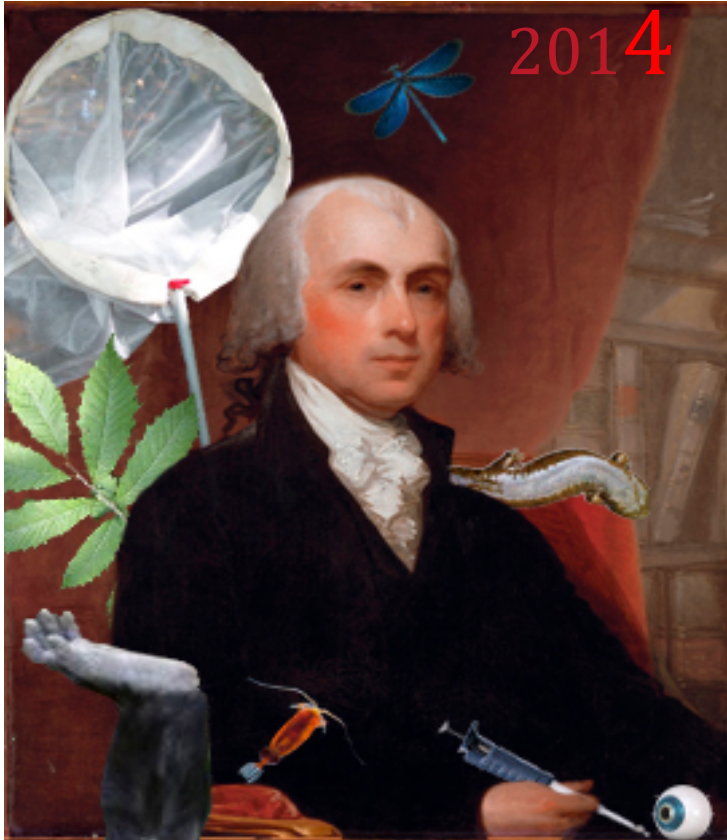


# BIOSYMPOSIUM



**James Madison University  
Biology Department**

## **Keynote Presentation**

**Michael Lipford**

VA Nature Conservancy

*“Conservation Challenges, Virginia Solutions”*

**Friday, April 11, 12:20-1:10, Bioscience 1007**

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
- ❖ Betty Jo Loving Butler '58 Endowment for Undergraduate Research Scholarship
- ❖ Farrell Summer Research Scholarship
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- ❖ Summer Research Scholarship (Anonymous Donor)

*\*Student authors whose research has been supported by one of these gifts are noted with an asterisk.*

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**Schedule: Thursday April 10<sup>th</sup>**

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<b>SESSION 1</b>	<b>12:30-2:00pm</b>	<b>POSTERS</b>	<b>2nd Fl. Foyer</b>
P1	Mycah McNett, Margaret King, Jessie Doyle, Joseph Noel, Nathan Robinson, Rebecca Sanders, Ty Steve, Erin Thady, Dr. Bruce Wiggins	<b>A Comparative Stream Water Quality Survey of Smith Creek Using Benthic Macroinvertebrates</b>	
P2	Allyse Harris, Matthew Wallace, William Noftz, Joseph Balsamo, Dr. Mark Gabriele	<b>Layered Lateral Superior Olivary Projection Patterns in the Inferior Colliculus in EphA4 Mutant Mice Prior to Hearing Onset</b>	
P3	Sina Kipry, Dr. Roshna E. Wunderlich	<b>Midfoot pressure distribution during bipedal and quadrupedal walking in <i>Pan Troglodytes</i></b>	
P4	Joseph Balsamo, Allyse Harris, William Noftz, Dr. Mark Gabriele	<b>Exploring multimodal inputs to the developing auditory midbrain</b>	
P5	Matt Hartmann, Chris Chrzan, Miriam Kabore, Katelyn Moore, Dr. Corey Cleland	<b>Rat Hind Limb Withdrawal Response to Heat Stimuli Depends on Initial Paw Posture but not Stimulus Location</b>	
P6	Michael Yeager, Victori Callahan, Dr. Marta Bechtel	<b>Characterization of Normal Human Articular Chondrocytes Gene Expression Following Dengue Viral Envelope Protein Treatment</b>	
P7	Lina Chhuy-Hy, Michael Yeager, Dr. Marta Bechtel	<b>The Effect of Lacritin on Primary Rabbit Cornea Cell Proliferation, Wound Healing, and the prevention of Apoptosis</b>	
P8	Jessie Ward, Kelsey Samms, Olivia Vito, Dr. Heather Griscom	<b>The Effect of Soil, Light, and Herbaceous vegetation on <i>Castanea dentata</i> seedling growth: Implications for Reintroduction</b>	
P9	Tara Gallagher, John Marafino, Brandi Volkens, Nick Minahan*, Jason Floyd, Abby Perlin, Caroline Dilworth, Dr. Kevin Caran, Dr. Kyle Seifert (graduate poster)	<b>The Antibacterial Activity of Novel Cationic Amphiphiles</b>	
P10	Allison Welborn, Dr. Conley K. McMullen	<b>A Floristic Survey of Smith Creek Restoration Area, Rockingham County, Virginia</b>	
P11	Ty Steve, Jessie Doyle, Dr. Bruce Wiggins	<b>National and Regional Geospatial Models To Predict The Levels of Methyl Mercury In Fish</b>	
P12	Brian Schwenk, Dr. Dean Cocking	<b>Landscape heterogeneity of airborne Hg (mixed dissolved, particulate, and vaporous) and a sampling of total Hg concentration in macroinvertebrates within Rockingham Co., VA</b>	
P13	Wes Deaver, Kevin Hatala, Heather Dingwall, Brian Richmond, Dr. Roshna Wunderlich	<b>Foot Strike Patterns in Habitually Unshod Children Compared to Adults</b>	
P14	Jennifer Kurasz, Curtis Kapsak, Jesmine Roberts-Torres, Dr. James Herrick	<b>Our Little MinION: Introducing the Next Generation of Sequencing Technology</b>	



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**Schedule: Friday April 11<sup>th</sup>**

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**SESSION 3 10:00-noon ORAL PRESENTATIONS BIOSCI 2007**

- 10:00 WELCOME
- 10:15 T9. Jonathan Schmitz, Dr. Jon Monroe  
**Differential effect of pH on two functional  $\beta$ -amylases in *Arabidopsis thaliana***
- 10:30 T10. William Shoemaker, Dr. Eria Rebollar, Dr. Reid Harris  
**Horizontal and pseudo-environmental transmission of the probiotic bacterium *Janthinobacterium lividum* occur in larval stage green frogs, *Lithobates clamitans***
- 10:45 T11. Michael Partin, Kevin Libuit, Dr. James Herrick  
**Isolation of Methicillin-Resistant *Staphylococcus* (MRCoNS) from a Manure-contaminated Stream in the Shenandoah Valley of Virginia**
- 11:00-11:15 :::::::::::::::::::: BREAK ::::::::::::::::::::
- 11:15 T12. Lauren R. Desrosiers, Dr. Dave Anderson  
**Investigation of the Mechanism of Action of the Steroidal Antiestrogen SR16388 Using Triple Negative Breast Cancer Cells**
- 11:30 T13. Nurlybek Mursaliyev, Dr. Christopher Berndsen, Nathan Wright, Dr. Jonathan Monroe  
**Biochemical characterization of an inactive beta-amylase9 (BAM9) in *Arabidopsis thaliana***
- 11:45 T14. Charlie Watt, Corey Swanson, Dillon Miller, Alaina Esposito, Dr. Christine May  
**Background color matching in juvenile brook trout: Evaluating the extent of phenotypic plasticity and habitat preferences in artificial stream channels**
- 12:00-12:20 :::::::::::::::::::: BREAK ::::::::::::::::::::

**Keynote Presentation:**

*“Conservation Challenges, Virginia Solutions”*

Michael Lipford, VA Nature Conservancy

***Bioscience 1007, 12:20-1:10***

1:10-2:00

LUNCH  
Second floor foyer

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## Schedule: Friday April 11<sup>th</sup>

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### SESSION 4 FRI 2:00-4:00 POSTERS 2nd Floor Foyer

- P15 Corey Swanson, Charlie Watt, Dillon Miller, Alaina Esposito, Dr. Christine May  
**Background color matching and substrate preference by juvenile brook trout (*Salvelinus fontinalis*)**
- P16 Alexandra Deal, Taelor Weaver, Shelby Snowden, Dr. Terrie Rife  
**Imaging First Exons 1h and 1k in the Rat Nitric Oxide Synthase 1 Gene**
- P17 Nicole M. Roberto, Anisa C. Tracy, Dr. Steven G. Cresawn  
**Next Generation Sequencing and Genomic Comparisons of Novel Mycobacteriophages**
- P18 Bridget Mitchell, Samantha Heitsch, Rebecca Gaita, Dr. Corey Cleland  
**Stimulus and Postural Determinants of the Escape Response of Crickets to Localized Heat Stimuli**
- P19 Katie McCullar, Maria Wilkins, Dr. Janet Daniel  
**Investigating the Effect of Various Salt Concentrations on Plant Growth in STP1 K/O and WT *Arabidopsis thaliana***
- P20 Emma Bales, Oliver Hyman, Andrew Loudon, Gregory Lipps, Eric Chapman, Kenneth Roblee, John D. Kleopfer, Kimberly A. Terrell, Dr. Reid Harris  
**Pathogenic chytrid fungi *Batrachochytrium dendrobatidis*, but not *B. salamandrivorans*, detected on hellbender salamanders**
- P21 Mary Katherine Garbarini, Cy Lampugnale\*, Kelcy Jackson, Kelly Burke, Dr. Justin Brown  
**The Role of Ambient Temperature in the Recovery from Major Surgical Stress**
- P22 Morgan Donovan, Kristen Reitano, Dr. Janet Daniel  
**The Role of STP1 in Glycophytic Plants**
- P23 Ahmed Alotaibi, Sally Coleman, Pritpal Saggu, Dr. Raymond Enke  
**Characterizing the Role of Epigenetic Gene Regulation in the Developing Chicken Retina**
- P24 Oumar Sacko, Dr. Idelle Cooper  
**Color variation and mating success in the Ebony Jewelwing Damselfly, *Calopteryx maculata***
- P25 Emily Grunwald, Victoria Shuklis, Sophia Brown, Dr. Raymond Enke  
**Histological Analysis of the Developing Chicken Eye**
- P26 Stephen Simonetti, Will Shoemaker, Dr. Eria Rebolgar, Dr. Reid Harris  
**A test of horizontal and vertical transmission among tadpoles of a bacterial probiotic that can inhibit a lethal fungal pathogen**
- P27 Spencer C. Burnett, Clara R. Thiel, Dr. Conley K. McMullen  
**Vascular plants of the E.J. Carrier Arboretum**
- P28 Catherine Torres, Dr. Amanda Storm, Dr. Jonathan Monroe  
**Expression and Characterization of BAM2 and BAM6 from *Arabidopsis thaliana***
- P29 Peter D Rossbach\*, Dr. Carol Hurney  
**Examining tail development models in the four-toed salamander, *Hemidactylium scutatum***
- P30 Kristen Fadel, Dr. Amanda Storm, Dr. Jonathan Monroe  
**Constructing BAM9 promoter-GUS plasmids for analysis of BAM9 expression in *Arabidopsis***
- P31 Kimberly Okafor\*, Ian Brown, Dr. Steve Cresawn  
**Cytotoxicity screening in a Mycobacteriophage genome**
- P32 Hayley A. Norian, Dr. Steven G. Cresawn  
**A Correlational Study of Mycobacteriophage Host Specificity and Protein Family Conservation**
- P33 Shvan Kareem\*, Courtney Matson\*, Erin Wallace, Dr. Kim Slekar, Dr. Tim Bloss  
**The effects of misfolded protein stress on different cell types in *C. elegans***
- P34 Jeff Kopsick, Dr. Eva Strawbridge

