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Abstracts

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Anthropology Section

An Examination of Midwestern American Indian Female Crania in FORDISC 3.0 with Regard to an Isolated Calotte Found in Indiana

Susan Spencer Helfrich, University of Southern Indiana, and Della Collins Cook, Indiana University

Isolated crania are a common find in the Midwest and are often broken and incomplete. These fragmentary finds are particularly difficult to identify using traditional forensic techniques. We present on a calotte from Greene County, Indiana, submitted to us as a forensic case. It was missing the face and skull base, allowing for only seven measurements to be entered into FORDISC 3.0 (GOL, XCB, WFB, UFBR, ASB, FRC, PAC). Discrepancies in FORDISC 3.0 results for the Greene County calotte prompted an examination of results for ancient American Indian female crania from the Schild site (AD 700-1250) in west-central Illinois. We learned that (1) ancient Midwestern American Indian females tend to be misclassified in FORDISC 3.0; (2) the likelihood of having a result with a posterior probability above 0.800 increased as the number of measurements entered into FORDISC 3.0 increased; (3) an increased number of measurements entered into FORDISC 3.0 do not guarantee a more accurate result. We propose that the application of FORDISC 3.0 in cases such as the Greene County calotte is unreliable, and should not be used to exclude an ancient American Indian identification. Radiocarbon dating and DNA analysis could provide conclusive evidence that the calotte was ancient, but these techniques are expensive and are not always successful due to the degradation of bone collagen and DNA in wet environments.

Can dental metrics be used to distinguish between European and Native American individuals?

Paige Wojcik, Indiana University

Odontometrics have been used to evaluate ancestry, kinship, and sexual dimorphism. Tooth crown size is highly heritable and is therefore frequently used to determine biological distance between individuals within and between populations. On a broad scale, the teeth of Native Americans tend to be larger than those of Europeans. Non-metric dental traits more commonly found in Native American dentitions account for some of this variation in crown size.

This study attempts to determine whether the maximum mesiodistal and the maximum buccolingual crown diameters of permanent teeth can be used to distinguish between individuals of Native American and European ancestry in a mixed sample. The sample consists of 40 Native American maxillary and mandibular dental casts (including Navajo, Pima, and Yuma individuals) and 10 maxillary and mandibular dental casts from European individuals.

The maximum mesiodistal crown diameters and the maximum buccolingual crown diameters of permanent teeth were measured to the nearest 0.10 mm with Mitutoyo calipers using the technique described in Moorrees (1957) and Moorrees (1964). Each measurement was repeated twice to ensure minimal intra-observer error. The data were randomized and then sorted by the mesiodistal or buccolingual crown diameter of each tooth. Although the European individuals occasionally cluster towards the smaller end of the spectrum when the mesiodistal and buccolingual crown diameters are sorted in size order, the individual dimensions of any given tooth cannot be used to distinguish between individuals of Native American or European descent. European teeth cannot be differentiated from Native American teeth when mesiodistal crown diameter is plotted against buccolingual crown diameter of any given tooth. Analyses of the variation in the Native American dentitions demonstrate the challenges in using odontometrics to evaluate continental ancestry.

Anthropology Section Continued.....

Elliptical Fourier Analysis of the Human Nasal Aperture

Justin R. Maiers, Krista E. Latham, Stephen P. Nawrocki, Jessica Byram, University of Indianapolis,

In the analysis of human skeletal remains, the nasal aperture is often used in discrete, non-metric analyses, especially in regards to ancestry assessment. The shape itself is complex, so the final assessment of the shape has traditionally been based largely on the subjective judgment of the observer. This means the final decision of the qualities of that shape are subject to the Gestalt of the observer, and do not necessarily accurately describe actual shape. Elliptical Fourier analysis can transform the complex shape of the nasal aperture into a numeric variable. Once transformed, not only can the complex shape be consistently and precisely recreated, but it can be quickly and easily compared against data from other nasal apertures.

A total sample of 840 individuals were sampled for this study. Samples were collected from the Pretoria bone collection in Pretoria, South Africa and the W.M. Bass collection at the University of Tennessee in Knoxville, TN. This study sampled males and females ranging from 18-100, all with known age, sex, and ancestry.

Elliptical Fourier analysis was instrumental in quantifying a complex shape, which allowed this study to focus on statistical comparisons instead of qualitative descriptions. Principal component 1 was the strongest contributor to the observable variation at ~84%. The shape was significantly affected by sex, ancestry, continent of origin, and the interaction of ancestry and country of origin. This presentation will explain how those different variables affect the shape of the nasal aperture.

How long has tuberculosis (TB) infected humans?

Krystiana Krupa, Indiana University

The Mycobacterium tuberculosis complex (MTC) consists of several species that cause TB in humans and other organisms, with origin estimations ranging from ~40,000 years ago, coinciding with human migration out of Africa, to a relatively recent ~6,000 years ago. Because TB species have infected humans for thousands of years, they offer insights into the co-evolution of bacteria and their human and animal hosts. The events associated with the peopling of the Americas provide a unique window into this evolutionary scenario. Initially, it was believed that Native American populations had not been exposed to TB until European Contact (EC), because TB was one of the diseases that ravaged Native American people at the time of contact, which suggested they had no immunity. Skeletal markers of TB infection exist, often on the ribs and spine, and these markers are seen in ancient Native Americans from at least a thousand years ago, confirming that TB was present in the Americas before EC. TB ancient DNA (aDNA) isolated from Peruvian mummies was found to be most similar to *M. pinnipedii*, an MTC species that previously showed no evidence of infecting humans. The authors suggest sea mammals, the natural hosts of *M. pinnipedii*, as a novel route for the disease to be transmitted to humans. Is this how TB first came to the Americas, or was this a secondary acquisition of a disease already present in humans? Through next-generation aDNA analysis, this is the research question that this project will address. Preliminary analysis of portions of the gyrase-B and 16s genes from North American samples suggests they are quite different from the Peruvian samples. Sequence differences between ancient samples and modern *M. pinnipedii* at these two genes analyzed as part of a pilot study suggest it is very unlikely that our samples are derived from *M. pinnipedii*. In fact, phylogenetic analyses suggest that the Midwestern samples from the pilot study are likely ancestral to all modern strains of TB, both human- and animal-infecting. If true, this would have important implications for interpreting the evolution and differentiation of the various modern strains within the MTC. Further analyses of these potentially ancestral strains will be crucial for how we understand the evolutionary history of TB. All of the work for this project will utilize samples from Midwestern pre-EC burial sites in Illinois, which date to the Middle and Late Woodland and Mississippian periods (~1000 BCE – 1500 CE). These periods cover approximately fifteen hundred years before European contact. The first sustained settler contact with Midwestern Native American groups would have occurred well after these periods, which makes them good sites for examining the pre-contact disease environment. Next-generation DNA sequencing provides a new cutting-edge method to analyze a much larger portion of the genome; and while one of the caveats of aDNA research is that often only small fragments are available, next generation sequencing (NGS) relies on small fragments for analysis, making it ideal for this project.

Anthropology Section continued.....

