Abstract Submissions

Anthropology

Presentation

A Comparison of the Effects of Subsistence Patterns on Dental Macrowear

Sidney Thompson, BA, University of Indianapolis Biology Department, 1400 E Hanna Avenue, Indianapolis, IN 46227; Jeremy J. Beach, Ph.D., Indiana University School of Liberal Arts at IUPUI, Department of Anthropology, 425 University Boulevard, Indianapolis, IN 46202

Dental macrowear, or obliteration of hard dental tissues, has long been used by bioarchaeologists as a method of dietary assessment in both prehistoric and historical populations. Examination of macrowear levels within and between populations allows researchers to interpret changes and differences in subsistence patterns. This study aims to examine the dental macrowear, along with dental caries and antemortem tooth loss, of Orendorf, an indigenous population from the Central Illinois River Valley, and to compare those findings to a Mongolian sample from the Late Bronze-Early Iron Age and Xiongnu period (1200-300 BC and 500 BC-155 AD, respectively).

The Orendorf settlement in the Central Illinois River Valley was at its peak in the middle-Mississippian period (1200-1250 AD). Previous archaeological thought proposed that settlements in the Cahokia Hinterlands shifted to an agricultural subsistence economy, with maize at the epicenter. However, recent floral analyses have questioned the timeline, degree, and patterns of maize adoption and intensification in the Central Illinois Valley (e.g., VanDerwarker et al. 2016).

The Xiongnu, a mobile pastoral community with dairy and meat-based subsistence, were previously thought to have been partially sustained by agricultural foods from sedentary China. Comparing this population with the Late Bronze-Early Iron Age sample — little documented interaction with China — the senior author demonstrated both had similar amounts of dental macrowear, and thus similar maintained a diet of similar consistency (Beach 2017).

By comparing the macrowear of a Mississippian population to a Mongolian population, this study will examine the effects of different subsistence patterns (agricultural and pastoralism, respectively) on the dentition of the two populations.

Presentation

**Linguistics, Ancestry, and Race in the Field of Forensic Anthropology**

**Alba E. Craig**, BA, University of Indianapolis Human Identification Center; **Krista E. Latham**, PhD, D-ABFA, University of Indianapolis Human Identification Center.

Forensic anthropologists operate within and between the boundaries of a holistic, theoretical science and the practical applications of the US medicolegal system. While it is recognized that humans display normal variation that is not categorical in nature, forensic anthropologists still provide medicolegal authorities with an assessment of ancestry to aid in the identification of human remains. Though they should be aware of the differences between assessed skeletal ancestry and social race, the scientific literature suggests otherwise. The terminology for social race and that of ancestry are often used interchangeably in reference to human identification.

Select forensic anthropology articles were reviewed demonstrating a distinguishable change in how ancestry is defined and discussed. Various authors elected to emphasize that the exact terminology used in their publication may not encompass all individuals classified within the group. If this terminology cannot fully grasp the variation existing within one group, an emphasis towards changing the terminology should be the next step.

This presentation serves as a pathway through which questions, such as the following, should be considered: Why are researchers narrowing the definition of Hispanic and Black into distinct, smaller subpopulations while still using the definition of White to encompass individuals from various backgrounds? Why don’t forensic anthropology reports address these distinctions? Should the standardization of racial terminology used in forensic anthropology reports be considered? What is the obligation of the forensic anthropologist to consider the broader cultural ramifications associated with the use of certain racial terminologies in their reports?

Ultimately, this presentation will emphasize that the recognition of the undeniable relationship between social race and biological ancestry should be used as a way to open discussion about the use of suitable terminology to reach a more holistic way to define an individual’s ancestry and racial identity based on skeletal traits.
The Effects of an Automated Dishwashing Cycle on the Perseverance of DNA

Holley Gilmour, BS, University of Indianapolis Biology Department; Arden J Mower, BSc, University of Indianapolis Biology Department; Jessica Rivosecchi-Fulton, MS, University of Indianapolis; Daisy J Hernandez, University of Indianapolis; Rusvi Patel, University of Indianapolis; Krista E. Latham, PhD, D-ABFA, University of Indianapolis Biology Department; Lindsey Williams, BS, Strand Diagnostics; and Gay Bush, PhD, Strand Diagnostics.

A dishwashing cycle creates an environment that should accelerate DNA degradation by exposing the biological material to high temperatures, high moisture contents, and enzymatic detergents. However, this scenario has not been scientifically assessed to determine if DNA can persist on used eating utensils after undergoing a normal cycle in an automated dishwasher. If it is demonstrated that DNA can persist in these conditions, investigators should consider the “clean” utensils inside of a dishwasher as a potential source for DNA profile generation at a crime scene. This study aims to investigate DNA persistence on utensils within a dishwasher by transferring DNA from a participant’s mouth onto a spoon that had been decontaminated by exposure to UVR. The utensil was then placed into a decontaminated dishwasher caddy located within a dishwashing appliance. Eight trials were completed, for a total sample size of sixteen. A decontaminated spoon and a decontaminated spoon that underwent a one hour dishwashing cycle were sampled to test the effectiveness of UVR exposure and decontamination of the dishwashing appliance. DNA was purified from the swabs using the QIAmp DNA Mini Kit, and quantified utilizing Quantifiler Trio (Life Technologies Corporation, Carlsbad, CA) on an Applied Biosystems® (Foster City, CA) 7500 Real-Time PCR System. DNA was detected in 56% of the samples. However, the sample yields were below the amplification threshold and therefore DNA amplification was not attempted. Quantifiable DNA was not detected in either negative control, therefore it can be concluded that the DNA collected from the utensils was deposited by the participants and withstood the applied heat, humidity and detergents of the dishwashing cycle. This presentation will discuss the study results, the impact of heat, humidity and detergent on DNA preservation and potential applications of this study data to forensic investigations.
Poster

Expression of Osteoarthritis in the Elbow of a Modern Cadaver Sample: A Pilot Study

Rebekah Judkins and Stephen Nawrocki
University of Indianapolis

Osteoarthritis (OA) is an age-related degenerative condition that includes cartilage destruction and bony changes resulting from normal joint use during life. It is the second most common skeletal condition in the United States, affecting more than 30 million adults. Because of its wear-related nature, OA is highly correlated to chronological age and has the potential to be used in age estimation during skeletal analysis. This study investigates OA expression on all joint surfaces in the elbow of 43 modern cadavers. OA was scored at each joint surface – distal humerus, proximal ulna, proximal radius – using a 4-point scale, which measures: 1) degree of lipping, 2) circumference affected by lipping, 3) cartilage destruction, 4) bone exposed from cartilage destruction, and 5) eburnation. Cartilage destruction and bone exposure can only be scored on soft tissue, so they are not applicable in skeletal analyses. No significant differences were found between left and right sides, so these scores were averaged for each surface. Those averages were then summed for each individual to obtain a single score for each category. Ordinal regression and ANCOVA were used to interpret the effects of age and sex on OA severity. Similar to our research on the knee, sex does not seem to have a significant effect on the individual surfaces or on the elbow as a whole. However, when left and right are separated, sex is significant for cartilage destruction of the right radius. Age, however, has a significant effect on humerus lipping circumference, humerus cartilage destruction, radius cartilage destruction, and radius bone exposure. Additionally, the effect of age is suggestive for several other surfaces. Descriptive statistics indicate that certain soft tissue features have the highest rates of OA in this sample, suggesting that dry bone samples are not sufficient for interpreting OA progression in human remains.
A prehistoric Native American quarry into a massive stalagmite, known as the Pillar of the Constitution in Wyandotte Cave, Crawford County, Indiana, is unique in eastern North America. This speleothem is comprised of aragonite with thinly banded Mg-calcite, a composition known to be distinct from aragonite deposits in other caves in the region. Native American artifacts of Wyandotte aragonite are rare in museum collections. Artifacts made of Wyandotte aragonite have been identified at only eleven sites, primarily from Middle Woodland Hopewellian contexts dating ~1-400 CE. With two exceptions, the artifacts are complete or fragmentary platform pipes or reel-shaped, two-hole gorgets. An additional artifact suspected of being made of this material is a fragment of a reel-shaped gorget that was recently observed in a private artifact collection. It was collected from the Crib Mound site (12SP1-2), a Hopewell site with earlier and later components that has been lost to erosion along the Ohio River. The artifact was loaned for analysis to investigate its raw material and physical attributes. Raman spectroscopy identified the material as calcite (CaCO3), and microscopic examination revealed distinctive acicular fabric and banding typical of Wyandotte aragonite that has inverted to calcite. Specific gravity was found to not be distinctive of artifacts of Wyandotte aragonite once this material has been removed from the cave and recrystallized as calcite, undoubtedly reflecting variable depositional contexts and groundwater chemistry. The pattern of wear around the holes of the Crib Mound gorget informs about its likely function, as supported by experimental archaeology. A new AMS radiocarbon assay from the aragonite quarry provides additional information.
In 1814, Father George Rapp and approximately 1,000 fellow Germans established New Harmony, Indiana, in the frontier wilderness of the Wabash River Valley. The millenarian group, known as Harmonists, was renowned for communal living, hard work, and celibacy. The society became wealthy selling their goods to local settlers and exporting products to distant markets. The group resided in Indiana until 1825.

Harmonist settlements contained a commonly shared public garden. The garden at New Harmony was prominently, and symbolically, located adjacent to Rapp’s house. It contained an orangery that featured moveable walls that allowed it to be seasonally opened and closed and where citrus trees were grown. The New Harmony State Historic Site requested that we examine artifacts believed to be related to the Harmonist orangery. The objects included wood levers, seven wooden “rollers,” and saddles that precisely fit each roller. It was thought that the artifacts were used to open and close the orangery structure by rolling a large timber-framed structure that held large glass panels. Using wood and tree-ring analysis, along with primary sources, we attempted to determine if it is feasible that the artifacts were contemporary to the Harmonists, how the moving parts of the building functioned, and explain the Society’s interest in growing such plants.

A manufacture date was established by comparing the tree-ring growth patterns in the rollers and saddles with accurately dated regional tree-ring series. We identified the wood species to determine if the objects were constructed from regional forests or imported to Indiana, and if the physical qualities of the wood were appropriate for moving a framed structure. Archival sources describing eighteenth century precedents, maps, and written descriptions of the structure, were examined to place the Harmonist orangery within historic discourses of frontier Indiana, late 18th century glasshouse structures, and the Harmonists as transplanted German separatists.
**Osteoarthritis of the knee and its relationship with age at death in a modern cadaver sample**

**Amelia Lasbury** and Stephen Nawrocki, University of Indianapolis

Most elderly individuals eventually develop some degree of osteoarthritis (OA), a degenerative disease of the synovial joints. However, the accuracy of using OA to determine age at death for unidentified individuals in forensic casework is largely unverified. This study builds on our previous work with a modern Euroamerican anatomical cadaver sample. The knees of 67 individuals of known sex and age at death (43 to 97 years) were scored using 4-point semi-continuous ordinal scales. Variables included (1) the severity of peri-articular lipping, (2) the total circumference of lipping, (3) the degree of hyaline cartilage destruction, (4) the degree of subchondral bone exposure, and (5) the degree of subchondral eburnation (‘polishing’). Traits (3) and (4) are not normally available on dry bone specimens. Each trait was assessed individually and also summed with other traits, and the left and right sides were averaged for some tests. ANCOVA and ordinal regression were then used to determine the simultaneous effects of sex and age on the severity of arthritis. Unlike our previous work with a smaller sample, the sex of the individual does seem to play a role on the development of OA independently of age at death. Females display higher levels of OA than males and a tighter correlation with age. Right knees display higher levels of OA than left knees, but the effects of sex and side are secondary to the effects of age. Regression equations developed to predict the age of unknown individuals from the three arthritis variables available on dry bone display moderate success, with eburnation alone being the most sensitive (r-squared = ~0.45), especially in females. However, the lack of soft tissue indicators such as cartilage destruction significantly limits the anthropologist’s ability to estimate age from arthritic expression on dry bone.

HotTopics

**Compliance Science: How the Center for Archaeological Investigations Utilizes Forest Service Partnerships to Train the Next Generation of Researchers**

Ryan M. Campbell
Associate Director of the Center for Archaeological Investigations, Southern Illinois University

Angela R. Doyle
Heritage Program Manager/Tribal Liaison, Hoosier National Forest

Since 2014, the Center for Archaeological Investigations at Southern Illinois University (CAI) has brought undergraduate and graduate student researchers to the
forests of southern Indiana to collect archaeological data as part of a series of cooperative agreements with the Hoosier National Forest. These student researchers have explored thousands of acres of Indiana’s protected lands and helped evaluate a wide variety of archaeological sites including prehistoric Native American campsites, early African American and Euroamerican farmsteads, and early industrial sites like a nineteenth-century gristmill. In this talk, we review some of the surveys and site evaluations that these students have helped to complete, while highlighting how these types of projects create experiential learning opportunities that can bring students into contact with the methods used by professionals to document and interpret archaeological resources. These methods can include broadly applicable skills such as archival research, data processing, and technical writing, and skills more specific to archaeological research such as mapping, excavation methods, and artifact analysis. These projects also allow us to introduce these early career professionals to technical skills like geophysical survey, data processing with GIS software, photogrammetry, and more. Unlike traditional archaeological field schools, the research conducted through this partnership provides students with paid learning opportunities that can take them through all steps of archaeological research from the planning stages through the preparation of reports. The work prepares them for real-world jobs outside of academia in cultural resource management and other environmental compliance fields by giving them a strong foundation in the practical skills needed to complete this type of research.

**Botany**

**Poster**

**Screening Neurospora crassa knockouts for genes important to cold adaptation II: Recovery**

Jacob Nava, Allison Pariso, Tiffany Blacklidge, Mariam Zaki, Kirsten Treptow, Abishek Sehgal, Hailey Seiler, Cody Holmgren, Maya Leon, Valerie Vulpita, Megan Kelly, Abby Bridwell, Ngocmi Vo, and Michael Watters

Valpariso University, Valparaiso Indiana

Many organisms go through an adjustment in order to adapt themselves to changes in the environment. The fungus *Neurospora* normally maintains a fixed distribution of branching at extremes of temperatures, while select mutants show altered branching at temperature differences. This helps reveal genes important the *Neurospora*’s maintenance of homeostasis in the face of changing temperatures. In addition, both *Neurospora* and *E. coli* go through transient changes in response to cold shock, providing a second window on the maintenance of homeostasis.
Identifying genes important to the maintenance of morphological homeostasis will help improve our understanding of this process. Three groups of knockouts were selected to be screened: genes previously suspected to be involved in hyphal development as well as knockouts resulting in morphological changes; transcription factors; and genes homologous to E. coli genes known to alter their expression in response to cold shock. These have all previously been examined for their morphological variation during the apical phase of the response to cold shock. We conducted a screen of these same mutants for any morphological variation during the recovery phase of cold shock. The results, especially compared with the previous cold-shock results, provide an additional window on branching homeostasis in fungi. A total of 344 knockout strains were screened for morphological differences in growth at different temperatures. We report here the morphologies of strains subjected to this screen.

Poster
New species of Urocystis (Basidiomycota, Urocystidales) on Anemone acutiloba from Indiana

Huda Mahmood & Kyryll Savchenko

Butler University, Dept. of Biological Sciences, 4600 Sunset Ave., Indianapolis, IN 46208

Smut fungi are one of the largest groups of plant pathogenic fungi with more than 3,000 species found in every country of the world. One of the main genera of smut fungi, Urocystis is easily transmitted from plant to plant, due to the spores being very lightweight. The systematics and biodiversity of Urocystis are unknown in many parts of the world, including the United States. There are many different economically important plant species that are affected by smut fungi, including plants such as corn, wheat, sugarcane, sorghum, and sunflowers. However, smut fungi are not only restricted to crops, but they are also commonly found on wild and ornamental plants as well.

Smut fungi are known to be host specific. Although, smut fungi are posed as a threat to economically favorable crops, agriculture, and medical advances, the genetics between the host plants and pathogens are still unknown. On Urocystis Anemones specifically, there are about 31 currently known species (Vánky, 2012). To be able to classify species correctly, we conducted the morphological and molecular analysis that revealed a novel species of Urocystis on Anemone acutiloba. From the phylogenetic analysis, it can be seen that the new species is closely related to Urocystis anemones from Anemone nemorosa from Europe as well as may have originated from pathogens of grasses, likely due to a host jump event.
New Records of Macroscopic Mushrooms for Marion County, Indiana

Torey Kazeck & Kyryll Savchenko

Butler University, Dept. of Biological Sciences, 4600 Sunset Ave, Indianapolis, IN 46208

Ever since the ability to sequence DNA entered mycological studies, fewer mycologists are trained in fieldwork. As a result, there are very few new specimens to replace the aging specimens in the public herbaria, and work done on aged specimens is more difficult and more expensive. This has led to a resurgence of interest in macroscopic, mycological fieldwork to gather new materials to study as well as specimens that are currently undocumented. Furthermore, fungi, in general, are known for being understudied, unclassified, and unnamed. The latest, collective list of macroscopic mushrooms found in Marion County was published in 1947 by John O. Cottingham and was updated almost yearly until 1956, where the number of species totaled 387. As a result, new studies updating this list are necessary, and this study is the first step in doing so. In this study, macroscopic mushrooms were collected and identified based on morphological characteristics from Holcomb Gardens at Butler University in Marion County, Indiana. Twenty-one species were identified as new to Marion County: Bjerkandera adusta, Cerrena unicolor, Crepidotus crocophyllus, Cyathus striatus, Gymnopus luxurians, Hohenbuehelia angustata, Lentinellus micheneri, Mycena leptophylla, Mycorrhaphium adustum, Pachyella clypeata, Phellinus igniarius, Pluteus chrysophlebius, Poronidulus conchifer, Ramaria botrytis, Rhizomarasmius pyrrocepalus, Thelephora terrestris, Trametes pubescens, T. versicolor, T. villosa, Tremella foliacea, and Xylaria cubensis.

Presentation
Sourcing and Dating the Timber of The Captain’s House Using Tree-Ring Growth Patterns (Guilford, Dearborn County, Indiana)

Alexandra N. Ludwig, Biology Department, Hanover College, Hanover, IN
Christopher Baas, Department of Landscape Architecture, Ball State University, Muncie, IN
Darrin L. Rubino, Biology Department, Hanover College, Hanover, IN

Local Guilford, Indiana (Dearborn County) lore recalls a colorful farmer named Captain (John R. Kuhn). According to legend, his two-story, three-room, timber-frame house was built in the early 1800s. The small house is covered in board and batten siding and appears to be built almost entirely of coniferous wood. This is intriguing considering no conifers are native to the region except for red cedar and
because the local landscape was rich in hardwood timber readily available for construction. The objectives of this investigation were to accurately determine the build date of the Captain’s House, identify the species of timber used in its construction, and to determine the origin of the coniferous wood. Forty-five samples from 3 different timber species were collected from locations throughout the structure using standard dendrochronological techniques. After sanding the samples, individual tree rings were measured to the nearest 0.01 mm and assigned a calendar date by crossdating, a method of comparing tree-ring growth patterns in timber of unknown age to those of a known age. Dated tree-ring chronologies were created for hemlock (n = 29) and white oak (n = 7) samples; white pine samples did not crossdate reliably. The oak chronology consists of 605 rings (1652 – 1820) and matches the regional tree-ring signal well. The hemlock chronology consists of 2527 rings and spans from 1613 to 1874. We established an 1875 construction date for the Captain’s House, which is supported by historic Dearborn County archival sources. To determine the origin of the hemlock timber, dendroprovenancing was performed by comparing the timbers’ growth patterns with white pine and hemlock chronologies from throughout eastern North America. Crossdating suggested a strong match to trees from northwest Pennsylvania.

Poster

**Mass Flowering of *Aplectrum hyemale* in Hougham Woods Biological Field Station in Central IN**

**Bridget L. Crews, Gunnar Dittrich**, and Alice Long Heikens
Franklin College

Puttyroot, *Aplectrum hyemale*, is a perennial orchid that produces a basal leaf in the fall, overwinters, and flowers in spring as the leaf dies. It is found throughout the Midwest in relatively, undisturbed mesic woods, including Hougham Woods Biological Field Station in Johnson County, Indiana. The plant population biology of these orchids in Hougham Woods has been studied since 2011. While the population has remained relatively stable during this study (305-389), the number flowering plants has ranged from 0-96. Typically, only 5.5 percent of this population flowers each year. However, in 2019, there was abundant flowering with 27% of the 344 plants flowering and producing an average of 7 flowers/peduncle. Environmental data needs to be examined to determine what triggered the mass flowering event in 2019.
Characterizing the antimicrobial and anticancer activities and several associated bioactive compounds of *Argemone mexicana*

Danielle Orozco-Nunnelly (Valparaiso University)
**Theodore Lefeber** (Valparaiso University)
Teodora Najdeska (Valparaiso University)
Kelly Davidson (Valparaiso University)
Estefany Boncangel (Valparaiso University)
Jeffrey Pruet (Valparaiso University)

Commonly called the Mexican prickly poppy, *Argemone mexicana* is a stress-resistant member of the Papaveraceae family of plants that has been used in traditional medicine for centuries by indigenous communities in Mexico and Western parts of the United States. This plant has been used to treat a wide variety of ailments, including skin diseases and intestinal infections, with reported antimicrobial and anticancer properties. However, these properties are poorly understood, with few associated bioactive compounds yet identified. Herein, we describe the germination conditions of *A. mexicana* and preliminarily characterize the antimicrobial and anticancer activities of different parts (seeds, leaves, inner vs. outer roots) of the plant. We show that when comparing 1 mg of each sample normalized to background solvent alone, the *A. mexicana* methanol outer root and leaf extracts possess the strongest antimicrobial activity, with greatest effects against the gram-positive bacteria tested, and less activity against the gram-negative bacteria and fungi tested. Additionally, we report that when using the MTT colorimetric assay, the outer root and leaf methanol extracts and the seed hexane extract have pronounced inhibitory effects against T84 human colon cancer cells. Using normal-phase column chromatography and subsequent mass spectrometry analysis of the outer root and leaf methanol fractions, we have begun to chemically characterize several candidate antibacterial compounds. These preliminary results warrant further research into defining the bioactive chemicals produced in the roots, leaves and seeds of *A. mexicana* and are especially significant given the growing global concern of antibiotic-resistant ‘superbugs’ and lack of new antimicrobial and anticancer drug discovery.

Presentation
**Characterization of antimicrobial activity within Bryophytes during pathogen challenge**

**Viktor Stjepic** and Philip Villani, Butler University

Over time, organisms have evolved immune systems to defend themselves against pathogens. A focus is brought to one portion of the immune system: the innate
defense. The innate immune system is the first line of the body’s defense such as an organisms’ tissue or skin. Within these tissues are chemical components called antimicrobial peptides (AMP’s). AMP’s are proteins that rest on the cell membrane and fight pathogens through electrostatic repulsion (such as similar poles of a magnet). While these AMP’s are well studied in humans, the research on AMP’s is scant in Bryophytes (moss). The objective is to provide the support that AMP’s exist in moss through three research goals. The first objective of the research aimed to extract antimicrobial peptides from moss using a fungal elicitor. The extract was then plated on a bacterial lawn to measure defense. The second objective measured antimicrobial defense relative to the moss’s ecological niche (i.e. soil, rock, and tree). The third objective, through using the NCBI database, located putative genes in a model moss species relative to plants with antimicrobial genes that were characterized previously. Preliminary data showed a statistical probability that antimicrobial peptides are present in moss and have varying strengths relative to their ecological niche.

Presentation

**Identifying Construction Dates of the Huddleston Farm House and English Barn Using Tree-Ring Analysis**

**Darrin L. Rubino**, Alexandra Ludwig, Biology Department, Hanover College, Christopher Baas, Department of Landscape Architecture, Ball State University, and J.P. Hall, Department of Architecture, Ball State University

The Huddleston Farm served as a resting spot for nineteenth century travelers following the National Road (US 40 today). Currently, several buildings remain on this Wayne County property which is included on the National Register of Historic Places; the farmstead now serves as a museum. The main goal of this investigation was to use dendrochronology, tree-ring analysis, to determine the construction date for the Farm’s English barn and I-house which served as the Huddleston’s home and as an inn. Various sources differ on construction dates for the structures. Additionally, dating the buildings would lead to the creation of local tree-ring chronologies (series of accurately dated and measured tree rings) for a region that has received little dendrochronological study. Cores were collected from throughout the buildings using a borer and power drill. Sampling focused on timbers for which a death/felling date could be determined (presence of bark or a waney edge). The samples were prepared for dating following standard dendrochronological methodology (sanding, skeleton plotting, ring measurement). Dates were assigned to individual tree rings by crossdating (matching the patterns of ring widths in the timbers) with accurately dated regional chronologies. Fifteen samples (obtained from 14 timbers) in the barn yielded a beech chronology spanning from 1694 to 1840 (1475 rings). From the house/inn, beech (4 samples from 3 timbers) and tulip poplar (2 samples from 1 timber) produced
a chronology spanning from 1741 to 1842. Crossdating was successful for all timbers except for one beech in the house. Tree-ring analysis suggests that the barn was constructed prior to the house and that the rear ell of the house/inn is original and not a latter addition. These results will be used to enhance historical interpretation of the Huddleston Farm.

Cell Biology

Presentation

**Cotreatment of rifampin with ML141 enhances clearance of intracellular *Staphylococcus aureus***

**Melissa D. Evans**, Robert E. Sammelson, Ph.D., Susan A. McDowell, Ph.D.
Departments of Biology and Chemistry
Ball State University, Muncie, IN 47306

*Staphylococcus aureus* is a leading cause of severe infections worldwide, including infective endocarditis, skin and soft tissue infections, and bacteremia. Furthermore, *S. aureus* is a leading cause of persistent pulmonary infections in pediatric and adult cystic fibrosis patients. *S. aureus* can invade host cells and reside within the intracellular environment for extended periods of time. Treatment of intracellular bacterial infections is difficult because most antibiotics are unable to effectively penetrate the host cell plasma membrane. Rifampin, a lipophilic antibiotic, can readily penetrate the host cell plasma membrane and kill intracellular bacteria. However, high dose rifampin treatment is associated with rapid resistance development and severe adverse side effects. Rifampin often is administered with other antibiotics in an effort to combat the development of adverse side effects and resistance, but the continued emergence of antibiotic resistant bacteria is a concern. Prior research in our lab found that a host-targeted therapeutic, ML141, reduces invasion of *S. aureus* into host cells. We hypothesized that decreasing the number of bacteria invading the host cell using ML141 will enable a lower dose of rifampin to be used to clear the reduced intracellular bacterial population. We found that cotreatment of ML141 with rifampin enhanced clearance of intracellular *S. aureus*, through non-bactericidal and non-cytotoxic mechanisms.
Preliminary Analysis of Parotoid Gland Histology of the Fowler’s Toad, *Anaxyrus fowleri*

**Arden Mower, BS1; Sonal Baidwan, MS1; Emily Vasconcelles, MS1; Dr. Kevin Gribbins, PhD1**

1University of Indianapolis, 1400 E Hanna Ave, Indianapolis, IN, 46227

Parotoid glands are large, external poison glands located on the dorsum of toads, spanning from the caudal head to the shoulders. Toads stimulate these glands to produce bufotoxins when threatened, which produces an alkaline milky secretion. Few studies have documented the histology of parotoid glands, which would provide information on their physiological mechanisms and modes of action. This study was conducted to histologically examine the parotoid glands of *Anaxyrus fowleri*. Toads were obtained from Columbus, IN. They were euthanized using MS22 and glands were dissected and stored in formalin or Trumps. The glands were later dehydrated in ethanol, embedded in plastic, sectioned, and mounted onto slides. The samples were stained using basic fuchsin and toluidine blue. Slides were then examined using light microscopy. Large poison glands containing many protein granules were visualised with three distinct syncytial layers, which have been previously cited in similar studies of other toads. The poison glands are lined by a single layer of myoepithelium. Other expected structures included mucus glands embedded in the dermis with ducts travelling to the surface of the epidermis and blood vessels travelling underneath the poison glands. Two previously undocumented cells/structures were observed in connection to the poison glands, including foot-like cellular processes that originate from the epithelium that is juxtapositioned to the deep blood vessels. The poison glands were then examined using transmission electron microscopy to observe the organelles within the secretory epithelium of the glands in order to better deduce their function within these toad’s parotoid glands.

Poster

**Mutations of Ubiquitin Proteasome System Genes Protect Cells from the Effects of Anisomycin**

**Julia M. Niekamp**, Avery M. Runnebohm, Brian M. Voas, Conner G. Bailey, Rachel M. Vachon, David A. Huston, Eric M. Rubenstein

Department of Biology, Ball State University

The rates of protein synthesis and degradation are carefully controlled to maintain protein molecules at the appropriate abundance and in the appropriate conformations. The highly conserved ubiquitin proteasome system (UPS) maintains protein
homeostasis. This balance can be disrupted; for example, the antibiotic anisomycin, which is produced by the bacterium *Streptomyces griseolus*, interferes with translation by inhibiting polypeptide elongation. Anisomycin has been found to impair new memory formation in animal studies and has been proposed as a therapeutic for disorders associated with traumatic memory formation (such as post-traumatic stress disorder). Previous work revealed that *Saccharomyces cerevisiae* strains lacking specific protein quality control genes exhibit growth advantages in the presence of anisomycin. This was surprising, since anisomycin is expected to promote formation of aberrant, truncated protein molecules. Yeast strains lacking a limited panel of genes involved in protein quality control and regulated protein degradation were also tested for resistance to anisomycin studied, and no clear pattern to which mutants exhibited a growth advantage was observed. Ongoing efforts are aimed at comprehensively evaluating the roles of UPS genes in anisomycin resistance. Toward this end, yeast lacking each of 95 genes involved in protein degradation are being cultured in the presence and absence of anisomycin. Following growth assays, data will be analyzed to identify commonalities in genes conferring similar responses to anisomycin. Since the UPS is overwhelmingly conserved between yeast and humans, this work may contribute to an understanding of the cellular response to anisomycin and its potential therapeutic role for memory-associated disorders.

Poster

**Substrate specificity of Ste24 in targeting translocon-clogging proteins at the endoplasmic reticulum**

**Brian M. Voas**, Eric M. Rubenstein
Department of Biology, Ball State University

Cells continuously create and degrade proteins. Some proteins are made in the cytoplasm and need to be transported into the endoplasmic reticulum. To complete this task, proteins move through a channel called the translocon in a process called translocation. Mutations in the translocon are associated with multiple human diseases, including diabetes and immune disorders. Translocation may occur cotranslationally or post-translationally. Occasionally, the mRNA encoding a protein will aberrantly lack a stop codon, which results in translation of the 3’ untranslated region and ribosomal stalling if a stop codon is not fortuitously encountered. If the protein is ER-targeted, the protein will also stall within and clog the translocon. The ubiquitin ligase Rkr1 (which is conserved from yeast to humans) recognizes when a ribosome is stalled, and ubiquitylates the polypeptide chain stalling in the ribosome and translocon, resulting in its proteasomal degradation. By contrast, the protease Ste24 (also conserved from yeast to humans) cleaves proteins that are stalled in the translocon post-translationally. The aim of this study is to test the hypothesis that Ste24 acts upon co-translationally stalled polypeptides, using budding yeast as a
model organism. The hypothesis is being tested via western blot analysis in which abundance of model translationally stalled translocon-clogging protein is compared in wild type cells, cells lacking Rkr1, and cells lacking Ste24. Initial findings suggest that Ste24 does not act upon co-translationally stalled proteins at the translocon. These results indicate a high substrate specificity for the enzymes that target translocon-clogging proteins.

Presentation

**ER stress impairs degradation of translocon-clogging proteins by a novel mechanism**

Courtney L. Broshar*, Bryce W. Buchanan*, Adrian B. Mehrtash**, Avery M. Runnebohm*, Brian J. Snow*, Laura N. Scanameo*, Mark Hochstrasser**, Eric M. Rubenstein*

* Department of Biology, Ball State University, ** Department of Molecular Biophysics and Biochemistry, Yale University

Endoplasmic reticulum (ER) stress arises when ER proteins misfold and accumulate. Multiple diseases are associated with elevated levels of ER stress, including certain cancers, neurodegeneration, and heart disease. Cells respond to ER stress by activating four signaling pathways that function to restore ER homeostasis. Misfolded and otherwise aberrant ER-resident proteins are degraded through one of several branches of ER-associated degradation (ERAD). One branch of ERAD (called ERAD-T, for ERAD of translocon-associated proteins) is the destruction of proteins that clog the translocon, the channel through which proteins must pass to enter the ER. We found that ER stress caused by protein misfolding specifically impairs ERAD-T in the model organism *Saccharomyces cerevisiae*. We compared the effects of ER stress caused by protein misfolding and ER stress caused by disruption of lipid homeostasis on ERAD-T; ERAD-T was unaffected by lipid perturbation. Several different forms of cellular stress also did not impair ERAD-T. This suggests that ERAD-T is specifically impaired by misfolded ER-resident proteins. We tested whether the four characterized ER stress-response signaling pathways are required for impairment of ERAD-T during ER stress. Surprisingly, we found that none of these pathways impair ERAD-T during stress, suggesting the existence of a novel ER stress-responsive mechanism that impairs ERAD-T. We speculate that ERAD-T impairment is protective to cells experiencing ER stress, preventing the movement of additional proteins into an already stressed ER. Understanding how cells respond to ER stress may inform the treatment of diseases associated with stress.
Presentation

The Role of Nuclear Proteins in Unclogging Endoplasmic Reticulum Channels

Samantha M. Turk, Danielle L. Overton, Cade J. Orchard, Christopher J. Indovina, Avery M. Runnebohm, Sarah M. Engle, Sheldon G. Watts, Julia M. Niekamp and Eric M. Rubenstein

Department of Biology, Ball State University

Proteins are essential to life. They perform a variety of functions within the cell, from cell regulation to DNA synthesis. Just as important as protein synthesis is the process of protein degradation. When a protein is no longer necessary, is damaged, or behaves aberrantly, it must be degraded to prevent organismal harm. One way a protein behaves aberrantly is by persistently engaging with (or clogging) the translocon. The translocon is a protein channel that allows proteins to move across the membrane of the endoplasmic reticulum. One protein known to clog translocons in humans is a component of low-density lipoproteins (or “bad cholesterol”). A ubiquitin ligase in yeast known as Hrd1 polyubiquitylates the translocon-clogging protein, tagging it for degradation by the proteasome. The proteasome detects polyubiquitylation and degrades tagged proteins, breaking them into shorter fragments. Ubiquitin ligases rarely work alone; they function with accessory proteins called cofactors. Further, yeast lacking Hrd1 still exhibit residual degradation of translocon-clogging proteins, suggesting the existence of alternative degradation pathways. A previous master’s student performed a genome-wide screen to identify genes that may play a role in degradation of translocon-clogging proteins. With over 150 genes identified in the screen, small-scale reporter assays were performed, confirming potential roles for 42 genes in protein degradation. The confirmed genes are currently being biochemically validated by cycloheximide chase to visualize protein degradation over time. With the process of protein degradation being conserved in both yeast and humans, validated genes may represent therapeutic targets for patients with elevated levels of cholesterol.

Poster

Investigation of the role of FSHR-1 in neuromuscular signaling in C. elegans

Alyssa Ritter, Amy Godfrey, Allyson Munneke, and Jennifer R. Kowalski
Butler University

Neuronal communication is critical for organism function and involves a balance of excitatory (stimulating) and inhibitory neuronal signals (E:I balance). Caenorhabditis elegans roundworms are simple models used to study E:I balance. FSHR-1, follicle hormone steroid receptor, is conserved in mammals where it regulates gonad function;
in *C. elegans*, it controls E:I balance at neuromuscular junctions (NMJs), where muscle contraction is governed by signals from excitatory and inhibitory motor neurons. Although FSHR-1 is expressed in *C. elegans*’ nervous system and intestine, the cell(s) where FSHR-1 acts to control neuromuscular signaling are unknown. We hypothesized *fshr-1* is sufficient in neurons to restore normal E:I balance at the NMJ. In rescue experiments using pharmacologically-induced paralysis to measure muscle contraction, worms without *fshr-1* had decreased muscle contraction; replacing *fshr-1* in all neurons or under its own promoter restored wild type contractions. Worms expressing *fshr-1* in inhibitory neurons showed partial recovery, and animals expressing *fshr-1* either in intestine or excitatory neurons displayed increased contractions relative to wild type worms. We saw similar results in swimming assays, except worms with excitatory neuron *fshr-1* expression had over-rescued contraction in the paralysis assay but no rescue of swimming behavior. This may imply *fshr-1* acts in multiple neuron types or other tissues to affect muscle contraction. *C. elegans* and human nervous systems are similar, so learning more about FSHR-1 may help research into human neurological disorders. Future directions will use these assays to explore *fshr-1* function in diverse physiologic states (e.g. oxidative stress) in which FSHR-1 has been implicated.

**Poster**

**Modification of the Endoplasmic Reticulum Translocon Impairs Protein Translocation**


Department of Biology, Ball State University

The endoplasmic reticulum (ER) is the entry point for most proteins residing and functioning in the eukaryotic endomembrane system. The primary mechanism by which proteins enter the ER is via the translocon complex. Dysfunction in this complex can block access into the ER, which is detrimental to cellular health. The translocon is highly conserved and has been intensely studied in *Saccharomyces cerevisiae*. Analysis of translocon function in protein trafficking, localization, and interactions in yeast has been facilitated by the use of epitope tags. We have found that a tag on the translocon pore subunit previously suggested not to impair translocon function subtly affects translocation of proteins into the ER in yeast cells. Intriguingly, this tag also suppresses a phenotype associated with defective protein quality control pathways, consistent with a functional link between translocation and quality control. Ongoing work includes characterizing the effects of placing different epitope tags on different translocon subunits, with the goal of identifying tags that affect translocon function the least.
**Notch receptor 3 signaling regulates autocrine and paracrine communication in the myeloma tumor niche**

Hayley M. Sabol, David L. Halladay, Nori Kurihara, G. David Roodman, Teresita Bellido, Jesus Delgado-Calle

1Marian University College of Osteopathic Medicine, 2Indiana University, Department of Medicine, Division of Hematology/Oncology, 3Department of Anatomy, Cell Biology, and Physiology, 4Department of Medicine, Division of Endocrinology, 5Indiana Center for Musculoskeletal Health, Indiana University School of Medicine, 6Richard L. Roudebush Veterans Affairs Medical Center, Indianapolis, IN, US.

