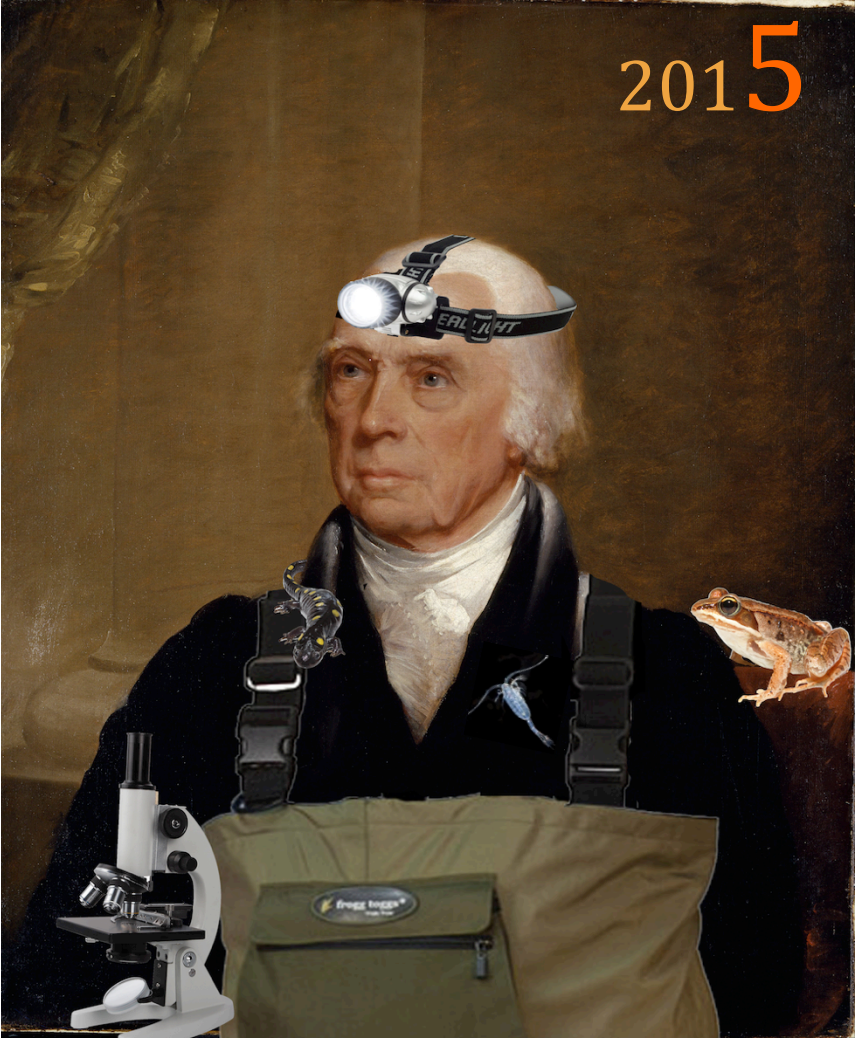


BIOSYMPOSIUM

2015



James Madison University
Biology Department

Thursday, April 17 - Friday, April 18, 2015

**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
- ❖ Elizabeth McConnell Bliss Endowment for Undergraduate Research Scholarship
- ❖ Trelawny endowment
- ❖ Jeffrey E. Tickle '90 Family Endowment Scholarship
- ❖ Taliaferro Scholarship
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- ❖ Chappellear Scholarships
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Schedule: Thursday April 17th

SESSION 1	12:30-2:00pm	POSTERS	2nd Fl. Foyer
P1	Emily Grunwald*, Sophia Brown, Dr. Raymond Enke	Transcriptome Analysis of the Developing Chicken Retina	
P2	Yaw Owusu, Mrs. Andrea Pesce, Dr. Beaux Berkeley	Student Attitudes on the Use of Calibrated Peer Review in Bio114	
P3	Morgan Hedden, Courtney Stout, Nicholas Dunham, Dr. Raymond Enke	Epigenetic Analysis of Retina-specific Dnmt1, Dnmt3A and Dnmt3B Knockout mice	
P4	Lindsey Toothman, Steven McBride, Ben Holland, Dr. Pradeep Vasudevan, Dr. Joanna Mott	Antibiotic Resistance of <i>Enterococcus</i> spp. Collected from Organic Turkey Litter	
P5	Shannon Fox, Ryan Samuel, Dr. Susan Halsell	Determining the Molecular Mechanisms of Cold Nociception in the <i>Drosophila</i> model system	
P6	Shahzeb Khan, Dr. James Herrick	Isolation of Bacteria Native to Cooks Creek, a Stream in the Shenandoah Valley of Virginia	
P7	Katie McCullar, Dustin Phillips, Maria Wilkins, Dr. Janet Daniel	Investigating Phenotypic Differences Between STP1 K/O and WT <i>Arabidopsis thaliana</i> in Response To Various Salt Concentrations	
P8	Kevin Tomlinson, Dr. Jon Kastendiek	The Effect of Addition of Sapling Protection Tubing toppers on Survivability of Saplings at Smith Creek Restoration Site	
P9	Naomi Gilbert, Dr. Morgan Steffen	Diversity of the UreC Gene in Bacteria Associated with Toxic Cyanobacterial Blooms	
P10	Justin Lawhorne*, Dr. Tim Bloss	The Effects of Endoplasmic Reticulum (ER) Stressors on Cell Morphology and Viability in the Presence and Absence of the Nascent Polypeptide Associated Complex (NAC)	
P11	Jasmine Roberts-Torres*, Jennifer Kurasz, Michael Partin, Kevin Libuit, Dr. James Herrick	Isolation and Characterization of <i>Staphylococcus</i> spp. from Manure-contaminated Streams in the Shenandoah Valley	
P12	Wes Deaver, Wes Geyer, Dr. Michael Renfroe	Antioxidant Concentrations in Younger and Older Leaves of <i>Lavandula</i> and <i>Salvia</i>	
P13	Clara Thiel*, Tyler Mullins*, Dr. Michael Renfroe	Hydrophilic and Lipophilic Analysis of Antioxidants in Culinary Herbs and Spices	
P14	Ryan Samuel, Shannon Fox, Stephen Jurko, Haley Nisson, Harold Burke, Kendyl Combs, Kaleigh Jaeger, Dr. Susan Halsell	Analysis of Cold Behavioral Response to Reverse Genetic Screening of Innexin Gap Junction Proteins in <i>Drosophila</i> neurons	

Schedule: Friday April 18th

SESSION 3 10:00-noon ORAL PRESENTATIONS BIOSCI 2007

- 10:00 WELCOME
- 10:15 T2. Lina Chhuy Hy*, Taylor Derby, Christopher Watson, Dr. Marta Bechtel
Optimizing Primary Rabbit Cornea Cell Isolation for Tissue Engineering Studies
- 10:30 T9. Joseph Balsamo, Dr. Mark Gabriele
Development and Organization of Multimodal Brain Circuits: Applications for Tinnitus Relief?
- 10:45 T10. Michael Rudloff, Dr. Nathan Wright
Biophysical Characterization of Naturally Occuring Titin M10 Mutations
- 11:00-11:15 : BREAK:
- 11:15 T12. Stephen Simonetti, Dr. Reid Harris, Dr. Eria Rebollar, Dr. Idelle Cooper, Will Shoemaker
Pseudo-Environmental and Horizontal + Pseudo-Environmental Transmission of a Probiotic *Janithinobacterium lividum* Between Green Frog Tadpoles
- 11:30 T13. Emily C. Thyroff, Dr. Heather P. Griscom
Experimental Greenhouse and Field Trials on American Ginseng, *Panax quinquefolium*: Implications for Restoration in Appalachia
- 11:45 T14. Courtney Matson, Erin Wallace*, Dr. Tim Bloss
Characterization of Cell-specific Responses in *C. elegans* Experiencing Misfolded Protein Stress: How Do Some Cell Types Save Themselves While Others Die?
- 12:00-12:20 : BREAK:

Keynote Presentation:

Karah Nazor

The McCallie School

*One Scientist's Journey from the Field to the Lab
and to the Classroom:
Birds, Prions, Boys and Cnidarians*

Bioscience 1007, 12:20-1:10

1:10-2:00 LUNCH RECEPTION FOR PRESENTERS - Second floor foyer

Schedule: Friday April 18th

SESSION 4	FRI 2:00-4:00	POSTERS	2nd Floor Foyer
P31	Breanna Lee, Dr. Carol Hurney	Hox13 expression in embryonic and post-embryonic <i>Hemidactylum scutatum</i> tails.	
P32	Benito Blanchfield-Felice, Kelly Burke, Elli Flora*, Kelcy Jackson*, Dr. Justin W Brown	The Role of Brain Stem Neurotransmitters in the Thermoregulatory Response to Hypoxic Stress	
P33	Brittany Manning, Dr. Conley K. McMullen	Sampling, databasing, and digitizing the Flora of the E.J. Carrier Arboretum	
P34	Ariel Childs, Aubrey Siebels, Kate Reiman, Dr. Corey L Cleland	The Escape Response of Crickets Depends on the Direction of Looming Stimuli	
P35	Olivia Vito, Ben Pisano, Megan Budnik, Anna Nordseth, Dr. Heather Griscom	The Effect of Slope Position and Gap Size on Chestnut Hybrid Performance	
P37	Catherine Torres, Dr. Jonathan Monroe, Dr. Amanda Storm	The Characterization of BAM6 from <i>Arabidopsis thaliana</i>	
P38	Megan Hines, Dr. Jonathan Monroe, Dr. Amanda Storm	Searching for Potential Binding Partners of β-amylase2 using Yeast 2-Hybridization	
P39	Amanda Crandall, Jessie Doyle, Rhiannon English, Mycah McNett*, Megan Moore, 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	
P40	Erin Thady, Amanda Crandall, Jessie Doyle, Rhiannon English, Mycah McNett*, Megan Moore, Joseph Noel, Nathan Robinson, Becca Sanders, Ty Steve, Dr. Bruce Wiggins	Effects of Restoration Practices on Stream Health in the Shenandoah Valley using Macroinvertebrate Survey and GIS Analysis	
P41	Monica Paneru, Stephanie Masters, Suma Haji, Sybelle Djikeng, Caroline Dilworth, Brandi Volkers*, Jason Floyd, Nick Minahan, Tara Gallagher, John Marafino, Jade LaDow, Kyle Bonifer, Dr. Kevin Caran, Dr. Kyle Seifert	The Colloidal, Antibacterial, and Cytotoxicity Properties of Tris-cationic, Triple-headed Amphiphiles	
P42	Kelsey Savage, Dr. Eria Rebolgar, Dr. Reid Harris	Comparative Analysis of Anti-Bd Bacteria from Six Malagasy Frog Species of Ranomafana National Park	
P43	Tiffany Bridges, Dr. Eria Rebolgar, Dr. Reid Harris	Ability of Skin Bacteria on the Panamanian frog, <i>Craugastor fitzingeri</i>, to Inhibit the Fungal Pathogen <i>Batrachochytrium dendrobatidis</i>	
P44	Roxana Behrooz, Dr. Mark Gabriele	Exploring Perineuronal Net Labeling as a Neurochemical Marker for Auditory Midbrain Modularity	
P45	Rebecca Gaita, Alex Silva, Dr. Corey Cleland	The Escape Response of Crickets to Heat Stimuli Utilizes a Translational rather than Turning Strategy	
P46	Jocelyn Medrano, Kendyl Combs, Isabel Lamb, Katrina Gobetz	Trelawny Research in Nociception in <i>Drosophila melanogaster</i>, Photoreceptor Differentiation in Developing Chick Retina, and Development of Auditory Neuropathways in Mice	