***C. elegans* locomotion in 3D-Printed Environments**

- P35 Gregory M. Steffensen, Zackary A. Zayakosky, Caroline I. Dilworth, Joshua Gerard T. Mata, Kristen L. Hoffman, Chloe I. R. Wines, Tessa L. Nester  
**Research Experiences of the Trelawny Learning Community 2013-14**
- P36 Michelle Barber, Jessica Wagner, Tyler Mullins, Clara Thiel, Rachael Schneider, Russi Tran\*, Harley Burton, Dr. Michael Renfroe  
**Analysis of Antioxidants in Selected Herbs and Spices**
- P37 Jena Butler, Harold Burke, Haley Nisson, Stephen Jurko, Benjamin Williamson, Dr. Susan Halsell  
**Brrrr! Analyzing Cold Nociception in *Drosophila* using Behavioral and Optogenetic Assays**
- P38 John Stephen Fisher, Kyle Snow, Dr. Christine May  
**Sedimentation and its effects on the dynamic relationship between brook trout and stoneflies, an undergraduate research project and outreach opportunity**
- P39 Laura Johansen, Dr. Susan Halsell  
**Genetic and Phenotypic Characterization of RhoA Alleles in *Drosophila melanogaster***
- P40 Kelsey Savage, Molly Bletz, Dr. Eria Rebollar, Dr. Reid Harris  
**Comparative analysis of anti-Bd bacteria from five Malagasy frog species of Ranomafana National Park**
- P41 Heather Gagné, Molly Bletz, Dr. Eria Rebollar, and Dr. Reid Harris  
**Tests of whether bacterial species from Malagasy frogs inhibit multiple strains of the lethal pathogen *Batrachochytrium dendrobatidis***
- P42 Kevin Tomlinson, Dr. Jon Kastendiek  
**The effect of addition of seedling protection tubing toppers on survivability of saplings at Smith Creek Restoration Site**
- P43 Rebecca Dickey, Molly Bletz, Dr. Eria Rebollar, and Dr. Reid Harris  
**Invasive parthenogenetic decapod *Procambarus fallax f. virginalis* as a possible host for the fungal pathogen *Batrachochytrium dendrobatidis***
- P44 Lina Chhuy-Hy, Michael Yeager, Dr. Marta Bechtel  
**The Effect of Lacritin on Primary Rabbit Cornea Cell Proliferation, Wound Healing, and the prevention of Apoptosis**

**T1** Katie Sipes, Dr. Charles Wolgamuth, Dr. Eva Strawbridge, Dr. Tim Bloss  
**Watch Those Worms Wiggle**

Viscosity is a measurement of a fluid's resistance to the rate of deformation. An example of a liquid with high viscosity is tree sap, or a homogeneous mixture of mud. Both of these liquids run very slowly when acted upon by gravity. In contrast, water has a very low viscosity and flows readily. So how does a liquid's characteristics effect the locomotion of a swimming organism? Do higher viscosities change the dynamics that an organism implements in order to move in a solution? The Reynolds number is defined as the ratio of inertial to viscous forces and is given by  $\rho(V)L$  divided by  $\mu$ , where  $\rho$  is the fluid density,  $V$  is characteristic velocity,  $L$  is the characteristic length of the system, and  $\mu$  is kinematic viscosity. In particular, I am interested in measuring these different scales in a system where the worm *C. elegans* is swimming in fluids of different viscosities. We will compare these measurements to different modes of locomotion.

**T2** Abdalla Sheikh, Dr. Chris Lantz  
**Role of interleukin-3 in blood-stage malaria infection caused by *Plasmodium berghei* ANKA**

We investigated the contribution of interleukin-3 (IL-3) in the host response to malaria infection by inoculated wild-type (WT) and IL-3-deficient (IL-3<sup>-/-</sup>) mice with *Plasmodium berghei* ANKA. We show that infected female IL-3<sup>-/-</sup> mice, but not male mice, presented with lower numbers of circulating parasitized red blood cells, were more anemic, and had increased splenomegaly compared to corresponding WT mice. However, there was no difference in the survival between infected WT and IL-3<sup>-/-</sup> mice of either sex. Together these data indicate that IL-3 might act to suppress immune responses to *P. berghei* ANKA in female mice, but these actions are not enough to prolong survival. Our findings that gender influences disease indicators suggest hormonal and immunological differences in the sexes. These findings are in contrast to similar studies by our laboratory using *P. berghei* NK65 in which it was shown that IL-3 plays a critical role in suppressing protective immunity in both male and female mice, and prolongs survival in male mice. We believe that the studies presented here, together with those reported for *P. berghei* NK65, will help elucidate the relative contribution of IL-3 in morbidity and mortality associated with both rodent and human malaria.

**T3** Anna Young, Dorottyta Boisen, Dr. Ken Roth, Dr. Chris Lantz  
**Using qPCR to determine levels of TNF expression in *Plasmodium berghei* infected wildtype and IL-3 deficient mice**

Interleukin-3 (IL-3) is a signaling molecule that plays a role in hematopoiesis and immune function. The role of the cytokine IL-3 in *Plasmodium berghei*-infected wild type and IL-3 deficient (knockout) mice has been under investigation for some time in our laboratory. In fact, after seeing increased survival and significantly larger spleens in IL-3 knockout mice infected with *P. berghei* compared with wild type mice, we have dug deeper into its other effects. For example, tumor necrosis factor alpha (TNF- $\alpha$ ) is a cytokine involved in the inflammatory response. The effects of IL-3 deficiency on the levels of TNF- $\alpha$  have been studied via ELISAs. Results from these ELISAs have shown no differences between serum concentrations of IL-3 knockout and wild type TNF- $\alpha$ . Our goal is to investigate the differences in messenger RNA levels of TNF- $\alpha$  and other cytokines between both genotypes. First, RNA will be isolated from splenocytes of wild type and knockout mice. Levels of mRNA for TNF- $\alpha$  and other cytokines will be compared using reverse transcription and real-time quantitative PCR (qPCR). Ultimately, we wish to determine what role, if any, IL-3 plays in the expression of these cytokines during *P. berghei* infection.



**T4**

Amelia Tavangar, Dr. Chris Lantz

**Interleukin-3 suppresses protective immunity to *Plasmodium berghei* NK65****infection**

Malaria is a serious and sometimes fatal mosquito-borne disease caused by protozoa of the genus *Plasmodium*. It characteristically causes periodic chills, rigors, and high fevers followed by profuse sweating, which occur at regular intervals. Most symptoms are caused by the lysis of infected red blood cells, which leads to anemia. *P. berghei* NK65 is a lethal rodent strain of malaria, the manifestation of which is similar to the human-infecting *P. falciparum*. The role of interleukin-3 (IL-3), a hematopoietic growth factor and immunoregulatory cytokine, in blood-stage *P. berghei* infection was investigated in male wild-type (WT) and IL-3-deficient (knockout [KO]) mice. It was determined that IL-3 KO mice were more resistant to infection than WT mice, as evidenced by lower peak parasitemia and prolonged survival. Furthermore, infected IL-3 KO mice had increased splenomegaly compared to corresponding WT mice. Hepatomegaly and liver damage is another common indicator of infection. It was recently observed that infected male IL-3 KO mice greater hepatomegaly than their WT counterparts. Alanine aminotransferase (ALT) is a liver enzyme that can be detected in blood following liver damage. Experiments are currently in progress to determine if IL-3 influences the degree of parasite-induced liver injury as measured by serum ALT levels.

**T5**

Michelle K. Clower, Ashton S. Holub, Dr. Grace A. Wyngaard

**Unusual Changes in Germline DNA Contents and the Fate of Excised DNA in a Copepod that Possesses Chromatin Diminution**

*Mesocyclops edax* (Crustacea: Copepoda) possesses, chromatin diminution, a programmed excision of enormous amounts of DNA from genomes of presomatic cells. According to the classic model, the germline genome in unaltered in size and organization. DNA excision occurs during the 5th embryonic cleavage division. The excised DNA is resorbed into the nucleoplasm, suggesting it may serve a purpose following the diminution. Observations of embryonic cleavage divisions before and after diminution revealed that excised DNA is not immediately resorbed into the nucleoplasm. Rather, the few large masses of excised DNA are broken down into many smaller masses over several cleavage divisions, ensuring availability of DNA material for subsequent cycles of DNA replication. Chromatin diminution is also a marker of the timing of germline-soma differentiation. Observations of germline DNA contents during embryogenesis revealed yet another anomaly. The undiminished embryonic germ cell contains only half as much DNA as expected! Additionally, the germline divides asynchronously relative to the somatic line, contradicting the classic model of chromatin diminution in copepods. Timing and mechanisms of germline-soma differentiation are essential to understanding the developmental biology of *M. edax*. Quantifying nuclear DNA contents throughout development, including the juvenile and adult will be necessary.

**T6**

Ashton Holub, Dr. Grace Wyngaard

**Hypothesized Utilization of Excised DNA during Copepod Development: the Yolk Genome Model**

Embryonic chromatin diminution is the process of excising DNA from the presomatic genome, resulting in an individual that possesses both a small somatic genome and a large germline genome. *Cyclops kolensis* (Crustacea: Copepoda) possesses an obese germline genome containing 75 Gb of DNA; diminution excises 74 GB from presomatic genome, resulting in a somatic genome of 1 Gb. Much of this DNA is comprised of transposable elements and simple repeat DNA sequences. We hypothesize that this enormous amount of excised DNA functions as a yolk genome which supplies the necessary components for DNA replication through development to the first larval stage. We present a quantitative model in which we show that the amount of excised DNA is sufficient to complete the post diminution DNA replication throughout embryogenesis up to and including the 11th cleavage division when the embryo hatches into a feeding larvae. This model supports the hypothesis that juveniles and adults sequester in their germline genome the nucleotides or necessary components to build nucleotides (nitrogen, phosphorous, etc...) that embryos need to complete embryogenesis.

Thus, chromatin diminution may be advantageous for copepods that inhabit nitrogen and phosphorus depauperate environments.

**T7**

Brittany Leigh Hatchett, Dr. Terrie Rife

### **Gene Expression in the Kidney of Rats Affected by Metabolic Syndrome: A Meta-Analysis**

Metabolic syndrome is a clustering of risk factors that make a person more susceptible to cardiovascular disease and diabetes, and subsequently kidney disease. The overall metabolic health of American society is decreasing at an alarming rate. Metabolic syndrome is difficult to study due to its multi-factorial nature, which can vary from study to study. This work utilizes a meta-analysis to examine the trends in changes in gene expression that occur in rat kidneys from three different models of metabolic syndrome. Studies used for this analysis were GSE4800, GSE7193, and E-MEXP-1695. It was determined that 597 genes were significant based on meta-Z statistic cut off values, including 551 upregulated genes, and 46 downregulated genes. Approximately 45.3% of the significant genes had already been associated with metabolic syndrome. Six novel genes were chosen to examine in further detail using real time PCR. These genes include downregulated genes RGD1309350, Rnf40, and Klk1c9 and upregulated genes Stk32c, Ampd3, and Mgmt. Preliminary qPCR results verified the trends found through meta-analysis for Klk1c9, Stk32c and Mgmt, but not for the remaining three genes. Future work involves continuing to verify the gene expression with PCR.

**T8**

Julianne Naples, Anna Marie Young, Brendon R. Perry, Dr. Chris Lantz

### **Prevention of genetic drift and sub-strain divergence in Interleukin-3-deficient mice**

Our laboratory is using Interleukin-3 (IL-3)-deficient (knockout [KO]) BALB/c mice to study the role of IL-3 in blood-stage malaria caused by *Plasmodium berghei*. In order to maintain these mice on a stable inbred background and avoid substrain development caused by spontaneous mutations, it is necessary to backcross mutants to the parental wild-type (WT) inbred strain periodically. This is important because it ensures that any observable pathological difference between *P. berghei*-infected wild-type and IL 3 KO mice is attributable to IL-3. To begin, IL-3 KO mice were first crossed with WT mice. The resulting heterozygous (-/+ ) F1 mice were then bred, generating a mixture of WT, -/+, and the desired homozygous (-/- ) mutants. These WT and mutant mice cannot be distinguished phenotypically but can be genetically identified by DNA analysis since -/- mice lack exon 3 of the IL-3 gene and contain a neomycin cassette. Tail DNA was extracted, subjected to PCR using specific primers, and amplified products of 800 and/or 1200 base pairs (bps) were visualized by agarose gel electrophoresis. The results indicate that our breeding scheme was successful since we were able to identify several IL-3 KO mice.

