Calculation of the heat of combustion of different grades of gasoline.

The price of gasoline can vary dramatically with the octane rating or grade. It is suggested that higher grades of gasoline produce more engine power by producing more heat during the combustion process. The relationship between the grades of commercially available gasoline to the heat of combustion was studied using bomb calorimetry. To determine the efficiency of different grades of gasoline, the heats of combustion, $\Delta H_{\text{combustion}}$, were determined for several different commercially-available grades of gasoline. Calibration of the bomb calorimeter was achieved using standardized benzoic acid to calculate the calorimeter constant. The $\Delta H_{\text{combustion}}$ for each gasoline sample was obtained by generating a heating curve and analysis using industry standard procedures. Preliminary results show little difference in the heat of combustion versus the different grades of gasoline. For commercially available mid-grade gasoline, the $\Delta H_{\text{combustion}}$ was determined to be 6129.40 (±1) cal/g, while the $\Delta H_{\text{combustion}}$ for the highest grade of the same commercially available gasoline was determined as 6636.57 (±1) cal/g, with a difference of only 507.17 (±1) cal/g. This suggests that although the price of different grades of gasoline can vary drastically, the heat produced does not vary as drastically. Further studies are currently being developed to compare other grades of gasoline to these preliminary results.

Analysis of Ebola SIR variant model.

The recent outbreak of Ebola in West Africa is a tragic example of how quickly an unchecked virus can run through a population. It shows the need for strong and rapid measures for containment of possible infection. While the first case in Guinea appeared in December 2013, no great measures were taken to contain the disease until March 2014; the Ebola virus is still spreading in Guinea today. The first case in Mali was reported in October 2014, with measures taken to combat the spread enacted that month. Consequently, Mali has been declared Ebola-free since January 18, 2015. This research proposes a set of ODEs based on the well-known SIR model to model the spread and transmission of the disease. The model will be calibrated to observations of the outbreak using Markov chain Monte Carlo algorithms. In this model, we include a parameter that represents the country's ability to seek contacts with infected individuals and to put them into quarantine. This model also accounts for delays that may occur in organizations' reactions to epidemics and the speed at which they can set up quarantine protocols. We vary both of these and show how the speed and efficacy of quarantine could affect the number of infected.
This project was supported by the NIGMS of the NIH grant as part of the West Virginia INBRE (P20GM103434).

CHRISTIAN SHIMER, DARRYL JOHNSON, Department of Computer Sciences, Mathematics and Engineering, Shepherd University, Shepherdstown, WV 25443; FRANK LIAO, Boonsboro High School, Boonsboro, MD; QING WANG, Department of Computer Sciences, Mathematics and Engineering, Shepherd University, Shepherdstown, WV 25443; DAVID J. KLINKE, Department of Chemical Engineering, and Department of Microbiology, Immunology and Cell Biology, West Virginia University, Morgantown, WV 26506. **Modeling of Japan’s population by a Markov Chain Monte Carlo method.**

According to reports, Japan has experienced net population loss due to falling birth rates and almost no net immigration in recent years, and this trend will continue in the coming decades. This project is aimed at developing the Japanese population growth model through a system of differential equations, and then calibrating parameters against published census data using a Markov Chain Monte Carlo (MCMC) method. MCMC creates a probability distribution as its desired equilibrium distribution (basically the trajectory of the population), and it is desired because it provides a rapid convergence to probable areas of parameter space. The model has been formulated trying to accommodate all major factors that affect each sub-population. It might be used to predict the future population and develop population management strategies that could avoid the declining trend in the long run.

Funding supporting this project was received from West Virginia Higher Education Policy Commission Division of Science and Research SURE Grant Program and the NIGMS of the NIH grant as part of the West Virginia INBRE (P20GM103434).

---

**Aquaculture**

CHRISTINE LEPINE, PETER VILA, Institute of Environmental and Physical Sciences, Shepherd University, Shepherdstown, WV 25443; LAURA CHRISTIANSON, KATA SHARRER, and STEVE SUMMERFELT, The Conservation Fund’s Freshwater Institute, Shepherdstown, WV 25443. **Impact of hydraulic retention time on nitrogen removal in a woodchip bioreactor receiving aquaculture effluent.**

Four 1:10 pilot-scale models (L x W x D: 3.8 x 0.75 x 0.61m) were constructed at The Conservation Fund’s Freshwater Institute, Shepherdstown, WV, USA to assess the practicality of denitrification (i.e., nitrate-removing) woodchip bioreactors for field-scale implementation in an aquaculture setting. Hydraulic retention time (HRT), a main design parameter of bioreactors, controls the degree of denitrification by regulating the flow rate through the system; the four models were operated at differing HRTs (12, 22, 39, and 58 hours). Denitrification was measured by weekly water quality sampling of total nitrogen (TN) and nitrate-nitrogen (NO₃-N); sulfate (SO₄²⁻) and dissolved oxygen (DO) were measured concurrently. After 125 days of operation, all four bioreactors demonstrated high nitrogen reductions of 59-100% of NO₃-N removal. The 12 hour HRT treatment had the lowest removal efficiency (59%), but the highest removal rate (64 g N/ (m³·dy)) since it received the highest nitrate loading rate. The 22-hour HRT bioreactor had slightly higher removal efficiency (76%) but a lower removal rate (44 g N/
The 39-hour and 58-hour HRT treatments resulted in nearly 100% nitrate removal; in these two systems, sulfate reduction was observed as nitrate became a limiting factor. To avoid toxic hydrogen sulfide (H₂S) production from sulfate reduction, this work recommends HRTs of 12-24 hours. Early evidence of excessive bacterial growth was observed and may eventually cause clogging and disruption of flow through the system. Further analysis over a longer time period is needed to ascertain whether this growth impacts bioreactors’ longevity.

Botany

MITCHELL HAINES and ANDREAS BAUR, Department of Chemistry, Biology, and Geoscience, Fairmont State University, Fairmont, WV 26554. **High performance liquid chromatography method development for the analysis of selected phenolic acids in plant extracts.**

Several plants with anti-inflammatory or anti-cancer activity, including some traditional West Virginia herbs, were analyzed for the phenolic acids gallic, caffeic, and vanillic acid, since the literature reports a connection between these medicinal properties and phenolic acids. Plant samples (wild grape, calendula officinalis, elderberry, butterfly weed, lemon balm, apple mint, ox-eye daisy, bee balm, flowering dogwood, poke weed, jewel weed, and purple coneflower) were extracted using methanol and water (1:1, v/v) and analyzed by HPLC with fluorescence detection (column: ZORBAX Agilent SB-C18 column (4.6 x 150 mm, 5 μm particle size), gradient: 25%-80% methanol in 0.02% aqueous TFA, flow rate: 0.5 mL/min, excitation wavelength: 268 nm, emission wavelength: 360 nm). There was a large region spanning retention times from 3-6 minutes on all chromatograms that made determining a gallic acid peak (retention time 3.6 min) impossible. There were a lot of unexpected peaks on the chromatograms from other substances in the plant samples which fluoresced at the chosen wavelengths. The results for vanillic and caffeic acid were inconclusive.

This project was funded by the College of Science and Technology's SURE program at Fairmont State University.

Cell Biology

GRANT BASAGIC and BRUCE ANTHONY, Department of Chemistry/Biochemistry, West Virginia Wesleyan College, Buckhannon, WV 26201. **Alcohol-induced single-strand breaks recruit elevated XRCC1 proteins associated with DNA damage repair in neuronal stem cells.**

Fetal Alcohol Spectrum Disorders (FASD) are associated with both facial and neurological phenotypes that are a result of changes in both proliferation and cell death within the neural crest stem cells. During development, cell losses in the region are attributed in part from oxidative stress, although the mechanism is still not known. Under normal DNA stress conditions, single-stranded DNA breaks recruit the DNA repair protein XRCC1, which is involved in the repair of DNA damage by forming repair complexes with Pol-β, Ligase III, and PARP. We suggest that alcohol causes a premature entry into S-phase that induces both double-
and single-stranded DNA damage. Recruitment of repair mechanisms such as XRCC1 complexes would be the first line of response to damage. Under these conditions we suggest that XRCC1 protein expressional changes would be elevated and recruited to multiple DNA damage sites.

To examine the effects of alcohol on this repair mechanism, we used rat cortical neuronal stem cells (derivatives of neural crest cells) with alcohol doses of 300 and 400 mg/dL over 8 h exposure times. Cells were fixed in Hanks balanced salt solution and stained for DNA (DAPI and/or propidium iodide) and counter-stained with antibody to XRCC1 with a FITC secondary. Microscopic examination shows alterations in both DNA integrity and XRCC1 expression. This suggests a mechanism of DNA damage associated with alcohol and suggests an unsuccessful mode of DNA repair that likely leads to apoptosis.

MEGAN BRADFIELD and ROBERT WARBURTON, Department of Chemistry, Shepherd University, Shepherdstown, WV 25443. Antibody epitope mapping of murine H-2K\textsuperscript{b} MHC molecules as a means to study the effects of mutation on B-cell recognition.

The specific aim of the research presented here was to determine if a directly-conjugated fluorescent (FITC) stained monoclonal antibody, used in a single-step binding assay, would be able to determine cell surface expression of wild type and mutant murine MHC Class I molecules, wild-type H-2K\textsuperscript{b} and mutant H-2K\textsuperscript{b\textsuperscript{m19}}. It was determined that the efficacy of a one-step stain was comparable between the two H-2K\textsuperscript{b} molecules but not as effective as what was previously reported in the literature for a two-step system. It is hoped that future work will reinforce the results of this study and provide information on differences between the two MHC molecules relating structures, point mutations and epitope recognition by antibodies as a means to investigating differences in B-cell recognition.

Funding supporting this project was received from West Virginia Higher Education Policy Commission Division of Science and Research SURE Grant Program.

LAUREL BRAGG and BRUCE ANTHONY, Department of Chemistry/Biochemistry, West Virginia Wesleyan College, Buckhannon, WV 26201. Detection of isoform changes in exons 5 and 11 of the Spastin gene associated with hereditary spastic paraplegia.