Multiple myeloma (MM) is a hematological cancer characterized by expansion and accumulation of malignant plasma cells in the bone marrow (BM). Interactions between MM cells and cells of the bone/BM niche activate Notch signaling, which promotes tumor growth and bone destruction. The goal of this study was to characterize the effects of the genetic deletion of Notch receptor (NR) 3 in MM cells on cell proliferation, cell death, gene expression, and in co-culture with osteocytes. NR3 mRNA and protein expression was undetectable in cells transduced with shRNA-NR3, whereas the expression of NR1, NR2, and NR4 remained unchanged compared to control cells. NR3 silencing decreased the mRNA expression of Notch target genes and cyclinD1. NR3-silenced cells displayed a reduction in cell proliferation/number compared to control cells. There were modest increases in cell death in NR3-silenced cells. The mRNA expression of Rankl was decreased, which was accompanied by an increase in OPG. Conditioned media (CM) from control cells increased osteoclast formation in a dose dependent manner, while the number of osteoclasts stayed the same in CM from NR3-silenced cells, showing that NR3 silencing decreases the osteoclastogenic potential of MM cells. Co-culture with osteocytes increased MM cell proliferation and NR3 signaling inhibition reduced the increase in proliferation induced by osteocytes. Silencing NR3 partially prevented the upregulation of Notch target genes and CyclinD1 induced by co-culture with osteocytes. This suggests NR3 signaling contributes to the communication between MM cells and osteocytes, but is not the only NR involved. Treatment with GSI prevented the increase in proliferation induced by osteocytes. Using *ex vivo* cultures, we found less tumor burden in bones bearing NR3-silenced cells compared to control cells. Our findings support that autocrine NR3 signaling in MM cells drives proliferation, favors survival, and enhances the osteoclastogenic potential. Further, paracrine NR3 signaling contributes to the pro-proliferative effects of osteocytes on MM cells.
The G protein-coupled receptor FSHR-1 regulates synaptic vesicle and active zone protein localization at *C. elegans* neuromuscular synapses

**Morgan Buckley**, Julie Kolnik, Sarah Olofsson, and Jennifer R. Kowalski

G protein-coupled receptors (GPCRs) are a class of multi-functional cell membrane proteins that are targets of many current drug therapies. In the nervous system, GPCRs are receptors for diverse signaling molecules and can impact neuronal communication. Follicle Stimulating Hormone Receptor (FSHR-1) is a conserved GPCR found in the nervous system of *Caenorhabditis elegans* roundworms. At *C. elegans* neuromuscular junctions (NMJs), a balance of excitatory and inhibitory signals regulates muscle contraction. Previous data showed that the gene encoding FSHR-1 is required for normal neuromuscular function, as blocking *fshr-1* expression causes reduced muscle contraction. This is likely due to decreased synaptic vesicle (SV) release, as SV accumulation occurs at motor neuron presynapses in mutants lacking *fshr-1*. Active zone (AZ) proteins are also located at presynapses where they regulate synapse structure and SV release. We imaged NMJ synapses within wild type and *fshr-1*-deficient worms expressing fluorescently labeled AZ proteins (UNC-10::GFP, SYD-2::GFP, and CLA-1::GFP) in motor neurons and measured relative AZ protein quantity. *fshr-1* mutants exhibited robust increases in UNC-10::GFP and modest increases in SYD-2::GFP synaptic and axonal abundance in excitatory motor neurons. However, levels of CLA-1::GFP were unchanged. Together these data suggest FSHR-1 signaling could result in direct or indirect effects on the AZ proteins UNC-10 and SYD-2 to regulate SV release and neuromuscular signaling. Future experiments will determine the cell types where FSHR-1 acts to control these effects. Given the conservation of *C. elegans* and human nervous systems, determining the role of FSHR-1 may aid understanding of human neurological disorders.

Destabilizing the Stabilizer: Evaluating Microtubule Destabilizing Proteins and Cellular Energetics in Acquired Paclitaxel Chemoresistance

**Keeley Cleghorn**, Indiana State University and Catherine E. Steding, Indiana State University

Breast cancer remains one of the most diagnosed cancers in American women. It is expected that approximately 40,000 women will succumb to advanced, metastatic disease in the next calendar year. Advanced and recurrent breast cancers frequently exhibit clinical resistance to therapeutic interventions with many exhibiting resistance against multiple drugs or therapies. Chemoresistance can be classified as either innate or acquired both of which result in dramatically different consequences following
therapeutic intervention. Work in our laboratory has emphasized evaluating acquired chemoresistance against the microtubule-stabilizing drug, paclitaxel, and has demonstrated the ability to generate cells significantly more resistant than cells treated with solvent controls. These paclitaxel-resistant cells provide a critical model system for evaluating mechanisms of acquired chemoresistance. Findings from preliminary investigation of these chemoresistant cells indicated both novel growth patterns and dose-dependent reactions to paclitaxel. Observed changes in cellular behavior imply a previously unreported mechanism of chemoresistance including involvement of unique microtubule associated proteins previously only implicated in other cancers. These findings demonstrate potential markers for chemoresistance and pave the way for further analysis of the role of microtubules and microtubule associated proteins in the cellular behavior of cancer.

Presentation

**Exploring the signaling pathway of the G protein-coupled receptor FSHR-1 in regulating neuromuscular function in C. elegans**

**Abigail Shores**-Butler University  
David Ryskamp-Butler University  
Sarah Oloffson-Butler University  
Allyson Munneke-Butler University  
Amy Godfrey-Butler University  
Dr. Jennifer Kowalski-Butler University

A required balance of excitatory and inhibitory neuronal signals (E:I balance) in the nervous system is responsible for many functions, including speech and cognition. Nerve cells utilize many of the same regulatory molecules as other cells to control E:I balance, including membrane-integrated G-protein coupled receptors (GPCRs) which require binding of a ligand to activate a relay protein pathway. Previous data show that, in the model roundworm *C. elegans*, the GPCR FSHR-1 controls E:I balance at the neuromuscular junction to regulate movement, likely through promoting synaptic vesicle release. The human receptor, FSHR, works in gonadal tissue similar to worm FSHR-1; however its possible role in the nervous system is unknown. Considering knowledge of the gonadal functioning mammalian FSHR and worm FSHR-1 signaling pathways, as well as the known *C. elegans* NMJ relay proteins, we hypothesize that *C. elegans* require a neuronal GSA-ACY-PKA pathway to control synaptic vesicle release and E:I balance at the NMJ. Thus, we predict that loss of function of genes involved in this pathway will result in abnormal muscle contraction, and that because FSHR-1 can work in multiple cell types, these phenotypes may differ among cells. Using behavioral assays, we found/confirmed that the genes acy-3, unc-10, unc-2, and egl-36 are all required at the *C. elegans* NMJ and are poised to explore their activity downstream of FSHR-1 signaling. Clarifying the mechanisms of FSHR-
Signaling in the nervous system will provide new knowledge likely relevant to mammalian FSHR biology, potentially providing insight into diseases in which E:I imbalances occur.

**HotTopics**

**OMM Virtual Reality Application: Advancing the Osteopathic Medical Education**

**Anthony J. Perugini, OMS II1,2Y; Sergej Stjepic, OMS III1,2Y; Bradley J. Boget, OMS III1,2Y; Garren P. Gebhardt, DO, MS2,3; David L. Dufeau, PhD1,2**

13D Visualization Laboratory, Marian University College of Osteopathic Medicine, Indianapolis, IN, USA; 2 Department of Biomedical Sciences, Marian University College of Osteopathic Medicine, Indianapolis, IN, USA; 3 Department of Clinical Affairs, Marian University College of Osteopathic Medicine, Indianapolis, IN, USA;

Y Contributed equally to this work

**Objective:** The methods of instruction, mainstays of the disciplines of Anatomy and Osteopathic Manipulative Medicine (OMM) in the past, are discussed here; additionally, the presentation of exciting innovations in virtual reality, haptic feedback, and instructional simulation that current and future medical students stand to benefit from. The advancements at the *Marian University Visualization Laboratory* with respect to instructional 3-Dimensionally rendered anatomic models for the instruction of OMM are also discussed.

**Design/Methods:** Data from two-dimensional Computerized Tomography (CT) images were analyzed, interpolated, and rendered to create three-dimensional bones and muscles. These renderings were used to create educational simulations of physiologic kinetic three-dimensional movements and musculoskeletal pathologic correlates taught in Osteopathic medical schools across the United States. The movements modeled were: forward and backward rotation of the left innominate and Fryette motion (Type I and II) in the first and second lumbar vertebrae. These movements were uploaded to Sketchfab 3D visualization website, creating three-dimensional spatially manipulatable skeletal models of pathology. A brief application to the osteopathic educational experience, compared to standard didactic modalities, will demonstrate education, diagnosis, and treatment of a innominate musculoskeletal pathology using these virtual simulations to highlight the applications they have to the future of osteopathic medical education.

**Results:** Renderings of 3-Dimensional anatomic models were realized and used to construct narrated instructional video as well as static, labeled, virtual reality
compatible 3-Dimensional anatomic models. These renderings have been used in the instruction of Osteopathic medical students at Marian University College of Osteopathic Medicine (MUCOM).

**Conclusion:** Academic disciplines that require a higher degree of mastery and understanding of visuospatial concepts can be greatly aided via forthcoming technological advances. Moreover simple readaptation and adoption of currently available technology, towards these pursuits, is paramount and currently ongoing at institutions such as MUCOM and other institutions of higher learning nationwide.

**Poster**

**Depletion of Notch target genes impairs fertility**

**Joseph Ballard, Nick Fox, Erika Sorensen-Kamakian**

Wabash College

Notch signaling regulates stem cells and differentiation during normal animal development and when dysregulated can lead to cancer. In the model organism *C. elegans*, functional Notch signaling is essential for several aspects of reproduction and fertility. 1) Within the somatic tissue, Notch signaling is required to form the egg-laying apparatus via vulval development. 2) Within the germline (the lineage of cells responsible for producing gametes), Notch signaling, initiated from the somatic gonad, functions in both the distal and proximal ends. In the distal germline, a single-celled niche initiates Notch signaling to maintain a population of germline stem cells (GSCs) in an undifferentiated, self-renewable, and totipotent (capable of giving rise to all cell types) state. GSCs function to create a continuous supply of gametes (here sperm and oocytes) that can give rise to a new animal. In the proximal germline, recent reports suggest that the gonadal sheath initiates Notch signaling and functions to maintain proper ovulation. Although Notch clearly plays an important role in animal fitness, key *in vivo* molecular effectors/targets of Notch signaling are still largely unknown. Here we describe that Notch target genes play a role in several aspects of fertility. *mir-61* and *mir-250* reinforce GSC maintenance. These microRNAs (miRNAs) act post-transcriptionally to limit expression of target genes. Loss of these miRNAs reprograms germ cells into somatic cells (*i.e.* neurons) suggesting that these microRNAs repress somatic gene expression to maintain GSCs in a totipotent state. Furthermore, we also identify a role for cell cycle genes *lin-9* and *mcm-6*, Notch signaling targets, in ovulation. Loss of these targets impairs ovulation and results in the formation of polyploid oocytes. We propose that *C. elegans* offers a simplified model for how Notch signaling and its effectors regulate fertility broadly.
Workshop
Expansion Microscopy: a broadly accessible method for super-resolution microscopy using standard light microscopes

Douglas H. Roossien, Ph.D.
Ball State University, Department of Biology

The advent of super-resolution fluorescent microscopy has enabled glimpses into the cell at resolutions below the ~200 nm diffraction limit of a standard light microscope. This new technique preserves the high-contrast and molecular specificity advantages of fluorescent microscopy while offering resolution previously achievable only with electron microscopes. However, super-resolution microscopy is both cost-prohibitive and technically challenging for most laboratories. In this 60 minute workshop, we will discuss an alternative technique called Expansion Microscopy (ExM). The concept behind ExM is to improve resolution by physically expanding the sample rather than by optical magnification. The chemicals required for ExM are affordable and the procedure is accessible to undergraduate and graduate students. Despite the simplicity, there are nuances worth considering when implementing ExM into a research laboratory. The intent of this workshop is to introduce background, theory, and tools necessary to apply ExM to a broad range of systems. We will first discuss the theory and basic chemistry behind ExM. This will be followed by a brief introduction to the variety of ExM protocols that have been published and discuss the range of current and potential applications. We will then focus on procedural details, which will include common pitfalls and tips for troubleshooting. Participants are encouraged to bring questions about their own interests and application for ExM. Dr. Douglas Roossien was one of the early end-users of ExM through a collaboration with Dr. Ed Boyden, the pioneer of ExM at MIT, which resulted in a 2016 Nature Biotechnology paper. He has previously facilitated two laboratory workshop modules on ExM as part of an NSF-sponsored education program. Dr. Roossien started his own laboratory in 2019 at Ball State University, where they employ ExM to study the development of complex morphologies in the Drosophila brain.

Poster
The Role of the Wnt Signaling Pathway in Chemoresistance

Danielle Muse, Keely Cleghorn, Kathryn Besserman, Katelyn Sidloski, Emily Dircks, Dr. Catherine Steding

As both technology and research continue to progress within the field of personalized molecular medicine, novel advancements to the breast cancer platform are announced almost daily. In spite of this, thousands of women still succumb to advanced, metastatic breast cancer each year. It has been shown that an atypical Wnt signaling
pathway response plays a significant role in breast cancer development and chemoresistance; however, the mechanisms behind the development of true acquired chemoresistance remain poorly defined. Wnt signaling manages many vital cell processes including proliferation and differentiation through the use of various enzymes. PORCN is a membrane-bound enzyme that is essential in the Wnt pathway particularly for activities such as Wnt transportation. Preliminary evidence in the laboratory demonstrated a potential role for PORCN in chemoresistance against the microtubule stabilizing drug, paclitaxel. Altering the function of PORCN may impact the proliferative ability of breast cancer cells in the presence of such therapeutics. Through up-regulation and down-regulation of the Wnt pathway with an activator and an inhibitor, respectively, analysis of PORCN activity and overall Wnt activity in chemoresistance is possible. Our results demonstrate the significant importance of these molecules to acquired chemoresistance and supports further investigation into the role of transportation and signaling in the acquisition of chemoresistance.

Poster

**Effect of TNF-α on C. albicans Morphological Change in Liquid Media**

Chase Jones*, Alyssa Graziani*, Michael K Watters and Patrice G Bouyer (*equally contributed Author) Valparaiso University

*C. albicans* must undergo a morphological change in order to infiltrate the epithelial cell layer and pose a health threat due to this activity. This morphological infectious state is known as filamentous due to the elongated protrusion that stems from the base yeast form. To date, the intestinal cues responsible for the change in *C. albicans* morphology are not clearly defined. Candidiasis is observed in Crohn’s disease, where elevated level of inflammatory cytokines such as tumor necrosis alpha (TNF-α) is reported. We hypothesize that TNF-α may represent an intestinal cue responsible for the shift in morphology in *C. albicans*. We tested our hypothesis in three different liquid media: Minimal (MM), Yeast Extract Peptone Dextrose (YEPD), and Spider media (SM). In our experimental conditions, we found that Fetal Bovine Serum (FBS) induced filamentous growth 66% of the time in SM, 16% of the time in YEPD and none in MM (n = 18 plates for each condition). While both YEPD and SM saw significant impact on filamentous growth with FBS, SM showed greater significance so this condition was used in the TNF-α study. In a preliminary study, we found that TNF-α on its own did not cause filamentation (n = 15). Yet, TNF-α totally inhibited FBS-induced filamentous growth, while FBS induced 20% filamentous growth in our control (n=15). In conclusion, we found that FBS-induced filamentous growth is dependent on the type of liquid media used. In addition, our preliminary experiments show that TNF-α prevent filamentous growth and may represent a protective mechanism during candidiasis observed during Crohn’s disease.
Introduction:
Candida albicans (C. albicans), an opportunistic pathogen, lives symbiotically within the intestine of its human host. Temperature and chemical factors have been shown to induce a morphological change in C. albicans from yeast to filamentous form turning C. albicans pathogenic. In this study, we investigated the intestinal cues that might be responsible for the change. We found that different solid media impact the morphological phenotype so we focused on characterizing these before further testing. We tested Estradiol (E2) because of its known linkage to sepsis and higher levels during infections. Experiments were conducted to compare solid agar plates of YEPD, Minimal Media (MM), and Spider Media (SP) for C. albicans growth to choose the best one for further testing with E2 and other factors that could be prone to causing morphological changes.

Methods:
C. albicans was inoculated through streak method on different solid media (YEPD, MM, SP) and incubated at 30°C. The effect of 0.1nM E2 on C. albicans morphology was also tested. Morphological changes were assayed through bright-field microscopy.

Results:
Using the three different medias, we found three distinctive phenotypes: A,B,C. Out of 6 experiments of 14 MM plates, the expressed phenotype was 86% A and 14% inconclusive of the time. 8 experiments of 17 SP plates showed 100% of phenotype B. 6 experiments of 14 YEPD plates presented phenotype C 92% of the time and 8% inconclusive. For E2 trials, 2 experiments, 6 MM plates showed 50% phenotype A and 50% inconclusive. 4 experiments, 10 SP plates had phenotype B 100%. YEPD 2 experiments, 2 plates had phenotype C at 100%.

Conclusion:
We have established experimental conditions of media controls for further testing whether E2 and other cues, such as inflammatory cytokines, have inhibitory or positive effects on the growth of C. albicans.
Role of adipose triglyceride lipase and CGI-58 in lipid droplet breakdown during *Coxiella burnetii* infection

Michael J. Newman, Rachel Silliman, Minal Mulye
Division of Biomedical Sciences, Marian University College of Osteopathic Medicine

*Coxiella burnetii* is an obligate intracellular bacterium responsible for causing culture negative endocarditis. The manifestation of this condition several years following initial infection exhibits *Coxiella*’s ability to persist long-term in alveolar macrophages. Our overall goal is to identify the strategies *Coxiella* employs for successful survival in the host. In our previous studies, examination of *Coxiella*-infected alveolar macrophages revealed accumulation of host lipid storage organelles called lipid droplets (LDs). Host LD alteration was dependent on *Coxiella* Type 4 Secretion System (T4SS) which releases bacterial proteins in the host cytoplasm and manipulates several host cell processes. Further, manipulating LD metabolism significantly altered *Coxiella* growth. Specifically blocking the activity of the LD breakdown enzyme adipose triglyceride lipase (ATGL) completely inhibited *Coxiella* growth suggesting that ATGL-mediated LD catabolism is important for bacterial intracellular survival. Based on our preliminary studies, we hypothesize that *Coxiella* manipulates host cell ATGL to promote LD breakdown and support intracellular bacterial growth. To test this, we quantitated ATGL gene expression level in uninfected, wild-type *Coxiella*-infected and T4SS-mutant *Coxiella*-infected alveolar macrophages. Compared to uninfected cell, *Coxiella*-infected cells demonstrated increased ATGL gene expression in a T4SS-dependent manner suggesting importance of ATGL during *Coxiella* infection. Since ATGL activity depends on co-activator CGI-58 binding, we determined CGI-58 gene expression levels in differentially infected cells. However, no differences were observed in CGI-58 suggesting that *Coxiella* specifically manipulates ATGL via its T4SS and not its co-activator. Ongoing studies are determining if *Coxiella* manipulates ATGL protein level and activity. Completion of our studies will identify the mechanism *Coxiella* employs to manipulate host LD homeostasis to promote its intracellular survival.
Poster

Neuromedin U (NMU) regulates osteoblast differentiation and activity

Yu-Tin Hsiao, (a), (b) Kelli J. Jestes,(a), (b) Krista L. Jackson,(a), (b) Tara Zukosky,(c) Maria E. Squire,(c) Julia M. Hum, (a), (b) Jonathan W. Lowery(a), (b), (d)
(a) Division of Biomedical Science, Marian University College of Osteopathic Medicine, Indianapolis, Indiana, USA
(b) Bone & Mineral Research Group, Marian University, Indianapolis, Indiana USA
(c) Department of Biology, The University of Scranton, Scranton, Pennsylvania, USA
(d) Indiana Center for Musculoskeletal Health, School of Medicine, Indiana University, Indianapolis, Indiana, USA

Osteoporosis is a disease of low bone mass that places individuals at enhanced risk for fracture, disability, and death. Osteoporosis rates are expected to rise significantly in the coming decades yet there are limited pharmacological treatment options, particularly for long-term management of this chronic condition. The drug development pipeline is relatively bereft of new strategies, causing an urgent and unmet need for developing new strategies and targets for treating osteoporosis. Here, we examine a lesser-studied bone remodeling pathway, Neuromedin U (NMU), which is expressed in the bone microenvironment along with its cognate receptors NMU receptor 1 (NMUR1) and 2 (NMUR2). We independently corroborate a prior report that global loss of NMU expression leads to high bone mass and test the hypothesis that NMU negatively regulates osteoblast differentiation. Consistent with this, in vitro studies reveal NMU represses osteoblastic differentiation of osteogenic precursors but, in contrast, promotes osteoblastic marker expression, proliferation and activity of osteoblast-like cells. Phospho-profiling arrays were used to detail differential signaling outcomes that may underlie the opposite responses of these cell types. Collectively, our findings indicate that NMU exerts cell-type-specific responses to regulate osteoblast differentiation and activity.

Presentation

Cotreatment of rifampin with ML141 enhances clearance of intracellular *Staphylococcus aureus*

Melissa D. Evans1, Robert E. Sammelson, Ph.D.2, Susan A. McDowell, Ph.D.1
Departments of Biology1 and Chemistry2
Ball State University, Muncie, IN 47306

*Staphylococcus aureus* is a leading cause of severe infections worldwide, including infective endocarditis, skin and soft tissue infections, and bacteremia. Furthermore, *S. aureus* is a leading cause of persistent pulmonary infections in pediatric and adult
cystic fibrosis patients. *S. aureus* can invade host cells and reside within the intracellular environment for extended periods of time. Treatment of intracellular bacterial infections is difficult because most antibiotics are unable to effectively penetrate the host cell plasma membrane. Rifampin, a lipophilic antibiotic, can readily penetrate the host cell plasma membrane and kill intracellular bacteria. However, high dose rifampin treatment is associated with rapid resistance development and severe adverse side effects. Rifampin often is administered with other antibiotics in an effort to combat the development of adverse side effects and resistance, but the continued emergence of antibiotic resistant bacteria is a concern. Prior research in our lab found that a host-targeted therapeutic, ML141, reduces invasion of *S. aureus* into host cells. We hypothesized that decreasing the number of bacteria invading the host cell using ML141 will enable a lower dose of rifampin to be used to clear the reduced intracellular bacterial population. We found that cotreatment of ML141 with rifampin enhanced clearance of intracellular *S. aureus*, through non-bactericidal and non-cytotoxic mechanisms.

**Presentation**

**The effect of a major G-quadruplex helicase on toxic transcription in ALS.**

**S.N. Sandwith** 1, M.E. Sharlow.1, Y.J. Tseng 5, H. Raimer, 6, E. D. Routh 2, B. E. Beerbower 3, A. E. Chambers 1, A. E. Richardson 1, J. Wang 4, S. A. Goutman 5, E. L. Feldman 5, M. A. Smaldino 1, Y. H. Wang, 6, J. P. Vaughn 2, P. K. Todd 5, P. J. Smaldino 1

1Department of Biology, Ball State University, Muncie, IN
2Department of Cancer Biology, Wake Forest School of Medicine, Winston-Salem, NC
3Department of Plant Sciences, North Dakota State University, Fargo, ND
4Department of Biochemistry and Molecular Biology, John Hopkins School of Medicine, Baltimore, MD
5Department of Neurology, University of Michigan, Ann Arbor, MI
6Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, NC

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that results in the breakdown of motor neurons, leading to severe deficiencies in eating, breathing, and moving; 80% of all ALS patients succumb to the disease within 5 years of diagnosis. The most common ALS-linked mutation occurs in the C9ORF72 gene (*C9*), consisting of a GGGGCC-sequence which, becomes repeated hundreds to thousands of times, compared to healthy individuals who have C9 sequence folds into extensive G-quadruplex (G4) DNA structures, which form within guanine-rich DNA and RNA sequences. G4s generally provide negative regulation on transcription. G4-helicases such as G4R1 (aliases: DHX36 and RHAU), potentially provide positive regulation on
these processes via unwinding G4 DNAs. C9-repeat G4-DNA structures are partially unwound and transcribed, and the resulting RNAs coalesce into toxic RNA foci that sequester RNA binding proteins. It is unknown if G4R1 binds to the C9-repeat expansion or if it affects C9-repeat transcription. We hypothesized that G4R1 directly binds to and unwinds C9-G4 DNA and increases the transcription of toxic C9-repeat RNAs. To test this, we expressed and isolated recombinant G4R1 (rG4R1) and performed gel mobility shift assays with synthetic C9-G4 DNA. Furthermore, we used a C9-repeat in vitro transcription assay to determine if rG4R1 affects C9-repeat transcription. We found that G4R1 directly and preferentially binds to C9-G4 DNA and affects the transcription of toxic C9 RNA. These observations are consistent with a model where G4R1 plays a fundamental role in ALS pathogenesis.

Presentation
Role of RNA-binding proteins Rbfox11 and Rbfox2 in neuronal development and behavior in zebrafish

**Michael A. Berberoglu**, Duaa Hijaz, Romeo Karris, Allison Pariso, Trupti Patel, Margaret Schlicker, Yonahga Stickles
Valparaiso University; 1610 Campus Drive East, Valparaiso, Indiana 46383 USA

RNA-binding proteins RBFOX1/Rbfox1 and RBFOX2/Rbfox2 have been implicated in neurodevelopmental and neuropsychiatric disorders including autism, anxiety, schizophrenia, and epilepsy in mammals and have also been shown to play a role in cerebellar development. However, the role of Rbfox proteins in neuronal development and function has not been well-studied. We have recently identified expression of Rbfox1l (Rbfox1-like) in a restricted population of neurons within the adult zebrafish dorsal telencephalon (Ma et al., 2019). These Rbfox1l-positive neurons span Dm and Dc, which are thought to represent amygdala-like and putative cortical-like regions, respectively. We would like test the hypothesis that Rbfox1l regulates neuronal development and/or function in the telencephalon using rbfox1l CRISPR mutants (unpublished mutants will be obtained in collaboration with Dr. Sharon Amacher at The Ohio State University, OSU). In addition, we will determine the developmental stage when Rbfox1l is first expressed in the zebrafish brain, and test the hypothesis that Rbfox1l is involved in autistic-like and anxiety-related behaviors by performing behavioral tests in the mutants. In contrast to mammals, zebrafish have a remarkable capability to regenerate tissue of the brain and spinal cord after injury. Our preliminary data (OSU) suggest that adult viable rbfox2 CRISPR mutant zebrafish (rbfox2 mutants will be obtained in collaboration with Dr. Sharon Amacher at OSU) have a smaller cerebellum, as has been observed in Rbfox2 knockout mice. We will test the hypothesis that Rbfox2 is necessary for regeneration of the adult zebrafish cerebellum and for adult regenerative neurogenesis by performing a needle-stick injury paradigm together with BrdU pulse-chase experiments to assess neuronal
production. Our work aims to better understand the role of Rbfox RNA-binding proteins in neural development and behavior.

Presentation

Inhibiting Apoptosis as a Novel Treatment for Retinitis Pigmentosa using a Transgenic Zebrafish Model.

Emre Coskun; Purdue University, Logan Ganzen; Purdue University, Yuk Fai Leung; Purdue University.

Retinitis pigmentosa (RP) is the most common cause of night blindness, it affects 1 in 4,000 people worldwide, and it has no cure. RP is a heterogenous disease that can be inherited through many mutations in the human rhodopsin gene, including the Q344X mutation. This mutation is associated with autosomal dominant RP in humans. The goal of this project is to use a transgenic zebrafish model expressing the Q344X mutation in the human rhodopsin gene to discover an effective treatment for RP. The Q344X rhodopsin is truncated, which leads to its mislocalization and ultimately results in rod photoreceptor cell degeneration. Rod degeneration in the Q344X larvae show onset by 5 days post fertilization (dpf) and progresses significantly by 7 dpf. The 7 dpf Q344X larvae experience a diminished behavioral response to dim light preceding a light offset as analyzed by the Visual-Motor Response (VMR) assay. This suggests that the behavioral phenotype of Q344X is due to rod degeneration. Previous research proposed that rods degenerate through apoptosis. Therefore, the Selleckhem Apoptosis Compound Library was selected to identify compounds that can inhibit apoptosis and save rods. My hypothesis is that compounds that inhibit apoptosis would decrease rod degeneration and enhance VMR in the Q344X mutant. The Q344X larvae are treated with these compounds between 5-7 dpf and the VMR is analyzed at 7 dpf. So far, 6 compounds have enhanced VMR in the Q344X mutant. A TUNEL assay and immunostaining against active caspase-3 on retinal cryosections will determine the extent of apoptosis in the rods of Q344X larvae treated with identified compounds. The rod number will be quantified using the fluorescence from a Tg(-3.7rho:EGFP) reporter for rod visualization. Identified compounds will provide insight into specific target molecules involved in rod apoptosis and can be further developed as RP treatments in the future.
G4R1 (DHX36/RHAU) facilities *in vitro* transcription of toxic RNAs linked to ALS.

M. E. Sharlow 1, S. N. Sandwith 1, Y. J. Tseng 5, H. Raimer 6, E. D. Routh 2, B. E. Beerbower 3, A. E. Chambers 1, A. E. Richardson 1, J. Wang 4, S. A. Goutman 5, E. L. Feldman 5, M. A. Smaldino 1, J. P. Vaughn 2, Y. H. Wang 6, P. K. Todd 5, P. J. Smaldino 1

1Department of Biology, Ball State University, Muncie, IN
2Department of Cancer Biology, Wake Forest School of Medicine, Winston-Salem, NC
3Department of Plant Sciences, North Dakota State University, Fargo, ND
4Department of Biochemistry and Molecular Biology, John Hopkins School of Medicine, Baltimore, MD
5Department of Neurology, University of Michigan, Ann Arbor, MI
6Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, NC

G-quadruplex (G4s) DNA structures form within guanine (G) rich sequence and negatively regulate transcription. An aberrant excess of G4s underpins a substantial number of neurodegenerative diseases, an observation that is especially evident in amyotrophic lateral sclerosis (ALS). ALS causes muscle atrophy and the loss of motor control, including the inability to eat, speak, move, or breathe. This fatal disease currently has few treatment options, and patients often succumb to the disease within five years of diagnosis. The most common inherited ALS mutation occurs in *C9ORF72* (C9), consisting of a GGGGCC-repeat expansion. Healthy individuals have ~2-23 GGGGCC repeats in C9, while C9 ALS patients have between 100-1000+ C9 repeats. These repeats form extensive G4 DNA structures which are transcribed into toxic RNAs. Expanded repeat RNAs coalesce into foci that sequester RNA binding proteins and trigger aberrant translation of toxic proteins. We propose that these structures are fundamental drivers of neurodegeneration in C9 ALS. G-quadruplex helicases unwind G4 structures and may play important roles in C9 ALS. G4 resolvase 1 (G4R1; aliases DHX36 and RHAU), a G-quadruplex helicase, tightly binds and unwinds G4 DNA. Thus, G4R1 has the potential to increase the abundance of toxic RNAs by unwinding C9-repeat G4 DNA. We hypothesized that G4R1 unwinds C9-repeat DNA, allowing RNA polymerase to further transcribe toxic RNA sequences. We tested this hypothesis using an *in vitro* transcription system that included a C9-repeat plasmid as a DNA template. We demonstrate that G4R1 affects transcription of C9 repeat RNA, suggesting a potential role in ALS pathogenesis.
Zoledronate (ZOL) is used to treat bone resorptive diseases such as osteoporosis and cancer metastases to bone. ZOL is associated with medication-related osteonecrosis of the jaw (MRONJ), a condition known to cause bone destruction and maxillofacial region lesions. Geranylgeraniol (GGOH) is a potential rescue agent that provides an alternate route in the mevalonate pathway when the pathway is inhibited by ZOL. This study measured the viability of normal human oral keratinocytes (NHOKs) when exposed to ZOL and when GGOH was added under varying conditions to rescue the ZOL-induced viability loss. Viability was measured by an MTT assay in which viable cells convert water-soluble (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into an insoluble, formazan product. Quantitation was performed by measuring absorbance of the product at 570 nm (630 nm reference) after 72 hours of treatment incubation. NHOKs grown on uncoated 96-well plates showed significantly higher corrected absorbance values (p < 0.035) than NHOKs grown on poly-L-lysine-coated plates; consequently, successive experiments were performed on uncoated plates. We tested rescue of NHOKs when 10 microM GGOH was added 15 minutes prior to ZOL, 24 hours prior to ZOL, or simultaneously with ZOL. In the 15 minute-prior variation, 25 and 50 microM ZOL significantly decreased viability but GGOH did not rescue. The 24 hour-prior and the simultaneous addition variations displayed significant ZOL-induced viability loss at 10, 25, and 50 microM. Only when ZOL and GGOH were added simultaneously did significant rescue occur. These results inform successive NHOK experimentation on the relative efficacy of rescue agents to rescue from ZOL-induced viability loss. This work was partially funded by an Indiana Academy of Science senior research grant.
Nitrogen-containing bisphosphonates (NBPs) are a common medication used to treat bone resorptive diseases. A complication that can arise from NBP use is medication-related osteonecrosis of the jaw (MRONJ). MRONJ involves deconstruction of bone and soft tissue in the maxillofacial region. Zoledronate (ZOL) is the most potent NBP. The goal of this study was to compare the ability of geranylgeraniol (GGOH) to the ability of sodium citrate in rescuing osteoblasts from ZOL-induced cell death. The study utilized MTT viability assays of MC3T3-E1 cells (a murine osteoblastic cell line). Sodium citrate is a calcium chelator and theorized to chelate ZOL-bound intracellular calcium, thus displacing ZOL from the cell. Geranylgeraniol (GGOH) rescues osteoblasts and oral keratinocytes from ZOL-induced death effects in vitro, as reported by others previously. Cells were incubated for 72 hours after the simultaneous addition of 50 microM ZOL with either 2.5, 5, or 10 milliM sodium citrate. Only at 10 milliM was a rescue observed (1.53-fold increase in viability over ZOL-treated, p=0.08). In parallel, cells were incubated for the same time after the simultaneous addition of 50 microM ZOL with 0.5, 5, or 10 microM GGOH. GGOH mildly rescued at all three concentrations tested (p<0.05, p<0.001, p<0.01 for each of 0.5, 5, and 10 microm, respectively). all concentrations were determined by dose-response curve experiments in our lab in addition to literature values. the preliminary results suggest sodium citrate may rescue from zol-induced death in osteoblasts; however, additional control experiments are required to confirm successful sodium citrate-mediated chelation of intracellular calcium. this work was partially funded by an indiana academy of science senior research grant and a university scholar award from indiana wesleyan university.
Medication-related osteonecrosis of the jaw (MRONJ) is characterized by an 8-week period of exposed necrotic bone and a lack of soft tissue regeneration in the mouth. This condition can be brought on by treatment with nitrogen-containing bisphosphonates as well as other classes of drugs. Zoledronate (ZOL) is a nitrogen-containing bisphosphonate (N-BP) used to treat post-menopausal osteoporosis and cancers that have metastasized to bone. This study used an MTT cell viability assay in normal human oral keratinocytes (NHOKs) to produce dose-response curves at 48 and 72 hours for ZOL as well as three candidate rescue compounds. Geranylgeraniol (GGOH), known to rescue NHOKs from ZOL-induced death effects in vitro, was tested as well as phosphonoformic acid (PFA), and sodium citrate. The two latter compounds were chosen based on characteristics that are thought to counteract the effects of ZOL. NHOKs were seeded into uncoated 96-well plates for growth to confluence, then treated over a time course of 48 and 72 hours to determine ZOL and rescue agent concentrations for use in future viability rescue assays. At both time points, 50 and 100 microM ZOL caused significant viability loss. The maximal doses of the rescue agents that did not cause significant viability loss at 48 hours were 10 microM GGOH, 0.5 milliM PFA, and 2 milliM sodium citrate. In contrast, at 72 hours the following doses significantly reduced viability (each p<0.01 compared to the respective vehicle control): 1 microM GGOH (85% viability), 0.025 milliM PFA (83%), and 0.2 milliM sodium citrate (85%). These dose levels inform successive experiments to determine the relative efficacy of each agent in rescuing from ZOL-induced death in NHOKs. This work was partially funded by an Indiana Academy of Science senior research grant and a University Scholar Award from Indiana Wesleyan University.
Generation of novel triple transgenic mice to study frontal temporal dementia.