Mastodon Extinction in the Context of Dental Microwear Texture: Preliminary Findings

Sarah Fann & Christopher Schmidt, University of Indianapolis

The extinction of the American mastodon (*Mammuth americanum*) is thought to have been caused by dramatic climate changes or human overhunting. The purpose of this study was to determine if mastodon diets, as indicated by dental microwear texture, changed prior to their extinction. In a previous study of mastodon macrowear we found no differences between earlier (greater than 20,000 years ago) and more recent (around 15,000 years ago) groups. Microwear texture, however, may be able to elucidate more subtle dietary differences. We studied 10 animals from Missouri (n = 4), Illinois (n = 1), and Indiana (n = 5). We used a white-light confocal profiler to generate 3D reconstructions of lower molar buccal lophs (usually loph 2). Data collection commenced at 100x and covered an area of approximately 242 X 182 microns². We leveled and 'cleaned' the data using SolarMap®; cleaning involved removing debris from the data so that they are not included in the surface calculations. Data analysis employed Toothfrax® and Sfrax® scale-sensitive fractal analysis software, which computed three texture variables: complexity (surface undulations), anisotropy (similarity of feature orientation), and textural fill volume (the amount of surface removed). Animals with hard diets tend to have high complexity and textural fill volume values. Again, we compared animals dating to around 15,000 years ago (n=7) to individuals older than about 20,000 years (n = 3). Qualitative results indicate the diet of the more recent animals was harder and less fibrous (having greater complexity and lower anisotropy). However, ANOVA found no differences. We suspect our small sample size affected the quantitative findings. We feel, therefore, that it is worthwhile to continue this avenue of study because the data suggest mastodon diets may have fluctuated just prior to their extinction.

Sourcing Ceramics, Clays, and the Movement of People through Ceramic Compositional Data from 11th Century Illinois, Indiana, and Kentucky (Poster)

Rebecca M. Barzilai, Indiana University

A constructed Mississippian mound center where day-to-day activities were interwoven with the performance of religious experiences tied to the location and activities enacted there, the Emerald Site in Lebanon, Illinois has a high density of non-domestic structures dating primarily to the Edelhardt and Lohmann Phases (1000-1100 CE) and ceramic types signaling the presence of diverse peoples from throughout the Midwest. Associated with the rise of the Native American City of Cahokia circa 1050 CE, the ceramic styles found at the Emerald Site indicate peoples from throughout the Midcontinent of North America traveling to the location to participate in ritual and religious activities. This poster presents data from recent petrographic and geochemical testing of ceramics from the Emerald Site and descriptions of clay and compositional groups of source clays. Additionally, building on previous research characterizing Mississippian and Late Woodland ceramics in the Lower Ohio River Valley, this poster presents data from a baseline study of the movement of peoples and objects throughout the Midcontinent around 1000 CE. Funded by a Summer Research Grant from the Glenn A. Black Laboratory of Archaeology and an Indiana Academy of Science Senior Research Grant, Yankeetown Phase, Late Woodland, and Mississippian ceramics were compositionally characterized and compared to compositional data of clays from Southwest Indiana, Illinois, and Missouri.

The Detection of Nicotine in Skeletal Remains Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry: A Feasibility Study (Poster)

Savannah Leach Newell, Indiana University

Tobacco played an important role in the lives of prehistoric North American populations. Ethnographic and archaeological evidence indicate its usage in Shamanism (due to its hallucinogenic properties), in medicine (due to its analgesic properties), and as a daily habit for pleasure (not far off from modern use). Pipes in burial contexts and analyses of pipe dottels have been used previously to determine smoking practices. This project aimed to look at the feasibility of using liquid chromatography/tandem mass spectrometry to detect nicotine and

its primary metabolite, cotinine, in skeletal remains as more direct evidence of tobacco consumption in the past. Both nicotine and cotinine were successfully extracted from the teeth of a modern smoker. Based on the presence of cotinine, it can be shown the nicotine was processed during life and not via contamination. However, based on amount recovered in internal standards, material was lost in the process and further analyses are required before archaeological remains can reliably be tested.

Botany

***Asimina triloba* patch density and reproduction in four urban forest fragments**

Aaron J. Rudolph, Vamsi J. Nalam, and Jordan M. Marshall, Indiana University-Purdue University Fort Wayne

Asimina triloba (L.) Dunal (pawpaw) is a common moist hardwood forest tree species found throughout the eastern United States. Clonal reproduction in *A. triloba* contributes to patchy distribution within forests. However, fruit production is also common with flowers pollinated by carrion beetles and flies. The objectives of this study were to describe the habitat characteristics where patches of *A. triloba* occurred within four forest fragments and to quantify the reproductive strategies within these patches. Forest fragments were selected in Allen County, Indiana. Forest and environmental characteristics were recorded through overstory surveys and measurements of soil compaction, soil moisture, and canopy cover. Within each *A. triloba* patch, height and diameter were measured for each individual, as well as flower number and subsequent fruit numbers. Additionally, a subset of *A. triloba* individuals were selected and sampled for dendrochronological analysis. A separate subset of *A. triloba* individuals were sampled for genetic analysis via inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) to signify successful sexual reproduction and subsequently genotypic diversity. The overstory surveys and environmental characteristic data aligned with previous studies regarding conditions and forest types in which *A. triloba* is typically found. Patches ranged in number from 100 to over 700 *A. triloba* individuals with patches being dominated by smaller individuals across all patches. Age structure data indicated that *A. triloba* is opportunistic in growth strategy. We found a majority of clonal indistinct genotypes with sparse instances of visibly distinct genotypes. While it is often described as shade intolerant, we observed *A. triloba* tolerated high shade by persisting in smaller size classes until canopy gaps opened at which time growth greatly increased. *A. triloba* is well equipped to persist in a fragmented environment by utilizing a growth strategy "waiting" for gap formation and both sexual and asexual reproductive strategies.

Crown Geometry of Plantation-Grown American Sycamore (*Platanus Occidentalis*) and its Simulation 2: Trunk Primary Growth.

John R. Schutt, James A. Rhodes State College, Lima, OH

A prior talk showed that a soil moisture model could be used to generate indices that could predict the length of the shoot primary growth. This talk continues the discussion of crown geometry by looking at the relationship between total extension growth and the number of nodes, the placement of nodes, and node phyllotaxy. Node density was most highly related to the length of the branch, although fertilizer, the amount of soil water, and tree genetics also had effects ($F = 843.7$, $R^2 = 0.913$.) Node position was unsuccessfully modeled by linear, quadratic, and cubic models. Relative node distance was then predicted from relative node position three distinct phases of growth were identified: increasing internode distance, almost constant node distance, and then rapidly declining node distance. Phyllotaxy analysis of the trunk nodes showed no preferred orientation azimuth. The distance between node azimuth was calculated to be either close to 140 degrees or it showed a phyllotaxy reversal with an angle near 220 degrees. Lastly, two types of proleptic branching were observed. Some trees were observed to form more Type I proleptic branches early in their lives ($\text{ChiSq} = 13.8$, $p = 0.0002$) and this trait appears to be genetically inherited. The number of Type I proleptic branches was also strongly associated with branch length ($\text{ChiSq} = 7.2$, $p = 0.0072$.) Lastly, Type I proleptic branches were found to be positioned lower on the trunk compared to Type II branches.

Screening for genes important to cold acclimatization in *Neurospora crassa*

Victor Manzanilla, Holly Howell, Alexander Mehreteab, Eric Rose, Nichole Walters, Nicholas Seitz, Jacob Nava and **Michael Watters**

The environmental conditions that life must contend with can vary widely. Organisms have evolved a wide range of mechanisms for contending with these changing conditions. For relatively simple, sessile organisms (such as fungi), they must adapt themselves to the extremes of the environment. When subjected to rapid drops of

temperature (cold shock), *Neurospora* responds with a dramatic, but temporary shift in its branching pattern. The fungal response, seems to parallel the physiological response to similar cold shock which has been observed in *E. coli* in which a set of genes is activated transiently in order to adjust the cell for growth in the cold. While the *Neurospora* response has been described morphologically, it has yet to be examined to any serious extent genetically. This project aims to begin the genetic characterization of the cold shock response and the associated acclimatization to cold environments. We report here the results of two screens of mutants from the *Neurospora* knockout library for their response to cold shock. In the first, strains with knockouts in genes previously suspected to be involved in hyphal development as well as knockouts resulting in morphological changes are tested for their response to cold shock. In the second, strains with knockouts of genes homologous to *E. coli* genes known to alter their expression in response to cold shock are examined for their morphological response. Several were identified with altered responses. The genes impacted in these mutants are listed and discussed.