Hereditary spastic paraplegias (HSPs) are an inherited set of diseases whose main phenotype is progressive stiffness and contraction in lower limbs as a result of peripheral nerve damage. This genetic disorder follows inheritance including autosomal dominant or recessive, or X-linked recessive. The mode has a direct impact on the chances of inheriting the disorder. HSP is caused by defects in protein transport mechanisms that aid movement through the cell. Long nerves are affected due to long distances of transport, and are particularly sensitive to defects. Genes that contribute to the disorder include 72 spastic gait disease-loci and 55 spastic paraplegia genes (SPGs). Several genetic mutations have been identified which underlie various forms of HSP, and genetic testing is available and used to confirm clinical diagnosis. Testing does not include all genes or identify all the mutations of genes known to cause HSP. Many cases involve a mutation in the SPG4 (Spastin) gene, which controls the spasticity of the lower extremities. The extent of mutation or variance is yet unknown.

We wish to examine exon variants of the SPG4 gene at two distinct locations to see if splice variances contribute to the disease. Buccal swabs were collected from several consenting
patients (control and affected) and PCR was used to amplify differences in exons 5 and 11. A decrease in expression levels of the tested gene indicates missing genomic sequence, increases mean extra DNA. We suggest this to be a possible diagnostic tool for specific forms of HSPs.

MICHAEL FAGIOLA, Department of Chemistry, Shepherd University, Shepherdstown, WV 25443; TAICHENG ZHOU, YIZENG YANG, and JONATHAN P. KATZ, NIH Center for Molecular Studies in Digestive and Liver Diseases and the Division of Gastroenterology; Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104. **Regulation of mesothelin and kallikrein-related peptidase 5 in human esophageal keratinocytes by the zinc-finger transcription factor Krüppel-like factor 4.**

Krüppel-like factor 4 (KLF4) is a key transcriptional regulator that functions in a number of cellular processes including proliferation, differentiation, and carcinogenesis. In the esophagus, KLF4 is typically induced in differentiating keratinocytes and plays a role in esophageal keratinocyte proliferation, migration and differentiation functions. KLF4 deletion in murine esophageal epithelia alters keratinocyte morphology and delays keratinocyte differentiation at 5 weeks and 3 months of age; these mice eventually developed a premalignant condition known as squamous cell dysplasia. To determine the targets of KLF4 in esophageal epithelial cells, we performed gene expression analyses on esophageal epithelia of 3 month-old mice. Among the most differentially regulated genes were MSLN and KLK5, two genes implicated in squamous cell differentiation and carcinogenesis. We hypothesized that KLF4 binds to the promoters of MSLN and KLK5 and acts as a direct transcriptional regulator of these genes during proliferation and/or differentiation in human esophageal keratinocytes. In studies using human primary esophageal keratinocytes in culture, we found that KLF4 was induced during keratinocyte differentiation and that KLF4 loss altered differentiation. Taken together, these findings are consistent with an important role for KLF4 in human esophageal keratinocyte differentiation. By quantitative real-time PCR and Western Blot, respectively, KLF4 loss in human esophageal keratinocytes also resulted in decreased mRNA and protein levels for MSLN and KLK5. Further, by chromatin immunoprecipitation, we found that the MSLN and KLK5 gene promoters were directly bound by KLF4. Thus, KLF4 is important for human esophageal keratinocyte differentiation and directly regulates MSLN and KLK5.

The authors acknowledge support from NIH NIDDK R01-DK069984 (to J.P.K.), and by the Undergraduate Student Scholars Program at the University of Pennsylvania Center for Molecular Studies in Digestive and Liver Diseases (NIH R25-DK066028).

JAMES GAINER and BRUCE ANTHONY, Department of Chemistry/Biochemistry, West Virginia Wesleyan College, Buckhannon, WV 26201. **Alcohol-induced alterations in protein-protein interactions of transcription factors associated with proliferation changes in FASD and Alcoholism.**

Excessive alcohol consumption during pregnancy (Fetal Alcohol Spectrum Disorders, FASD) or in adult alcoholism, induces significant developmental and neurological alterations that affect neuronal development or brain plasticity, respectively. It has been shown that alcohol exposure leads to alterations in neuronal stem cell proliferation, apoptosis and cell migration. Our interest was to begin delineation of the mechanism(s) associated with altered growth
patterns of neuronal stem cells associated with FASD and alcoholism.

Recent studies on protein expression demonstrate that alcohol induced increases in expression of cell cycle proteins that affect the G1/S phase transition and, likely, DNA replication. These include the E2F1 family of transcription factors that regulate transcription of S-phase-specific genes at the start of DNA replication. In addition, alcohol is shown to induce overexpression of the E2F1 dimerization partner(s) DP1 family and the retinoblastoma protein (RB), both of which demonstrate importance in regulation of transcription from E2F1 consensus sites. However, the mechanism of altered E2F1 activity is poorly understood. We examined E2F1 interactions with both DP1 and RB by co-immunoprecipitation of E2F1 and Western blotting. We used cortical neuronal stems cells from a rat in cell culture with a moderate (400 mg/dL for 8 h) alcohol exposure. We examined protein interaction changes for both DP family isoforms as well as the RB protein. Our antibody capture of E2F facilitated all eight isoforms. We suggest that alterations in RB binding to E2F alter transcription of S-phase-specific genes and increases transcription of S-phase specific genes in a premature fashion.

ASHLEY HUREY and BRUCE ANTHONY, Department of Chemistry/Biochemistry, West Virginia Wesleyan College, Buckhannon, WV 26201. **Alcohol increases double-stranded DNA breaks and reduces nucleosome formation in a dose-dependent manner in rat cortical stem cells.**

In Fetal Alcohol Spectrum Disorders (FASD), alcohol induces both physical and neurological changes attributed to alterations in proliferation, differentiation, migration and cell death in neural crest stem cell populations. It has been demonstrated that oxidative stress in these cells contribute to both single-stranded (SS) and double-stranded (DS) DNA breaks that ultimately increase apoptosis in a large number of cells. Recent data on histone H1 expression suggests effects on nucleosome formation and changes in condensation of chromatin. To date, the extent and type of DNA damage incurred by alcohol is poorly understood. It is thought that dose and duration of alcohol exposure play roles in the extent of damage and the number of cells lost. Our hypothesis is that alcohol-induced DNA damage is dose-dependent and affects predominantly DS DNA breaks. This in turn would affect chromatin structure and nucleosome formation.

To examine these changes, we used ELISA to detect histone H1 formation in nucleosome formation that will lead us to interpretations on the extent of DS DNA damage. We used rat cortical stem cells (derivative of neural crest stem cells) that were treated with two alcohol doses (300 and 400 mg/dL) for 8 h. Extracts of DNA were tested on H1 antibody-coated plates and developed using horseradish peroxidase (HRP). Increased expression will determine the extent of DNA DS breaks and the binding of DNA to nucleosomes in both alcohol and control samples. We suggest a dose-dependent increase of DS DNA damage associated with a decrease in DNA associated with nucleosome formation.

ANDREW LEICHLITER, MARQUEZ GIBSON, ALICE MAGRO and ALBERT MAGRO, Department of Biology, Fairmont State University, Fairmont, WV 26554. **Temozolomide’s effectiveness upon the invasiveness of glioblastoma cells.**

Temozolomide (TMZ) is the current adjuvant drug of choice in the clinical, chemotherapeutic treatment of glioblastoma multiforme. Glioblastoma multiforme is a type of...
brain cancer that is very invasive and highly malignant. We have previously determined that TMZ does not effectively induce apoptosis in a variety of glioblastoma cell lines. In light of TMZ’s clinical effectiveness devoid of apoptotic effects, the purpose of this study was to determine the efficacy of TMZ as an inhibitor of invasiveness. The efficacy of TMZ to inhibit invasiveness was compared to the naturopathic products thymoquinone (TMQ) and epigallocatechin gallate (EPIGAL), and also to the antibiotic staurosporine (STAURO). TMQ, EPIGAL and STAURO are all known to have in vitro antineoplastic properties. The effect of TMZ upon growth and invasiveness was tested using the established glioblastoma cell lines U87MG, U118MG, LN18, M059J and T98G. A three dimensional in vitro matrigel spheroid assay, which measures the changes in the radius and volume surrounding the invading cellular spheroid, was used. It was determined that TMZ was not effective as an inhibitor of invasiveness as measured by the spheroid assay. The data raise the question as to why TMZ is effective as an adjuvant drug in the treatment of glioblastoma multiforme. Overall, the data indicate that there may be in vivo effects other than those manifested by in vitro apoptotic and spheroidal testing that are more relevant with regard to some chemotherapeutic agent’s ability to inhibit the growth and invasiveness of glioblastoma cells.

KELLY MACHUCA and BRUCE ANTHONY, Department of Chemistry/Biochemistry, West Virginia Wesleyan College, Buckhannon, WV 26201. Alcohol-induced changes to replication fork helicase complex (MCM) may induce DNA damage in rat neuronal stem cells.

Fetal Alcohol Spectrum Disorders (FASD) and adult alcoholism exhibits phenotypes associated with alterations in proliferation and cell losses in stem cell populations derived from the neural crest. Although the mechanism associated with cell losses is not well understood, our recent data suggests that cells enter S-phase prematurely, experience both double- and single-stranded DNA breaks early in replication, and are followed by apoptosis from poor repair of multiple DNA breaks. We also show that alcohol induces overexpression of histone H1, normally aligned with nucleosome packaging early in replication. To determine if apoptosis induction is associated with early DNA replication, we have chosen to examine expression of the MCM3 protein, which is part of the helicase complex associated with DNA replication forks. It is feasible that the cells experience a lack of function or stalling of the replication forks, hindering the replicative properties of the cells, followed by cell death. To test this, we used rat cortical stem cells with an alcohol dose of 400 mg/dL for 8 h. Both control and alcohol-induced cells were double-stained using either DAPI or propidium iodide for DNA stain, along with antibody against MCM3 and FITC secondary antibody. Microscopic analysis suggests alterations in MCM3 expression and the formation of stalled replication forks. This will help in understanding the mechanism in which alcohol induces DNA damage and apoptosis for future design of treatment paradigms.

PATRICK MILKOWSKI and BRUCE ANTHONY, Department of Chemistry/Biochemistry, West Virginia Wesleyan College, Buckhannon, WV 26201. Effects of alcohol exposure on the binding of transcriptional factor E2F1 to DNA consensus sequence.

Fetal Alcohol Spectrum Disorders (FASD) are associated with alterations in proliferation and increased apoptosis in neuronal stem cells exposed to alcohol. Chronic alcohol abuse also shows alterations in self renewal and apoptosis in neuronal stem cells. Alcohol exposure is
shown to cause an expressional change in cell cycle proteins that monitor and initiate cell cycle checkpoint progression at the G1-S phase. Improper regulation is thought to alter DNA replication processes, contributing to the decreased proliferation and increased apoptosis seen in these cells. Our preliminary studies show that alterations in protein expression of E2F and DP1 transcription factor families may induce altered gene expression and allow for premature G1-S phase transition and altered DNA replication. To date, there is little understanding of the mechanistic changes that occur in altered proliferation and apoptosis associated with alcohol exposure. Our interest is to understand how changes in E2F1 expression might relate to functional changes in transcriptional activation.