Michael Reisinger1, Abbie Scott1, Hasna Alashi1, Yuh-Wah Wang2, Peter K Todd3, Bart Pederson1,4, Melissa A. Smaldino1, James P. Vaughn4, Philip J. Smaldino1

1Department of Biology, Ball State University, Muncie, IN
2Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA
3Department of Neurology, University of Michigan, Ann Arbor, MI
4Indiana University School of Medicine, Muncie, IN
5Nanomédica, Inc., Winston-Salem, NC

Frontal temporal dementia (FTD) is the most common early onset dementia. There are currently no disease-modifying treatments available. A hexanucleotide repeat expansion mutation (GGGGCC)n in the gene C9orf72 (C9) is the most common inherited cause of FTD. C9 repeat expansions sequences form G-quadruplex structures as DNA and RNA. Expanded repeat RNAs coalesce into foci that sequester RNA binding proteins. These repeats also trigger aberrant translation of toxic proteins. We propose that these structures are fundamental drivers of neurodegeneration in C9 FTD. G4 resolvase 1 (G4R1; aliases DHX36 and RHAU) is the major human G-quadruplex helicase with dual specificity for DNA and RNA G-quadruplexes. Thus, G4R1 has the potential to increase the abundance of both toxic RNAs and dipeptide repeat proteins by unwinding C9 DNA and RNA G-quadruplexes, respectively. Studies that examine the roles of G4R1 in C9-FTD have been limited to cell line models. A mouse model of C9-FTD is currently available, which harbors a C9 expansion of 450 GGGGCC-repeats. These mice exhibit an age-dependent decrease in spatial memory and increase in anxiety levels analogous to C9-FTD, and has allowed us to develop a system where the role of G4R1 in C9-FTD can be studied in vivo. We have generated a novel mouse line containing three transgenes (loxP-G4r1-loxP, Cre-ERT, and C9(GGGGCC)450), allowing for inducible knockout of G4r1 in a C9-FTD mouse model. By using this model, we expect to be able to test the hypothesis that reduction of G4r1 will ameliorate progression of C9-FTD.
Determining the Effects of Knocking Out the Major G-quadruplex Helicase, G-quadruplex Resolvase 1 (G4R1) in Mice

Hasna Alashi1, Michael Resinger1, Abbie Scott1, Tyler Osborne1, Bart Pederson1,2, Melissa A. Smaldino1, James P. Vaughn3, Philip J. Smaldino1
1Department of Biology, Ball State University, Muncie, IN
2Indiana University School of Medicine, Muncie, IN
3Nanomedica, Inc., Winston-Salem, NC

Abstract
G-quadruplexes are secondary DNA and RNA structures found in guanine-rich sequences. They negatively regulate major cellular processes including replication, transcription, regulation, translation and telomere extension. In neurons, G-quadruplexes regulate local translation of hundreds of mRNAs at the dendritic synapse. In addition, G-quadruplexes can confer neuroprotective effects to cells during certain cellular stresses. Disruption of G-quadruplex processes underlies a number of neuromuscular diseases such as amyotrophic lateral sclerosis (ALS), frontal temporal dementia (FTD) and Huntington’s disease (HD). G-quadruplex Resolvase 1 (G4R1; aliases DHX36 and RHAU) is the major human G-quadruplex helicase with dual specificity for DNA and RNA G-quadruplexes. G4R1 is highly expressed in neurons and has been shown to effect dendrite formation in culture neurons. We hypothesize that G4R1 is important for maintaining normal neuronal development and maintenance, and consequently normal motor and cognitive functions. To test this hypothesis, we have developed a whole-body (G4R1-/- (+)) inducible-mouse model. We expect that G4R1 knockout in a mouse model will cause impaired motor and cognitive function similar to the ones observed in neuromuscular diseases. Considering the prevalence of disrupted G-quadruplexes processes in neuromuscular diseases, we expect this work to have broad-reaching impacts on our understanding of the basic mechanisms of neuromuscular biology and disease.
Duloxetine Improves Light Sensation in a Zebrafish Model of Retinitis Pigmentosa

Rebecca James, Purdue University; Logan Ganzen, Purdue University; Yuk Fai Leung, Purdue University.

Retinitis Pigmentosa (RP) is a degenerative eye disease that affects the vision of 1 in every 4000 people. RP is caused by rod photoreceptor degeneration in the retina, which has deleterious effects on dim-lit vision. This project investigates the effects of the serotonin–norepinephrine reuptake inhibitor duloxetine as a potential treatment for RP. In order to examine the effect of this drug on RP, a zebrafish model with a Q344X rhodopsin RP mutation was used in a behavioral assay to screen for a visual motor response (VMR). The assay measured the swimming displacement of larvae after a light stimulus is turned off. Increased swimming activity indicated an increased VMR. The mutant larvae utilized in this assay begun experiencing rod degeneration at 5 days post fertilization (dpf), which increased by 7dpf. Therefore, RP mutants in this assay do not possess the healthy rods required to elicit a strong VMR in response to scotopic light. When the RP mutants were treated with duloxetine on 5dpf, a strong VMR response was generated in multiple separate trials. The average displacement value for each trial was calculated: RP mutants had an average VMR of 0.0396cm and duloxetine-treated RP mutants had an average VMR of 0.0649cm. A Welch’s Two Sample t-test comparing these average values yielded a p-value of 0.03035, indicating a significant difference between duloxetine-treated Q344X larvae and untreated Q344X larvae. VMR assays on zebrafish treated with duloxetine show that there is a statistically significant difference in the startle response between treated and untreated mutants.

Assessing the Combinatory Effects of Ellagic Acid, Garcinol, and Benzyl Isothiocyanate on Breast Cancer Cell Proliferation

Mary Grace Ertel, Spencer Klimek and Kimberly M. Baker
University of Indianapolis

The natural phytochemicals ellagic acid, garcinol, and benzyl isothiocyanate have anticancer properties and the use of these dietary agents in combination may be effective for the chemoprevention and therapy of breast cancer. In this study, we evaluated the antiproliferative effects of ellagic acid, garcinol, and benzyl isothiocyanate, individually and in combination, using MCF-7 breast cancer cells. We found that ellagic acid, garcinol, and benzyl isothiocyanate each inhibited MCF-7 cell proliferation in a dose dependent manner. Furthermore, we found that co-
administration of lower doses of ellagic acid and garcinol, led to a greater inhibition of cell proliferation than either agent alone at the same dose and indicate an additive effect. These results suggest that combinatorial treatments using ellagic acid, garcinol, and benzyl isothiocyanate may be an effective chemotherapeutic strategy against breast cancer.

Presentation

**Portable Sensory Device that Improves the Gait Cycle of Patients with Proprioception Loss**

Emily Clark, Research and Development Division, Health & Science Innovations, Inc.; Saketh Boyapalli, Research and Development Division, Health & Science Innovations, Inc.; Luis Palacio, Research and Development Division, Health & Science Innovations, Inc.

Proprioception is our perception and awareness of the position and movement of our bodies. Those with proper proprioception can sense limb movement without visual recognition [Gandevia/Proske, 2016]. Patients with proprioception loss have difficulty walking and their gait cycles can be improved by utilizing a portable sensor device that tracks their movements. In this project, the variables tested will include the dorsiflexion of the foot and the circumduction of the leg [Cowan/Hillman, 2014]. The motion sensors used shall track these movements and an app will analyze the information and provide it to the user. Through reliance of the app and small cognitive adjustments made through physical therapy, improvements in patient gait cycles over time are predicted. In order to track the lack of foot dorsiflexion and excess of leg circumduction, a combination of surface electromyography (EMG), accelerometer and gyroscope sensors will be used. The surface EMG sensor will detect amounts of foot dorsiflexion by recording muscle activity of the tibialis anterior muscle [Lee, 2018]. The accelerometer will record the distance and direction of the linear motion in the x, y, and z axes [Neyyan, 2017]. The gyroscope will detect the degree of leg circumduction by measuring the rotation and angular velocity of the leg about the x, y, and z axes [Neyyan, 2017]. Since only two gait cycle variables are being tested with this project, in the future, more variables of the gait cycle will be added. Variables will later include, upward and downward slope of the terrain, along with different phases of the gait cycle. This ensures a more accurate representation of an individual's gait cycle. Additionally, the app will need to be more accessible for users, and shift toward an easier to understand user-interface. This device and its data will benefit the user and physical therapist, allowing for more accurate treatment and diagnosis.
**Chemistry**

**Presentation**
**Rieske Business: Development of a Directed Evolution Platform for Rieske Dioxygenase Engineering**

**Jordan T. Froese (Ball State University)**, Brian O. Bachmann (Vanderbilt University)

Today, fossil fuels are so commonly employed by the chemical industry as feed stocks for reactions, and as sources of the heat and pressure that drive them, that the chemical industry’s use of petroleum currently accounts for 14% of all greenhouse gas emissions, and the chemical industry is set to become the single largest driver of global oil consumption by 2030. In the Ball State Laboratory for Biocatalysis Research, we strive to develop new green-chemical tools that can contribute to alleviating the chemical industry’s reliance on fossil fuels.

With the recent advances in the fields of Synthetic Biology and Directed Evolution, the potential for engineering enzymes as robust, selective, and environmentally benign chemical catalysts has exploded. In the Ball State Laboratory for Biocatalysis Research, we have developed a novel fluorescence-based assay system that has allowed us to pursue the engineering of Rieske dioxygenases. Rieske dioxygenases, with their unique ability to perform oxidative dearomatization to produce chiral diol metabolites, have long been utilized as green-chemical catalysts by synthetic chemists, although their utility has been limited by their substrate scope and selectivity. By applying our novel assay system, we are developing new catalysts based on Rieske dioxygenases, which will expand the chemical utility of this class of enzymes.

**Poster**
**Purine Scaffolds Toward a New Class of Antibacterial Agents Targeting Gram-Negative Drug-Resistant Bacteria**

**Roslyn Lampkins**, Timmothy Miller, Phillip Wittel, Katarina Rodriguez, and Tirtha Bhattarai
University of Evansville

Antibiotic resistance is one of the greatest challenges currently facing our world. The number of people succumbing to drug-resistant infections per year in the U.S. and Europe has reached approximately 50,000 and is expected to reach 10 million by the year 2050 if left unchecked. Our group’s work involves synthesizing, characterizing,
and computationally investigating a purine-derived molecule that we designed to exhibit antibacterial activity against gram-negative bacteria. This molecule is expected to be rigid, flat, and polar which will allow it to enter the bacterium by penetrating its two cell membranes and porin protein channels. Once inside the bacterium, molecular recognition design features will allow this agent to strategically bind with the ATP binding site of the DNA gyrase enzyme (GyrB), a target that has been successfully less explored than the “cleavage/ligation” active site of the DNA gyrase enzyme. This binding is expected to inhibit the enzyme’s biological activity of replicating bacterial DNA by essentially cutting off its ATP fueled energy supply, which will lead to the bacteria’s inability to divide and replicate, ultimately resulting in the death of the cell.

Presentation

An Interesting observation in Analogy to the Beer-Lambert Law – the Discovery of Critical Phase-Transition Concentration (CPC)

Cody Leasor and Zhihai Li*
Department of Chemistry, Ball State University, Muncie, IN 47304

In this presentation, a unique feature in the adsorption and molecular assembly discovered by classic electrochemical cyclic voltammetry (CV) and electrochemical scanning tunneling microscopy (EC-STM) techniques will be reported. The aromatic carboxylic acid (ACA) composed of one phenyl ring and a single -COOH, benzoic acid (BZA) behaves drastically different from other ACAs. In this work, we have systematically varied the BZA concentration, and found that the number of current peaks in cyclic voltammograms (CVs) started to change from one to three at a concentration that we refer to “critical phase-transition concentration (CPC)”. Further, we discovered that the peak position in the CVs shifted as a function of solution concentration, resulting in larger peak separations between either anodic or cathodic peaks as concentration increases. Interestingly, just as the Beer-Lambert law can be used to determine the solution concentration via the absorbance-concentration relationship, similarly, the linear relationship in the plot of peak separation vs. concentration (ln[BZA]) also can be used to determine an unknown concentration of BZA solution.
Dye Sensitize Solar Cell Preparation for Solar Energy Conversion

Kristina Crumpacker, Maggie Sutton, Nilupa Siriwardana, Amir Hosseini and Zhihai Li*
Department of Chemistry, Ball State University, Muncie, IN 47306

Dye Sensitize Solar Cells (DSSCs) are one of the most efficient third-generation solar technology available, absorbing more sunlight per surface area than standard silicon-based solar panels. The mechanism by which solar cells convert light into energy can be likened to photosynthesis due to the way in which it mimics nature’s absorption of light energy. Exploring how different parameters can affect the energy conversion efficiency of DSSCs is important in developing high-efficiency solar cells. In this poster, we will show our recent development on how to fabricate lab-based solar DSSCs, including preparation of anode and cathode electrodes, sealing of electrodes, introducing of dye molecules and injection of electrolyte, using standard dye N-719 for testing and developing the cell preparation procedure.

Reference

Towards Aqueous Batteries: Electrochemistry of Silver Molybdenum Oxides

Paul F. Smith, Brendan Godsel, Allen Huff, Julie Pohlman-Zordan

There have been recently renewed efforts to expand the capabilities of the aqueous “Zinc ion” battery, which can potentially deliver the same energy as the lithium ion analog for only 25% of the cost and without using flammable components. To attain this, electrodes are required which must increasingly support faster electron transport. Towards this goal, the development of cathodes containing Ag+ is pursued for fundamental interest because, when reduced, the product can contain a network of atomically dispersed, conductive Ag0 particles that support fast electron transport throughout the bulk. Silver vanadium oxide (SVO) cathodes remain the dominant material in implantable cardiac defibrillator batteries due to this reason. However, it has recently been shown that the formation of silver metal can be intriguingly dependent on the intercalating cation, with silver metal forming at different discharge depths for the same cathode in sodium vs. lithium batteries. Hence, given the extensive optimization of the SVO cathode in Li batteries, it cannot be assumed that
in zinc systems there exists a well-established view of how much Ag is required, where it should be located, and, if formed in-situ, what the initial structure of Ag+ should optimally resemble. This presentation aims to address this issue by reporting the results from testing, under identical conditions for the first time, a compositional range of silver molybdenum oxides as free-standing electrodes in zinc-ion batteries. This talk will discuss requirements for phase-purity, how the structure gives the ability to tailor the Ag+ reduction potential by up to ~200 mV, and the descriptors that most strongly associated with highest energy density.

Presentation

Mechanistic Studies of Ipomoeassin F for Its Cell Growth Inhibition Activity

Wei Shi, Kwabena B. Duah, Lauren Andrews, Princess Walker

Ipomoeassin F is a natural product isolated from leaves of a morning glory plant. It showed potent activity for inhibiting cancer cell growth. However, its mechanism for cell growth inhibition has so far remained unexplored. Here, we report our chemical biology studies that revealed Sec61α, an ER membrane protein, as the physiologically relevant primary target of ipomoeassin F. We confirmed that the natural product inhibits Sec61-mediated protein translocation after binding to Sec61α. Such discovery may offer new opportunities to explore the Sec61 protein translocation complex as a potential therapeutic target for drug discovery.

Presentation

Aromaticity of Moebius Molecules

Shannon Lieb and Lauren Bauerle, Butler University, Indianapolis, IN 46121

Novel organic compounds have been studied for their own sake. Examples of these are cubane and prismane. Both were thought to be too highly strained to be synthesized. Another type of novel compound that has recently been studied is a Möbius strip. This investigation is for fused benzene rings twisted into a Moebius strip. A comparison of the particle on a ring solution for the open, flat, aromatic fused benzene rings to the corresponding Moebius strip shows that the Moebius strip is aromatic; whereas, the cylinder form is antiaromatic. Conversely adding an antiaromatic cyclooctatetraene in place of one of the benzenes makes the Moebius compound anti-aromatic and the cylinder form aromatic. The aromaticity of theses compound was tested using a computed NMR spectrum using B3LYP/6-31+G**.
Engineered Plasticity in TM0077, a serine hydrolase from *Thermotoga maritima*

**Geoffrey C. Hoops (Butler University)**
McKenna Albers (Butler University)
R. Jeremy Johnson (Butler University)
Mark Macbeth (Butler University)

TM0077 is a highly thermostable serine hydrolase enzyme isolated from *Thermotoga maritima*, which naturally displays a large degree of substrate plasticity for the alcohol moieties of ester substrates, but a narrow specificity for small acyl moieties of those same esters. The strong preference for small acyl substrates is governed by a hydrophobic wall in the binding pocket, formed principally by two residues: proline-228 and isoleucine-276. We extensively mutated the cis-Pro228 residue and subjected the variant enzymes to steady state kinetic analysis using a custom library of fluorogenic ester substrates. Several variants retained catalytic activity and furthermore displayed expanded plasticity for the acyl moiety of the ester substrates, as measured by relative catalytic efficiency.

**Poster**

The Importance of Brain Glycogen for Neural Metabolism and Motor Learning During Hypoglycemia

**Logan T. Vaughn**, Justin J. Crowder, Bryce W. Buchanan, Staci A. Weaver, and Bartholomew A. Pederson
Ball State University

Diabetes is a metabolic disease that affects millions of Americans. A common treatment of this disease is insulin therapy to lower blood glucose levels. Approximately 300,000 thousand Americans are hospitalized each year as a result of insulin-induced hypoglycemia, a severe lowering of blood glucose. Hypoglycemia can cause several detrimental effects, including cognitive impairment and neural stress/degeneration. Glycogen in the brain has been proposed to serve a protective role during hypoglycemia by serving as a source of fuel for neural cells when their preferred fuel, glucose, is limited. The goal of this project is to determine the effect glycogen in the brain has on neural stress and motor learning during insulin-induced hypoglycemia. To address this goal, a brain specific GYS1 knockout mouse model (BGSKO mice) along with control mice expressing GYS1 were examined. Severe insulin-induced hypoglycemia caused significant decreases in brain levels of glycogen and ATP along with increased levels of aspartate. These findings are consistent with isoelectric conditions under which neural damage occurs. To assess the role of brain glycogen during motor learning, BGSKO and control mice were subjected to multiple
trials on an accelerating rotarod. Experiments in fed and fasted metabolic states suggest that glycogen in the brain is not required for motor learning, but may be important during hypoglycemia.

Poster

**Design, Synthesis and Characterization of 6,7-dimethoxy-1-arylidene-2-tetralones as Novel Anticancer Compounds**

Carlos E. Enci and Mohammad Hossain
School of Sciences, Indiana University Kokomo, Kokomo, Indiana 46904, USA

Abstract:
A series of 6,7-dimethoxy-1-arylidene-2-tetralones (1) has been synthesized as novel anticancer compounds. This series of compounds is expected to display greater toxicity to cancers than the corresponding normal tissues as they contain α,β-unsaturated carbonyl group. In addition, this series of compounds contains two methoxy groups which are expected to enhance potency of the compounds by causing even greater toxicity to cancer cells. Each of the compounds in series 1 was synthesized in 5-step process starting from a commercially available compound, 4-(3,4-dimethoxyphenyl)butyric acid, in good overall yields. All compounds were characterized using 1H NMR. The synthetic procedures of these compounds and their characterization will be presented.

Poster

**Synthesis and Investigation of Conjugated Azo Dyes**

Jacob Dittmer, Manchester University
Dr. Susan Klein*, Manchester University

Data was collected on the color changing properties of azo dyes, specifically when introduced to different forms of energy. Each azo dye used was synthesized and purified through column chromatography and solid extraction techniques, with the purity indicated by TLC plates. Spartan calculations were conducted to determine the likely structure of each dye. Dyes were characterized by IR and H NMR. It was determined that several dyes were highly soluble at pH 10, which allowed all tests to be conducted under basic conditions. Beer’s Law studies were conducted and each dye was subjected to various types of energy (heat, light, and sound). UV-Vis spectra
were measured over two days, using sound, heat, and light as the three forms of energy tested.

Poster
**Battery Application of Silver Molybdate Structures**

**Brendan Godsel**  
**Allen Huff**  
**Julie Pohlman-Zordan**  
**Paul F. Smith**  
Valparaiso University

Rapid and substantial advancements in technology have dramatically increased the demand for faster rechargeable energy sources such as lithium, sodium or zinc batteries. The inexpensive and nonflammable characteristics of zinc have made it popular in the field of electrochemical research but the effectiveness of its battery potential is dependent on the material it is paired with to react during operation. The high conductivity of silver and large oxidation number of molybdenum make varying structural conformations of silver molybdates (Ag2MoxOy) strong candidates for battery application. Their inspiration is derived from the battery in the implantable cardiac defibrillator (ICD), which uses a silver vanadium oxide cathode to last for several years at a time. In theory the higher oxidation number of molybdenum can improve the capacity of the current ICD design. To determine the best possible silver molybdate structure as a battery cathode, we analyzed a compositional range with varying structures, symmetries, and Ag:Mo ratios.

Poster
**Detection of Crystalline Active Ingredient in Over-the-counter Tablets by Electrospray Laser Desorption Ionization Mass Spectrometry Imaging**

**Salah Khan**, Mariann Van Meter, Brynne Taulbee-Cotton, Adam Shuder, Patrick A. McVey  
Marian University, Indianapolis, IN

The Food and Drug Administration requires crystallinity of the active pharmaceutical ingredient (API) to be minimal in tablet blends. Crystalline API causes agglomeration and clustering, which lowers its bioavailability. This can cause issues with dosage, especially for patients who split pills in half or require exact drug loads. Ideally, the API exists in the amorphous form, which maximizes bioavailability and is more stable in the tablet blend. Agglomeration in over-the-counter (OTC) medications is difficult to determine via the optical methods commonly employed. In this study, Mass Spectrometry Imaging (MSI) was utilized to directly observe agglomeration in tablets.
for the first time. The use of electrospray laser desorption ionization MSI (ELDI-MSI) to determine the amount of crystallinity in pharmaceutical preparations was successful in imaging the active ingredient concurrently with surfactants, binders, and fillers in the tablet blend. ELDI-MSI is a matrix-free method laser ablation method with no sample preparation required while operating at atmospheric pressure, which is ideal for tablet formulations. The results of this study showed that OTC acetaminophen tablets had a very low amount of crystallinity and agglomeration. OTC tablets compared favorably to a standard blend of 2.5% crystalline API drug load, which is under the FDA proposed limit. Additional active ingredients and tablets morphologies are currently under examination using ELDI-MSI.

Poster

Effect of Nuclear Radius on the Ratio of Conformers for the [Fe-Fe] Hydrogenase Mimic Compounds and the Application to its Electrochemical Properties

William Davis, Jacob Wade, Jesse Tye*, Zhihai Li*
Department of Chemistry, Ball State University, Muncie, IN 47304

It is documented that certain bacteria can produce sizeable quantities of hydrogen gas using an [Fe-Fe]- or [Fe-Ni]-hydrogenase, with the [Fe-Fe]-hydrogenase being the most productive enzyme to directly produce the dihydrogen molecule. The [Fe-Fe]-hydrogenase catalyst is very efficient and productive at producing hydrogen gas, in some cases yielding up to 6000 catalytic turnovers per second, which is comparable to the noble metal catalysis methods. The [Fe-Fe] hydrogenase mimic compounds have two main structural classes: the bridged dithiolate class and the unbridged dithiol class of compounds. The work presented here focuses on the unbridged class of compounds and how changing the nuclear size at the dithiol region of the compound changes the ratio of the different conformers (syn-syn, syn-anti, syn’-syn’) as well as its effect on the electrochemical properties of the catalyst. The work herein will take the unbridged dithiol and replace one of the sulfur atoms with a much larger selenium atom. This size difference will cause some inhibition of inversion at the R-group attached to the selenium atom relative to the thiol. There is a significant amount of research being done on the dithiol compounds, however there has been very little work done on the seleno-thiol analog on the unbridged class.
Poster

Electrospinning Chitin and PVA for Improved Mechanical Properties

Sarah Franklin, IUPUI, Department of Chemistry
J.D. Mendez, IUPUC, Division of Science

The purpose of this experiment was to determine the material properties of an electrospun product based on the ratio of Chitin/Chitosan suspended within a concentration of PVA (Polyvinylacetate). A variety of concentrations of PVA and ratios of Chitin to Chitosan were used. Perfection of the electrospinning process to create this product was also determined. Material properties were determined by tensile testing the product to obtain a Young’s Modulus and by SEM analysis to determine the fiber structure.

Presentation

Ultraviolet and Infrared Spectroscopic Investigations of the interactions between phenylbutazone and nanoparticles (C60 & C70).

Joseph Kirsch* and Nagma Tai, Department of Chemistry, Butler University, Indianapolis, Indiana 46208

* PhD, Professor of Chemistry at Butler University, jkirsch@butler.edu

Ultraviolet and Infrared Spectroscopies were used to investigate the interactions between phenylbutazone and nanoparticles (C60 & C70) in toluene and heptane solvents. The ultraviolet spectra showed shifts from 238.0 nm for phenylbutazone to 236.9 nm for phenylbutazone treated with C70 and to 234.0 nm for phenylbutazone treated with C60 in heptane and toluene solvents. The infrared spectra showed a shift of the carbonyl absorption of phenylbutazone from about 1735 cm\(^{-1}\) to about 1715 cm\(^{-1}\) on treatment of the phenylbutazone with both C60 and C70 nanoparticles in the toluene and heptane solvents. A number of studies indicate that the nanoparticles are good electron acceptors. The shift to lower nm’s in the uv spectra and lower cm\(^{-1}\) in the ir spectra are consistent with electron donation from the phenylbutazone to the nanoparticles.

Workshop
WORKSHOP ABSTRACT

Title: Diving into Bio, Material, & Chemical Analysis – From Concept to Technique

Emil Khisamutdinov, Zhihai Li, Wei Shi, Elsayed Zahran
Department of Chemistry, Ball State University, Muncie, IN 47304

The Goal of this workshop is to offer a unique opportunity for middle & high school educators, undergraduates in science majors, instructors at Primarily Undergraduate Institutions (PUI), as well as students who are interested in STEM, to allow them dive into the state-of-the-art analytical methods and techniques. These advanced techniques are popular and essential for biotech, material characterizations, and chemical analysis. Students and teachers will learn from concepts to working principle, from instrumental techniques to applications from an inter-disciplinary perspective.

Nowadays, the development of analytical instruments and techniques has a substantial impact on the chemistry, biology, materials, and interdisciplinary research. We will selectively introduce several analytical techniques that play an important role in the fields mentioned above. These instrumental techniques include atomic force microscopy (AFM), nuclear magnetic resonance (NMR) and x-ray photoelectron spectroscopies, as well as agarose and polyacrylamide gel electrophoresis techniques. The workshop is designed to provide undergraduate and graduate students and public attendees with a tutorial understanding of the basic principle of these techniques. Furthermore, data from each group will be presented to show the application of these techniques in research.

Emil Khisamutdinov received his Ph.D. from the Center for Photochemical Sciences, Bowling Green State University, OH. He held postdoctoral position at University of Kentucky College of Pharmacy before joining chemistry department Ball State University (BSU) as an Assistant Professor. He is a biochemist by training and studies structural aspects of nucleic acids. In particular, Dr. Khisamutdinov’s laboratory is focused on designing Nucleic Acid-based nano-architectures for various applications ranging from biosensing to drug delivery. Dr. Khisamutdinov is author of multiple research articles, book chapters, and patents on this theme.

Zhihai Li received his Ph.D. from RWTH Aachen University (Germany) in Prof. Thomas Wandlowski group. He worked as a postdoc at Arizona State University, and then at Temple University in Prof. Borguet’s lab before he joined Ball State University. His research belongs to analytical, materials, and physical chemistry, with a focus on the electrochemical scanning tunneling microscopy and its applications in molecular assembly and molecular devices. Recently, his research also involves the
synthesis of nanomaterials and applications in energy conversion, AFM characterization of nanomaterials and biological molecules.

Wei Shi earned his Ph.D. degree in bioorganic chemistry from the University of Alberta, Canada. Following that, he conducted his postdoctoral research in Chemical Biology at Johns Hopkins University, School of Medicine. He is currently an assistant professor in the Department of Chemistry at Ball State University, where his group focuses on applying concepts and techniques of organic synthesis and medicinal chemistry to design and prepare bioactive small molecules. Afterwards, chemical probes derived from these molecules will be utilized to understand function of biological components in cells.

Elsayed Zahran received his Ph.D. from University of Kentucky. He was a research assistant professor at the University of Miami before joining Ball State University (BSU) in 2018 as an Assistant Professor. Dr. Zahran has published more than 20 papers in high impact peer-reviewed journals. Dr. Zahran research focuses on the development of new approaches for the detection and remediation of environmental contaminants via the design of novel nanostructured materials with unique architectures that provide efficient catalytic activity. These materials demonstrate applications in degrading environmental contaminants, producing clean fuel from renewable resources, and developing analytical sensors.

Poster

**Pd/Cu2O/BiOBr Nanostructured Photocatalysts with Tailored Heterojunctions for Enhanced Degradation of Organic Contaminants.**

Abelline Fionah, Sarah Abaddi, and Elsayed M. Zahran

Persistent organic pollutants have been a global threat. The need for a cost-effective and environmentally benign method for the degradation of these contaminants has led to tremendous research in the field of photocatalysis, especially using metal oxides. Bismuth based semiconductors have emerged as very promising visible light-activated catalysts. In this poster, we introduce a method to control the heterostructure of Cu2O/BiOBr via a low-temperature hydrothermal procedure. Controlling the reaction conditions via sequential and one-pot methods enabled the preparation of well-interfaced Cu2O nanocubes over BiOBr nanosheets. The size, composition, morphology, and structure of the material were characterized by TEM, SEM, EDS, XRD, XPS, and HPLC-IC. The activity, recyclability, and role of scavengers of the photocatalysts were evaluated in the degradation of rhodamine B and methyl orange organic dyes. Tuning the composition of the heterostructures led to substantial activity in the photocatalytic degradation of both cationic and anionic organic contaminates. Cu2O/BiOBr photocatalyst was employed in the photocatalytic degradation of glyphosate, a widely used herbicide that has shown adverse effects on the human health. The composite photocatalyst demonstrated complete degradation of
glyphosate within 10 minutes of light irradiation. Palladium nanodomains were anchored selectively on the surface of Cu2O via a light activated deposition approach. The multicomponent Pd/Cu2O/BiOBr photocatalyst demonstrated enhanced photocatalytic degradation activity in comparison to the bare material.

Poster

Synthesis and evaluation of amide-based dithiolethiones as anti-neuroinflammatory agents

Dennis Brown, PhD Manchester University
Paula Avila, BS Manchester University
Karrington Craig BS Manchester University

Multiple sclerosis is an immune-mediated disease of the central nervous system characterized by demyelination of neurons. Dithiolethiones, a class of sulfur-containing heterocycles, have established anti-inflammatory activity. Herein, we describe the synthesis and pharmacological characterization of a series of amide-based dithiolethiones. Synthesized compounds were assayed for ability to suppress pro-neuroinflammatory cytokines IL-12 and IL-23 induced by LPS in the BV2 cell line. Structure-activity data revealed tolerance toward a range of amides. Active morpholine analog S-13 reduced various other inflammatory cytokines and mediators. Mechanistic studies showed S-13 increased the expression of anti-inflammatory Nrf2 and HMOX proteins. Taken together, our data demonstrate that amide-based dithiolethiones are promising anti-neuroinflammatory agents.

Poster

Degradation of Glyphosate by Pd/m-BiVO4/BiOBr Nanocomposite: Mechanistic Study and Catalyst Stability

Sarah Abaddi, Nicholas A. Ensinger and Elsayed M. Zahran

Glyphosate is the most widely used synthetic organophosphate herbicide for weed control, commercialized as Roundup. Although glyphosate is classified as low toxicity pesticide, several reports demonstrate side effects on non-target plants and organisms which has resulted in increased adverse effects on higher order organisms. This research describes the light-activated catalytic degradation of glyphosate by Pd/m-BiVO4/BiOBr photocatalyst. The photocatalytic activities of the bare m-BiVO4/BiOBr and the Pd-decorated particles, with various Pd content, were elucidated via the degradation of rhodamine B. These materials were employed in the photocatalytic degradation of glyphosate and subsequently aminomethylphosphonic acid under simulated sunlight irradiation. The kinetics of the photocatalytic degradation reaction were studied by monitoring the concentration of glyphosate and
its byproducts by ion chromatography. Complete degradation of glyphosate by $Pd/m$-$BiVO_4/BiOBr$ was achieved within 5 minutes of the reaction. Our mechanistic studies of the reaction displayed that holes and superoxide radicals are the main species involved in the degradation of glyphosate. The catalyst stability study revealed that the nanocomposite maintained over 75% of its activity after six cycles of the degradation of glyphosate. Such results could lead to the development of sustainable catalytic technologies to minimize the global impact of glyphosate.

Presentation

**Exploration of Small Angle Scattering Methods for Studying Structural Changes of Proteins in Solution**

**Jason Kim**, Research and Development Division, Health & Science Innovations, Inc.;
Luis A. Palacio, Research and Development Division, Health & Science Innovations, Inc.;
Taylor N. Segally, Research and Development Division, Health & Science Innovations, Inc.;
Christopher B. Stanley, Biology and Soft Matter Division, Oak Ridge National Laboratory;
Soenke Seifert, Chemical and Materials Science X-ray Science Division, Argonne National Laboratory;
Horia I. Petrache, Department of Physics, Indiana University Purdue University Indianapolis.

Glycoproteins have different functions and their structures change when placed in different physiological conditions and in response to other enzymatic processes. In particular, *Alpha-1-Antirypsin* (A1AT) has been shown to have a protective role for lung cells against emphysema. The goal of this study is to better understand the stability of A1AT's native conformation when its environment deviates from physiological conditions. The protein environment variables include osmotic stress, crowding, and presence of lipid membranes. Two different experimental methods were used to collect data: Small Angle X-ray Scattering (SAXS) and Small Angle Neutron Scattering (SANS). The data was then analyzed by fitting shape dependent and shape independent models in order to quantify structural and conformational changes of the envelop of the protein. Previous approximations, using radius of gyration analysis (shape independent), have shown that there is a phase transition for A1AT under osmotic stress. Two of the challenges of the analysis is determining a consistent methodology to examine structural changes of a protein in solution, and how account for the scattering signal of a secondary solute (e.g. Polyethylene Glycol or PEG) that polymerizes (non-linear and non-homogeneous behavior) at a different rate according to its concentration level. This work explores these two challenges in the analysis phase of the investigation. Two types of methodologies are used and
compared for the former analysis challenge: fixed momentum vector range, and optimization of fit. While the latter analysis challenge for SAXS has not been solved, SANS provides a solution through contrast matching of the desired solute (i.e. D2O to H2O ratio).

Ecology

Poster
Variation in Roost Use by the Indiana Bat (Myotis sodalis) Across its Distribution

Ashley L. Johnson and Scott M. Bergeson.
Department of Biology, Purdue University Fort Wayne, Fort Wayne, IN 46804

Myotis sodalis (Indiana bat) is listed as an endangered bat species according to the US Fish and Wildlife Service. These bats are endangered due to a combination of White Nose Syndrome, pesticides, climate change, and habitat loss. With the bat population declining, preservation of the summer roost habitat is key to helping the species survive. Current management for the species is informed primarily by research conducted in the Midwest, despite the species using a variety of roosts in different landscapes throughout the Eastern United States. Our objective was to investigate how variable Indiana bats’ roosting habitat was across the species’ distribution. We hypothesized that there would be differences in roost characteristics and roosting landscapes used by Indiana bats located in different EPA Level 3 Ecoregions. We collected roost habitat data by sending out standardized forms to researchers in various states. MANOVA tests for roost and landscape characteristics revealed that characteristics were not significantly different between roosts located in different ecoregions whereas landscape characteristics were significantly different. Thus, female Indiana bats use similar roost characteristics across the species’ distribution: diameter at breast height (45.73cm±1.99), tree height (19.67m±.98), and roost height (10.72m±.74). However, the landscapes that bats roosted within differed by the size of the largest habitat patch, the total amount of edge habitat, and the density of edge habitat. To manage the Indiana bat in the future, there is importance in understanding the landscape characteristics in the region to know what the species will use and thus what to conserve for.
The restoration and population status of eastern wild turkeys (*Meleagris gallopavo sylvestris*) in Indiana.

Steven E. Backs, Indiana Division of Fish and Wildlife, 562 DNR Road, Mitchell, IN 47446, USA.  
Emily B. McCallen, Indiana Division of Fish and Wildlife, 5596 E State Road 46, Bloomington, IN 47401, USA.

The eastern wild turkey (*Meleagris gallopavo sylvestris*) was extirpated from Indiana around 1900. Restoration using wild-trapped birds from wild populations in other states began in 1956 to re-establish source populations in Indiana. Restoration was completed in 2004. Criteria to evaluate suitable habitat for establishing self-sustaining wild turkey populations were developed for Indiana and used to determine restoration priorities. Indiana’s wild turkey restoration spanned 49 years (1956 to 2004) during which 185 releases occurred totaling 2,795 wild-trapped turkeys (809 males; 1986 females) obtained from six states and wild turkeys now occur in all 92 counties. Based on spring harvest data from 2015-2019, estimates of winter (pre-breeding season) populations of wild turkeys range by county from < 0.01 to 5.45 wild turkeys/km². Regional mean population estimates vary from highs in the southeast (2.55 birds/km²) and southcentral (1.88 birds/km²) where the forest cover is more contiguous, followed by the southwest (1.46 birds/km²), north (1.42 birds/km²), and west-central (1.35 birds/km²) with the lowest estimated densities in the more intense agricultural east-central region (0.12 birds/km²) where forest cover is < 10%. A regulated annual spring harvest was initiated in 1970 and a regulated fall harvest began in 2005.