Using tree-ring growth patterns to date the construction of the Reverend Frederick and Sophia Wyneken house in Adams County, Indiana

Taylor Davis and Darrin L. Rubino, Hanover College, and Chris Baas, Ball State University

Tree rings are an effective way to date the construction of historic buildings. Our goal was to determine the construction date of the nineteenth century home of the German pioneer religious leader, F. C. D. Wyneken. Using standard dendroarchaeology methods, we collected a total of 59 samples from 5 timber species. The samples were sanded so individual rings could be identified. The rings were then measured to the nearest 0.01 mm at 45X magnification. Through the use of crossdating, a method of comparing tree-ring growth patterns in timber of known age to those of an unknown age, each ring was assigned a calendar date. We successfully dated 12 white oak, 36 ash, 4 tulip poplar, 5 red oak, and 2 American elm samples. We were able to produce a chronology, a series of accurately measured and dated tree rings, spanning from 1663 to 1857. We hypothesized that this side-hall house was built after the 1858 growing season as the samples indicated a death date (presence of bark or wane) of 1858. Our hypothesized date of construction agrees with the historical records of Wyneken's service to the Lutheran Church and movement throughout the country. The data collected from this project was a valuable addition to our tree ring database because we were able to establish chronologies in northeastern Indiana, an understudied geographical area in the state.

Cell Biology Section

Investigation of the Cold-Regulated promoter RD29A in *Arabidopsis thaliana* and *Glycine max*.

Adib Behrouzi, Jennifer Robison, Stephen Randall, Indiana University-Purdue University of Indianapolis

The inability of plants to tolerate extreme environmental conditions is a concern for the agricultural industry. Plants around the world are often exposed to cold, drought, and high salt concentrations that can be damaging, causing significant losses in yield, resulting in significant economic losses to the producers. Previous work shows that the *Arabidopsis thaliana* promoter RD29A is responsive to a variety of abiotic stresses, and the RD29A gene is involved in cold-stress adaptation. An RD29A reporter construct (AtRD29A::GUS/GFP) was constructed and transgenically introduced into *Arabidopsis thaliana* (At) and *Glycine max* (Gm). Homozygous AtRD29A::GUS/GFP transgenic lines (four *Arabidopsis thaliana* and three soybean) have been identified. Preliminary examination of *Arabidopsis* seedlings showed GUS activity in trichomes, roots, and leaf tips. Tissue and organ expression and cold responsiveness of RD29A::GUS/GFP in a cold-tolerant plant (*Arabidopsis thaliana*) was compared the cold-intolerant soybean plant (*Glycine max*). To evaluate cold regulation, a single heterozygous line was examined quantitatively using GUS assay and the substrate 4-methyl-umbelliferyl- β -D-glucuronide (MUG) after 2 day cold (4°C) or control (22°C) conditions. The GUS assay showed a strong increase in cold-driven RD29A expression of GUS, supporting that RD29A is cold-regulated. To confirm these results, examination will be done of the other transgenic lines (homozygous AtRD29A::GUS/GFP *Arabidopsis thaliana*) in cold and control conditions. Studying this promoter may help the field to determine how cold-tolerance occurs in plants.

Cell Biology Section continued.....

A Robust, High Throughput Behavioral Assay for Novel Therapeutic Compounds in Zebrafish Retinitis Pigmentosa Model (Poster)

Khaled Noui-Mehidi, Logan Ganzen, Yuk Fai Leung, Purdue University

Retinitis pigmentosa (RP) is a family of retinal degenerative diseases affecting 1 in 4000 people globally. Histologically, RP manifests primarily as rod photoreceptor cell death, and the subsequent induction of cell death in the neighboring cone photoreceptors. Clinically, this presents as night blindness, progressive loss of peripheral vision, and eventually complete vision loss. There are currently no effective treatment options available.

The Q344X rhodopsin mutation was identified as a genetic precursor for autosomal dominant retinitis pigmentosa and isolated from a human patient. The Q344X nonsense mutation truncates the rhodopsin polypeptide immediately upstream from the VXPX ciliary trafficking motif. Mutant rhodopsin accumulates within the inner segment where it catalyzes a mislocalized phototransduction cascade. This cascade ultimately results in apoptosis.

In this research, we characterize the behavior of a transgenic zebrafish line expressing the Q344X rhodopsin mutation using an optimized visual motor response (VMR) assay. The VMR is a startle behavior elicited by a sudden light stimulus. We have optimized a low light assay that distinguishes between the VMR behavior of Q344X rhodopsin mutants and wild type controls. Our VMR characterization associates a behavioral phenotype with rod degeneration in affected retinæ, and the distinction serves as a benchmark with which to appraise the therapeutic potential of drug treatments.

We have screened the ENZO Redox drug library, a collection of 84 compounds known to modulate oxidative pathways. We selected this particular library in accordance with the hypothesis that oxidative stress is a mechanism of photoreceptor cell death in RP. From this library, we have successfully identified compounds that reconcile the behavioral phenotype of Q344X zebrafish with wild type control, and therefore present therapeutic potential.

An RNA interference screen to identify cardiogenic genes regulated by Forkhead domain transcription factors (Poster)

Leah E. Carter, Kennedy A. Camden, Harjas Singh, John M. Dalloul, Linda D. Garza, Takoda M. Sons, Andrew J. Kump, Manoj Panta, Srivathsan V. Raghavan, and Shaad M. Ahmad, Indiana State University

While mutations in four genes that encode (Fkh/Fox) domain transcription factors (TFs) give rise to human congenital heart defects, and at least eight Fkh TFs are required for proper cardiac development in mammals, relatively little is known about the molecular mechanisms or the downstream targets by which these Fkh-mediated developmental functions are brought about. Given the remarkable conservation of genes and molecular processes in heart development between mammals and *Drosophila*, we are building on our initial discoveries on the roles of Fkh TFs in *Drosophila* cardiogenesis to remedy this gap in our understanding. Our laboratory had previously identified the cardiogenic roles of two *Drosophila* Fkh genes, *jumeau* (*jumu*) and *Checkpoint suppressor homologue* (*CHES-1-like*). Mutations in these genes result in numerous heart defects, including disruptive changes in cardiac cell types and numbers, errors in cardiac progenitor cell specification and division, and defects in cardiac cell positioning. Collectively, these two *Drosophila* Fkh TFs integrate diverse cardiogenic processes by regulating a large number of downstream target genes, thus raising the question of what these target genes are, and what their individual functions might be during heart development. By comparing the transcription expression profiles of flow cytometry-sorted mesodermal cells from wild-type embryos and *jumu* loss-of-function, *CHES-1-like* loss-of-function, and *jumu* and *CHES-1-like* dual loss-of-function mutants, we have identified a large number of Fkh-regulated genes. We are presently assessing which of these downstream Fkh targets play a critical role in heart development by examining the effect of knocking down their gene function by RNA interference: cardiogenic Fkh targets exhibit reproducible and significant heart malformation defects in the *Drosophila* embryo. Subsequently, detailed phenotypic analysis at

the tissue, cellular and subcellular levels, combined with epistasis, genetic interaction and rescue experiments will be utilized to elucidate the pathways and molecular mechanisms by which these cardiogenic genes function.