The goal of this experiment is to determine how the binding of E2F transcriptional factors to DNA consensus is affected by exposure to alcohol. Both control and alcohol-exposed nuclear proteins were used in an Electrophoretic Mobility Shift Assay (EMSA). DNA probes included 1) non-consensus site 2) single consensus sequence and 3) two consensus sequences in tandem. We suggest that E2F1 binding to a normal consensus sequence is altered in alcohol-exposed stem cells. This suggests a mechanism in which changes in functional E2F1 transcription alters DNA synthesis and increases apoptosis associated with moderate alcohol exposure.

TINA NGUYEN, Department of Biology, Shepherd University, Shepherdstown, WV 25443; MARY DAVIS, MACKENZIE NEWMAN, and HAN GANG YU, Department of Physiology and Pharmacology, West Virginia University School of Medicine, Morgantown, WV 26506. Mechanism of leptin resistance-induced cardiac arrhythmias.

Cardiovascular disease, such as heart disease, is the leading cause of death in the United States. Approximately 70% of overweight/obese individuals die from heart disease. Premature ventricular contraction (PVC), a type of cardiac arrhythmia, and prolonged QT syndrome occur at an exponentially higher rate in overweight/obese patients. Yu’s lab has recently discovered the expression of functional leptin receptors (ObRb) in cardiomyocytes, suggesting that leptin may exert a direct local effect on cardiac electrical properties, as opposed to the canonical adrenergic pathway. Using next-generation sequencing RNA-Seq, our lab has generated a database of mRNA expression from obese Zucker rats (OZR) and lean Zucker rats (LZR). Through Ingenuity Pathway Analysis, we have identified significantly-decreased gene expression of Akt3 and changes in ion channel transcript levels in OZR compared to LZR. In addition, confocal microscope imaging showed internalization of leptin receptors in cardiomyocytes when exposed to a high concentration of leptin. These data suggest a novel mechanism for leptin resistance toward modulating cardiac ion channels known to affect natural pacemaking, such as Ca,1.2.

Supported by NIH Grant 5P20RR016477 to the West Virginia IDeA Network for Biomedical Research Excellence.

Chemistry

JENNIFER ADAMS and MARK FLOOD, Forensic Science Program, Fairmont State University, Fairmont, WV 26554. Cross-reactivity of various cleaning products with luminol.
Luminol is used at forensic crime scenes to detect trace amounts of blood by reacting with the iron found in hemoglobin. However, luminol emits chemiluminescence when mixed with several different oxidizing agents, as has been shown by its cross-reactivity with some chemicals containing copper and some bleaches. This experiment tests the ability of various household cleaning products to react with luminol and yield a false positive test result when testing for traces of blood at a crime scene. All-purpose cleaners, cleaners containing bleach, wood cleaners, wood polishes, and environmentally-friendly cleaners were tested to determine their reactivity with luminol. Testing was conducted on a non-porous surface and a porous wood surface. Varying chemiluminescence was shown upon exposure to a UV light. The amount of chemiluminescence depended on the cleaner used. Based on the fact that several cleaners cross-reacted with luminol, alternative methods of testing for trace amounts of blood at a crime scene might be best, to ensure the validity of the test and make sure the luminol is reacting with blood and not being contaminated by another source.

BRITTANY ARAGON, JAMES WEEKLEY, and MATTHEW SCANLON, Department of Chemistry, Biology, and Geoscience, Fairmont State University, Fairmont, WV 26554.  
**Gunshot residue (GSR) analysis using atomic absorption spectroscopy.**

Discharged firearms expel airborne particles known as gunshot residue (GSR) which provides evidence that a person has discharged a firearm. Gunshot residue is known to contain lead, barium, and antimony. A modified testing method was developed using atomic absorption spectroscopy (AA) to find the mass percent of Pb and Sb in gunshot residue. Samples were collected by swabbing the hands of people immediately after firing a gun. The swabs were washed with 8 mL of nitric acid, and the washings tested using the AA. The results were compared to a calibration curve constructed from standards for lead and antimony ranging from 20 to 30 ppm. The mass percent of lead and antimony in the GSR samples are currently being analyzed. Being able to analyze precise concentrations of the components of GSR may help determine the type of ammunition that was discharged.

MICHAEL BATES, JOSH BENFIELD, JON SERRA, and THEUNIS VAN AARDT, Department of Natural Science and Mathematics, West Liberty University, West Liberty, WV 26074. **Synthesis of trans-pterocarpans.**

The objective of this research project is to synthesize trans-pterocarpans, a flavonoid that does not occur naturally. Trans-pterocarpans are of particular interest because they may display increased steroidal activity when compared to the naturally-occurring cis-pterocarpans. First, a Williamson ether synthesis is performed using benzyl chloride on the hydroxyl functional group of 2-hydroxy-4-methoxy-acetophenone. The protected acetophenone is then reacted with thallium trinitrate in order to rearrange the molecule into a protected phenylacetic acid compound. The protected phenylacetic acid is then combined with a similarly-protected benzaldehyde via an aldol condensation. These aldol products are then converted to trans-pterocarpans through cyclization of the 5-membered ring followed by cyclization of the 6-membered ring using protection and deprotection methods. As of now, several methods of synthesizing the ether product are under investigation.
TIA LeMASTER and KEVIN L. EVANS, Department of Science and Mathematics, Glenville State College, Glenville, WV 26351.  **Effect of UV radiation on anti-Markovnikov hydrobromination of alkenes.**

Alkyl bromides are key intermediates in numerous multi-step organic syntheses and are commonly synthesized from the hydrobromination of alkenes. The regiochemistry of the hydrobromination of alkenes is controlled with the absence or presence of radicals. The addition of a peroxide results in a radical mechanism which yields predominately the anti-Markovnikov alkyl bromide. The objective of this research is to develop an efficient synthesis of the anti-Markovnikov alkyl bromide by generating hydrobromic acid *in situ* by the hydrolysis of phosphorous tribromide. The reaction of 1-octene with phosphorous tribromide, silica gel, and benzoyl peroxide in hexanes has been studied with varying reaction conditions. The benzoyl peroxide promotes the desired radical mechanism, whereas, the silica gel promotes the electrophilic addition mechanism. By having conditions that result in the mechanisms competing, the effects of varying reaction conditions become more prominent. Results of the ratio of 1-bromooctane (anti-Markovnikov product) to 2-bromooctane (Markovnikov product) with varying quantities of peroxide and varying exposure times to shortwave UV radiation will be presented.

Baley Loy, Department of Biological Sciences, Shepherd University, Shepherdstown, WV 25443; Jordan Mader, Department of Chemistry, Shepherd University, Shepherdstown, WV 25443.  **Preparation of p-mercaptopstyrene/divinylbenzene copolymer foam and its removal of arsenic from groundwater.**

Arsenic pollution and contamination is a large concern in many countries and affects many people worldwide. Exposure to toxic arsenic species can lead to serious short-term and long-term medical conditions and abnormalities. Development of a low-cost arsenic filter system was studied by creating thiol-functionalized polymer foams. Thiol (-SH) groups have an affinity for heavy metals such as arsenic. Functionalization of the different density polystyrene/divinylbenzene copolymer foams was conducted by a series of reactions including electrophilic aromatic substitutions (EAS) (halogenation and nitration), thioacetate substitution to form the protected polymer, and deprotection to achieve functionalized polymer.

Foams with densities of 30, 60, 90, 100, 120, and 500 mg/cc were made using either a mechanical stirrer or gas-tight syringes. Stability and functionalization were studied using thermogravimetric analysis (TGA) and attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy. An arsenic testing kit was used with the functionalized polymer to test the efficacy of the thiol groups. Arsenic levels were reduced between 25 and 250 ppb when tested with 30, 60, 90, and 120 mg/cc functionalized polymer after one hour of contact time. Stable functionalized polymers were developed and preliminary testing of arsenic reduction occurred, but further tests need to be conducted.

This work was supported by the West Virginia Higher Education Policy Commission Division of Science and Research SURE Grant Program, the Shepherd University Chemistry Department, and the Shepherd University School of Natural Sciences and Mathematics.
Synthesis of trans-pterocarpans from 2-hydroxy benzaldehyde.

The objective of the research project is to synthetically produce trans-pterocarpans, a non-naturally occurring flavonoid. In order to achieve this, a synthetic method must be developed to produce the desired stereo outcome of the pterocarpan product. First, a Williamson ether synthesis is performed using MOMCl to protect the hydroxyl functional group of a benzaldehyde molecule, to be later combined via an aldol condensation with a similarly protected phenylacetic acid. The aldol products can then be converted to trans-pterocarpans by sequential cyclization of the 5-membered ring and the 6-membered ring using protection and deprotection procedures. At this point, the protection of 2-hydroxy-4-methoxy benzaldehyde has been completed via Williamson ether synthesis with MOMCl with a maximum yield of 92%. The product has been verified via NMR, including H1, C13, DEPT, HETCOR, and COSY spectrums, as well as GC-MS. Preparative TLC plates were used in separation of the reaction mixture of the ether synthesis. Protection of salicylaldehyde is also being performed using the same methods. Once a sufficient amount of protected benzaldehydes has been prepared, the next step will be to perform the aldol condensation and confirm the ester product.

Analysis of GSR particles post-firing and post-laundering.

Clothing was tested for gunshot residues (GSR) succeeding discharged weapons and laundering of worn garments with different types of detergents with warm water in a conventional washing machine. Each garment of the same type of fabric was tested post-firing and post-washing to determine if GSR particles were present if washed in different types of detergents in concentrations of 60 mL, 120 mL, 180 mL, and 240 mL of detergents: Purex dirt lift action, Oxi Clean max force and Ultra Tide plus bleach. Results demonstrated that nitrite particles were present post-firing on all garments. Results concluded that the type of detergent did affect the particles of GSR. The increased concentrations affected the amount of GSR particles observed post-laundering by the color intensity of the Gunpowder Particle Detection Kit that provided a sensitive method to visualize particles containing chemical nitrates. The detergent containing bleach products did not show any residual evidence of GSR particles post-laundering. Testing of GSR particles on clothing does signify that a person was near a discharged weapon, but cannot conclude whether or not they were discharging the weapon.