- P47 Joshua Mata, Shelby Snowden, Dr. David McLeod, Dr. Katrina Gobetz
Methodology of Preserving Animal Specimens and its Importance in Biology Education and Research
- P48 Christina Myers, Caylin Murray, Dr. Joseph Harsh
Teaching to Learn Like the Experts: Eye-tracking Analysis of Graphing Interpretation Practices along the Novice-expert Continuum
- P49 Michelle Wallagora, Jasmin Rose, Sarah Maher, Dr. Rose
Quantifying and Comparing Shape Change in the Pharyngeal Arch Cartilages of Salamanders
- P50 Nicole Roberto*, Elizabeth Lucas, Dr. Steve Cresawn
Next Generation Sequencing and Genomic Comparisons of Mycobacteriophage
- P51 Heather Maher, Dr. David McLeod
How Much have we Underestimated Southeast Asia's Biodiversity? New Species in the *Limnonectes khulii* Complex
- P52 Debbie Stratton, Dr. Corey Cleland
The Effect of Physical and Demographic Factors and Training on the Time-to-Adoption of Domestic Cats in a Local Shelter
- P53 Nicholas Dunham, Morgan Hedden, Dr. Raymond Enke
Characterization of Epigenetic Patterns in the Post-Mortem Human Retina
- P54 Jennifer Cahill*, Dr. Chris Rose
Isolating T4 and T3 Effects on Cartilage Growth and Shape Change in *Xenopus* Tadpoles
- P55 Kimberly M Seamon*, Bart Gumpert, Chris Chrzan, Matthew Hartmann, Corey L Cleland
Rat Hind Limb Nociceptive Withdrawal Response to Heat Stimuli Depends on Initial Paw Posture but not Stimulus Location
- P56 Giavanna Verdi, Taylor Berrena, Jacob Edwards, Corey Cleland
The Nociceptive Withdrawal Response to Mechanical Stimuli of the Rat Foot Depends on Initial Foot Position but not Stimulus Location
- P57 Victoria Callahan, Michael Yeager, Dr. Marta Bechtel
Pro-Inflammatory Effect of the Dengue Virus Envelope Protein in Cultured Human Articular Chondrocytes
- P58 Roneka Spady, Dr. David McLeod
Dermeid Beetles as a Carcass Cleaning Agent: An Integrated Pest Management Procedure
- P59 Adrienne Muetterties*, Dr. Patrice Ludwig
Resource Type Affects Offspring Survival and Phenotype in the Dung Beetle, *Onthophagus taurus*
- P60 Nathan Evans, Dr. Patrice Ludwig
Habitat Preference Modelling for the Endangered Northeastern Beach Tiger Beele, *Cicindela dorsalis dorsalis*; Implications for Rising Sea Level
- P61 Gail Moruza, Gregory Mansou, Dean Cocking
Background Mercury Presence in Air, Soil, Duff, and MacroInvertebrate Compartments of "Uncontaminated" Forest Ecosystems Located in Rockingham Co.
- P62 Alexandra Deal, Taelor Weaver, Michelle Huth, Dr. Terrie Rife
Cloning Reporter Genes to Investigate Mechanisms behind Nitric Oxide Synthase I Misregulation
- P63 Rhiannon English, Sheikh Hossain, Samantha Webster
Trelawny Research in Water Quality with Macroinvertebrates, BAM Genes in *Arabidopsis thaliana*, and Artificial Oyster Reefs.
- P64 Haley Davis, Josh Donohue, Dr. Chris Lantz
Evaluation of Interleukin-3 in Blood-stage Immunity against Murine Malaria *Plasmodium yoelii*

T1 Kristen Fadel, Dr. Amanda Storm, & Dr. Jonathan Monroe

Investigation of BAM9 expression in *Arabidopsis*: Construction of promoter-GUS transgenic plants

β -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 β -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 out the inserts. The insert was ligated into the recipient vector, pCAMBIA 1305.1, and the plasmid was transformed into *Agrobacterium tumefaciens* cells and infiltrated into *Arabidopsis* using a floral spray method. Putative transgenic plants were selected on media supplemented with hygromycin and will be tested by PCR. 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.

T2 Lina Chhuy Hy*, Taylor Derby, Christopher Watson, Dr. Marta Bechtel

Optimizing Primary Rabbit Cornea Cell Isolation for Tissue Engineering Studies

Currently there is no standardized protocol for isolating primary stromal cells from cornea tissue. Protocols in current literature show a wide range of collagenase concentrations (0.02% to 2.0 mg/mL), and incubation times ranging from 1 hr to 4 hr at 37°C. We have optimized a protocol that demonstrates higher cell yields and increased viability per cornea than achieved with current protocols. This was achieved by investigating six different collagenase concentrations (50, 100, 200, 400, 600, and 800 Unit/mL). Each collagenase concentration was incubated with freshly excised cornea tissue at 37°C in a humidified incubator with 5% CO₂ for 18 – 48 hours. Two of the six concentrations (200 and 400 Unit/mL), yielded the highest cells/cornea with no significant difference ($P > 0.05$) based on three independent trials. Cell viability was determined over a three day cell culture time course, and quantified by hemocytometer using trypan blue dye exclusion. Results indicate optimum cell yield and viability are obtained with either 200 Unit/mL or 400 Unit/mL collagenase treatments at 18 hours. This work will prove helpful to investigators employing the rabbit cornea model system for tissue engineering studies.

T3 Sepideh Asgari, Dr. Chris Lantz

Interleukin-3 Regulates the Production of Interferon-gamma during Blood-stage Malaria infection.

Malaria is a mosquito-borne disease caused by protozoan parasite of the genus *Plasmodium*. About 3.2 billion people are at risk for exposure and greater than 200 million new cases are reported each year. We have previous shown that the cytokine interleukin-3 (IL-3) plays a critical role in suppressing protective immunity to lethal *P. berghei* NK65 infection as indicated by the ability of infected IL-3-deficient (knockout [KO]) mice to survival longer and experience lower parasitemia than corresponding wild-type (WT) mice. To determine if IL-3 influences the course and outcome of infection by regulating levels of other cytokines, we examined protein levels of the inflammatory cytokine interferon-gamma (IFN-g) in WT and IL-3 KO mice prior to infection and at various days post-infection. Although levels of IFN-g in serum and spleen were undetectable in uninfected animals, infection induced increased production of IFN-g in both genotypes beginning at approximately day 3 post-infection. Interestingly, infected WT mice appeared to have higher levels of IFN-g in both the serum and spleen than did corresponding IL-3 KO mice. These data indicate that the observed ability of

IL-3 to influence the host response to malaria infection may in part be due to IL-3-dependent regulation of IFN- γ production.

T4 Curtis Kapsak*, Kevin Libuit, Dr. James Herrick

Successful Sequencing of a Transmissible Antibiotic Resistance Plasmid using the MinION, a New Nanopore-based 3rd Generation DNA Sequencer

Oxford Nanopore Technologies® (Oxford, UK) developed the MinION™, a compact and portable nanopore-based sequencer capable of producing real-time data powered directly by a computer via USB. To support this project, they have launched the MinION Access Program to allow the Herrick lab and ~500 other groups worldwide to beta test this device. Our latest sequencing run was performed on a single, antibiotic resistance plasmid that was captured exogenously from local stream sediment. The circular plasmid DNA was isolated, prepared, and sequenced using the MinION for a 13-hr period and generated 39463 reads. Sequence data was aligned (using LAST) to the Comprehensive Antibiotic Resistance Database, and over 4,436 of the 13330 2D reads (template and complement strand in one read) mapped to known genes found in a range of microbes. Due to our selection for resistant recipients on tetracycline-infused agar, we expected to see hits to a tetR gene and found 185 reads mapped to the gene, tetG, whose source is a *Pseudomonas* sp. plasmid pPSTG1. Most notably, 45 of those reads mapped to blaCARB-3, an antibiotic resistance gene encoding for carbapenemase beta-lactamase, which confers resistance to a last resort, clinical antibiotic. Our future experiments will include sequencing different plasmids and eventually sequencing the plasmid metagenome from our sample sites.

T5 Emma Bales*, Dr. Oliver Hyman, Dr. Reid Harris

Pathogenic Chytrid Fungus *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.

T6 Christophe Langouet, Katelyn Thomas, Dr. Nusrat Jahan

Oh Rats... Statistics: Relationship between Renin-Angiotensin System and Sex

Renin-angiotensin is a blood pressure regulator and plays an important role in the development of cardiovascular disease and kidney damage. Previous studies indicate a higher frequency of men developing renal damage potentially related to the renin-angiotensin system (RAS). The Bauer et al. (2011) study seeks to find the impact of sex and gene expression on renal damage development. Microarray studies were performed using double transgenic rats (dTGR), expressing both human renin and angiotensin genes with comparison to control Sprague-Dawley (SD) rats in order to investigate sex-dependant responses in the RAS system. Three comparisons were made between female dTGR versus control rats, male dTGR versus control rats, and female versus male rats, each being the difference between the dTGR and their control counterparts. Bauer et al. identified differentially expressed genes within each sex and between the sexes, then investigated pathways related to significant genes using gene ontology (GO) terms. We analyzed their gene expression data, controlling false discovery rate at 0.01. Significant genes were further analyzed with a two-way ANOVA

to investigate the effects of sex on the RAS system. Multivariate analysis, such as cluster analysis, was also performed on the significant genes to investigate genomic interrelationships.

T7 Michelle Clower*, Ashton Holub*, Dr. Grace Wyngaard

Embryonic Development in a Zooplankton that Undergoes Chromatin Diminution: A Quantitative Model of Changes in DNA Contents (Part 1)

Mesocyclops edax (Crustacea: Copepoda) undergoes chromatin diminution, a programmed excision of DNA from the presomatic cell lineage. The function of chromatin diminution in this microcrustacean copepod is unknown. Previous work on chromatin diminution in copepods studied only two cell stages in development: immediately before and after the diminution. Such a complex phenomenon is unlikely to be confined to just these 2 cell stages. In an effort to more thoroughly describe and understand chromatin diminution, we have examined all cell stages of embryogenesis for changes in chromosome morphology and nuclear DNA contents.

Mesocyclops edax were preserved and stained using the Feulgen reaction for DNA quantification. Densitometric image analysis revealed some new findings. (1) DNA content of the polar body does not necessarily contain the haploid amount of DNA and varies within and among embryonic cell stages. (2) Typical chromosomal structure is not apparent during the first and second cell stages, perhaps due to delayed fusion of male and female pronuclei or delayed formation of the kinetochore. (3) The primordial germ cell and primordial endoderm cell divide asynchronously with one another and the presomatic cells. (4) Diminution in *M. edax* occurs at the 5th cleavage division, contrary to the published report.

T8 Ashton Holub*, Michelle Clower*, Dr. Grace Wyngaard

Embryonic Development in a Zooplankton that Undergoes Chromatin Diminution: A Quantitative Model of Changes in DNA Contents (Part 2)

Mesocyclops edax (Crustacea: Copepoda) undergoes chromatin diminution, a programmed excision of DNA from the presomatic lineage. *M.edax* embryogenesis starts with a 1 cell embryo containing 15 pg of DNA per nucleus. At the 5th cleavage division, 15 of the 16 cells destined for the somatic lineage undergo diminution, excising 12 pg of DNA each. This results in somatic genomes yielding 3 pg of DNA per nucleus. The 16th cell, the primordial germ cell, the precursor to the germ line, remains undiminished retaining all 15 pg of DNA. The excised DNA forms "droplets", the fates of which were followed throughout embryogenesis until the first larval stage. Rather than immediately being resorbed into the nucleus, the excised droplets remain in the embryo until the 512-1024 cell stage (10th cleavage division). Initially the excised droplets contained an average of ~ 0.68 pg DNA. Preliminary results suggest that over subsequent cleavage divisions the average size of the droplets increases, with an average size of ~ 1.94 pg at the 512-1024 cell stage. However, the number of droplets per embryo decreases suggesting a reformation of excised droplets. Understanding the fate of these excised droplets after chromatin diminution will aid in understanding its function in development.

T9 Joseph Balsamo, Dr. Mark Gabriele

Development and Organization of Multimodal Brain Circuits: Applications for Tinnitus Relief?