Cell Biology Section continued.....

Antioxidant Protein Expression in Glutathione-Deficient *Saccharomyces cerevisiae* is Altered by Cysteamine Treatment

Sarah Labra, Anne Rodgers, Allison Hendershot, Nichole Thompson-Van Hook, Thaddeus Harmon, Corey Mills, Sarah Mordan-McCombs, Franklin College

Cellular production of reactive oxygen species (ROS) occurs naturally as a result of oxidative phosphorylation during mitochondrial production of ATP. ROS, if left in their active form, can attack cellular proteins, lipids, and nucleic acids, causing premature aging and apoptosis. Properly functioning cells produce multiple antioxidant enzyme pathways to neutralize ROS, including superoxide dismutases, thioredoxins, catalase, and glutathione-glutaredoxins. Loss of function in antioxidant pathways is hypothesized to be a precursor to neurodegenerative diseases such as Parkinson's and Alzheimer's Disease. Previous research in our lab indicated that *Saccharomyces cerevisiae* which are null for some members of the glutathione-glutaredoxin system show decreased survival in the presence of hydrogen peroxide; however, pre-incubation with the thiol cysteamine rescued viability upon exposure to the same concentrations of hydrogen peroxide. We hypothesized that cysteamine may function as a glutathione substitute in these cells, due to the presence of a thiol group in the chemical structure. To test this hypothesis, cells lacking gamma-glutamyl synthetase, GSH1, which catalyzes the first step in glutathione synthesis, were treated with cysteamine and exposed to hydrogen peroxide at a 1 mM concentration. Expression levels of glutathione-glutaredoxin system proteins were assessed after 60 minutes of peroxide exposure to determine if cysteamine treatment could restore wild-type protein expression concurrent with the observed increase in survival. We found that deletion of GSH1 greatly altered protein expression of glutathione-glutaredoxin system proteins, but that addition of cysteamine was able to partially restore normal expression when cells were treated with hydrogen peroxide.

Cardiogenic role of *no ocelli*, a gene encoding a Zinc finger transcription factor (Poster)

Manoj Panta, Andrew J. Kump, Srivathsan V. Raghavan, Harjas Singh, Kennedy A. Camden, Leah E. Carter, John M. Dalloul and Shaad M. Ahmad, Indiana State University

The identification of regulatory genes and an understanding of the molecular mechanisms they mediate during organogenesis can aid the development of stem cell therapies and synthesized organs for the treatment of genetic as well as acquired diseases. *Drosophila* serves as an excellent model organism for this initial analysis of regulatory pathways because of (i) its amenability to genetic and genomic dissection, and (ii) the remarkable conservation of genes and regulatory pathways between it and vertebrates, including humans. In a preliminary screen to identify novel cardiogenic regulators for a detailed investigation of molecular pathways involved in heart development, we discovered the transcription factor-encoding gene *no ocelli*. Mutations in the *no ocelli* gene lead to cardiac defects. The normal *Drosophila* heart is composed of 108 contractile cardiac cells arranged in repeated units known as hemisegments. However, in mutant embryos where the *no ocelli* gene function is disrupted, multiple hemisegments are frequently missing. *A priori*, the absence of entire hemisegments suggests that *no ocelli* is required during an earlier step in cardiogenesis by regulating at least one of these three pathways: (i) proper migration of the cells that will become specified as cardiac progenitors in response to particular position-specific signals, (ii) the signal transduction process itself that mediates this specification of the cardiac progenitors, or (iii) the subsequent differentiation of the cardiac progenitors into the cardiac cells of the hemisegments. Our results to date indicate that *no ocelli* does not play a role in the third pathway, the specific differentiation into cardiac cells. Further analyses of the other two potential pathways as well as detailed investigations of the cardiogenic role of *no ocelli* is ongoing.

Cathepsin K Gene Expression Measurement in Equine Chondrocytes (Poster)

Christian Cuevas, Luke Hayden, Seth Baker, and Dan Jones

Osteoarthritis (OA) is a common “wear-and-tear” disease characterized by thinned cartilage, fragmentation, and bone spurs. In humans, OA affects 33.6% of those over 65—an estimated 26.9 million U.S. adults (2005 figures). One model for study of human OA is the joint of the performance horse. It is estimated that equine OA affects 60% of horses. One of the active genes in OA is cathepsin K (CTSK), an active degrader of the cartilage matrix. The purpose of this study was to establish an experimental in vitro equine chondrocyte (EC) model for characterizing CTSK gene expression changes in response to various factors and correlating those changes with CTSK promoter epigenetics. ECs purchased from Ohio State University College of Veterinary Medicine were successfully propagated. Genomic DNA, RNA, and proteins were isolated from untreated and IL-1-treated cells with IL-1B treatment to mimic cytokine conditions in OA. One percent agarose gel electrophoresis and A260/A280 absorbance analysis confirmed DNA and RNA yield and purity. Non-isotopic Northern blot analysis to detect CTSK message from untreated and IL-1B-treated ECs was not conclusive. Western blot detection of recombinant CTSK was successful but EC samples failed to yield signal. ECs were expected to respond to IL-1B by decreasing soluble glycosaminoglycan (sGAG) formation; however, the treated monolayer cells did not show a significant change in sGAG levels, indicating that the model did not reflect the known physiological effect. Potentially the cultured ECs had dedifferentiated. Therefore future experiments with equine ECs will require an earlier passage monolayer or 3D pellet culture.

Cell-instructive Polymer/Bioactive Glass Composite-Based Scaffolds for Bone Regeneration (Poster)
Helena Lysandrou, Jessica Zuponcic, Meng Deng, Purdue University

Every year in the United States, over 500,000 patients need bone defect repairs, which include bone-grafting procedures. Current bone grafts involve autografts and allografts which are constrained by limited supplies and donor site morbidity. Scaffold-based bone tissue engineering has emerged as a prospective strategy for bone regeneration to overcome these limitations by using biomaterials, cells, and growth factors such as bone morphogenetic proteins (BMPs). However, the clinical utility of BMPs is hindered by supraphysiological dose requirements and ectopic ossification. Therefore, there exists a great need for the development of novel biomaterials-based therapies to promote bone healing without high doses of BMPs. Our previous work has demonstrated that calcium and phosphate ions exhibit osteoinductive properties causing stem cell BMP production. In this study, a three-dimensional (3D) porous scaffold system was sintered from composite microspheres containing biodegradable poly(lactide-co-glycolide) (PLGA) and an ion-releasing novel bioactive silicate glass (BSG). Such a composite combines both the benefits of PLGA mechanical properties with the bone-bonding bioactivity of BSG. The composite scaffolds were optimized to mimic structural and mechanical properties of natural bone and evaluated for bioactivity and cellular responses of human mesenchymal stem cells (hMSCs). Mechanical testing revealed that the optimized composite scaffolds had a compressive elastic modulus in the range of trabecular bone. Cell attachment and viability on the composite scaffolds were validated using confocal microscopy. During a 3-week cell culture, the composite scaffolds significantly promoted osteogenic differentiation of hMSCs leading to enhanced mineralization as compared to control PLGA scaffolds, which further confirmed the osteoinductive potential of the signaling ions provided by the BSG. By demonstrating osteoinductive properties and bioactivity, our composite scaffolds are a step closer towards potential replacements for current bone grafts.