A survey of embedded computer systems and their applications.

Embedded computer systems are nowadays seen everywhere in enormous forms, from everyday devices to computer systems themselves. Consumer electronics, such as TVs, refrigerators, and utility meters, are now “smart” or computerized. Many cars and other automotives have
embedded processors to enable telecommunication and semi-automated driving. Even computer peripherals are now equipped with silicon chips so they could conduct certain tasks without the involvement of “main” processor. However, the answer seems unclear when people ask what embedded computer systems are, what distinguish them from regular computer systems, and what special design requirements are for embedded systems. In this poster, we provide an overview and classification of embedded systems and their contemporary application, and present a classification of current embedded systems and their design requirements.

ANDREW PHILPOTT, ASHLEY HOFFMASTER, DANIELLE GRODI, and ERICA HARVEY, Department of Biology, Chemistry and Geoscience, Fairmont State University, Fairmont, WV 26554. **Molecular dynamics simulations of detergent micelles.**

Molecular dynamics simulations are being performed to model the micelle properties of three detergents: 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), dodecyl maltoside (DDM), and dodecylphosphocholine (DPC). The goal is to see which type of detergent micelle best mimics the natural lipid environment for proteorhodopsin, a light-activated membrane protein that acts as a proton pump. The detergents are built as individual molecules in a program called Avogadro, and multiple molecules are assembled into a spherical micelle configuration using a program called Packmol. VMD software is used to visualize the molecules and to add water molecules and ions to the micelles. The molecular dynamics simulations are run in NAMD software to see how the micelle behaves in water over time. Our longest simulation to date is 2 ns with the DPC system. Difficulties encountered include different atom naming and numbering conventions in molecule and micelle structure files versus force field topology and parameter files. All software being used in this project is freely available and runs in a Unix-based operating system or on the Windows operating system, although Linux is recommended for easier use of some of the programs.

This work has been supported by the NSF Research Infrastructure Improvement/NanoSAFE-HPC project at West Virginia University (WVU), Fairmont State University, and the NASA Space Grant Consortium, and has been carried out in collaboration with Blake Mertz, Assistant Professor of Chemistry at WVU.

JOSEPH ROXBY and NATALIA OMELCHENKO, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. **In pursuit of cyberconsciousness.**

The rising processing power of computers offers a possibility that our real-life personalities can exist outside of a biological entity. Here we attempted to outline a scaffold for such digital copies, using a synthesis of knowledge of philosophy/ psychology and neuroscience.

This analytical work relies on several assumptions: the neurons and their connections are fully responsible for our personalities and behaviors; the decisions we make result from information processing that can be simulated; the modern fields of neuroscience and psychology/philosophy deliver complementary ideas and data.

We propose that there are at least five essential personality parameters that give weight to behavioral outcomes, some of which may be unique to humans: 1) fear tolerance, the ability to overcome fears and the resistance to developing them in the first place; 2)
impulse tolerance, the ability to resist immediate impulses to gratify desires or correct homeostatic imbalances; 3) emotional tolerance, the ability to resist the effects of emotion in favor of rationality; 4) traditional tendency, the measure of likelihood that either a traditional or novel option will hold more sway; 5) self-conception, the rewarding value we assign to projected outcomes. The parameters can be evaluated using both surveys and brain imaging (the topics that will be discussed in the presentation in details) and can provide a rough scaffold of our personalities.

We believe that someday it may be possible to fine-tune an artificial personality in such a way that its decision-making process would be nearly indistinguishable from its biological counterpart.

Ecology

ASHLEY GEORGE and CAROL Z. PLAUTZ, Department of Biology, Shepherd University, Shepherdstown, WV 25443. Cloning of key steroidogenic pathway elements in the mollusk *Lymnaea palustris*.

The increasing use of herbicides in agriculture poses a threat to aquatic organisms. Studies using the gastropod mollusk *Lymnaea palustris* and chronic Roundup have demonstrated decreases in reproductive output, alterations in steroidogenic acute regulatory protein (StAR, the rate-limiting step in steroid biosynthesis) levels, and a decrease in testosterone levels. This study aims to clone genes encoding key enzymes in the steroidogenic pathway of *L. palustris*. One of the most relevant genes to pursue, since testosterone levels are clearly affected, is the gene coding for the protein aromatase (CYP 19), which serves as the switch between testosterone and estradiol. After designing degenerate primers for aromatase (using *Alligator mississippiensis*, *Mus musculus*, *Homo sapiens*, *Xenopus laevis*, *Danio rerio*), two methods of nucleic acid extraction and amplification were compared: adult tissue-specific snail RNA/RT-PCR, and snail embryo genomic DNA/PCR. Extraction of genomic DNA and PCR amplification yielded a product of ~600 bp. Following ligation into a vector, transformation, and sequencing, sequence analysis results will be shared. Cloning of the *L. palustris* aromatase gene will provide tremendous insights into invertebrate steroidogenesis, and ultimately facilitate determination of transcriptional changes or altered regulatory mechanisms caused by herbicide disruption.

LORI M. PETRAUSKI, GREGORY A. GOOD, THOMAS RODD, GEORGE CONSTANTZ, and JAMES T. ANDERSON, Department of Wildlife and Fisheries Resources, West Virginia University, Morgantown, WV 26505. Using archival data to analyze patterns of bird breeding and flowering dates of wildflowers to create a baseline phenological database for the West Virginia University Natural History Museum.

The objective of this study is to use archival phenological information to analyze temporal patterns of bird breeding, to evaluate the factors that affect full flowering in wildflowers, and to create a general database of phenological data across species in cooperation with the West Virginia University Natural History Museum. There has been little research on phenology in West Virginia, and no central phenological database for the state exists. In order to create a long-term historical database, we are utilizing archival sources to uncover historical
phenologic data. We are collecting information from diaries of nature-minded citizens, natural history observations found in local history centers, herbarium specimens, field station records, and field notes taken by nature clubs. Using this phenological information, we are analyzing patterns and the environmental variables that affect species specific to West Virginia.

We are using a variety of techniques to recover, collect, and organize this historical phenologic data. Outreach materials including flyers, brochures, newspaper and magazine articles, an online blog, and social media are being used to reach citizens who are in possession of relevant data. This type of public outreach for data collection is unique in the field of phenology and an important resource in establishing a reliable baseline dataset for future climate change research and citizen science programs.

EVAN VENSKOSKE, CLARISSA DAMIS, and ZACHARY LOUGHMAN, Department of Biology, West Liberty University, West Liberty, WV 26074. Isopod diversity survey in the northern panhandle of West Virginia.

A survey of terrestrial isopods was conducted in the northern panhandle of West Virginia. The survey included many sites in Hancock, Brooke, Ohio, and Marshall Counties. Isopods were collected by hand at each of these sites. A total of 1158 isopods was collected, belonging to seven genera and ten species. Of the seven genera, *Armadillidium* was the most numerous and *Porcellio* was found in the most sites.

**Engineering**

SERDAR BILGILI, HALEY MORELLA, and V’YACHESLAV AKKERMAN, Department of Mechanical and Aerospace Engineering, West Virginia University, Morgantown, WV 26506. Effect of internal flame structure on the flame dynamics.

Internal flame effects are incorporated into the theory of flame acceleration, where the flame structure is described by means of the Markstein number, Mk, which characterizes the flame response to curvature and stretch. This quantity is a function of the primary thermal-chemical combustion parameters such as the Lewis, Le, and Zeldovich, Ze, numbers as well as the thermal expansion coefficient $\alpha$. Mk can be positive or negative, thereby moderating or promoting the flame acceleration. Herein, the Bychkov theory is combined with the Matalon-Matkowsky formulation [Journal of Fluid Mechanics 124 (1982) 239]. While the difference between these two approaches is recognized (the former deals a strongly elongated flamefront, while the latter is devoted to the planar one), we reduce such a discrepancy by splitting the analysis into two parts: first, we identify the diffusional-thermal (in)stability limits within the Ze-Le-\(a\) space; second, the Markstein number is incorporated into the formulae for the flame acceleration rate. Two formulae are compared: one is based on a simplified theory, while the other is the outcome of a model equation. Both the simplified theory and the model equation are proposed, first, in the present work. It is shown that the internal flame structure may provide a substantial correction to the flame acceleration rate only when the flame propagation Reynolds number is small enough. However, such a correction moderates fast with the increase in the Reynolds number. Overall, the effect of the internal flame structure is quantitative, but it does not change the qualitative scenario of the flame acceleration.
JENNIFER CLYMER, Department of Forensic Science, Fairmont State University, Fairmont, WV 26554. **Comparison of round-nose and hollow-point blood spatter analysis.**

The difference between bloodstain patterns of round-nose and hollow-point bullets of three different types of guns were compared. The three guns used were an Anschutz .22 competitive rifle, a 9 mm Ruger pistol, and a .38 special Smith and Wesson revolver. The Anschutz was shot at 50 yards, the Ruger at 7 yards, and the Smith and Wesson at 7 yards. Each gun was shot with a round-nose and a hollow-point bullet. The target was a balloon filled with fake blood. The results concluded that a hollow-point bullet makes a bigger blood stain pattern. The balloon ‘exploded’ and made a very large pattern. Also, there was blood found feet away from the point of impact. A round-nose bullet does the opposite. When the balloon was hit with a round nose, the blood just leaked out. There was a pool of blood directly below the point of impact. The type of bullet, a round-nose or hollow-point, affects the blood stain pattern.

Health Sciences

HOLLY CHILES and NATALIA OMELCHENKO, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. **Improving the clinical evaluation: two-point discrimination threshold is strongly influenced by stress.**

In order to test for proper nerve function in the extremities, many physicians use the two-point determination test (also known as a compass test), a measure of tactile spatial resolution. The test requires placing two separate points on a person’s finger, and subsequently increasing the lengths between the points until the subject can differentiate between each individual point. In this study, we attempted to identify the factors that may influence the threshold in 15 healthy volunteers (5 males, 10 females, all between the ages of 19-25). Afterwards, the baseline measures of the threshold were obtained (threshold is 1.666 mm +/- 1.047, p= 0.490). In contrast, the stress lead to a significant decrease in the number of errors made (average threshold is 0.933 mm +/- 0.594 SD, p = 0.014). Our data indicates that the vividness of somatosensory perception is influenced by stress, but not by mild cold exposure. The findings may be potentially important for accurate interpretation of outcomes of two-point determination tests in clinical settings (average threshold is 1.4 mm +/- 0.986 SD). The experiment was repeated twice again after 1) the volunteers placed their hand into ice cold water for 2 minutes ; and 2) performed the Stroop Color–Word Interference Task for 2 minutes (it is an accepted method to generate mild stress in adults). Our data indicate that the exposure to cold did not affect the two-point determination threshold.