Tinnitus is a debilitating condition in which sufferers experience phantom auditory percepts in the absence of external stimuli. While believed to be of auditory origin, recent evidence suggests that somatosensory stimuli are capable of influencing tinnitus in complex ways. One integrative area that receives converging auditory and somatosensory connections is the auditory midbrain, or inferior colliculus (IC). To date, our understanding for the organization and mapping of this multimodal center is lacking. Studies show tinnitus may result from maladaptive plasticity of established auditory maps. The present study provides a first step in understanding the development and alignment of auditory and somatosensory maps and how each may be potentially harnessed to normalize the other in pathological states. A series of expression studies for a variety of neurochemical markers and guidance molecules suggest an early modular/extramodular organization in the IC prior to hearing onset. Here, we

demonstrate somatosensory innervation to the IC mimics the developmental time course for previously described auditory inputs. Taken together, discretely defined molecular IC compartments appear to provide the anatomical substrate for converging auditory and somatosensory projection patterns. Understanding this multimodal organization and innervation scheme may prove critical for designing better, noninvasive techniques that reconfigure altered neural maps to previous non-pathological arrangements.

T10 Michael Rudloff, Dr. Nathan Wright
Biophysical Characterization of Naturally Occurring Titin M10 Mutations

The giant human muscle proteins titin and obscurin are important for sarcomeric organization, stretch response, and sarcomerogenesis in myofibrils. The extreme C-terminus of titin (the M10 domain) binds to the N-terminus of obscurin (the Ig1 domain) in the M-line, an interaction that is critical for sarcomere stability. The high-resolution structure of human M10 has been solved, along with M10 bound to one of its two known molecular targets, the Ig1 domain of obscurin-like protein. Multiple M10 mutations are linked to limb-girdle muscular dystrophy type 2J (LGMD2J) and tibial muscular dystrophy (TMD), however the effect of the M10 mutations on protein structure and function has not been thoroughly characterized. Here, all four naturally occurring human M10 missense mutants have been engineered and biophysically characterized *in vitro* with the hypothesis being the mutations ablate this critical interaction. Two of the four mutated constructs are severely misfolded and cannot bind to the obscurin Ig1 domain. One mutation, H66P, is folded at room temperature but unfolds at 37 °C, rendering it binding incompetent. The I57N mutation shows no significant structural, dynamic, or binding differences from the wild-type domain suggesting this mutation may not be directly responsible for muscle wasting disease.

T12 Stephen Simonetti, Dr. Reid Harris, Eria Rebollar, Dr. Idelle Cooper, Will Shoemaker
Pseudo-Environmental and Horizontal + Pseudo-Environmental Transmission of a Probiotic *Janithinobacterium lividum* Between Green Frog Tadpoles

The purpose of this research was to identify the ability of a probiotic, *Janithinobacterium lividum* (*J.liv*), to be horizontally transmitted from individual to individual and/or pseudo-environmentally transmitted from individual to environment and back to individual 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 hypothesized that pseudo-environmental and horizontal transmission will occur in tadpoles, which I based this hypothesis on unpublished data collected by Will Shoemaker. The experimental design had two treatments and their controls. The first treatment, which tested for pseudo-environmental and horizontal transmission, included two tadpoles in a container, swimming freely, with only one tadpole inoculated with rifampicin-resistant *J.liv*. The second treatment, which tested for pseudo-environmental transmission, included two tadpoles in a container, physically separated by a net, with only one tadpole inoculated with *J.liv*. Tadpoles were swabbed at different time points, and plated on rifampicin plates to determine the success of transmission. It was found that in both treatments, the tadpoles given the *J.liv* bath were able to retain *J.liv* on their skin immediately after the inoculation bath but the pseudo-environmental treatment had overall better transmission of probiotic from one tadpole to another along with overall retention of *J.liv* on tadpoles skin. These results suggest that not all individuals have to be inoculated to apply the probiotic to the entire frog population, which has the potential to greatly facilitate disease mitigation efforts.

T13 Emily C. Thyroff, Dr. Heather P. Griscom
Experimental Greenhouse and Field Trials on American Ginseng, *Panax quinquefolium*: Implications for Restoration in Appalachia

Panax quinquefolium, American ginseng, is one of the most valuable non-timber forest products. Despite a broad distribution range, it is not abundant anywhere due to overharvesting and deer browse. This study included experimental field and greenhouse trials to determine optimal growing conditions for future restoration. Three soil series and two aspects (represented by soil moisture in greenhouse) were manipulated. We

hypothesized that there would be significant differences in ginseng performance due to soil and aspect. We predicted that ginseng would grow best in limed loam soil and on northern sites and perform the poorest in sandy loam soil and southern sites. We found that soil type had a significant effect on height and leaf area in greenhouse and field trials (< 0.05). On average, field ginseng from the limed loam soil was 6 mm taller and 1250 mm² greater leaf area than ginseng from sandy loam; whereas survival was greatest in loam soil. Surprisingly, we found no effect of aspect or soil moisture on ginseng growth or survival. Having a better understanding of the ecology of ginseng, especially soil series, will help create a ginseng habitat model for national forests and private lands.

T14 Courtney Matson, Erin Wallace*, Dr. Tim Bloss

Characterization of Cell-specific Responses in *C. elegans* Experiencing Misfolded Protein Stress: How Do Some Cell Types Save Themselves While Others Die?

To maintain viability, cells must resolve misfolded protein stress; the inability to do so often triggers cell death, most notably in neurons during neurodegenerative disease. The NAC is a highly conserved translational chaperone essential for protein folding and localization to organelles throughout the cell. In *C. elegans*, depletion of the NAC initiates misfolded protein stress specifically in the ER, inducing a response that up-regulates chaperone HSP-4 in an attempt to prevent cell death. This up-regulation is robust but not uniform, and deficient in regions containing neurons. We are characterizing this non-uniform stress response to determine if HSP-4 up-regulation is cell-specific and correlates with survival. We've developed a protocol that characterizes stress response in NAC-depleted *C. elegans* expressing two different fluorescent proteins concurrently, and determined NAC-depleted gut cells marked with red fluorescent protein up-regulate robust levels of HSP-4 fused to green fluorescent protein, and this up-regulation is polarized to the anterior and posterior of the gut. Future studies will focus on HSP-4 up-regulation in NAC-depleted animals expressing either neuron-specific or muscle-specific fluorescent proteins. Our current results are consistent with observations in mammalian systems showing intestinal cells are resistant to misfolded protein stress, and may provide a compelling explanation for this resistance.

P1 Emily Grunwald*, Sophia Brown, Dr. Raymond Enke

Transcriptome Analysis of the Developing Chicken Retina

Purpose: Global changes in gene expression are critical during development of the vertebrate retina. The purpose of our study is to analyze and validate an RNA-Seq data set measuring differential gene expression of retinal mRNAs at two critical time points during chick embryonic development.

Methods: For RNA-sequencing, chicken retinas were harvested at embryonic day 8 (E8) and E18. Genomic DNA and total RNA was extracted from tissues and a cDNA library was made from mRNA using the Illumina TruSeq library preparation kit. The cDNA library was then sequenced using the Illumina HiSeq 2500 platform and computational analysis was performed using the Tuxedo bioinformatics pipeline. Gene ontology (GO) analysis was used to identify candidate genes of interest to further study using quantitative reverse transcriptase PCR (qRT-PCR).

Results and Ongoing Experiments: 30-60 million 150 bp reads were sequenced per sample identifying 1,077 upregulated and 1,416 downregulated genes in the E18 retina. GO analysis revealed enrichment of differentially regulated genes associated with phototransduction and the Notch signaling pathway. Our current experiments are using qRT-PCR to validate and further characterize differentially regulated genes in these pathways. Additional experiments are aimed at characterizing differential DNA methylation on regulatory sequences of these genes during development.

P2 Yaw Owusu, Mrs. Andrea Pesce, Dr. Beaux Berkeley

Student Attitudes on the Use of Calibrated Peer Review in Bio114

The incorporation of writing assignments in most science courses is met with the challenge of using too much time to accurately grade students' papers or grading discrepancies between teaching assistants and instructors. Because of this, the innovation of the Calibrated Peer Review (CPR) software has come as a huge relief to most instructors as the software is designed to reduce the workload on instructors and more importantly, enhance the writing skills of most students. This study serves to investigate the attitudes of students toward the use of CPR in an introductory level biology course: Organisms (BIO 114). The students were divided into treatment and comparison groups, and asked a series of five questions about the use of CPR. Students' evaluation of the use of CPR showed poor results; while some of them responded that effective writing is necessary for biologists, the majority felt as though the software did not help them in completing their assignments. Further evaluation of student learning outcomes should be done to see if learning is correlated with attitude.

P3 Morgan Hedden, Courtney Stout, Nicholas Dunham, Dr. Raymond Enke

Epigenetic Analysis of Retina-specific Dnmt1, Dnmt3A and Dnmt3B Knockout Mice

Purpose: Epigenetics plays a key role in the development of the eye. Studies have shown that DNA methylation is instrumental in gene expression in the developing retina. Our collaborators at the University of Pittsburgh Medical Center constructed mice with retina-specific targeted mutations in DNA methyltransferase (Dnmt) genes encoding Dnmt1, Dnmt 3A and Dnmt 3B enzymes in order to discover the role of DNA methylation in retinal development.

Methods: DNA was extracted from tail and retina of Dnmt triple flox mice and triple knockout mice as well as brain and retina from wild type mice using a Qiagen Allprep Kit. Genomic DNA was bisulfite converted using a Zymo EZ DNA MethylGold kit in order to distinguish cytosines from 5-methylcytosines within the genome. Primers designed for bisulfite (BS) PCR and subsequent pyrosequencing using a Q24 Pyrosequencer were used for DNA methylation analysis. mRNA gene expression analysis will also be conducted with RT-PCR from mRNA from the same tissues.

Results: Temperature gradient BS PCR reactions were used to determine the optimal annealing temperatures for primers designed to amplify and analyze 32 murine genomic regions for DNA methylation. Primers amplifying murine retina-specific genes RHO, RBP3, and GNAT1 as well as the B1 retrotransposon repeats, and the H19 imprinted locus were successfully optimized and chosen as pilot regions for DNA methylation analysis. Ongoing Experiments: DNAs from wt, Dnmt triple knock out, and floxed control mice will be assayed for differential DNA methylation using quantitative gene specific bisulfite pyrosequencing to analyze DNA methylation at multiple genomic loci.

P4 Lindsey Toothman, Steven McBride, Ben Holland, Pradeep Vasudevan, Dr. Joanna Mott
Antibiotic Resistance of *Enterococcus* spp. Collected from Organic Turkey Litter

While *Enterococcus* spp. are generally commensal organisms, multidrug resistant strains has led to an increase in enterococci nosocomial infections. Poultry litter, a common reservoir for *Enterococcus* spp., may contribute to the increase of fecal bacteria in water ways. If these bacteria are resistant to antibiotics this may lead to an increase of resistant strains in the environment. The purpose of the study was to examine the levels of antibiotic resistance of *Enterococcus* spp. collected from organic turkey litter. Enterococci were isolated from litter at 1, 2, 4 and 6 weeks after turkeys were first housed on the litter. Species identification was performed using the BIOLOG Microbial Identification System. The most abundant species identified were *E. faecium* and *E. durans*, comprising 75% of the isolates. Antibiotic resistance profiling was conducted using a 12 antibiotic disk arrangement. The proportion of isolates that were resistant to erythromycin, doxycycline, rifampin, tetracycline, and ciprofloxacin significantly increased by week 6 compared to week 1 ($p < 0.05$). Tetracycline resistance increased with each time interval of collection using a Fisher's exact test, with an α -value of 0.0083 ($p < 0.0083$). Further studies are needed to investigate survival of these bacteria in litter dispersed in the environment.

P5 Shannon Fox, Ryan Samuel, Dr. Susan Halsell
Determining the Molecular Mechanisms of Cold Nociception in the *Drosophila* Model System

Nociception refers to an organism's nervous system reaction to noxious stimuli that could cause damage to tissues. When pain signals are propagated for extreme lengths of time, humans experience chronic pain, a debilitating condition that involves the persistence of pain signals in the nervous system months after any form of trauma, injury or infection. The similarities between the nervous systems of *Drosophila* and mammals makes the fruit fly an ideal organism to study how the mammalian nervous system responds to pain signals. Previous studies have shown class III dendritic arborization (da) neurons to function in cold nociception in *Drosophila*, however little is known about which protein channels are involved. Through a combination of behavioral and optogenetic assays, we will observe flies with knockout genes encoding innexin channel proteins in class III da neurons to study their role in cold nociception. A cold environment will be simulated by placing flies on aluminum plates chilled to 6°C via a thermocycler. Cold reaction will be numerically quantified by 'cringe' rate, or change in length of third instar larva per second. By doing this reverse genetic screening of knockout innexin protein and wild-type flies, we can conclude the role of innexins in pain signaling.