Cells Lacking Quality Control Mechanisms Exhibit Improved Growth in Presence of Proposed Anti-cancer Drug (Poster)

Rachel M Vachon, David A Huston, Eric M Rubenstein, Ball State University

Just as protein synthesis is vital to life and cellular health, so is the destruction of proteins. Eukaryotic cells have quality control mechanisms in place to detect and destroy aberrant proteins before they cause harm. In some cases, overzealous destruction of aberrant but partially functional proteins may worsen disease systems. *Saccharomyces cerevisiae*, commonly known as a baker's yeast, is an ideal model organism to study protein quality control. The entire genome of yeast has been sequenced and is easily manipulated, yeast has a short doubling time, and many cellular processes in yeast are highly conserved with those in human beings. Experiments have been performed to characterize the protein quality control mechanisms that are responsible for the degradation of aberrant proteins formed in the presence of a proposed anti-cancer medication in *S. cerevisiae*. Serial dilutions of yeast strains, each with mutations in one or more different quality control proteins, were pipetted on agar media lacking or containing the proposed anti-cancer drug. Yeast were incubated at 30°C, and growth was monitored and recorded by photographic imaging for three days. Surprisingly, yeast strains lacking a specific quality control mechanism grew better than wild type cells in the presence of the drug, suggesting that the drug may result in the production of one or more aberrant proteins that retain some function,

but are degraded when the quality control mechanism is present. Ongoing investigation of the quality control mechanism that functions in the cellular response to the anticancer drug may enhance the understanding of cancer biology and human therapies.

Cell Biology Section continued.....

Characterization of *Apoptosis* and *Necrosis* Induced by *Reveromycin A*, a Potential Bone Anti-Resorptive Agent, in Normal Cells of the Joint (Poster)

Patricia Martinez, Austin Greer, Haley Svrčina, Nathan Granger, and Dan Jones, Indiana Wesleyan University

Previous in vitro studies in our laboratory demonstrated that reveromycin A (Rev A), a natural compound, induced apoptosis in osteoclasts at an osteoclast-relevant pH of 5.5 through the intrinsic pathway without accompanying necrosis. In vitro and in vivo work by Woo et al. revealed that Rev A does not induce apoptosis in the bone-forming osteoblast, indicating that this antibiotic may have clinical potential as a bone anti-resorptive. The current study was conducted to discover whether Rev A induces any death effects in normal cells of the joint. Normal murine chondrocytes and rabbit synoviocytes were each treated with 10 μ M Rev A, the dosage used in our published osteoclast study, at pH 7.4 for 6 hours. No significant apoptosis as measured by caspase 3 activity in cytoplasmic extracts nor necrosis measured by LDH release occurred. We further investigated these effects at an acidic pH of 6.8 to reflect inflamed joint tissue pH conditions and over an extended time course at different doses (0.1, 1.0, and 10 μ M). 10 μ M Rev A-treated normal chondrocytes showed no apparent apoptotic nor necrotic changes. In a similar fashion, 0.1 μ M and 1.0 μ M Rev A-treated normal synoviocytes demonstrated no apparent apoptotic nor necrotic changes compared to untreated controls. However, 10 μ M Rev A-treated normal synoviocytes showed a four-fold increase in apoptosis as compared to the untreated control at 48 hours ($p < 0.005$). These results suggest that physiologically relevant Rev A doses do not cause significant death in chondrocytes but do appear to negatively affect synoviocytes.

Chemotherapeutic Resistant Breast Cancer Cell Lines (Poster)

Keeley Williams, Megan Osmon, Marcus Voges, Ashley Lynch, and **Catherine E. Steding**, Indiana State University

Approximately 40,000 American women succumb to metastatic breast cancer on a yearly basis. These staggering numbers make it a leading cause of cancer related death. Treatment for breast cancer traditionally involves the use of a combination of surgery, radiation, or chemotherapy (anti-cancer drugs designed to kill rapidly dividing cells such as cancer). Although effective therapeutics exist including the highly effective microtubule stabilizing drug, paclitaxel, cancer cells can express an innate resistance to the therapeutic or even acquire resistance during treatment. Resistance to a chemotherapeutic drug means that the cancer cells can still grow in the presence of the drug rendering the treatment ineffective. Acquired resistance has been observed in nearly every single chemotherapeutic agent utilized. Previous work in the laboratory emphasized the generation of resistant cell lines in an effort to better understand the changes cells undergo during the process of acquiring resistance. Findings of the initial studies demonstrated that the development of resistance in vitro can be obtained under controlled conditions over time. Cells were found to exhibit clinically relevant levels of resistance. The goals of this project are to further explore the mechanisms of acquired resistance to paclitaxel. Additionally, this project seeks to ascertain whether there are key differences in gene expression associated with generation of resistance versus maintenance of that resistance. Preliminary analysis of both RNA and protein was completed in an effort to link gene expression with functional consequences in the cells. Initial findings indicate that there are fundamental changes in gene expression associated with acquired resistance. It is probable that the differences in gene expression correlate with the changes in cellular behavior observed throughout the generation of the cells. Further exploration of the changes in the cells over time will lead to additional insights into the complicated system of acquired resistance.

Cold Response Biomarker Identification in Diploid Strawberry

Zach Deitch and S. Randall, Indiana University Purdue University Indianapolis, N. Osuagwu and R. Wilson, Hedmark University College, Hamar, Norway, I. Fattash, , American University of Madaba, Madaba, Jordan , M. Alsheikh, Graminor Breeding Ltd., Ridabu, Norway, and Norwegian University of Life Sciences, Ås, Norway

Strawberry (*Fragaria* spp.) has high variability in its susceptibility to freezing injury, which is of great concern for agriculture in temperate regions. In order to breed cultivars for frost and freezing tolerance, identification of molecular markers associated with low temperature tolerance is advantageous. Previous work identified seven putative dehydrins in *Fragaria* and demonstrated that cold tolerance positively correlated with dehydrin protein expression levels. In an effort to understand the cold-regulated expression of dehydrins as a function of cold exposure time, two dehydrins were purified and positively identified by mass spectroscopy and determined to be COR47-like (SKn) and XERO2-like (YnSKn). Utilizing RT-qPCR and western blots, the levels of RNA and proteins corresponding to the two dehydrins were examined in strongly cold tolerant (ALTA) and lesser cold tolerant (FDP817, NCGR1363) *Fragaria* cultivars. The *Fragaria vesca* genotypes/accessions (ALTA, FDP817, and NCGR1363) were cold acclimated at 2 degrees Celsius and triplicate samples of crown and leaf tissue were taken at 10 different time points over 42 days. The COR47-like (SKn) and XERO2-like (YnSKn) dehydrins both had higher transcript accumulation in the more cold tolerant line with dehydrin protein levels mimicking that response. It is concluded that dehydrin transcripts are potential biomarkers for identifying low temperature tolerance in diploid strawberry. The specificity of individual dehydrin contribution to cold tolerance must still be determined.