KELSEY ROBERTSON and NATALIA OMELCHENKO, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. **Improving the clinical evaluation: palpatory sensibility is strongly influenced by wearing gloves.**

Palpation allows examination of variations in tissue and organs by feeling them from the surfaces of the body and is commonly used in diagnosis. To identify the factors that affect the palpation threshold, we studied the palpation capacity in 15 healthy volunteers. Each volunteer
was presented with 12 boxes filled with sheets of paper (from 18 to 60 sheets). Some of these boxes contained a short nylon monofilament on the bottom. The boxes were given in a random order and the volunteer was asked to identify the presence of a monofilament by touching. The test was repeated three more times after the volunteer 1) had the hand into the ice cold water for 2 minutes; 2) performed the Stroop Color–Word Interference Task for 2 minutes (an accepted method to generate a mild stress in adults); and 3) put on latex gloves (Sigma brand gloves that were appropriate for the volunteer size). The majority of volunteers were effectively recognizing the object under the paper (average threshold is 3.867 pages, +/- 2.295 SD). The palpation sensibility was not affected by exposure to cold (average threshold is 4.400 pages, +/- 2.923 SD, p=0.49) or stress (average threshold is 3.2 pages, +/- 2.111 SD, p=0.19). However, wearing the gloves decreased abilities of volunteers to correctly determine the content of the boxes (average threshold is 5 pages, +/- 2.323 SD, p=0.020). Our data indicate that the palpatory ability is strongly influenced by wearing gloves. Nonetheless, regardless of the impact on palpatory threshold, the health care workers must continue to wear gloves if there is a risk of exposure to body fluids or other potentially infectious materials.

**Microbiology**

REBECCA BARNES, DEANNA M. SCHMITT, TRICIA L. GILSON, and JOSEPH HORZEMPA, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. *The role of the erythrocyte cytoskeleton protein spectrin during invasion by Francisella tularensis.*

*Francisella tularensis* is classified as a Category A bioterrorism agent by the Centers for Disease Control and Prevention. Inhaling as few as ten of these bacteria can cause acute, lethal pneumonia. Studies using mouse models of infection have shown that erythrocyte invasion is a novel feature of *F. tularensis* during infection; however, the mechanism by which this bacterium enters red blood cells is unknown. Cytoskeletal rearrangement of other host cells such as macrophages and hepatocytes is required for entry of *F. tularensis*. Therefore, in this study, we evaluated the role of the major erythrocyte cytoskeletal component, spectrin, in red blood cell invasion. To test this, human erythrocytes were treated with venom from the Blue-bellied black snake (*Pseudechis guttatus*), which disrupts the spectrin in the cytoskeleton. Treatment with this venom significantly reduced the number of intra-erythrocytic bacteria suggesting that spectrin is involved in erythrocyte invasion. Importantly, the concentration of venom used in this study did not reduce the number of intact erythrocytes. This is the first study showing the involvement of an erythrocyte protein during invasion by *F. tularensis*.

AMANDA CORBEL, Department of Biology, Shepherd University, Shepherdstown, WV 25443; SEAN O’BRIEN, ELENA CHERTOVA, MacTRUBEY, JULIAN BESS, JR., and BRANDON KEELE, AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702. **Imaging Human/Simian Immunodeficiency Virus infectivity using fluorescently-functional fusion partners.**
Non-human primate models for HIV have enabled investigations into the early stages of infection that would not be possible in human models. However, longitudinal studies tracking the course of virus infection suffer from the need to sacrifice the subject for autopsy to probe viral dissemination and pathogenesis. The stochastic nature of early infection, animal-to-animal variability, and large sample sizes being cost-prohibitive make proper interpretation of the data and insightful conclusions difficult. The ability to visualize the early phase of infection would circumvent the need for autopsy and allow for the true longitudinal studies within the same animal. To address this need, we tested a battery of fusion partners of fluorescent functionality for their impact on viral viability. Virus with the SNAP-tag fusion partner remained infectious. Future studies will focus on characterizing the SNAP-tag for in vivo imaging.

MacKENZIE L. DAVIS and JOSPEH HORZEMPA, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. Resorufin acetate has potential for use as a novel antibiotic against tularemia.

Francisella tularensis is a pathogenic bacterium and is the causative agent of the disease tularemia. Tularemia can be passed to humans through contact with infected animal tissues, biting insects, contaminated food and water, and by inhaling the bacteria. Due to the virulence and ease of aerosolization, the Centers for Disease Control and Prevention classified F. tularensis as a category A bioterror agent. Therefore, it is essential to develop multiple effective treatments to combat F. tularensis. Resazurin is a compound commonly used to test cell viability. In previous experiments, resazurin and the reduced form, resorufin, were effective in killing F. tularensis in vitro. However, both compounds were ineffective as treatments during in vivo F. tularensis infections of mice. We hypothesize that resazurin is being metabolized to an inactive form when introduced to the host. To prevent this, resazurin / resorufin was modified in an effort to identify a compound that maintained its antibacterial properties, but was more stable in the mammalian host. Therefore we investigated the antimicrobial nature of resorufin acetate. Resorufin acetate (44 μM) completely inhibited the growth of F. tularensis on chocolate II agar plates and in broth culture. Therefore, the addition of an acetate group to the hydroxyl of the resorufin phenol did not diminish the antimicrobial properties of this compound. Future investigations will determine whether resorufin acetate is effective in treating tularemia in mice.

ALEKSANDR FLORJANCZYK and JOSPEH HORZEMPA, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. A Penicillium sp. isolate inhibits the growth of the American chestnut blight fungus, Cryphonectria parasitica.

The American chestnut (Castanea dentata) used to be the dominant canopy tree along the eastern seaboard of the United States. Cryphonectria parasitica, the causative agent of chestnut blight, was introduced from Asia in the very early 1900s, and obliterated the chestnut population within 50 years. We sought to identify microbes that were capable of producing factors that were fungicidal or inhibited growth of C. parasitica in the hopes developing a biological control of chestnut blight. We isolated a filamentous fungus that significantly inhibited the growth of C. parasitica upon co-cultivation. Extracellular fractions were capable of inhibiting C. parasitica growth, indicating that a potential fungicide was produced by the novel isolate. Sequence analysis of 16S rRNA identified this inhibitory fungus as Penicillium sp., with the nearest identity to P. expansum. Future work will explore whether amending soil with P. expansum, or
treated chestnuts with *P. expansum* extracts could prevent chestnut blight. A more
unconventional future approach may be to engineer *C. dentate* to express the inhibitory factor
produced by the *Penicillium* isolate.

TRICIA L. GILSON, DEANNA SCHMITT, and JOSEPH HORZEMPA, Department of Natural
Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. **The role of
IglC in erythrocyte invasion by Francisella tularensis.**

*Francisella tularensis* is a gram negative bacterium whose virulence has been associated
with its ability to replicate within phagocytic cells of the immune system, such as macrophages.
More recently, investigation revealed that *F. tularensis* invades erythrocytes. Preliminary studies
from our laboratory suggested that the type VI secretion system (T6SS) is required for red blood
cell invasion. In homologous systems, the T6SS is synthesized by the polymerization of Hcp. *F.
tularensis* lacks a clear homolog to Hcp. However, modeling predictions reveal that IglC of *F.
tularensis* may have a similar three-dimensional structure to the Hcp of other bacteria. To
further confirm the role of the T6SS and to examine the contribution of IglC in this process, we
generated an IglC-null deletion mutant. *F. tularensis* IglC-null mutant bacteria exhibited a
substantial defect in their ability to invade erythrocytes. Complementation of this mutation is the
focus of ongoing investigations. These results provide further support that the T6SS is required
for erythrocyte invasion by *F. tularensis."

Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence and NIH Grant 5K22AI087703.

JESSE HALL, SAMANTHA KNOWLTON, KATHLEEN LOUGHMAN, DEVIN SINDELDECKER, COURTNEY WOOD, TRICIA GILSON, DEANNA SCHMITT, and JOSEPH HORZEMPA, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. **The molecular mechanism of the temperature-
dependent antibiotic resistance of Francisella tularensis.**

Aminoglycosides such as gentamicin (Gm) are commonly used to treat bacterial
infections such as tularemia, the disease caused by *Francisella tularensis*. In addition to being
pathogenic, *F. tularensis* is found in environmental niches such as soil where this bacterium
likely encounters Gm producers (*Micromonospora* sp.). *F. tularensis* LVS and Schu S4 show
increased resistance to Gm at ambient temperature (26 °C) compared to mammalian body
temperature (37 °C). Temperature regulates a number of *F. tularensis* LVS genes, notably,
those presumed to be involved in resistance to beta-lactams, drug efflux, and LPS modification
are induced at 26 °C. To gain further insight into the mechanism of this temperature-dependent
resistance, we examined the effect of CCCP, an inhibitor of the proton motive force that powers
efflux pumps and the uptake machinery of small molecules. CCCP increased Gm resistance at
both ambient and mammalian temperatures. This indicated that the increased resistance at 26 °C
was likely due to diminished uptake of Gm, and not enhanced efflux. To evaluate uptake of Gm
by *F. tularensis*, fluorescent marker Texas Red (Tr) was conjugated with Gm, yielding Tr-Gm.
Bacteria incubated at 26 °C showed reduced fluorescence compared to those at 37 °C when
exposed to Tr-Gm, further suggesting that uptake of Gm was reduced at 26 °C. Moreover,
unconjugated Gm competitively inhibited uptake of Tr-Gm, demonstrating the specificity of the
Tr-Gm uptake. *F. tularensis* also exhibited enhanced beta-lactamase activity at 26 °C.
Collectively, our data suggest that *F. tularensis* augments antibiotic resistance at lower temperature by increasing beta-lactamase expression and reducing permeability to aminoglycosides.