P6 Shahzeb Khan, Dr. James Herrick
Isolation of *Aeromonas* Bacteria Native to Cooks Creek, a Stream in the Shenandoah Valley of Virginia

The genus *Aeromonas* is comprised of 16 known species. It is common to aquatic environments and certain species are opportunistic human pathogens acquired via open wounds or ingestion. The purpose of this study was to isolate and characterize one or more strains of *Aeromonas* to be used as model native stream bacteria in plasmid capture studies. Our study site was Cooks Creek, a stream near Harrisonburg Virginia that passes through agricultural pastureland and a small town before joining with the North River. Both stream and water samples were collected in November of 2014. Water samples were directly plated

while sediment samples were plated, after cells were released using 0.1% sodium pyrophosphate, onto ampicillin dextrin agar, which is selective for *Aeromonas*. Seven colonies that appeared yellow on the agar were further plated onto trypticase soy agar. Of these, five were found to be Gram negative, catalase positive, oxidase positive, and indole positive using standard microbiological tests. The complete 16S rRNA genes of these presumptive *Aeromonas* were successfully amplified and will be sequenced to verify the genus identification. As further verification and to help speciate our isolates, we plan to amplify and sequence the *Aeromonas*-specific *ropD* gene. One or more *Aeromonas* strains will then be cured of their plasmids and, using an antibiotic gradient plate assay, rendered resistant to rifampicin in order to use them as recipients for exogenous antibiotic resistance plasmid capture.

P7 Katie McCullar, Dustin Phillips, Maria Wilkins, Dr. Janet Daniel
Investigating Phenotypic Differences Between STP1 K/O and WT *Arabidopsis thaliana* in Response To Various Salt Concentrations

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 phenotypic differences in the growth of wild type (WT) and STP1 k/o plants when exposed to varying concentrations of sodium chloride using hydroponics and agar plates. The hydroponics system is used to expose the plants to 0mM or 50mM concentrations and measurements are taken of root length, stalk length and number of leaves at four weeks. The average root lengths of STP1 k/o were longer than WT at 50mM after four weeks when grown using hydroponics. We also investigated a dose response using agar plate concentrations from 0-200 mM. Root length was not correlated with NaCl concentration. While increased average root lengths were seen at 25mM and 100mM NaCl, at concentrations greater than 100mM, both WT, and STP1 k/o decreased in average root lengths. Overall, STP1 k/o average root lengths grown on plates were longer than WT root lengths, at two and four weeks. In total, results suggest that the STP1 k/o plant exhibits an alternate phenotype when grown in higher NaCl concentrations. This may indicate that STP1 k/o phenotype includes a resistance to increased salt concentrations.

P8 Kevin Tomlinson, Dr. Jon Kastendiek
The Effect of Addition of sapling 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 2012, only 7 of the original 4749 oak saplings were found to have grown above the deer browse line of 40 cm above the protective tubing. The plastic mesh tubing toppers were added to a total of 93 individual oak trees with 34 pairs based on location in fall of 2013. There was no significant difference in the initial heights between the treated and untreated saplings for both paired (t-test, 0.067) and unpaired (t-test, 0.095) evaluations. After one growing season, the topped trees were significant taller as seen in both paired (t-test, 2.55×10^{-6}) and unpaired (t-test, 3.24×10^{-7}) evaluations. In the fall of 2014 another 61 ash and 20 maples were added to the study. These species showed similar results to the first year of oak protection. However, addition of the toppers may have had an effect on mortality of the saplings. In summary, the addition of saplings protection tubing toppers improved saplings growth after one year.

P9 Naomi Gilbert, Dr. Morgan Steffen
Diversity of the UreC Gene in Bacteria Associated with Toxic Cyanobacterial Blooms

There have been reports of numerous occurrences of toxic cyanobacterial blooms (cHABs) plaguing lakes worldwide. The source of these harmful blooms is unclear, despite the strong evidence of nutrient loading due to agricultural and industrial practices in communities surrounding lake habitats. Recently, studies have shown a correlation between the widely used fertilizer, urea, and bloom occurrences due to excessive sources of nitrogen available to toxin-producing species of cyanobacteria. We attempt to extend upon this evidence to

identify urea as a potential, primary driver of toxic blooms. The purpose of this research is to identify and classify the community during peak blooms that have the ability to degrade urea. Samples of bloom biomass collected from Lake Erie and Lake Taihu during summer bloom events will be screened for the presence of ureC, a gene encoding the alpha subunit of the urease enzyme. Subsequent sequence analysis of positive samples will reveal the diversity of organisms capable of degrading urea in these two lakes. The outcome of this genomic approach will provide the first examination of the individuals in the community with the ability to degrade urea, and establish a foundation for further investigation into the influence of urea on bloom formation.

P10 Justin Lawhorne*, Dr. Tim Bloss

The Effects of Endoplasmic Reticulum (ER) Stressors on Cell Morphology and Viability in the Presence and Absence of the Nascent Polypeptide Associated Complex (NAC)

C. elegans is a ubiquitous roundworm and well characterized model organism used to study various highly conserved biological mechanisms, including misfolded protein stress response and control of cell death. An important player in both of these mechanisms is the NAC, a heterodimeric translational chaperone that manages protein folding and localization to prevent the aggregation of misfolded proteins and the induction of cell death. The NAC's role in this process is not well understood; my goal is to characterize this role in the context of different inducers of ER misfolded protein stress. Utilizing RNA interference to knockdown the nascent-polypeptide associated complex (NAC) in conjunction with different classes of endoplasmic reticulum stressors, I am correlating the mechanism of induced stress with the morphological defects and cell deaths that often result. Specifically, I am characterizing these effects in wild type worms, as well as worms depleted of one or the other of the two NAC subunits. In time, we will gain a better understanding of the role of the NAC during the management of misfolded protein stress, which could lead to new potential treatments of diseases known to have misregulated NAC function, such as Alzheimer's disease and liver cancer.

P11 Jesmine Roberts-Torres*, Jennifer Kurasz, Michael Partin, Kevin Libuit, Dr. James Herrick

Isolation and Characterization of *Staphylococcus* spp. from Manure-Contaminated Streams in the Shenandoah Valley

The genus *Staphylococcus* is comprised of 41 known species; of these, eighteen are capable of colonizing humans. While *Staphylococcus* has been studied in clinical, agricultural, and beach settings, its presence in freshwater environments has yet to be fully established. Studies on contamination of poultry and other meats by *Staphylococcus* led us to hypothesize that these bacteria may be introduced into (and possibly persist in) stream waters and sediments via runoff from farms and agricultural operations. We isolated eleven *Staphylococcus* strains from water and sediment samples collected from Muddy Creek in Hinton, Virginia, which is impacted by fecal runoff from livestock farms and processing plants. Ten of the eleven isolates exhibited resistance to oxacillin/methicillin, and all were coagulase-negative (MRCoNS). More recently, two coagulase-negative putative staphylococci were isolated from two additional streams in the Shenandoah Valley; these were not, however, resistant to oxacillin/methicillin. The genetic basis of resistance in the MRCoNS isolates is being investigated using *mecA* and *SCCmec* PCR, and their species will be identified via 16S rRNA gene sequencing. The presence of MRCoNS in freshwater environments raises concerns regarding human and animal health, as they may be opportunistic pathogens or act as reservoirs of transmissible antibiotic resistance genes for other pathogenic bacteria.

P12 Wes Deaver, Wes Geyer, Dr. Michael Renfroe

Antioxidant Concentrations in Younger and Older Leaves of *Lavandula* and *Salvia*

Antioxidants are compounds that can absorb free radicals, molecules implicated in agine effects and various diseases. We measured hydrophilic and lipophilic antioxidants in sage (*Salvia officinalis*) and lavender (*Lavandula angustifolia*), plants with known health benefits, using a spectrophotometric ABTS decoloration assay. Antioxidant reducing power was

measured in trolox equivalents (TE). In this investigation, we measured and analyzed the antioxidant levels of younger leaves (closer to the apical meristem) and older leaves (closer to the roots) from specimens with the greatest number of nodes and healthy leaves. Based on prior research, we predicted that less mature leaves would possess higher levels of antioxidants than more mature ones. After sampling upper, middle, and lower leaves from plants grown in a greenhouse, we calculated TAA (Total Antioxidant) content. After data collection and analysis, we discovered that in both plants, TAA values were greatest in the most mature leaves at the bottom of the plants. These statistically significant findings contradict our initial prediction that less mature leaves would have higher antioxidant values, and increase our understanding of the specific antioxidant properties of consumer food products.

P13 Clara Thiel*, Tyler Mullins*, Dr. Michael Renfroe

Hydrophilic and Lipophilic Analysis of Antioxidants in Culinary Herbs and Spices

Selected culinary herbs and spices were analyzed for hydrophilic and lipophilic antioxidant content. Antioxidants are important dietary components for humans and have been linked to protection from cancer, aging, arteriosclerosis, and other disorders related to human physiology and oxidative stresses on tissues. In this study, we analyzed commercial samples of dried herbs and spices, including cilantro, oregano, thyme, rosemary, gourmet rosemary, basil, Mediterranean 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 spice antioxidant extract. 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 both hydrophilic and lipophilic antioxidant content were observed among the spices, and the TAA, the total antioxidant content, was determined. It was determined that gourmet rosemary had the highest amounts of antioxidants and that chives had the lowest. These results may be important to dieticians developing specialized diets for clients.

P14 Ryan Samuel, Shannon Fox, Stephen Jurko, Haley Nisson, Harold Burke, Kendyl Combs, Kaleigh Jaeger, Dr. Susan Halsell

Analysis of Cold Behavioral Response to Reverse Genetic Screening of Innexin Gap Junction Proteins in *Drosophila* neurons

Nociception refers to an organism's perception and reaction to potentially damaging noxious stimuli. Nociception is clearly beneficial, but debilitating chronic pain occurs in humans when pain signals abnormally persist months after any form of trauma, injury or infection (NINDS 2015). Better understanding of nociceptive processes should aid in developing treatments for chronic pain. Evolutionary similarities between mammalian and *Drosophila* nociception makes the fruit fly an ideal organism to study the molecular components of nociceptor neurons. Previous studies have shown *Drosophila* class III dendritic arborization (da) neurons function during cold nociception; however little is known about which channel proteins and synaptic proteins are required (Mogil 2004). Using both behavioral and optogenetic assays to study larval cold nociception behavior, the effects of tissue specific down-regulation of innexin gap junction proteins by RNAi expression will be studied. To date, studies of two of the eight *Drosophila* innexin genes is underway. By performing reverse genetic screening of innexin protein knockouts, we will gain insight into the role innexins play in nociception.

P31 Breanna Lee, Dr. Carol Hurney

Hox13 Expression in Embryonic and Post-embryonic *Hemidactylum scutatum* Tails.

The salamander *Hemidactylum scutatum* adds axial tail segments throughout its life, both embryonically and post embryonically. This is uncommon, as most vertebrates stop adding axial tail segments at the end of embryonic development. The mechanism that causes continual addition of vertebral segments is unknown, but may shed light on vertebral

development in vertebrates. During embryonic development, Hox proteins act sequentially to initiate signaling cascades in several tissues, primarily somites and their derivatives. Segmentation and segmental identity is controlled via these cascades along the anterior-posterior axis with Hox13 expressed most posteriorly in all vertebrates. Hox13 is suspected to be involved in vertebral segmentation throughout the life cycle of *H. scutatum*. Through the use of RT-PCR techniques, Hox13 was cloned and sequenced from *H. scutatum* larval tail tissues. Ultimately these studies will help determine if Hox13 plays a role in post-embryonic tail development.