Presentation

*Does the “naming” of an urban green space provide extra protection over time from human disturbance or development?*

Stephanie Freeman-Day- PhD Student in Environmental Science at Indiana University School of Public and Environmental Affairs  
Dr. Burnell C. Fischer, PhD- Clinical Professor Emeritus at Indiana University School of Public and Environmental Affairs

With human populations concentrating in urban areas and overall increasing awareness of vital ecosystem services that urban forests and other urban green patches provide, public interest grows in protecting urban forest patches. However, the desirability of urban land brings pressure to expand development into forested urban patches. We investigate whether the naming (formal or informal) of a patch is
associated with that patch being protected from future development. Our focus began
with Indiana University’s Bloomington campus and is expanding to include the more
complex history and structure of urban forested patches in the City of Bloomington,
Indiana and Indianapolis. The first step of the project methodology was examining
historical maps stored in Indiana University’s archives and tracking by decade,
beginning in the 1900s, named and unnamed green patches through to present time.
We also have studied Master Plans and historical university records for language
designating patches to be protected. Early research has found 17 significant sites that
are suggested for preservation in the 2010 Master Plan. Some of the sites listed have
been included in the historical maps since the beginning of the study period, an
example being Dunn’s Woods which was indicated by drawing in the early 1900s but
not yet formally named on a map until the late 1980s. To date, results show an ebb
and flow of patches being identified by name on maps, often with names of patches
being removed during periods of high development and added after public pushback
to protect green spaces. Newly designated patches are rare. The result of our work will
be a case study applicable to other settings and of interest to urban planners and
groups active in preserving urban green infrastructure.

Presentation

Conservation Through the Use of Art

Cheyenne Olson and Kamal Islam
Department of Biology, Ball State University, Muncie, IN 47306, USA.

Described as a plentiful species in 1972, the crawfish frog (Lithobates areolatus) was
listed as state endangered in Indiana in 1984 due to rapid population declines- likely
caused by mining, farming, and urban sprawl. This cryptic, ground-dwelling species
spends most of its time in or around burrows made by burrowing crayfish. These
burrows are used for shelter, and for locations where they hunt for insects and spiders,
and where they warm themselves. Artistic endeavors may be one way to draw
attention and showcase the plight of this cryptic species. Scientific evidence is often
not enough to draw the hearts of the masses into working towards a common
conservation goal. Thus, it is time to use art to achieve scientific aims such as the
protection of species and their environments. Throughout history, art has been able to
bridge gaps between scientific and socio-cultural understanding as it can appeal to the
emotions and heart of the individuals that it touches while still allowing for the
dissemination of important information. This presentation aims to look at the potential
influences that art can have in the field of conservation biology and how this can be
used to help animals with localized populations and restricted distributions, such as
the crawfish frogs of southern Indiana. This presentation will also focus on some
natural history aspects of these frogs, as well as art pieces that have been created to
raise awareness about these frogs and their status in Indiana. 
**Tags:** Crawfish frog, Conservation, Endangered species, art

**Poster**

**Effects of elevated CO2 on growth and quality of cancer-fighting vegetables: A meta-analysis**

**Xianzhong Wang**
Indiana University-Purdue University Indianapolis

A number of common vegetables have been shown to contain compounds that have cancer-fighting properties. As a result, these vegetables are being increasingly recommended and consumed as part of a healthy diet to help reduce the risk of cancer. How these vegetables respond to global environmental changes, such as elevated carbon dioxide (CO2) in the atmosphere, will have a wide range of implications for the well-being of humans in the future. In this meta-analysis, I examined the effects of elevated CO2 on the biomass production of six common vegetables grown at a level of CO2 concentration projected for the end of the 21st century. The response of cancer-fighting compounds, such as antioxidants, flavonoids and vitamin C, to elevated CO2 was also analyzed in this study. It was found that biomass production of all six vegetables examined increased significantly in response to CO2 elevation. Magnitude of increase in biomass production ranged from 17.6% in sugar beet to 136.0% in lettuce. Content of cancer-fighting compounds, particularly vitamin C, also tended to increase at elevated CO2. Results from this meta-analysis demonstrated that common vegetables will have a higher yield and contain greater amount of cancer-fighting compounds, thus higher quality, when grown in a future environment of higher CO2.

**Poster**

**Impact of Microplastic Fiber Pollution on Ramshorn Snail (Panorbella campanulata) Reproduction and Mortality**

**Cole J. Philips**
**Kalleb Miller**
**Laurie S. Eberhardt**
Department of Biology, Valparaiso University

Microplastic pollution is an emerging problem in aquatic environments becoming a global challenge. Many studies have shown impacts of microplastics on marine organisms, but less is known about the impact of exposure to microplastics on freshwater organisms. Mollusks are one group of organisms that appear to be especially sensitive to exposure to microplastics. We explored how snail mortality and
reproduction were impacted by exposure to polyester microfibers. The polyester microfibers were obtained by grinding small pieces of a blue fleece jacket. We exposed freshwater snails (*Planorbeella campanulata*) to these microfibers for a seven week period in individual 1.1L laboratory mesocosms. The mortality of parent snails, along with the amount of eggs sacs, eggs, and offspring were recorded in both experimental and control treatments. We found a higher rate of mortality in adult snails during the experiment. At any given time the fiber treatment group was recorded to have 0.33 more adult deaths in each mesocosm. The fiber treatment group also had significantly more offspring per adult by the end of the experiment. To test for the effects of competition in the mesocosms, we repeated the experiment with fewer initial adult snails, and found the same result in reproductive behavior and mortality. We concluded that exposure to polyester microfibers stimulated reproduction, possibly by hormonal stimulation from the microfibers or blue dye in the original fleece material. It is also possible that reproduction and mortality are linked through increased stress caused by reproduction. Our results suggest that microplastic pollution can be having negative effects on freshwater organisms.

Poster
**The effect of polypropylene on the formation of byssal threads produced by *Dreissena polymorpha* (zebra mussels)**

Anna-Christina Z. Crisman, Thomas G. Paul, Laurie S. Eberhardt
Department of Biology
Valparaiso University

The presence of microfibers and microplastics in the environment is an ever-growing concern as proliferation of these particles poses potential ecological hazards in both aquatic and terrestrial ecosystems. Accumulation of microplastics (plastic particles smaller than 5 mm) in aquatic environments and the subsequent exposure of these particles to organisms have been shown to have negative effects on aquatic biota. As an invasive, filter-feeding bivalve found across Indiana freshwater ecosystems, the zebra mussel (*Dreissena polymorpha*) serves as a good model organism for studying microplastics’ effects on physiological and behavioral functions of affected organisms. We have studied the impacts of microplastic exposure on a freshwater mollusk, the zebra mussel. We collected zebra mussels from Stone Lake, Indiana, in late fall of 2019. Individual zebra mussels were exposed to polypropylene rope fibers (concentration of rope fibers in the environment of one zebra mussel was ~400 microfibers per L) for 24-hour trials and assessed the effects by production of byssal threads and plaques. These are produced by the zebra mussel for anchorage and in response to predation threats. Results from a comparison between unexposed control mussels (n=70) and mussels exposed to rope fibers (n=70) revealed no significant difference in motility nor the number of byssal threads.
produced. Despite using microplastic concentrations that were higher than that found in the Great Lakes, a 24 hour exposure time may still not have been enough to significantly impact the animals. Continued research on the attachment strength of *Dreissena polymorpha* exposed to rope fibers will provide clearer evidence of any direct effect of these microplastics on the ecologically important mussel species.

Presentation

**New records for rare herps in northern Indiana: How secretive wildlife can go undetected**

**Brock Struecker**, Cardno

A number of amphibian and reptile species are listed as endangered or special concern in the state of Indiana. Many of these species have been surveyed for at various preserves around the state but intensive surveys for herps are not commonly funded. Thus, surveys conducted in areas not previously investigated have the potential to uncover new records for rare or secretive species. Incidental and targeted surveys for amphibians and reptiles were conducted throughout northern Indiana in 2019. Surveys included visual encounters and searching natural and artificial cover objects. Surveys resulted in newly documented locations for three state listed species including four-toed salamanders (*Hemidactylium scutatum*), Kirtland’s snakes (*Clonophis kirtlandii*), and eastern massasaugas (*Sistrurus catenatus*). One new location for each species was documented. Two Kirtland’s snakes were observed in La Porte County, three eastern massasaugas were documented in LaGrange County, and two four-toed salamanders were found in Porter County. All properties are protected and some have been surveyed for amphibians and reptiles in years prior to 2019. These observations show that there are locations in the state where rare wildlife are able to persist without detection. Additionally, some areas may lack sufficient survey effort to confirm the presence/absence of rare species, especially for species that are difficult to survey for and have low rates of detection. Documenting the occurrence of rare wildlife may have implications for habitat loss in the future as anthropogenic change continues to increase.

Presentation

**Soil transfer for seed bank preservation and non-native species management**

**Adam R. Warrix**, R. Nelson Snider High School, adam.warrix@fwcs.k12.in.us
Tessa M. Aby, Purdue University Fort Wayne, abytm01@pfw.edu
Jordan M. Marshall, Purdue University Fort Wayne, marshalj@pfw.edu

Soil transfer is used as a management technique to relocate seed bank and established plants. By moving soil from a known location, there is a potential of reducing non-
native plant abundance. The goal of this study was to compare plant communities in a donor and recipient location at Eagle Marsh Nature Preserve and test the hypothesis that soil transfer is an effective management strategy in forb dominated ecosystems for facilitating native plant colonization in disturbed areas. Soil was collected via front-end loader and moved via dump truck from an area with an established native plant community (Donor) to a disturbed area (Recipient). Prior to soil transfer, we conducted plant surveys in the Donor site (2014) and recipient location (Pre-Recipient, 2014). After soil transfer, we conducted plant surveys at the recipient location in the following growing season (Recipient, 2015), in the third growing season (Established, 2017), and in the fifth growing season (Stabilized, 2019). Sørensen similarities were 0.50 and below for all survey pairings, except for the Established and Stabilized surveys (0.62). While there were overlapping species between surveys, very few were present in the Pre-Donor and occurring in later surveys. In overall, native, and non-native species richness and diversity calculations, the Recipient survey was typically the lowest (i.e. growing season after soil transfer). Additionally, there were steady increases in the Established and Stabilized surveys following the Recipient in overall and native species richness and diversity. NMDS ordination displayed dissimilarity between the Pre-Donor and Pre-Recipient surveys. The Recipient, Established, and Stabilized surveys converged back towards the Pre-Donor survey along axis 1, but equally diverged from the Pre-Donor survey along axis 2. The lack of similarities in species and recovery of non-native species after transfer supports our interpretation that this technique may not be effective for forb-dominated meadow communities.

Presentation

Summer Mist Net Surveys for *Myotis sodalis* & *Nycticeius humeralis* Before, During, and After Construction of a Highway in Southwestern Indiana

Josiah Kleinhenz, Darwin Brack, Dale W. Sparks, Virgil Brack, Jr.

The federally endangered Indiana bat (*Myotis sodalis*) and state-endangered evening bat (*Nycticeius humeralis*) form summer maternity colonies in woodlands of North America. Land uses that convert woodland habitats, including agricultural, residential, commercial, and industrial developments, contributed to long-term population declines for these and many other species. Our goal is to understand how development of a highway and associated conservation influenced the presence, abundance, and diversity of bats near the project in southwestern Indiana. Bats were captured at 22 mist net sites within a 4-mile wide, 60-mile long corridor during summers of 2010 through 2017. Numbers of Indiana and evening bats declined after construction and remained low during the first 3 years of highway operation, which also coincided with the arrival of the fungal disease White Nose Syndrome (WNS). Capture rates of both bats increased during the fourth year of operation (2016; greater than one standard
deviation during the study period). During the fifth year of operation (2017), the capture rate of Indiana bats was within one standard deviation above the mean. Capture rates of evening bats remained over one standard deviation above the mean. The decline in Indiana and evening bat captures 2013 to 2015 and increase in 2016 and 2017 is mirrored, to a lesser extent, in the abundance and diversity scores of all bat captures during these years. A variety of restoration, conservation, and mitigation measures were implemented to avoid and minimize impacts to bats, to the degree feasible. Since we do not have a control lacking conservation measures, we cannot directly credit the conservation program for population rebounds. However, we believe the survival of Indiana and evening bat communities in the face of both the highway project and WNS provides important circumstantial evidence supporting the conservation effort.

Poster

**Impacts of Grass-specific Herbicide Application on the Establishment of Interseeded Forbs in a Tallgrass Prairie Restoration: Year Seven Results**

**Jacob Hurd, Adam Thada, and Robert Reber, Taylor University**

The Avis Prairie in Grant County, IN is a 10-hectare, grass-dominated prairie restoration. This restoration was established in 1993. It is managed using annual spring burns and has become dominated by warm-season grasses that have decreased forb diversity. In April 2013, five native forb species (*Pedicularis canadensis, Eryngium yuccifolium, Rudbeckia hirta, Baptisia alba, and Parthenium integrifolium*) were seeded into a restoration dominated by *Andropogon gerardii* (Big bluestem). A grass-specific herbicide (Clethodim) was applied once (in May) or twice (May and June) during the 2013-15 growing seasons. Seedling counts, and coverage estimates and visual obstruction measurements (VOM), were conducted in 2013-15 and in 2018-19. Initial VOM and coverage estimates showed that *Andropogon gerardii* was significantly reduced in the herbicided plots. This trend continued into 2019. Prairie natives (species in the original restoration seed mix) and regionally native species are exhibiting less of a positive response to herbicide treatment. In the first three years, the density of *Rudbeckia hirta, Parthenium integrifolium, Baptisia alba* was higher in the herbicided plots. The density of *Rudbeckia hirta* declined across all treatments in 2018-19. *Eryngium yuccifolium* was present at significantly higher densities in the treated plots in 2019. *Pedicularis canadensis* has not been present in any of the growing seasons. VOM measurements in 2019 were conducted twice in June and once at the end of July for both the grasses and the forbs. The treated plots produced significantly greater forb VOM values. VOM measurements were not significantly lower in the herbicide plots, although coverage of *Andropogon gerardii* was significantly reduced. Four growing seasons after the last herbicide application, the
use of a grass-specific herbicide has continued to increase the density of select interseeded forbs and has lowered the coverage of competitive grasses.

Presentation

**Results of the 2019 Biodiversity Survey at The Center at Donaldson, Marshall County, Indiana**

**Adam Thada**, The Center at Donaldson

The Indiana Academy of Science conducted its 2019 biodiversity survey (“bioblitz”) at The Center at Donaldson in western Marshall County, Indiana. The 1,000+ acre site straddles the border between the Northern Lakes Natural Region and the Kankakee Sand Section of the Grand Prairie Natural Area, consisting of lake, wetland, prairie, and woodland habitats, all situated in an agricultural matrix. This 24-hour survey brought together approximately 40 scientists and naturalists from across the state and region. Species totals by taxa will be reviewed, including new county records and observations of scientific importance.

**Entomology**

Presentation

**Indiana’s Big River Mayflies (Insecta: Ephemeroptera): July & August 2019 Sampling**

**Luke M. Jacobus**
Indiana Univ. Purdue Univ. Columbus

The first field phase of a two-year project exploring the mayfly fauna of Indiana big rivers was conducted during July and August 2019. Twenty-two locales were visited along the White, Wabash and Ohio Rivers, sometimes on more than one occasion, as part of 31 sampling events. Aquatic sampling was conducted by hand-picking rocks, driftwood & debris; and by using a D-frame dipnet, an Ekman grab, a Petite Ponar sampler, and a shovel & sieved bucket. Hand-picking and dipnetting were the most productive. Aerial sampling was conducted by hand picking winged specimens from a reflective, white sheet, over which a UV light had been suspended or from car headlights. At least 25 different species were collected over the two-month period. *Heptagenia elegantula* (Heptageniidae) was rediscovered in the White River for the first time in 83 years (previously reported as *H. diabasia*), and *Tortopsis primus* (Polymitoryidae) was confirmed from the Wabash for the first time in 45 years. One *Caenis* species (Caenidae) from the Ohio River does not match any current morphological species concept documented for the region.
Presentation

A (True) Bug's Life: An investigation into the diversity of true bugs (Insecta: Hemiptera) found in the Yellowwood State Forest

Ciara J. Mergler, Hanover College
Glené Mynhardt, Hanover College

True bugs (Order: Hemiptera) represent a diverse and specialized group of insects integral to the health of natural ecosystems that is nevertheless understudied in the vast majority of environments found in Indiana. In collaboration with the Indiana Forest Alliance (IFA), a non-profit organization dedicated to studying and protecting native fauna and flora of Indiana state forests, this study focuses on the diversity of true bugs found in the Yellowwood State Forest Back Country Area. From May to October 2016, specimens were collected using Townes-style Malaise traps. These traps were placed at differing habitats with varying elevations, plant composition, and disturbance levels and trap catches were collected and rotated a total of six times throughout the collection period. While true bugs are largely understudied and identification efforts are on-going, this study has so far identified nearly 50 unique species belonging to 15 families. The majority of this research into the identification of true bugs is ground-breaking as information regarding hemipterans is predominantly corroborated by research conducted in the first half of the 20th century and is outdated for modern considerations of biodiversity. Further research into the identification of true bugs is essential for fully understanding the relative diversity of this order – the majority of which are not often collected via Malaise traps – and the role they play in the ecosystem at a large scale.

Poster

The Effects of Carrion Clothing Color on Blow Fly Oviposition

Harley Conn, Monique Le Donne, Heather Wendland, Kristi Bugajski, Ph.D., Valparaiso University

The use of blow fly maggots is crucial in medical-legal research. Blow flies are the most widely used insects to determine post-mortem interval (PMI), because they are typically the first insect to arrive at the carcass after death. Conditions such as temperature, weather, and season can alter the accuracy of PMI, thus these aspects should be considered when making a PMI determination. Students in the Bugajski lab designed and carried out a project that examined the effects of clothing color on blow fly oviposition by different species. Two trials were conducted. In each trial, three pigs dressed in white cotton and three pigs dressed in black cotton were laid out in a fenced grass lot for approximately ten days. Pigs were checked twice a day to monitor blow fly oviposition and larval instars. Maggots were collected for identification once
they reached the third instar stage. Statistical analysis of data is currently being conducted and will be presented at the conference.

Presentation

**Effect of Stab Wounds on Blow Fly Oviposition**

*Raenah Bailey, Shelby Leucata, Maranda Powell, Kristi Bugajski*

Forensic Entomology is the use of insects in criminal investigations. Entomologists are often asked to provide estimations of a post-mortem interval (PMI) in cases involving unknown times of death. Blow flies are the most important insects in these investigations and information regarding their behavior is important for accurate PMI estimations. Blow flies will lay their eggs in mucous membranes and other natural openings of the body. Wounds present another opening, and researchers have predicted that blow flies would lay their eggs in wound openings. Our research group looked at the effect of wounds on blow fly oviposition by stabbing deceased pigs and observing where eggs were laid. Six frozen pigs were thawed for approximately 24 hours before placement in the field. In the first round, three of the pigs were inflicted with a one inch incised surface wound to the abdomen, and three pigs were unwounded and served as controls. In the second iteration, three of the pigs served as controls, and three pigs were stabbed in the abdomen with a 1’ knife blade. Pigs were observed multiple times a day for a period of one week. The different life stages of the blow fly were noted including eggs, larvae, migrating larvae, and adult flies to detect differences between wounded and control pigs. Larvae were collected and are being identified to species. The species composition and statistical results will be presented at the conference. Stab wounds are a common occurrence in forensic science and information pertaining to blow fly oviposition in wounds is important for accurate PMI estimations.

Presentation

**Genetic identification of *Culex pipiens* subspecies to determine population composition and the influence of temperature on activity in Jefferson County Indiana.**

*Hanna M. Werner; Hanover College*
*Monique Huynh; Hanover College*
*Glene Mynhardt; Hanover College*
*Pamela R. Pretorius; Hanover College*

Two subspecies of *Culex pipiens* are of interest as potential vectors of West Nile Virus, *Culex pipiens pipiens*, the northern house mosquito, and *Culex pipiens quinquefasciatus*, the southern house mosquito. Historically, species were identified
morphologically using the DV/D ratio of male genitalia, which is challenging for the *Culex pipiens* complex. *C. p. pipiens* and *C. p. quinquefasciatus* females are morphologically identical, which makes it difficult to differentiate between the two subspecies. Female specimens were identified to genus morphologically. Using molecular techniques paired with the nuclear gene angiotensin-converting enzyme 2 (*ACE2*), the two subspecies can be identified through genetic variation. Both subspecies were collected using a CDC Gravid Trap, model 1712, placed in two locations, including on the Hanover College Campus and at the Margret Seifert Water Treatment Plant. Collection locations were chosen based on the habitat of mosquitoes; seasonally wet, forested areas. Traps were set each week between the months of June and October 2017 and 2019. Of the 470 specimens collected, 51.27% were identified to *Culex pipiens*, based on morphology. Of the *C. pipiens* specimens collected between June 2017 and August 2019, 20.49% were genetically identified as *C. p. pipiens*, 27.05% as *C. p. quinquefasciatus*, and 52.46% as hybrids. Collection data suggest that, as temperatures increased, the number of *C. p. pipiens* collected also increased, while *C. p. quinquefasciatus* decreased; thus, temperature may differentially influence the reproductive activity of the subspecies. The hybrids follow patterns of both subspecies. Non-*Culex* genera collected at the same time fluctuated with temperature as well, suggesting temperature influences mosquito activity. Molecular identification of specimens collected later in the sampling period is still ongoing.

**Workshop**

**Bumble Bees of Indiana**

**Robert P. Jean**, Environmental Solutions & Innovations, Inc., 1811 Executive Dr., Indianapolis, IN 46241

This workshop will focus on the identification, ecology, and natural history of bumble bees (Apidae: *Bombus* spp.) in Indiana. Come and discover the basics of bumble bees, how to identify them, and how to help them flourish. Pollinators, bumble bees in particular, have received much attention lately as several species are thought to be declining and the rusty patched bumble bee (*Bombus affinis*) has been listed under the Endangered Species Act; however, training in bumble bee identification and biology have been largely lacking. This workshop will introduce one to the diversity of bumble bees in Indiana, the characteristics for identifying them in hand and on the wing, and basic management strategies that can potentially benefit bumble bees. This course will require the use of stereo microscopes and handling of preseved bumble bee specimens. In addition, this workshop will look at the historical changes in bumble bee communities in Indiana over the last several decades. The intent of this workshop is increase understanding of bumble bee identification, ecology, and biology to help further monitor bumble bees throughout Indiana.
Pretty fly: An estimate of Diptera diversity in the Yellowwood State Forest Back Country Area

Zoë Madison Henry and Glené Mynhardt, Hanover College

Knowledge of insect diversity in Indiana’s forested ecosystems is limited and baseline data on species richness are largely absent or incomplete. In particular, the Diptera (true flies) are often overlooked due to their immense diversity and the difficulty of identifying species. This study is the first to estimate the diversity of flies in an Indiana deciduous forest ecosystem. Samples were collected from the Yellowwood State Forest Back Country Area (YWSF) between the months of May and October of 2016 with the use of Malaise traps, which are effective in capturing flying insects. Identification efforts have resulted in a total of 39 different fly families and a conservative estimate of at least 170 species that assume a variety of trophic guilds at the family level. These include 8 parasitic, 14 predaceous, 19 scavenging, 8 nectar and pollen feeding, 4 plant associated, and 2 fungivorous families. Among the scavenging families collected, 11 of them are associated with dead or decaying wood (saproxylic). Similar studies conducted in North American deciduous forests have found that saproxylic and fungivorous flies are the most diverse, but data from this study suggest that predators are the second most species-rich trophic group of families present. Species-level identifications are still in progress and may provide more accurate information about the ecological structure of the YWSF. Tentative data from this study suggest that the YWSF hosts a rich fly diversity and highlights the importance of insect surveys and taxonomic training in order to establish baseline data for future studies.

Estimating Beetle Diversity in Indiana: Challenges and Pitfalls

Samuel Stryker and Glené Mynhardt
Hanover College

Since 2014 efforts have been in place to establish baseline diversity estimates of both flora and fauna in Indiana’s south-central forest ecosystems. In collaboration with the Indiana Forest Alliance (IFA), estimates of Coleoptera (beetle) diversity in the Yellowwood State Forest Back Country Area are presented. Beetles were collected using Malaise traps between May-October of 2016. Additional sampling was done prior by hand and aerial net, Lindgren funnel, window traps, fermenting traps, aquatic sampling, mercury-vapor and halogen lights, and UV lights in 2014 and 2015. Additional samples were collected as bycatch from moth pheromone traps in 2017. A total of 63 beetle families were identified with the majority being captured in Malaise traps.
traps (n = 43) and moth pheromone traps (n = 35). A total of 22 families were collected in only one trap type, twelve of which were captured only by Malaise trap, however other trapping methods were not utilized as extensively as Malaise traps were. A Jaccard Similarity Index was used to identify the degree of overlap between families caught by each method. The largest percentage overlap was between specimens collected by hand and by UV light at 52% similarity, followed by Lindgren funnels and window traps sharing 46% similarity. While efforts to identify specimens are still ongoing, this study suggests that, while Malaise traps are an effective means of collecting the majority of major beetle families typically found in deciduous forest ecosystems, multiple collection methods are necessary to estimate the total beetle diversity in a sampling area.

**Engineering**

Presentation

**Geological and Environmental Studies of Newton and Tippecanoe Counties, Indiana**

Claire Snitovsky and Terry R. West, Department of Earth Atmospheric and Planetary Sciences, Purdue University, West Lafayette, Indiana

The objective of this study was to determine the current conditions and geologic aspects of several environmental challenges in Tippecanoe and Newton counties, Indiana. Through inquiry and site investigations, facts were obtained on environmental topics, including sanitary landfills, sewage treatment plants, septic tank fields and combined sewer overflow problems. Visits to the West Lafayette Waste Water Treatment Plant, Lafayette Water Reclamation design office, Tippecanoe County Commissioners office and the Newton County Sanitary Landfill provided first-hand information on environmental topics. The well-run Newton County Landfill was contrasted to the failed Tippecanoe County Landfill, while all options of the combined sewer overflow problem in Lafayette were explored. Study of the CSO problem included an examination of the new underground storm water storage facility and the attempt to mitigate the eleven overflow points of storm water into Wabash River. The work was accomplished through a summer research project by the first author supervised by Dr. West.
Environmental Quality

Poster
Building and Characterizing Constructed Wetlands For Stormwater Treatment

Sean Jackson
Michelle Marincel Payne
Rose-Hulman Institute of Technology

By simulating a natural wetland environment, a constructed wetland can be used to treat wastewaters. In the Cook Laboratory for Bioscience and Research I built a simulation of a constructed wetland to test phosphate removal using limestone fines. Phosphate is a major contributor to water pollution if in excessive amounts. These high concentrations can disrupt the ecosystem by causing critically low oxygen levels and killing many aquatic organisms. The objective of the project was to build constructed wetlands, characterize the hydraulics of the system, and begin characterization of how limestone can remove phosphates and by how much.

I have constructed two separate wetlands, the first is a free water surface which has standing water. The other constructed wetland is subsurface flow. Each system consists of two small boxes and one large box. The smaller boxes are two by two feet by one foot deep, the larger box is two by three feet by one foot deep. I characterized the hydraulics with the goal of a retention time of eighteen hours and confirmed it with a dye test.

I also began characterizing the sorption of phosphate to limestone. I generated isotherms for the sorption of phosphate to limestone fines and found a linear correlation of removal for a range of 0.2 mg to 7.5 mg of limestone for water containing 15 mg/L phosphate.
Nanoparticles are defined as entities having at least one dimension measuring less than 100 nanometers. So-called engineered nanoparticles (ENPs) play important roles in optics, electronics, pharmaceuticals, and personal care products. ENPs escape to the environment during manufacture and use; the majority of released ENPs eventually enter freshwater and marine environments. Little is known regarding their effects and fates in surface water. Many aquatic plants tolerate high levels of pollution and may serve as useful indicators of ENP fate and toxicity. Cattail (Typha latifolia) and sedge (Carex Camosa) were cultivated in a growth chamber for 15 weeks; mesocosms were doped with Ag, TiO2, ZnO, BiVO4 and Cu2O nanoparticles at a rate of 1.5 mg/l per week. Leaf chlorophyll content and shoot and root length were measured throughout the study. At weeks 8 and 15, plant tissue was analyzed for carbonic anhydrase activity and concentrations of nanoparticle metals using ICP-AES. Sedge tolerated ENPs better than did cattails; many cattails died by week 10. Sedge tolerated TiO2 and BiVO4 ENPs better than other nanoparticles. Chlorophyll content declined
considerably in both sedge and cattail exposed to ZnO. Sedges exposed to Ag exhibited lowest shoot length and those treated by TiO2 had the highest shoot length. Cattail shoot length increased for all ENP treatments except for Ag. Cattail root length was greatest in the ZnO treatment compared to the other ENP treatments. Nanoparticle size and morphology were determined using transmission electron microscopy. Findings from this study will provide useful insights regarding the ability of common aquatic plants to tolerate and remediate nanoparticle-contaminated aquatic ecosystems.

Presentation

**Modeling the impacts of acidic drainage on macroinvertebrate assemblages today and in the past**

Kathryn Mudica, Jen Latimer

West-central and southern Indiana contain coal seams that were a prime area for both underground and surface mining. Unfortunately, even after mining was discontinued, mine spoilage continues to affect nearby surface water systems. Coal Creek, located in Vigo County, Indiana, is a freshwater stream that passes through a large area of historic underground and surface mining. While surface spoilage is often visible and remediated, underground seepage is less easily detected. Macroinvertebrate communities have been used to assess organic pollution in freshwater systems but their response to metal pollutants or changes in pH are not as well documented. The goal of this study is to develop a stream biotic index using macroinvertebrates that could also be used to assess other Indiana streams that have similar stressors.

Hester-Dendy type multi-plate macroinvertebrate collectors are being used to collect macroinvertebrate communities along Coal Creek. The objective is to assess the effects of slow acidic seepage on macroinvertebrate communities. This field assessment will then be used to create a training model in open source R-Studio and BUGS programs. Historic macroinvertebrate data from Hoosier Riverwatch will be modeled to assess creeks and streams throughout Indiana within the proximal risk of underground seepage. This pilot study could allow for the assessment of stream ecology impacted by acid seepage across Indiana without further costly data collection.
Presentation

Environmental Philanthropy and Outdoor Recreation: Analyzing Environmental Funding and Facilities in Indiana

Patricia Snell Herzog, PhD, Indiana University Lilly Family School of Philanthropy

Section: Environmental Science

This paper examines environmental philanthropy and outdoor recreation in Indiana. To conduct the analysis, data are wrangled from two public databases. The first data source is county-level data reported in the Indiana Statewide Comprehensive Outdoor Recreation Plan 2016-2020 (SCOORP 2015). These data include county-level population size (U.S Census 2014), state and federal acres of land dedicated to outdoor recreation facilities, recommended number of acres for recreational facilities (based on population size), and the deficit or dearth of available acres relative to recommended acres. The second data source is from the National Center for Charitable Statistics (NCCS 2015). These data include county-level aggregated IRS tax data compiled from the 990s of legally tax-exempt entities with physical addresses in each location. These nonprofit organizations report their revenue and expenditure streams, and the included National Taxonomy of Exempt Entities (NTEE) classifications allow for a categorization of organizations into their primary mission emphasis. The particular focus for this analysis is on environmental organizations. The research question for this analysis is: Do counties with more philanthropic expenditures for environmental causes meet the recommended acres for outdoor recreational facilities for their population size? Theories on civil society and the three-sector system of government, enterprise, and philanthropy present competing hypotheses. Intuitively, philanthropic sector would seemingly support the greater availability of outdoor recreational spaces. Alternatively, crowd out theories posit that private spending on public goods can crowd out public spending, in which case public outdoor recreational facilities may be less prevalent in counties with greater private spending. This analysis adjudicates between these alternative hypotheses and thus contributes to broader understanding of the symbiotic or synthetic relationship between funding and facilities.
Earth Science

Presentation

**Surface magnification, a sixth window to recognition and attribution of structural alterations in the recent, archeologic and geologic record.**

**Bruce Rothschild**, Carnegie Museum

Recognition of alteration of physical structures has been predicated upon direct analysis of (1) chemical and (2) physical properties, indirect assessment through (3) various radiologic techniques and (4) the history of their development. Application to skeletons was subsequently complemented by their (5) direct visual (macroscopic) examination, when overlying structures have been physically removed.

However, recognition of areas of tendon attachment has still resisted macroscopic and even histologic attempts to map their distribution and delineation of fleshy muscle attachments has been totally elusive. Controversy further exists as to exactly what macroscopic bone appearances represent pathologic reaction.

The solution to these two issues is provided by a sixth technique, direct magnification (e.g., 200x) viewing of bone surfaces. The character of tendon and muscle attachments was evaluated in three modern (alligator, rabbit and turkey) and one fossil (**Tyrannosaurus rex**) species. Further, bone surfaces were examined on a large set of individuals with disorders known to produce periosteal reaction and in whom such reaction was independently verified and compared with unaffected individuals.

Surface microscopy identifies characteristics which clearly identify the full distribution not only of tendon attachments, but also that of fleshy muscles. Patterning muscle attachments in extinct animals has previously been predicated on analogy and phylogenetic bracketing. Now, we have a mechanism for their exact placement, by a technique validated in alligators, turkeys, rabbits and **Tyrannosaurus rex**. Contrary to preconceived notions, surface microscopy revealed that linear fiber prominence is not a sign of periosteal reaction, but simply characteristic of normal healthy bone. This is analogous to previous paleontological reports, in which such prominence has been attributed to normal aging. Confusion as to prevalence of periosteal reaction can now be resolved by exclusion of linear fiber prominence as a character as well as any perceived alterations localized below the original bone surface.
Presentation

**The Continental Divide of Northern Indiana**

**Mose Nasser**

The surface area of Indiana is approximately 36,330 Sq. miles (Indiana Geological Survey Website, IndianaMap, Measurement Tool) with a little talked about continental divide that separates the Great Lakes Drainage Basin from the Mississippi Drainage Basin. The continental divide goes from west to east starting in Lake County with a northeastern portion that splits and goes south from Nobles to Adams County. The area north and northeast of the divide covers 10% of Indiana’s surface and includes the Southwestern Lake Michigan Watershed, Southeastern Lake Michigan Watershed and Western Lake Erie Watershed. To the south of the divide water flows through the remaining 90% of the state’s land mass towards the Mississippi River Drainage Basin. That area includes the Upper Illinois Watershed, Wabash Watershed, Patoka-White Watershed, Great Miami Watershed, Middle Ohio-Little Ohio Watershed, Lower Ohio-Salt Watershed and Lower Ohio Watershed (Indiana Geological Survey Website, IndianaMap, Hydrology Watershed HUC06). Furthermore, through the Indiana Geological Survey website, water quality of the major rivers, length of the rivers, bedrock aquifers, unconsolidated aquifers and the surface area of each watershed were reviewed. This information will be presented in the form of maps in a paper. The author would like to thank Tracy Branam of Indiana Geological Survey & Water and Lindsey Beckley of the Indiana Historical Bureau for conversations and information provided to make this project happen.

Presentation

**Size-Related Shape Changes in Tridactyl Footprints Attributed to Non-Avian Theropod Dinosaurs**

**James O. Farlow** (Department of Biology, Purdue University Fort Wayne) and James A. Hyatt (Department of Environmental Earth Science, Eastern Connecticut State University)

Non-avian theropod dinosaurs (meat-eaters and their close kin) spanned a huge size range, from chicken-sized and smaller animals to dragons bigger than elephants. Three-toed (tridactyl) footprints attributed to these dinosaurs are common in Mesozoic continental sedimentary rocks, and an extensive ichnotaxonomy has been created to classify them. However, because many body proportions of theropods can be expected to change across their great size range, some of the shape differences upon which ichnotaxa are based may reflect size differences, rather than the actual phylogenetic relationships of trackmakers. We therefore investigated size-related shape changes in footprints attributed to theropods, measuring linear and angular parameters in tracks.
from ichnofaunas of different ages and geographic origin. Three related shape parameters distinctively change with increasing footprint size: 1) projection of the middle toe beyond the limits of the two peripheral toes, relative to the length of the footprint behind the tips of the two peripheral toes, initially increases, but flattens out at larger sizes; 2) width across the tips of the two peripheral toes, relative to the overall length of the print, also initially increases, but also flattens out at larger sizes; 3) the angle formed by the long axes of the two peripheral toes initially increases, but flattens out or even decreases at larger sizes. Such size-related changes must be considered before concluding that shape differences among footprints of different size bear a phylogenetic signal.

Poster

**Trace element distribution in zinc ore from a Mississippi Valley Type deposit in central Tennessee**

Samantha Ricci, Indiana State University; Connor Pieratt, Indiana State University; Derick Unger, Mine Development Associates; and Sandra Brake, Indiana State University

Samples of sphalerite (ZnS) were collected from the Cumberland Mine in central Tennessee and analyzed via a scanning electron microscope with an energy dispersive system (SEM-EDS) to determine trends in trace element distribution within the deposit. The Cumberland Mine is classified as a Mississippi Valley Type deposit located on a flank of the Cincinnati Arch. Ore mineralization is hosted in Lower Ordovician carbonate rocks and occurs as sulfide replacement in limestones and as open-space fillings in dolomitic breccias. Reddish brown sphalerite is the dominant ore mineral along with minor galena, pyrite, calcite, fluorite, and barite. This study specifically evaluates the substitution of trace elements into the sphalerite structure for the purpose of identifying temporal variations in the geochemistry of ore-bearing fluids with regard to the two ore types. Samples of sphalerite were cut, sanded, and smoothed prior to SEM-EDS analysis. Analytical results show that the highest concentrations of Ge, Cu, Cd, and Ga occur in sphalerite located in the breccia ore. Significant positive correlations exist between Fe and Cu, Fe and Ge, and Ge and Cd in replacement ores and between Ge and Cu in breccia ores, suggesting a relationship between these elements during substitution into the sphalerite structure for the different ore types. The overall results suggest the possibility of two geochemically distinct mineralizing events.
An Analysis of Soil and Blood Lead Samples in Delaware County, East Central, Indiana

Benjamin Azar, Carolyn Dowling, Jessi Haeft, Klaus Neumann, Abdul Elnajdi

Approximately 7 percent of residents tested in Delaware County had elevated blood lead levels, which is more than double the national average. Lead (Pb) is a neurotoxin and exposure to it can cause irreversible brain damage from its capacity to replace calcium in bodily processes and serious permanent health and societal effects, such as developmental delays, learning difficulties, and seizures. Potential sources of lead in the environment include lead-based paint, leachate in drinking water, industrial byproducts, and contamination in soil.

