



# CCURI

COMMUNITY COLLEGE UNDERGRADUATE  
RESEARCH INITIATIVE

# Fall Student Colloquium 2013

Blue Bell, PA

November 1-3, 2013

Heather Post

*"What we have to learn to do, we learn by doing"*

*~ Aristotle*

# CCURI Student Colloquium

November 1-3, 2013

Montgomery County Community College  
Blue Bell, PA

EXPRESSION, PURIFICATION AND STRUCTURE OF SOME GLUTAMATE RECEPTORS  
[iGluRs]

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Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that are essential for synapsis in the nervous system. They are essential in learning and memory; they are also implicated in brain diseases such as Parkinson's disease, Alzheimer's disease, epilepsy and schizophrenia. The structure and dynamics of these receptors with different ligands (agonists and antagonist) are interesting for developing new drugs or treatments for brain diseases. In this particular study, the expression of the protein by bacteria, the purification process and then the use of the pure protein to determine structure and dynamics with different ligands is the main objective.

## THE CLONING AND SEQUENCING OF GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GAPDH) IN OKLAHOMA WILDFLOWERS

Michael G. Adair, Andrew K. Brown, Lina M. Guerrero, Patrice A. Samuels, Mylissa A. Stover,  
Diana Spencer, Ph.D.

Tulsa Community College Department of Biotechnology

The enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a housekeeping gene classically known for its role in glycolysis. Recent studies indicate possible GAPDH roles in a variety of activities as diverse as apoptosis, neurodegenerative disease, and DNA replication and repair. The study of the multidimensional nature of the functions of this enzyme in higher plants is unexplored and presents a significant area of study. Our research goal was to determine the gene sequence for the NAD<sup>+</sup>-dependent subunit of the GAPDH in the cytosol (GAPC) in native Oklahoma wildflowers, and compare the sequences through phylogenetic relationships. The genomic DNA of the selected plants were extracted, amplified, ligated into a pJet 1.2 plasmid vector and transformed. The purified plasmids were digested and electrophoresed to verify insert size. Portions of the plasmids were sequenced. Through bioinformatics tools analysis, sequences were edited and assembled. The longest reading frames were determined, and the amino acids were analyzed for protein structure. Future plans include a phylogenetic comparison of the multiple sequences.

A STUDY OF WATER QUALITY OF MANSKER CREEK IN SUMNER COUNTY AND  
THE OLD HICKORY WATERSHED AS INFLUENCED BY AGRICULTURAL AND  
RESIDENTIAL AND INDUSTRIAL LAND USES

Genna Batchelder and Maria White and Parris Powers  
Volunteer State Community College

Water samples were collected and water quality assessments were made at three sites on Mansker Creek in Sumner County as part of the Old Hickory Lake Watershed in the Cumberland River Basin in Tennessee. Stream water was sampled in order to study effects of land use and development on the stream water quality impacted by agricultural, residential and industrial land use. Levels of inorganic ions ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ , and  $\text{SO}_4^{2-}$ ), DO, hardness and temperature were collected and examined based on land uses (agricultural, wooded, mining and residential areas) in the drainage areas for respective sites. Stream site assessments also included channel width, buffer width, flow rates, turbidity, canopy and outflows. Evidence suggests that stream water quality was affected by the section of the drainage area and storm water impact in respective land uses.

EFFECT OF ARKANSAS RIVER WATER ON VIABILITY OF MAMMALIAN CELLS  
GROWN *IN VITRO*

Hunter Bearden, James Brown, Mang Chang, Scotty Karvia, Jeremy Sabo,  
Patrice Samuels, Mylissa Stover, Amanda Winn, Mieko Zarubin, and Dusti Sloan  
Tulsa Community College, Department of Biotechnology

Chemicals used to treat wastewater may have negative effects on the environment. Mammalian cells were exposed to media made from water collected downstream from a wastewater facility. Viability assays revealed that cells experience a loss in viability, compared to control, when grown in media made with downstream water. However, there was also a significant difference in viability of cells, compared to control, when grown in media made with upstream water. Therefore, detrimental chemical contaminants are present all along the river.

## RADIO ASTRONOMY AT M.D.R.S

Larry Benton, Dr. Bernard Smith, and Kyle Flaherty  
McLennan Community College

Radio astronomy is a crucial method for observing solar activity and celestial events. R.A.A.M will cover the application of radio astronomy in a Martian environment with a focus on the hardware. Radio astronomy could be useful to a manned mission on Mars by providing a way to monitor solar activity, and a different view point to supplement Earth based observation. Initially industrial and commercial equipment was analyzed for its use in the context our research. After the initial research was complete, existing equipment was to deliver the lowest total noise figure and highest resolution. At the Mars Desert Research Station the equipment was tested for quality and physical resiliency.

# INVESTIGATING THE SEASONAL DIETARY VARIATION OF BIG BROWN BATS (EPTESICUS FUSCUS) USING DNA BARCODING

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Delaware Technical Community College

Most investigations of the diets of insectivorous bats have relied heavily on morphological identification of insect parts found in feces. More recently, PCR and high throughput sequencing have been used to obtain a more thorough identification of consumed insects. This study takes advantage of the advances in DNA barcoding to investigate prey choice of a maternity colony of big brown bats (*Eptesicus fuscus*) within the White Clay Creek Preserve (WCCP), PA. Amplification and cloning of insect DNA isolated from feces collected monthly revealed changing patterns in diet during the summer. Most big brown bat studies have identified predominately beetle (Coleoptera) and moth (Lepidoptera) fragments. In contrast, the majority of sequences obtained from WCCP colony guano align with crane flies (Diptera: Tipulidae and Limoniidae). Comparison of sequences between different months indicates that the bats are feeding on different Diptera families; in all probability this coincides with the emergence patterns of local crane fly populations. Concurrently with feces collection, light traps were used to sample insect communities but this method yielded low crane fly counts. Future research will focus on collecting crane flies in the preserve to correlate emergence with prey discrimination and choice of feeding habitat.