Deciphering the dynamics of alternative pre-mRNA processing of glutaminase in ovarian cancer (Poster)
Patrick LaFontaine, Bettine Gibbs and Chioniso Patience Masamha. Butler University

Ovarian cancer has the highest mortality rate of all gynecologic malignancies and is the fifth leading cause of cancer death among women in the United States. Ovarian cancer is often diagnosed at an advanced stage where metastatic disease is present and leads to an overall poor prognosis. Currently there is a need for adequate reliable tumor specific biomarkers for early disease detection for ovarian cancer. An emerging hallmark of cancer is the metabolic reprogramming of transformed cells to support the energy and biosynthetic needs of the rapidly dividing and metastatic tumor phenotype. Included in these changes are increased glucose and glutamine uptake and incorporation into metabolic pathways. Recent studies suggest that highly invasive ovarian cancer cells show a remarkable dependence on glutamine, making glutamine metabolism a potential therapeutic target in ovarian cancer. There are several clinical trials focused on inhibiting glutaminase, the enzyme that catalyzes the first step of glutaminolysis and converts glutamine to glutamate. Two genes code for glutaminase in the human genome, glutaminase 1 (GLS1) is located on chromosome 2 and glutaminase 2 (GLS2) is located on chromosome 12. GLS1 is the enzyme that is most associated with tumor metabolism, and has two documented isoforms of GLS1, GAC and KGA, which result from alternative splicing. Different tumors express either or both isoforms. Under normal cellular conditions, the production of the KGA isoform is repressed by miR-23 through binding to the long 3' UTR of KGA, silencing the expression. We recently found that depletion of a 3' end processing factor, CFIm25, results in alternative polyadenylation, and the formation of a novel truncated 3' UTR isoform which can evade miR-23 regulation. Additionally, the depletion of CFIm25 also results in a preferential splicing into KGA instead of the GAC isoform. The objective of this study is to determine the different glutaminase isoforms in metastatic ovarian cancer cells and how they are regulated for therapeutic targeting using isoform specific siRNAs. We have developed isoform specific amplicons which can measure GAC, along with long and short form KGA. We show that highly metastatic ovarian cancer cell lines express high levels of the GAC isoform through qRT-PCR. The cisplatin resistant cells line SKOV3 also expresses KGA in addition to GAC, which has been detected at both the mRNA and protein level. The use of GLS1 inhibitors resulted in decreased cell viability of these highly metastatic ovarian cancer cell lines. Based on our previous findings, we hypothesize that expressing both KGA and GAC isoforms facilitates the chemotherapeutic drug resistance of highly metastatic ovarian tumors.

Determination of the roles of two *spalt* genes in cardiogenesis (Poster)

John M. Dalloul, Kennedy A. Camden, Leah E. Carter, Linda D. Garza, Paaras Kumar, Harjas Singh, Takoda M. Sons, and Shaad M. Ahmad, Indiana State University

Heart diseases, many of which can be traced back to congenital heart defects, affect a major portion of American society. Detailed examinations of the genetic mechanisms involved in forming a normal, functioning heart may lead to novel therapies for heart diseases. *Drosophila melanogaster* is an excellent model organism for such studies: the remarkable conservation of genes and regulatory pathways between mammals

and *Drosophila* implies that cardiogenic genes identified in *Drosophila* are likely to have human orthologs with analogous functions. A gene that we have begun studying is *spalt major (salm)*, which encodes a Zinc finger transcription factor. In our initial assays of *Drosophila* lacking all *salm* function, we found two distinct and significant cardiac phenotypes: (i) one in which cardiac hemisegments, i.e. repeated subunits of the heart, were missing, and (ii) the other in which there were erroneous numbers of a specific type of heart cell – the cardinal cell – within a hemisegment. We currently have several hypotheses to explain the phenotypes observed in our *salm* mutants. For example, *salm* could be involved in the specification of the cardiac progenitors, as errors in this process could lead to the missing hemisegments that we observed. *salm* could also be playing a role in the cell division of these cardiac progenitors; errors in this process could result in the incorrect numbers of cardinal cells we detected. Intriguingly, even more severe cardiac phenotypes were detected with another *salm* mutation. Another closely related Zinc finger transcription factor encoding gene, *spalt related (salr)*, that provides some redundant function and arose by gene duplication, lies adjacent to *salm* in the genome, raising the possibility that this second mutation disrupts the function of both *spalt* genes. We are presently testing this hypothesis, and are attempting to further analyze the cardiogenic functions of both *spalt* genes.

Effect of inflammatory cytokines and high fat diet on inositol-1,4,5-trisphosphate (IP3) receptors binding protein released with IP3 (IRBIT) expression in intestinal cells (Poster)

Natasa Petreska, **Kelli Cook**, Kenneth Bridgmon, Chase Jones, Jesse Smallwood and Patrice G Bouyer, Valparaiso University.

IP3 upon binding to the IP3 receptor (IP3R) causes the release of intracellular calcium from the endoplasmic reticulum, which drives many cellular responses (e.g., cell spreading, exocytosis). In addition to releasing calcium, IP3 also causes the release of IRBIT from the IP3R. Over the past decade IRBIT has been described as a protein that regulates calcium release due to interaction with the IP3R, the activity of the Na-HCO₃ cotransporter, the cystic fibrosis transmembrane regulator and the Na/H exchanger (NHE3). Lack of reabsorption of Na⁺ by NHE3 in the intestine is responsible for diarrhea. Recently it was shown that IRBIT and NHE3 expression was decreased in a mouse model of diabetes and the loss of NHE3 expression induced diarrhea in this model. Insulin treatment restored IRBIT and NHE3 expression, resulting in a decrease of diarrhea. Besides insulin, very little is known about factors regulating IRBIT expression in intestinal epithelial cells. In this work, we set to study the effect of inflammatory cytokines and high calorie diet on IRBIT expression due to the fact that diabetes is associated with chronic inflammation and high caloric intake. To test the effect of inflammatory cytokines we used the human colonic crypt cells T84. Exposing T84 cells to interleukin 13 or tumor necrosis factor alpha for 72 hours decreased IRBIT expression by 36% (P < 0.001, n = 5), 44% (p < 0.001, n = 3) respectively. finally, we compared the expression of irbit in mice fed with low fat milk (control) versus high milk fat (37%). we found that in the duodenum of 3 mice with a high fat diet a substantial increase of irbit expression compared to the control. our work is the first to demonstrate that inflammatory cytokines and dietary fat can alter irbit expression.

Effects of EGCG Treatment on Bone Deficits in Ts65Dn Down Syndrome Mice (Poster)

Raza Jamal, Roshni Patel, Amanda Yacoub, Jonathan LaCombe, Randall Roper

The triplication of chromosome 21 in Down syndrome (DS) causes cognitive deficits, skeletal defects, and increased chances of congenital heart disease. Trisomy 21 impacts bone structure and strength in all individuals with DS. Our previous work showed increased dosage of the trisomic gene *Dyrk1a* negatively impacts bone strength. Epigallocatechin-3-gallate (EGCG) has been shown *in vitro* to reduce Dyrk1a kinase activity. In previous studies, a 10 mg/kg/day dose of EGCG rescued bone mineral density (BMD) and some of the trabecular bone deficits associated with DS in trisomic mouse model. We hypothesized that a higher dose of EGCG would have additional benefits on trisomic cortical bone. In this study, 200 mg/kg/day EGCG was given via oral gavage for 3 weeks to 6-week-old Ts65Dn DS and control mice. Femurs were extracted and analyzed using MicroCT to quantify BMD, trabecular and cortical bone parameters. Our data indicate that a higher dose of EGCG does not rescue the bone phenotypes associated with DS. Our data also show that euploid and Ts65Dn mice treated with EGCG have lower BMD and cortical values than control mice. A high dose of EGCG, therefore, may be detrimental to skeletal parameters in both trisomic and normal mice. We will use an HPLC-based kinase activity assay to measure Dyrk1a protein kinase activity, and find if changes in skeletal parameters correlate with the levels of Dyrk1a activity.