P32 Benito Blanchfield-Felice, Kelly Burke, Elli Flora*, Kelcy Jackson*, and Dr. Justin W Brown

The Role of Brain Stem Neurotransmitters in the Thermoregulatory Response to Hypoxic Stress

Sudden Infant Death Syndrome (SIDS) is a leading cause of infant mortality. Alterations in brainstem development of Serotonin (5HT) and GABA are linked to its cause. The sympathetic premotor neurons located in the Nucleus of the Raphe Pallidus (NRP) in the brainstem have both 5HT1A and GABA-A receptor subtypes that mediate protective cardiovascular responses to environmental stress. It is hypothesized that alteration in these receptors at the NRP will also impair protective thermoregulatory responses to hypoxic stress. Using aseptic techniques, male Sprague-Dawley rats (225-325g) were instrumented with radiotelemetry probes to non-invasively measure core temperature (T_c). Using a stereotaxic device, a steel cannula was inserted into the brainstem which allowed microinjection at the NRP. After recovery (1 week), rats were housed in a thermal gradient which allowed them to select their ambient temperature (ST_a) and thereby facilitated behavioral thermoregulation. Once acclimated the gradient and to handling, 30mM of either a 5HT1A agonist (8OH-DPAT or "DPAT"), antagonist (WAY), a GABA-A agonist (Muscimol), antagonist (Bicuculine) or ACSF (control vehicle) was then microinjected into the NRP immediately before exposure to 6% O₂ for 60 min. In rats injected with ACSF T_c decreased as much as 2.7±0.2°C while the T_c of those injected with DPAT or WAY decreased by 4.0±0.4°C and 2.8±0.1°C respectively. Those injected with Muscimol and Bicuculine decreased T_c by as much as 3.3±1.0°C and 2.1±1.1°C respectively. The maximum decrease in ST_a in rats injected with ACSF was 7.1±1.5°C while the ST_a of those injected with DPAT and WAY decreased by up to 10.5±1.8°C and 5.0±0.8°C respectively. Rats injected with Muscimol and Bicuculine decreased T_c by up to 12.5±0.1°C and 6.8±0.9°C respectively. These preliminary data suggest activation of inhibitory receptors, like 5HT1A or GABA-A, exacerbates the hypothermic response to hypoxic stress. Inhibition of these receptors by injection of WAY or Bicuculine did not seem to affect the hypothermic response. Importantly, the ST_a responses to hypoxic stress help facilitate T_c changes suggesting coordination between behavioral and autonomic mechanisms of thermoregulation in a protective hypothermic response to hypoxic stress. This suggests the 5HT1A and GABA-A receptors may be at least involved in mediation of protective thermoregulatory responses to hypoxic stress and, if poorly developed in an infant, may contribute to the etiology of SIDS.

P33 Brittany Manning, Dr. Conley K. McMullen

Sampling, Databasing, and Digitizing the Flora of the E.J. Carrier Arboretum

This research project intends to create a digital database, complete with images, of the vascular plants both native and cultivated that inhabit the E.J. Carrier Arboretum. The specimens collected and mounted over the past three decades will be updated to reflect any changes in nomenclature, photographed, and organized into an online database. The data will be in the form of a map of the arboretum with specimens' images and descriptions linked to the location of their collection. The data can then be used by professors, students, and the general public as a reference for identification and study of vascular plants in the arboretum as well as the greater Rockingham area.

P34 Ariel Childs, Aubrey Siebels, Kate Reiman, Dr. Corey L Cleland

The Escape Response of Crickets Depends on the Direction of Looming Stimuli

Crickets tend to exhibit an escape response when delivered a wind stimulus. Once stimulated, crickets tend to turn and then run or jump away. Looming stimuli better approximate the combined stimulus modalities associated with attack by an airborne predator. The goal of this study was to describe the escape response of the cricket (*Acheta domesticus*) to looming stimuli, delivered from 8 separate, lateral angles. The direction of "attack" by a looming ball varied by 45 degree increments around the cricket whose response was recorded by a high-speed video camera (Redlake/IDT, 650 fps) placed overhead. The top of the head, thoracic-abdominal junction and the tip of the abdomen were tracked over time (Proanalyst) to provide the two dimensional locations and orientation of the abdomen and the head/thorax. In response, crickets initiated a turn away from the stimulus and either walked (89%) or jumped (11%) away. The direction of the turn was away from the stimulus 98% of the time. The response direction varied with stimulus laterality (slope = -0.57; 1.0 would be directly away from the stimulus; $p < 0.0005$). Demonstrating that the direction of the crickets' escape turn from looming stimuli depends strongly on the laterality from which the stimulus is delivered.

P35 Olivia Vito, Ben Pisano, Megan Budnik, Anna Nordseth, Dr. Heather Griscom
The Effect of Slope Position and Gap Size on Chestnut Hybrid Performance Relative to Tulip Poplar and Chestnut Oak: Implications for Reintroduction

Optimal sites in the forest environment must be identified for successful reintroduction of American Chestnut, *Castanea dentata*, hybrids. Understory light levels, slope position, aspect, soil type and competition may all affect chestnut performance with some factors having a more pronounced effect. We focused on how understory light levels and slope position affect growth and survival of tree seedlings. The experimental study was conducted in an oak-hickory secondary forest in the Ridge and Valley province of Virginia. In 2008, pure and hybrid *C. dentata*, *Liriodendron tulipifera* and *Quercus prinus* seedlings were planted in two gap sizes, 200 m² (30-45% of full sunlight) and 800 m² (60-75% of full sunlight) and on two slope positions, one with xeric soil (upper slope) and one with mesic soil (mid-slope) for a 2 x 2 factorial design. Height (cm) and diameter (mm) were recorded at the end of every growing season to quantify relative growth rate and percent seedling survival. After six years, treatment (a combination of gap size and slope position) had a significant effect on diameter ($p = 0.008$) and survival ($p < 0.0001$) but not on height ($p > 0.05$). While average height and diameter measurements of *Castanea* hybrids were highest in large, mesic treatments (437cm tall, 50.5mm diameter), survival was highest in small, xeric treatments (70.1%). Therefore, the recommended locations for chestnut reintroduction within oak-hickory forests in Virginia are small gaps on upper slopes due to a combination of lower rodent predation (by girdling) and being better adapted to environmental conditions than competitors (*L. tulipifera*).

P37 Catherine Torres, Dr. Jonathan Monroe, Dr. Amanda Storm

The Characterization of BAM6 from *Arabidopsis thaliana*

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

Our own study of starch accumulation in *Arabidopsis* supports a minimal role of BAM6 in young plants, but also indicates that this BAM may be playing a more dominant role in older plants. To further investigate the role of BAM6 we have isolated BAM6 protein by ligating the mature protein coding sequence (cDNA) into pETDuet-1, an E. coli expression vector with a His-tag for easy purification. Through the expression of this protein we will be able to characterize its catalytic properties to provide information on its potential roles in the degradation of starch. Additionally, the characteristics of BAM6 will be compared to the other active chloroplast targeted BAMs, BAM1 and BAM3, to examine how the role of BAM6 is unique.

P38 Megan Hines, Dr. Jonathan Monroe, Dr. Amanda Storm

Searching for Potential Binding Partners of β -amylase2 using Yeast 2-Hybridization

BAM2 is a noncatalytic chloroplast-targeted member of the β -amylase gene family that currently has an unknown function in starch hydrolysis. Young plants lacking BAM2 exhibit no phenotype, but older plants lacking BAM2 exhibit a starch-excess phenotype. In addition, the BAM2 gene is conserved among most land plants, indicating selective pressure to maintain it. However, as BAM2 is not catalytically active, it may have a regulatory function. Many regulatory proteins interact with other proteins, so to test for potential protein binding partners for BAM2, the yeast two-hybrid system is being used. A plasmid containing the BAM2 gene from *Arabidopsis thaliana* and plasmids from a cDNA library of mRNA isolated from mature *Arabidopsis* leaves and roots will be cotransformed into strain Y190 yeast (*Saccharomyces cerevisiae*). If the proteins encoded by the BAM2 gene and by the cDNA library gene interact, transcription will be activated and the reporter genes will be expressed. Plasmids from true positive results will be sequenced and analyzed to determine the validity of the interaction in nature. Meaningful positive results may reveal the function of BAM2 in the process of starch hydrolysis.

P39 Amanda Crandall, Jessie Doyle, Rhiannon English, Mycah McNett*, Megan Moore, 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 2006, 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. In 2010, a study was initiated that utilized the presence and taxa of macroinvertebrate inhabitants to determine the success of the restoration. Both the restoration area (Rainbow Hill Farm) and an upstream unrestored area (Depoy Farm) were analyzed using the HBI, VA-SCI, and Shannon Index. We hypothesized that Rainbow Hill would have a healthier stream quality than Depoy, and that the stream health within the restored area would improve over time. Statistical analysis of the indices failed to support our hypothesis. There was no significant difference in the H' of the restored area from 2010-2014 ($p=0.920$), or in the VA-SCI ($P=0.873$). No significant change was shown in the HBI from 2010-2014 ($p=0.671$). Likewise, the upstream p -values for the HBI, VA-SCI, and H' were 0.945, 0.120, and 0.089 respectively, showing no significant change with time.

P40 Erin Thady, Amanda Crandall, Jessie Doyle, Rhiannon English, Mycah McNett*, Megan Moore, Joseph Noel, Nathan Robinson, Becca Sanders, Ty Steve, Dr. Bruce Wiggins

Effects of Restoration Practices on Stream Health in the Shenandoah Valley using Macroinvertebrate Survey and GIS analysis

The Shenandoah Valley encompasses some of the highest agricultural producing regions in Virginia. The Conservation Reserve Enhancement Program (CREP) is a land conservation program that aims to preserve lands impacted by humans. Several farms were targeted by the CREP program to undergo restoration projects in which cattle were fenced out or a riparian buffer was planted adjacent to the stream. This study aims to look at the temporal effects of restoration and impact of the surrounding environment on water quality. It was hypothesized that the greater the length of time that the CREP program has been established would have a positive effect on the water quality of a stream. This was quantified by analyzing benthic macroinvertebrate assemblage through sample productivity, Hilsenhoff Biotic Index (HBI), Virginia Stream Condition Index (VA-SCI), and the Shannon Diversity Index. Additionally, GIS analysis was used to evaluate the effects of land use on water quality. There were no significant differences between the year since restoration and the metrics. Significant differences were found between the percentages of forested and agricultural land with the HBI. While the results indicate that time since restoration does not have a significant effect on water quality, surrounding land use may impact it.

P41 Monica Paneru, Stephanie Masters, Suma Haji, Sybelle Djikeng, Caroline Dilworth, Brandi Volkers*, Jason Floyd, Nick Minahan, Tara Gallagher, John Marafino, Jade LaDow, Kyle Bonifer, Dr. Kevin Caran, Dr. Kyle Seifert

The Colloidal, Antibacterial, and Cytotoxicity Properties of Tris-cationic, Triple-headed Amphiphiles

In order to stay ahead in the antibiotic arms race with bacteria, new antimicrobials must constantly be developed. One promising alternative to traditional antibiotics is the development and use of amphiphiles, compounds with at least one hydrophilic head group and one hydrophobic tail. For nearly a century, varieties of amphiphiles have been utilized as antimicrobials in cosmetics, disinfectants, and antiseptics.

Three novel series of triple-headed amphiphiles (M-P,_{n,n,n}; M-T,_{n,n,n}; M-T,T,_n) with variations in the third head group composition (Pyridinium or Trimethyl ammonium), tail length (where n for C_nH_{2n+1} ranges from 10-20 hydrocarbons), and number of tails (one or two) were synthesized. The antibacterial activity of individual amphiphiles against six bacterial strains and of binary combinations against two representative strains was determined by standard antimicrobial microdilution techniques. The lowest concentration of amphiphile resulting in < 50% hemolysis (EC₅₀) was also determined. Notably, three compounds (M-P,_{10,10,10}; M-P,_{12,12,12}; M-T,_{12,12,12}) killed multiple bacterial strains at concentrations ranging from 1-16 μM and were not cytotoxic to blood cells, with EC₅₀ values that were two- to 65-fold higher than bactericidal concentrations. In addition, several combinations of amphiphiles exhibited synergistic antibacterial activity.