## AN INTEGRATED STUDY OF A HOT SPRINGS MICROBIAL COMMUNITY IN THERMOPOLIS, WY

Austin Buckingham, Ruth Law, Georgianna Holley, Nathan Yeomans, Suzanne M. Smaglik  
Central Wyoming College

The biogeochemical characterization of the Big Spring in Thermopolis, Wyoming has been an ongoing study since 2005. The spring is characterized as a stable pH neutral, low nutrient, high alkalinity, high hydrogen sulfide and no dissolved oxygen environment. Minerals are deposited rapidly within the microbial mats, trimming the filaments and forming thick carbonate and sulfate terraces that drip over the cliffs into the Big Horn River. This year's studies emphasized the genomics of the 52°C hot springs microbial thermophile population. Thermophiles are thought to be related to the first life on Earth, and understanding the extreme environments in which they live may help us understand the evolution of early life on, and perhaps give us clues in searching for Earth life beyond our own planet. As an integrated field and lab project, the students in a sophomore-level Earth Systems Science course at Central Wyoming College, were able to extract DNA from the microbes 12 samples, of geographic variability, to determine the types of bacteria present in this environment.

In preparation for amplification (PCR) and genetic sequencing the DNA was extracted using the PowerSoil DNA Isolation Kit from Mo Bio Laboratories, Inc. Once the DNA extraction process was completed the samples were placed into a -70°C freezer for storage until sent for off-site processing. The 16S rRNA genomes were sequenced by Research Testing Laboratory (RTL) of Lubbock, Texas. One-step PCR and Reads 454 MSC sequencing was performed utilizing Roche 454 FLX instruments and Titanium reagents to produce a tag-encoded FLX amplicon pyrosequencing analysis, and identified by comparisons with the NCBI (v.1-1-11) archives. Results of sequencing show a microbial community populated by over 200 possible species, with moderate to high variability between sites. Dominant species include *Chloroflexus* sp and *Spirulina* sp with others identified as *Pedobacter saltans*, *Thiofaba tepidiphila*, *Sulfurovum* sp, *Venenivibrio stagnispumantis*, *Enterobacter asburiae*::*Enterobacter* sp, *Flavobacterium* sp, *Sporobacterium* sp. In addition to the microbial analysis, the distribution of microbe mats were mapped, water geochemistry was analyzed (and was consistent with earlier work) and underwater photography of the source spring was collected.

## DEVELOPMENT OF A BIOSENSOR FOR COLIPHAGE

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The American Public Health Assay (APHA) for human waste contaminated water is hampered by a dependency on a laboratory, culturing coliform bacteria and an assay turn-around time exceeding one day. An assay for coliphage (enteric bacteriophage) detection, as surrogate for coliforms (enteric bacteria), has been developed which employs the lytic coliphage release of the host indicator cell  $\beta$ -galactosidase enzyme ( $\beta$ -gal) activity and the conversion of a colorimetric substrate. While significantly shortening the APHA time to 3 hours, this assay requires laboratory equipment to separate phage-induced released  $\beta$ -gal enzyme from intact cells before addition of the substrate. To optimize this assay we have taken advantage of the  $\alpha$ -complementation feature of  $\beta$ -gal. We have engineered a cell line to express the 17- C-terminal amino acid “ $\alpha$ -fragment” of  $\beta$ -gal ( $LacZ\alpha$ ) and are developing a method to purify the  $\beta$ -gal  $LacZ\Omega$  from the *E. coli* M-15 mutant DH5 $\alpha$  cell line. We predict upon lysis of a population of transformed cells by coliphage in the presence of purified  $\beta$ -gal- $\Omega$  fragment and substrate, the gene products will complement in trans, reconstituting the  $\beta$ -gal activity which should be proportional to the amount of coliphage present. Alpha-complementation of  $\beta$ -gal activity will thus eliminate a separation step in the assay and make it compatible with a hand-held field device. In addition we are testing the introduction of a cassette of coliphage cell lysis genes under the control of a coliphage promoter to accelerate the enzyme fragment release thus further reducing test time. Our goal is to design a fast-acting hand-held coliphage biosensor device that can be used by someone without special technical expertise. We anticipate our device being useful in rapidly reporting the contamination of recreational waters in the U.S. or assuring potable water in the water sources in the developing World.

# OPTIMIZATION OF CELLULOSIC PRETREATMENT METHODS FOR THE PRODUCTION OF BIOETHANOL

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Second generation cellulosic feedstocks were used in a study of different pretreatment methods for eventual ethanol production. Chemicals like high base and high acid were used. Physical pretreatments like pressure, heat, and agitation were also tested. Findings suggest that heat and acid are the best methods to breakdown pine and switchgrass. Other variables made little if any difference in glucose production.

## OPTIMIZATION OF GFP ISOLATION AND PURIFICATION

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Green Fluorescence Protein (GFP) is widely used in cellular biology as a useful biomarker with numerous applications. GFP is naturally produced in the species of jellyfish *Aequorea Victoria*. It is a small protein (24 kDa) that is easily incorporated into many cell types and is commonly used as a protein tag to study protein production and function since it has the unique ability to emit green fluorescence under UV light. In this study GFP was expressed in *E.coli* cells and methods for GFP extraction and purification were tested to optimize the GFP isolation procedure.

Various sonication conditions were used to optimize cell lysis and several ethanol concentrations tested to optimize GFP precipitation from the cell lysate. Further GFP purification was achieved using column chromatography methods including Hydrophobic Interaction Chromatography (HIC) and Ion Exchange Chromatography (IEX). Data leading to the optimization of the GFP protein purification scheme will presented.