After undergoing appropriate training and with IRB protocols in place, we analyzed the blood lead levels (BLL) by using blood lead data provided by Regenstrief Institute, Indianapolis, IN. Through ArcGIS Pro and SPSS, spatial patterns were analyzed across multiple metrics including BLL test results (0 to 5 µg/dL, >10 µg/dL, and >25 µg/dL), age group, season, and the interaction between each variable.

To assess the idea that the elevated BLLs are caused by lead contamination in soil, we developed a comprehensive, biased sampling approach. Soil samples were collected from at-risk public areas, indicated by mapped BLL values exceeding 10 µg/dL. Soil sample analysis was conducted via EPA method 3050B for bio-available lead by GeoChemical Testing in Somerset, Pennsylvania. A map of soils containing bio-available lead was generated and overlain on the BLL map. These layers were analyzed alongside the statistical results to form an understanding of the extent of soil contamination. These maps and associated data can be utilized in the future development of remediation plans for the Delaware County region.

HotTopics
Historical Land Survey Documents Accessibility, GIS Pilot Project for Indiana: Marion County, Clark's Grant, and Indian Reserves (Field Notes and Plats)

Lorraine Wright
Cadastral, Boundaries, PLSS Workgroup Co-Chair
Indiana Geographic Information System

Historical land survey field notes and plats, from the late 1700's to the mid 1800's, are the basis for the current Public Land Survey System (PLSS). The PLSS is used to described land parcels today. The goal of the Historical Documents Pilot Project was to research, locate, digitized (scan) and make the documents publicly available, via a
Geographic Information System (GIS) application. The Original version of survey field notes and plats were used to transcribe three different versions (at different times) for the Federal, State, and County government. It is important to have all four versions for research and comparison purposes due to discrepancies. The project is available through ArcGIS Online. A GIS map was used to link all four versions to the: 1) Survey Townships, 2) Lots, 3) Indiana Reserves, etc. (described from notes and plats). The pilot created a new, easily accessible, resource never before available. The pilot demonstration was completed using a Story Map application. The Story Map describes the project and shows step by step instructions on how to use the ArcGIS Map.

Deputy Surveyors instructions: "You will be careful to note in your field book *** all rivers, creeks, springs and smaller streams of water, with their width and the course they run in crossing the lines of survey, and whether navigable, rapid, or mountainous, the kinds of timber and under-growth with which the land may be covered, all swamps, ponds, stone quarries, coal beds, peat or turf grounds, uncommon, natural or artificial productions, such as mounds, precipices, caves, etc., all rapids, cascades or falls of water, materials, ores, fossils, etc.; the quality of the soil and the true situation of all mines, salt-licks, salt-springs and mill seats, which may come to your knowledge." (Trails and Surveys, Part II., Pioneer Surveying In Indiana, P. 403)

Poster

**Modeling the effectiveness of wetland restoration in mitigating extreme streamflows under future climate change in the Ohio River watershed**

Alexandria Silowsky, Dr. Bangshuai Han, Ball State University

Anthropogenic climate change is projected to precipitate a shift to drier growing seasons and wetter winters in the agriculturally-dominated Ohio River watershed. Wetlands exhibit a hydrologic buffering effect which regulates discharge in connected streams and rivers, and therefore wetland restoration efforts may play an important role in mitigating the climate change effects on water variability. Indiana has lost approximately 85% of its wetlands in the last 200 years, mainly to the growing demand for agricultural production. The Soil & Water Assessment Tool (SWAT) is an open-source hydrologic model which accounts for land use and climate change projections, widely used to assess the implications of land management practices on surface and subsurface hydrology. The newly constructed version, SWAT+, improved algorithms for simulation of water-dominated areas by separation of water areas and adding the concept of landscape units, which may facilitate better simulation of spatially explicit wetlands and floodplains. This investigation will predict hydrologic conditions in the Ohio River watershed from the present through the year 2100 under a variety of climate change and wetland restoration scenarios. Findings of this investigation will fill a glaring gap in current understanding of the climate change
effects on hydrology. This research has the potential to inform land management decisions with respect to flood and drought mitigation, wetland restoration, and agricultural production based on projections of water resource availability and distribution by the year 2100.

Poster

A possibly-new species of diatom in the genus *Staurosirella* from Marl Lake, Wisconsin

**Levi Gambill** & **Jeffery R. Stone**
Department of Earth & Environmental Systems, Indiana State University, Terre Haute, IN 47809

During the initial analysis of materials from the sediment record from Marl Lake, Wisconsin, a diatom species belonging to the genus *Staurosirella* was observed with characteristics that do not seem to conform to any known species. *Staurosirella* is a genus of rectangular shaped, pennate diatoms that is generally considered to be character-poor and typically live in benthic habitats. The taxa that is presented here, along with SEM and morphometric data, closely resembles *Staurosirella lapponica*, but it can be distinguished using a few key characteristics. The species’ populations that were observed from Marl Lake have a greater valve size range, with substantially longer and slightly narrower valves than the published metrics for *S. lapponica*. Additionally the striae density tends to be less variable and coarser than the reported ranges for *S. lapponica*. We believe these characteristics are enough evidence to separate the observed species as a new species to be described within the *Staurosirella* genus.

Poster

Reassessment of Water Quality of the Prairie Creek Reservoir in Eastern Indiana

**Md Shahin Alam**1, **Dan Deifenbaugh**1, **Banshuai Han**1
Department of Environment, Geology and Natural Resources, Ball State University

Prairie Creek Reservoir is a municipal park in eastern Indiana, which also serves as a secondary drinking water source for the city of Muncie. Prairie Creek's watershed is primarily dominated by agriculture and is subject to heavy nutrient runoff similar to other watersheds throughout the Midwest. Previous studies have identified Prairie Creek Reservoir as an eutrophic water body. Conservation plans have been implemented in the region since 2007 to improve the water quality in the reservoir by protecting the water source from excessive nutrient inputs. During the summer of 2019, the water quality of the Prairie Creek Reservoir was studied to assess the
effectiveness of soil and water conservation efforts in the area. Water was sampled biweekly from epilimnion and hypolimnion depths at both the north and center of the reservoir following standard methods. Water quality parameters including temperature, pH, turbidity, conductivity, and dissolved oxygen, and Secchi Depth were measured on-site. Nutrient levels including nitrate, ammonia, total nitrogen, and reactive orthophosphate were analyzed in the laboratory. Noticeable improvement in water quality has been observed. Average nitrate as N decreased from 0.280 mg/L in a previous study conducted in 2006 to 0.238 mg/L, a decrease of 15% throughout the reservoir. Average orthophosphates as P decreased from 0.058 mg/L in the 2006 study to 0.034 mg/L throughout the reservoir, a decrease of 41% throughout the reservoir. The results are echoed by a significant increase of Secchi Depth. Improvements in the water quality of the Prairie Creek Reservoir could possibly be attributed to changes in land use and other conservation practices in the area, but more studies are needed to make a conclusive determination.

Presentation

**Geological Specimens Recovered from Excavations of the Harmonist Shoemaker’s Shop used by David Dale Owen as a Laboratory from 1835 to 1843 in New Harmony, Indiana**

Connor P. Rose, William S. Elliott, Jr., and Michael R. Strezewski, University of Southern Indiana

From 1975 to 1977, excavations were conducted at the Harmonist Shoemaker’s shop in New Harmony, Indiana, led by John Elliott of Indiana State University Evansville. The shoe shop was built around 1820 and was renovated by David and Richard Owen in 1833-1834 to serve as a geology laboratory, lecture hall, and museum/storage space for David Owen’s fossil and mineral collections. During this time, Owen was tasked with surveying areas of the Midwestern United States, working in Tennessee from 1836 to 1837, Indiana from 1837 to 1838, and northern Illinois, Wisconsin, and Iowa from 1839 to 1840. Throughout his work, Owen collected geological specimens to conduct geochemical investigations, add to his collections, and/or trade for new specimens. After moving his geology lab in 1843, the building was razed. Unfortunately, until recently, no further study of Elliott’s excavated materials had been undertaken. Given that many geological specimens were recovered during these excavations, the resulting material undoubtedly has much to say about Owen’s pioneering work.

At the present time, 353 specimens have been examined, including 92 fossils. It is likely that the total number of specimens is over 500. Of the cataloged samples, fossils include ammonites, bivalves, brachiopods, bryozoans, crinoids, gastropods, nautiloids, petrified wood, rugose and tabulate corals (e.g. *Halysites*), and stromatolites. Minerals
identified are amphibole, apatite, azurite, barite, calcite, galena, malachite, molybdenite, and quartz. Rocks include banded iron formation, chert, concretions, geodes, granite, oölitic limestone, fossiliferous limestone, sandstones, schists, slates, and travertine. The geological specimens from this excavation were most likely collected by David Dale Owen, and probably discarded when his laboratory was moved to the Harmonist Granary in 1835. This study aims to link geological specimens with Owen’s work from 1833 to 1843. Fossils will be especially useful to identify the provenance of the geological specimens.

Poster

**Development of a Virtual Field Resource for Geoscience Education**

Ryan Young, William S. Elliott, Jr., and Anton Maria, University of Southern Indiana

Geology is a natural science that is enhanced through hands-on experiences, but opportunities for field work are decreasing due to budgetary constraints, reduced logistical support, and safety concerns. Thus, a virtual field trip may provide an alternative for students to experience new geological settings. A web-based learning environment provides a unique and interactive opportunity for students to learn about geology over multiple scales, such as geologic maps, field relationships through photographs, hand sample images, and photomicrographs of thin-sections paired with geochemical data. Moreover, virtual field trips accessible on University web pages may also serve as an asset to the recruitment of environmental science and geology majors, along with educating the general public. Additionally, the web-based learning environment has several advantages, such as the capability to instantly update information, and the ability to expand resources through visuals, such as interactive maps, graphs, and photographs.

In this study, a virtual field resource was developed for Mt. Desert Island and Acadia National Park in Maine. This virtual field resource highlights eleven localities and rock specimens with accompanying photomicrographs of thin-sections. Geochemical analysis of selected samples using an X-Ray Fluorescence Spectrometer are used to link elemental composition to geological specimens. The web pages are designed for a student, or a visitor, to explore on their own using interactive tools. This approach follows “just-in-time” methods, in which the learning is self-paced.

While there is no replacement for the real-world experience of field geology, a virtual resource allows programs to have an adaptive learning experience. In addition, the virtual field trip provides the advantage of having a multi-scale web-based teaching tool. This resource may be catered to a specific set of sub disciplines (i.e. Hydrology, Paleontology, or Geochemistry). Furthermore, virtual trips may permit the exploration of diverse settings (i.e. National Parks, State Parks).
Poster

Phosphorus and Metal Geochemistry of a Coal Seam: A case study from Coal Creek

Ravin Gaines, Douglas Hanna, Dr. Jennifer Latimer
Indiana State University

West Central and Southwestern Indiana coal seams, which are part of the Illinois Basin, were formed during the Pennsylvanian period, 320-299 million years ago, and span an area of 6,500 acres. In most areas, coal seams are overlain by more recent Pleistocene deposits making coal an historically important economic resource due to the close proximity of the deposits near the surface. To date, over 2 billion tons of coal has been mined in Indiana, and coal mining and processing has contributed significantly to the economy of the region. Unfortunately, many areas have ongoing water quality concerns related to acidic drainage from abandoned coal mines. While much of the coal is covered by glacial till, in many areas it is exposed as lenses or due to faulting and cutouts. Many of these exposed areas occur along streams and can be readily observed along stream banks. The purpose of this project is to study the geochemistry of the exposed coal along Coal Creek in Vigo County, Indiana. Many studies have investigated the quality and mercury content of Indiana coal and the environmental impacts of coal mining on water quality, but the goal here is to quantify metals and phosphorus. Coal samples will be powdered and analyzed for total metals using a portable X-Ray Fluorescence Analyzer and detailed phosphorus geochemistry. This data will help us to better quantify historic fluxes of metals and nutrients to streams from past mining practices.

Presentation

Accuracy of digital surface models versus digital elevation models using the Modesitt Mound Group

Cindy Hendry, Indiana State University
Dr. Stephen Aldrich, Indiana State University
Dr. Alex Badillo, Indiana State University

A DEM (Digital Elevation Model) from USGS (United States Geographic Survey) is the standard bare-earth terrain model that many analysts use. As new technology appears, so do new methods of making digital models. These new methods have allowed for cost-effective digital modeling, opening the industry of mapping to a broader range of practitioners. The question is, how do these other models compare to the standard DEM in terms of accuracy, precision, and dependability? Photogrammetry is the science of creating an accurate 3D model from photographs. The DSM (Digital Surface Model) created by photogrammetry has yet to be
statistically compared to the DEM. Comparing these two methods could validate the science of photogrammetry and bring this needed science to natural resource and environmental industries previously unable to utilize this scientific resource. To compare the two models, I will be using a Phantom IV drone, a multi-band RTK (Real-Time Kinetic) GNSS (Global Navigation Satellite System) unit, and terrestrial imagery methods. Working from complicated to relatively simple, I will attempt to show that all three methods have a place and purpose in the scientific world. My study site is the Modesitt Mound Group along the Wabash River. This unique site has vegetation ranging from trees to understory vegetation along with varied terrain. Archaeologists have not excavated the mounds, but vandals, looking for artifacts in the 1920s, dug into the tops of many of them, giving a volcanic appearance. There have been archaeological excavations in the plaza area near the mounds which is also part of my study area. These mounds are on private property and have not been digitally mapped before. The variations in the Modesitt Mound Group will allow the methods included in this study to be statistically analyzed in diverse ways. Although the focus of this study is not archaeological, the data from this study can be used for future archaeologic work at the site.

Presentation

Making Soil Maps Accessible for the Arequipa Region, Peru

Darrell G. Schulze, Purdue University
Martín J. C. Villalta Soto, Universidad Nacional de San Agustín de Arequipa, Arequipa, Peru
Zachary Brecheisen, Purdue University
Tim R. Filley, Purdue University
Martha E. Jimenez-Castaneda, Purdue University
Lucia Zuniga, Purdue University
Erika Foster, Purdue University

Soil maps can be difficult, if not impossible, for many people to understand and use. Soil differences are depicted as polygons of similar soils, often on a base map that provides little or no additional context. If the legend gives only the soil classification, then it provides no information unless the user is an expert in soil classification. This was the situation that confronted us for a general soil map of the Arequipa region of southern Peru. Instead of a single map, we parsed the information imbedded in the legend to created 3 separate maps, each of which is fairly simple, but which together present the same information in a more accessible format. The Soil Orders map groups soils according to the major differences in the kinds of soil horizons or features, parent materials, and soil climate. The Soil Moisture Regimes map shows the distribution of arid soils along the coast and moister soils in the mountains. The Pedogenic Features map shows the distribution of a variety of more specific features,
including a number of limiting conditions. All the maps have popups that provide information in both English and Spanish, along with links to additional information. An additional map consists of Sentinel-2 satellite imagery with points that can be queried to provide physical and chemical information about the soils at various sampling locations. The maps can be accessed on the SoilExplorer.net website or with the Soil Explorer app for iOS and Android devices so that users anywhere can learn about soils of the Arequipa region of Peru.

Poster

**Flood-generating mechanisms, seasonality and the impact of the Pacific Decadal Oscillation on Michigan streamflow**

**Todd Grote** - Geosciences Program, School of Natural Sciences, Indiana University Southeast, New Albany, Indiana 47150

The Pacific Decadal Oscillation (PDO) is an ocean-atmosphere teleconnection that expresses variation in sea surface temperature at centers of action in the tropical and extratropical Pacific Ocean, and is known to impact meteorological and hydrological processes. This study first examines flood seasonality and generating-mechanisms throughout the upper and lower peninsulas of Michigan, and secondly examines the relationship of the PDO to peak annual flows (floods), mean annual flow and mean monthly flow. Streamflow composites were created based on the positive (warm) and negative (cool) phases of the PDO, and differences in composite means were compared to address hydrologic variability. Climate Division precipitation and temperature records were partitioned in the same manner.

The results demonstrate distinctly different flood-generating mechanisms between Michigan’s upper and lower peninsulas. Snowmelt-related flooding dominates peak annual flows during the spring (March-May) in the Upper Peninsula, a region where no winter (Dec-Feb) peak flows occur have occurred. The Lower Peninsula exhibits a more mixed flood hydroclimatology with late winter and spring floods dominating the flood series, but also mid-winter flooding. Of greater interest and importance, the PDO appears to exert influence on monthly and seasonal streamflow, with the strongest signal occurring during the cooler months of the year. Cool PDO phases are generally associated with below average streamflow, with some exceptions. The opposite generally holds true for warm PDO phases. Variability within the records is likely due to to individual watershed conditions such as storage and eventual release of water from seasonal snowpack, storm type and track, antecedent landscape conditions, and basin morphometry and geomorphology. However, mean annual and peak annual flows show little consistent relationship to PDO phase. Nonetheless, these results are useful for understanding impacts of climate variability on the
Microbiology & Molecular Biology

Poster

**Identification of *Escherichia Coli* O157:H7 Outer Membrane Proteins Which Mediate Adherence to Bovine Endothelial Cells**

**Vishnu Iyer** (University High School)
Prabha Bista (Department of Comparative Pathobiology, Purdue University)
Sanjeev Kumar Narayanan (Department of Comparative Pathobiology, Purdue University)

*Escherichia coli* (*E. coli*) O157:H7 is a human pathogenic bacterium known to cause foodborne outbreaks of bloody diarrhea and hemolytic uremic syndrome. Cattle are the primary reservoirs of *E. coli*, which are shed in the feces and transmitted to humans through contaminated milk products and uncooked meat. The purpose of the study was to identify bacterial outer membrane proteins (OMPs) involved in the colonization of bovine endothelial cells. It was hypothesized that similar *E. coli* O157:H7 OMPs would be involved in adhesion to both human and bovine endothelial cells. In addition, identification of bovine specific bacterial OMPs can lead to development of effective therapeutic invention strategies to prevent spread of infection. An optimized “pull-down” technique was developed to selectively anchor biotin-labeled bovine endothelial cell surface proteins (CSP) onto a streptavidin bead matrix followed by incubation with *E. coli* OMPs. After washing the beads, bound OMPs were eluted and subsequently analyzed by peptide sequencing. A total of 90 proteins were identified including significant hits of OmpA, OmpX, Omp slp and flagellin, in addition to several chaperone proteins, which represented a group of previously well characterized mediators of adhesion of *E. coli* to human cells. This is the first study in the literature to demonstrate the role of these OMPs for attachment of *E. coli* to bovine endothelial cells.
DNA introduced to a crime scene through indirect transfer is of concern for forensic scientists, as increasingly sensitive technology has improved the detection of DNA unrelated to the criminal event. DNA can be deposited by direct and indirect means on surfaces, often simultaneously. The person contacting the object may transfer their DNA through direct contact or act as a vector for foreign DNA.

DNA profiles obtained from mugs when lips covered in shared ChapStick® contact the rim. One participant (primary user) opened a new tube of ChapStick® in the morning, used it three times throughout the day, then shared the tube with a second participant (secondary user). The primary user then reapplied the ChapStick®. Five minutes later, both participants “drank” five times from a mug. The rim was swabbed for subsequent DNA profiling to test the following null hypotheses: 1) mixed DNA profiles will not be detected in samples obtained from the mug rim of the primary ChapStick® user, and 2) mixed DNA profiles will not be detected in samples obtained from the mug rim of the secondary ChapStick® user.

All samples generated DNA profiles. For 18.75% of the samples, mixed profiles were detected that were consistent with both participants. Two of these originated from swabs of the secondary user’s mug, and one from the primary user’s mug. Both null hypotheses can be rejected. The results of this study demonstrate that the simultaneous direct and indirect transfer of DNA is possible from the lips of an individual who is wearing shared lip balm. Therefore, the DNA of a person who never drank from a cup can be detected on the rim.
Deodorant as a Potential Source of DNA Profiles for References in Criminal Investigations

Alba Craig, BA, University of Indianapolis Biology Department; Samantha Beck, BA, University of Indianapolis Biology Department; Megan Patton, BA, University of Indianapolis Biology Department; Angela Zimmer, MS, University of Indianapolis Biology Department; Jessica Gall, University of Indianapolis Biology Department; Krista E. Latham, PhD, D-ABFA, University of Indianapolis Biology Department; Cynthia Cale, MS, Houston Forensic Science Center.

In forensic contexts, identifying personal items from suspects or victims that frequently yield single-source profiles would simplify the collection of objects for obtaining reference samples. This study investigates if solid deodorant sticks are a good source of biological material for the generation of a DNA profile. The current study tests the following null hypotheses: (1) single-source DNA profiles will not be obtained from solid deodorant and (2) DNA quantity will not predict the completeness of a profile. For nine consecutive days, two participants were each given deodorant decontaminated by exposure to UV light to use once in the morning after showering. The deodorants were wet swabbed each day after usage using sterile technique; one swab was used per deodorant, per day. The deodorants were swabbed on the exposed deodorant stick and the inside of its plastic cap.

Samples were extracted utilizing the QIAamp DNA Mini Kit (QIAGEN Hilden, Germany). Samples were further processed through DNA analysis workflow utilizing Applied Biosystems™ (Carlsbad, CA) products: samples were quantified with Quantifiler™ Trio DNA Quantification Kit on a 7500 Real-Time PCR System; samples were amplified using Globalfiler™ Amplification Kit on a ProFlex™ PCR System and analyzed on a 3500xL Genetic Analyzer.

DNA quantification results ranged from 0 to 1.7 ng/μL; most samples yielded no detectable DNA profiles. Two samples had only one allele consistent with the deodorant user. In a third sample, 11 out of 34 alleles detected were consistent with the user. The first null hypothesis can be partially rejected as consistent alleles were present, but no full profiles were obtained. Based on the data, the second hypothesis can be rejected as there’s no relationship between quantification and DNA profiling results. In conclusion, solid deodorant is not a good source for generating single-source profiles that could be useful as references in criminal investigation.
Poster

Investigating Third- and Fourth-Generation Cephalosporin Resistance of *Escherichia coli* Plasmids in Northwest Indiana Water Systems

Angad Sidhu, Ayesha Khan, Palak Patel, Dr. Jenny Fisher; Indiana University Northwest

The One Health concept illustrates the connection between the health of humans, animals, and their shared environments, along with the transmission of disease when this delicate balance is disrupted. The prevalence of antibiotic resistant bacteria has caused a major healthcare crisis worldwide, and it has become crucial to monitor the spread of these organisms. Plasmids that harbor antibiotic resistance genes can be transferred among bacterial populations and serve as one mechanism for the spread of resistance and the increase in multiple resistant organisms. Our research investigates plasmids with extended-spectrum beta-lactamase (ESBL) genes that confer resistance to drugs such as third- and fourth-generation cephalosporins found in *Escherichia coli* isolated from the environment. Environmental water samples were collected and analyzed by membrane filtration and plating on selective and differential agar amended with cephalosporin antibiotics. Colonies characteristic of *E. coli* were selected, purified, and stored as isolates for further testing. Plasmids were extracted from the confirmed resistant isolates, followed by molecular methods to amplify known ESBL genes *blaCTXM*, *blaSHV*, and *blaTEM*, with visualization by gel electrophoresis. Concurrently, Minimum Inhibitory Concentration (MIC) and Kirby-Bauer tests were performed on *E. coli* isolates to determine the degree of resistance to cephalosporins and resistance to other classes of antibiotics, respectively. We have thus found a consistent resistance to both the third- and fourth-generation cephalosporins, cefotaxime and cefepime, along with isolates that display multidrug resistance.

Presentation

A One Health Approach to Detecting Emerging Antibiotic Resistance in Northwest Indiana

Jenny C. Fisher, Biology Department, Indiana University Northwest

Antimicrobial resistance is a critical problem for the health care profession worldwide, but the evolution of multi-drug resistant strains dubbed “Superbugs” presents an even more extreme level of concern. Superbugs are pathogens that have acquired resistance to almost all classes of preferred antibiotics, and the threat of pan-resistance is an imminent possibility. Emerging mobile resistance to last resort drugs like colistin may mean that some common infections, particularly secondary, healthcare acquired infections, become untreatable. Who are these Superbugs, and how did
they gain this remarkable suite of abilities? This talk will explore the genetic and ecological mechanisms that facilitate the acquisition and maintenance of new antibiotic resistance traits. I will track the epic rise of the Superbugs like *Neisseria gonorrhea*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Clostridioides difficile*. Each of these organisms took a unique path to notoriety, but all pose a significant human health risk. While the outlook seems dire, novel approaches such as phage- and microbiome-based therapy are a promising alternative to traditional antibiotics and might provide treatment options in the future.

**Poster**

**Characterizing the role of Candida albicans Ume proteins**

**Ben Evans, Douglas Bernstein, Ball State University**

*Candida albicans* is a polymorphic fungus found in the gut of most humans and is the most commonly isolated human fungal pathogen. The ability to undergo various morphological changes is a *C. albicans* major virulence factor. *C. albicans* typically grows in the yeast morphology. However, environmental or chemical stimuli, such as human body temperature and presence of serum, induce a morphological shift towards filamentation. Filamentous cells are capable of invading host tissues as well as forming complex biofilms. A complex network of transcription factors regulates formation of invasive filaments. *C. albicans* Ume6 is a transcriptional regulator known to control induction and duration of hypha-specific gene expression. Ume6 contains a conserved zinc finger domain hypothesized to be important for DNA binding. Preliminary data from our lab suggests Ume6 N-terminus and zinc finger domain are insufficient to induce filamentation. Ume6 contains a conserved C-terminus we hypothesize to be important for DNA binding and filamentation. Using CRISPR-mediated genome editing we inserted stop codons or missense mutations into the C-terminus of Ume6. Resulting mutants were screened for filamentation defects and decreased virulence. In addition, *C. albicans* Ume7 is an uncharacterized homolog of Ume6. Preliminary data from our lab suggests Ume7 is not involved in regulation of filamentation however, the *Saccharomyces cerevisiae* homolog of Ume7 regulates expression of early meiosis genes. We hypothesize the putative zinc finger domain of Ume7 is involved in regulation of white/opaque switching a morphological transition necessary for *C. albicans* mating. We inserted stop codons upstream of Ume7 in the white/opaque switching strain, WO-1 and changes in white/opaque switching were assessed. Our data suggests that the Ume family of transcription factors play a variety of roles in *C. albicans* morphology.
Ochratoxin A Production by *Aspergillus Carbonarius* on Nyjer and Flax Seeds

Dawit Gizachew1, Sandra De La Torre1, Wei-tsyi Ting2

1Department of Chemistry & Physics 2Department of Biological Sciences, Purdue University Northwest, Hammond, IN 46323

Food safety is a major concern globally including in the USA. Mycotoxins are secondary metabolites of toxigenic strains and species of molds which can contaminate crops before or after harvest. The Food and Agriculture Organization of the United Nations (FAO) estimated that approximately 25% of the cereals produced in the world are contaminated by mycotoxins. Consumption of mycotoxin contaminated food can cause acute to chronic diseases. The symptoms may range from vomiting, birth defect, neurotoxicity, kidney damage (nephrotoxicity), liver cancer, and death. Feeding moldy feed to animals can result in mycotoxin contaminated milk, meat and eggs. Many countries have adopted regulations to limit mycotoxin exposure. Ochratoxin A (OTA) is produced by several species of *Aspergillus* such as *A. carbonarius*. OTA is one of the most widespread mycotoxins in food commodities worldwide and is among the most toxic compounds that affect both human and animal health. Nyjer and flax seeds are important oilseeds that are used for both human and animal consumption, but they are highly susceptible to fungal growth and mycotoxin contamination. The oilseeds are used in a variety of ways for both human and animal consumption and are important sources of cooking oil. In the last two decades, oils extracted from plant seeds have gained popularity because of their health benefits over animal-based fats. Oilseed cakes, which are residues of oil extraction, are used by farmers to increase productivity of their animals. Ground oilseeds and oilseed cakes (animal feed) are stored for extended periods of time, which may allow mold growth and the production of mycotoxins such as ochratoxins.

We determined water activities and temperatures that support or reduce the fungal growth and production of OTA by *A. carbonarius* on ground nyjer and flax oilseeds. The results of this study will provide guidance to minimize OTA production on ground nyjer and flax seeds during storage and processing.
Poster

**Profiling the Antibiotic Resistome in Rural and Urban Soil Environments in Indiana**

Noah Schuster1, Victoria Nguyen1, and Elizabeth Orta1
1DePauw University, Greencastle, IN, 46351

The soil is a very large reservoir for microbial diversity; therefore, profiling the soil resistome can help determine the composition and distribution of antibiotic resistant bacteria and their genes in the soil. This can provide information to further understandings of antibiotic resistance evolution and development. We hypothesize that larger numbers of resistance genes would be present in urban environments when compared to rural environments. Soil was collected from twelve locations, with six locations in a rural environment and the other six in an urban environment. Soil from each location was characterized on texture and pH, followed by enrichment with tryptic soy broth containing either tetracycline, carbenicillin, or chloramphenicol and incubated for three days at room temperature. Following DNA extractions from each enrichment, the presence of tetracycline resistance genes was evaluated. In total, 25 tetracycline resistance genes were found to exist throughout 11 soil locations and the most frequently detected genes were both tet(L) and tetA(P), followed by tet(M), tet(O), tet(B), and tet(A). In addition, 13 beta-lactamase genes were found to exist throughout 11 locations and the most frequently detected gene was blaTEM-1, followed by cmy-13 and ampC. Overall, 14 tetracycline resistance genes and 7 beta-lactamase genes were in rural soils while 11 tetracycline resistance genes and 6 beta-lactamase genes were in urban soils. Taken together, our data to-date suggests the number of tetracycline resistance genes and beta-lactamase genes are equally distributed in rural and urban environments. No clear relationship between soil texture or pH versus the number of genes present in each location exists. We are planning to assess chloramphenicol enrichments and determine the microbial communities in each location using metagenomics. This study will help identify reservoirs of antibiotic resistance and further elucidate its spread between soil bacteria; furthermore, we might help reveal new patterns of molecular mechanisms for resistance.
Glycogen is a multi-branched polysaccharide of glucose that serves as a form of energy storage in many organisms with the highest concentrations being in the liver and muscle. However, glycogen in the brain, where it plays a role in learning, has been hypothesised to act as a neuroprotectant during physiological stressors such as hypoglycemia and hypoxia. Symptoms of hypoxia include rapid breathing, shortness of breath, lack of cognitive function and motor coordination, neurodegeneration, and cellular death. Hypoxia can be caused by asthma attacks and other lung-related diseases, obstructive sleep apnea, heart problems, anemia, usage of strong pain medicines that challenge breathing, and environmental factors such as altitude and varying oxygen quality and concentrations. To test the importance of brain glycogen during low oxygen conditions, mice (8-10 days old) with or without functional glycogen synthase in the brain were subjected to carbon dioxide- or nitrogen-induced hypoxia. The effects on physiological states, brain glycogen levels, and survival time were evaluated. These studies showed that mice lacking brain glycogen exhibited characteristic physiological response to hypoxia, but expired ~50% sooner than mice with brain glycogen. These results show that brain glycogen is important for survival in hypoxic conditions. Future studies will examine whether brain glycogen may protect against hypoxia-induced cognitive impairment and neurodegeneration.

Forkhead (Fkh/Fox) domain transcription factors (TFs) mediate multiple cardiogenic processes in both mammals and Drosophila. We showed previously that the Drosophila Fox genes jumeau (jumu) and Checkpoint suppressor homologue (CHES-1-like) initially specify cardiac progenitors by transcriptionally activating FGF and Wnt receptors and subsequently mediate three categories of cardiac progenitor cell division—asymmetric, symmetric, and cell division at an earlier stage—by regulating Polo kinase activity, with the latter two categories being regulated with the assistance of Myb. These observations raised three intriguing
developmental questions: (i) whether additional Fox-regulated genes are involved in mediating either of these processes, (ii) whether these downstream genes are regulated exclusively one of these Fox TFs or by both, and (iii) whether, in the case of cardiac progenitor cell division, the regulated gene mediates all three categories of division, or a subset thereof. By comparing transcriptional expression profiles of wild-type and jumu loss-of-function embryos, we identified nebbish (neb), a kinesin-encoding gene transcriptionally activated by jumu, but not by CHES-1-like. Phenotypic analysis of mutations show that neb is required for only two categories of jumu-regulated cardiac progenitor cell division: symmetric and cell division at an earlier stage. Synergistic genetic interactions between neb, jumu, Myb, and polo and the rescue of jumu mutations by ectopic cardiac mesoderm-specific expression of neb demonstrate further that neb is an integral component of a jumu-regulated subnetwork mediating cardiac progenitor cell divisions. Our results thus illustrate how an individual regulator can utilize different combinations of downstream effectors to control distinct developmental processes.

Poster

Increased catalase activity during the propane metabolism in the soil bacterium Rhodococcus sp. ENV425

Esmeralda Perez, Carina Rajagopal, Hisako Masuda
School of Sciences, Indiana University Kokomo

Catalase converts toxic hydrogen peroxide into water and molecular oxygen, thereby protecting cells from oxidative damage. It is ubiquitously found in all kingdoms of life. In cells, hydrogen peroxide is produced as a result of fortuitous reduction of molecular oxygen by electrons in the electron transport chain. In our previous work, a catalase gene was identified in close proximity to the genetic loci encoding propane metabolic enzymes in soil bacteria Rhodococcus strain ENV425. Moreover, the level of catalase was significantly increased in cells grown on propane as a sole source of carbon. In this study, whether or not total cellular catalase activity differs depending on the carbon source was investigated. Our results show that the catalase level was increased in strain ENV425 but not in another propanotroph Mycobacterium smegmatis. This result suggests a unique regulation of catalase expression in strain ENV425 and we propose the possibility of metabolic hydrogen
Poster

**Utilizing Wastewater as a Preemptive Screening Method for Emerging Colistin Resistance in Northwest Indiana**

*Ayesha Khan, Palak Patel, Angad Sidhu, Jenny Fisher*
Institution: Indiana University Northwest

Recent reports have highlighted the emergence of global resistance to colistin, a “last resort” antibiotic for humans, but one commonly added to animal feed in Europe and China. Acquired colistin resistance is mainly attributed to bacterial plasmids harboring the *mobile colistin resistance (mcr)* gene. Having an early detection system for emerging resistances is crucial for clinical preparedness. Thus, we hypothesize that sewage collected from a large population can provide early insight into the resistances present in local environmental, commensal, and pathogenic bacterial populations. For this study, we used untreated wastewater from Northwest Indiana to identify bacteria that have acquired colistin resistance through plasmids bearing the *mcr* gene. We first processed wastewater through membrane filters, which were plated onto selective media containing colistin. After isolating colonies from the plates, we performed minimum inhibitory concentration tests to profile the extent of the isolates’ resistance. Plasmids were extracted from the isolates and used for downstream molecular analysis. We performed a polymerase chain reaction (PCR) for different *mcr* variants on these plasmids and used gel electrophoresis for visualization. The results indicated a variable degree of resistance among the wastewater bacterial populations. Resistance was typically associated with plasmid-based *mcr* genes. Some resistant isolates lacked an *mcr* containing plasmid, implying that alternative modes of resistance exist. Plasmids provide a vehicle for resistance conferring genes to be transferred to pathogens and other resistant organisms, potentially creating a superbug. Moreover, our data suggest that *mcr*-harboring colistin-resistant bacteria have spread into the Northwest Indiana human population and may present an additional challenge for local healthcare providers.
Adolescent Intermittent Ethanol Increases the Sensitivity to the Reinforcing Properties of Ethanol and the Expression of Select Cholinergic and Dopaminergic Genes within the Posterior Ventral Tegmental Area

Sheketha R. Hauser, Christopher P. Knight, William A. Truitt, Robert Aaron Waeiss, Ian S. Holt, Gustavo B. Carvajal, Richard L. Bell, and Zachary A. Rodd

Background: Although not legally allowed to consume alcohol, adolescents account for 11% of all alcohol use in the United States and approximately 90% of adolescent intake is in the form of an alcohol binge. The adolescent intermittent ethanol (AIE) model developed by the NADIA consortium produces binge-like EtOH exposure episodes. The current experiment examined the effects of AIE on reinforcing properties of EtOH and genetic expression of cholinergic and dopaminergic factors within the posterior ventral tegmental area (pVTA) in Wistar male and female rats and in male alcohol-prefer-ring (P) rats.

Methods: Rats were exposed to the AIE or water during adolescence, and all testing occurred during adulthood. Wistar control and AIE rats were randomly assigned to groups that self-administered 0 to 200 mg% EtOH. Male P rats self-administered 0 to 100 mg%.

Results: The data indicated that exposure to AIE in both Wistar male and female rats (and male Prats) resulted in a significant leftward shift in dose–response curve for EtOH self-administration into the pVTA. TaqMan array indicated that AIE exposure had divergent effects on the expression of nicotinic receptors (increased a7, reduction in a4 and a5). There were also sex-specific effects of AIE on gene expression; male only reduction in D3 receptors.

Conclusion: Binge-like EtOH exposure during adolescence enhances the sensitivity to the reinforcing properties of EtOH during adulthood which could be part of biological sequelae that are the basis for the deleterious effects of adolescent alcohol consumption on the rate of alcoholism during adulthood.