Effects of Endoplasmic Reticulum Stress on Protein Quality Control (Poster)

Bryce Buchanan, Eric (VJ) Rubenstein, Ball State University

Protein quality control is critical for cellular health. The Endoplasmic Reticulum (ER)-Associated Degradation (ERAD) protein quality control system degrades aberrant proteins at the ER membrane. ERAD can be divided into multiple pathways, including ERAD-L (lumen), -M (membrane), -T (translocon), -C (cytosol), and -RA (ribosome associated). In general, each ERAD pathway degrades proteins with aberrations in specific positions relative to the ER membrane. In ERAD-L and -M, the ubiquitin ligase Hrd1 recognizes proteins with aberrations in ER luminal or transmembrane segments. Hrd1 also functions in ERAD-T, in which proteins that persistently or aberrantly associate with the translocon are targeted for destruction. In ERAD-C, the ubiquitin ligase Doa10 recognizes soluble or transmembrane proteins with cytosolic aberrations. In ERAD-RA, the ubiquitin ligase Rkr1/Ltn1 recognizes translationally stalled ER-targeted proteins. ER stress occurs when misfolded proteins accumulate in the ER. Multiple human diseases, including Parkinson's disease and diabetes, are characterized by elevated levels of ER stress. The extent to which ER stress contributes to pathology is not clear. In this study, we systematically evaluate the effect of ER stress on ERAD in the model organism *Saccharomyces cerevisiae* by comparing the abundance and rate of degradation of a panel of model substrate proteins. Our data suggest that the ERAD pathways are differentially affected by ER stress. A better understanding of the effects of ER stress on protein quality control pathways may contribute to an improved understanding of the consequences of ER stress on cellular physiology and facilitate the discovery of therapeutic targets for diseases characterized by increased levels of ER stress.

Elucidating internalization mechanism of the Na-K-2Cl cotransporter 1 and its fate in the endocytotic pathway during protein kinase C activation in epithelial cells (Poster)

Kenneth Bridgmon, Jesse Smallwood, Chase Jones, Natasa Petreska, Kelli Cook and Patrice G Bouyer, Valparaiso University.

Gut clearance (i.e., fluid secretion) is an important mechanism for host defense. Fluid secretion flushes luminal toxins and prevents bacterial attachment to intestinal epithelial cells, which otherwise would harm the host. In the colon, transepithelial chloride fluid secretion drives fluid secretion. The basolateral Na-K-2Cl cotransporter 1 (NKCC1) is the main protein pumping chloride inside the cell for its secretion by apical chloride channels. Previous studies have demonstrated that activation of the protein kinase C (PKC) causes a rapid internalization of NKCC1, thus decreasing chloride secretion. To date, the protein kinase C downstream targets involved in NKCC1 internalization and the fate of NKCC1 in the endocytic pathway is unknown. Using the human colonic crypt cells T84, we demonstrate that T84 cells express α -adducin and Myristoylated, Alanine-Rich C Kinase Substrate, two substrates of the PKC involved in protein internalization in other cells. In presence of phorbol 12-myristate 13-acetate (PMA), an activator of the conventional and novel PKC, we demonstrate that α -adducin is strongly phosphorylated in T84 cells. Next, we hypothesized that upon activation by PKC, α -adducin binds to NKCC1. In T84 cells subjected to PMA, we show that phospho α -adducin co-immunoprecipitates with NKCC1. Next, we used Mardin Darby Canine Kidney (MDCK) cells stably expressing eGFP-NKCC1. In this model, using immunocytochemistry we show that NKCC1 colocalizes with α -adducin at the plasma membrane during PKC activation. Finally, we tested the fate of NKCC1 in the endocytic pathway. In MDCK cells exposed to PMA, we found that NKCC1 colocalizes with LAMP1, a marker of the lysosome. In conclusion, our data suggest that α -adducin participates to NKCC1 internalization during PKC activation and NKCC1 is targeted for degradation.

Identification of novel cardiogenic regulators (Poster)

Kennedy A. Camden, Leah E. Carter, John M. Dalloul, Linda D. Garza, Harjas Singh, Takoda M. Sons, and Shaad M. Ahmad, Indiana State University

A comparison of the molecular mechanisms governing heart development in vertebrates and *Drosophila* reveals a remarkable conservation of all major regulatory components, including both elements of signaling pathways and transcription factors (TFs). Moreover, many of these conserved regulators of heart development are found to be mutated in various types of congenital heart disease. Thus, the identification of new regulatory genes involved in cardiac development, and detailed investigations of their functions in *Drosophila*, are likely to provide considerable insight into the related mechanisms controlling cardiogenesis in vertebrates, including human. With this in mind, we have used two distinct approaches to identify candidates for previously uncharacterized cardiogenic genes in *Drosophila*. First, using an integrated strategy combining genetic perturbations, genome-wide gene expression profiling of purified mesodermal cells, statistical meta-analysis, and embryological methods, we identified 70 previously uncharacterized genes specifically expressed in the *Drosophila* embryonic heart. Second, using a combination of machine learning, array-based ChIP data, and experimental analyses to computationally classify, predict and validate cell-type specific cardiac enhancers and the critical transcription

factor binding sites responsible for their activities, we putatively identified the potentially cardiogenic TFs binding to these sites. We have begun carrying out an RNA interference (RNAi)-based knockdown screen of prioritized candidate regulatory genes from both of these approaches. To date, this RNAi-based screen has identified 24 genes which exhibit cardiac defects when knocked down, demonstrating their role as cardiogenic regulators. More detailed analyses of these novel cardiogenic regulators are now being pursued.

Cell Biology Section continued.....

Identifying gene expression changes that reduce age-related bone loss

Aaron Hudnall and **Jonathan W. Lowery**, Marian University

Osteoporosis, a disease of low bone mass that results when bone resorption exceeding the rate of bone formation, places individuals at enhanced risk for fracture, disability, and death. By 2020, an estimated 1.3 million senior adults in Indiana will either have or be at high risk for developing osteoporosis. There is an urgent and unmet need for novel targets in treating osteoporosis, which requires a better understanding of the endogenous mechanisms regulating bone formation. We reported that deletion of the *Bmpr2* gene in the skeletons of mice causes substantially elevated bone mass in young adulthood due to increased rate of bone formation. Unpublished work indicates that the age-related decline in bone mass of *Bmpr2* mutant mice is reduced approximately three-fold compared to control mice due to a sustained increase in bone formation rate to at least 35 weeks of age with no alteration in bone resorption. Using research funds awarded by the Indiana Academy of Science, we performed genome-wide transcriptome profiling on mRNA isolated from the bones of 35-week-old control and *Bmpr2* mutant mice to identify changes in gene expression that accompany abnormally sustained bone formation rate. These results revealed that 696 genes are expressed at least 2-fold higher in *Bmpr2* mutant bones than control bones while 1229 genes are expressed at least 2-fold lower in *Bmpr2* mutant bones than control bones. Bioinformatic analyses were used to categorize the 1925 altered genes by pathway, known function, and transcriptional regulatory network and future studies will advance these findings through a prioritized, candidate-driven approach to identify potential targets for reducing age-related bone loss. Secondary confirmation of two such targets using quantitative RT-PCR (*Pak4* and *Frat1*) reveal striking consistency between RNA-Seq and RT-PCR: *Pak4* – elevated 280% and 200% by RNA-Seq and qRT-PCR, respectively; *Frat1* – reduced 63% and 65% by RNA-Seq and qRT-PCR, respectively.