## GEOBIOCHEMICAL AND GEOPHYSICAL CHARACTERISTICS OF WHITE SULPHUR SPRING, THERMOPOLIS, WY

Holley, Georgianna A., Yeomans, Nathan S., Law, Ruth M., Coleman, Cheryl L., Buckingham, Austin R., And Smaglik, Suzanne M.  
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White Sulfur Spring (WSS) is located approximately 500m north of the Big Spring (main outflow) in Hot Springs State Park, along the eastern bank of the Big Horn River in Thermopolis, Wyoming. Unlike the Big Spring, which exists as a 20-m diameter high-flow pool, WSS trickles out of the side of the cliff, below travertine terraces, only a meter above the river level, at a slightly higher temperature of ~54oC. Rather than forming a pool, the outflow from WSS is a constantly flowing 10-cm wide, 1- to 3-cm deep channel, flowing out of a small cave and directly into the river. The channel is lined with a smooth, white mineral coating with bright green bacterial growth restricted to the edges of the channel, rimmed with gold/yellow bacterial growth away from the flow.

The clear flow emanates from the back of this cave onto a floor coated with gray sand and minerals. Because of the abundant vapor, the interior cave walls and the entrance way are decorated with sulfur, calcite and gypsum crystals. The conditions in the cave are harsh and require safety equipment for exploration. The water vapor and liquid in the cave is highly acidic (thought to be sulfuric acid based on the damage done to cotton fibers), although water taken outside of the cave was near neutral. Photographs of the crystal-coated walls and underground stream were taken inside the cave. Water and microbe samples were collected both inside and outside of the cave. The microbial samples were prepared in the lab for DNA sequencing and water was analyzed in the field and laboratory. The results of sequencing the 16S rRNA gene has identified a community different from that of the neighboring Big Spring. The dominant species at WSS is *Thiofaba tepidiphila*, with, *Chloroflexus* sp, and smaller amounts of *Sporobacterium* sp, *Flavobacterium* sp, and *Venenivibrio stagnispumantis*.

The WSS cave appears to be formed along a crack in the travertine terraces, slightly off of vertical. The entrance is about 2-m high by ½-m wide, opens to about 1-m wide just inside the entrance, and runs only about 5 meters back into the rock face. In addition to the geobiochemistry, a resistivity study was conducted above the cave to determine its pathways of openings and water flow. The results of the resistivity study show a complicated system. Other geophysical methods will be deployed to assess the nature of this complexity.

## DNA BARCODING: DETERMINING THE COI GENE SEQUENCE OF INSECTS IN THE GOLDENROD – GALL FLY COMMUNITY

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Interspecific community interactions of predation and parasitism can be challenging to track in complex systems. In the case of the goldenrod – gall fly system, it is unclear whether two bird predators, the downy woodpecker (*Picoides pubescens*) and black-capped chickadee (*Poecile atricapillus*) preferentially prey on goldenrod gall fly (*Eurosta solidaginis*) larvae present in goldenrod galls or larvae of the hymenopteran parasitoid (*Eurytoma gigantea*) of the gall fly. The parasitoid larva consumes the gall fly larva and overwinters in the gall. Gall fly larvae are larger than parasitoid larvae, and as a consequence provide a greater return of energy. Galls containing gall fly larvae are larger with thicker walls than galls occupied by parasitoid larvae, requiring a greater investment of energy by the avian predators to break through the wall to reach the larvae. Previous research on avian predation suggested that downy woodpeckers and black-capped chickadees could determine whether a gall fly or wasp larva was present without opening the gall, which would allow birds to bypass galls containing lower energy wasp larvae. However, the determination of the gall occupant, a gall fly or wasp larva, removed due to avian predation has been based on characteristics of the gall chamber. This is clearly an indirect measure of occupancy and damage occurs to the gall when birds break into the gall to reach the larva. Our goal was to amplify a portion of the mitochondrial cytochrome c oxidase subunit I (COI) gene used as a standard barcoding sequence used to identify animals to the species level. We hypothesized that recovery and amplification of only gall fly DNA would indicate that the gall contained only a gall fly larva at the time of avian predation. Amplification of the COI gene from two sources, the gall fly and the parasitoid, or of the parasitoid alone would indicate the presence of a parasitoid larva at the time of avian predation. To address this question we had to isolate DNA from galls which had been empty for several months. Also, we first had to determine the COI sequence for the gall fly, the parasitoid, and another occasional occupant of the goldenrod galls, the beetle *Mordellistena convicta*. We were able to amplify the COI gene for the three species of insects found in goldenrod galls. The gall fly sequences obtained represented only the third location in Minnesota. The COI sequence of the parasitoid had not previously been published, and only a few populations of the beetle had been sequenced in North America. We were unable to amplify the COI gene from goldenrod galls after several attempts to extract DNA from galls preyed on by avian predators. We suspect the presence of PCR inhibitors in the galls prevented amplification. Our next step is to attempt to extract DNA from galls using several alternative methods.

# ANTHROPOGENIC INFLUENCE ON CORAL DISEASE DENSITY IN ROATAN, HONDURAS

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Coral reefs across the world are facing degradation. Over the past three decades, coral reefs worldwide have experienced major changes in structure and function due to both anthropogenic and natural impacts (Coral Disease Handbook). According to Porter, these issues include direct and indirect anthropogenic disturbances (1992). Some bacteria linked with causation of coral disease are associated with humans (Coral Disease Handbook). Little research has been conducted regarding anthropogenic impact on the frequency of coral disease. The objective of this study was to determine the human role in contributing to the frequency of coral disease in Roatan, Honduras. If humans increase the risk of coral disease, then dive sites with higher diving frequency may have a greater frequency of coral disease than sites with lower dive frequencies. Five dive sites, located on the west coast of Roatan, were studied twice during the research process. A 40m<sup>2</sup> transect line was used to determine the frequency of coral disease. Total Human Impact Values (THIV) were determined based on dive site frequencies and environmental factors determined by the Roatan Institute for Marine Sciences Education Coordinator, Jennifer Keck. These data suggested no correlation between the frequency of coral disease and Total Human Impact Values. These results could have been due to the late spring research dates, as some coral disease does not appear until much later in the summer. An in-depth study of the water current in the area should be conducted to further elucidate the environmental impact. Future studies should focus on comparing specific coral disease frequencies to each of the Human Impact Values.