**T9**

Jonathan Schmitz, Dr. Jon Monroe

### **Differential effect of pH on two functional $\beta$ -amylases in *Arabidopsis thaliana***

Starch, a polymer of glucose, is a source of stored energy and carbon for plants. Starch accumulates in chloroplasts during the day and is broken down at night. Many enzymes are involved in this degradation, but the main players are in the  $\beta$ -amylase family of enzymes. In *Arabidopsis*, the  $\beta$ -amylase (BAM) family consists of nine proteins, six of which are plastid targeted. One of these plastidic BAMs is BAM1, which was suggested to be catalytically active during the day in guard cells to aid in the opening of stomata. During the day, the pH of the stroma is 8.0 and it drops to 7.0 at night. It was previously observed that assays using a synthetic substrate produced a narrow activity curve in which BAM1 was nearly inactive at pH 8.0, suggesting that BAM1 would not be active during the day. We repeated their assays comparing BAM1 and -3, another plastidic BAM, using Lintner soluble starch, which is similar to the natural substrate of BAM1. These assays produced broader pH curves showing that BAM1 was not only active at pH 8, but was twice as active as BAM3 at pH 8, relative to the maximum activity at pH 6.

**T10** William Shoemaker, Dr. Eria Rebollar, Dr. Reid Harris

**Horizontal and pseudo-environmental transmission of the probiotic bacterium**

***Janthinobacterium lividum* occur in larval stage green frogs, *Lithobates clamitans***

Amphibian populations are in a state of global decline, due in part to the disease chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* (Bd). Bioaugmentation via cutaneous inoculation of an anti-Bd bacterium is a promising treatment option for the mitigation of chytridiomycosis. Due to the fact that this proposed treatment uses living organisms and a maximally effective treatment is defined as one that disperses throughout an amphibian population, it is essential to understand how anti-Bd bacteria transfers between individuals. In this study we examined whether horizontal and pseudo-environmental transmission occur and the difference between them using the anti-Bd bacterium *Janthinobacterium lividum* in larval stage of *Lithobates clamitans*. A porous divider was used to discern between modes of transmission and was measured using culture based techniques. We found that transmission occurred and that the potential for horizontal transmission did not increase the microbial load of *J.lividum* in *L.clamitans* over pseudo-environmental transmission alone. Anti-Bd bacteria may be transferred independent of direct contact between unrelated individuals, facilitating disease prevention.

**T11** Michael Partin, Kevin Libuit, Dr. James Herrick

**Isolation of Methicillin-Resistant *Staphylococcus* (MRCoNS) from a Manure-Contaminated Stream in the Shenandoah Valley of Virginia**

*Staphylococcus* is comprised of 41 known species, of which 18 can colonize humans. Despite the prevalence of infectious *Staphylococcus* within hospital settings and agriculture, there are few reports of *Staphylococcus* in natural bodies of water. A recent study by the US FDA found substantial contamination of 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 stream waters and sediments. In this study, we sought to determine whether *Staphylococcus* occurs and persists within Muddy Creek, a stream located in Hinton, Virginia that runs through various agricultural fields and adjacent to a poultry processing plant. Mannitol Salt Agar (MSA) was used to isolate eleven coagulase-negative staphylococci both from water and sediment. Sequencing of the 16S rRNA gene and use of The Biolog Microbial ID System identified five different agriculturally related coagulase-negative *Staphylococcus* spp. Ten of the eleven isolates were oxacillin resistant (now used to identify phenotypic methicillin resistance) using a Kirby Bauer disk diffusion test and shown, using PCR amplification, to harbor the *meC*A gene known to confer methicillin-resistance. Overall, isolating ten methicillin-resistant coagulase-negative staphylococci (MRCoNS) from agriculturally impacted stream water and sediment represents a potential environmental and health concern.

**T12** Lauren R. Desrosiers, Dr. Dave Anderson

**Investigation of the Mechanism of Action of the Steroidal Antiestrogen SR16388 Using Triple Negative Breast Cancer Cells**

The novel anti-angiogenic compound, SR16388, developed by chemists at SRI International is being studied in tumor cells and cancer animal models for its effectiveness in preventing tumor growth in vitro and in vivo. The mechanism and targets of this small-molecule inhibitor, however, remain unknown. Chemical proteomics, utilizing mass spectrometry coupled with two-dimensional liquid chromatography, provides a strategy for the evaluation of drug impact on the proteome of the cell throughout the course of drug action. Potential SR16388 targets have been identified as a result of this technique, but more can still be learned about proteome changes as SR16388 effectively kills MDA-MB-231 cells.

**T13** Nurlybek Mursaliyev, Dr. Christopher Berndsen, Dr. Jonathan Monroe

**Biochemical characterization of an inactive beta-amylase9 (BAM9) in *Arabidopsis thaliana***

Beta-amylases play an important role in nighttime starch degradation in chloroplasts producing maltose, which is exported to the cytosol for further metabolism. There are nine

known beta-amylase-like genes (BAMs) in *Arabidopsis*. One of the non-catalytic, plastidic BAMs, BAM9, is conserved in flowering plants, suggesting that it plays an important role in plastid function. *Arabidopsis* plants lacking BAM9 appear to accumulate starch, so we suspect that BAM9 may function to regulate starch metabolism under certain conditions. Amino acid sequence alignments revealed three loops surrounding the active site of BAM9 that are not conserved compared with active BAMs, suggesting that BAM9 may not bind to starch. Starch binding assays supported this hypothesis. The alignments also showed that the active site residues of BAM9 were not all identical to those in the active BAMs suggesting that some other molecule may bind to BAM9 in the active site. Residues in the deeper half of the active site were conserved among the BAM9 orthologs suggesting that these proteins might bind a small carbohydrate, such as maltose. However, experiments with isothermal titration calorimetry showed BAM9 has a low affinity for maltose.

**T14** Charlie Watt, Corey Swanson, Dillon Miller, Alaina Esposito, Dr. Christine May  
**Background color matching in juvenile brook trout: Evaluating the extent of phenotypic plasticity and habitat preferences in artificial stream channels**

Phenotypic plasticity in fish is commonly used as a means of crypsis and as a means of intraspecific communication. The ability of resident trout to alter their coloration to match the local substrate, and how this is affected by stressors, is poorly understood. Experiments conducted in artificial stream channels with native brook trout tested for the potential of color matching by varying light and dark substrates, and included treatments with individuals as well as with paired cohorts to test for the effect of stress on this phenomenon. Fish displayed a broad ability to adapt their coloration to match a range of substrates. When the channels were divided into half-light and half dark substrates, trout showed an overwhelming preference to reside over dark substrates. In trials when fish were paired, the baseline colors of the dominant fish seemed to match light substrate more effectively than subordinate fish, perhaps owing to an inherent difference in their ability to handle stress; however, there was substantial variation among cohorts depending upon their aggressiveness. This ongoing research provides new insight into a form of camouflage not typically associated with salmonid fishes, and how it is mediated by social hierarchies.

**P1** Mycah McNett, Margaret King, Jessie Doyle, Joseph Noel, Nathan Robinson, Rebecca Sanders, Ty Steve, Erin Thady, 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 Virginia Stream Condition Index (VA-SCI), 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 would have a healthier stream quality than the upstream area, and that the stream health would improve over time in the restored area. Although it seemed that the stream was improving after the third year, it appears now that the trend is not improving. There were no significant differences found in the means of the indices among the years (2010 - 2013). For each of the three indices there were no significant trends. There was no significant change in the diversity of the restored area ( $H'$ ) from 2010 to 2013 ( $p=0.315$ ), or in the VA-SCI index ( $p=0.536$ ). The pollution tolerance index (HBI) showed no significant change ( $p=0.481$ ) over the 4-year period. Likewise, the upstream  $p$ -values for the HBI, VA-SCI, and  $H'$  were 0.665, 0.737, and 0.449 respectively, showing no significant change over time. More samples must be taken in the coming years to see if the stream is improving.

**P2** Allyse Harris, Matthew Wallace, William Noftz, Joseph Balsamo, Dr. Mark Gabriele  
**EphA4 Mutant Mice Prior to Hearing Onset**

Processing sophisticated auditory tasks requires complex circuitry. These pathways are highly organized, peripherally to centrally. The focus of our laboratory is understanding how central auditory connections develop prior to experience. The present study examines the establishment of projection maps from the lateral superior olivary nucleus (LSO) to inferior colliculus (IC). Previous results from our lab show LSO to IC projections are arranged tonotopically before hearing onset, and frequency-specific inputs segregate into characteristic axonal layers. Here we test the influence a signaling molecule, EphA4, exerts in establishing early topography. Eph receptor tyrosine kinases and ephrin ligands are proteins involved in axonal targeting development and topographic map formation in other systems. Recent studies reveal EphA4-positive LSO neurons as well as an EphA4 expression gradient along the IC tonotopic axis during early development when axonal layers emerge. Anterograde fluorescent tract-tracing approaches are used to compare ordered LSO-IC projection patterns in wild-type and EphA4 mutants. We hypothesize that EphA4 signaling is necessary for LSO point-to-point mapping and pattern formation in IC. Results indicate no qualitative errors in EphA4 mutants concerning the targeting ability of LSO projections to IC and forming axonal layers. These findings provide insights regarding Eph-ephrin involvement in constructing complex auditory circuits.

**P3** Sina Kipry, Dr. Roshna E. Wunderlich  
**Midfoot pressure distribution during bipedal and quadrupedal walking in *Pan Troglodytes***

Evidence suggests that australopithecines and *H. floresiensis* may have possessed a rigid lateral midfoot with a mobile medial midfoot characterized by a primitive, possibly weight-bearing, navicular. Few analyses have examined the function of the midfoot in apes and humans. We examined midfoot plantar pressure using an EMED-SF pressure mat during bipedal (BW) and quadrupedal walking (QW) in two male chimpanzees and during BW in 21 barefoot humans. Peak pressures, contact time, and timing of contact were compared in medial and lateral midfoot in chimpanzees during BW and QW and humans BW. Following heel strike, medial and lateral midfoot contact start times did not differ significantly, suggesting these regions are

touching down simultaneously. Medial and lateral peak pressures were similar during BW and QW, although medial midfoot pressures were significantly higher during BW than QW ( $p=0.018$ ). This differs from humans in which medial midfoot pressures are significantly lower than lateral midfoot pressures. Our data supports the suggestion that the navicular tuberosity bears weight during midstance of QW and BW in chimpanzees, this supports a weight-bearing function in early hominins with a thin navicular tuberosity. Further functional understanding of navicular tuberosity morphology will require integration of tibialis posterior function into kinetic models.

**P4** Joseph Balsamo, Allyse Harris, Will Noftz, Dr. Mark Gabriele

#### **Exploring multimodal inputs to the developing auditory midbrain**

The nervous system is responsible for interpreting sensory information and determining an appropriate motor response. Essential to the formation of this response is integration of different sensory modalities within the brain. The lateral cortex of the inferior colliculus (LCIC) is one such multimodal center within the auditory midbrain. This study aims to determine the development and patterning of somatosensory inputs arising from the dorsal column nuclei and spinal trigeminal nucleus (Sp5) to the LCIC. The convergence of multiple systems in the LCIC requires a precise circuitry and likely involves close-contact signaling mechanisms. The Eph-ephrin signaling family provides guidance cues in other regions of the developing auditory and somatosensory systems. Within the LCIC, EphA4 and ephrin-B2 exhibit modular expression patterns while ephrin-B3 is expressed in extramodular domains. In addition to establishing the neural topography of somatosensory inputs to the LCIC, this study seeks to determine any changes in connectivity in Eph-ephrin mutant mice. By understanding the development of connections between sensory systems, better models for therapy and intervention can be formulated.

