpaired t-test). Average root lengths of STP1 k/o and WT plants grown in 0 mM NaCl were 9.64 mm and 14.61 mm, respectively ( $p=.12$ , unpaired t-test). PLATE RESULTS: Average root lengths of 3 week old STP1 k/o and WT plants grown in 50 mM NaCl were 19.16 mm and 11.91 mm, respectively. Average root lengths of 3 week old STP1 k/o and WT plants grown in 0 mM NaCl were 23.26 mm and 19.09 mm, respectively. Taken together, these results suggest that STP1 k/o root growth is more resistant to salt stress than WT.

**P27** Katie Garbarini, Ben Boward, Chelsea Cockburn, Michael Ferras\*, Cy Lampugnale, Ashley Miller, Abby Pyper, Dr. Justin Brown

**Exploration of the Role of Ambient Temperature in the Recovery From Major Surgical Stress.**

Laboratory animals are routinely surgically instrumented for experimental protocols. However, the guidelines for recovery remain ambiguous. Many times, animals recover at normal room temperature ( $\sim 22^{\circ}\text{C}$ ) which equates to a cold stress (Brown and Le 2011). Previous data suggest that rats prefer ambient temperatures ( $T_{\text{amb}}$ ) of  $\sim 27^{\circ}\text{C}$ . We hypothesize that if  $T_{\text{amb}}$  during recovery were elevated to an animal's preferred temperature, then the animal experience less stress and recover more quickly.  $T_{\text{amb}}$  was maintained at various levels while measuring water and food intake, body weight, and circadian changes in motor activity (MA) and core temperature ( $T_{\text{c}}$ ) for one week after abdominal and cranial surgical stress. Rats struggled to recover to normal circadian rhythms for  $T_{\text{c}}$  and MA when maintained at  $T_{\text{amb}}$  of  $24^{\circ}\text{C}$  and  $30^{\circ}\text{C}$ . When rats were maintained at  $27^{\circ}\text{C}$ , MA returned to normal circadian rhythms almost immediately while  $T_{\text{c}}$  rhythms returned at  $\sim 3$  days. Rats housed at  $30^{\circ}\text{C}$  may have been exposed to a heat stress which affected surgical recovery. We suggest animals be maintained near  $27^{\circ}\text{C}$  following surgical instrumentation to minimize thermal stress during recovery. This reduction in thermal stress will facilitate recovery from surgery and enable more reliable data collection from these animals. \* Student research supported by Elizabeth McConnell Bliss Endowment for Undergraduate Research Scholarship

**P28** Michelle Clower, Rebecca Turner, Dr. Grace Wyngaard

**Genome Size: A Tool to Aid in Delineating Putative Species of Copepods (Crustacea: Copepoda)**

Species delineation is often a challenging task, especially when such data on interbreeding are not available. The task of defining a species is critical in studies of biodiversity, in the interpretation of large bodies of ecological data and as a basis for reconstructing phylogenies. Traditionally species delineation relied on crossbreeding and morphological criteria. In the past 10 years, DNA sequence barcodes (most often mitochondrial cytochrome oxidase I) have increasingly been used, as they are convenient and often easily obtained. However, this COI barcode sequence



has proven problematical to PCR amplification in many microcrustacean copepods. But, even in cases in which such barcodes can be amplified and sequenced, it remains to be shown how much nucleotide divergence corresponds to differentiation at the species level. In either case, additional kinds of characters may supplement the species criteria and aid in delineating species. Genome size, measured as the number of pg or Gb of DNA per nucleus in a genome, is a species specific characteristic. Genome sizes will be measured using image analysis densitometry in several species of *Mesocyclops* (Crustacea: Copepoda) from Sudan, Australia, Brazil and Europe that possess variable morphologies and DNA barcode sequences. Variation in genome size among widely separated geographical populations of a putative species may indicate that additional examination of species criteria and boundaries are warranted. Additionally, because genome size is also an important fitness characteristic, variation in genome size may yield insights into environmental and evolutionary constraints.