Key Words: Ventral Tegmental Area, Adolescent Alcohol, Nicotinic Receptors, Reward.
HotTopics

**Antibiotic Resistance in Gram-Negative Bacteria: How Does Central Indiana Fare?**

**Karen Bush**
Indiana University Bloomington
Bloomington, IN

Antibiotic resistance is considered to be one of the most urgent conditions in modern medicine. Organizations such as the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC) and the United Nations (UN) have all identified a set of antibiotic-resistant pathogens that pose critical or urgent threats to humans. Among these are enteric Gram-negative bacteria that are resistant to almost all beta-lactam antibiotics such as cephalosporins and the carbapenems, as well as to aminoglycosides and colistin which are viewed as drugs of last resort to treat seriously ill patients. Most of these organisms carry plasmids bearing multiple resistance genes that can be readily transferred among species, making control of resistance more difficult. Although nine new antibiotics have been approved in the last five years to treat infections caused by these bacteria, not all infections can be successfully treated, and, resistance may still emerge to the new agents. My laboratory has been identifying resistance mechanisms in multidrug-resistant enteric bacteria isolated from hospitals and healthcare facilities in central Indiana between 2009 and 2019. We have also tested many of the newly approved drugs as they were moving through clinical development. In this discussion, comparisons will be made showing how the population of resistant bacteria in Indiana compare to those found in the rest of the world, and how new antibiotics behave against some of the most deleterious of these bacteria. Although the results are overall encouraging, the rapid spread of these organisms and the paucity of new drugs on the horizon are worrisome.

Poster

**Isolation and characterization of a *Citrobacter freundii* bacteriophage**

**Kaitlyn Comstock** and Natalia B. Hubbs, PhD

Viruses outnumber cells 10:1 with bacteriophages (phages) being one of the most abundant subgroups. Bacteriophages infect bacterial hosts by inserting their genome into the host and hijacking its machinery to replicate the phage. Recently, there is an interest in using phage therapy as treatment for bacterial infections due to increases in antibiotic resistance. *Citrobacter freundii (Citrobacter)* causes a variety of nosocomial infections of the urinary and respiratory tract, however, *Citrobacter* has become increasingly multi-drug resistant. The primary focus of this work was to isolate and characterize a bacteriophage from the environment that infects *Citrobacter*; the phage,
JAY-1, was isolated from primary effluent provided by the Madison Wastewater Treatment Plant. JAY-1 was isolated and characterized by enrichment techniques, plaque assays, and electrophoresis. The preliminary data indicates a limited host range of the phage. In the future, more tests will be performed to understand the phage’s mechanism of infection. This host-phage interaction will allow for a better understanding of human health and environmental factors regarding treatment of *Citrobacter*.

Presentation

**Stringent response regulation of virulence genes in *Vibrio cholerae***

David Raskin, Marian University College of Osteopathic Medicine
Huajun He, Marian University College of Osteopathic Medicine
Arunima Mishra, Loma Linda University
Zachary Lundy, Marian University College of Osteopathic Medicine

*Vibrio cholerae* is the etiologic agent of the epidemic diarrheal disease cholera. *V. cholerae* is a Gram-negative bacterium that produces several virulence factors, including toxin-coregulated pilus (TCP) and cholera toxin (CT). TCP is necessary for *V. cholerae* to colonize the host GI tract, while CT is primarily responsible for the watery diarrhea. TCP is required early in infection in order to colonize the small intestine and CT is expressed later in order to produce diarrhea and escape the host, although it is not well understood how differential timing of CT and TCP expression occurs. Expression of these factors is controlled by the ToxR regulon. The ToxR regulon includes ToxT, a transcription factor that directly initiates transcription of the CT and TCP genes; and the upstream regulators TcpP and ToxR, integral membrane transcription factors necessary for expression of *toxT*. TcpP and ToxR activity requires TcpH and ToxS, respectively. ToxR can also induce expression of the CT genes independent of ToxT. We found that the stringent response, the low nutrient stress response, regulates components of the ToxR regulon. We made deletions in the three *V. cholerae* stringent response genes (*relA*, *spoT* and *relV*) and analyzed the stringent response-defective mutants for expression of the *toxRS*, *tcpPH* and *toxT* genes in conditions in which the ToxR regulon is induced. We found that stringent response induced *toxT* and *tcpPH* expression, while it repressed *toxRS*. We also found that stringent response defective mutants showed loss of TCP expression and were unable to colonize infant mice. Stringent response had minor affects on CT expression. This work showed that stringent response produced differential regulation of ToxR regulon components and may be involved in the differential timing of CT and TCP expression. We are continuing to investigate the interaction between stringent response and the ToxR regulon and how CT and TCP can be differentially expressed.
Identification and Characterization of Bacteria that Can Metabolically degrade Nylon-11

Jocelyn Gatz-Schrupp and Hisako Masuda
School of Sciences, Indiana University Kokomo

Nylon-11 is a synthetic polymer used commonly in many manufacturing industries. Because of its highly crystalline structure, it is resistant to chemical and physical decomposition. Therefore, when released into environments such as the ocean, it persists without degradation for a long period of time. This poses a significant threat to human and animal health. In this study, we show that pre-treatment of nylon-11 with organic solvents was effective in increasing the bioavailability of nylon-11. Using this modified nylon 11, we successfully isolated four bacterial strains that can grow on media with nylon-11 as a sole source of nitrogen. In this presentation, we will discuss our current progress in the characterization of these bacteria and the enzymes involved in nylon degradation in these strains.

Importance of lipid droplets in immunosuppression during Coxiella burnetii infection

Kyle Reed, Mahek Ali, Morgan E. Harrison, Minal Mulye
Marian University College of Osteopathic Medicine, Indianapolis, IN

Coxiella burnetii, the known causative agent of Q fever, is an obligate intracellular bacterium capable of infecting host alveolar macrophages and residing within these cells for several years. While causing mild symptoms at initial infection, C. burnetii retains the ability to survive long-term by disseminating and causing serious complications such as culture-negative endocarditis. Previous studies in endocarditis patients demonstrated that C. burnetii suppresses the host immune response via production of prostaglandin E2 (PGE2) and modulation of anti-inflammatory cytokine IL-10 and proinflammatory nitric oxide (NO). C. burnetii is also found in lipid droplet-rich foam cells suggesting the importance of host cell immune response and lipid metabolism during bacterial infection. Previous studies in our lab revealed that C. burnetii manipulates host cell lipid storage organelles, lipid droplets (LDs), via its type 4 secretion system (T4SS) which secretes effector proteins into the host cell cytoplasm to modulate cellular processes. Further, we observed that breakdown of LDs is essential for C. burnetii growth suggesting the importance of LD-derived lipids during bacterial infection. LD breakdown releases arachidonic acid, a PGE2 precursor. Since PGE2 plays an immunosuppressive role during C. burnetii infection, we hypothesize that LD-derived PGE2 production leads to modulation of IL-10 and
NO. To assess this, we determined IL-10 and NO production enzyme iNOS gene expression in mouse alveolar macrophages by quantitative Real-Time PCR (RT-qPCR). Compared to uninfected cells, iNOS gene expression decreased while IL-10 expression remained unchanged. Further, we performed ELISA to determine IL-10 protein level and identify the contribution of LDs to IL-10 production. Compared to uninfected cells, IL-10 production in C. burnetii-infected cells remained unchanged. Surprisingly, compared to untreated cells, blocking LD formation and breakdown with specific inhibitors resulted in increased IL-10 production. This suggests that LDs play an important role in IL-10 production in C. burnetii-infected cells. However, if IL-10 contributes to immunosuppression is yet to be determined. Ongoing studies are identifying the importance of LDs in NO production. Future studies will determine the changes in the overall cytokine profile in the presence/absence of LD breakdown.

Poster

**Human cathelicidin (LL-37) exhibits non-strain specific inactivation of a panel of recombinant respiratory syncytial virus (RSV) isolates**

**Karina N. Latsko**, Nathan A. Junod, Andrew T. Jacob, Caitlin E. Haas, Katelyn R. Castiglia, and Christopher C. Stobart

Department of Biological Sciences, Butler University, Indianapolis, IN

Respiratory syncytial virus, or RSV, is a small negative-sense single stranded RNA virus that is responsible for both upper and lower respiratory tract infections that predominantly affects infants, the elderly, and those who are immunocompromised. Due to its poor ability to elicit an immune response in humans as well as its overall instability, it has been difficult to create a functional vaccine for RSV. However, new interesting therapeutic options have been recently introduced. Antimicrobial peptides (AMPs), are tiny molecules that have existed in many organisms for many years and recent research has shown them to be of great importance in the immune response of some organisms and have even shown direct antimicrobial activity. Cathelicidin, or LL-37 is one of the more well studied AMPs in terms of its structure and function; it is a host defense peptide that is typically expressed in the epithelial cells. As past research has suggested that AMPs can protect cells from detrimental effects, we used cathelicidin to determine the efficacy of inactivation of the recombinant RSV strains. Past studies have displayed cathelicidin’s ability to damage the viral envelope and therefore inactivate RSV, but all have only done research with the lab strain of RSV, A2. This study demonstrates the effectiveness of human cathelicidin in inactivating various recombinant viral strains of RSV and aims to provide the foundation for future treatments of active RSV infections.
**Poster**

**Evaluation of a panel of recombinant respiratory syncytial virus (RSV) strains to human beta-defensin inactivation**

**Andrew T. Jacob**, Nathan A. Junod, Karina N. Latsko, Caitlin E. Haas, Katelyn R. Castiglia, and Christopher C. Stobart

**Department of Biological Sciences, Butler University, Indianapolis, IN**

Human beta-defensins, a class of antimicrobial peptides, are proteins that have been found to be a significant part of the natural immune response to various pathogens. Respiratory syncytial virus (RSV) is a respiratory pathogen capable of causing serious upper and lower respiratory infections in infants and children, and it is the number one viral cause of infant mortality. There is currently no effective vaccine for the virus, as past attempts at creating one have been unsuccessful as a result of the virus’s low immunogenicity, its ability to effectively mutate, and underlying instabilities of potential vaccines. Previous studies have shown that antimicrobial peptides may have an effect on viral replication and spread of RSV. Our study considers human beta-defensins hBD-1, hBD-3, and hBD-4. The antiviral activity of antimicrobial peptides and the tendency of RSV to have differences based on viral strain lead us to believe that glycoproteins on the surface of the virus may act in the peptides’ inactivation of the recombinant viral strains. By testing beta-defensins on a panel of recombinant RSV strains, we hope to find strain-specific vulnerabilities of the virus to peptide inactivation. The study’s evaluation of the role that strain-specific differences play in relation to peptide inactivation may inspire new treatment options when dealing with patients suffering from RSV.

**Presentation**

**Evaluation of the conservation and functionality of the interdomain loop of the coronavirus mouse hepatitis virus (MHV) protease nsp5**

**Benjamin C. Nick**, Mansi C. Pandya, Sean M. Callahan, Megan E. Franke, Emily F. Hasik, and Christopher C. Stobart

**Department of Biological Sciences, Butler University, Indianapolis, IN**

Human coronaviruses are associated with a variety of diseases ranging in severity from the common cold to severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). Activity of the coronavirus protease, nsp5, is required for virus replication and remains a primary target for efforts to produce an inhibitor to block active infections. Nsp5 is comprised of 3 distinct domains with domains 1 and 2 forming the enzymatic active site and a third domain involved in dimerization.
Between domains 2 and 3 is a 15-residue interdomain loop (IDL) of unknown function. We hypothesize that this loop plays a critical role in the structure and function of nsp5. To test this hypothesis, we used site-directed mutagenesis to evaluate whether the IDL could tolerate mutagenesis. Recovered viruses were tested for replication and temperature-sensitivity. We identify a novel critical “horseshoe” region within the IDL associated with three residues which failed to permit mutagenesis and an additional two residues associated with temperature-sensitivity upon changes to alanine. These studies provide new insight into a unique structural feature of the nsp5 protease of coronaviruses and may identify a new putative target for coronavirus inhibitor design.

Poster

**Deletion of mucin domains in the G glycoprotein of respiratory syncytial virus (RSV) results in attenuation, increased immunogenicity, and reduced thermal stability**

Molly K. Roe1, Stacey A. Lapp2,3, Christopher C. Stobart1, and Christina A. Rostad2,3

1Department of Biological Sciences, Butler University, Indianapolis, IN
2Department of Pediatrics, Emory University, Atlanta, GA
3Children’s Healthcare of Atlanta, Atlanta, GA

Respiratory syncytial virus (RSV) is one of the leading causes of lower respiratory tract infections in infants, with cases ranging minor upper respiratory tract infections to pneumonia with respiratory failure, yet there remains no vaccine. RSV G, a glycoprotein on the virus’s surface that facilitates attachment to the host cell, is antigenic and could be a strong therapeutic target, but its contribution to viral stability, growth kinetics, and shielding of RSV F (a second surface glycoprotein facilitating viral fusion that is a well-studied antigenic target) is unclear. Here we explore the phenotype of viruses expressing varying mutations in their G protein (which consists of a small, central conserved region and two variable mucin domains). To explore the effects of these different regions, RSV viruses were generated in strain A2-line19F with mKate2 label with either wildtype full-length G (membrane-bound and secreted), truncated G with removed mucin domains (membrane-bound and secreted), and truncated G with removed mucin domains (secreted only), and desired protein expression was confirmed via western blotting. ELISAs were also performed to explore G’s effect on F. Viral growth kinetics and thermostability were measured in HEp2 and Vero cells. Removal of G mucin domains (AZ-G155) led to increased F binding by ELISA despite similar F expression levels by Western blotting, and complete removal of surface glycoprotein (AZ-G155S) led to increased F expression. AZ-G155 was the most attenuated, followed by AZ-G155S and then A2L19F
respectively. No significant difference in kinetics was apparent in Vero cells, making this cell line likely suitable for rescue of a vaccine candidate. Thermostability at 37°C was likewise measured, with A2L19F having the highest stability, followed by A2G155 and A2-G155S respectively. Viral growth kinetics and thermostability were also measured in two thermosensitive (unstable) strains with G-null mutations: A2 and 220. In these cell lines, significant differences were found in viral growth kinetics, but not in thermostability. The G protein appears to increase viral growth kinetics, as well as contribute in some degree to thermostability. Removal of the mucin domains can affect F expression depending on the strain-specific stability of F, suggesting some shielding.

Poster

**Current Update on the Survey of Antibiotic Resistance and Associated Genes in Natural Communities of Bacteria in an Urban Wetland Ecosystem (Marian EcoLab) in Indiana**

Azeem Ahmad1, Samina Akbar2

1Department of Biology, 2College of Osteopathic Medicine, Marian University 3200 Cold Spring Road, Indianapolis, IN46222.

Approximately 2 million infections from antibiotic resistance (AR) bacteria occur annually in the United States, resulting in a minimum of 23,000 deaths. According to the Center for Disease Control (CDC), spread of antibiotic resistance (AR) among gram-negative bacilli (GNB) is arguably the most worrisome. In recent years, considerable research focus has been on AR from specific sources which has led to good understanding of pathways and drivers in the environment. However, much less is known about AR in urban wetlands and its dispersal to opportunistic pathogens in natural habitats. In this study, we did initial survey of AR bacteria and ARGs in a pristine wetland ecosystem. We hypothesized that bacteria in wetland environments could serve as a major reservoir of resistance genes as a result of introduction (and progressive accumulation), in the environment, of antimicrobial agents etc. Spatial and temporal detection of these resistant bacteria will help in source-tracking of antibiotic resistance and identifying in situ factors for selection of ARGs and may even help in detecting a hidden “resistome in natural environments. To test this hypothesis, we first isolated bacteria of the *Enterobacteriaceae* group and analyzed their AR profile against 20 currently used antibiotics in health clinics. Preliminary results show prevalence of multidrug resistance (MDR) and suggest widespread mixing, evolution and dispersal of antibiotic resistant bacteria. Molecular analysis of isolated bacteria indicate presence of MDR genes both on the chromosomes and on extra-chromosomal elements in these bacteria as well as presence of type 1 integrons. We are currently determining the incompatibility profiles of plasmids present in these
bacteria as well as further characterizing the MDR genes identified in these bacteria. We will provide a current update on these experiments. This study will focus on deficiencies in state-wide policy for controlling AR in wetland ecosystems.

Poster

**Mitochondrial Dysfunction in Motor Neurons: An Electron Micrograph Study in Aging**

**Max Sheng**, Marian University College of Osteopathic Medicine  
**Heewon Choi**, Marian University College of Osteopathic Medicine  
**Xinglong Wang**, Case Western Reserve University

Objective: To investigate the correlation between Mitochondria-ER contacts (MERCs) in cortical motor neurons and their relation to aging.

Background: Neurons are highly differentiated cells with a high-energy demand and their loss is often seen in relation to mitochondrial dysfunction. One of the important factors for maintaining mitochondrial health and dynamics are MERCs. MERCs were shown in the last decade to help facilitate a flux of information transfer between the mitochondria (mt) and endoplasmic reticulum (ER), specifically playing roles in both lipid metabolism and calcium interchange. Several proteins are known to be involved in facilitating mt-ER tensionary such as Mitofusin2 (mfn2). Mt-ER cross talk are vital to maintaining mitochondrial stability as they are involved in inflammasome formation, activation of autophagy for removal of damaged mitochondria and redox signaling control.

Design/Methods: 4 groups of mice animal models were used and sacrificed at 6, 12, 24, and 36 months respectively. Mice brain tissue were fixed in 4% w/v formaldehyde, dehydrated in ethanol and embedded in LR white resin. Thin sections were blocked with PBC and processed for transmission electron microscopy. Imaging analysis was performed with an open-source image analysis program called ImageJ. MERCs on EM were characterized as "tight tensionary" if its distance was 0-50nm, "loose tensionary" if 50-100nm and no tensionary if >100nm.

Results: Cortical and hippocampal sections of mice age 6 months had the greatest amount of tight mt-ER tensionaries (88% of all tensionaries) followed by a gradual decline as mice grew older to 12, 24, and 36 months. Neuron EM sections at 36 months showed very few mt-ER tensionaries with the majority being loose tensionaries. There was no major difference seen between cortex versus hippocampus sections.
Conclusions: These findings indicate that mitochondrial health and stability are critical to neuronal function and a decrease in MERCs is seen with aging.

Poster
Molecular Dynamics Investigation of the Physical Binding of the NNK Diazonium Ion to Exon 5 of TP53

David Wahl*, Christos Deligkaris*, Evan Millam**

Department of Geology and Physics*, Department of Chemistry**, University of Southern Indiana, Evansville, IN, 47712

The diazonium ion metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a strong carcinogen found in tobacco smoke. NNK diazonium ion has been shown to damage DNA through the formation of pyridyloxobutyl (POB) adducts. The current study focuses on the physical interaction of a tumor suppressor gene (TP 53, exon 5) with NNK diazonium ion. TP53 is mutated in nearly 50% of all human tumors and 40% of lung cancer cases. Our work explores the physical interactions that precede bond formation. Density functional theory was used to generate improved force field parameters for the diazonium ion. Molecular dynamics simulations were performed on two systems: NNK diazonium ion and TP53/NNK diazonium ion. Results of the MD simulations that will be presented include an analysis of the various conformations NNK diazonium ion explores and distances of its reactive carbon atoms to reactive atoms of TP53 bases. This will provide insight into the possible reaction mechanism of POB adduct formation.

Presentation
Role of cyclooxygenase inhibitors in blocking Coxiella burnetii intracellular growth.

Steven Wooten, Constantine Kim, Michael Shain, Minal Mulye

Department of Biomedical Sciences, Marian University College of Osteopathic Medicine

Coxiella burnetii is an obligate intracellular bacterial pathogen and a causative agent of culture-negative endocarditis. While Coxiella initially infects alveolar macrophages, it may disseminate and cause endocarditis up to 20 years after initial infection, which can be fatal if untreated. Even for those receiving treatment, the mortality rate is 19%. Further the treatment has several restrictions and poses significant side effects. Hence, designing better treatments is crucial. To this end, it is important to determine the pathogenesis of Coxiella endocarditis. The occurrence
of *Coxiella* endocarditis several years after initial infection suggests that the bacterium survives long term by subverting host immune response. Our overall goal is to elucidate the specific host immune pathways *Coxiella* modulates to achieve long-term intracellular survival and identify potential therapeutic targets. Previous studies in endocarditis patients demonstrated an increase in lipid immune mediator prostaglandin E2 (PGE2) which contributed to immunosuppression to promote bacterial growth suggesting importance of PGE2 in *Coxiella* survival. Our preliminary ELISAs in *Coxiella*-infected alveolar macrophages demonstrated increased PGE2 production. Since PGE2 production is directly correlated to the enzyme cyclooxygenase (Cox) expression and activity, we determined expression of the constitutive *Cox*-1 and the inducible *Cox*-2 genes. Compared to uninfected cells, our gene expression analysis revealed *Cox*-2 but not *Cox*-1 upregulation in *Coxiella*-infected alveolar macrophages. Since FDA-approved *Cox*-2 inhibitors are readily available, we will test whether blocking *Cox*-2-mediated PGE2 production using the inhibitor celecoxib alters *Coxiella* growth. Compared to untreated cells, our preliminary qualitative microscopic analysis demonstrated *Coxiella* growth inhibition in celecoxib-treated alveolar macrophages. Ongoing studies are determining optimal inhibitor concentrations and quantitating changes in *Coxiella* growth after celecoxib treatment. Future studies will elucidate the mechanisms *Coxiella* employs to regulate *Cox*-2 expression and PGE2 production to induce immunosuppression.

**Poster**

**The Effect of Different Temperatures/Humidity Ranges on Cultures of Hericium Erinaceus**

**Bradley Enneking**  
Ivy Tech Community College

*Hericium Erinaceus*, also known as Lion’s Mane, is an edible and medicinal mushroom known for its medicinal benefits that grows on dead logs/trees in North America and Asia. Mushrooms are cultivated by combining a colonized spawn with an uncolonized substrate in a growing chamber and allowing the mycelium from the colonized spawn to feed on and colonize the uncolonized substrate until it finally has enough nutrients to produce a fruiting body, otherwise known as a mushroom. Four grow bags that contained rye berries as the spawn, a blend of different types of sawdust for the substrate, and a liquid culture of Hericium Erinaceus were prepared and placed in two different greenhouses (two bags to each greenhouse) that contained different temperatures and humidity ranges for 7 weeks. One greenhouse had temperature ranges between 15.5-23.8° C with a humidity value of 80% (greenhouse A). The other greenhouse had temperature ranges between 29.4°-32.2° C with a humidity value of 95% (greenhouse B). Observations regarding growth stalling and the presence of mold/bacterial contaminations were made over the 7 week fruiting
period. The results indicated that the grow bags subjected to greenhouse A had no mold/bacterial contaminants present and experienced no growth stalling, producing a total of 1309.02 grams of mushrooms. The grow bags subjected to greenhouse B produced five different types of mold and one bacterial contamination. These bags also experienced four weeks of growth stalling and only produced 48.37 grams of mushrooms total. Overall, the results from this experiment indicated that higher temperatures and humidity values do in fact stall growth of the culture and promote the growth of mold/bacterial contaminations. Growth stalling took place due to the mycelium network trying to fight off the mold and bacterial contaminations. Mold/bacterial contaminations over took the culture due to fact that mold and bacteria thrive in environments with high temperatures and moisture contents.

Poster

**Role of RNA-binding proteins Rbfox1l and Rbfox2 in neuronal development and behavior in zebrafish**

**Duaa Hijaz, Romeo Karris, Allison Pariso, Trupti Patel, Margaret Schlicker, Yonahga Stickles, and Michael A. Berberoglu**

Valparaiso University; 1610 Campus Drive East, Valparaiso, Indiana 46383 USA

Rbfox proteins are RNA-binding proteins that play a significant role in the alternative splicing of neuronal transcripts in the central nervous system (CNS). Rbfox proteins are required for proper brain development and function. In humans, RBFOX1 has been implicated in a variety of neurological disorders, including autism, anxiety, epilepsy, and schizophrenia. Rbfox2 is involved in cerebellar development in mammals. The zebrafish is used as a model system for studies in neurobiology given their neuroanatomical conservation with mammals, and remarkable capability to regenerate parts of their CNS. Rbfox1l (Rbfox1-like) and Rbfox2 have been identified in neurons of the adult zebrafish brain. Rbfox1l was found in a restricted population of dorsal telencephalic neurons, and Rbfox2 was found broadly throughout the brain. Both genes have been found in Purkinje cells of the cerebellum. We will use *rbfox1l* and *rbfox2* mutant zebrafish (in collaboration with Ohio State University) to better understand the role of *rbfox1l* in behavior and determine whether *rbfox2* is necessary for regeneration of the cerebellum. Understanding the role of the Rbfox proteins in neural development, regeneration, and behavior may lead to substantial advancement in the research field and health care.
**Poster**

**High-throughput gene expression analysis of in vitro and in vivo mammalian cardiogenesis identifies common developmental gene expression signatures**

Mirian Alvarez-Dubon, Ishmeet Kaur, Dwayne Tally, Joseph Dalloul, Andrew Williamson, Rusty Gonser, **Jeff Kinne**, and Kristopher Schwab

The development of the embryonic heart requires the step-wise activation of a complex gene regulatory network that guides the specification and differentiation of pluripotent cells to cells of the heart. Published high-throughput gene expression data sets of mouse embryonic heart development and human in vitro cardiomyocyte differentiation provides us the opportunity to investigate important biological processes. We have identified early developmental and early cardiac gene expression signatures that are shared between the two experimental systems: the mouse heart and in vitro cardiomyocyte differentiation. Using our CarDGEA (Cardiac Development Gene Expression Analysis) tool, we have created early embryonic and early cardiac gene lists that comprehensively identify gene expression during the early stages of cardiomyocyte differentiation.

**Poster**

**Investigating the sex-specific, tissue-specific, and segment-specific regulation of the branchless gene in Drosophila**

**Author list**

Naureen Aslam Khattak1,2, Rusty A. Gonser1,2, Shaad M. Ahmad1,2

**Detailed Affiliations**

1Department of Biology, Indiana State University, Terre Haute, IN, USA
2The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN, USA

One of the interesting challenges in developmental biology is to understand how different cues are integrated to determine where, when, and under what conditions a particular gene is expressed. A striking example of such a gene is *branchless (bnl)*, which is expressed in a sex-, segment-, and tissue-specific manner in the *Drosophila* genital disc. Analysis of the regulation of the heretofore unidentified *bnl* gene enhancer would therefore provide insight into how these sex-, segment, and tissue-specific cues are integrated. We hypothesize that the integration of sex-, segment-, and tissue-specific manner cues are achieved by transcription factors (TFs) specific for each of these cues binding to and regulating one or more *bnl* gene enhancers. Using ChIP-seq and Dam ID binding data, we have mapped clusters of binding sites of Doublesex (the TF providing sex-specific cues), abdominal-B, and caudal (two TFs providing segment-specific cues) in the
neighborhood of the \textit{bnl} gene to identify several putative \textit{bnl} gene enhancer(s). These putative enhancers will be assessed using enhancer-reporter transgenic constructs to identify the true \textit{bnl} enhancer(s): only the latter will recapitulate the sex-, segment-, and tissue-specific expression patterns of the endogenous \textit{bnl} gene. We will then functionally analyze the roles of each of these TFs on the validated \textit{bnl} enhancer-reporter construct(s) by examining the effects of mutating the TF binding sites (\textit{cis}-assays) or removing the TF in loss-of-function clones (\textit{trans}-assays). Collectively, our results will shed light on the interactions and integration between different regulatory inputs and further our understanding of \textit{Drosophila} sexual differentiation.

Poster

\textbf{Forkhead/Fox domain transcription factors mediate proper positioning of cardiac cells by both ensuring correct cardiac progenitor cell divisions and restricting the expression of ECM genes}

\textbf{Manoj Panta}1, 2, Andrew J. Kump1, 2, Neal Jeffries3, Shaad M. Ahmad1,2.
1) Department of Biology, Indiana State University, Terre Haute, IN; 2) The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN; 3) National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

The development of a complex organ requires the specification of appropriate numbers of its constituent cell types as well as the correct positioning of these cell types within the organ. We have previously shown that the Forkhead/Fox domain transcription factors (TFs) Checkpoint suppressor homologue (CHES-1-like) and Jumeau (Jumu) determine the correct number of different cardiac cell types by regulating cardiac progenitor cell divisions. Here we show that \textit{CHES-1-like} and \textit{jumu} are also required for the correct positioning of these cardiac cell types: null-mutations in either gene result in the incorrect location of cardiac cells within individual hemisegments. Statistically, we demonstrate that some of these positioning defects are due to steric constraints: contralateral hemisgments with differing numbers of cardial cells as a consequence of cell division defects show significantly more positioning errors than those with equal numbers in Fox mutants. However, our analysis also indicated that steric issues were not responsible for all cardiac positioning defects: contralateral hemisgments with equal numbers of cardiac cells from Fox mutants still exhibit significantly more positioning errors than those from wildtype embryos. In order to find downstream targets utilized by these Fox TFs to bring about correct positioning, we compared genome-wide transcription expression profiles of purified mesodermal cells from wild-type embryos and Fox mutants. Our analysis of expression profiling data detected a significant overrepresentation of upregulated extracellular matrix (ECM) protein-encoding genes in the Fox mutants compared to wildtype, raising the possibility that Jumu and/or CHES-1-like may be bringing about the correct positioning of cardiac cells by restricting the expression of
ECM genes in the mesoderm. Our preliminary analysis of a subset of these Fox TF-repressed targets showed that ectopic overexpression of the ECM protein-encoding genes in the mesoderm of otherwise wildtype embryos phenocopies the cardiac cell positioning defects in CHES-1-like and jumu mutants.

Poster

*Ptip, a H3K4 Methyltransferase Complex Member, is Essential for Proper Cardial Cell Division in Drosophila Heart Development*

**Adam Farmer**, Andrew Kump, Manoj Panta, Shaad Ahmad, and Kristopher Schwab, Indiana State University

Epigenetic modifications of chromatin allow stable and heritable gene expression patterns regulating cell-specific phenotypes and functions. The methylation of the tail of histone 3 at lysine residue 4 (H3K4) is an epigenetic landmark of both open chromatin and actively transcribed genes. Ptip (Pax transactivation domain-interacting protein) recruits an evolutionarily conserved MLL3/4 COMPASS-like complex and is required for the deposition of H3K4 methylation marks on chromatin. Furthermore, *Ptip* and proper H3K4 methylation is essential for gene expression in embryonic development and cell differentiation. Previous studies suggest that *Ptip* is necessary for the epigenetic regulatory functions of heart development and cardiomyocyte function, however a detailed study of *Ptip* in cardiogenesis remains to be completed.

*Drosophila melanogaster* (the fruit fly) provides an excellent model system to study heart development allowing the quantitative analysis of single cardiac cells within a genetically amenable system. The embryonic fruit fly heart is a symmetrical tube consisting of linearly arranged cardiac cells that are repeated in a hemisegmental pattern. The cardiac cells of the heart tube are produced by cardiac progenitor cells that undergo many developmental processes including cellular specification, migration, differentiation, and cell division. Cardiac cell division consists of both symmetrical and asymmetrical cell divisions that generate the diverse cell fates that produce the proper organization of the heart tube. Given the requirement of *Ptip* for cardiomyocyte function and heart development, we investigated *Ptip* function within the *Drosophila* embryonic heart by evaluating *Ptip* hypomorphic embryos for cardiac defects. Quantitative analysis of individual embryonic heart segments identified numerous symmetrical and asymmetrical cell division defects within the *Ptip* hypomorphic embryos which disrupt normal heart morphology. Our data demonstrates that *Ptip* is required for proper cardiac cell division within the embryonic heart suggesting an epigenetic role for *Ptip* in regulating gene expression through H3K4 methylation.
A significant bottleneck in “Big Data” is the availability of user-friendly tools to effectively analyze high-throughput gene expression microarray data and RNA sequencing (RNA-seq) of biological processes in spatiotemporal context, such as a time, developmental stage, or tissue series. The incredible amount of quality high-throughput gene expression data available offers scientists and scientists-in-training to investigate their biological process or mechanism of interest to support their own research interests. However, many of the available gene expression analysis tools require significant computer programming and data science experience to utilize effectively.

We have developed an integrated gene expression analysis software suite that allows the analysis of a sample series consisting of a development time course using a variety of statistical, clustering, and mapping software. To demonstrate the usefulness of the software, we analyzed gene expression datasets from two different experimental models of mammalian embryonic heart development: a developmental stage series of the embryonic mouse heart and the in vitro differentiation of human cardiomyocytes from human pluripotent stem cells (hPSCs) with the goal of identifying common developmental and cardiac gene expression profiles. These commonly gene expression profiles identify previously known developmental regulatory genes, including pluripotency and cardiac genes, as well as uncharacterized genes that remain to be investigated. Furthermore, we have developed a unique set of developmental expression signatures that characterize both the differentiation of hPSCs into cardiomyocytes and the gene expression profiles of the early stages of mouse heart development. devDGEA will allow scientists to effectively perform their own in silico data analyses to develop new hypotheses, validate existing data, and investigate experimental reproducibility.
The Microscopy of Microecosystems Present in Activated Sludge

Breanna Amelunke¹, Jay Thompson², and Natalia B. Hubbs, PhD¹

¹Department of Biology, Hanover College
²Madison Sewage Disposal Plant

The Madison Wastewater Treatment Plant (MWWTP) is a government-based facility that provides water treatment to sewage and releases it into the Ohio River. Wastewater treatment is essential for several reasons: it is important for public health, for the safety of the environment, and for agriculture. The duties of this facility include maintaining wastewater standards by keeping the pH, phosphorous, dissolved oxygen, and bacteria levels within safe parameters set by the government. In collaboration with the MWWTP and Hanover College, we developed a research-based internship with several key projects to combine my interests in cell and microbiology to the application of the real-world microbiology that happens at the plant. One of the key projects involved learning how to use different microscopy techniques to characterize and observe microorganisms present throughout different locations in the plant. Using the aforementioned techniques, several different kinds of microbes were observed, including bacteria, amoebas, algae, worms, tardigrades, gastrotrichs, and water fleas. The sample locations included primary effluent, aerobic digestion tanks, anoxic zone, and the digesters.

Cytogenetic Analysis of Lymphoid Malignancies Using Mercator and CytoGPS

Dwayne Tally¹, Zachary Abrams², Caitlin Coombes², Suli Li², and Kevin Coombes²

¹The Center for Genomic Advocacy at Indiana State University, ²Department of Biomedical Informatics at Ohio State University

Lymphoid malignancies are commonly classified clinically by location and morphology. However, we hypothesize that useful classifications can be obtained from cytogenetic abnormalities alone. The public Mitelman Database of Chromosomal Aberrations and Gene Fusions in Cancer has curated karyotypes from the literature since 1970. It is the largest database of published cases of cancer
karyotypes in general and of Lymphoid malignancies. We previously developed CytoGPS (http://cytogps.org/), a tool that converts text karyotypes into binary vectors using a Loss-Gain-Fusion model. To test our hypothesis, we applied CytoGPS to the lymphoid malignancies in the Mitelman Database. The first step was to extract the data from Mitelman for a total of 69,174 patients. Then we ran CytoGPS to convert the data to the Loss/Gain/Fusion (LGF) model, stored in 14 JSON files. A total of 22,741 samples were associated with B cell malignancies. Afterwards we analyzed the data using two r packages developed by Dr. Coombe’s lab, Thresher and Mercator. We found that there were 134 clusters, we assigned samples to clusters using Partitioning Around Medoids (PAM). We visualized the results using t-distributed Stochastic Neighbor Embedding (t-SNE). We calculated high-frequency events and displayed them in a heatmap. This heatmap shows a clear distinction among lymphoid malignancies between multiple gain events and loss events. This is an important finding since it shows a pattern of multiple loss or multiple gains within an individual case. Through this computational cytogenetic research, we were able to find patterns that have not been previous documented.

Poster
Passing of the TORCH: A Medical, Historical, and Social Comparison between Rubella and Zika

Nhat M. Pham, M.S. - Marian University College of Osteopathic Medicine
Peter S. Marcus, M.D. - Ascension St. Vincent Hospital Indianapolis

In 1941, Australian ophthalmologist Norman McAlister Gregg classified Congenital Rubella Syndrome (CRS) and demonstrated for the first time that a virus could be teratogenic. Previously, it was thought that the protective nature of the placenta could prevent maternal-fetal transfer of exogenous factors such as drugs and viruses. Around the same time that CRS was classified, zika virus (ZIKV) was first discovered in Uganda. Thought for decades to only cause a mild disease, the true pathologic nature of zika emerged in 2015. After an overwhelming outbreak in the Western Hemisphere, zika became the newest classified viral teratogen, capable of causing serious fetal malformations. A comprehensive comparison between rubella and zika reveals noteworthy correlations. In both viruses, the risk of fetal malformation is highest in the first trimester and tapers down in subsequent trimesters. Rubella’s pathogenesis is more systemic and presents with a classic triad of cataracts, deafness, and cardiac abnormalities. Zika is more neuropathologic, targeting specifically neural progenitor cells, and presents most notably with microcephaly. Differences are also observed in each virus’s mode of transmission. While rubella is strictly a human disease, commonly transmitted via respiratory droplets, zika is an arbovirus and maintains a wide range of animal hosts and vectors. Interestingly, a historical comparison of the incidence rates in the United States finds zika to have been
controlled at an exceptionally faster rate than rubella. This correlation is significant given the success and effectiveness of rubella vaccines since the 1970s while zika still does not have any specific therapies or vaccines. This finding suggests that broad-based social efforts at the population level may be more significant at affecting public health than narrow-based medical interventions at the individual level. This presentation will reflect on the progress and history of medicine within the past century and demonstrate the need for continued vigilance within the scientific community.