**P5** Matt Hartmann, Chris Chrzan, Miriam Kabore, Katelyn Moore, Dr. Corey Cleland

#### **Rat Hind Limb Withdrawal Response to Heat Stimuli Depends on Initial Paw Posture but not Stimulus Location**

Rats withdraw their hindlimb in response to heat applied to the paw, known as the Nociceptive Withdrawal Response. Previous studies in spinalized, unanesthetized nonhuman mammals showed the direction of response depends on stimulus location. The goal of this experiment is to determine if stimulus location, or other factors, determines the direction of withdrawal in the intact, unanaesthetized rat. Rats were placed on a glass plate through which an infrared laser was directed to heat a small (1mm) localized portion of the plantar foot. The withdrawal was recorded with three conventional camcorders on the left/right/underneath the rat. The rat withdrew and rapidly (~40ms) replaced its paw. To determine if the location of the stimulus influences response direction, the rat's paw was stimulated in five locations. Unexpectedly, we found no dependence on stimulus location. However, we noticed the initial position of paw varied in both location and angle. Correlation between initial and final locations and angles revealed a highly significant linear relationship ( $p<0.001$ ). Results suggest, in contrast to previous studies, that initial posture plays a greater role in the programming of the withdrawal than stimulus location.

**P6** Michael Yeager, Victori Callahan, Dr. Marta Bechtel

#### **Characterization of Normal Human Articular Chondrocytes Gene Expression Following Dengue Viral Envelope Protein Treatment**

Dengue Virus (DENV) is part of the *Flaviviridae* viral family and is endemic to more than 100 sub-tropical or tropical countries in the world. Infection with any single DENV serotype causes Dengue Fever (DF) as well as severe arthralgia. Arthralgia has been associated with the presence of pro-inflammatory cytokines in cartilage tissue. Our lab has observed that when normal human articular chondrocytes (NHAC) are treated with Dengue Envelope protein (DENV-sE), there is a change in gene expression for a number of pro-inflammatory cytokines. Two preparations of DENV-sE protein, Biesecker DENV [DENV-BsE] and Kielian DENV [DENV-KsE] were used to treat NHAC. Following treatment, NHAC lysates were collected, total RNA isolated, cDNA synthesis generated, and quantitative real-time PCR (qR-T PCR) performed. Of the cytokines evaluated, gene expression patterns peaked after approximately two hours

following treatment with DENV-BsE preparation, while treatment with the DENV-KsE preparation resulted in a higher level of cytokine gene expression that peaked approximately 4 hours following treatment. Our preliminary data suggests that purity of a DENV-sE protein preparation may have an impact on gene expression patterns in NHACs. Future studies will investigate additional DENV-sE preparations, and will expand the cytokine profile to include gene expression patterns associated with wound healing.

**P8** Jessie Ward, Kelsey Samms, Olivia Vito, Dr. Heather Griscom

**The Effect of Soil, Light, and Herbaceous vegetation on *Castanea dentata* seedling growth: Implications for Reintroduction**

The American chestnut, *Castanea dentata*, historically a keystone species in eastern U.S. forests, was nearly eliminated by 1950 due to the invasive fungus *Cryphonectria parasitica*, chestnut blight. To reintroduce this species, the optimal growth conditions must be determined. Pure and hybrid *C. dentata* seedlings were planted with major competitors, *Liriodendron tulipifera* and *Quercus prinus*, in two gap sizes (to manipulate light) and two slope positions (to vary soil conditions). Height (cm) and diameter (mm) were recorded at the end of every growing season for five years to quantify relative growth rate and survival of seedlings. Treatment had a significant effect on diameter ( $p=0.003$ ) and survival ( $p<0.0001$ ) but not height ( $p>0.05$ ). After five years, *C. dentata* establishment was more successful (in terms of diameter growth and survival) in small, xeric treatments due to a combination of rodent predation in mesic conditions and competition from *Rubus spp.* in large gaps. *L. tulipifera* had significantly greater growth in large and small, mesic plots, therefore have a competitive edge over *C. dentata*. The recommended condition for reintroduction of *C. dentata* is small, xeric because they have an increased ability to compete with *L. tulipifera* as well as a higher chance of survival.

**P9** Tara Gallagher, John Marafino, Brandi Volkens, Nick Minahan\*, Jason Floyd, Abby Perlin,

**The Antibacterial Activity of Novel Cationic Amphiphiles**

The rising levels of antibiotic resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), underlines the need for the development of novel antimicrobial agents. Quaternary ammonium compounds (QACs), a class of cationic amphiphiles, were first discovered in 1900 and used by surgeons as pre-operative hand soap as early as 1935. Notably, these compounds have been widely used as antiseptics and disinfectants for over 50 years with minimal evidence of developed resistance in target bacteria. Synthetic QACs show promise as therapeutic alternatives to antibiotics for the treatment of bacterial infections and disinfection of hard surfaces. The Caran Lab at James Madison University has synthesized two novel series of amphiphiles for this study. Both series consist of three quaternary ammonium head groups and one or two hydrophobic tails with varying lengths. The critical micelle concentration (CMC), Krafft temperature, and minimum inhibitory concentration of these amphiphiles has been determined. The synergistic capabilities, biofilm disruption and cytotoxicity of these compounds will also be investigated.

**P10** Allison Welborn, Dr. Conley K. McMullen

**A Floristic Survey of Smith Creek Restoration Area, Rockingham County, Virginia**

Smith Creek Restoration Area, in Rockingham County, Virginia, comprises a 1.5 mi section of creek and a 66 ft buffer zone along either side. Prior to the beginning of this study, for ca. 200 years, the property had been used for agriculture/pasture. As a result, the creek had become unsuitable as a habitat for native brook trout, and most of the native plants in the immediate area were lost. In 2006, the land bordering the creek was made available for a restoration study, and thousands of trees were planted in the hopes of eventually returning the area to its natural condition. The purpose of the research described here is to catalog the native vascular plant species that occur in this area, thus providing baseline data for future studies that might occur as the landscape changes due to the tree plantings and the absence of agricultural and grazing pressures. Results thus far will be discussed.

**P11** Ty Steve, Jessie Doyle, Dr. Bruce Wiggins

**National and Regional Geospatial Models To Predict The Levels of Methyl Mercury**

**In Fish**

Departments of Health across the country are becoming more alarmed with the levels of methyl mercury (MeHg) in the water and the risk it poses to newborns and unborn children. As mercury is deposited into the water from the atmosphere, anaerobic bacteria can methylate it. The ability to predict the levels of MeHg in watersheds would be beneficial to the health of consumers and to ecosystems. In an effort to create a model to predict the levels of MeHg in streams across the continental United States, a study using 5 independent variables to analyze their predicting power of MeHg was performed. Model variables include: percent wetlands, sulfur deposition, stream density, organic carbon levels, and atmospheric mercury deposition levels. These variables were calculated for each sub watershed using ArcGIS, and analyzed using multiple regression to determine their relationship with known levels of MeHg in fish. Regional sub watersheds were combined for national analysis. National Analysis produced a significant, but non-predictive model ( $R^2=0.001$ ,  $p<0.001$ ) with organic carbon, and mercury deposition being significant variables ( $p=0.012$ ,  $p<0.001$  respectively). However, when a single region (the sub-Mississippi) was modeled, it showed a high predictive model, ( $R^2=0.420$ ,  $p=0.008$ ). This suggests that regional analysis may be needed for better predictive power.

**P12** Brian Schwenk, Dr. Dean Cocking

**Landscape heterogeneity of airborne Hg (mixed dissolved, particulate, and vaporous) and a sampling of total Hg concentration in macroinvertebrates within Rockingham Co., VA.**

Distribution of Airborne Hg is known to occur at the landscape level. Air samplers consisting of inverted plastic Petri plates containing a sticky organic have been used to examine its presence at a variety of locations in Rockingham Co, VA. The assumption is that this deposition serves as a relative index of the different locations. The total Hg content when plates were exposed to the air was measured. Five contaminated locations, which had consistently above average Hg concentrations, and a low Hg control site, were selected for more extensive study in 2010-2012. Separate insect traps were deployed with the intent of obtaining greater invertebrate biomass for analysis. A comparison was made of the relative Hg content of the invertebrate tissue to the relative airborne Hg measured by the plate samplers. More extensive analysis of the data was completed in 2013 to discern patterns of Hg presence. Some invertebrates from the contaminated locations had higher Hg concentrations than those at the low Hg control site. This supports 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 due to local sources is to be determined.

**P13** Wes Deaver, Kevin Hatala, Heather Dingwall, Brian Richmond, Dr. Roshna Wunderlich  
**Foot Strike Patterns in Habitually Unshod Children Compared to Adults**

Two different patterns of footstrike mechanisms during running have been observed in habitually unshod populations. Kalenjin adults run barefoot with primarily forefoot striking, yet Kalenjin children rearfoot strike 12% of the time and use more variable footstrike patterns. Daasanach adults rearfoot strike 72% of the time, midfoot strike 24% of the time, and forefoot strike 4% of the time during running. As Kalenjin children do not follow footstrike patterns of the adults, we sought to determine whether or not Daasanach children showed similar footstriking patterns as adults in order to assess the extent to which running footstrike patterns are learned or trained. We studied footstrike patterns in Daasanach children ages 3-15 using 2-D video and plantar pressure data. We compared ankle and foot-ground angles and qualitatively categorized footstrike patterns in contrast to Daasanach adults as well as Kalenjin runners. Daasanach children showed rearfoot striking 35% of the time, midfoot striking 55% of the time, and forefoot striking 10% of the time during running. Habitually unshod Daasanach children exhibit footstrike patterns different from adults, suggesting that running footfall patterns may be trained or learned rather than innate to humans or certain environments.



**P14** Jennifer Kurasz, Curtis Kapsak, Jesmine Roberts-Torres, Dr. James Herrick

**Our Little MinION: Introducing the Next Generation of Sequencing Technology**

Nanopore sequencing was first conceptualized over 20 years ago, but technical difficulties limited its practicality. Recently, Oxford Nanopore Technologies® developed the MinION™ device, soon to be the world's first commercially available nanopore sequencer. A compact and portable machine, it produces real-time data directly to any computer, via USB. The Herrick lab will be among the first group to test this device. The MinION utilizes a protein nanopore with a channel small enough that only a single strand of DNA can pass through. A potential difference across the pore-containing membrane creates an ion current through the channel. As the DNA strand passes through, each base produces a distinct disruption to the flow of current. Sequence data is recorded as one long read rather than numerous short reads, as is currently the norm. While the MinION in its present form is most useful for DNA analysis, research and development is taking place so that it could eventually be used to sequence RNA and proteins. This new technology potentially offers advantages in cost, speed, and clarity of sequencing, but it also has limitations. Here, we compare this device to the leading next generation sequencers, as well as traditional Sanger sequencing.

**P15** Corey Swanson, Charlie Watt, Dillon Miller, Alaina Esposito, Dr. Christine May

**Background color matching and substrate preference by juvenile brook trout (*Salvelinus fontinalis*)**

Phenotypic plasticity in fish is commonly used as a means of camouflage and as a means of intraspecific communication. Camouflage, also referred to as background matching or crypsis, is an evolutionary tactic many species use as a defense mechanism to reduce the chance of detection by a potential predator. Experiments conducted in artificial stream channels at James Madison University with juvenile brook trout tested for the potential of color matching by varying light and dark substrates, and included treatments with isolated individuals and with paired cohorts to test for the effect of stress on this phenomenon. Experiments tested the response of individuals when only light or dark substrate was available, and then tested for a preference when both were available. This was done over substrates of varying sizes and of the same size. The baseline or resting colors that was established for dominant and subordinate individuals in paired trials showed trends that displayed the tendency for dominant fish to match to light substrate more effectively, possibly due to lower stress levels. There was a strong preference for dark substrate when given the choice. This ongoing research provides new insight into a form of camouflage not typically associated with salmonid fishes.