## A PILOT STUDY: MACROALGAL PREFERENCES FOR *Diadema antillarum* ON THE MESOAMERICAN REEF IN ROATAN, HONDURAS

Clara Hernandez, Breanna Grisham, Samuel Warren  
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Caribbean coral reefs have been observed to alternate between states of coral dominance and macroalgal dominance (Mumby 2009); events known as phase-shift events. One factor that has been associated with macroalgal overgrowth has been the decline of *Diadema antillarum* populations after mass mortality events (Ogden 1987; Hughes 1994; Mumby 2009). *Diadema* were among the top algal grazers before their mass mortality and within a two year span after *Diadema* die-off, macroalgae such as *Dictyota* sp., *Lobophora variegata*, *Anphiroa fragilissima*, *Jania adherens*, *Laurencia obtusa*, *Turbinaria turbinata*, and *Sargassum* sp., grew more rapidly than former dominant species (Ogden 1987). This study of *Diadema* dietary preference was conducted to help illuminate the role of *Diadema* in controlling algal overgrowth for potential phase-shift species. Urchins were placed in a flow through tank that was partitioned into three areas. Alga was placed on opposite sides of each urchin in a “Y” pattern and secured. The urchins were observed to determine which algal species was favored among the urchins. *Diadema* preferred *Styopodium zonale* to *Padina* sp. and *Dictyota* sp. *Styopodium zonale* has not been observed as a problem alga in phase-shift events (Lirman and Biber 2000). Each of the specific phase-shift algal species needs to be tested for palatability to *Diadema*. In addition, future studies should focus on algal species documented in phase-shift events and collect *Diadema* at depths associated with algal dominated coral reefs to determine the role of *Diadema* in phase-shift events.

ANALYSIS OF BORRELIA IN LIPOTENA CERVI (DEER KEDS) AS A MODEL OF INFECTION IN THE WHITE TAILED DEER POPULATION OF NEW YORK STATE

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Lyme disease or Lyme borreliosis is the most prevalent tick-borne disease in America. The etiological agent of Lyme disease is the *Borrelia* spirochete. Transmission of Lyme disease in North America is through the deer tick, which takes blood meals from infected white tailed deer. The hypothesis of this study is that *Lipotena cervi* (ked flies), which also take blood meals from the white tail deer, carry this bacteria. PCR analysis was used to detect the presence of the *B. burgdorferi* flagellin B (flaB) gene in DNA from ked flies. Of 40 ked flies tested, 8 were positive for the flaB gene, yielding an infection rate (20%) that is very similar to that found in ticks taken from the regional deer population (25%). Gel electrophoresis displayed that the PCR product was approximately 30 base pairs smaller than the predicted size for *B. burgdorferi*. This is also consistent with variation in the flaB gene among strains of this bacteria and may indicate a unique strain of *B. burgdorferi* in ked flies. It is possible that the strain of *Borrelia* cycling in ked flies may be different from that in regional deer tick populations.

## NEW FAR-INFARED LASER EMISSIONS FROM OPTICALLY PUMPED $^{13}\text{CHD}_2\text{OH}$

Sumaya Ifland, Mark McKnight, Patrick Penoyar, and Michael Jackson  
Edmonds Community College and Central Washington University

The purpose of this project, carried out at Central Washington University, was to study the far-infrared lasing properties associated with the isotopic form of methanol,  $^{13}\text{CHD}_2\text{OH}$ . This was accomplished using a  $\text{CO}_2$  laser lines were observed and recorded along with their wavelengths and operating characteristics. This work demonstrates the ability for laser to stimulate far-infrared laser emissions from  $^{13}\text{CHD}_2\text{OH}$ . Forty three new  $^{13}\text{CHD}_2\text{OH}$  to serve as an effective source of optically pumped far-infrared laser radiation.

## EFFECTS OF HABITAT FRAGMENTATION ON ANIMAL DIVERSITY: ELM CREEK PARK RESERVE VERSUS NORTH HENNEPIN COMMUNITY COLLEGE

Molly Kerhin, Kong Lo, Jesse Miller, Charlie King, Dominique Rumpel, Derek Schwab and  
Lisa Tracy

North Hennepin Community College

With changes in land use, including expansion of suburbs and industrialization, more landscapes are being disturbed. This alters animal habitat by decreasing resources, cover and fragmenting habitats. For instance, Elm Creek Park Reserve, established in 1957, is 4,900 acres of mostly regenerating habitat. In contrast, about four miles from Elm Creek Park lies North Hennepin Community College, which serves over 10,000 students. Within this campus is a fragmented forest of fewer than thirteen acres. The purpose of this study was to use camera traps to compare the biodiversity of relatively large vertebrate mammals and birds in these two locations. Our Environmental Science class monitored two regenerating habitats and two relatively undisturbed habitats in Elm Creek Park with camera traps as well as one location on the college campus from April 4th to April 18th. Freshly road-killed white-tailed deer were provided by the county roads department to be used to attract avian and mammalian predators to the motion and heat sensitive cameras. We hypothesized that there would be higher animal diversity within Elm Creek Park than at the North Hennepin campus. In addition, we characterized the tree community present in both relatively undisturbed areas of Elm Creek Park, both areas at Elm Creek Park with more recent secondary succession, and the North Hennepin Community College campus. We hypothesized that the undisturbed habitats at Elm Creek Park would have larger trees and more tree diversity than the regenerating woodlots in Elm Creek Park and the North Hennepin Community College campus. Tree diversity and tree diameter were significantly higher in Elm Creek Park Reserve than on the college campus although quite variable within Elm Creek Park. Animal diversity was highly variable among the five locations monitored with camera traps. The college campus had the third highest animal diversity among the five sites. Overall, consistent patterns of biodiversity and the presence and number of individuals of particular species were difficult to ascertain. The unexpectedly high biodiversity observed in the fragmented habitat at North Hennepin Community College may have been due to the presence of ecotones (edge habitats) not present in the more extensive and less fragmented land of Elm Creek Park. The habitat at the college had smaller trees and lower tree diversity; however, the college habitat had denser cover, which may have contributed to a relatively high animal biodiversity. In the future we would increase the sample size so that more powerful statistical comparisons could be made.