Poster

**Bacteria Fitness in Antibiotic resistant E. Coli**

**Eyovel Eyassu, Dr. Samina Akbar, Dr. David Strom, Dr. David Raskin, Marian University**

Drug-Resistant bacteria are increasingly a concern in the modern world. Even with new drugs and novel cellular targets, resistance still develops. There are many mechanisms to account for increased resistance in bacteria. The goal of our research is to explore methods of decreasing resistance in antibiotic-resistant bacteria. We hypothesized that antibiotic-resistant Escherichia coli (E. coli) have a higher fitness cost than non-plasmid containing E.Coli. We first measured fitness cost by calculating doubling times. We observed no difference from wild type E.coli and plasmid containing E.Coli. Next, we wanted to see how fast the drug-resistant bacteria will lose their plasmids. We plated Amp resistant pUC18 in a drug-free environment after a few days resulting in few colonies when plated on Amp present plate. We also carried out a co-culture experiment that plated pUC18 along with pBAD18 (kanamycin-resistant) which resulted in pUC18 being out-competed by pBAD18. In future studies, we will use this difference to identify pharmaceutical interventions that will more quickly reverse the fitness cost of drug-resistant E. coli by reverting back to wild type E. coli that are again sensitive to antibiotics.

Poster

**Role of peroxisome proliferator-activated receptor g (PPARg) in Coxiella burnetii infection**

**Celina Spencer, Adelaide Calhoun, Minal Mulye**

Division of Biomedical Sciences, Marian University College of Osteopathic Medicine

Peroxisome proliferator-activated receptors (PPARs) are important nuclear receptors in regulating macrophage lipid metabolism and modulating cellular inflammatory responses. In recent years, host cell PPARs have been linked to bacterial pathogenesis and macrophage infections. Specifically, bacteria-induced manipulation of lipid
droplets (LD), host lipid storage organelles, are directly associated with PPARg activation. For example, the intracellular pathogens *Mycobacterium bovis* and *M. leprae* breakdown the macrophage LDs to release free fatty acids. These free fatty acids subsequently activate PPARg to induce an anti-inflammatory immune response, thus sustaining Mycobacterial intracellular growth. Our previously published studies report that the intracellular bacterium *Coxiella burnetii* induces host cell LD breakdown to support its growth in macrophages. *Coxiella* is known to cause non-culturable endocarditis that can present up to 20 years post initial infection, suggesting its ability to survive long-term in the host by subverting the host immune response. Our overall goal is to identify the strategies that *Coxiella* employs to manipulate host immune response. This could ultimately aid in developing therapeutic targets to inhibit *Coxiella*’s intracellular growth. Since LD breakdown is important for intracellular *Coxiella* growth through the release of free fatty acids (PPARg agonists), we hypothesize that *Coxiella* infection activates host cell PPARg to induce an anti-inflammatory environment which supports bacterial growth and infection. To test this hypothesis, we quantified PPARg gene expression in *Coxiella*-infected macrophages using quantitative Real Time PCR (qRT-PCR) at different times post-infection. Compared to uninfected cells, infected macrophages demonstrated increased PPARg gene expression suggesting that *Coxiella* induces PPARg activation. Ongoing studies are focused on identifying PPARg transcriptional activity in *Coxiella*-infected cells and the downstream targets. Future studies will determine if PPARg activity is dependent on host cell LD metabolism and identify potential PPARg antagonists as therapeutic targets to inhibit *Coxiella* intracellular growth.

**Mathematics**

**Presentation**

**Using Temporal Dynamics to Understand Spatial Patterns**

**Kevin L. S. Drury, Ph.D.** Huntington University, Department of Mathematics and Computer Science

Understanding dynamics in both space and time is notoriously challenging. When progress is made, it is usually via partial differential equations, most of which are insoluble. Certain classes of interactions, such as those among some chemical species, biological species, or economic entities, result in distinct spatial patterns, that repeat throughout the domain. Within such repeating patterns, we study a class that is cyclical in time. Specifically, entities present at location (x,y) at time t0, say of type A, are replaced at time t1 by species of type B. Species B is replaced by species C in its turn at time t2. Finally, at time t0+3=t3, species A once again occupies location (x,y). We quantify the time scale of t0 to t3 using the temporal correlation length. Our
goal is to show that this correlation length in time is related to the spatial correlation, i.e., the distance between clusters of type A, for example, in the domain. This provides an accessible approximation to the spatial dynamics of the full partial differential equation model.

Presentation

Social Media Influencers as Super Infectors

Jacob Allred Huntington University
Alexandria Marris Huntington University
Jin Zou Huntington University
Kevin Drury Huntington University

Individuals with large social media followings can act as conduits for rapid dissemination of ideas. The spread of information through such networks can be modelled using frameworks developed a century ago to model the spread of disease. We therefore refer to the spread of an infection, understanding that infection abstractly refers to any contagious process. We first use an agent-based model to illustrate the spread of the infection through space and time. Using the simple rules of that model, we then develop an associated 3-dimensional ordinary differential equation model. Such higher dimensional models can be difficult to analyze and understand, however. Furthermore, because in the context of ideas transmitted through social media, we are primarily interested in the rate of spread of the first wave of the infection, we collapse the 3-dimensional model into one dimension and show that it faithfully represents the dynamics of the more complicated model. Finally, we analyze the 1-dimensional model to quantify infection rates as a function of influencer status.

HotTopics

Opportunities and Challenges in Modeling Re-Emerging Infectious Diseases

Ana Bento, Ph.D.
Indiana University-Bloomington

Using mathematical models to understand and forecast disease re-emergence, I will use measles re-emergence as my case study but will point out how these models can be generalizable.
Physics & Astronomy

Presentation

Characteristics of High Mass-Ratio Binary Neutron Star Mergers

Allen R. Murray, and Aaron R. Warren, Purdue University Northwest

We report results of numerical relativity simulations for irrotational binary neutron star (BNS) mergers with total ADM masses 2.50 and 2.75 Msol and mass-ratios q = 1.75, 2.00, and 2.25 (highest mass-ratio yet simulated). These simulations employ a seven-segment piece-wise polytropic SLy equation of state (EOS) with a thermal component. The effect of the mass-ratio on the dynamical mass ejecta and gravitational wave emissions is studied. Characteristics of the ejecta including the mass, kinetic energy, and velocities are estimated. Comparisons with derived fits of these quantities based on simulations of mergers with mass-ratio equal to or less than 1.75 are performed to assess their accuracy for mass-ratios above 1.75. Gravitational waveforms, spectral characteristics, and spectrograms of the GW strain are calculated, including multipoles up to l = 5. The mass-ratio has a specific imprint on the GW multipoles in the late-inspiral-merger signal, and it affects qualitatively the spectra of the merger remnant. Also, unequal mass BNSs produce more ejecta than equal mass systems and we estimate luminosity peaks and light curves of macronovae events associated to the mergers. For q ~ 2 the luminosity peak is delayed for several days and can be up to four times larger than for the q = 1 cases. The kilonova emission associated with the q ~2 BNS is more persistent in time and could be observed for weeks instead of a few days (q = 1) in the near-infrared. Finally, we estimate the flux of possible radio flares produced by the interaction of relativistic outflows with the surrounding medium. Also in this case a large q can significantly enhance the emission and delay the peak luminosity. Overall, our results indicate that BNS mergers with large mass ratio have EM signatures distinct from those produced in near-equal mass cases.

Presentation

The Impact of Poisson Solver Parameters on Long Term Evolutions of Binary Stars

Patrick M Motl Indiana University Kokomo

We discuss a variety of factors determining the behavior of a combined Fourier analysis plus Alternating Direction Implicit (ADI) implementation to solve Poisson’s equation in cylindrical coordinates. The boundary value of the potential is found through integration of Green’s functions over the density distribution. We have varied
(1) the distance to the grid boundary, (2) the number of terms used in the Green’s functions and (3) the number of ADI iterations in simulations of a detached binary initially on a circular orbit. Numerical errors resulting from the Poisson solver are reflected in epicyclic and apsidal motion of the binary and cumulative numerical errors result in motion of the system center of mass over long term evolutions. In light of constraints on computing resources, we explore optimal parameters for the Poisson solver for long term, direct numerical simulations.

Poster
Low-Cost Single-Photon Avalanche Detectors

Purdue Fort Wayne

Christopher Jacobs
Jucoen Yeater
Dr Masters

Single photon experiments typically require expensive equipment to detect photons, align laser beams, and specific devices to cause necessary events to occur. We are investigating whether off the shelf avalanche diodes can function sufficiently well as Single Photon Avalanche Diodes (SPADs) which would allow single photon investigations to be performed for a broader audience.

Poster
Spooky Interferometry: An Investigation of Nonlocality in Quantum Mechanics

Justin Smethers
Dr. Mark Masters

I experimentally demonstrate a phenomenon in quantum mechanics known as spooky action at a distance – the idea that an object’s physical characteristics can be affected without any direct interaction. This effect is demonstrated through single-photon interferometry. I send single photons through a beam splitter and into an interferometer, resulting in an interference pattern. This result implies the photons have two different polarizations simultaneously, as there’s no other way for an interference pattern to emerge. I then measure the polarization and the subsequent disappearance of the interference pattern. I conclude that the photons are forced to have only one polarization, or in other words, the polarization of the photons changed as a result of being measured.
Poster

3-D Printed Air Bearings

Samuel Wyss - Purdue University Fort Wayne - Physics Department
Mark F. Masters Ph.D. - Purdue University Fort Wayne - Physics Department

Generally, one thinks air bearings must have a very smooth surface. However, we have found that it is possible to create 3-D printed air bearings. This research examines the viability and manufacturing process of fabricating 3-D printed air bearings. Three properties of the bearings were tested: operating pressure, operating angle, and surface roughness. The operating pressure was tested using an aquarium pump and a pressure sensor. The operating angle was tested with a stepper motor fitted to an adjustable stage, and surface roughness was examined using an optical microscope and a scanning electron microscope. All of the tested bearings were compared to a traditionally machined acrylic bearing. 3-D printed bearings varied by plastic-type, socket surface geometry, and socket depth, and sidewall geometry.

Poster

Observations of U Leporis

Larson Pavey, Tyler Smith: Purdue University Fort Wayne Department of Physics.

U Leporis is a pulsating variable that demonstrates periodic variation in apparent magnitude consistent with RR Lyrae stars, a category of intrinsic variables suitable as standard candles for determining distances. RR Lyrae stars are understood to be low-mass stars (0.6 to 0.8 M\text{\odot}) found at the intersection of the horizontal branch (HB) and the instability strip of the Hertzspring-Russell diagram (Catelan & Smith, 2015). These older (> 10 Gyr) giant (4-6 R\text{\odot}) stars, post-helium flash, are able to fuse helium in their cores as hydrogen fusion occurs in a shell surrounding the core. RR Lyrae stars were first used to determine the distance to globular clusters, though today more RR Lyrae stars are known in other parts of the Milky Way galaxy and are used to study the structure of the galactic halo and disk.

The purpose of this study is to use images from a remote telescope network to determine the light curve of an RR Lyrae star. The period determined from the light curve is used to calculate distance, which can be compared to recent measurements taken by Gaia. U Leporis is a field star too dim to see with the naked eye (m ~ 10.9) but present in the evening winter skies of Fort Wayne, IN (RA 04 h 56 m 17.96, Dec -21° 13’ 01.5’’). The 0.4 meter SBIG telescopes of the Las Cumbres Observatory remote telescope network made over fifty observations from October 2 – 14, 2019. Filters were used to obtained images for four bandwidths, two visible (B, V) and two near-infrared (ip and zs).
Analysis of the images was completed using Autovar. This python program calculates the apparent magnitude of $U\ Lep$ within each image, then tests several period values to determine which value generates the most accurate light curve for each filter. The period of the four light curves ranged between $0.58006 \pm 0.01208$ and $0.58276 \pm 0.01050$ days. Range in the apparent magnitude of the stars decreased slightly as filter wavelength increased, consistent with observations made of other RR Lyrae stars.

Using the periods of each of the light curves and accounting for metallicity ($[\text{Fe}/\text{H}] = -1.78$), the average distance to $U\ Lep$ was determined to be $977 \pm 10$ pc. This result compares favorably to that obtained by Gaia using parallax ($1042 \pm 40$ pc). Thus, this method of using RR Lyrae stars as standard candles holds and $U\ Lep$ is confirmed as an RRab variable.

Poster

**Laser-Stimulated Fluorescence for Examination of Fossils**

**John Neidich** and Mark Masters

Department of Physics, Purdue University Fort Wayne

Fossils have given us irreplaceable information, from our planet’s age to the creatures and environments that used to reside in our place. How might we safely study these priceless, fragile pieces of history? The answer lies in the light source. Traditionally, a black light is placed above a specimen, better defining worn-out pigmentations than ordinary lighting would.

The use of fluorescence is a nonintrusive method for evaluating fossils, utilizing the reaction of a specimen to a light source projected upon it. This results from a photon exciting an atom at the specimen’s surface and an electron of that atom effectively dropping back down from its temporarily raised energy level. The difference in energy is then emitted in the form of light at a wavelength greater than that of the original light source.

My experiment will use a mounted laser, travelling back and forth along its track, scanning over the sample area below. Due to its higher generated irradiance, or energy per unit area, a laser creates greater fluorescence than traditional ultraviolet and black lighting used in nonintrusive archaeology, allowing for further light penetration beyond a sample’s surface layer. This added benefit has allowed paleontologists to scrutinize even finer details of specimens, which were previously unseen. Improved irradiance also provides an increase in the light’s signal-to-noise ratio.

The backscattered photons resulting from fluorescence will produce varying amplitudes of light intensity depending on the energy output of the light source used. In the first, visual-based phase of this experiment, a photo sensor will be used in detecting the amount of light projected upon my samples, giving an output of voltage. A traditional black light can then be used to comparatively fluoresce the material.

The second phase of this experiment is structural-based. It evaluates an output signal from the photo sensor to calculate the resulting backscattered wavelengths, which are known to be intrinsic responses of chemical compounds. This is known as hyperspectral imaging, and it involves the individual spectra at each fluoresced point.
Poster

**Acoustic Levitator**

**Erica Miller** and Mark Masters

We report on our work with an acoustic levitator. An acoustic levitator creates areas of high and low pressure that produce resonating sound waves. These sound waves create a standing wave, with nodes (regions of low pressure) and antinodes (regions of high pressure). Suspended objects levitate at the nodes. This acoustic levitator uses two concave disks, each containing 36 transducers, tiny speakers. The polarity of each transducer was tested in order to connect all the positive and negative terminals in parallel. Both prongs of the transducers were soldered to their respective wires, and then connected to a driver. After hooking up a DC power supply, a square wave was sent to an amplifier which then powered all 72 transducers. Once the transducers are on, we can levitate small objects between the two concave disks. This acoustic levitator, we intend to suspend tiny solids, water droplets and other small objects. We will be measuring the trapping force on a variety of objects.

Presentation

**Visualization of orbital free models of kinetic energy density in solids**

**Brielle Tilson,** Antonio Cancio
Department of Physics and Astronomy, Ball State University, Muncie, IN 47306

The metaGGA class of functionals for describing the exchange-correlation (XC) energy in density functional theory (DFT) is conventionally constructed as a functional dependent on the density, density gradient, and kinetic energy density (KED). The addition of the KED makes metaGGA’s more accurate functionals than ones that use the density and its gradient alone but also more computationally expensive for some applications such as ab initio molecular dynamics simulations. The calculation of the XC energy in meta-GGAs can be made less expensive by replacing the explicit orbital dependence in the KED with expressions involving only the particle density and its gradient and Laplacian. We test the validity of recent deorbitalization strategies in the literature by visualizing their predictions for the KED and related quantities, and comparing these to exact calculations. For an effective test, we perform these calculations on semiconductor solids with varying ionicity and atomic number. We explore how well the KED can be represented by a single metaGGA model in terms of the scaled gradient, Laplacian of the density, and reduced density. The results show a near-universal linear correlation with Laplacian and gradient for regions outside of the atomic bond, which can be fit to a straight line when viewed at the optimal rotation. The calculations of exact KED and electron density are done with the ABINIT DFT plane-wave pseudopotential code.
Poster

**Observations of the Original RR Lyrae**

Institution: Purdue University Fort Wayne Department of Physics
Authors: Natalie Severeid and Travis Kreager
Presenter: Natalie Severeid and Travis Kreager

The purpose of this research was to record the light output of the variable star RR Lyrae through four optical filters and to use that data to generate the different light curves of each filter. RR Lyrae’s period could be determined by the light curves and also through the examination of the shape of the light curve itself to show how it pulsates over time. RR Lyrae was observed over a two week period through Los Cumbres Observatory’s global network of telescopes. The data gathered by the telescopes was processed through Autovar to generate a light curve graph of RR Lyrae. The four different light curves gave periods of about 0.567 days. The calculated period for the star was nearly identical to the period found by previous research done in other studies.

Poster

**NSVS 2854398, an Orange W Ursae Majoris Star**

Alexander J. Neal, Ball State University
Robert C. Berrington, Ball State University

We present a photometric analysis of the eclipsing variable binary star NSVS 2854398. A total of 402 images were taken in the Johnson B, V, and Cousins R band passes. All images were acquired using the Ball State University Observatory 20-inch telescope atop the Cooper Science Complex in July 2018. All images were reduced with the Image Reduction and Analysis Facility (IRAF). Differential ensemble photometry was performed using the AstroImageJ (AIJ) program. The system was modeled using the PHysics Of Eclipsing BinariEs (PHOEBE) program. With PHOEBE, a synthetic light curve was compared to the observed light curve to determine best-fit model parameters. Our analysis shows that NSVS 2854398 is consistent with an over-contact binary in thermal contact with a surface temperature of 4720 ± 190 K and an orbital period of 0.290 ± 0.003 days. Observed times of minimum light were determined and compared to a calculated time of minimum light.
Presentation

Automated Fabrication of Langmuir-Blodgett PVDF-HFP Thin Films to Improve Beta-phase Properties

Ashley S. Dale (IUPUI), Aaron Mosey (IUPUI), Ruihua Cheng (IUPUI)

Ferroelectric poly(vinylidene fluoride)-hexafluoropropylene (PVDF-HFP) thin films exhibit ferroelectric properties and are capable of maintaining a large internal electric field in the absence of an externally applied potential. These properties make PVDF-HFP a competitive candidate in the design of organic based electronics and spintronics. However, accessing these properties requires the PVDF-HFP thin film to be predominantly in the beta-phase. We have achieved beta-phase dominant PVDF-HFP thin films without additives or annealing through Langmuir-Blodgett (LB) deposition using a home-built automated desktop dipper as a component in a retrofitted LB trough system. Ferroelectric capacitors with a LB PVDF-HFP thin film dielectric and multilayer metallic top and bottom electrodes were characterized using a custom ferroelectric measurement system implemented in LabVIEW with a Keithley picoammeter/voltage supply. The ferroelectric capacitors use a unique step design to eliminate the edge effects during measurement of intrinsic switching dynamics. Ferroelectric hysteresis data shows distinct increase in electric coercivity of samples fabricated using our automated system. Additional characterization with scanning electron microscopy and atomic force microscopy shows a remarkable improvement in thin film surface morphology.

Poster

Bound Free Emission Spectra of Sodium Potassium Alloy

Anna Patterson
Dr. Mark Masters
Purdue University Fort Wayne

We are endeavoring to observe emission from a bound molecular state to and unbound molecular state (known as bound-free emission) in sodium potassium molecules. We have assembled a heat pipe oven, which allows us to vaporize the sodium potassium alloy. The heat pipe oven uses cooled windows with argon buffer gas to eliminate condensation of the metal on the windows. Laser light will be used to excite the molecules from their ground state into an electronically excited energy level. The excited molecules then emit over a broad continuum to the unbound molecular energy level causing the molecule to dissociate. We are determining atomic species vapor densities using equivalent width measurements as a preliminary experiment.
We present a photometric study of the eclipsing binary candidate NSVS 6103255. All observations took place throughout September and October 2019 using the 20-inch telescope located on the rooftop observatory at Ball State University. Images were collected using three wide-band filters: Johnson V, Johnson B, and Cousins R, and were reduced using the Image Reduction and Analysis Facility (IRAF). All photometry was performed using the AstroImageJ (AIJ) software package and is multi-aperture differential photometry. Light curves are presented and analyzed using the Physics of Eclipsing Binary (PHOEBE) software suite. Resulting best-fit models are presented and discussed.

We present multi-band aperture photometry of the eclipsing binary NSVS-6099331 from the Northern Sky Variability Survey (NSVS). All data was obtained using the Ball State University Observatory 20-inch telescope during August 2019 in the Johnson B, Johnson V, and Cousins R filters. All images were reduced using the ccdred images reduction package in the Image Reduction Analysis Facility (IRAF). Multi-aperture photometry was performed using AstroImageJ (AIJ). All photometry was analyzed in the PHysics Of Eclipsing BinariEs (PHOEBE) software suite, and best-fit model parameters are determined. PHOEBE provides a convenient Graphical User Interface (GUI) to the Wilson-Devinney. An orbital period of 0.404570 ±0.000294 days was found using the observed minus calculated (O-C) times of minimum. NSVS-6099331 is consistent with an over-contact eclipsing binary star in thermal contact with a surface temperature of T= 6912.2 ± 281.7 K.
Richard Gorby  
Dr. Robert Berrington  
Ball State University

"We present a photometric analysis of the eclipsing variable binary star NSVS 3350218. Photometric images were taken in the Johnson B, V, and Cousins R band passes. All images were acquired using the Ball State University Observatory 20-inch telescope atop the Cooper Science Complex. All images were reduced with the Image Reduction and Analysis Facility (IRAF). Differential ensemble photometry was performed using the AstroImageJ (AIJ) program. The system was modeled using PHysics Of Eclipsing BinariEs (PHOEBE) program. With PHOEBE, a synthetic light curve was compared to the observed light curve to determine best-fit model parameters. Our analysis shows that NSVS 3350218 is consistent with an over-contact binary in thermal contact with a surface temperature of 9616 ± 1132 K and an orbital period of 0.316774 ± 0.000382 days. Observed times of minimum light were determined and compared to a calculated time of minimum light."

Poster  
Photometric Study of the Spotted W-Ursae Majoris Variable Star NSVS 254037  

Kyle J Koeller, Ball State University  
Robert C. Berrington, Ball State University  

We present new differential ensemble photometry of an eclipsing binary star candidate NSVS 254037. All data was acquired using the Ball State University Observatory 20-inch telescope during September and October of 2018. We observed the variable star in three band-pass filters, Johnson B, Johnson V and Cousins R and all images were reduced using the Image Reduction and Analysis Facility (IRAF). The differential ensemble photometry was performed using AstroImageJ (AIJ). The light curves were analyzed using the Physics of Eclipsing Binaries (PHOEBE) software. Our analysis shows that the binary system is consistent with an over-contact binary in thermal contact with a surface temperature of 6632±366 K. With this model the orbital period of the star was found to be 0.3175±0.0015 days. An analysis of an orbital period of the system was performed using an observed minus calculated (O-C) times of minimum light. We report best-fit model parameters that were determined using the PHOEBE program.
Presentation

**Electrostatic interaction of negatively charged lipid vesicles with *adenosine triphosphate***

Azam Shafieenezhad, Andres T. Cavazos, Stephen R. Wassall, Horia I. Petrache
Indiana University Purdue University Indianapolis

*Adenosine triphosphate* (*ATP*) is an essential cellular component which provides the chemical energy needed in biological processes. Many such processes occur in the vicinity of lipid membranes. We use *Dynamic Light Scattering (DLS)* and *Deuterium Nuclear Magnetic Resonance (*²H NMR*) spectroscopy to study the effect of *ATP* on model lipid vesicles made of a negatively charged lipid molecule, *phosphatidylserine (PS)*. Based on their interaction with water, phospholipids are made of two general parts: a headgroup region that is polar and likes water (i.e. it is hydrophilic) and a hydrocarbon region made of two chains which are hydrophobic. In our study we determine the effect of *ATP* on these two parts. By *DLS* we characterize the electric potential of lipid vesicles from which we obtain information on surface charge. In contrast, by *NMR* experiments we obtain information on the organization of lipid hydrophobic chains. We present results as a function of temperature and discuss the thermodynamics of the system in terms of a mean-torque alignment field of lipid chains and its associated entropy. These results can help molecular biology studies as well as research on biomaterials.

Presentation

**Modelling the electronic kinetic energy density and *Pauli* potential by orbital free density functional theory.**

Bishal Thapa
Ball State University

In *Kohn Sham* density functional theory, the kinetic energy (*KE*) functional is described by fictitious Kohn-Sham (*KS*) orbitals. This causes a computational bottleneck for large systems that require many *KS* orbitals. Much recent research is going into Orbital-Free Density Functional Theory (*OFDFT*), which models the Kinetic Energy as a functional of density and other ingredients that are derived from density directly, avoiding the need for orbitals. There are reasonable *OFDFT* models for Kinetic Energy at the *meta-GGA* level, such the *Perdew-Constantin* model [1], that properly treat the nonnegativity constraint for the Pauli contribution to the KED, which describes the correction to the *von-Weizsäcker* KED, which describes the KE of a single electron pair. However, an issue arises of *Pauli* potentials that are not physically reasonable and difficult to find convergent solutions for. Our goal is to construct *meta-GGA* level models with potentials which vary smoothly.
In March of 2019 Eco Logic LLC was contracted by the City of Bloomington Parks and Recreation to have their Senior Ecologist map the plant communities of this approximately 1220 acre park located on the north side of Bloomington. This project was initiated to provide background information for construction of an improved hiking trail around the lake. The property lies on the border between the Brown County Hills and the Mitchell Karst Plain Natural Regions. The study mapped 16 distinct plant communities in the park based on ecologic land types and stages of succession. Other variables were also mapped, including invasive plant pressure, the maturity of the plant community, and the natural area value. A table of plant community characteristics was developed including soil parent material, soil types, landforms, aspect, dominant overstory tree species, characteristic understory species, susceptibility to erosion, herptofaunal habitat, and other habitat characteristics for each community. The study also included a terrestrial vascular plant inventory that documented 574 plant species found in the Indiana FQA calculator (Rothrock 2019). The frequency and plant communities for each species were also noted. The property had previously been inventoried several times, however this study documented 56 species not previously recorded in the park including twelve additional species in the genus Carex.
A floristic quality assessment of the entire property was performed yielding a very high FQI of 79.1 for all species and 87.9 for native species. The mean coefficient of conservative was 3.3 for all species and 4 for the native species. Further, separate floristic quality assessments were conducted by breaking out the dry communities, the mesic communities, the wet forested communities, and wet open communities, and the cultural communities.
Presentation

Quantitative Vegetation Analysis of Newfields Art and Nature Park: a Successful Example of an Urban Greenways Restoration Project

Marcia E. Moore, Butler University and Rebecca Dolan, Butler University

The Newfields Art and Nature Park lies between the Indianapolis Water Company's Water Canal and the White River, west of Michigan Road and north of West 38th Street. The site has a long history of disturbance of the native vegetation being cleared for agriculture or other purposes from at least 1941. Marcia Moore and Rebecca Dolan of the Friesner Herbarium, Butler University initially surveyed the Art and Nature park site between June of 2000 and May 2001. The site was highly disturbed, with few characteristic floodplain trees and little native understory, due to previous land use and a substantial presence of invasive non-native plants. During late summer of 2005 we conducted a Quantitative Vegetation Analysis whereby we surveyed five 100 meter long transects located throughout the A&NP, chosen to cover the range of plant community and cover types present based on visible inspection. The goal was to provide a baseline for comparison of pre-development vegetation with that following implementation of ecological restoration and to also compare the A&NP to other natural areas in the city. In 2019 we repeated our survey work during the same timeframe. We used Floristic Quality Assessment (FQA) to compare the herb-layer vegetation present during both sample years. Data from 2005 and 2019 were entered into the online Universal Floristic Quality Assessment Calculator using the Indiana, 2019 database. Based on our resurvey, the flora of Newfields A&NP is trending in the right direction. All FQA metrics increased between 2005 and 2019, indicating that ecological restoration underway at Newfields A&NP is on track to potentially restore the site to a high quality natural area.

HotTopics

Plain Ol’ Charlie Deam: The Making of a State Flora

Paul E Rothrock, Indiana University, Bloomington

Charles C. Deam published his landmark “Flora of Indiana” 80 years ago. He actively pursued a remarkably deep understanding of the State's rich and rapidly changing flora over a 35-year period. With the completion in 2019 of an electronic database of his Indiana University Herbarium specimens, we are able to provide additional perspectives on how he accomplished this feat, almost single handedly. From the database we can explore the temporal-spatial patterns of collecting activity and how his work on “The Flora” dovetailed with other obligations as State Forester and author of several milestone books. One can discern the personality of the man, personal biases in his collecting, and the drive behind his field work. Most interesting of all, we
can see how changes in Indiana’s transportation system dramatically affected his work. This anniversary is a fitting occasion for remembering the most important contributor to our understanding of Indiana’s natural history, Plain ol’ Charlie Deam.

Presentation

Floristic Quality Assessment of Reber Woods, Delaware County, Indiana

Donald Ruch, John Taylor, Taylor Davis, Sam Luginbuhl, Kemuel Badger: Department of Biology, Ball State University, Muncie, IN and Paul Rothrock: Indiana University’s Deam Herbarium, Indiana University, Bloomington, IN

Reber Woods, an 11.4 ha woodland and old-field complex owned by the Red-Tail Land Conservancy, is located in northwest Muncie, Delaware County, Indiana. The site consists of two woodlands (i.e., a small woodland with a large woodland pond on the west side and a larger woodland on the east side. The woodlands are separated by old-fields in various stages of succession and a ditch that drains to the north. A floristic quality assessment of the site was conducted during the 2018-19 growing seasons. Of the 333 taxa documented, 244 (73%) were native species and 89 (27%) were non-native. The taxa represented 82 families and 207 genera. The 10 families containing ~ 53% of the documented species were Asteraceae [37], Poaceae [35], Cyperaceae [35], Rosaceae [18], Fabaceae [12], Brassicaceae [11], Lamiaceae [9], Fagaceae (7), Plantaginaceae (6), and Polygonaceae (6). A detailed physiognomic analysis revealed that of the 333 taxa, 75 spp. are woody, 184 spp. are herbaceous vines or forbs, 70 spp. are graminoids, and four species were ferns and allies. The Floristic Quality Index (FQI) for native species is 49.3 and the mean Coefficient of Conservatism (mean C) is 3.2. The FQI for all species is 42.2 and the mean C is 2.3. An analysis of these matrices for the overall site and for the woodlands versus the old-fields will be presented.

Presentation

Forest Composition and Structure of Reber Woods, Delaware County, Indiana

John Taylor, Donald Ruch, Daniel Kern, Racheal Stenger: Department of Biology, Ball State University, Muncie, IN and Jake Gamble, Red-tail Conservancy Land Trust, Inc. Muncie, IN

Please schedule this presentation immediately following Don Ruch. Thanks!

Reber Woods, an 11.4 ha woodland and old-field complex owned by the Red-Tail Land Conservancy, is located in northwest Muncie, Delaware County, Indiana. The site has two small wooded areas. This study focused on the larger woodland on the east side. A full inventory of standing trees ≥ 20cm diameter at breast height (DBH)
was made, along with an interactive GIS map of each tree location. This information will be combined with aerial imagery and LiDAR data to monitor changes to the forest structure and species composition over time. We found 27 species of trees, with prominent groups (based on relative importance) being *Acer* (maples) 26.1%, *Carya* (hickories) 8.4%, *Fraxinus* (ash) 20.9%, *Quercus* (*Quercus* - white oaks) 6.7%, *Quercus* (*Lobatae* - red oaks) 16.1% and *Ulmus* (elms) 6.8%. These five groups accounted for approximately 85% of relative importance. Nearly all ash trees appeared to be dead. Demonstration of the interactive map, along with sampling methods and implications for future forest health will be discussed.

Presentation

**Efforts to Recover the State-Endangered Stout Goldenrod (*Solidago squarrosa*) in Indiana**

Michael Homoya, Roger Hedge, and Jason Larson
Indiana Department of Natural Resources, Division of Nature Preserves

*Solidago squarrosa* currently exists at Indiana’s only known extant site of occurrence, an area of rugged hills in Clark State Forest. Ecologists from the Indiana DNR Division of Nature Preserves have been monitoring the site for over 3 decades, and in 1995 the highest count ever made totaled 26 individuals scattered over 5 sub-populations. Numbers declined in subsequent years and by 2013 only 9 were found, split between 2 sub-populations. Fearing loss of the Indiana genotype 6 plants were dug in the fall of 2017 and brought into nursery culture. This resulted in several hundred seedlings produced, 150 of which were planted at the site in early 2019. Recovery efforts continue with additional augmentation and habitat management this year.

Presentation

**Natural Communities and Plant Species of Conservation Concern at Deer Lake, St. Joseph County, Indiana**

Scott A. Namestnik, Indiana Department of Natural Resources, Division of Nature Preserves, snamestnik@dnr.IN.gov
Roger L. Hedge, Indiana Department of Natural Resources, Division of Nature Preserves, rhedge@dnr.IN.gov

The approximately 25-acre Deer Lake, located west of South Bend in St. Joseph County, Indiana, is one in a series of kettle lakes within the glacial ridge that was deposited approximately 13,000 years ago at the intersection of the Lake Michigan lobe and the Saginaw lobe of the Wisconsin glaciation. These kettle lakes are at different successional stages due to their size, depth, surrounding substrates,
hydrological inputs and outputs, vegetation development and anthropogenic influences. Field surveys at Deer Lake were conducted primarily during summer 2018 and summer 2019, with data from past field surveys incorporated into the results. Deer Lake has developed into a globally and state imperiled (G2/S2) inland coastal plain marsh/muck flat community with numerous conservative vascular plant species, 13 of which are on the list of Indiana endangered, threatened or watch list species. A small portion of the shoreline of Deer Lake is owned by The Nature Conservancy, with the remainder in three parcels under private ownership. Deer Lake is a unique high quality natural area worthy of the highest form of protection.

Workshop
Fern Identification

Roger L. Hedge, Division of Nature Preserves, Indiana Department of Natural Resources

With approximately 80 species in Indiana, ferns are found in a variety of habitats from wetlands to xeric outcrops. These plants are challenging to identify but are important members of plant communities. Geared toward beginners, this workshop is for those who have an interest in fern identification but lack field experience with this group of plants. Basic vocabulary, fern morphology, important field characteristics, and distribution will be addressed for some common Indiana ferns. Please bring a hand lens.

Presentation
Sand myrtle (*Kalmia buxifolia*, Ericaceae) biogeographic history supports the Cape Fear Arch as a Glacial Refugium

Emily L. Gillespie; Butler University
Tesa Madsen-McQueen; University of California, Riverside
Torsten Eriksson; University of Bergen
Allison Welz; Butler University
Zack Murrell; Appalachian State University

Sand myrtle, *Kalmia buxifolia* (Bergius) Gift & Kron, exhibits a disjunct distribution in eastern North America, ranging from the NJ Pinelands (NJP) to the Sandhills/Cape Fear Arch (SCFA) and westward into the Southern Appalachian Mountains (SAM), with no present-day geographic connection between NJP and southern populations, and very little between the SAM and SCFA populations. This species exhibits considerable morphological variability, leading experts to recognize between one (current consensus) and three species historically. Here, we update efforts to understand the biogeographic history and genetic structure of this species within the
context of southeastern biogeography. We developed microsatellite markers using populations from each of the three regions and then applied these markers to a total of 11 populations of Sand myrtle and its closest relative, *Kalmia procumbens* (L.) Gift, Kron & P. F. Stevens ex Galasso, Banf. & F. Conti and evaluated various measures of population diversity and divergence. We evaluated phylogenetic signal using both chloroplast and nuclear markers and compared this to signals within the microsatellite data. Our updated dataset continues to reveal striking inter-population divergence in the south. Our analyses provides support for the hypothesis that the Cape Fear Arch of the Carolinas was a glacial refugium, from which Sand myrtle migrated northwestward into the SAM and northward into the NJP. Intermittent genetic connectivity between the NJP and SCFA populations appears to have continued for some time, but SAM populations have diverged considerably during the same period, raising new questions about the mechanism of divergence in the mountain populations.