**P29**

Melissa Maloof, Rachel Stottlar, Laura Johansen, Pria Chang\*, Katherine Sinclair, Maureen Filak, Fafa H. Koudoro, Rahul Warrior, Dr. Susan R. Halsell

**In vivo Time Lapse Confocal Analysis of the RhoA Head Involution Defect and Molecular and Genetic Characterization of Five Extant RhoA Mutant Alleles**

RhoA signal transduction functions in myriad morphogenetic processes throughout the *Drosophila* life cycle. Characterized RhoA mutant alleles are homozygous embryonic lethal with a characteristic defect in head involution. This work presents analysis of the head involution defect using time-lapse confocal microscopy. Cells and their actin cytoskeleton were visualized using an actin-binding GFP-moesin fusion protein driven by the spaghetti-squash promoter (SGMCA; Edwards et. al. 1997. *Dev. Biol.* 191, 103). Wild type and null RhoA mutants were analyzed. To date, data indicates the dorsal ridge forms normally and begins its anterior-ward movement, but is ultimately impeded on the dorsal side of the embryo when the procephalon and clypeolabrum fail to retract; this is consistent with the dorsal anterior hole observed in cuticle preparations of RhoA mutant embryos. Phenotypic and molecular characterization of five EMS induced RhoA mutations (233 and 246, Ward, Evans and Thummel, *Genetics.* 165:1397; 3.5.1, 4.4.2 and 7.23.1) will also be described. Four of the five alleles exhibit apparent complete loss of function phenotypes; they are 100% embryonic lethal and all show consistent anterior dorsal holes in the cuticle. In addition, three of these alleles have been sequenced and each shows a mutation consistent with a null phenotype. All are single G to A transitions and alter either the start codon, a splice donor site or introduce a premature stop codon. The fifth allele, however, may be a hypomorphic mutation. This is based on the observation that the mutation is not 100% lethal embryonically and the dead embryo cuticle phenotype is variable, with the majority of embryos exhibiting defects in the head skeleton by no dorsal anterior hole. Genetic and molecular characterization of this allele continues.

\* Student research supported by The Betty Jo Loving Butler '58 Endowment for Undergraduate Research Scholarship and The Farrell Summer Research Scholarship

**P30**

Nurlybek Mursaliyev, Chris Berndsen, Nathan Wright and Jonathan Monroe

**Structural Investigations of a Catalytically-inactive  $\beta$ -amylase9 from Arabidopsis**

$\beta$ -Amylases play an important role in nighttime starch degradation in chloroplasts hydrolyzing  $\alpha$ -1,4-glycosidic bonds to produce maltose, which is exported to the cytosol for further metabolism. There are nine known  $\beta$ -amylase-like genes (BAMs) in Arabidopsis, six of which encode plastid-targeted proteins, but only four of those are catalytically active. One of the non-catalytic, plastidic BAMs, BAM9, is conserved in flowering plants and green algae, suggesting that it plays a regulatory role in plastid function. Arabidopsis plants with a T-DNA insertion in exon 3 of the BAM9 gene appear to be normal, but accumulate small amounts of starch. Microarray data show that BAM9 expression peaks strongly at the night/day transition suggesting that the protein may function during starch synthesis. Because plants lacking BAM9 accumulate starch, we suspect that BAM9 may function to slow or block starch synthesis during the day under certain conditions. Compared with active BAMs, models of BAM9 reveal a more open sugar-binding side with conservation deep in the pocket, consistent with it binding to a disaccharide such as maltose. We are using isothermal calorimetry to investigate which sugars bind to a his-tagged, purified BAM9. We are also attempting to characterize BAM9 using CD spectra, and are using Ellman's reagent to

detect disulfide bonds. Models of BAM9 with and without maltose in the sugar-binding site revealed surface mobility in a structurally disordered region, consistent with it being a protein-binding site. Interestingly, this flexible surface is conserved in all of the BAM9 orthologs, including those from algae.