P42 Kelsey Savage, Dr. Eria Rebollar, Dr. Reid Harris

Comparative Analysis of Anti-Bd bacteria from Six Malagasy Frog Species of Ranomafana National Park

As Malagasy amphibians are facing an extinction crisis from the fungus *Batrachochytrium dendrobatidis* (Bd), it has become imperative to mitigate the threat. It has been shown that certain bacterial species are able to inhibit Bd on amphibians by producing antifungal metabolites. Community-based probiotics are used to combat chytridomycosis by inoculating an environment with Bd-inhibitory bacteria so that many amphibian species are treated at once. To minimize effects on non-target organisms, bacteria are selected that occur on the amphibians' skins. The purpose of this study was to determine which bacteria from an amphibian community in Madagascar are capable of inhibiting Bd. To identify anti-Bd bacterial isolates, inhibition assays of each isolate against Bd were conducted. Changes in optical density of isolates' filtrates with Bd were compared. After finding which bacteria were positive for inhibition, relationships between the anti-Bd bacterial species and frog species were assessed. Anti-Bd isolates were found on every frog species. Several bacterial isolates were able to inhibit Bd as found in previous studies. However, only two isolates of *Shingobacterium multivorum* were identified to inhibit Bd at the species level. From this

study, I recommend that more research be done to identify a possible probiotic that is more common.

P43 Tiffany Bridges, Dr. Eria Rebollar, Dr. Reid Harris

Ability of Skin Bacteria on the Panamanian frog, *Craugastor fitzingeri*, to Inhibit the Fungal Pathogen *Batrachochytrium dendrobatidis*

An emerging infectious disease caused by the fungus *Batrachochytrium dendrobatidis* (Bd) is leading to global amphibian declines and is threatening the biodiversity of amphibians. Although Bd continues to colonize areas throughout Central America, Bd-susceptibility varies among species. Some species such as *Craugastor fitzingeri*, a terrestrial frog native to Central America, continues to persist in Panama and is therefore considered a tolerant or resistant species. The microbial community on the skin of *C. fitzingeri* is known to consist of antimicrobial-producing bacteria such as actinobacteria and *Pseudomonas*. In this study, we will characterize the bacterial diversity residing on the skin of *C. fitzingeri* using 16S rRNA PCR amplification of culture-dependent samples. The relative abundance of the bacterial taxa will be compared to the quantification of all isolates using a culture-independent method. We will also identify Bd-inhibitory isolates, as potential candidates for probiotics, using challenge assays against Bd. We expect that the most relatively abundant isolates tend to be culturable and that the probability of having anti-Bd inhibitory capacities is higher among the most relatively abundant members of the resident microbiota. Identifying Bd-inhibitory isolates with a high relative abundance is a critical step in discovering effective probiotics for conserving Panamanian amphibians.

P44 Roxana Behrooz, Dr. Mark Gabriele

Exploring Perineuronal Net Labeling as a Neurochemical Marker for Auditory Midbrain Modularity

The inferior colliculus (IC) is the crossroads of lower auditory brainstem processing located in the midbrain. It consists of three subdivisions: the central nucleus (CNIC), lateral cortex (LCIC), and dorsal cortex (DCIC). The CNIC exhibits a well-documented frequency organization. In contrast, less is known concerning LCIC's compartmentalized arrangement. Perineuronal Nets (PNNs) are extracellular structures implicated in neuronal maintenance and strengthening of established synaptic connections. Their emergence correlates with closure of brain developmental critical periods, and interestingly, their degradation reopens the potential for plasticity in established circuits. PNNs are prevalent in the auditory system typically surrounding rapidly firing high-frequency neurons. PNN labeling reveals a modular LCIC organization in a variety of adult species. Wisteria floribunda agglutinin (WFA) is a lectin that labels PNNs by binding glycosaminoglycan (GAG) side chains. The present study ultimately aims at determining whether LCIC modules are WFA-positive in mouse and when such an arrangement is first detectable developmentally. As a first-step, serial dilutions were performed to identify WFA levels that optimize signal-to-noise ratio. Also assessed was the quality of WFA histochemistry on slides vs. free-floating sections. Results indicate a 1:1000 dilution and free-floating sections provide optimal morphological detail for continued study.

P45 Rebecca Gaita, Alex Silva, Corey Cleland

The Escape Response of Crickets to Heat Stimuli utilizes a Translational Rather than Turning Strategy

Animals respond to aversive stimuli with escape or withdrawal responses. In crickets, wind and looming stimuli, which simulate an approaching predator, are commonly used to evoke escape responses. The goal of this study was to describe the escape response of the cricket (*Acheta domestica*) to heat stimuli delivered to each of its six tarsi and determine the factors that control direction and magnitude of the response. Heat was delivered to the tarsus of each leg in 25 crickets with an infrared laser diode (980nm). The response was recorded by high-speed video (650 fps). The top of the head, thoracic-abdominal junction and the tip of the abdomen were tracked over time (Proanalyst, Xcitex) to provide the two dimensional locations and orientation of the abdomen and the head/thorax. In response to heat stimuli, crickets first retracted the stimulated tarsal. In contrast to previous studies, there was only a small turning response but there was a significant translation of the entire body directly

away (180deg) from the stimulus. These results demonstrate that crickets escape from heat as well as from looming, touch and wind stimuli, and allow for identification of common movement strategies by comparing the escape responses to the four different stimuli.

P46 Jocelyn Medrano, Kendyl Combs, Isabel Lamb, Katrina Gobetz
Trelawny Research in Nociception in *Drosophila melanogaster*, Photoreceptor Differentiation in Developing Chick Retina, and Development of Auditory Neuropathways in Mice

Three different labs study the development of sensory perception in organisms. The Gabriele lab studies the development of central auditory pathways in neonatal mice utilizing a variety of techniques, including fluorescent tract-tracing and immunocytochemistry approaches. These methods provide insights regarding the organization of major auditory structures as well as the patterns of connections that functionally link them. An ongoing interest in the lab is determining the role that a family of guidance molecules play in establishing early auditory projection maps. Experiments in mutant animals that lack certain signaling proteins aid in identifying molecular interactions that are necessary for normal circuit formation.

The Halsell lab uses RNA interference in *Drosophila melanogaster* to isolate and remove ion channel proteins suspected in the reception and reflexive response to cold stimuli. Video recordings of cold plate assays are processed and converted into quantitative data for statistical analysis.

The Enke lab is studying how genetics play a part in the differentiation of retina cells in different organisms. One section of the lab uses DNA Methylation to focus on the expression of rhodopsin, a pigment in the retinal rods of different use species. Another section is studying the retinal cells of developing *Gallus gallus* (chick) by observing the differences between the retinal cells present at different times of development in the egg. The third section of the lab is comparing cells of human eyes and producing organisms that resemble the eye using blood stem cells.

P47 Joshua Mata, Shelby Snowden, Dr. David McLeod, Dr. Katrina Gobetz
Methodology of Preserving Animal Specimens and its Importance in Biology Education and Research

The process of preserving specimens has two main components: fixation and preservation. Fixation is used to stabilize the proteins of the specimens to prevent decomposition. Preservation is storing the specimen in a substance that would prevent further deterioration and will allow it to be observed and used for an extended period of time. Fluid preservation is considered to be the best method of preserving animal specimens. Thousands of specimens are collected and preserved in museums and universities today which can then be used for research, such as to verify if a discovered animal is a new species, for the education of students, and to observe the past biodiversity that we've lost. Proper care of collections, such as maintaining fluid levels and storage temperatures, are also explored. The JMU biology department has a limited collection of zoological specimens that most students never see. Among these is Cletus the fetus, a stillborn giraffe (*Giraffa camelopardalis*) aged approximately 8 months. The history of this specimen and general giraffe development are further explained to illustrate proper collection techniques.

P48 Christina Myers, Caylin Murray, Dr. Joseph Harsh
Teaching to Learn Like the Experts: Eye-tracking Analysis of Graphing Interpretation Practices along the Novice-expert Continuum

Proficiencies in graph literacy, one's ability to compose and interpret graphical data representations, are held as a core skill in the sciences given the importance of communicating complex information. Even though the merits of graph literacy is well known and the ubiquity of graphs in our daily lives, prior studies have indicated an array of difficulties that undergraduates have in making sense of visual data (Bowen, Roth, & McGinn, 1998). Understanding the cognitive basis by which one makes sense of visual data would allow us to better approach the interpretation of graphs, and how this skill can be developed. Building on pilot data that has identified potential differences in the visual data analysis

approaches of students and scientists (Harsh & Maltese, 2013), this intended research will use eye tracking technology to compare how individuals along a continuum of expertise in the sciences interpret data. By focusing on the differences between experts and novices, we can better tailor our instruction to increase graph literacy. Eye movement data will be captured using a heads-free desktop eye tracker connected to a computer as participants complete an electronic survey that contains graphs that one may reasonably encounter in science (e.g. environmental website, textbook images). Pre-determined areas of interest (AOIs) will then allow us to statistically interpret variability among expert and novice scientists' attention and visual search.

P49 Michelle Wallagora, Jasmin Rose, Sarah Maher, Dr. Rose

Quantifying and Comparing Shape Change in the Pharyngeal Arch Cartilages of Salamanders

The amphibian pharyngeal arch (PA) skeleton is comprised of rod-, plate- and bar-shaped cartilages that support feeding and breathing in two habitats, an aquatic one followed by a terrestrial one. To accommodate its dual functions, the skeleton undergoes two periods of growth: larval and postmetamorphic, and two periods of development: embryogenesis and metamorphosis. Having multiple periods of growth and development suggests multiple opportunities for phylogenetic diversification. Functionally significant differences in PA skeletal shape arise in both embryogeny and metamorphosis, but how much allometric growth contributes to variation in larval and adult PA skeletons remains unclear. It is also unclear whether cylindrical cartilages adhere to fixed growth and static allometries in the way that bird and mammal long bones do. This study addresses these questions using developmental series of skeletally stained whole-mounts for 13 species of salamander belonging to eight families (Ambystomatidae, Salamandridae, Plethodontidae, Dicamptodontidae, Amphiumidae, Sirenidae, Proteidae, and Cryptobranchidae). PA skeletons including mandibles are dissected, photographed, and digitized to generate width and length measurements for all cartilages. The data for individual cartilages are averaged between sides, log-transformed and fitted to linear regression equations, the slopes of which are compared between larval and adult stages within species and between the same stages among species. Interspecific comparisons are also intended to reveal how variation in length of larval

P50 Nicole Roberto*, Elizabeth Lucas, Dr. Steve Cresawn

Next Generation Sequencing and Genomic Comparisons of Mycobacteriophage

Bacteriophages, viruses that infect bacteria, are the numerically dominant biological entities on planet Earth. They are enormously genetically diverse and collectively represent the largest source of novel genetic information known to science. 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.

Four mycobacteriophages were sequenced using the Ion Torrent Personal Genome Machine (PGM); all of which were isolated during the Viral Discovery course offered in the fall. The phages are referred to by their given names: Philus, Threshyone, Biggie, and Penny144. 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 Ion Torrent PGM. Here we report on these four mycobacteriophage genomes and draw comparisons among them with other previously sequenced phage genomes.

P51 Heather Maher, Dr. David McLeod

How Much Have We Underestimated Southeast Asia's Biodiversity? New Species in the *Limnonectes khulii* Complex

Rapid and extensive deforestation in Southeast Asia is taking its toll on the region's biodiversity. Species tied to forest habitats are the most likely to be negatively affected by deforestation, including the forest stream frogs of the *Limnonectes khulii* complex. This complex consists of more than twenty species that have been subsumed under a single

species for nearly 200 years. Recent molecular phylogenetic studies based on mitochondrial DNA reveal a significant divergence between a lineage from Cambodia and all other taxa in the *L. kuhlii* complex. Analyses of mensural data corroborate the molecular results and suggest that there are detectable differences in the morphology of both sexes of the new species when compared to its congeners. These findings will allow for the recognition of the Cambodian lineage as a new species.

P52 Debbie Stratton, Dr. Corey Cleland

The Effect of Physical and Demographic Factors and Training on the Time-to-Adoption of Domestic Cats in a Local Shelter

Animal shelters in the United States house a large number of dogs and cats, raising the question as to what factors influence adoption rates and how those rates can be improved. The specific aims of my research were to determine which factors affect adoption at a local animal shelter and determine whether teaching cats to “high-five” would improve adoptability. The results demonstrate that color, source and age significantly affected adoption. Regarding color, black was significantly less adoptable. High-five training was shown to be possible and preliminary results suggest an improvement in adoption rates.