IDENTIFICATION AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH  
WHITE-NOSE SYNDROME INFECTED WINGS FROM LITTLE BROWN BATS (*MYOTIS  
LUCIFUGUS*)

Stephen Krasucki, Taryn McKee, John McDowell and Virginia Balke  
Delaware Technical Community College

White-nose syndrome (WNS), an emerging infectious disease of bats, has led to the death of an estimated seven million little brown bats since 2007. Evidence indicates WNS is caused by the psychrophilic fungus *Geomyces destructans*, although the specific cause of death has yet to be determined. Scanning electron microscopy (SEM) of WNS infected wing tissue revealed not just fungus, but also abundant rod-shaped bacteria. DNA sequence analysis from infected bat wings indicate a different community of bacteria than those found on uninfected wings. Bacterial isolates were obtained from swabbed infected wings, but no growth was observed from uninfected wings. Isolates were characterized using Biolog plates, BOX PCR, and fluorescent pigment production. These results indicate a correlation between the presence of specific types of bacteria and *G. destructans* and raise the possibility they contribute to disease progression of WNS.

## SEROSURVEY OF A RURAL POPULATION FOR WEST NILE VIRUS IGG ANTIBODIES

Rachel Lamb, Roderick Printz, Tyler Graham, Russell Goff, and Steven McAllister  
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**Background:** West Nile virus (WNV) is an RNA arbovirus in the family Flaviviridae. Birds are the primary reservoir for the virus; mosquitoes act as the primary vector. Humans are terminal hosts. Most infections are asymptomatic and less than 1% of patients exhibit severe symptoms. Sequelae include paralysis, encephalitis, and death. Fremont County, Wyoming is a focus for WNV infections. Unexpectedly, the number of recent cases has declined precipitously. Two serosurveys, 21 months apart, were conducted to determine if the cause of this decline was correlated to previous exposures. **Methods:** Blood was drawn from 87 subjects in the first serosurvey in February, 2011 and 95 subjects in the second serosurvey in November, 2012. All subjects resided in Fremont County. Serum samples were then tested for WNV IgG antibodies using Enzyme Linked Immunosorbent Assays. The presence of high titers of IgG antibodies indicates past exposure to the virus. **Results:** IgG titers indicated approximately 16% (95% CI: 9% to 24%) of subjects in the first serosurvey had been exposed to WNV and approximately 11% (95% CI: 4% to 17%) in the second serosurvey. **Conclusions:** These results indicate that the recent decline in cases is not due to previous exposure of the population to West Nile virus. Future research will focus on the presence of the virus in the reservoir and vector population.

## A SURVEY OF MOSQUITO POPULATIONS FOR EVIDENCE OF LATERAL GENE TRANSFER

Chayse Langworthy, Andrew Lorence, Daren Card  
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Lateral gene transfer is an important evolutionary mechanism between organisms through a variety of processes. In prokaryotes it can be mediated by conjugation, transformation and transduction, while in eukaryotes, transposable elements and viruses play a key role. Additionally, complex evolutionary relationships driven by lateral gene transfer can exist in eukaryotic organisms infected with prokaryotes. One such evolutionary system is seen in mosquitoes carrying *Dirofilaria immitis* (the causative agent of heartworm) and WO phage-infected *Wolbachia pipientis*. The studies herein explored evidence of lateral gene transfer in this system of coevolution.

Mosquitoes were trapped using resting boxes or light traps and identified taxonomically. Mosquito genomic DNA was used to perform polymerase chain reaction (PCR) to detect the *W. pipientis* surface protein (*wsp*) gene. An 856 bp PCR product from *C. melanura* was cloned, sequenced and subjected to genomic analysis. DNA sequencing data showed a region of 29 bp at the beginning of the PCR product with 100% homology to the *wsp* gene. A region of 31 bp at the end of the DNA sequence had 92% homology to the *wsp* gene. In addition, the 856 bp PCR product was also comprised of unidentified DNA including a 92 bp stretch having 73% homology to *Aedes aegypti*. A detailed analysis is difficult since the genomic DNA sequence for *C. melanura* has not yet been reported. However this data supports that the 856 bp PCR product has mosquito origin, probably from *C. melanura*. The data also evidence lateral gene transfer of *wsp* into the *C. melanura* genome. Additional PCR data shows that *Anopheles quadramaculitis*, *Anopheles punctipennis*, *Culex pipiens* and *Culex territans* may also contain *W. pipientis* DNA. These observations are noteworthy since, evidence of *Wolbachia* infection and lateral gene transfer of *W. pipientis* DNA in *Anopheles* mosquitoes has not been previously reported.

## MEDICINAL HERBS IN HISPANIC CULTURE

Jessica Leos, Carrie Firmani and Patricia Phelps  
Austin Community College

This research focused on the study of medicinal herbs used in the Hispanic culture, focusing on Loquat tree leaves, known as Nispero, and Mexican oregano. The Loquat tree leaves have been studied very little and very few studies have been reported on Mexican oregano, specifically dried extract. The more intensely-studied Greek oregano provided a point of reference to compare these two herbs. Antimicrobial properties were evaluated by zone inhibition of bacterial lawns and minimal inhibition concentration of liquid culture by serial dilution. Antioxidant properties were evaluated by the DPPH and total phenolic assays. The herbs studied showed both antimicrobial and antioxidant properties.

MONITORING THE POPULATION OF BIG BROWN BATS IN WHITE CLAY CREEK  
PRESERVE, PA

Kristabel Madera, Jennifer Sykes, Virginia Balke  
Delaware Technical Community College

Over the past three years Delaware Technical Community College faculty and students have been researching big brown bats (*Eptesicus fuscus*) at the White Clay Creek Preserve, Pennsylvania. Starting in early May, after the bats finish hibernating, researchers begin doing emergence counts on a maternity roost. When enough bats have returned to the roost, mist nets are used to capture bats. Measurements on the bats were done to determine the reproductive status, body mass index (ratio of mass to forearm length), and when the juveniles begin to emerge. The population of big brown bats in the preserve has been increasing and shows no indication of being affected by white nose syndrome. Using special equipment, walking transects were done along the trails of the preserve to see which habitat the big brown bats are using. The bats are found more frequently along the creeks and open areas next to tree lines.