**P31** Jessie Doyle, Margaret King, Mycah McNett, Joseph Noel, Rebecca Sanders, Ty Steve, Dr. Bruce Wiggins

### **A Comparative Stream Water Quality Survey of Smith Creek Using Benthic Macroinvertebrates**

In 2005, a restoration project began at Rainbow Farm, which is located on Smith Creek, a 35-mile tributary of the North Fork of the Shenandoah River. Trees were planted in the fields surrounding the stream, and cattle were fenced out. To determine the effectiveness of this restoration, measurement of the presence and types of macroinvertebrates that inhabit the stream was initiated in 2010. Several indices, including the Hilsenhoff Biotic Index (HBI), the Rapid Bioassessment Protocol of the Environmental Protection Agency (EPA), and the Shannon diversity index ( $H'$ ) were used to analyze stream health in the restored area and upstream in an unrestored area. We hypothesized that the restored area will have a healthier stream quality than the upstream area, and that the stream health would improve over time in the restored area. Although there were no significant differences found in the means of the indices among the years (2010 - 2012), there were trends showing the restored areas of the stream becoming healthier. Diversity in the restored area ( $H'$ ) increased from 2010 to 2012 ( $p=0.004$ ), as did the EPA index ( $p = 0.066$ ). The pollution tolerance index (HBI) decreased ( $p= 0.059$ ) over the 3-year period. In contrast, the upstream  $p$ -values for the HBI, EPA, and  $H'$  were 0.782, 0.776, and 0.657 respectively, showing no significant change over time. All of the overall trends suggest that the stream is becoming healthier. More samples must be taken in the coming years to confirm this.

**P32** Jacob Mesler, Emily Cate, Dr Patrice Ludwig

### **A Comparison of Developmental Thresholds for Horn Length in Male Dung Beetles.**

Morphological variations among members of the same sex indicate multiple behavioral adaptations for obtaining mates. The dung beetle *Onthophagus taurus* possesses a noticeable dimorphism between males, in which those males above a specific body size develop large horns, while those below this body size do not develop horns. Each morph's success is determined by sexual selection; males with horns, called majors, guard females and use their horns to combat other majors. Generally the major with the larger horns will win the ability to copulate with the female. However, males without horns, called minors, do not fight and instead dig tunnels that allow them to copulate with a female by circumventing the guarding major. This trait is controlled by a developmental switch regulated by the amount of food present in the larval stage, resulting in an identifiable inflection point when plotting horn length to body size. Horn length was compared to body size in the Shenandoah Valley allowing for the determination of the body size required to activate the developmental switch. This inflection point differed from those measured in other areas by different studies. This difference indicates that environmental factors are relevant in determining the body size required to trigger the switch. This result suggests that food quantity during the larval stage alone does not account for the morphology of the resulting adult beetle.

**P33** Shelby Burns, Renzo Renteria, Dr. Alex Bannigan

### **Creating a Digital Online Catalog of Microscope Slides in the Biology Department**

The Madison Digital Image Database (MDID) is a helpful resource to view collections of image entries from different projects made by students and faculty at James Madison University. We have created and are maintaining and updating a microscopy slide catalog database on MDID. This slide catalog database will be of use for faculty and students in the Biology department searching for microscopy slides or images of biological specimens for educational classroom visual aids or biological research. Each slide catalog data entry includes a slide image taken with an explanation of appropriate microscopic techniques utilized, a description of the specimen, as well as other relevant details which define and organize the collection. Students contributing to the microscopy slide catalog used laboratory procedures such as correct brightfield,

differential interference contrast (DIC), phase contrast and fluorescence microscopy techniques. We aim to expand this project to digitize and catalog slides specific to different classes taught in the Biology Department.