**P16** Alexandra Deal, Taelor Weaver, Shelby Snowden, Dr. Terrie Rife

**Imaging First Exons 1h and 1k in the Rat Nitric Oxide Synthase 1 Gene**

Nitric Oxide Synthase (NOS) is an enzyme that catalyzes the conversion of L-arginine, NADPH, and O<sub>2</sub> to citrulline, NADP<sup>+</sup>, and nitric oxide (NO). Nitric oxide is a secondary messenger that regulates many bodily functions including vasodilation, long-term memory, muscle movement, and glucose regulation. Misregulation of the neuronal NOS (NOS1) gene is linked to type 2 diabetes, schizophrenia, Parkinsons, Alzheimers, and Huntingtons disease. The human NOS1 gene has been fully characterized, but the gene must be characterized in the rat model in order to research NOS1 mis-expression. The human NOS1 gene consists of twelve different first exons, which all splice to a common second exon to begin translation. Previous research has identified three rat-human first exon orthologs in the rat NOS1 gene, and our lab has identified two additional orthologs. This study aims to verify the tissues in which these two newly identified rat first exons are expressed. RNA has been isolated from rat brain, kidney, testis, intestine, heart, and skeletal muscle. Reverse transcription, PCR, and Southern Blot techniques are being utilized to generate an image depicting where the two orthologs are expressed. Overall, these experiments will allow researchers to better utilize rat models to study NOS1 in disease pathogenesis.

**P17** Nicole M. Roberto, Anisa C. Tracy, Dr. Steven G. Cresawn  
**Next Generation Sequencing and Genomic Comparisons of Novel**

**Mycobacteriophages**

Next Generation sequencing has made it possible to efficiently sequence entire bacteriophage genomes. Having the full sequence provides a way to more effectively compare these highly diverse and mosaic bacteriophage genomes. Bioinformatic comparisons using pairwise nucleotide identity provide a powerful tool for elucidating virus/host interactions. We aim to use this tool to explore the host range of Mycobacterium ulcerans, the causative agent of the neglected disease, Buruli ulcer. Four mycobacteriophages were sequenced using an Ion Torrent Personal Genome Machine (PGM); three of which were isolated during the Viral Discovery course offered in the fall and named OrionPax, Burke, and Rover14. Each was isolated from soil collected in the US while the fourth mycobacteriophage, Espe, was isolated from soil collected in Uganda. Genomic DNA was isolated for each of the four mycobacteriophage and then used to create a shotgun DNA Library using enzymatic shearing. These samples were barcoded, pooled, and sequenced on the PGM. Here we report on these four novel mycobacteriophage genomes.

**P18** Bridget Mitchell, Samantha Heitsch, Rebecca Gaita, Dr. Corey Cleland  
**Stimulus and Postural Determinants of the Escape Response of Crickets to**

**Localized Heat Stimuli**

In crickets, wind or looming stimuli, which might normally be produced by an approaching predator, evoke an escape response in which the cricket turns and then runs or jumps away. Although in mammals aversive heat stimuli have been used routinely to evoke withdrawal responses, there have been no studies on the crickets response to heat stimuli. The goal of this study is describe the escape response of the house cricket (*Acheta domestica*) to heat delivered to each of its six tarsi and determine factors that control response direction and magnitude. Heat was delivered to the tarsi in 20 crickets with an infrared laser, the response was quantified by high-speed video (650 fps) and features were tracked in software. In response to heat stimuli, crickets first retract the stimulated tarsus, turn by pivoting about a point toward the rear of the animal, and finally either walk (86%), jump (9%) or remain stationary (5%). Turning was always away from the stimulus location. These results demonstrate that crickets escape from heat as well as from looming or wind stimuli, and offer the opportunity to identify common movement strategies by comparing the escape responses to the three different stimuli.

**P19** Katie McCullar, Maria Wilkins, Dr. Janet Daniel  
**Investigating the Effect of Various Salt Concentrations on Plant Growth in STP1**  
**K/O and WT *Arabidopsis thaliana***

*Arabidopsis thaliana* is a commonly used model organism in the study of plants and is described as a glycophytic plant. In this experiment, the role of sugar transport protein-1 (STP1) in relation to salt resistance is investigated by observing the growth of wild type (WT) and STP1 k/o plants when exposed to varying concentrations of sodium chloride on agar plates. Plant growth was monitored by measuring root length, stalk length and number of leaves at two and four weeks on plates with salt concentrations varying from 0 mM to 100 mM. On average, plated STP1 k/o plants exhibited increased root growth as well as a faster growth rate compared to the WT at both two weeks and four weeks, except on 25 mM NaCl at four weeks. At two weeks, STP1 k/o plants showed a higher percentage of plants with 4 leaves compared to WT plants, except on 25mM NaCl. At four weeks, STP1 k/o plants showed a higher percentage with 4 leaves or more on all concentrations compared to WT plants. These results suggest that the STP1 k/o gene causes an alternate phenotype, which may lead to a higher salt stress resistance.

**P20** Emma Bales, Oliver Hyman, Andrew Loudon, Gregory Lipps, Eric Chapman, Kenneth Roblee, John D. Kleopfer, Kimberly A. Terrell, Dr. Reid Harris

**Pathogenic chytrid fungi *Batrachochytrium dendrobatidis*, but not *B. salamandrivorans*, detected on hellbender salamanders**

Recent worldwide declines and extinctions of amphibian populations have been attributed to chytridiomycosis, a disease caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (Bd). Until recently, Bd was thought to be the only *Batrachochytrium* species that infects amphibians. A newly described second species, *Batrachochytrium salamandrivorans* (Bs), is known to infect salamanders, but very little is known about its distribution and host range. In this study, we surveyed wild hellbender salamanders (*Cryptobranchus alleganiensis alleganiensis*) for the presence of both Bd and Bs. We aimed to determine the prevalence of Bd and Bs in wild hellbender populations. Skin swabs were collected from 92 individuals across four states (NY, PA, OH, and VA) and tested for the presence of Bd and Bs using Duplex qPCR. Bd was found in 22 of the 92 samples (24% prevalence with 95% confidence intervals of  $0.16 \leq 0.24 \leq 0.34$ ) with samples from all states testing positive. All samples that tested positive showed low loads of Bd (0.508 0.910 zoospore G.E.). Bs was not detected in any samples, suggesting it is not present on eastern hellbenders. Our findings are consistent with previous studies that found Bd on eastern hellbenders. Further studies are needed to determine the role of diseases on hellbender declines.

**P21** Mary Katherine Garbarini, Cy Lampugnale\*, Kelcy Jackson, Kelly Burke, Dr. Justin Brown  
**The Role of Ambient Temperature in the Recovery from Major Surgical Stress**

Surgical trauma is a significant physiologic stressor on the body. A result of surgical intervention is a decrease in body core temperature (T<sub>c</sub>) due to the use of anesthesia. The use of anesthesia eliminates accurate control of T<sub>c</sub> by affecting hypothalamic thermoregulatory centers. After surgery is complete, the animal attempts to raise T<sub>c</sub> back to normal levels. It is possible that the added effects of this hypothermic response and other physiologic stressors hinder subsequent surgical recovery. The use of external heating sources is often required until the effect of anesthesia dissipates. Current guidelines suggest that animals recover from surgical stress on a heating pad to support the recovering animal for one hour post-surgery. Rodents are then returned to the normal animal holding areas where ambient temperature (T<sub>amb</sub>) should be maintained within the range of 20C-26C. The T<sub>amb</sub> of the majority of vivariums is approximately room temperature (22C) to make the environment more comfortable for human workers. However, previous data suggest that a rodents preferred ambient temperature is actually closer to 27C. This study presents the preliminary analysis of the effect of T<sub>amb</sub> on surgical recovery outcomes and indications of thermal stress when rats are housed at 21C, 24C, and 27C.

**P22** Morgan Donovan, Kristen Reitano, Dr. Janet Daniel  
**The Role of STP1 in Glycophytic Plants**

*Arabidopsis thaliana* is a commonly used model organism for glycophytic plants. Although much of its genome is characterized, the purpose of the sugar transport genes (STPs) is not well understood. During the investigation of STP1s role in *Arabidopsis thaliana*, hydroponics was employed to optimize plant germination and accumulate plant mass for microscopy studies and cell/molecular investigations. AtWT and AtSTP1 k/o specimens were grown in Gibeauts solutions of 0 and 50 mM NaCl for 4 weeks. Upon harvesting, each plants root length was measured. On average the AtSTP1 k/o plants had longer roots than AtWT plants in both the 0 and 50 mM NaCl growth conditions. After four weeks in Gibeauts solution of 0 mM NaCl AtSTP1 k/o and AtWT plants had average root lengths of 45.82 mm and 32.27 mm, respectively (p-value 0.035). After four weeks in Gibeauts solution of 50 mM NaCl, AtSTP1 k/o and At WT plants had average root lengths of 27.48 mm and 21.88 mm, respectively (p-value 0.292). In this study we propose that AtSTP1 k/o plants have uncontrolled root growth, which supports the theory that STPs play a major role in sugar sensing and orchestrating cellular activities in glycophytic plants.

**P23** Ahmed Alotaibi, Sally Coleman, Pritpal Saggu, Dr. Raymond Enke

**Characterizing the Role of Epigenetic Gene Regulation in the Developing Chicken**

**Retina**

The retina is a light sensing layer of neuronal tissue that lines the inside our eyes. Retinal neurons convert light into an electro-chemical signal that is transmitted to the brain and translated to what we perceive as the visual world. Our research is focused on characterizing mechanisms of epigenetic gene regulation in the developing chicken retina, specifically DNA methylation. In order to achieve this aim, we analyzed DNA methylation on retina-specific genes to determine if there is a correlation between methylation and gene expression. Transcription and methylation analysis were conducted for the retina-specific genes, RBP3, OTX2, PDE6c, and GNAT2, using retina harvested from day 6-16 chicken embryos (E6-E16). Purified genomic DNA was subject to quantitative bisulfite pyrosequencing DNA methylation analysis. RNA extracted from the same tissues was used for gene expression analysis using quantitative reverse transcriptase PCR (qRT-PCR). Our results demonstrate an inverse correlation between DNA methylation on retina-specific genes and their levels of transcription. Retina-specific genes demonstrated the highest levels of DNA methylation at the earliest time points analyzed with loss of methylation occurring at later embryonic time points. Transcriptional activation of OTX2, PDE6c, and GNAT2 coincided with decreased methylation. RNA expression analysis of RBP3 is ongoing.

**P24** Oumar Sacko, Dr. Idelle Cooper

**Color variation and mating success in the Ebony Jewelwing Damselfly, *Calopteryx maculata***

The roles of ecological and sexual selection in driving phenotypic variation within species have been the focus of long-standing debates in evolutionary biology. Wing pigmentation in Jewelwing damselflies (*Calopteryx*) differs between closely related species, presumably to reduce hybridization, but the role of that trait in mate choice within species is unknown. To identify how wing pigmentation and size may be under mate choice *within* a single species, we compared wing pigmentation and size with mating success in *Calopteryx maculata* damselflies. While there was no difference in wing traits between mated or unmated females, mated males had significantly longer and more pigmented wings and held territories more successfully. These findings indicate that male wing traits are under sexual selection in this species. Wing size and pigmentation may be a visual cue during courtship or beneficial during male territorial defense.

**P25** Emily Grunwald, Victoria Shuklis, Sophia Brown, Dr. Raymond Enke

**Histological Analysis of the Developing Chicken Eye**

The eye is a complex organ that detects and converts light into an electro-chemical signal transmitted to the brain where the signal is translated to what we perceive as the visual world. Our research aim is to provide a histological framework of the developing chicken eye to use as a model for studying development and disease of the vertebrate eye, particularly the retina. To achieve this, chicken embryos were harvested at developmental stages from embryonic days 6 (E6) through E18. For our analysis, a developmental series of whole chicken embryos and whole eyes was documented. Additionally, paraffin embedded and cross-sectioned whole eyes were used for histological analysis of the developing retina. E6-E20 retinal cross-sections were stained with hematoxylin and eosin (H+E) and compared for developmental milestones. Our results demonstrate differentiation of some distinct retinal layers by E10 with characteristic inner and outer nuclear layer (INL+ONL) formation by E12. By E18, three days prior to hatch, the chicken retina is fully laminated with photoreceptors extending mature inner and outer segments that contact the retinal pigment epithelium (RPE). Future studies will focus on mechanisms of developmentally regulated gene expression in the cone and rod photoreceptors that make up the ONL.