# A STUDY OF STREAM DYNAMICS AND SILTATION BASED OF FLOW-RATES, TURBIDITY AND STREAM MODELING

Lanz Russell and Robert Twardzik and Parris Powers  
Volunteer State Community College

Field measurements of flow rate and turbidity siltation data were made at a bend of Lower Station Camp Creek. After a rain creek stage effects of channel width, mean depth, mean flow velocity and turbidity were examined from field data. Detailed observations over several days after a rain event provides the ability to determine sediment load, siltation and storm water impact to the Old Hickory watershed as part of the Cumberland River Basin. Using modeling techniques, siltation can be measured and studied. Examining such issues provides insights into understanding remediation needs for the management of stormwater.

## EXPRESSION OPTIMIZATION OF HIS/GST-TAGGED DIHYDROFOLATE REDUCTASE (GST- DHFR-HIS) IN RECOMBINANT E. COLI

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Dihydrofolate reductase (DHFR) is an essential enzyme in DNA synthesis. It aids in creating thymine by converting Dihydrofolate into tetrahydrofolate (Dihydrofolate reductase ). DHFR was the first enzyme targeted for cancer related chemotherapy because the cancer drug aminopterin binds a thousand time more tightly that folate rendering the enzyme useless, ultimately causing cell death (Goodsell). There are numerous studies aimed to increase the protein solubility of DHFR in different vectors, but even today increasing the output of soluble proteins remains an active field of research. The Bio-Rad Protein Expression and Purification Series kit yields 50% soluble DHFR, while the other 50% forms visible inclusion bodies leaving plenty of room for optimization. To increase the amount of soluble protein the temperature at which it is sub-cultured after induction was manipulated at various temperatures lower than 37oC. By incubating the E. coli at lower temperatures after induction it was thought that even though the overall protein production would be reduced the amount of soluble protein would be increased. By decreasing the rate of over all protein production it is thought that the cell will not become overwhelmed with the target protein and reduce the amount of inclusion bodies formed. For this 37oC was used as a control and the experimental temperatures used were 30oC, 20oC, and 4oC.

## POTENTIAL INFLUENCE OF FISH BITES ON YELLOW BAND DISEASE IN CORALS OFF THE COAST OF ROATAN, HONDURAS

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Over the past several decades, reef-building corals have been in a steady decline due to infectious diseases, which are thought to be a key contributor to mass mortality of coral reefs (Bruno et al. 2007). Yellow band disease is a widespread coral disease known to affect the primary Caribbean reef builders, *Montastraea faveolata*, *M. franksi* and *M. annularis*, by attacking the zooxanthellae (Weil et al. 2009). There has been a lack of studies regarding the transmission of coral disease in the Caribbean. Many fish species such as, *Sparasoma viride*, Stoplight Parrotfish, *S. guacamaia*, Rainbow Parrotfish, *Stegastes fuscus*, Dusky Damselfish, and *S. partitus*, Bicolor Damselfish display feeding or algal farming behaviors which result in extensive bite coverage, particularly on *Montastraea* corals. Through these behaviors, fish may transmit the pathogen for yellow band and other coral diseases or may increase coral susceptibility to infection due to the resulting wounds. This study was conducted to determine the relationship between coral disease and fish bite coverage on the Mesoamerican Reef in Roatan, Honduras. As fish bite coverage may be contributing to increased coral disease frequency, corals with greater fish bite coverage will have a greater coverage of coral disease. The study was conducted at 5 locations off the west coast of Roatan at depths averaging 40 ft. A 0.5m x 0.5m quadrat was placed on randomly selected corals of the genus *Monstastraea* that were affected by yellow band disease. There was no correlation between yellow band disease and fish bites in accordance to size and number for individual diseased corals. However, mean fish bite coverage was positively correlated with coral disease coverage per study site with an exception of one dive site. This dive site, known as Fish Den, had strong currents which resulted in fewer samples collected, which may have affected the results for this site. Long-term studies documenting seasonal changes for yellow band coverage and comparing relative abundance of coral biting fishes and coral disease may provide vital information on potential disease transmission routes and source agents. By further studying the biting behaviors of these fish species, there may be seasonal behavioral differences which may correlate with peak coral disease months.

## EFFECTS OF PRAIRIE FIRE ON PLAINS POCKET GOPHER ACTIVITY

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Crow-Hassan Park Reserve, located in Rogers, MN, is a large restored prairie home to many different species of plants and animal. The reserve is a popular site for horseback riders and nature-lovers. To simulate the natural prairie environment, Crow-Hassan park officials periodically perform prescribed burns on certain “burn areas” which are mostly divided by dirt roads. Prescribed burns increase plant growth, an important food source for plains pocket gophers (*Geomys bursarius*) and other wildlife. The plains pocket gophers is a fossorial, herbivorous mammal present in Minnesota prairies and sandy, non-prairie environments. Gophers create dirt mounds while tunneling for food and making dens. Preliminary observations suggested that some burn areas within Crow-Hassan Park appeared to contain more gopher mounds than other areas. This study attempted to determine whether prairie fires have an effect on the mound-forming activity of plains pocket gophers. We hypothesized that gopher activity, as measured by number of recent gopher mounds present at a site, would decrease with the length of time since the most recent burn. Each of two sites were sampled for each burn period: never burned or burned in 2011, 2012 or 2013. Nine circular locations 30 m in diameter were observed in each of the eight sites. The number of pocket gopher mounds was determined with each mound categorized according to a scale measuring vegetated cover on the mound, specifically, no vegetation, 1-25% vegetated cover or greater than 25% coverage. Percentage coverage with vegetation was used to provide an estimate of the time since the mound was created. We assumed that less vegetated cover indicated a more recently formed gopher mound. Prairie fires appeared to benefit local gopher populations. Plots burned in 2011 had an average of 20.7 gopher mounds per plot, which was the highest density among the burn treatments. This observation is contrary to our prediction that more recent burn sites would exhibit greater pocket gopher activity due to the flush of new plant growth following a burn. It may be that a longer period of time to allow greater diversity and density of plant growth contributed to our observation. However, plots burned in 2012 had a larger proportion of recently formed mounds than plots burned in 2011 even though the total number of gopher mounds was lower. Plots that had never been burned had the lowest level of pocket gopher activity with only 6.2 gopher mounds per plot. Although our observations would have to be considered tentative due to limited sample size it does appear that pocket gopher burrowing activity is influenced by time since the area was burned. The relationship between burn period and presence of pocket gophers is complex, and may vary depending on other conditions not examined in our study.