**P34** Kyle Bonifer, Brandi Volkers, Dr. Kyle Seifert

**Antimicrobial Analysis of the Meta-Pyridine Series of Novel Amphiphiles**

Throughout the history of antibiotic use, drug resistance has made the development of novel and potent drugs a necessity. Our research is centered on the development of novel amphiphiles comprised of non-polar hydrocarbon tails varying in length, and multiple polar head groups. These series of amphiphiles were assayed for antimicrobial effectiveness against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli* by performing standardized minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. MIC and MBC values for these amphiphiles were in the low micromolar range. Specifically, the compound M-P,12,12 (meta-pyridine, 12 carbon chain, 12 carbon chain) was most effective for all strains tested, with MIC and MBC values of 2 $\mu$ M for *S. aureus*, 2 $\mu$ M for *E. faecalis*, 4 $\mu$ M for *E. coli*, and 8 $\mu$ M for *P. aeruginosa*. In addition to being the most effective compound, the MIC values of M-P, 12, 12 were less than control compounds DTAB, CTAB, and Lysol. The amphiphilic compounds have also shown efficacy in synergy with one another, having FIC (fractional inhibitory concentration) values below the 0.50 value necessary to indicate synergy. For example the combination of M-P,12,12 and mX,14,14 were able to have value of 0.19 for *E.coli* and M-P,12,12 and M-P,14,14 have an FIC value of 0.31 for *S.aureus*. Our most promising compounds have also shown to significantly reduce biofilm formation in *P.aeruginosa*, reducing to nearly zero when compared to positive biofilm controls.

**P35** Nicholas Minahan, Jason Floyd, Dr. Kyle Seifert

**Antimicrobial Analysis of the Xylylene Series of Novel Amphiphiles**

Antibiotic resistance is advancing ubiquitously via multiple modes of horizontal gene transfer (HGT), and has given rise to highly resistance life-threatening human pathogens such as *Mycobacterium tuberculosis* and methicillinresistance *Staphylococcus aureus* (MRSA). To date, our collective aim has been to develop a new class of potent, broad-spectrum antimicrobials, based on novel series of amphiphiles that will be difficult for organisms to counteract or to resist via mutation. In order to determine the biological activity our amphiphiles, minimum inhibitory concentration (MIC) assays and minimum bactericidal concentration (MBC) assays were performed for each compound to determine the inhibitory efficacy of each compound against known bacterial pathogens. Notably, our amphiphiles have shown greater inhibitory and bactericidal activity than control compounds DTAB, CTAB and benzalkonium chloride (Lysol), falling within the low micromolar range. Although our xylylene series (oX, mX, pX) amphiphiles do not match the inhibitory or bactericidal activity of our mesitylene series (M) or paraquat series (PQ) amphiphiles, they achieve striking synergy in combination against both Gram-positive and Gram-negative organisms. Synergy of our amphiphiles is determined by the fractional inhibitory concentration (FIC) index, where an FIC index of  $\leq 0.5$  indicates synergy. For example, amphiphiles mX,14,14/mX-1,14 of the xylylene series have an FIC index of 0.19 for Gram-negative *Escherichia coli* and 0.50 for Gram-positive *Staphylococcus aureus*. All the while, our xylylene series amphiphiles maintain MIC and MBC values as low as 4 $\mu$ M for *Staphylococcus aureus* and 8 $\mu$ M for *Escherichia coli*.

**P36** Lena Husnay, Dr. Wunderlich, Dr. Rittenhouse, Dr. Daniel

**Effects of Omega-3 Fatty Acids on Bone Mineral Density and Overuse Injuries in Female Athletes**

Overuse injuries such as stress fractures occur with repeated microdamage to musculoskeletal tissues and are especially prevalent in female athletes. Geometric features of bone as well as bone mineral density (BMD) have been associated with stress fracture risk. While Omega-3 polyunsaturated fatty acids (PUFAs) have been associated with reducing inflammation, increasing BMD and reducing incidence of osteoporotic fractures in the elderly, few studies have addressed the role of Omega-3 PUFAs in injury risk reduction in young female athletes. Studies indicate that Omega-3 PUFAs act on cytokines to inhibit osteoclasts while also promoting