**P26** Stephen Simonetti, Will Shoemaker, Dr. Eria Rebollar, Dr. Reid Harris

**A test of horizontal and vertical transmission among tadpoles of a bacterial probiotic that can inhibit a lethal fungal pathogen**

The purpose of this research is to identify the ability of a probiotic, *Janithinobacterium lividum*

(*J.liv*), to be horizontally transmitted (individual to individual) and/or pseudo-environmentally (individual to environment to individual) transmitted between *Lithobates clamitans* (green frog) tadpoles. The probiotic *J.liv* is able to kill the pathogenic fungus known as *Batrachochytrium dendrobatidis* (Bd), which is known to be causing massive amphibian decline worldwide. I hypothesize that pseudo-environmental and horizontal transmission will occur in tadpoles; I base this hypothesis on unpublished data collected by Will Shoemaker. There will be two different treatments and their controls. The first treatment, which is testing for pseudo-environmental and horizontal transmission, will include two tadpoles in a container, swimming freely, with only one tadpole inoculated with *J.liv*. The second treatment, which is testing for pseudo-environmental transmission, will include two tadpoles in a container, separated by a net, with only one tadpole inoculated with *J.liv*. Tadpoles will be swabbed at different time points and plated on rifampicin plates to determine the success of transmission. Successful results would be a major breakthrough in stopping this massive amphibian decline since it means that not all individuals have to be inoculated to apply the probiotic to the entire frog population.

**P27** Spencer C. Burnett, Clara R. Thiel, Dr. Conley K. McMullen  
**Vascular plants of the E.J. Carrier Arboretum**

This research project is part of an ongoing effort to collect and correctly identify vascular plant species located within the confines of the Edith J. Carrier Arboretum at James Madison University, Harrisonburg, Virginia. This floristic survey consists of collecting, identifying, and pressing vascular plants that inhabit the arboretum. The pressed specimens will be mounted on herbarium sheets, cataloged, and stored in the JMU Herbarium (JMUH), located on campus. During Spring Semester 2014, while waiting for the beginning of the growing season, a list has been compiled from the previous floristic surveys and entered into a database. This information will be combined with newly collected specimens to create an updated list of the varying flora throughout the arboretum. The data, in addition to the herbarium specimens, can be used by professors, students, and the general public to aid in the identification and study of the vascular plants inhabiting the arboretum as well as the Rockingham area in general.

**P28** Catherine Torres, Dr. Amanda Storm, Dr. Jonathan Monroe  
**Expression and Characterization of BAM2 and BAM6 from *Arabidopsis thaliana***

Starch is the primary form of energy storage in plants and is also a foundational dietary energy source. Experimental evidence supports that the family of  $\beta$ -amylase (BAM) proteins are responsible for the hydrolysis of starch in *Arabidopsis thaliana*. There are nine genes in the  $\beta$ -amylase family and identifying the unique roles of each of these beta-amylases is an intriguing research question. BAM2 and BAM6 are targeted to the chloroplast, however previous research implies that BAM2 is inactive while no research has been published on the expression and characterization of BAM6.

Our own study of starch accumulation in *Arabidopsis* supports a minimal role of BAM2 and BAM6 in young plants, but also indicates that these beta-amylases may be playing a more dominant role in older plants. To further investigate the role of BAM2 and BAM6 we want to obtain the purified protein. PCR will be used to isolate the mature protein coding sequence (cDNA) using specific primers. The cDNA will then be ligated into pETDuet-1, an *E. coli* expression vector. Through the expression of each of these proteins we will be able to characterize their properties and roles in the degradation of starch throughout development.

**P29** Peter D Rossbach\*, Dr. Carol Hurney  
**Examining tail development models in the four-toed salamander, *Hemidactylium scutatum***

Tails are common among most vertebrates. The embryonic tails of most vertebrates grow into adulthood while other vertebrates absorb their embryonic tails. Interestingly, some species of salamanders have the ability to add axial segments to their tail post-embryonically. In the salamander *Hemidactylium scutatum*, postembryonic growth of the tail is due to the development of new caudal vertebrae, as well as the growth of pre-existing segments. The mechanisms that drive the postembryonic segmentation in salamanders are relatively unexplored and may offer insights on the molecular nature of tail development in vertebrates.

By comparing the expression of Wnt8 and My5 in the developing tails of *H. scutatum* embryos, larvae, and adults, I found evidence that larval and adult tail growth could be utilizing the same mechanisms for tail segmentation that are used in embryonic tail development. Specifically, the expression of Wnt8 in the growing tails suggests that both larval and adult *H. scutatum* continue to segment through the use of a molecular oscillating clock and tail organizer, which induce the tail to undergo somitogenesis and, with the aid of Myf5, develop skeletal muscle in the tail.

**P30** Kristen Fadel, Dr. Amanda Storm, Dr. Jonathan Monroe  
**Constructing BAM9 promoter-GUS plasmids for analysis of BAM9 expression in *Arabidopsis***

$\beta$ -amylase nine (BAM9) is a plastidic, catalytically inactive protein in *Arabidopsis thaliana*. The objective of this study is to investigate its function in starch metabolism by learning more about the BAM9 promoter. This can be done by constructing plasmids containing portions of the BAM9 promoter fused to a GusPlus reporter gene, which will drive the expression of  $\beta$ -glucuronidase. In order to do this, five primer pairs were created to amplify 0.5, 1.0, 1.5, 2.0, and 2.5 kb of the BAM9 promoter, each using the same downstream primer. The PCR products were cloned into a cloning vector and the resulting plasmids were then digested to cut the inserts out. The inserts will be ligated into the recipient vector, pCAMBIA 1305.1, and the plasmid will be transformed into *E. coli*. After verifying that the sequence is correct, the constructs will be transformed into *Agrobacterium tumefaciens* cells and infiltrated into *Arabidopsis* using a floral spray method. Transgenic plants will be selected on media supplemented with hygromycin. To detect GUS activity, samples from various tissues at different developmental stages will be incubated in a GUS staining solution. Patterns of BAM9 expression may lead to more detailed hypotheses regarding its function.

**P31** Kimberly Okafor\*, Ian Brown, Dr. Steve Cresawn  
**Cytotoxicity screening in a Mycobacteriophage genome**

*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. Mycobacteria phages are being explored in this lab as a possible treatment to this disease since antibiotics have been proven unsuccessful and phages have been historically known to act as a natural growth control to mycobacteria for millions of years. In this project, we aim to isolate genes from bacteriophages with known anti-mycobacterial activity. LeBron, a bacteriophage known to infect *M. smegmatis* was focused on to establish a positive control. The genome was sequenced and annotated using Phamerator and BLAST. We were able to determine genes most likely to encode for the lysis cassette and developed primers for the region to be amplified in PCR. The gene has been successfully amplified, and are now cloning the region into an inducible plasmid using the Gateway Cloning Method before the plasmid is induced into *M. smegmatis*. Then, we will evaluate the cytotoxicity of the cloned genes via plating on anhydrotetracycline induced plates. This process will be repeated to identify and collect genes in the viral genome known to produce anti-mycobacterial affects.

**P32** Hayley A. Norian, Dr. Steven G. Cresawn  
**A Correlational Study of Mycobacteriophage Host Specificity and Protein Phamily Conservation**

Mycobacteriophages are bacteriophages that infect the genus *Mycobacterium*, including pathogens such as *Mycobacterium tuberculosis* and *Mycobacterium ulcerans*. Full genome sequences of 654 mycobacteriophages are currently available. A mere 20.25% of the 69,581 genes encoded by these phages have at least one known homologue in NCBI, leaving roughly 80% of known mycobacteriophages genes without a predicted function. The host range of 204 mycobacteriophages, initially isolated on *Mycobacterium smegmatis* strain mc2155, was recently determined on *M. tuberculosis* and *M. smegmatis* strains Jucho and MKD8. Three different levels of infectivity were observed: phages that were incapable of infecting the host, phages that were capable of infecting the host, and some that were at a plating efficiency less than one relative to mc2155. The phages that are capable of infecting a host are of particular interest. With so many uncharacterized genes encoded by these phages, we will take a

computational approach by performing an association study using the Phamerator software to study the relationship between the complement of protein families in specific genomes and host range of the corresponding phages.

**P33** Shvan Kareem\*, Courtney Matson\*, Erin Wallace, Dr. Kim Slekar, Dr. Tim Bloss

#### **The effects of misfolded protein stress on different cell types in *C. elegans***

Proper management of protein folding and localization during the life of a cell is essential for its survival, and many human diseases are associated with disruption of this management. The nascent polypeptide-associated complex (NAC) is a two-subunit complex essential to the proper folding and localization of proteins during translation, and loss of this complex has been associated with significant cell stress and cell death. Specifically, NAC depletion generates misfolded protein stress, engaging the unfolded protein response (UPR), a cell survival mechanism that mitigates the damage misfolded protein stress generates in the endoplasmic reticulum (ER). We are using the nematode *C. elegans* as a model organism for the study of the NAC, depleting it to observe cell stress and cell death phenotypes associated with the resulting misfolded protein stress and induction of the UPR. *C. elegans* development has been thoroughly investigated, and proteins expressed in specific cell lineages can be fluorescently tagged and followed throughout the life of the worm. In addition, cell stress proteins associated with UPR induction are available as fluorescently tagged proteins. Using microscopy techniques, we are investigating the co-localization of UPR-induced proteins with cell-specific markers in NAC-depleted worms to better understand the differential responses of specific cell types to misfolded protein stress.

**P34** Jeff Kopsick, Dr. Eva Strawbridge

#### ***C. elegans* locomotion in 3D-Printed Environments**

Using the 3D printers in the JMU Maker Lab, we have developed a protocol for printing 3D environments for the study of *C. elegans* swimming in different geometries and fluids of different viscosities. *C. elegans* offers an unprecedented middle ground between the macro and microscopic. This project involves experimental and theoretical components as well as techniques in image segmentation and processing. From these techniques, we aim to quantify how significant the role of environment is to the swimming gait of nematodes. If we can quantify a significant difference between an isolated droplet and a droplet within our environment, we will begin to model the worm's fluid mechanics.

**P35** Gregory M. Steffensen, Zackary A. Zayakosky, Caroline I. Dilworth, Joshua Gerard T. Kristen L. Hoffman, Chloe I. R. Wines, Tessa L. Nester

#### **Research Experiences of the Trelawny Learning Community 2013-14**

The Trelawny research group for the 2013-14 academic year includes 10 students and peer mentors. Group discussions this year focused on what it means to be a scientist, and how to work well with a team while developing and evolving individual interests. This year, TLC students were observers and assistants in the labs of Drs. Mott, Bloss, Enke, Kastendiek, Rife, Seifert, Cooper, and Gobetz. The group took macroinvertebrate samples from the Purcell Park reach of Blacks Run, in our multiple-year survey of the Blacks Run site. The survey was taken late in the season, and < 200 samples were collected. Between site contamination, cold weather, and low leaf litter levels, populations were low. The purpose of this study was not only to evaluate local water quality of Blacks Run, but to assess impacts of the restoration effort. Long-term data is essential in determining success or failure of this expensive restoration project. Thus, TLC will continue to monitor this reach of Blacks Run. Students also gathered owl pellet samples from a barn at the Smith Creek field site. Bones in these pellets were identified by species, as part of a survey of rodent and insectivore populations. These projects enabled TLC to develop teamwork skills, learn about local ecology, and gain field experience; the members of the group have gone on to apply these skills in various fields of research.