## OPTIMIZING A PROTOCOL FOR THE DETECTION OF BATRACHOCHYTRIUM DENDROBATIDIS, AN AMPHIBIAN PATHOGEN

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*Batrachochytrium dendrobatidis* (Bd) is a water-borne fungal pathogen that poses a growing threat across the globe to frogs, toads, and some other amphibian species. Bd infects susceptible amphibians and leads to the disease chytridiomycosis, which may delay development, cause physical abnormalities, and even mortality. Bd has been found on every continent except Antarctica even though it was unknown as a pathogen of amphibians until about 20 years ago. Our study had several objectives. The first goal was to modify existing protocols to create a simple and inexpensive process to extract DNA from Bd- infected tadpole and adult bullfrogs, *Rana catesbiana*, and pure cultures of Bd maintained in the lab. We were able to amplify DNA using two sets of PCR primers without a DNA extraction step, which reduced the cost of sampling and increased the number of samples one could run on a limited budget. None specific amplification did not occur and primer concentrations were optimized to limit primer-dimer formation. A second objective was to address our hypothesis that water associated with aquatic plants shipped from areas in which Bd is endemic may be a source of inoculum to regions where Bd is not present. In addition, some suppliers of aquatic plants for home ponds use frogs to maintain water quality in production tanks. Water hyacinth and bullfrog tadpoles were ordered from a supplier located in a region of the U.S. where Bd is known to be present. None of the 28 bullfrogs were positive for Bd. The water was filtered using published protocols and PCR also failed to amplify Bd DNA in the shipping water. A third objective was to collect epidermal swabs from the digits, oral cavity, and lower abdomen of frogs in the field. Almost 50 leopard frogs, *Rana pipiens*, were collected in three locations. Bd DNA was not amplified from any of the samples. Negative samples may be difficult to interpret because the target DNA may be present in quantities too low to amplify efficiently or inhibitors to PCR amplification may be present. Hence, the fourth objective, which was to test the limits of Bd detection measured as number of zoospores present. We made a dilution series of Bd zoospores from a lab culture. Both end-point PCR and real-time PCR were successful at low concentrations of zoospores. We detected Bd concentrations as low as 4 spores per ten microliters, which was as low as possible in the most successful of the published research. We detected Bd from both filtered water and nonfiltered water samples. Because we detected zoospores at low concentrations we believe that samples found to be Bd negative were accurate. Our overall goal is to develop a simple, inexpensive, mostly fool-proof protocol to allow other colleges to sample amphibians and aquatic environments for Bd to better understand the current distribution and spread of an important pathogen of amphibians.

## DETERMINING THE EFFECTS OF LAND USE CHANGE ON WATER RESOURCES

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This project is part of an internship funded through the NASA EPSCoR program through Oklahoma University. The NASA internship goal was to learn the concepts of hydrology and chemical transport and how they are impacted by land use changes, and gain skills on remote sensing and hydrologic modeling techniques. The research study goal was to determine the effects of land use change on water resources. Remote sensing involves obtaining land use data from satellite images and using ground truthing data for validation. The hydrologic model used is the Soil and Water Assessment Tool (SWAT), which is a watershed-scale model developed to predict the impact of land management practices on water, sediment, and agricultural chemical yields in watersheds with varying soils, land use, and management conditions over time. The study area was Lake Creek sub-watershed (LCSW) located within the Fort Cobb Reservoir Experimental Watershed (FCREW) in southwestern Oklahoma. The LCSW (136 km<sup>2</sup>) was chosen to study impact of land use changes on water resources. The USDA Agricultural Research Service weather station network (MICRONET) weather and State Soil Geographic (STATSGO) (Figs. 1b) data were collected and processed. Land use in Lake Creek consists of winter wheat (38.5%), grass (37.9%), peanuts (9.3%), grain sorghum (4.7%), forest (5.2%), water (0.1%), and urban/roads (4.0%) (Fig. 1a). Fertilizer application for corn was 46-0-0 (N-P-K) @ 200kg/ha; peanuts 30-80-0 @300kg/ha, and wheat 46-0-0 @ 40kg/ha. A rapid geomorphic assessment revealed stream bank instability for some streams and contributes sediments to streams.

## STRESS-TOLERANCE PROTEINS IN DEINOCOCCUS HOPIENSIS

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*Deinococcus hopiensis* is a bacterium that was recently discovered in the Sonoran desert of Arizona. Very little is known about *D. hopiensis* and only a handful of articles exist referencing the existence of the bacteria. (Bagaley, et al., 2005). *Deinococcus radiodurans*, however, is a well characterized bacteria found in a wide variety of environments, that has shown to be resistant to environmental stressors such as radiation and desiccation. (Gao et al., 2003, Hua et al., 2003, Ohba et al., 2009) The ability of *D. radiodurans* to withstand such extreme conditions is attributed to a DNA repair mechanism which involves several genes including, *ppri*, *ppra*, *pprm*, and *reca*. ( Gao, et al., 2003; Hua et al., 2003; Kota & Misra, 2006; Lu et al., 2009; Ohba et al., 2009). Sequence database searches have revealed that *D. hopiensis* also contains *ppri*, *ppra*, *pprm*, and *reca* genes. Both *D. radiodurans* and *D. hopiensis* also contain a Late Embryogenesis Abundant (*lea*) protein, which is known to confer salt tolerance in plants and marine bacteria. (Liu et al., 2009)

The goals of our project are to characterize the effects of biological stressors on *D. hopiensis* and *D. radiodurans*, and to transform the genes of interest from both species into *E. coli* to determine how the same genes from different bacteria affect the ability of this commonly studied bacterium to withstand the environmental stresses.