ASHLEY HAUGHT, TAYLOR ROGERSON, TRICIA GILSON, DEANNA SCHMITT, and JOSEPH HORZEMPA, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. **Inhabiting the intracellular space of an erythrocyte protects *Francisella tularensis* from the low pH environment associated with the gut of a tick.**

*Francisella tularensis* is a highly infectious bacterium that causes the disease, tularemia. Humans acquire tularemia through inhalation, oropharyngeal exposure, or tick bites. A unique feature of *F. tularensis* during infection is its ability to invade erythrocytes. Recent evidence indicated that residing within an erythrocyte enhances the ability of *F. tularensis* to colonize ticks. We hypothesized that the intracellular environment of the erythrocyte protects *F. tularensis* from the low pH or the different proteases associated with the digestive tract of the feeding tick. To test whether residing within an erythrocyte protects this bacterium from the acidic environment of the tick’s gut, intra-erythrocytic *F. tularensis* or bacteria that had been liberated from erythrocytes were subjected to media that ranged in pH. Results from these investigations indicated that inhabiting an erythrocyte protected *F. tularensis* from an acidic solution (pH of 3.6, which is similar to that of the intracellular space of tick gut cells). Similarly, intra-erythrocytic *F. tularensis*, or bacteria that had been liberated from erythrocytes were incubated with proteases found in the gut of ticks that commonly transmit this bacterium. These proteases included Cathepsin D, Legumain, and Papain. These investigations indicated equivalent survival between extracellular bacteria and those inhabiting erythrocytes, suggesting erythrocyte invasion does not provide additional protection from proteases associated with the tick gut. Results presented here indicate that erythrocyte invasion may protect *F. tularensis* bacteria from an acidic pH, promoting tick colonization.

LEXIE KEDING, DEANNA SCHMITT, and JOSEPH HORZEMPA, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. **A high throughput screen of a natural product library for the discovery of novel antibiotics.**

Antibiotic resistance is considered one of the greatest public health and clinical threats encountered during the twenty-first century. Discovery of novel antibiotic agents is imperative to combat the trend of decreasing antibiotic efficacy. The National Center for Natural Products Research (NCNPR) has assembled a library of organic compounds derived from plants, fungi, marine, and algae species with potential antibacterial properties. In partnership with NCNPR, we have developed a rapid and effective method for screening large quantities of organic compounds for antibacterial sensitivity against *Neisseria sicca*. *N. sicca* is a nonpathogenic bacterium related to the pathogenic *N. gonorrhea* and *N. meningitidis* and serves the purpose as a laboratory surrogate. Bacteria were inoculated into microtiter plates in which each well contained liquid growth medium. A small volume of each compound was added to a corresponding well in the microtiter dish. After incubation at 37 °C, bacterial growth was measured using a plate reader. Wells lacking treatment or containing antibiotics known to be effective at inhibiting *N. sicca* were used to identify normal or inhibited growth levels,
respectively. Preliminary results have identified two compounds exhibiting significant growth inhibition. Future investigations will involve the characterization and a detailed analysis of the antimicrobial efficacy of these compounds.

EVAN LAU, E. JOSEPH NOLAN, HANNAH E. CREWDSON, ZACHARY W. DILLARD, MARCUS A. KINKER, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074; HONGWEI YU, Department of Biochemistry and Microbiology, Marshall University, Huntington, WV 25755. **Microbial diversity and abundance of the sputum of younger Cystic Fibrosis patients using Illumina MiSeq multiplex 16S rRNA sequencing.**

Cystic fibrosis (CF) is the most common genetic disease among individuals of European ancestry. The microbiota of sputum of CF patients, especially in younger patients before later stage infections where mucus- and alginate-producing bacteria tend to dominate is relatively under-studied. It is not known whether the presence and abundance of pathogenic microbial taxa is linked to disease progression in CF patients. In this study, we used multiplex sequencing of 16S rRNA genes to reveal microbial diversity and abundance in younger CF human sputum (age 10-16), focusing on pathogenic taxa. Our data indicate that the vast majority of the microbiota in these CF patients consists of potentially pathogenic *Staphylococcus aureus* (other *Staphylococcus* sp. were absent) followed by members of the Proteobacteria and Firmicutes, based on multiplex sequencing of the 16S rRNA gene. This is in sharp contrast to the presence of the less-abundant but more diverse Pseudomonads, which may explain the lack of serious respiratory infections seen in later stage CF patients. Our results correspond with previous work based on microbial cultivation and laboratory observations. Further work is underway to sequence the *mucA* and *algU* genes in more CF patients to test our hypothesis that *mucA* gene mutations only occur a relatively small number of Pseudomonads in CF patients in early stages of infection.

LEANNE MAZZELLA, DEANNA SCHMITT, and JOSEPH HORZEMPA, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. **Erythrocyte invasion by Francisella tularensis increases colonization of Ixodes ticks.**

*Francisella tularensis* is a highly-infectious bacterium that causes the severe disease tularemia. Humans acquire tularemia through inhalation, oropharyngeal exposure, or tick bites. A unique feature of *F. tularensis* is its ability to invade erythrocytes. In this study, we investigated if red blood cell invasion enhances colonization of ticks, which could lead to increased transmission of disease. To test this, we used a predominant vector for this bacterium, *Dermacentor variabilis*. Ticks become infected with *F. tularensis* as nymphs and bacteria are maintained until adulthood. Subsequently, *F. tularensis* is transferred to humans during a blood meal. First, we sought to inoculate *D. variabilis* nymphs and adults *in vitro* through capillary feeding. Our experience was consistent with reports that indicate these ticks do not actively feed from capillary tubes, and require a live animal host to initiate feeding. Therefore, an alternative tick species, *Ixodes scapularis* was used as a model for this experiment, since these ticks can also transmit *F. tularensis* and actively feed from capillary tubes. Here we show that red blood cell invasion increases bacterial colonization of *I. scapularis*. 
Survival of *Francisella tularensis* in a nectar surrogate and the colonization of mosquitoes.

*Francisella tularensis* is a highly-infectious bacterium that causes tularemia. In parts of Europe, mosquitoes are capable vectors for transmission of *F. tularensis*. Presumably, mosquitoes become colonized with *Francisella* following extraction of a blood meal from an infected mammal. Mosquitoes feed intermittently on nectar between blood meals. Our long-term goal is to determine whether mosquitoes deposit *F. tularensis* into plant nectar during these intermittent feedings. And if so, can the bacteria present in the nectar colonize a feeding mosquito, producing a disease vector? Therefore, we first sought to determine the viability of *F. tularensis* in nectar by inoculating sucrose solutions, a nectar alternative, in comparison to water and a bacterial growth medium (TSBc). Bacteria were capable of surviving in sucrose and TSBc over a long period of time, relative to water. Mosquitoes (*Aedes aegypti*) feeding from this sucrose became colonized with *F. tularensis*, as was determined by plating insect homogenates at various time points. The presence of *F. tularensis* was confirmed by PCR using *Francisella*-specific primers on the viable bacteria recovered from the mosquitoes that fed from the colonized sucrose.

Antimicrobial properties of trans-2-octenal and trans-2-decenal, the major aldehydes responsible for the scent of *Halyomorpha halys*, the Brown marmorated stink bug.

Some insects release scented compounds as a defense against predators. Such compounds in earwigs have also been shown to have antimicrobial activity. Trans-2-octenal and trans-2-decenal are the major aldehydes responsible for the scent of *Halyomorpha halys*, the Brown marmorated stink bug. Previous research has shown that these aldehydes are antifungal and produce an anti-predatory effect, but have never been tested for antibacterial activity. We hypothesized that these compounds functioned similarly to the analogous multifunctional action of earwig compounds, so we tested whether these aldehydes could inhibit the growth of bacteria. Disk diffusion assays indicated that these aldehydes significantly inhibited the growth of methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, *in vitro*. An *in vivo* model using wax worms and meal worms coated in stinkbug aldehydes was utilized to determine if the aldehydes induced an antibacterial effect on live insects. Application of these aldehydes significantly reduced the colonization of bacteria on insects. These results suggest that stinkbug aldehydes are indeed antibacterial. These compounds could potentially be used as novel antimicrobial treatments. Furthermore, aldehydes have been shown to destroy bacteria more efficiently when coupled with antibiotics, thereby decreasing minimum inhibitory concentrations necessary to kill microbes.

This work was funded by a West Liberty University faculty development grant and an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health (P20GM103434).
Sexual assault: Participants’ knowledge, attitudes, and behavior.

Our study assessed participants’ knowledge related to sexual assault, and focused on specific attitudes and behaviors of women and men regarding sexual assault. Participants were 86 undergraduates (52 women and 34 men). Participants completed 10 questions measuring knowledge of facts about sexual assault, and 11 statements designed to assess participants’ attitudes about sexual assault issues, then read state legal definitions of sexual assault, sexual abuse, and sexual harassment, and indicated the degree to which they had experienced, and had perpetrated, each of the three.

Single-sample t-tests showed participants did not have accurate knowledge about sexual assault, either significantly overestimating or underestimating nine of the 10 items. Independent-groups t-tests showed men averaged significantly higher on seven of the attitude items, e.g., “Women should wear less suggestive clothing to reduce their chances of being raped,” while women averaged significantly higher on one item. Finally, independent-groups t-tests showed women reported significantly higher levels of having been victims of sexual assault, abuse, and harassment.

Our results have clear implications for sexual assault and awareness programs. First, our participants varied widely, and generally inaccurately, in knowledge related to sexual assault. Second, men’s and women’s responses to the attitude items show substantial differences in most cases, with men more strongly supporting sexual assault myths, and perpetuating gender stereotypes about factors related to sexual assault. Finally, our results indicate that men need to become aware of, and control, behaviors related to sexual assault, abuse, and harassment.

Interpreting a date rape situation with a male victim and a female perpetrator.

We explored women's and men's interpretations of a date rape situation involving a female perpetrator and a male victim. Seventy mostly-white undergraduate participants (39 women and 31 men) read a passage describing a typical college party involving alcohol and sexual innuendo, but with an ambiguous ending. The scenario indicated that the female character (named Brooke) was more interested in having sex than the male character (named Kenny). Participants completed the story in writing, then read an addition to the scenario which indicated that sexual activity had occurred. Finally, participants responded to the following items using a five point scale rating from 1 - Strongly Disagree to 5 - Strongly Agree: Kenny wanted to have sex; Kenny was raped; Kenny is to blame; Brooke is to blame; if Kenny dressed more conservatively this wouldn't have happened; if Kenny were sober this wouldn't have happened; if Kenny said fine after many attempts at saying no it would have been consensual sex.
Independent-samples $t$-tests showed male participants were significantly more likely to blame the female perpetrator, although significantly less likely to view what happened as rape. Both male and female participants evaluated the male victim as being as aggressive, powerful, and in control as the female perpetrator. Men were not significantly more likely than women to indicate that the scenario involved sexual assault, abuse, or harassment. Overall, gender stereotypes appeared to influence participants’ interpretations more than the characteristics of the situation did.