P53 Nicholas Dunham, Morgan Hedden, Dr. Raymond Enke

Characterization of Epigenetic Patterns in the Post-Mortem Human Retina

The vertebrate retina is a stratified layer of neuronal cells that serves a highly specialized purpose: manufacturing signals that are sent to the brain and perceived as vision. To have these retinal cells develop and function properly, many retina-specific genes undergo epigenetic regulation. One particular epigenetic modification, DNA methylation is essential for the regulation of genes by the recruitment of repressor proteins in the promoter region. Our experiments characterize DNA methylation levels near the promoter regions and transcriptional start sites (TSS) of retina-specific genes in post-mortem human retina. Previous studies have demonstrated mRNA and protein expression in the human retina, but retina-specific epigenetic regulation of gene expression has yet to be fully characterized in this system. We hypothesize that the mRNA expression of retina-specific genes is inversely correlated with cell-specific patterns of DNA methylation in the promoter region and around the TSS. Preliminary data demonstrates lowered levels of DNA methylation in promoter regions of the retina-specific genes, RCVRN, RHO, and CRX, in post-mortem human retina compared to non-retinal tissues using bisulfite pyrosequencing. Our future studies will analyze DNA methylation as well as mRNA transcription of additional retina-specific loci.

P54 Jennifer Cahill*, Dr. Chris Rose

Isolating T4 and T3 Effects on Cartilage Growth and Shape Change in *Xenopus* tadpoles

Investigators of how thyroid hormones (TH) regulate frog metamorphosis often apply TH to induce metamorphic changes precociously in tadpoles. However, precociously induced remodeling of skeletal tissues might not resemble natural remodeling for many reasons. Remodeling might be induced before larval tissues become competent to fully respond to TH or before they attain the shapes at which natural remodeling starts. Further, induced remodeling means applying exogenous TH at fixed concentrations, which does not simulate the changing T3 and T4 levels during metamorphosis. To test how precocious induction affects remodeling, this study quantifies the stage-dependency of size and shape changes induced in Meckel's cartilage (MC) and ceratohyal (CH) by TH. We treated *Xenopus* tadpoles at early, mid and late tadpole and early metamorphic stages (NF 46, 53, 57 and 59/60) with 50 nM T4 or T3 or no hormone and measured the changes in body size and size and shape of the MC and CH. Specimens at NF 53, 57 and 59/60 were pretreated with methimazole to arrest them and methimazole and iopanoic acid were applied during experiments to block endogenous TH production and prevent T4 or T3 being converted to other forms of TH. Animals were photographed before and after treatments, cleared and stained for cartilage and bone, and their MC and CH dissected and photographed. Body sizes and final cartilage sizes and shapes were quantified from photographs; initial sizes and shapes were estimated from allometric relationships for controls. Cartilage dimensions responded similarly to both

T3 and T4 at all stages, though the magnitude of change and impact on shape varied with stage and TH type.

P55 Kimberly M Seamon*, Bart Gumpert, Chris Chrzan, Matthew Hartmann, Dr. Corey L Cleland

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

Rats rapidly withdraw their hindlimb in response to noxious heat stimuli applied to the plantar surface of their paw, an example of the Nociceptive Withdrawal Response (NWR). Previous studies in spinalized or anesthetized non-human mammals showed that the direction of response depends on stimulus location. The goal of this study was to determine if stimulus location, or other factors such as initial posture or response latency, determines the direction of withdrawal in the intact, unanesthetized rat. Rats were placed on a glass plate through which an infrared laser (980 nm) heated a small (1mm) portion of the foot plantar surface. The withdrawal response was recorded with three camcorders. In response to the stimulus, the rat withdrew and rapidly (~40ms) replaced its paw on the glass, at which point the final location and angle of the paw were recorded. The rat's paw was stimulated in five. Unexpectedly, we found no dependence on stimulus location. However, we explored if initial position and paw angle influenced final location and angle. Correlation between initial and final locations and angles revealed a significant linear relationship ($p < 0.001$). These results demonstrate that initial posture plays a greater role in the programming of the NWR than stimulus location.

P56 Giavanna Verdi, Taylor Berrena, Jacob Edwards, Dr. Corey Cleland

The Nociceptive Withdrawal Response to Mechanical Stimuli of the Rat Foot Depends on Initial Foot Position but not Stimulus Location

Rats rapidly withdraw their hind limb in response to noxious stimuli, an example of the Nociceptive Withdrawal Response (NWR). Previous studies have shown that the direction of response depends on stimulus location. The goal of our studies was to determine if mechanical stimulus location or initial posture determine the direction of withdrawal in the intact, un-anesthetized rat. Rats were placed on a wire mesh and a stimulus was applied to one of five locations along the rostral-caudal and lateral-medial directions on the plantar surface with either a "Von Frey" monofilament or a 30 gauge needle. The resulting withdrawal response and initial position were recorded with two camcorders (60 fps), on the left and underneath the rat. In response to the stimulus, the rat withdrew and rapidly replaced its paw on the mesh, at which point the final location was recorded to determine the direction and distance of response. We found that neither the direction nor magnitude of response depended on stimulus location, but strongly depended on the initial position of the paw. For example, if the paw was initially rostral the movement tended to be caudal and if the paw was initially medial it would move lateral.

P57 Victoria Callahan, Michael Yeager, Dr. Marta Bechtel

Pro-Inflammatory Effect of the Dengue Virus Envelope Protein in Cultured Human Articular Chondrocytes

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 pro-inflammatory cytokine expression in articular cartilage tissue. To fully understand the effects of Dengue envelope protein (DENV sE) in normal human articular chondrocytes (NHACs), our lab has cultured NHACs in the presence of DENV sE to assess the levels of proinflammatory cytokines in DENV-treated NHACs. Experiments were performed over a six-hour timecourse to evaluate early gene expression patterns. Analysis of gene expression was completed using quantitative Real-time PCR.

P58 Roneka Spady, Dr. David McLeod

Dermeid Beetles as a Carcass Cleaning Agent: An Integrated Pest Management Procedure

Beetles of the family Dermestidae feed on organic matter, including decaying flesh — a feature often employed by natural history collections to prepare skeletal materials. The purpose of this research is to develop and test a protocol to manage and control the potentially destructive aspects of this pest species. We have established a 4-layer system of pest management and are actively monitoring the effectiveness of our system to ensure that none of the beetles are able to escape. Here, I present the first five months of this research.

P59 Adrienne Muettterties*, Dr. Patrice Ludwig
Resource Type Affects Offspring Survival and Phenotype in the Dung Beetle, *Onthophagus taurus*

The aim of this experiment is to test the extent to which two resources, commonly used by the sexually dimorphic burrowing dung beetle *Onthophagus taurus*, differ in their effects on adult body size and male horn development. This experiment is based on those done by Moczek (1998) and Hunt and Simmons (2004). Pairs of beetles were placed in breeding tubes with thawed dung, and the brood balls were collected and weighed. With the use of digital imaging and ImageJ, horn length and thorax width were measured for each emerging offspring. Statistical analyses, t-test and chi square, tested the following hypotheses: there is a difference in the number and mass of brood balls that produce live offspring; there is a difference in the thorax size and horn length of resulting male offspring based on resource quality. Preliminary results showed more brood balls were produced from cow dung, whereas those from horse dung had a greater mass. Average horn length of major males was greater than minor males and females, with no difference in average thorax width among all sexes. The initial results implicate that the form in which the resource is available may affect parental energy investment in offspring.

P60 Nathan Evans, Dr. Patrice Ludwig
Habitat Preference Modelling for the Endangered Northeastern Beach Tiger Beetle, *Cicindela dorsalis dorsalis*; Implications for Rising Sea Level

The Chesapeake Bay serves as a case study for conditions that estuarine environments may experience in the future due to the rise in ocean levels as a function of global climate change. The Northeastern Beach Tiger Beetle (*Cicindela dorsalis*) is a federally threatened shore species that previously had a range from the coasts of Virginia to Massachusetts, but is currently only found along the Virginian portion of Chesapeake Bay and Martha's Vineyard, Massachusetts. The primary threats to *C. dorsalis* are erosion and human traffic. The objective of this study is to determine the current habitat preferences and predict where future habitats due to climate change. We use a current distribution map (http://www.fws.gov/northeast/Endangered/tiger_beetle/pdf/Tigerbeetle2_92711.pdf) and measured the beach width at each known site in Google Earth using latitude and longitude coordinates then imported these into ArcMap. We used MAXENT modeling to predict the potential habitat range using environmental layers (precipitation, temperature, beach width), which contain known locations *C. dorsalis*. The objective of this study is to model and predict the potential habitat range of *C. dorsalis*; this information could be used to help relocate *C. dorsalis* with poor habitat and strengthen metapopulations. The results show the potential range and this knowledge can be used to relocate populations.

P61 Gail Moruza, Gregory Mansou, Dr. Dean Cocking
Background Mercury Presence in Air, Soil, Duff, and MacroInvertebrate Compartments of "Uncontaminated" Forest Ecosystems Located in Rockingham Co.

Forest ecosystems in the Shenandoah Valley of Virginia are not directly exposed to major sources of mercury (Hg) contamination. Therefore it was assumed to be suitable as a low level control in prior studies. Subsequently, the presence of low level Hg from regional background sources has been demonstrated. This study examines various trophic levels and compartments within two late successional forests located in Rockingham County. In addition to litter, duff and airborne inputs, a variety of invertebrates including, but not limited to, flies, beetles, hymenopterans, and spiders, were digested in hot concentrated nitric and sulfuric acid and analyzed for total Hg concentrations using a Perkin Elmer Flow Injection

Spectrophotometer dedicated to Hg analysis. The results demonstrated that Hg is present within the various compartments and patterns emerged. For example, samples of Japanese Beetles had mean concentrations of 0.31 µg Hg/g dry biomass, while soil samples contained only 0.03 µg Hg/g dry weight. None of the concentrations attained at these sites are great enough to be considered a health hazard and no adverse effects on the biota were observed. Mercury is identified as merely associated with these organisms; this study does not confirm whether the association is superficial or internal.

P62 Alexandra Deal, Taelor Weaver, Michelle Huth, Dr. Terrie Rife
Cloning Reporter Genes to Investigate Mechanisms behind Nitric Oxide Synthase I Misregulation

The nitric oxide synthase I (NOS1) gene is misregulated in many neurodegenerative diseases including Alzheimer's Disease (AD) and Parkinson's Disease (PD). AD is characterized by intracellular neurofibrillary tangles comprised of misfolded tau, and PD is characterized by intracellular inclusions called Lewy bodies, composed of insoluble α -synuclein protein. The damaging mechanisms of tau and α -synuclein have yet to be determined. Both proteins have DNA-binding properties, and they may bind preferentially to purine-pyrimidine repeats and affect the expression of the NOS1 1f promoter. The human NOS1 promoter 1f has a polymorphic purine-pyrimidine repeat made up of thymines and guanidines (TGn), and shorter repeats have been associated with both AD and PD. We hypothesize that tau and α -synuclein may influence NOS1 gene expression through interaction with the TG repeat. To test for this interaction, three NOS1 promoter deletions will be made and cloned into luciferase reporter gene plasmids. We currently have two of the three desired constructs. These plasmids will be put into human cell lines either with or without tau and into cells with normal or increased alpha-synuclein. Luciferase assays will show the region of the promoter where tau and alpha-synuclein may regulate NOS1 gene expression.

P63 Rhiannon English, Sheikh Hossain, Samantha Webster, Dr. Katrina Gobetz
Trelawny Research in Water Quality with Macroinvertebrates, BAM Genes in *Arabidopsis thaliana*, and Artificial Oyster Reefs.

In Dr. Wiggins' lab the purpose is to understand the role that land use plays in water quality. Benthic macro invertebrates are sensitive to sediment and nutrient pollution; therefore the diversity that exists in their population serves as an indicator of water quality. The lab is also using GIS to measure topography of the areas located near the streams; the landscape that surrounds a body of water can have a large impact on the water quality. Plants use solar energy and Carbon dioxide to make their own food source, however they also need to breakdown this food source, starch, to produce energy. An enzyme known as Beta-amylase aids in breaking down starch, there are nine genes that translate into these enzymes. However not much is known about these nine genes and their specific physiological functions and the Monroe lab is trying to answer these questions. In Dr. Ludwig's lab the purpose is to develop artificial oyster reefs, that can replace damaged natural reefs. In the lab we are trying to create an artificial concrete that will be able to provide the oysters the nutrients needed to grow, which they naturally obtain from old oyster shells. My part is to collect data to maintain the tanks for optimal oyster growth.