**P36** Michelle Barber, Jessica Wagner, Tyler Mullins, Clara Thiel, Rachael Schneider, Russie Tran\*, Harley Burton, Dr. Michael Renfro

#### **Analysis of Antioxidants in Selected Herbs and Spices**

Selected herbs and spices were analyzed for the hydrophilic antioxidant content. Antioxidants are important dietary components for humans and have been linked to protection from cancer, aging, arteriosclerosis, and other disorders related to oxidative stresses on tissues and human physiology. In this study, we analyzed commercial samples of dried herbs and spices, including cilantro, oregano, thyme, rosemary, basil, and chives. Analyses were performed using a trolox-equivalent antioxidant capacity assay (TEAC), based on the change in absorbance at 730 nm of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was used to develop a standard curve to establish the trolox equivalents for reduction in absorbance by ABTS following injection of the antioxidant extracts from the spices. Resultant data were analyzed by analysis of variance followed by a test of the significance of difference of means by a Dunnett T3 test. Significant differences in hydrophilic antioxidant content were observed among the spices. Preliminary research has been conducted to examine variation in fresh spice and herb plants. These results may be important to dieticians developing specialized diets for clients.

**P37** Jena Butler, Harold Burke, Haley Nisson, Stephen Jurko, Benjamin Williamson, Dr. Susan Halsell

#### **Brrrr! Analyzing Cold Nociception in Drosophila using Behavioral and Optogenetic Assays**

Nociception is a conserved, innate physiological response to a harmful stimulus present in abundance throughout the kingdom Animalia. By studying nociception in *Drosophila melanogaster*, we may be able to identify mechanisms conserved with humans that can be targeted for pharmacological treatment, such as those suffering from chronic pain. It has been shown that class III dendritic arborization (da) neurons play a role in *Drosophila* cold nociception; however, it is not known which channels within these neurons are involved as cold nociception has not been thoroughly studied in *Drosophila*. Using a combination of behavioral and optogenetic assays, we will knock out genes encoding DEG/ENaC channels, particularly pickpocket (ppk) family members, in class III da neurons to study their role in cold nociception. Cold behavioral assays will be executed by placing the larvae on thin metal plates and observing the resulting cringe cold response at certain temperatures. By comparing the physiological response between wild-type and genetically altered flies, we can determine the importance of genes encoding channels to nociceptive cold response.

**P38** John Stephen Fisher, Kyle Snow, Dr. Christine May

#### **Sedimentation and its effects on the dynamic relationship between brook trout and stoneflies, an undergraduate research project and outreach opportunity**

Sedimentation has been a reoccurring problem in streams of the Shenandoah Valley and throughout the U.S. for many years. We aimed to investigate the effects that sedimentation has on the predator-prey relationship between brook trout and stoneflies, both native to the Shenandoah Valley. Using artificial stream channels at James Madison, seven soft-bodied stoneflies and three armored stoneflies were released in the channels, after which a cohort of three brook trout entered the channel and preyed upon the stoneflies for 72 hours. Results indicate that sedimentation did have an effect on stonefly survivorship at high levels of sedimentation (45% and 100% fine sediment by surface expression); however, it was limited to soft-bodied stoneflies because armored stoneflies possess an effective defense mechanism that prevents the juvenile brook trout from consuming them. After experimentation, multiple outreach events were planned with the National Wildlife Federations Campus Ecology Fellowship program to communicate the findings to the public and help educate citizens on the impacts of non-point source pollution, particularly elementary and middle school aged children.



**P39**

Laura Johansen, Dr. Susan Halsell

**Genetic and Phenotypic Characterization of RhoA Alleles in *Drosophila******melanogaster***

Neural tube defects such as spina bifida and anencephaly are among the most common birth defects, seen in nearly 300,000 cases per year across the globe. These defects are the result of incomplete cellular movement and failures in proper morphogenesis during the embryonic stage. The Rho signal transduction pathway is involved in many cellular processes including morphogenesis and is highly conserved in humans as well as model organisms like *Drosophila melanogaster*. In *Drosophila*, mutant alleles coding for the RhoA protein have been implicated in causing embryonic lethality resulting from failed involution of the head and primitive brain. Two extant alleles, RhoA3.5.1 and RhoA4.4.2 are being analyzed for their specific morphologic effects via embryonic cuticle preparations, as well as their genetic sequences via PCR amplification of the RhoA gene in mutant embryos. RhoA4.4.2 previously has exhibited a typical loss-of-function phenotype, while RhoA3.5.1 has demonstrated possible hypomorphic activity. If successful, the experiment hopes to fully characterize these alleles and possibly identify one of the first hypomorphic RhoA alleles in *Drosophila melanogaster*. This research may be used in order to investigate the role of the Rho signal pathway in the etiology of human neural tube defects.

**P40**

Kelsey Savage, Molly Bletz, Dr. Eria Rebolarr, Dr. Reid Harris

**Comparative analysis of anti-Bd bacteria from five Malagasy frog species of*****Ranomafana*****National Park**

As Malagasy amphibians are facing an impending extinction crisis from the lethal fungus *Batrachochytrium dendrobatidis* (Bd), it is imperative to proactively mitigate the threat. Bd sporangia develop in the skin of infected amphibians, causing the skin to thicken, leading to ionic imbalance and heart failure. It has been shown that some bacterial species are able to inhibit Bd growth on amphibians by producing antifungal metabolites. Community-based probiotics can be used to combat the disease on a multitude of amphibians by inoculating an environment with anti-Bd bacteria. It is important to minimize effects on non-target organisms by selecting anti-Bd bacteria originally found on the amphibians. The purpose of this study will be to determine which bacteria from an amphibian community in Madagascar are capable of inhibiting Bd. To identify anti-Bd bacterial isolates, inhibition assays of bacterial samples against Bd will be conducted. Changes in optical density of isolates culture filtrates with Bd will be compared to controls. After finding several isolates that are positive for inhibition, as in previous studies, relationships between the anti-Bd bacterial species and the diversity of the anti-Bd bacteria found on each frog sampled will be assessed. A unique pattern of anti-Bd bacterial communities associated with certain amphibian species is expected, as bacterial communities can vary between species due to each host producing a different array of antimicrobial peptides.

**P41**

Heather Gagné, Molly Bletz, Dr. Eria Rebolarr, and Dr. Reid Harris

**Tests of whether bacterial species from Malagasy frogs inhibit multiple strains of the lethal pathogen *Batrachochytrium dendrobatidis***

Amphibians, the vertebrate class most threatened with extinction, are experiencing declines on every continent where they are found. Many of these declines are attributed to habitat destruction, pollution, and the infectious disease chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (Bd). Bd is a rapidly spreading pathogen that has been found in almost every part of the world, save for a few localities such as Madagascar and New Guinea, the homes of a multitude of endemic species. Naturally occurring bacteria on amphibian skin have been found to inhibit the growth of Bd. Potential probiotic bacteria collected from Malagasy amphibians in August of 2013-February 2014 are currently being isolated and stored to test for their inhibitory nature against a single Bd strain. The inhibition will be measured through challenge assays, where Bd is exposed to the cell free supernatant from a bacterial culture. If a bacterial species is found to inhibit Bd, I will test whether it can inhibit multiple

strains, since multiple strains can exist in the natural environment. Eventually, these probiotic bacteria can be used in the field in the form of probiotic baths and added to the environment.

**P42** Kevin Tomlinson, Dr. Jon Kastendiek

**The effect of addition of seedling protection tubing toppers on survivability of saplings at Smith Creek Restoration Site**

This study aims to determine if additional protection from deer browsing will increase growth and survival of trees planting at the Smith Creek Restoration site. In 2005, 9,033 tree seedlings were planted in four-foot seedling protection tubes. In 2012, only 656 seedlings were found to have grown above the deer browse line of 40 cm above the protective tubing. Of these 656 seedlings only 7 were oak seedlings. Oaks are of particular concern due to their high ecological significance because they provide shade and habitat for surrounding wildlife. The plastic mesh tubing toppers were added to a total of 93 individual oak trees with 34 pairs based on location. In both paired and unpaired t-tests there was no significant difference ( $p > 0.05$ ) in the initial heights of the treated and untreated seedlings. After one growing season, it was found that there was a significant difference in heights. Furthermore, another t-test was performed on the difference in heights which also showed a significant difference. These t-tests indicate that the addition of seedling protection tubing toppers was able to improve the oak seedlings growth. This study will be expanded to ash and maple seedlings to see if a similar result will be found.

**P43** Rebecca Dickey, Molly Bletz, Dr. Eria Rebollar, and Dr. Reid Harris

**Invasive parthenogenetic decapod *Procambarus fallax f. virginialis* as a possible host for the fungal pathogen *Batrachochytrium dendrobatidis***

The chytrid fungus *Batrachochytrium dendrobatidis* (Bd) has been implicated in the declines of hundreds of amphibian species worldwide. Given that Bd is known to have non-amphibian crayfish hosts of the genus *Procambarus*, it is possible that the enigmatic crayfish species *P. fallax f. virginialis* could act as a host for Bd. *Procambarus fallax f. virginialis* is a parthenogenetic form of the slough crayfish and has invaded parts of Europe and Asia. The purpose of this experiment will be to assess the ability of *P. fallax f. virginialis* to contract Bd, which will indicate whether it is a possible vector or reservoir species for Bd in environments shared with vulnerable amphibians or other native crayfish species. To accomplish this we will attempt to infect lab-reared *P. fallax f. virginialis* with Bd and confirm infection by examination of the intestinal walls via light microscopy and qPCR of carapace swabs and intestinal cells as described in previous research papers. Understanding the pathogens host range of non-amphibians is crucial to preventing and managing future outbreaks in areas that are currently free of Bd, such as parts of Madagascar.

**P44** Lina Chhuy-Hy, Michael Yeager, Dr. Marta Bechtel

**The Effect of Lacritin on Primary Rabbit Cornea Cell Proliferation, Wound Healing, and the prevention of Apoptosis**

Cornea is the transparent front part of the eye that covers the iris, pupil, and anterior chamber. Cornea accounts for approximately two-thirds of the eye's total optical power. The three cell layers made up cornea are: epithelial, stromal, and endothelial layers. Possible damages to the corneal epithelium could be chemical, physical, or microbial factors that could lead to changes in the phenotype of cornea which can lead to the loss of vision. Our studies will focus on the interaction between lacritin on cornea function and performance. Lacritin is a 12.3 kDa glycoprotein encoded in human by the LACRT gene. Lacritin is a secreted protein found in tears and saliva. By understanding the mechanism of lacritin on corneal epithelium, we might be able to understand the proliferation and/or wound healing processes of cornea. In our lab we use freshly isolated rabbit cornea tissues as models because they can serve as a great model for the human cornea tissue studies later on. However, before this study continues we must be able to obtain sufficient cell yield of each type of cell. These freshly isolated cells behave better than cell line in vivo study.