WILLIAM NACE and NATALIA OMELCHENKO, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. Do food and physical exercise alter cognitive performance in the morning?

A growing body of evidence supports the influence of exercise and diet on cognitive functions. The aim of the present study was to evaluate if morning exercise and breakfast alters cognitive performance in the morning.

Attention (the Stroop Task), working memory (the Digit Span Test), and the reaction time (the Ruler Drop Test) evaluations were performed over period of three days in six healthy volunteers during morning hours (8:00-10:00 am). After a volunteer described his/her morning routing on the first day of testing, the individual was asked to alter his/her exercise regimen for the second day of testing, and then return to the original exercise regimen but alter the food intake for the third day of testing.

Our data indicated that change in routine by itself did not alter cognitive performance ($p=0.433$). However, the volunteers seem to perform notably better on attention tests on the days when they had breakfast (14.1% improvement, $p=0.0687$) or exercised (5.5% improvement, $p=0.0824$). Working memory and reaction time seem to be less influenced by food intake or exercise regimen, with only working memory capacity showing a trend toward improvement following food intake (15.7% improvement, $p=0.0913$).

Although the sample size is too small for definite conclusion, our results indicate that food and physical exercise likely influence attention and possibly working memory in the morning. Since appropriate mourning routine seems to affect cognitive performance, the data of the study might be used to optimized the morning activities of the students and improve the learning outcomes in early-morning classes.

DEREK SCHOPPERT and CAROL Z. PLAUTZ, Department of Biology, Shepherd University, Shepherdstown, WV 25443. Exploration of memory formation and disruption in the pond snail, Lymnaea palustris.

Given the biological and chemical complexity of humans, the use of a model organism is often ideal in experimentation; one such organism that has demonstrated suitability for the study of memory formation is the pond snail Lymnaea palustris. For this study, operant conditioning was used to test alterations in long-term memory (LTM) formation following exposure to a common widely-used herbicide, Roundup. Snails were subjected to three 45-minute training sessions with a one hour break between the first two sessions and a 24-hour break before the third; in all sessions, a tactile stimulus is applied upon attempted pneumostome opening. LTM formation can be assessed by a significant decrease in mean pneumostome openings from the first to the third session. Animals pre-soaked and trained in the presence of Roundup exhibited
significant learning (from session one to two) as in control groups, but an inability to form LTM. LTM formation is related to up-regulation or activation of protein kinase C (PKC). Previous studies in our lab have demonstrated diminished PKC expression following conditioning with exposure to Roundup. The PKC inhibitor GF109203X was applied prior to and during conditioning to further establish a link between PKC and LTM in *L. palustris*.

KATHERINE VECCHIO, MEGANN BOONE, and BRUCE ANTHONY, Department of Chemistry/Biochemistry, West Virginia Wesleyan College, Buckhannon, WV 26201. **Alcohol-induced alterations in DNA and RNA synthesis in rat cortical stem cells relate a possible mechanism for slowed stem cell growth and increased apoptosis.**

Fetal alcohol spectrum disorders (FASD) are associated with a mother who consumed alcohol while pregnant. FASD symptoms include physical effects, along with behavior and learning problems, all associated to reduced proliferation and increased cell death of neural crest stem cells during embryo development. Under normal growth conditions, Cyclin D1 expression is transiently increased during late G1 phase and signals cells to proceed to S-phase. D1 initiates a cascade of events necessary for transcription of genes required for proper DNA synthesis. It has been shown in mouse embryos that alcohol increased Cyclin D1 protein expression two-fold, which in turn parallels increased piconosis in 40% of alcohol-exposed neuronal stem cells. We suggest that alcohol induced premature G1/S phase transition by D1 overexpression, which, in turn, altered DNA synthesis and increased apoptosis.

Through the use of flow cytometry, we examined both RNA and DNA profiles with gating focuses on G1 and S phases, respective. We then examined by antibody/fluorescent staining the overlapping expression of Cyclin D1 levels on these gated populations. Under normal growth conditions, Cyclin D1 expression is limited to the G1/S phase transition. Alcohol alters this expression pattern and suggests a continued expression in S phase cells. Profiles allowed an examination of apoptosis percentage as well. Understanding this mechanistic misregulation may eventually lead to a full understanding and treatment options for fetuses exposed to alcohol.

Science Education

JENNI PERKINS, JULIA OLIVETO, SEAN HARWELL, and ERICA HARVEY, Department of Biology, Chemistry and Geoscience, Fairmont State University, Fairmont, WV 26554. **Solar Army, West Virginia Brigade: Beta-testing the Solar Hydrogen Activity Research Kit (SHArK) and new techniques for metal oxide distribution.**

In collaboration with researchers at the University of Wyoming and California Institute of Technology, we are searching for cheap, abundant metal oxide combinations to act as photoelectrocatalysts for solar energy conversion. Two student-friendly instruments used to test samples of metal oxides for a photocurrent are SHArK (Solar Hydrogen Activity research Kit) and SEAL (Solar Energy Activity Lab). Fairmont State University is working with both systems to improve the procedures necessary to collect data and to help distribute the project to K-16 students throughout West Virginia. SEAL, the more developed system, provides insightful sample results during SHArK’s beta-testing phase. Beta testing has involved assembling and
running the SHArK in varied configurations using LEGO pieces and other commonly available materials, and troubleshooting the data acquisition boards and software provided by collaborators at the University of Wyoming. Test materials include a small commercial solar panel as well as mixed metal oxides prepared by pipetting metal salt solutions onto conductive glass plates and baking them in a kiln. Current progress on alternative methods (silkcreening and inket printing) to distribute metal salts onto the conductive plates will also be presented. Students who become involved in the Solar Army have the opportunity to work on a highly relevant problem, think critically about the technical aspects of the project, learn basic stoichiometric solution preparation and dilution techniques, and improve their technical writing and presentation skills.

Work this academic year has been supported by the NASA Space Grant Consortium.

QING WANG and ZHIJUN WANG, Department of Computer Sciences, Mathematics, and Engineering, Shepherd University, Shepherdstown, WV 25443. **Shepherd CME S-STEM program: enhancing academic performance and career preparation of CME students.**

The Scholarships in Science, Technology, Engineering, and Mathematics (S-STEM) Program at the Shepherd CME Department (grant number: DUE - 1259713) has been funded by the NSF since June 2013. The overall goal of the program is to increase the number of STEM graduates by providing financial, academic, and social supports, thereby enabling qualified students to overcome some of the barriers that prevent them from meeting their educational goals. The program has supported 19 students since Fall 2013. In this poster, major activities will be presented, and survey and report results will be discussed, along with initiatives that increase, retain, and support students in the computer science, mathematics and engineering fields.

**Stream Ecology**

EVAN COLLINS, Department of Biology, Wheeling Jesuit University, Wheeling, WV 26003. **Standardization of *E. coli* coliform counting for the determination of stream health from combined sewer outflows along Wheeling Creek.**

Five sites stretching the length of Big Wheeling Creek in Wheeling, WV were chosen and spanned from Elm Grove down to the mouth of the creek at Tunnel Green Recreational Park. These sites were chosen based on their proximity to a Combine Sewer Overflow (CSO) which discharges when approximately 2.5 mm of rain have fallen in a given area. A total of 230 samples were taken from the creek, 115 *E. coli* Fecal Coliforms (EFC) and 115 Non-*E.coli* Fecal Coliforms (NEFC), from a sampling period of 23 days. Once the EFC and NEFC were enumerated, a percentage was taken to give a ratio of EFC vs. Total Coliform Counts (TCCs) revealing a very low correlation of 0.0422; this was not enough to state whether the EFCs are a good enough predictor for stream health. It also showed that the EasyGel medium used was not an accurate medium for effective stream sampling, despite its simplicity. Also, the EFC levels in the creek were not dependent on the CSOs opening after greater than 2.5 mm of rainfall. As for acceptable levels of coliforms, the months of July and August had the most samples, and consequently both months exceeded the EPA's limit of 10% of EFCs greater than 400 CFUs/100 mL.
Chronic toxicity of crude 4-methylcyclohexanemethanol to the crustacean Ceriodaphnia dubia.

On January 9, 2014, more than 10,000 gallons of an organic solution contaminated the Elk River in West Virginia, which serves as a water source for over 300,000 residents in the Charleston area. The main constituent of the solution was 4-methylcyclohexanemethanol (MCHM). Prior understanding of crude MCHM’s toxicological effects on aquatic organisms has been limited. In this experiment, chronic toxicity tests were performed using Ceriodaphnia dubia to test the chemical’s aquatic toxicity at various concentrations, according to the Environmental Protection Agencies standard operating procedures. Chronic reference toxicant tests were run for quality assurance. Two tests were conducted with MCHM; a range-finder test and a narrow-range test. No Observed Effect Concentration (NOEC) values were found to be 6.25 mg/L, while the Effect Concentration 50% (EC50) was 25 mg/L. This experiment and other recent studies have shown that the toxicity of MCHM to aquatic organisms is greater than previously thought. Tests performed prior to 2014 do not support the endpoints gathered from this study regarding MCHM toxicity to any daphnid species.

Funding for this experiment was provided by the class action council of Goode vs. American Water.

Determination of the effects of acid mine drainage remediation in the Three Fork Creek drainage.

Acid mine drainage has become a more serious issue as years go by and the maintenance of closed and abandoned coal mines has decreased. The high sulfur content of the seams of coal that run through West Virginia create the perfect conditions for acid mine drainage (AMD). This research focuses on the success of attempts to remediate the acidification of the three Fork Creek Watershed through the use of limestone dosers. The dosers automatically dispense a regulated amount of a limestone slurry solution to counteract the AMD. Extensive stream data has been collected at sites above and below the treatment sites to serve as an assessment of the effectiveness of the stream remediation, including pH, total dissolved solids, turbidity, and dissolved oxygen (mg/L). Local macro-invertebrates were collected and used to determine the health of the river through the FBI Index. Water samples were also collected and Daphnia magna were used to test the ability of a semi-sensitive organism to survive and reproduce. Through all of the collections and analyses, the Three Fork River System is found to be positively affected by the remediation efforts, which will aid in the overall maintenance of the natural ecosystems of the state of West Virginia.

This project was supported by a Fairmont State University SURE grant.

Diffusion of CO2, a novel method to analyze CO2 in acid mine drainage.