P64 Haley Davis, Josh Donohue, Dr. Chris Lantz
Evaluation of Interleukin-3 in Blood-stage Immunity against Murine Malaria *Plasmodium yoelii*

Malaria is a mosquito-borne infection caused by the parasitic protozoan *Plasmodium*. This disease infects over 200 million people and causes nearly 600,000 deaths per year. Our laboratory previously examined malaria infection caused by *Plasmodium berghei* NK65, a lethal rodent strain that induces symptoms similar to those observed in humans. The role of a known hematopoietic growth factor and immunoregulatory cytokine interleukin-3 (IL-3) in this infection was investigated by comparing IL-3-deficient or "knockout" (KO) mice to wildtype (WT). Those results indicate that IL-3 plays a critical role in suppressing protective immunity to *P. berghei* NK65. However, the extent to which IL-3 contributes to host defense against blood-stage malaria infection caused by other *Plasmodium* species remains to be determined. Furthermore, the role of IL-3 in chronic malarial infections has not been explored. Preliminary studies indicate that IL-3 does not influence levels of blood parasitemia in mice chronically infected with *P. yoelii* 17XNL and 17X. Further studies are in progress with other strains of *P. yoelii* to determine if IL-3 influences additional indicators of disease in both acute and chronic malaria infection.

James Madison University Biology Department
Second Annual
GRADUATE SYMPOSIUM, BIOSCI 2007
Thursday April 17th, 5:00pm

OPENING RECEPTION – 2007, 5:00

WELCOME

- 5:15 G1. Tara Gallagher, John Marafino, Dr. Kevin Caran, Dr. Kyle Seifert
The effect of amphiphile structure on colloidal, antibacterial and hemolytic properties
- 5:30 G2. Barry Edgar, Dr. Heather Griscom
The Effect of Controlled Burns on Abundance of Woody Species at Buck Mountain, West Virginia
- 5:45 G3. Alaina C. Esposito, Dr. Christine L. May, Dr. Patrice M. Ludwig, Dr. Lihua Chen
Using Capture-Mark-Recapture Techniques to Estimate Detection Probabilities for a Rare & Cryptic Species
- 6:00 G4. Cheyenne Weeks-Galindo, Dr. Steve Cresawn
Corroboration of In silico Generated Genome Annotation of a Lytic Mycobacteriophage with RNA-Seq Analysis of Late Transcripts
- 6:15-6:30 :::::::::::::::::::: BREAK ::::::::::::::::::::
- 6:30 G5. Dehat Jalil and Dr. Terrie Rife
A Z-DNA forming polymorphism in the Nitric oxide Synthase I Promoter and its Relevance to Neurodegenerative Disease
- 6:45 G6. Jonathan Sandoval, Dr. Bloss
Characterization of the *C. elegans* Nascent Polypeptide Associated Complex (NAC) Function under Stress
- 7:00 G7. Steven McBride, Lindsey Toothman, Dr. Pradeep Vasudevan, Dr. Joanna Mott
Effect of Converting to Organic Turkey Rearing Practices on Antibiotic Resistance of Enterococci from Used Litter
- 7:15 G8. Ben Williamson, Dr. Susan Halsell, Dr. Daniel N. Cox
Molecular characterization of noxious cold detection in *Drosophila* larvae

G1 Tara Gallagher, John Marafino, Dr. Kevin Caran, Dr. Kyle Seifert
The effect of amphiphile structure on colloidal, antibacterial and hemolytic properties

Antibiotic resistant bacteria were first reported in the 1940s, several years after the clinical introduction of penicillin. Since then, antibiotic resistance has contributed to increased bacterial infection, mortality rates, and treatment costs. One promising alternative to traditional antibiotics is the development and use of amphiphiles, compounds with at least one hydrophilic head group and one hydrophobic tail. For nearly a century, cationic amphiphiles have been utilized as antimicrobials in disinfectants, mouthwash, contact lens solution, and other products. In this study, three novel series of triscationic amphiphiles with modifications in tail length, number of tails, and head group composition were synthesized. The effect of structure on colloidal properties and antibacterial activities were investigated, resulting in the identification of several antibacterial compounds that are non-hemolytic. These amphiphiles have promise as novel antibacterials that could be used in a variety of applications.

G2 Barry Edgar, Dr. Heather Griscom
Effect of Controlled Burns on Abundance of Woody Species at Buck Mountain, West Virginia

Each year the USFS prescribes burns within the GWJNF. Burns are prescribed both in the growing and dormant season. The goal of the burns is to reinstate the natural fire regime, and consequently, return the forests to their original species composition. Currently in GWJNF, Appalachian pine-oak forests are experiencing an increase in fire-intolerant species while *Quercus* spp. and herbaceous groundcover are declining. A vegetation survey was conducted on Buck Mountain, WV to determine if there was a significant difference between dormant and growing season burns compared to a no-burn control with regard to woody vegetation. We were interested in regeneration of oaks and the endangered *Gaylussacia brachycera* (box huckleberry). Sixty plots were established within a site burned twice (one growing, one dormant season), a site burned twice (both dormant), and a site protected from fire (control). We hypothesized that burns would have an effect on abundance of woody vegetation. We predicted that oaks and *G. brachycera* would increase after burns due to the decrease in competition from fire-intolerant species. We found that *Quercus prinus*, and *G. brachycera* were more abundant after dormant season burns. The results of this study suggest that seasonality is an important component when determining burning season.

G3 Alaina C. Sposito, Dr. Christine L. May, Dr. Patrice M. Ludwig, Dr. Lihua Chen
Using Capture-Mark-Recapture Techniques to Estimate Detection Probabilities for a Rare & Cryptic Species

The critically endangered James Spiny mussel (*Pleurobema collina*) is a species of freshwater mussel endemic to the James and Dan River basins. In the last 20 years, *P. collina* has experienced a substantial decline in numbers and currently only occupies 10% of its original habitat, however little information is currently known about this species to assist in conservation. A 230-meter reach of transitional habitat in Swift Run was selected for repeat observations to estimate detection probabilities using a Capture-Mark-Recapture framework. In June 2014, visual scouting began to locate and tag *P. collina* (as well as other mussel species found) with 12mm Passive Integrated Transponders (PIT tags). Repeat surveys were conducted on a bi-weekly basis to relocate all tagged individuals, record their current position, visibility on the surface, as well as relevant habitat characteristics that may have influenced their behavior or detectability. Preliminary results show that most *P. collina* are visually detectable <7% of the time, and that water depth, season, mussel size and community composition are good predictors of detection probabilities. Multi-state model analysis has generated parameter estimates for transition, detection and survival

probabilities for all species of tagged mussels, however not all parameters are significantly different between species.

G4 Cheyenne Weeks-Galindo, Dr. Steve Cresawn

Corroboration of In silico Generated Genome Annotation of a Lytic Mycobacteriophage with RNA-Seq Analysis of Late Transcripts

This study analyzes the novel mycobacteriophage *Sparkdehlily*. *Sparkdehlily* was isolated in the Fall 2014 semester of BIO 203: Viral Discovery. The viral genome was sequenced and then annotated in the Spring 2015 semester of BIO 204: Viral Genomics. A major goal of this study is to corroborate the in silico generated annotation of the genome, specifically, the late gene transcripts will be analyzed. It is hypothesized that late transcripts will primarily consist of structural genes as the phage is beginning virion assembly at this stage of the infection. The timing of late transcription was estimated by performing a one-step growth experiment of *Sparkdehlily* using *Mycobacterium smegmatis* mc2155 as a host. Experiments to be conducted include RNA extraction during transcription of late viral genes, followed by RNA-Seq analysis to study the relative levels of transcription across the viral genome at the chosen timepoint(s).

G5 Dehat Jalil, Dr. Terrie 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. 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. Altered DNA conformation can occur in regions of DNA called promoters. 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 and alpha-synuclein on gene expression via luciferase assay. Here we find that larger purine-pyrimidine sequences exhibit higher luciferase expression than smaller purine-pyrimidine sequences. We also see that in the presence of alpha-synuclein, purine-pyrimidine sequences exhibit an overall decrease in luciferase expression.

G6 Jonathan Sandoval, Dr. Bloss

Characterization of the *C. elegans* Nascent Polypeptide Associated Complex (NAC) Function under Stress

In the presence of stress cells activate coping mechanisms to restore integrity and functionality. Failure to resolving stress leads to premature apoptosis whereas exaggerated protective responses leads to cells evading apoptosis. The Endoplasmic Reticulum (ER) specific Unfolded Protein Response (UPR) contributes to this "tipping point" by eliciting cell saving responses and cell killing responses as a function of the stress duration. Based on the complexity of the UPR, understanding factors that affect UPR activity will provide further insights into cellular responses in the presence of stress. The NAC complex, composed of a α - and β - subunit, regulates protein shuttling into the ER. Although removal of either α - or β -NAC is sufficient to activate the UPR, a difference in UPR induction with removal of either subunit remains unexplored. This study aims to explore differences in the degree of UPR activity employing *C. elegans* as a model system. Interestingly, α -NAC depletion leads to increased expression of ER specific chaperones suggesting the individual subunits of the *C. elegans* NAC may be functional when unbound and may serve as a target for regulating altered protective responses in the presence of misfolded protein stress.

G7 Steven McBride, Lindsey Toothman, Dr. Pradeep Vasudevan, Dr. Joanna Mott

Effect of Converting to Organic Turkey Rearing Practices on Antibiotic Resistance of Enterococci from Used Litter

Conventional turkey production employs antimicrobial compounds for prophylaxis, treatment, and growth promotion. Conversely, organically raised turkeys are not administered antimicrobial compounds. Increasing public interest in organic and antibiotic free foods has led to a growing market for their production. In this study the prevalence of antibiotic resistance in *Enterococcus* spp. isolated from the turkey litter of adult tom turkeys after they were sent to market, was compared before and after the farm transitioned to organic rearing practices. *Enterococcus* spp. were isolated on m-EI agar and identified to species phenotypically. Susceptibility to 12 antimicrobial compounds was determined using the Kirby-Bauer disc diffusion technique. The proportions of resistant bacteria were compared using chi-square test and Fischer's exact test. Isolates from the litter of organic birds differed in species composition and resistance profiles from those isolated from the conventionally farmed litter. Isolates from organic litter exhibited a reduction in the percentage of isolates resistant to gentamicin (- 47%), tetracycline (- 42%), and doxycycline (- 25%). These results suggest that changing to organic rearing practices affects the antibiotic resistance of bacteria in turkey litter.

G8 Ben Williamson, Dr. Susan Halsell, Dr. Daniel N. Cox

Molecular Characterization of Noxious Cold Detection in *Drosophila* Larvae

The perception of pain (nociception) is a genetically conserved process in animals. Pain continues to be a major health concern and current treatments prove insufficient. Greater understanding of the molecular processes underlying pain sensation could lead to new and more effective treatments. The aim of this study is to investigate the molecular mechanisms of cold nociception in *Drosophila melanogaster*. A subset of peripheral sensory neurons (Class III dendritic arborization (da)), are implicated in *Drosophila* larvae's response to noxious cold.

Previous literature has implicated TRP and DEG/ENaC subfamily members in mediating sensory responses to noxious heat and mechanosensation, including mechanosensory nociception. Though much is known about noxious mechanical and heat nociception in *Drosophila*, little is known regarding the molecular components mediating cold nociception. Here we focus on characterization of *Drosophila* DEG/ENaC family members as potential regulators of noxious cold-evoked sensory behavior. A novel behavioral assay, coupled with functional optogenetic studies and *in vivo* RNAi, has been utilized to investigate the role of select pickpocket (ppk) family members, which we have identified as exhibiting significant enrichment in class III da neurons. Our analyses reveal that these ppk family genes are required for noxious cold detection in larvae.

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

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