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**James Madison University Biology Department**  
**Second Annual**  
**GRADUATE SYMPOSIUM, BIOSCI 2007**  
**Thursday April 10<sup>th</sup>, 5:00pm**

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Program:

4:45-5:00

OPENING RECEPTION – 2009

WELCOME

- 5:00 G1. Sarah Auclair, Dr. Chris Lantz  
**Interleukin-3-deficient mice have increased resistance to blood-stage malaria infection**
- 5:15 G2. James W. Conrad, Janet Moore, Dr. Joanna Mott, Dr. Pradeep Vasudevan  
**Comparison of phenotypic methods to identify *Vibrio vulnificus***
- 5:30 G3. Dehat Jalil, Dr. Terrie K. Rife  
**A Z-DNA forming polymorphism in the Nitric oxide Synthase I Promoter and its Relevance to Neurodegenerative Disease**
- 5:45 G4. John N Marafino, Tara Gallenger, Dr. Kevin L. Caran, Dr. Kyle Seifert  
**Triscationic Amphiphiles with One or Two Tails**
- 6:00-6:15 ::::::::::::::::::::::::::::::: BREAK :::::::::::::::::::::::::::::::::::::::
- 6:15 G5. Steven McBride, Benjamin Holland\*, Dr. Pradeep Vasudevan, Dr. Joanna B Mott  
**The survival of *Enterococcus faecium* in turkey litter microcosms**
- 6:30 G6. William A. Noftz, Valerie Y. Acquesta, Dr. Lincoln C. Gray, Dr. Mark Gabriele  
**Converging midbrain afferent patterns and auditory brainstem responses in ephrin-3 mutant mice**
- 6:45 G7. Kyle Snow, Dr. Christine May  
**Effects of Fine Sediment on Juvenile Brook Trout Habitat Use and Social Interactions**
- 7:00 G8. Anisa C. Tracy, Nicole Roberto, Dr. Steven Cresawn  
**Exploration of genomic determinants for host range in phages of pathogenic and non-pathogenic Mycobacteria**
- 7:15 G9. Jamie Smith, Dr. Conley McMullen  
**The Relationship Between Riparian Zone Width and Floristic Quality Along Streams in Shenandoah County, Virginia**

Graduate Poster: P9 (Thursday noon-2pm) - Tara Gallagher, John Marafino, Brandi Volkers, Nick Minahan\*, Jason Floyd, Abby Perlin, Caroline Dilworth, Dr. Kevin Caran, Dr. Kyle Seifert  
**The Antibacterial Activity of Novel Cationic Amphiphiles**

**G1** Sarah Auclair, Dr. Chris Lantz

**Interleukin-3-deficient mice have increased resistance to blood-stage malaria infection**

Malaria is a mosquito-borne disease caused by protozoan parasites of the genus *Plasmodium*. Malaria causes an estimated 200 million infections resulting in over 600,000 deaths per year. The contribution of interleukin-3 (IL-3), a hematopoietic growth factor and immunoregulatory cytokine, to resistance to blood stage malaria was investigated by infecting IL-3-deficient or "knockout" (KO) mice with *Plasmodium berghei* NK65. Male IL-3 KO mice were more resistant to infection than wild-type (WT) mice, as evidenced by lower peak parasitemia and prolonged survival. Both male and female IL-3 KO mice had increased splenomegaly and were more anemic than corresponding WT mice. Anemia was compensated for by an increase in bone marrow and splenic erythropoiesis in IL-3 KO mice, as evidenced by higher levels of erythroid progenitors. Plasma levels of gamma interferon (IFN- $\gamma$ ) and CXCL9 (a monokine induced by IFN- $\gamma$ ) were found to be significantly reduced in IL-3 KO mice during early stages of infection. In contrast, granulocyte colony-stimulating factor (G-CSF) levels were significantly higher, and the percentage of peripheral blood neutrophils lower, in infected IL-3 KO mice than in their WT counterparts. Overall, our results indicate that IL-3 plays a critical role in suppressing protective immunity to *P. berghei* NK65 infection.

**G2** James W. Conrad, Janet Moore, Dr. Joanna Mott, Dr. Pradeep Vasudevan

**Comparison of phenotypic methods to identify *Vibrio vulnificus***

*Vibrio vulnificus* is an opportunistic human and animal pathogen found in marine/brackish waters. Selective and differential media have been developed to screen for *V. vulnificus*. Phenotypic characterization systems have been developed for rapid speciation of isolates. The objective of this study was to compare several selective and differential media (*Vibrio vulnificus* agar (VVA), CHROMagar vibrio (CAV), thiosulfate bile-salts sucrose agar (TCBS), and colistin polymyxin B cellobiose agar (CPC+)), as well as Biolog Gen III MicroPlate™, and PCR (*vvhA*) for their ability to identify *V. vulnificus* and screen for antibiotic resistance. One hundred forty two *V. vulnificus* isolates had been collected previously from the environment and infected patients. Only 4.2% of isolates were not identified by one or more selective/differential media and 3.5% were not identified using Biolog Microbial Identification System. Virtually all isolates were susceptible to all antibiotics tested. Additional isolates (84) were collected from local aquaria as well as several Virginia coastal sites. Most isolates were presumptively identified on VVA and CPC+ but not on CAV or by PCR. Selective media offers a fast method for rapid identification and reduction in the costs associated with identification. However, verification of isolates by PCR with species specific primers is still necessary.

**G3** Dehat Jalil, Dr. Terrie K. Rife

**A Z-DNA forming polymorphism in the Nitric oxide Synthase I Promoter and its Relevance to Neurodegenerative Disease**

Alzheimer's Disease patients develop changes in their DNA structure that are not observed in age-matched controls. Altered DNA conformation occurs in regions of DNA called promoters. These conformation changes occur best in alternating purine-pyrimidine sequences. Furthermore, these altered conformations can be stabilized by tau protein and alpha-synuclein, which have both been implicated in Alzheimer's disease and Parkinson's disease respectively. Formation of alternative DNA in these regions regulates gene transcription making it likely that the altered DNA found in Alzheimer's patients is changing gene expression. Here we look at the effect of different sized purine-pyrimidine sequences on DNA structure and its effect on gene expression in SK-N-MC cells. We also analyze the effect of tau on gene expression via luciferase assay.

**G4** John N Marafino, Tara Gallenger, Dr. Kevin L. Caran, Dr. Kyle Seifert  
**Triscationic Amphiphiles with One or Two Tails**

The past 10 – 15 years has seen a rapid increase in the amount of antibiotic resistant bacteria. This growth is primarily due to the overuse of broad-spectrum antibiotics in both nosocomial and community-based infections. Most outbreaks involving antibiotic-resistant bacteria are nosocomial. However, community-acquired infections are increasing. In the clinical setting, a foundation for infection control lies in controlling bacterial transference. This management becomes crucial as more bacteria become resistant to antibiotics. Thus techniques such as hand washing and surface sterilization are two, of many, important avenues that need to be carefully monitored. Amphiphiles have a number of applications in the medicinal and industrial fields in which they assist in controlling bacteria transference due to their bactericidal activity. This activity is primarily due to their structure. Amphiphiles are comprised of two distinct regions: a hydrophobic tail (typically a fatty hydrocarbon chain) and a hydrophilic head (which can be ionic or non-ionic). Altering the composition, number and juxtaposition of these groups can affect their characteristics, which in turn can alter function. Two novel series of amphiphiles, with either a single or double hydrocarbon tail and three cationic head groups was synthesized. Here, the colloidal and biological properties determined thus far are shown, including the critical micelle concentration (CMC), established by isothermal titration calorimetry, and minimum inhibitory concentration (MIC).

**G5** Steven McBride, Benjamin Holland\*, Dr. Pradeep Vasudevan, Dr. Joanna B Mott  
**The survival of *Enterococcus faecium* in turkey litter microcosms**

Application of turkey litter to agricultural lands is a common practice in the United States, creating a potential method of transmission of fecal bacteria from poultry into the environment. The purpose of this study was to: 1) determine levels of *Enterococcus* spp., in turkey litter from a commercial poultry house; 2) identify isolates to species; 3) determine their resistance to a range of antibiotics; 4) examine survival of *E. faecium* in turkey litter under a range of conditions. Turkey litter was collected from a poultry house in Shenandoah County, VA. Presumptive enterococci were isolated using selective media, species were identified phenotypically, and antibiotic resistance was determined by disc diffusion. Microcosms were used to examine survival of the most common species isolated from the litter. Three variables were tested in the microcosms: strain, temperature and litter moisture. Decay rates were determined by quantifying the bacteria in the microcosms over time. While enterococci levels were low ( $< 1.14 \times 10^1$ /gdw) in fresh shavings, high levels ( $4.07 \times 10^4$ cfu/gdw) were found in litter following the rearing period. *E. faecium*, *E. durans*, and *E. faecalis* were the most prevalent species identified and antibiotic resistance varied between isolates. Turkey litter was shown to act as a reservoir of antibiotic resistant bacteria with the potential to contaminate the environment if handled incorrectly.

**G6** William A. Noftz, Valerie Y. Acquesta, Dr. Lincoln C. Gray, Dr. Mark Gabriele  
**Converging midbrain afferent patterns and auditory brainstem responses in ephrin-B3 mutant mice**

Eph-ephrins are a family of molecular guidance proteins that provide cell-cell interactions necessary for topographic mapping and pattern formation in the developing nervous system. Studies in our laboratory have shown in mouse the transient expression of certain Eph-ephrin members in the developing inferior colliculus (IC) prior to hearing onset. EphA4 and ephrin-B2 expression is graded along the tonotopic axis of the central nucleus of the IC (CNIC), while occupying discrete modules in the lateral cortex of the IC (LCIC). In contrast, ephrin-B3 expression is absent in the CNIC, while highly expressed in extramodular domains of the LCIC, as well as at the midline of the brain. We utilize multiple-labeling approaches in control and ephrin-B3 mutants to explore the development of converging CNIC and LCIC afferent patterns. Tract-tracing studies describe the relative distribution patterns of crossed CNIC and ipsilateral auditory brainstem inputs to the target IC. Additionally, we performed auditory brainstem responses (ABRs) as a physiological assessment of the auditory circuitry for each of our experimental groups. ABRs show effects on auditory threshold, peak latency, and waveform fidelity in ephrin-B3 mutant mice. Taken together, these findings suggest an important role for ephrin-B3 in constructing fully functional auditory circuits prior to experience.

**G7**

Kyle Snow, Dr. Christine May

**Effects of Fine Sediment on Juvenile Brook Trout Habitat Use and Social****Interactions**

Streambed sedimentation is a persistent cause of impairment to the ecological condition of streams. The objectives of this study were to investigate the effects of fine sediment deposition on streambed cover use by juvenile brook trout as a velocity refuge during simulated winter flood conditions, and streambed cover use and aggression within cohorts of three brook trout during low flow conditions. Using artificial stream channels, experiments were conducted with wild juvenile brook trout collected from the North Fork Tye River, Nelson County, VA. Brook trout did not use streambed cover as a velocity refuge during winter flood simulations. Generalized linear mixed modeling was used to analyze cohort low flow conditions with sedimentation, temperature, and time as fixed effects and cohort as a random effect. Streambed cover use decreased as temperature and sedimentation increased. Aggression increased as sedimentation increased, decreased as temperature increased, and decreased throughout the day. These results suggest that streambed fine sediment deposition has potentially adverse effects on salmonid carrying capacity through a reduction of juvenile rearing habitat availability and increased social stress.

**G8**

Anisa C. Tracy, Nicole Roberto, Dr. Steven Cresawn

**Exploration of genomic determinants for host range in phages of pathogenic and non-pathogenic Mycobacteria**

The genus *Mycobacterium* is diverse, ranging from slow growing human pathogens such as *M. tuberculosis* to fast-growing saprophytes such as *M. smegmatis*. *M. ulcerans* is the causative agent of the neglected disease known as Buruli ulcer, an emerging pathogen first described in Buruli County, Uganda. Antibiotic resistance among Mycobacterial pathogens is growing at an alarming rate and is creating an urgent need for novel diagnostic and therapeutic approaches. Elucidating the details of virus/host interactions among the mycobacteria and their phages thus has the potential to affect how we think about the role of these bacteria in both clinical and environmental settings. Towards this end, we are analyzing the complete genome sequence of 596 mycobacteriophages isolated on *Mycobacterium smegmatis* mc2155. Here we report on six novel bacteriophages from Ugandan soil, in addition to three bacteriophages isolated from American soil, and using pairwise nucleotide identity and phamily composition of the genomes work to elucidate the genomic determinants for their ability or inability to infect the host *M. ulcerans*.

**G9**

Jamie Smith, Dr. Conley McMullen

**The Relationship Between Riparian Zone Width and Floristic Quality Along Streams in Shenandoah County, Virginia**

Riparian zones harbor an above average plant biodiversity. This biodiversity is increasingly threatened by invasive species and human disturbance such as deforestation from agriculture and urban development. This study will determine the relationship between the size of the riparian zone and the floristic quality of its vegetation. A bioassessment tool, which utilizes plant community characteristics will, be used to obtain an estimate of habitat quality. This estimate, in turn will determine whether larger riparian zones provide more protection from the impacts of disturbance and provide higher quality habitats for plants. Vegetation plot data is currently being processed from five streams in Shenandoah County, Virginia.

# BIOSYMPOSIUM 2014 - Author Index

## Numbers refer to (T)alks, (P)osters, (G)raduate talks

T1-T8: TH 2-4pm *Biosci 2007*

T9-T14: FRI 10am-noon *Biosci 2007*

P1-P13: TH noon-2pm *2nd Fl. Foyer*

P15-P44: FRI 2-4pm *2nd Fl. Foyer*

G1-G9: TH 5-7:30pm *Biosci 2007*

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