Presentation

**Floristic Quality Assessment of a Scraped Restoration Site in Lake County, Indiana**

**Adam H. Balzer**, Cardno, Inc.

Sundew Prairie is a 12-acre wetland mitigation site located in Lake County, Indiana. At the time of the baseline Floristic Quality Assessments in 2012 and 2013 the site was predominantly a heavily degraded scrub-shrub habitat dominated by glossy buckthorn (*Frangula alnus*) and quaking aspen (*Populus tremuloides*). The restoration of this site began in May, 2014 with the removal of approximately one foot of organic material throughout the majority of the site exposing the lower sand layer. Prior to the restoration, the baseline Floristic Quality Assessments conducted documented 286 species (248 native) with a Floristic Quality Index of 74.4 (80.3 native). During the 2012 and 2013 surveys, ten endangered, threatened, rare, and watch list species were found. After the work was completed in 2014, meander surveys were conducted biannually in June and September from 2015 through 2019. The final surveys in 2019 resulted in a Floristic Quality Index of 82.4 (89.2 native) from a total of 367 vascular plant species (318 native). The plant community existing at the conclusion of the mitigation was a forb dominant wet-mesic sand prairie with 15 state listed species documented.
Little Wabash River Nature Preserve (LWRNP) is a 14.2 ha property of important geological terrain associated with the Maumee Torrent during the Wisconsin glaciation. Flowing water carved out a geographically unique passage in the landscape, variance in terrain creating subsequent diversity of flora. The site is protected by ACRES Land Trust within the Little River watershed. To ensure a better understanding of the community structure, ecological and floristic surveys were conducted between April and October of 2019. The ecological survey consisted of 48 quadrats along 7 transects visited in May, July, and September, and included identifying understory, midstory, and overstory plant species. Additionally, we measured PAR, canopy cover, litter depth, and soil moisture in each quadrat. The floristic survey was done to catalogue all species and conducted in a non-random meander between the set transects, in order to ensure inclusion of community types and terrain gradation potentially omitted in the ecological survey. At the quadrat level, understory species richness was significantly related to the canopy cover for all three surveyed months. However, understory species diversity was related to the litter depth in May, and canopy cover in July and September. As expected for the region, swamp white oak was a dominant overstory species, especially in the lower areas of the property. Unexpected was the dominance of black walnut throughout large areas of the property – occurring in the greatest proportion of quadrats and at the greatest density. LWRNP is an excellent example of the diversity that can exist in a small, protected forest. While there are some common invasive species in need of control within the property, there are more interesting native species as well, adding diversity and interest to the community. Further management may be necessary by ACRES Land Trust to ensure continued succession dominated by native species.
The genus *Cantharellus*, commonly known as the chanterelles, are some of the most popular wild mushrooms in Indiana. Their fluted orange and yellow fruiting bodies are a natural delicacy, and the global trade in chanterelles exceeds $1 billion per year. They are also significant ectomycorrhizal partners for many tree species and thus integral to the ecology of forests throughout the state. Only a fraction of the true chanterelle diversity in North America is known to science, and most collectors still use European species names such as *Cantharellus cibarius*, which may not be present at all in North America. Chanterelles are challenging to classify based solely on morphological traits, as these can be highly variable between conspecifics. Our goal is to use DNA barcoding to re-assess the diversity of chanterelles in Indiana, using specimens collected as part of the North American Mycoflora Project. We extracted DNA from 183 Indiana *Cantharellus* specimens and used PCR to amplify the elongation factor 1 (EF1) and DNA-directed RNA polymerase subunit II (RPB2), and D1-D2 of the 28S ribosomal subunit (LSU) barcoding regions. Previous molecular studies of chanterelles indicate that these genes are ideal for delineating chanterelles at the species level, and our work with EF1 confirms this. From our complete EF1 data we have identified 14 different species of *Cantharellus* within our state, of which 8 have never been reported in Indiana. By adding RPB2 and LSU sequences to our existing phylogeny, we hope to demonstrate the surprising diversity of chanterelles in Indiana and gain insight into their ecology by investigating metadata such as collection location, tree associations, and seasonality. Depending on forthcoming sequence data, we may also be able to describe one or more new species of chanterelle found in Indiana, which would surely generate interest from citizen scientists in the state.
Science Education

Poster
“Biology Indianapolis Outreach”—Connecting Undergraduate Science Students with the Community

Erin Gerecke, Butler University

Involving undergraduate students within their local communities through discipline-specific coursework is a growing trend in higher education. For students in STEM disciplines, community engagement opportunities that align with their scientific interests and career goals allow them to apply their learning beyond the classroom and consider their own place in the broader communities in which they live. “Biology Indianapolis Outreach” is a course at Butler University that satisfies the university’s Indianapolis Community Requirement. As part of the course, students developed and then presented their own hands-on, biology-themed activities for local families at both a local science festival and a museum. To help them feel more comfortable communicating about science and engaging with different audiences in different contexts, I introduced students to science communication strategies that are more commonly associated with training for professional scientists or graduate students in STEM fields. I report here on course activities and outcomes relating to science communication and community engagement.

Presentation
George Donaldson
Pioneer Hoosier Naturalist and Conservationist

Nelson R. Shaffer and Wyatt Williams

George Donaldson was born near Glasgow, Scotland. He had amassed considerable wealth from slate mining and lime production and bought a beautiful karsted parcel near Mitchell, Indiana about 1865. The parcel was preserved and is now part of Spring Mill State Park. He insisted that trees not be cut or that animals on his land not be killed, very unusual practices in that place and time. His land contains virgin forests in Donaldson Woods, a section of which was named to honor Academy member Damion Schmelz.

Donaldson traveled and corresponded all over the world. He had a large, cosmopolitan natural history collection at his home called Shawnee Cottage that overlooked Donaldson Cave. The home burned while he was away and Donaldson never returned. The remains of Shawnee Cottage exist and should be excavated and any items found curated.
Donaldson died in 1898 and is buried in the necropolis at his native Glasgow, Scotland but he left a lasting mark on Indiana’s natural history.

Presentation

**Improving STEM retention through research and cohort building**

**Kristi Bugajski**, Valparaiso University

Commuter students face unique challenges, which impact their chance of success. In order to improve recruitment and retention of commuters, the Establishing Practices for Integrating Commuters (EPIC) program at Valparaiso University addresses several problems documented in previous research for that demographic. This is accomplished primarily through scholarships, social engagement, and providing substantial interdisciplinary research opportunities across four academic years and one summer. Additional support mechanisms include training in oral and written communication, travel to conferences, monthly meal gatherings, and annual retreats and field trips. This program provides direct financial support for at least 30 STEM majors; many other STEM students also benefit as non-scholarship recipients are incorporated into program activities. The scholarships help bring Valparaiso University within the reach of more first generation students as well as students from nearby economically depressed areas with large minority populations. Results from the EPIC program will fill important gaps identified in the literature on recruitment and retention programs in STEM at liberal arts and religiously affiliated institutions, especially considering commuter students. The assessment plan focuses on the three major objectives of the program: 1) improve student retention, especially among commuters, 2) increase the number of STEM graduates prepared for research intensive careers, and 3) increase the number of departments with research intensive majors and strengthen existing programs. Evaluation, both formative and summative, utilizes mixed-methods design, including in-person interviews, focus groups, archival student data, and survey research. Results from the first three years of the program will be presented, including challenges, lessons learned, and retention data.

Presentation

**Designing, Collecting and Analyzing Data Using the Survey 123 App**

**Josephine Shireen Desouza**, Ball State, University

The Survey 123 is a form centric web-based data collection mobile app for ArcGIS. As part of a larger environmental education project, the Survey 123 app was used by preservice teachers in a science methods course to collect data on citizens’ perceptions.
of the White River in Muncie, Indiana. The preservice teachers designed the questionnaire, and collected the data by interviewing people on the Ball State campus, and those in close proximity to the White River. The data that was collected was immediately available to ArcGIS to be analyzed. The results of the survey were presented in charts and graphs. This web-based data collecting tool, is very versatile and can be used by middle and high school students, in addition to their field based study in science, social studies and language arts to collect data. Through web-based hands-on Geospatial technology, teachers can help their students participate in decision making about REAL world data, which is a key component to finding solutions to societal issues. This presentation will provide the results of the survey and information on how to obtain a GIS account, and where to get online professional development to pursue a GIS program in schools. Science teachers will get an insight into designing projects of their own and the understanding of the capability of GIS technology in integrating data from science and other disciplines.

Poster

**The Histology of Embalmed Cadaver Tissues**

*Laura Scheid MS, Western Michigan University and Kevin Gribbins PhD, University of Indianapolis*

Dissection of human cadavers is a major component of gross anatomy courses. Such dissection labs allow students to study human anatomy directly and provides a greater understanding of human variation while allowing students to practice dissection skills. The embalming protocols that cadavers undergo prior to their use in such courses is very important as this process determines the overall quality of the tissues encountered during dissection. The embalming process ensures long-term structural preservation of organs and tissues, prevention of overhardening, prevention of dehydration and decay, inhibition of the growth and spread of fungi and bacteria, reduction of biohazards and chemical hazards, and retention of color of tissues and organs. The preservation protocols of the cadavers utilized at the University of Indianapolis have proven satisfactory for the purposes of gross dissection. However, these donations could be utilized further as a source of tissues used for learning and research at the histological level. As important as it is to gross anatomical dissection, the process of embalming a cadaver may lessen the quality of tissues and organs for histological study. The purpose of this study is to sample various organ and tissue types from a cadaver obtained for use in the gross anatomy lab at the University of Indianapolis. These specimens will be fixed, mounted in paraffin, and stained with hematoxylin and eosin. Once completed, they will be compared to a set of control slides made from the same tissue types dissected from mice tissues fixed traditionally for histological study. The quality of each of these categories of slides will be determined based on standards used by Gupta & Gauba (2011). These quality
observations will be compared with observations made on the control slides to determine whether tissues and organs obtained from cadavers are suitable for use in histological education.

HotTopics
The Necessity for Teaching and Learning Research Methods

Patricia Arnett Zeck Northwestern High School (retired)

Research methodology should be paramount in both elementary and secondary classes. Research skills must integrate with the teacher's knowledge of subject matter. When pre-service teachers have practiced and learned research methodology in college, the transition to one's own classroom becomes much easier. Labs from upper elementary through college should be relevant and formal enough to require statement of the purpose/problem, hypothesis, basic methods, data with age appropriate analysis, and conclusion. Instructors may require a literature section of 2-10 quality references. Both simple and more complex labs should still follow the format with good English and writing skills. In the K-6 classroom, math and reading receive so much emphasis that science and research may be relegated to two periods per week. Creative teachers can add English, reading, and math components into stories or current events that have a scientific theme. Middle school reports can add the math concepts of mean, median, and mode. By high school some basic statistics like Student t-test can be added via computer programs. Understanding graphs and charts will pay dividends in both lab analysis and the inevitable standardized testing. Teachers can supplement thought processes with study questions to promote critical thinking skills. Extra benefits include collaborative learning and compliance with state and national teaching standards. Students have increased understanding when doing labs rather than staring at apps or lecture notes. Not all students will become scientists but research and problem solving skills relate to all areas of the job market and indeed to life decisions. It is up to teachers through the educational experience to further the development for each student regardless of his/her future career plans. Aspiring teachers must avail themselves of the research opportunities in their college or university.
Zoology

Poster
Using molecular techniques to determine species relationships in Tapinocyba (Araneae: Linyphiidae)

Esther Mullins (University of Indianapolis), Marc Milne (University of Indianapolis), and Richard Bradley (Ohio State University)

Tapinocyba is a large genus of sheet web-weaving spiders in the sub-family Erigoninae (Araneae: Linyphiidae) that are approximately 1 - 1.5mm in size. Many species within this genus are either poorly known, known from one sex, or undescribed. One particular species, Tapinocyba emertoni, is only known from the male. However, recently we caught multiple female specimens with the known male. The goal of our research was to use phylogenetics to ensure that these females are, indeed, T. emertoni. A secondary goal was to estimate the relationship of T. emertoni to two related species: Tapinocyba simplex and Tapinocyba minuta. We extracted, amplified, purified, and sequenced the COI gene from these Tapinocyba species. We then created a phylogenetic tree based off of COI to match the female T. emertoni to the male and distinguish the relationships between all three species. We found that the morphological suspicions were confirmed. We also determined the phylogenetic relationship between the three known species. With over 40 known species in Tapinocyba and many undescribed species sitting on museum shelves, there is still much to do in this field. Hopefully, these results will serve as the first steps towards attempting to revise this complex genus.

Presentation
Prevention of the Age-Related Decrease in Nuclear Reduced Glutathione Levels in Kidneys from Aging Female Lewis Rats with Alpha Lipoic Acid Supplementation

Marianna Zamlauski-Tucker and Bingwei Ye, Department of Biology, Ball State University

The purpose of the present study was to investigate whether supplementation with alpha lipoic acid reverses the decrease in nuclear reduced glutathione (GSH) levels in kidneys from old rats. GSH is the major antioxidant inside cells, and a decrease in GSH levels is associated with increased oxidative damage caused by free radicals. There were three groups of female Lewis rats used in the study. The Young Control rats (n=4) were 3 months of age, the Old Control rats (n=4) were 22 months of age, and the Experimental rats (n=4) were 22 months of age. The Experimental rats
received alpha lipoic acid (100 mg/Kg body wt) by i.p. injection for one week. The kidneys were harvested from anesthetized rats, and the cortex and medulla were separated and homogenized. The nuclear fractions were isolated by differential centrifugation, and the GSH levels were measured using a spectrophotometric assay. The three groups were compared using ANOVA followed by the Tukey post hoc test. There was a significant decrease in nuclear GSH levels in kidneys from Old Control rats when compared to Young Control rats. Alpha lipoic acid supplementation reversed the decrease in nuclear GSH levels in kidneys from the Old Control rats so that the levels were not different from Young Control rats.

Poster

**Not just horse play: a study testing sociability and local enhancement in *Equus caballus* when choosing a feeding station in the presence of conspecifics**

**Kathryn N. Perry**
Dr. Brian G. Gall
Hanover College

The ability to learn in horses (*Equus caballus*) has made them a vital companion to humans as well as other horses. In this study, we tested a horses’ desire is to be with conspecifics and whether they have the capacity to exhibit local enhancement. This was done by placing food near a blank picture and a picture displaying a horse expressing either aggression or passiveness. A horse was then allowed to choose a feeding station. After the horse was done eating, the pictures were removed and the horse was again allowed to choose a feeding station. We found that the horses exhibited a significant preference for the horse face over the blank sign. However, once the signs were removed, they did not exhibit a preference for the location they chose the first time. From these results, we conclude that horses do not exhibit local enhancement but prefer to be with an aggressive or passive horse rather than being alone. These findings aid our ability to interact with horses and understand their need to be with conspecifics and their ability to learn. This can be applied in their training, travel and companionship.
Orientation Behavior of Riparian Long-jawed Orb Weavers (*Tetragnatha elongata*) after Displacement Over Water.

**Sidney J Goedeker** - Hanover College  
Theresa E Wyrnn - Hanover College  
Brian G. Gall - Hanover College

Many organisms, including numerous invertebrates, possess remarkable abilities to orient and navigate within their environment to achieve goals. While these goals are often in the context of finding food or acquiring a mate, avoiding predation or mitigating incidental displacement can also be strong sources of selection for orientation. We examined the orientation behavior of a riparian spider, the Long-Jawed Orb Weaver (*Tetragnatha elongata*), when displaced onto the surface of the water. When displaced, spiders move with alternating movements of the first three leg-pairs while dragging the most posterior pair of legs behind them. In addition, spiders often perform a series of orientation behaviors consisting of concentric circles before ultimately choosing a path of travel directly towards the nearest point to land. Finally, while the number of orientation behaviors increases with increasing distance from shore, distance had no affect on their direction of travel which was significantly oriented toward the closest point of land. These results indicate a complex ability to correctly orient toward land when displaced onto water, possibly to decrease the amount of time on the surface of the water and thus decrease predation risk.

Poster

Exploratory Analysis of the Intestinal Epithelial Histology of the American Bullfrog, *Lithobates catesbeiana* (Amphibia: Ranidae)

**Evan Brandenburg**, Zoelaine Viewegh, Mikia A. Davis MS, Kevin M. Gribbins PhD. University of Indianapolis

*Lithobates catesbeiana* tadpole metamorphosis contains a larval-to-adult organ remodeling system. Intestinal growth fits the remodel system as thyroid hormone naturally synthesized by the tadpole is released to simultaneously initiate both the degradation and apoptosis of larval epithelial tissue, as well as the development of the adult epithelium. The focus of this study was to determine the effect of Thyroid hormone (TH) on the remodeling of the tissues of the intestinal tract. Tadpoles were exposed to three levels of Thyroxine (T4), dissected, sectioned, and examined using light microscopy. We hypothesized that we would observe signs of apoptosis during the intestinal cell development between the treatment groups compared to the control. As the level of thyroid hormone increases, the tadpole’s intestinal tract transforms
from the simple epithelium found in larval intestines to the more complex ciliated columnar epithelium and dense basal lamina of an adult Bullfrog. The purpose of our study was to identify potential mechanisms such as macrophage invasion that contribute to the remodeling of amphibian intestines during metamorphosis. We found that higher levels of T4 corresponded with higher numbers of macrophages found throughout the intestinal tract. This indicates the entire intestinal tract undergoes simultaneous metamorphosis dependent upon the concentration of endogenous T4. This research can serve as a foundation to understand how thyroid hormone can trigger crucial organ remodeling processes such as cell apoptosis and cell proliferation during different stages of metamorphosis.

Poster

Histological Analysis of the Esophagus and Stomach of *Lithobates clamitans*

*Jennifer Lai Hipp, BS, University of Indianapolis Biology Department; Diana Mancuso, University of Indianapolis Biology Department; Kevin Gribbins, PhD, University of Indianapolis Biology Department*

Little research has been done on the histology of the digestive tract within the genus of frogs, *Lithobates*. There is no histological information on the digestive tract of *Lithobates clamitans*. Thus, the aim of this research was to identify and study the cell types found within the esophagus and stomach of the Green Frog. Studying the digestive tract of frogs may help our understanding of their life history, population fluctuations, and the impact of habitat modifications. Due to their permeable skin, frogs are considered an indicator species, meaning they will quickly react to changes to the environment. Green Frogs were collected in Columbus IN, stomachs and intestines were removed, fixed, embedded in plastic, sectioned, and analyzed via light microscopy. Goblet cells containing two different types of secretions were observed; however, the role of these secretions is unknown. Previous histological examination revealed a large number of chief cells and zymogen granules in the digestive tract, suggesting pepsinogen is secreted in the esophageal glands of the Green Frog as in other species studied to date. In the stomach of *L. clamitans*, parietal and mucous cells were observed in the gastric glands and when compared to other frogs marked differences in the number and size of the glands were found. Upon studying the cell types found in the esophagus and stomach of *L. clamitans*, it was determined that a closer examination using immunohistochemistry and transmission electron microscopy should be performed to gain a better understanding of these structures and their functions.
**A Histological Examination of the Green Frog’s (*Lithobates clamitans*) Ventral and Dorsal Skin**

**Alex Wong, BA, University of Indianapolis Biology Department; Jessica Gall, BS, University of Indianapolis Biology Department; Kevin Gribbins, PhD, University of Indianapolis Biology Department.**

The Green Frog (*Lithobates clamitans*) is an abundant species located throughout Indiana. Its ability to thrive and adapt in aquatic and terrestrial ecosystems is the result of its unique integument. In comparison to other vertebrates, the skin of frogs is described as naked, that is, lacking the covering of scales, feathers, or hair. The varying colors, textures, and structures identified on the frog’s skin plays a key role in the species ability to both evade predators as well as hunt smaller prey. However, the permeability of frog skin makes them highly susceptible to harmful toxins that arise from within their environment. Comparing the distinct microscopic structures and layers of the ventral and dorsal skin is essential for researchers to understand histopathological changes occurring to the integument upon exposure to a toxin or invader. Using histology, the goal of this preliminary research was to identify the integumental layers and features of the Green Frog and compare our results to the previous literature on amphibians’ skin. The primary structures observed in this study included poison and mucous glands, chromatophores, and the different strata of the epidermis and dermis. In addition, the presence of atypical integumentary features such as the Eberth-Katschenko (EK) layer and flask cells were explored. Understanding the frequency and location of these various histological skin characteristics is an important first step in evaluating the adaptive function of frog skin. Future studies will focus on the ultrastructure of the glands and epidermal layers to aid our understanding on the mode of skin secretion and molting.

**Keywords:** Green frog, integumentary system, histology, *Lithobates*

**Fowler’s Toad Ventral and Dorsal Skin Ultrastructure: A Histological Analysis**

**Sara Mire, BA; Sai Suresh Kumar, BS; Kevin Gribbins, PhD, University of Indianapolis Biology Department.**

Histological analyses allow for the visualization of tissues by utilizing different stains to identify intracellular, extracellular structures, or cellular architecture. There have been few histological studies completed within *Anaxyrus fowleri*, the Fowler’s Toad. No data exist that focus on the dorsal and ventral skin of these amphibians, with subsequent comparisons to other species within Bufonidae. Toads were collected in
Columbus, Indiana, dissected, integument samples were removed and fixed, embedded in plastic, sectioned, stained, and analyzed under light microscopy. The results of the dorsal skin analysis showed clearly defined layers of epidermis and dermis. Desmosomes were observed in the epidermis, as well as dermal mucous and granular glands, and chromatophores. The most interesting components of the dorsal skin were the large flask cells and their developmental stages. Previous literature has little information on these cells and the molting process in which they take part. Both dorsal and ventral skin sections displayed warts that are thought to act as a defense mechanism. The ventral skin displayed exocrine glands, myoepithelial cells, skeletal muscle, and microvilli. While the Fowler’s toad integumental histology parallels that of other amphibians, their epidermis is more defined with less glands than that of other amphibians. Further research will focus on the ultrastructure of the glands and flask cells to better understand their role in the toad skin.

Presentation

**Reproductive stage and roost characteristics influence Indiana bat thermoregulatory strategies**

Scott Bergeson, Purdue University Fort Wayne  
Joy O’Keefe, Indiana State University  
Mark Brigham, University of Regina

Due to increasing temperatures, the Midwestern U.S. is predicted to become climatically unsuitable for the federally endangered Indiana bat (*Myotis luberta*) by 2030–2050. However, bats may be able to buffer the impacts of climate change via thermoregulation. Our objective was to investigate how flexible Midwestern Indiana bats are in their roost and torpor use in response to varying weather patterns. We tagged 13 adult female and 4 juvenile bats, captured in Central Indiana from 2013–2015, with temperature sensitive transmitters and tracked them back to their roosts. We compared models testing the effects of weather and roost characteristics (38 individual roosts) on daily torpor patterns (104 full temperature days), with reproductive period as a random effect. Based on AICc results, weather and roost canopy closure models best explained variation in torpor duration and depth. Torpor duration decreased with increasing air temperature and increased with roost canopy closure; bats used torpor for less time (often remaining normothermic) on hot days and while using solar-exposed roosts. Torpor depth was positively related to air temperature, precipitation, and humidity; bats used deeper torpor on colder, more humid, and stormier days. Additionally, bats greatly reduced the time they spent torpid during an extended period of abnormally high temperatures (> 29.9 C). However, bats also continued to use solar-exposed roosts during this time period, suggesting that they were resilient to high ambient temperatures. These results suggest that Indiana bats are flexible in their response to weather variation. Due to their
flexibility, temperate bat species may be more resilient to the negative impacts of climate change and may be able to persist in landscapes that are climatically unsuitable for other endotherms, especially if efforts are made to provide roosts with an array of thermal characteristics.

Presentation

Mitochondrial DNA variation and historical biogeography of the Spottail darter (*Etheostoma squamiceps*) in Indiana

Rex Meade Strange, Department of Biology, University of Southern Indiana

The Spottail Darter (*Etheostoma squamiceps*) reaches its northern-most extent in southern Indiana where it is restricted to three drainage systems in the extreme southwestern corner of the state. Each of the Indiana populations is relatively isolated from each other, and the genetic relationships between them and populations occurring in neighboring states have not been previously determined. Sequence variation of the mitochondrial cytochrome *b* gene from specimens of *E. squamiceps* collected from across its distribution was examined to better understand the phylogeographic relationships of its Indiana populations. Specimens collected from southern Indiana, Illinois, and the Tradewater, Rough, and upper Green rivers of Kentucky shared a common set of closely related mtDNA haplotypes, and form a Northern Group within the species. In comparison, samples collected from the Pond, Barren, and Red rivers had unique and divergent haplotypes. The Northern Group shows evidence of reduced genetic heterogeneity relative to the more southerly populations, consistent with an assemblage of populations that has recently expanded. These results suggest that the contemporary distribution of *E. squamiceps* in southern Indiana was established following climatic and drainage changes that occurred at the end of the Pleistocene.

HotTopics

Cultivating student learning in biology through Annelid discovery and speciation

Julie Davis Good, PhD
Indiana Institute of Technology

In order to promote effective and efficient conservation practices, the current state of our urban ecosystems must be described and understood. Indiana Institute of Technology (Indiana Tech) initiated a unique, urban-based earthworm census project on our campus during Fall 2019. During this inaugural year, students applied their learning through hands-on work in the soil. Their initial investigation will lead to discovery in further areas of conservation and environmental research over the next four academic years.
The Annelid project is an ongoing classroom-based observational study conducted by students enrolled in BIO-1340 (General Biology I Laboratory) geared to identify earthworms (Family: Annelida) in urban areas of less-disturbed soil structure on campus. The resulting “earthworm census” will provide critical information that will support – and may extend – the historical surveys of Indiana soil (Reynolds, 1994; Reynolds, 2016). New data from Allen County are important at a time when invasive species are over-burdening our regional resources.

Following the initial earthworm surveys in Fall 2019, subsequent studies are planned over the next five years to accomplish the following: Compare freshly isolated earthworms with those reported in wider, regional historical surveys Collaborate with other Annelid experts to confirm speciation and to extend published resources regarding invasive annelid species not previously identified in this area Analyze soil chemistry in regions with disparate earthworm colonization Obtain preliminary data on the gut bacteria (e.g. “microbiome) of Indiana Tech earthworms; then compare local populations with populations in agricultural or preserved areasThe experience brought immense learning opportunities to students with limited prior introduction to “science” while also substantially enriching the experience of students arriving with broader preparation. The annelid project encompassed the entire Fall semester in order for students to achieve the actual progress of science. Students mastered experimental methods, practiced scientific logic, and – thereby – accomplished important research with their own hands acquiring laboratory skills distinct from the types of learning in the classroom setting.

Presentation

**How social challenges modulate steroid signaling in the female brain**

**George, Elizabeth M**1 and **Rosvall, Kimberly A**1
1Indiana University Bloomington

Little is known about the mechanisms underlying female-female aggression, despite increasing evidence of its adaptiveness in many species. While some male vertebrates socially modulate circulating testosterone (T) levels after aggressive interactions, the same hormonal response has not been found in many female vertebrates, perhaps because selection has favored mechanisms that minimize the potentially high costs of elevated T levels in females. We hypothesize that, rather than changing systemic T levels in response to competitive interactions, females instead modulate local sex steroid sensitivity and conversion, i.e. by upregulating sex steroid receptors and steroid-modifying enzymes in behaviorally relevant tissues like the brain. Here we tested this hypothesis in tree swallows (*Tachycineta bicolor*), a species in which females compete for limited nesting sites and aggression is at least partially mediated by androgens. We found that females do not rapidly increase, and actually decrease, T
levels after both real and simulated social challenges. In light of this result, we further explored whether social challenges induce changes in local steroid processing and binding within the brain. To test this, we exposed females to simulated territorial intrusions and collected neural tissue 2-3 hours later. We used qPCR to measure the expression of genes involved in steroid binding and processing in brain regions thought to mediate aggression, including nodes of the vertebrate social behavior network. Preliminary findings suggest that patterns of correlated gene expression among brain regions are socially responsive, with control females displaying greater connectivity or correlations among genes and brain regions than females that recently experienced a simulated fight. These findings will provide novel insight into mechanisms by which individuals can respond to social challenges without increasing T production.

Presentation

**Biodiversity of spiders within Virginia Caves**

**Brandon Rhim**
Marc Milne
Tom Malabad
Will Orndorff
Ellen Koerterge

There is little known about the diversity and distribution of cave spiders in the world. This pattern extends to Virginia where only minimal work has been done to understand cave spider biology. We attempted to gain a better understanding of these reclusive animals by surveying caves throughout Virginia between 2014 and 2019. We conducted cave surveys by looking for spiders hiding under rocks, crawling on walls, and living in webs. We collected and identified over 1,000 spiders over these five years. We found common cave species such as Phanettia 144ubterranean, Porrhomma cavernicola, and Nesticus tennesseensis. However, we also found spiders in genera not previously known from the state. Unsurprisingly, we also identified six likely undescribed species within a large, complex spider family: Linyphiidae. These spiders belong to the genera Anibontes, Centromerus, Islandiana, Lepthyphantes, and an unknown genus. This study underscores the hidden biodiversity of subterranean habitats within North America and helps emphasize the overwhelming need for their protection and conservation.
Behavioral Modifications of an Open-cup, Canopy-Nesting Passerine in Response to an Increase in Nest Predation Threat

Alexander Sharp: Department of Biology, Ball State University, Muncie, IN 47306-044
Kamal Islam: Department of Biology, Ball State University, Muncie, IN 47306-044

The Cerulean Warbler (*Setophaga cerulea*), a small, neo-tropical migrant, has gained significant attention in recent years as a species that has declined faster than any other North American songbird. Since 2007, we have been monitoring Cerulean Warbler populations within the Morgan-Monroe and Yellowwood state forests of Southern Indiana. Previous studies on other species with various nesting strategies suggest that parents will adjust their behavior in response to an avian nest predator, to either avoid detection, or to fend off the predator. These behaviors include mobbing, selective provisioning of nestlings, reduction in clutch size, increased vigilance and aggression on the nest, or reduced activity at the nest. Studies suggest that forest fragmentation positively impacts nest predators, and in-turn leads to an increase in nest predation.

The objective of this study is to determine if and how Cerulean Warblers change their behavior when a common avian nest predator, Blue Jay (*Cyanocitta cristata*), is in the vicinity of the nest. In the summers of 2018 and 2019, we conducted a study with 30 Cerulean Warbler nests found at our study sites. During the observation period, half of the nests were randomly assigned to a control group, and subjected to Ovenbird (*Seiurus aurocapilla*) vocalizations, and the other half of the nests were assigned to a treatment group, and subjected to Blue Jay vocalizations. We videotaped parents at the nest during the observation period to analyze their behavior. The results of this study will be presented. This study is the first to analyze the behavior of an open-cup, canopy nesting species in response to an avian nest predator. Data collected from this study will determine if Cerulean Warblers are capable of identifying species that pose a threat to their nest, and if they then alter their behavior to avoid detection of their nest.

A comparison of territory and home range estimates of Cerulean Warblers (*Setophaga cerulea*) in southern Indiana

Brandon Connare and Kamal Islam, Department of Biology, Ball State University, Muncie, IN 47306, USA

The Cerulean Warbler (*Setophaga cerulea*) is one of the fastest declining North American wood-warblers. Over the past 11 years, we have monitored a breeding population of this state-endangered species at Yellowwood and Morgan-Monroe state
forests in Southern Indiana as part of a larger 100-year project, the Hardwood Ecosystem Experiment. This long term study intends to determine the effects of a variety of forestry practices on local plant and animal communities, including the Cerulean Warbler. We have examined the relationship between two types of space use for this species, the territory and home range. Space use information can be imperative when devising management, conservation, and research strategies of a species. Current research suggests that the size of these space use areas vary regionally for Cerulean Warblers, and space use of this species in Indiana has not yet been studied in detail. Throughout their range, territory estimates of Cerulean Warblers have relied upon spot-mapping techniques, which tend to underestimate territory size, as well as neglect use of the larger home range. Our goal is to determine the size and relationship of Cerulean Warbler territories and home ranges using an alternative method, radio telemetry. In 2018 and 2019, we attached radio transmitters to 20 male birds in an effort to track and record each bird’s locations throughout its home range and territory. We compared the sizes of territory and home range estimates, as well as territory estimates in our study with previous published estimates that used spot-mapping techniques. We present the results from our study.

Presentation

The Status of Eastern Spadefoots (Scaphiopus holbrookii) in Indiana.

Nathan J. Engbrecht - Indiana Department of Natural Resources
Michael J. Lodato (retired, no professional affiliation)
Jason J. Mirtl - Indiana Department of Natural Resources
Zachary T. Truelock - Indiana Department of Natural Resources

The Eastern Spadefoot (Scaphiopus holbrookii) is a secretive, fossorial anuran that has been difficult to assess due to its cryptic habits. Historically, populations were known from just a few scattered sites in southern Indiana. Beginning around 2000, a sharp increase in new Eastern Spadefoot records began to emerge, and increased survey effort by the Indiana Department of Natural Resources and others revealed a number of additional populations. Data compiled from field surveys and other records over the past two decades bring the total number of occupied counties from seven to 26. Its geographic distribution demonstrates a close association with alluvial plains and narrow bands of windblown sand along southern Indiana’s big rivers, and also in areas containing karst and escarpment topography. Despite being a habitat specialist, Eastern Spadefoots have shown a striking ability to persist in cultivated agricultural settings, a feature that has undoubtedly contributed to its success across a large portion of southern Indiana.
Steroid Regulation of Gastrointestinal Development and Reproduction in the Marsh Rice Rat (*Oryzomys palustris*)

**Kent Edmonds**, Lluvia Gibson, and Lou Roederer, Indiana University Southeast

Environmental factors and subsequent hormonal changes can regulate the development of various physiological systems. Steroid hormones are known to affect significantly the reproductive system in seasonal breeders, but effects on the gastrointestinal (GI) tract have not been as extensively studied. The present studies examined whether corticosterone or estradiol (or lack thereof) affect GI development and reproduction in juvenile male and female rice rats. Rice rats were subjected to subcutaneous Silastic implants of corticosterone or estradiol from 21-56 days of age. The following masses were examined: body, testes, seminal vesicles, Harderian glands (males only), female reproductive tract (RTM), and wet (W) and dry (D) masses of the stomach (St), small intestine (SI), cecum (Ce), and colon (Co). In addition, small intestine and colon lengths were measured. Corticosterone had no effect on body mass or any reproductive or GI variable examined in males or females. Estradiol, on the other hand, increased female reproductive tract mass, WSt, WSI, DSt, and DSI masses relative to animals with empty implants, but decreased SI and Co lengths. These data show that estradiol most dramatically affects reproduction and GI development in females, but that corticosterone was without effect on any reproductive or GI endpoints in either sex. Ovariectomy decreased RTM mass, and increased the mass of the body, WSt, WCe, DSt, DSI, DCe, DCo, and SI length. It was hypothesized that changes in the GI tract may be a necessary mechanism for coping with likely seasonal changes in metabolic requirements potentially brought about by changes in steroid hormone levels.

Predatory Selectivity of Pond-Breeding Salamanders in Ephemeral Wetlands of Ohio and Illinois

**Brock Struecker** Cardno, Joseph R. Milanovich Loyola University Chicago, Mollie McIntosh Xavier University, Martin B. Berg Loyola University Chicago, Matthew E. Hopton US EPA

Larval amphibians are important components of ephemeral wetland ecosystems. Larval pond-breeding salamanders (genus *Ambystoma*) are especially important as they are the primary vertebrate predators in fishless, ephemeral wetland systems where they consume large amounts of aquatic invertebrate and vertebrate prey. Aquatic invertebrates also fulfill many important ecological roles and thus the importance of predation by larval pond-breeding salamanders in ephemeral systems is
magnified. We aimed to elucidate further the predatory selectivity of larval pond-breeding salamanders in ephemeral wetlands across two regions in the Midwestern United States. We compared salamander stomach contents to determine diet and wetland aquatic invertebrate diversity and abundance to identify if larval salamanders showed any predation biases for certain taxa or functional feeding groups. Our data suggest larval salamanders have a predation bias for and against certain taxa in Illinois and Ohio wetlands. Salamanders at both locations selected primarily for the collector-filterer functional feeding group and selected against invertebrate predators. These data provide cursory evidence for how larval predatory salamanders may affect ephemeral wetland ecosystems.

Presentation

**An Update on the Plains Leopard Frog (*Lithobates blairi*) in Indiana**

Zachary T. Truelock (Indiana DNR), Jason J. Mirtl (Indiana DNR), Nathan J. Engbrecht (Indiana DNR), and John Burris (no affiliation)

The Plains Leopard Frog (*Lithobates blairi*) is a widely distributed prairie species that reaches the eastern edge of its range in Indiana. A relic of the Prairie Peninsula, Indiana records for this species have been scarce and an approximately 30-year dearth of confirmed sightings has made it difficult to assess the species’ status in the state. Recent surveys, however, identified extant populations along riparian corridors in western Indiana, and preliminary data suggest the Wabash and Iroquois rivers may play an important role in the conservation of this state endangered species. Here, we present the results of recent surveys and provide descriptions of the frog’s habitat use and distributional pattern in the state.

Workshop

**Blood sample collection techniques in wildlife species: tips and tricks for success.**

Maraiah Russell BS, RVT
Little River Wetlands Project

Participants are limited to 20 people, with a fee of $10 to cover the cost of supplies. Room setup requirements are 1 table for each pair of participants. Table covers will be supplied by the speaker.

Obtaining a viable blood sample in any species can be a challenging prospect and success is dependent on many factors. These samples can be immensely helpful in a wide range of wildlife research projects, from assessing health status, disease surveillance, and environmental contaminant exposure to genetic analysis. In this 75 minute workshop we will discuss blood collection principles, techniques, sample
handling protocols, and how to adapt them to a variety of species. Proper animal restraint techniques and syringe safety precautions must be utilized to avoid injury to the animal and researcher, and are essential to obtaining a quality sample. This workshop is for anyone in the wildlife research field who would like to increase their knowledge of blood sample collection methods and gain confidence using sampling materials. Participants will get hands on practice with collection materials and non-living models and observe collection sites on a live turtle. No previous experience or materials required.

Poster

**Sexual dimorphism in visceral organ mass and energy content in Fowler’s Toad (Anaxyrus fowleri).**

**Cassandra L. Proffitt** and Michael S. Finkler, Indiana University Kokomo, Kokomo, IN 46904-9003,

Sexual dimorphisms in body size are common in many species of anurans, and have often been ascribed to different selective forces that influence reproductive success in males and females. However, little is known about dimorphisms in whole-organism physiology or in internal morphology in anurans. In this study, we investigated potential differences in visceral organ mass (heart, liver, kidneys, stomach, small intestine, and gonads) and somatic energy content (kJ) between reproductive male and female Fowler’s Toads (*Anaxyrus fowleri*) collected en route to breeding areas during the spring. Female toads were both longer and more massive than males. Neither dry liver mass nor dry kidney mass differed between the sexes once somatic body mass was accounted for. Females had proportionately larger dry stomach and dry small intestine masses than did females, whereas males had proportionately larger heart masses and remaining dry carcass masses (largely consisting of bone and muscle). The mass-specific energy content of the total carcass (sans the gonads) did not differ between males and females. However, the energy content of ovaries containing mature follicles was similar to that of the rest of the body in reproductive females. Our findings suggest that males exhibit larger relative size in organs that may promote increased chances of successful reproduction (e.g., calling activity, amplexus, and interference of other males). Females, on the other hand, appear to exhibit larger relative size in organs that aid in the processing of nutrients, possibly in support of oogenesis. In addition, our findings suggest that although the overall energetic cost of reproduction may be greater for females, there may be other energetic constraints within or outside of the reproductive period that limit the ability of males to allocate resources to growth and storage.