osteoblasts, thereby preventing bone resorption and stimulating formation. We examined the hypothesis that athletes with higher circulating and dietary levels of Omega-3 PUFAs will have higher BMD and lower risk of overuse injuries than athletes with lower levels of Omega-3 PUFAs. Athletes with history of overuse injuries had significantly lower percentages of circulating Omega-3 PUFAs ( $p < 0.05$ ) and significantly lower BMD ( $p < 0.05$ ) than athletes with no history of overuse injury. However, we found no relationship between BMD and circulatory Omega-3 PUFAs ( $r^2 = 0.006$ ). Omega-3 PUFA levels identified in our food frequency questionnaire were not significantly related to circulating levels. While these data suggest Omega-3 PUFAs may influence bone health and injury risk, the mechanisms by which these factors affect bone health in young athletes are complex. Understanding the relationships among dietary factors, musculoskeletal health and injury risk is essential to the development of prevention strategies for stress fractures and other musculoskeletal injuries.

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**James Madison University Biology Department**  
**First Annual**  
**GRADUATE SYMPOSIUM**  
**Thursday April 18<sup>th</sup>, 5:30pm**

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Program:

- 4:45-5:30    OPENING RECEPTION – Second floor foyer
- 5:30            WELCOME
- 5:35            Marcus Skafren\*, Bharath Balu, Chris Lantz  
A Novel RCC1-like Protein is a Crucial Regulator of the Intraerythrocytic Cycle of the Human Malaria Parasite, *Plasmodium falciparum*
- 5:55            Jade E. Ladow, Jhosdyn A. Barragan, Gabriel A. Fitzgerald, Kyle S. Bonifer, Kevin L. Caran, Kevin, P.C. Minbiole, and Kyle Seifert  
Synthesis and Structure-Function Analysis of a Novel Series of Multi-Headed Amphiphiles for Use as Antibacterial Compounds
- 6:15            Caylin Murray and Dr. Tim Bloss  
Getting the NAC of the Unfolded Protein Response: is it a Matter of Life or Death?
- 6:35            Andrew H. Loudon. Advisor: Dr. Reid Harris  
THESIS PRESENTATION FOR DEFENSE  
Microbial Dynamics and Core microbiome of Red-backed Salamanders (*Plethodon cinereus*)

## 1.

Marcus Skaflen\*, Bharath Balu, Chris Lantz

**A novel RCC1-like Protein is a Crucial Regulator of the Intraerythrocytic Cycle of the Human Malaria Parasite, *Plasmodium falciparum***

Malaria is a deadly disease that infects 300-500 million people every year, 1 million of those infections resulting in death. The disease is caused by an infection from the single celled protozoan *Plasmodium falciparum*. Over a decade ago scientists sequenced the genome in hopes of gaining insight that would better help them cure this fatal disease. They discovered an AT rich genome with 60% of the genes coding for proteins of unknown function with no significant homology to any known proteins.

We used the *piggyBac* transposon system and reverse genetics in a phenotypic screen for attenuated growth in blood culture to identify an unknown gene that plays a critical role in the proliferation of *P. falciparum*. After identifying the previously unknown gene we examined the blood stage, also known as the intraerythrocytic cycle, to determine the function of this important protein.

Our analysis revealed no difference in cycle time, egress, or invasion between the wild type and the mutant strain. We did identify a significant reduction in the number of merozoites, the replicated parasite, being formed in the mutant. Further analysis revealed a significant delay in the initiation of nuclear division and the localization of our protein to the microtubule organization center.

Using forward genetics, flow cytometry, microscopic analysis, and immunohistochemistry we have determined the function of a previously unknown protein in *P. falciparum*. We have identified a protein important for the regulation of merozoite formation and the initiation of nuclear division during the intraerythrocytic cycle and a possible new drug target.