In this study, we have focused on the late embryogenesis protein in *D. hopiensis*. We designed PCR primers to amplify the coding sequence of this gene from *D. hopiensis* genomic DNA. These primers were designed based on the Biobrick standard (Shetty et al. 2008), which facilitated the construction of an expression construct composed of a LacI-sensitive promoter, a ribosome binding site, the *lea* coding sequence, and a terminator sequence. This construct was transformed into competent *E. coli* (New England Biolabs 10-beta) cells for physiological testing. The expression construct allows us to control the expression of the gene of interest by induction with IPTG or lactose. Salt tolerance will be determined by measuring the growth rate of lactose-induced *E. coli* carrying the *lea* expression construct under different NaCl concentrations. Growth will be measured using a spectrophotometer to assess increased culture turbidity over time.

Currently, we have completed our first expression construct that contains the *lea* gene and successfully transformed this construct into *E. coli*. Following validation of our expression construct, we will test the effects of this gene on salt tolerance in *E. coli*. The next steps in this project include repeating the assays described above with the *lea* gene from *D. radiodurans*, and expanding our testing to include other genes implicated in resistance to desiccation and other environmental stressors. Ultimately, we hope to compare the stress-tolerance effects of genes from other species in the genus *Deinococcus* that have been discovered in Arizona soils.

## DETECTION OF MUTATIONS IN CANINE MDR-1 GENE USING NON-INVASIVE METHODS

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The Multiple Drug Resistance-1 gene (MDR-1) encodes P-glycoprotein (P-gp) which is found on hepatocytes, endothelial cells in the brain and the brush border of renal proximal tubular and gastrointestinal epithelium. This 170kDa protein is an ATP-binding cassette transporter used to export many structurally unrelated drugs and xenobiotics, including macrocyclic lactones, thus protecting cells from drug-related toxicity. These drugs are used to treat a wide spectrum of diseases in dogs, like cancer, bacterial infections and heartworm. In fact, one of the most broadly used macrocyclic lactone is ivermectin, which is used as a heartworm treatment and prophylactic (i.e. Heartguard).

Dysfunction of MDR-1 can lead to severe drug-mediated neurotoxicity that is frequently fatal in dogs. Clinical consequences can be seen in dogs that are homozygous or heterozygous for such MDR-1 mutations. Additionally, it is estimated that a very high percentage of 14 different herding breeds carry MDR-1 mutations. In fact, 57% of Collies and 46% of Australian Shepherds carry mutations in this gene. Thus in these breeds of dogs, mutations in MDR-1 have profound impacts on clinical approaches to diseases usually treated with macrocyclic lactones and other drugs transported by P-gp.

The goal of this research is to identify MDR-1 mutations that might compromise the function of P-gp. It will also establish systematic PCR-based methods to detect the identified MDR-1 mutations. These methods can be used clinically to screen dogs that will have high sensitivity to treatment with macrocyclic lactones and other drugs transported by P-gp.

A STUDY OF WATER QUALITY OF MANSKER CREEK IN DAVIDSON COUNTY AND  
THE OLD HICKORY WATERSHED AS INFLUENCED BY RESIDENTIAL AND  
INDUSTRIAL LAND USES

Maria White and Genna Batchelder and Parris Powers  
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Water samples were collected and water quality assessments were made at three sites on Mansker Creek in Davidson County as part of the Old Hickory Lake Watershed in the Cumberland River Basin in Tennessee. Stream water was sampled in 2013 in order to study effects of land use and development on the stream water quality impacted by agricultural, residential and industrial land use. Levels of inorganic ions ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ , and  $\text{SO}_4^{2-}$ ), DO, hardness and temperature were collected and examined based on land uses (commercial and residential areas) in the drainage areas for respective sites. Stream site assessments also included channel width, buffer width, flow rates, turbidity, canopy and outflows. Data suggest that stream water quality was affected by the section of the drainage area and stormwater impact in respective land uses.

## WATER SUSTAINABILITY

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I will be conducting research on water sustainability and water quality by conducting an investigation in a creek that runs from Millersville Tennessee all the way into a city called Hendersonville. I will map each location as well as test the water quantitatively. Testing the water quality with different styles of research analysis will lead into the main purpose of this investigation which is to provide the city with information in order to succeed as a community in the conservation of one of our most important sources of energy, water. My research will involve the testing of the water for nitrate, phosphate, ammonia, hardness, turbidity, oxygen levels, conductivity, pH levels in the water and in the soil surrounding each testing location. Finally sewage systems will be analyzed and urbanization as well as agricultural pollution that could possibly be the cause of the water pollution. This experiment will serve as a method to not only warn our community neighbors, but my main goal is to pursue adequate change and awareness into preservation of our water.

## SYNTHESIS OF ETHYLE CINNAMATE

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Ethyl cinnamate is a product that exists in natural storax and is found in the essential oil of cinnamon. It has many useful applications such as providing flavor to food, providing scent for fragrances and perfumes, and for pharmaceutical means. The purpose of the research project was to synthesize ethyl cinnamate from benzaldehyde utilizing skills acquired in organic chemistry. Ethyl cinnamate can be synthesized from benzaldehyde or the more common synthesis, cinnamic acid and ethanol. In this project benzaldehyde was used to synthesize cinnamic acid by utilizing the Perkin's reaction; combining acetic anhydride and anhydrous sodium acetate and refluxing the mixture using a condenser (Sethi. 2003). Ethyl cinnamate was then synthesized by reacting ethanol, cinnamic acid, and sulfuric acid (Marvel & King, 1929). Cinnamic acid was successfully synthesized from benzaldehyde utilizing the Perkin's reaction. Although a small percent yield, ethyl cinnamate was successfully synthesized by reacting the cinnamic acid with ethanol and sulfuric acid.