This research focuses on the success of attempts to remediate the acidification of the three Fork Creek Watershed through the use of limestone dosers. The dosers automatically dispense a regulated amount of a limestone slurry solution to counteract the AMD. Extensive stream data has been collected at sites above and below the treatment sites to serve as an assessment of the effectiveness of the stream remediation, including pH, total dissolved solids, turbidity, and dissolved oxygen (mg/L). Local macro-invertebrates were collected and used to determine the health of the river through the FBI Index. Water samples were also collected and Daphnia magna were used to test the ability of a semi-sensitive organism to survive and reproduce. Through all of the collections and analyses, the Three Fork River System is found to be positively affected by the remediation efforts, which will aid in the overall maintenance of the natural ecosystems of the state of West Virginia.

This project was supported by a Fairmont State University SURE grant.
Measuring the partial pressure of CO₂ in acidic solutions is of interest to those working to eliminate acid mine drainage (AMD). Infrared gas analyzers can be used with equilibration chambers, but would be prohibitively expensive for the non-profit organizations leading AMD treatment. We are developing a method to measure amounts of CO₂ in alkalinity-free solutions by enclosing them in a sealed chamber with an alkaline trap. Carbon dioxide will diffuse from the acidic solution to the trap, and would then be detected by quantifying the hydroxide that it neutralizes.

**Zoology**

HEATHER BROWN, RUTH CONLEY, and ELIZABETH ROBINSON, Department of Biology, Shepherd University, Shepherdstown, WV 25443. **Vocalizations with syllables and motifs in Aleuropoda insignis, the Madagascan Flat-horned hissing cockroach.**

*Aleuropoda insignis*, the Flat-horned hissing cockroach, is a species of from Madagascar and has been found to produce hissing sounds when disturbed and during social encounters (Conley and Kanneh 2013). Courtship behaviors have been described for *Gromphadorhina portentosa* and vocalizations have been reported for both *G. portentosa* and *Elieptorhina chopardi* (Nelson and Fraser 1979, 1984, Sueur and Aubin 2006). In this study, we observed behaviors and vocalizations of *A. insignis* during courtship encounters. Audiovisual recordings of male and female *A. insignis* cockroach pairs were made during a 30-minute time period in a behavioral arena. Overall, the vocalizations can be described as ‘whistles’ and appear to be different than the whistles produced by *Elieptorhina chopardi* (Sueur and Aubin 2006). Some of the hisses also have broadband components, yet appear to be different from the courtship hisses produced by *Gromphadorhina portentosa*. We used *Aleuropoda insignis* vocalizations preceding successful mating and categorized them into ‘syllables’ based on visual characteristics within spectrograms. Weiner’s entropy and Goodness of Pitch were calculated for each hiss using Sound Analysis Pro software and found to be significantly different between the ‘syllables’ (Tukey, p<0.05). The ‘syllables’ seem to be ordered into ‘motifs’, as described in many species of birds but has not been described in insects.

JENNIFER BROWN, RUTH CONLEY, and SHER HENDRICKSON, Department of Biology, Shepherd University, Shepherdstown, WV 25401. **Evolution of cockroach communication by hissing.**

In hissing cockroaches, a pair of spiracles along the abdomen allow these species to generate hissing noises. This spiracle is elongated from the trachea, creating a distinct sound. Vocalizations of hissing cockroaches come from this vocal apparatus (Nelson and Fraser 1979). In the scientific literature, only hisses from *Gromphadorhina portentosa* and *Elieptorhina chopardi* have been characterized. However, species of tribe Gromphadorhini (subfamily Oxyhaloinae), have been noted to produce hissing sounds (Conley, pers obs). *Gromphadorhina portentosa* vocalizations are described as “hisses”, while *Elieptorhina chopardi* vocalizations are described as “whistles” (Nelson and Frazer 1984; Sueur and Aubin 2006). These vocalizations are affiliated with courtship behavior. *Gromphadorhina portentosa* that do not hiss do not mate (Nelson and Fraser 1984). Hissing is also used during agonistic encounters to establish social
rank within the colony (Nelson and Fraser 1980; Clark and Moore 1995). Using vocal characteristics, we examined the evolution of hissing communication in four genera of hissing cockroaches. We recorded vocalizations from three genera (Gromphadorhina, Aleuropoda, and Princisia) and examined vocal characteristics reported in the literature for two genera (Gromphadorhina and Eliptorhina). Using a selected subset of spectral features, vocalizations from each group were scored. We then performed cladistic reconstruction on a prior phylogeny based on five gene loci (Inward et al. 2007). Results show similarities and differences among these taxonomic groups and provide indications of more primitive versus derived vocal patterns, based on signal complexity across genera.

SAMUEL CANFIELD, Department of Science, Glenville State College, Glenville, WV 26351; JENNIFER RUDGERS, Department of Biology, University of New Mexico, Albuquerque, NM 87131; JOSHUA LYNN, Department of Biology, University of New Mexico, Albuquerque, NM 87131; ROSS CONOVER, Department of Science, Glenville State College, Glenville, WV 26351. Northern pocket gopher activity across elevation gradients.

A specific behavioral adaptation (i.e., burrowing) can indicate species distributional range, but the influence of behavioral factors are often poorly understood. Minimal research has been conducted on the range factors of northern pocket gopher (Thomomys talpoides) and its relatives above 2700 m. We investigated whether burrowing was affected by elevation and determined that activity is best represented by a quadratic relationship; activity was highest from 3000-3400 m (P<0.001). Pocket gopher activity is correlated with elevational abiotic factors (temperature, precipitation). As the climate changes, the range shifts and should be measured again to understand intra and interspecific interactions.

DANIEL LUKICH and ZACHARY LOUGHMAN, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. Cambarus bartonii bartonii (Common crayfish) in Virginia’s James River basin: one species or two?

The distribution of Cambarus bartonii bartonii (Common crayfish) is broader than that of any other North American crayfish. It encompasses stream ecosystems from Canada to Georgia (Cordeiro et al. 2010). In this study, a Virginian population of C. bartonii with a characteristic red and reticulated phenotype (dubbed “Virginia A”) was analyzed to determine if sufficient morphological difference existed between it and type populations to elevate it to full species status. Twelve morphometric measurements from males (Form I) were compared between both populations to determine if phylogeographic distinctiveness existed between these two groups. Univariate and multivariate analyses of these variables did not show significant morphological difference between Virginia A and the type population. Thus, a phylogenetic approach is recommended for determining if taxonomic definition is appropriate for the Virginia A population.

ELIZABETH ROBINSON, RUTH CONLEY, and HEATHER BROWN, Department of Biology, Shepherd University, Shepherdstown, WV 25443. Courtship behavior of Aeluropoda insignis, the Flat-horned hissing cockroach.
To characterize the courtship behavior of *Aeluropoda insignis*, the Flat-horned hissing cockroach, we examined behaviors preceding copulation to generate an ethogram. These courtship behaviors and affiliated hissing sounds may form the basis of species recognition, since several species of the Oxyhaloinae (namely, tribe Gromphadorhini) have been noted to produce hissing sounds, and a vocal apparatus has been described for *Gromphadorhina portentosa* (Nelson and Fraser 1979). In our experiments, two specimens of opposite sexed *Aeluropoda insignis* were placed in a sound-insulated room with audio and video monitoring for a thirty-minute time frame. Videotapes were analyzed to create operational definitions for associated behaviors, with behaviors and behavioral sequences compiled across successfully-mating pairs of animals. The most common behaviors observed included hissing, antenna tapping, mutual antennation, and body posturing (telescoping of the abdomen) between one or both members of the pair. Abdominal telescoping or extension has been observed during agonistic behaviors among males of *G. portentosa* as well as within the courtship display (Clark and Moore 1994; Nelson and Fraser 1980). A G-test (goodness-of-fit) shows that these behaviors and specific pairs of behaviors are associated during courtship. The resulting courtship behavior is similar to that reported for *Gromphadorhina portentosa* (Nelson and Fraser 1980), with the exception of characteristics of the hissing vocalizations, indicating that vocalizations may be the basis for species recognition and reproductive isolation within the species.

ERIC TENNANT, SPENCER BELL, ZACHARY DILLARD, LUKE SADECKY, NICOLE SADECKY, ERIC TIDMORE, and ZACHARY LOUGHMAN, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. *Phylogenetic analysis of Cambarus robustus in the North, Middle, and South Forks of the Kentucky River.*

*Cambarus robustus* is a wide-spread species complex whose global range extends from Tennessee to southern Ontario. This species has been assessed as Least Concern (LC) by the IUCN due to this large geographic range and current populations remaining stable. Southern populations may ultimately constitute undescribed species and require heightened conservation efforts. A protocol using modern phylogenetic analysis was developed to determine if *C. (cf.) robustus* in the Kentucky River watershed were undescribed taxa or nominate *C. robustus*. *C. robustus* were collected from the North, Middle, and South Forks of the Kentucky River watershed. DNA was collected from leg tissue of the crayfish and prepared using a DNEasy Kit. Genetic sequences were run through a bootstrap analysis with 2000 iterations using program MEGAv6. *Cambarus (cf.) robustus* display extreme phenotypic plasticity in this watershed, with distinct phenotypes allied with specific basins. Morphological analysis has already proved consistent differences exist between basins. This project’s goal was to corroborate morphological investigation with genetic information. Ultimately, Middle Fork *C. (cf.) robustus* claded separately from all other basins genetically, and are morphologically and genetically divergent from the rest of the Kentucky River system. Additional sequences are needed from the Middle Fork to officially declare it taxonomically unique.

EVAN VENSKOSKE, DANIEL MEYER, NICOLE SADECKY, and HEATHER KALB, Department of Biology, West Liberty University, West Liberty, WV 26074. *Preliminary survey of turtles in the West Liberty Reservoir, West Liberty, WV.*
The reservoir at West Liberty University is home to a wide variety of animals, including Common snapping turtles, *Chelydra serpentina*, and Midland painted turtles, *Chrysemys picta marginata*. Little is known about the population size or life history of these turtles. This survey will help us determine the number, sex, age, and species present in the reservoir. A second objective is to determine when the turtles go into hibernation and when they become active the following spring. The academic school year is from September through early May. For inclusion of the turtles into research-focused courses, it would be useful for them to be active.

This poster presents very preliminary results based on September, 2014 through the middle of March, 2015. Hoop traps (four) were placed at various locations around the perimeter of the reservoir. All turtles captured were weighed, measured, photographed, and an ultrasound exam conducted on the females. A total of three adult male Common snapping turtles, one hatchling Common snapping turtle, and five (two females, three males) Midland painted turtles were captured. The presence of the hatchling indicates that there are female snapping turtles present, though they were not captured. No animals were recaptured. Both female Midland painted turtles had ovarian follicles, but no oviducal eggs.