\* Student partially supported by Jean D. Acton Scholarship

## 2.

Jade E. Ladow, Jhosdyn A. Barragan, Gabriel A. Fitzgerald, Kyle S. Bonifer, Kevin L. Caran, Kevin, P.C. Minbirole, and Kyle Seifert

**Synthesis and Structure-Function Analysis of a Novel Series of Multi-Headed Amphiphiles for Use as Antibacterial Compounds**

In the US, approximately two million patients a year acquire a hospital-acquired infection during their stay, leading to almost 100,000 deaths. An increasing number of these infections are caused by multi-drug resistant organisms. Development of novel, potent antimicrobial compounds could be used in eliminating or reducing potential pathogens in the hospital setting. As a continuation of our previous research on bicephalic amphiphiles, which correlated antibacterial activity with head group arrangement and chain length, the antimicrobial activity of a novel series of amphiphiles was investigated. This series of amphiphiles was assayed for antimicrobial effectiveness against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli* by performing standardized minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. MIC and MBC values for these amphiphiles were in the low micromolar range. Specifically, the compound M-P,12,12, (meta-pyridine, 12 carbon chain, 12 carbon chain) was most effective for all strains tested, with MIC and MBC values of 2 $\mu$ M for *S. aureus*, 2 $\mu$ M for *E. faecalis*, 4 $\mu$ M for *E. coli*, and 8 $\mu$ M for *P. aeruginosa*. In addition to being the most effective compound, the MIC of M-P,12,12 for *P. aeruginosa*, a difficult to treat organism, was 4 fold less than the MIC of Lysol for *P. aeruginosa* (8 $\mu$ M and 32 $\mu$ M, respectively). This research builds upon initial discoveries regarding structure and bioactivity to identify structures with the most potent antimicrobial activity while remaining safe.

### 3.

Caylin Murray and Dr. Tim Bloss

#### **Getting the NAC of the Unfolded Protein Response: is it a Matter of Life or Death?**

The nascent polypeptide associated complex (NAC) is a highly conserved heterodimeric component of the cytosolic chaperone network. Recently, depletion of the [*C. elegans*] NAC homolog ICD-1, has been implicated as a model for inducing mis-folded protein stress. Loss of ICD-1 appears to induce levels of mis-folded protein in the endoplasmic reticulum (ER) necessary to trigger a stress response known as the Unfolded Protein Response (UPR). Activation of the UPR results in an up-regulation of chaperones, a global down-regulation of translation, up-regulation of protein degradation, and expansion of the ER membrane. Mis-folded protein has been implicated in many, if not all, neurodegenerative diseases. In this study, we examine the effect of mis-folded protein stress in backgrounds where salient UPR proteins have been removed. Understanding the response to mis-folded protein stress generated by the loss of the NAC will provide insight into the putative role of the NAC in preventing the onset of neurodegeneration.

### 4. THESIS PRESENTATION FOR DEFENSE

Andrew H. Loudon (Advisor: Dr Reid Harris)

#### **Microbial Dynamics and Core microbiome of Red-backed Salamanders (*Plethodon cinereus*)**

Beneficial cutaneous bacteria on amphibians can protect against the lethal fungal disease chytridiomycosis, which has decimated many amphibian species. The stability of these bacterial communities likely influences health outcomes, but has not been investigated. We describe the diversity of bacteria on red-backed salamanders (*Plethodon cinereus*) in the wild, and the stability of these communities over time in captivity using culture-independent Illumina sequencing. In the field, there was no correlation between the diversity of salamanders' microbial communities and the diversity of their substrates' microbial communities. Salamanders were brought into the laboratory to test for the effect of an environmental reservoir (soil) in maintaining diversity and stability and were sampled every 7 days ending at day 28. In the laboratory, the alpha diversity of salamanders in the 'no bacterial reservoir' treatment decreased, whereas it remained constant in the 'bacterial reservoir treatment'. The treatment groups diverged from each other, yielding significant differences in beta-diversity. Eight OTUs defined a core community, i.e., present on >90% of salamanders through time, and a majority of these species are known to secrete antifungal metabolites, such as pseudomonads. As alpha diversity decreased without a soil reservoir, one core OTU from the phylum Verrucomicrobia dominated the community. This result suggests that the non-core community on the salamanders regulate the core community and that the non-core community is dependent on the soil reservoir. Bacterial community structure in both treatments changed when their salamander hosts were brought into the laboratory. However, diversity was more constant in the bacterial reservoir treatment. Defensive function of salamanders' cutaneous microbiota may depend on the diversity and stability of the core community.

# BIOSYMPOSIUM 2013

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