Identifying the Genes Required for the Degradation of Translocon-Associated Proteins in *Saccharomyces cerevisiae*

Christopher J. Indovina, Sarah M. Engle, Sheldon G. Watts, Eric M. Rubenstein, Ball State University

The endoplasmic reticulum (ER) translocon is a molecular channel responsible for moving secreted and endomembrane system proteins across the ER membrane. Proteins that stall within or otherwise aberrantly engage the translocon channel may impair transport of other proteins across the membrane. Proteins that persistently or aberrantly engage the translocon are targeted for degradation via the conserved ER-resident ubiquitin ligase Hrd1. A better understanding of this system may prove useful in treating elevated cholesterol in humans as, under certain circumstances, the major protein component of low-density lipoproteins (bad cholesterol) stalls within the translocon and prevents normal function of the channel. Protein co-factors that assist Hrd1 in targeting other substrates for degradation are dispensable for Hrd1-dependent degradation of translocon-obstructing proteins. We hypothesize that Hrd1 requires the function of a distinct cohort of protein co-factors to recognize and ubiquitylate proteins that aberrantly engage the translocon. We developed a growth-based screen to identify genes in *Saccharomyces cerevisiae* that mediate the degradation of proteins that aberrantly engage the translocon. We fused the His3 enzyme, which is required for histidine biosynthesis, to a model protein that aberrantly engages with the translocon. Thus, histidine prototrophy is an indicator of impaired protein degradation. We introduced this fusion protein into each strain of the non-essential yeast gene knockout collection and screened for growth in the absence of histidine. Approximately 100 genes were identified as potential regulators of degradation of translocon-associated proteins. Pending validation, these genes represent potential therapeutic targets for cholesterol-related pathology.

Increasing the Anticancer Effects of the Chemotherapy Drug 5-Fluorouracil by adding Vitamin E δ -Tocotrienol and Simvastatin (Poster)

Colin Marsh, Kilia Liu, Qing Jiang, Purdue University

5-Fluorouracil is widely used in the treatment of colon cancer, yet the results in the past have not been very successful. Response rates for 5-FU-based chemotherapy on advanced colon cancer are only 10-15%. Recent studies suggest that nuclear factor NF- κ B likely contributes to acquired resistance of 5-fluorouracil. Colon cancer is an inflammation-induced cancer, and therefore NF- κ B is activated when there is inflammation. NF- κ B is a transcription factor, and when NF- κ B is active, it binds to DNA and transcribes genes for growth factors, anti-apoptotic signals, and angiogenic factors to repair the inflicted damage. If NF- κ B can be inhibited in inflammation-associated cancer cells, it will improve the anti-cancer effectiveness of 5-FU. Studies have shown that vitamin E forms including delta-tocotrienol (δ TE) exhibited potent anticancer activities in various types of cancer cells, and especially colon cancer cells. The underlying mechanism is not fully understood, but it is evident that δ TE inhibits NF- κ B. When cancer is present in specific cells, those cells have a higher rate of lipid synthesis due to an increase in glucose and aerobic glycolysis. Previous data has shown that Simvastatin, a lipid lowering medication, has produced anticancer effects when combined with other substances. By combining δ TE with 5-FU and Simvastatin, the goal is to decrease the relative cell viability in order to find either additive or synergistic effects between the three compounds. The model used to produce this data is a cell culture model using HT-29, Stage 3 colon cancer, cells. The relative cell viability is determined via an MTT Assay. Preliminary data suggests positive effects when δ TE, 5-FU and Simvastatin are combined, but further experimentation needs to be completed to fully determine the degree to which the relative cell viability is reduced and to determine the underlying mechanism that exists when these three compounds are combined.

Intercellular Calcium Waves in the Interommatidial cells of Live *Drosophila melanogaster* Eyes (Poster)

Saki Mihori, Henry Chang, Donald Ready

Intercellular Calcium (Ca^{2+}) waves have been observed to propagate across glial cells of vertebrate CNS, including the retina, but the mechanisms and functions of these waves are incompletely understood. Our lab recently found that intercellular Ca^{2+} waves propagate across the honeycomb-like lattice of pigment cells that isolate and optically insulate each unit eye, or ommatidium, of the compound eye. Concurrently, our lab has found that flies become more sensitive to light stress at approximately one week of age. The purpose of my study is to test the hypothesis that Ca^{2+} waves release trophic factors that support photoreceptor health, predicting that waves may be absent or weaker in older, less stress-resistant flies. LGMR>GCaMP6 flies are immobilized on coverslips and time-lapse fluorescent imaging is done using a cooled CCD camera and the speed and intensity of the Ca^{2+} waves are measured using ImageJ.

Investigation of the FSHR-1 Receptor as a Potential Substrate of the Anaphase Promoting Complex at the *C. elegans* Neuromuscular Junction (Poster)

David Emch, Kyle Cherry, Amy Godfrey and Jennifer R. Kowalski

Healthy nervous system function requires a balance of excitatory and inhibitory (E:I) neuronal signaling. This E:I balance relies upon the ubiquitin system, a process that regulates many proteins within cells through enzymatic tagging with ubiquitin polypeptides to affect their activity and abundance. Proper function of ubiquitin enzymes like the Anaphase Promoting Complex (APC), a ubiquitin ligase, and its substrates is needed for regulation of E:I balance. E:I imbalances are responsible for many neurological diseases, so a better understanding of the APC and its substrates may lead to improved treatments for diseases like Parkinson's and epilepsy. Our previous data showed the APC acts in inhibitory *C. elegans* GABA neurons to promote GABA neurotransmitter release, but APC substrates relevant for this effect are unknown. Follicle Stimulating Hormone Receptor 1 (FSHR-1) is a possible APC substrate. FSHR-1 possesses the necessary recognition sequences for APC interaction, and both *fshr-1* and *fshr-1;apc* loss of function mutants exhibit decreased muscle contraction indicating FSHR-1 acts after the APC to control signaling. I am testing the hypothesis that by generating strains of worms expressing fluorescently tagged FSHR-1 and quantitatively measuring changes in neuronal FSHR-1::GFP concentrations when the APC is rendered non-functional compared to normal worms. If FSHR-1 is a target of the APC enzyme, I expect higher FSHR-1::GFP concentrations in APC LoF worms. Future studies will assess whether the APC recognition sequence in FSHR-1 is important for regulation by the APC.

Investigation of the neuronal functions of the SUMO enzyme UBC-9 in *C. elegans*

Victoria Kreyden, Kristen M. Rush, Morgan K Harrison, Jennifer R. Kowalski, Butler University

Proper neuronal communication is essential for nervous system function. Communication occurs at specialized junctions, called synapses, where chemical neurotransmitters released from presynaptic neurons bind receptors on postsynaptic cells. Synaptic proteins are tightly regulated; misregulation occurs in neurological diseases, such as epilepsy. One pathway that regulates synaptic proteins is the SUMO (small ubiquitin-like modifier) pathway, which adds small SUMO polypeptide tags to targets; however, the molecular mechanisms by which SUMOylation affects the amount of signaling are unknown. We investigated how SUMO enzymes control synaptic transmission in the *Caenorhabditis elegans* neuromuscular junction (NMJ). This synapse employs a balance of excitatory (acetylcholine) and inhibitory (GABA) signaling from presynaptic motor neurons to control contraction of postsynaptic muscle cells. We used behavioral assays, loss-of-function techniques, and overexpression studies to examine how the SUMO conjugating enzyme UBC-9 acts in specific neuronal types to control muscle contraction at the NMJ. We found inhibition of UBC-9 in GABA neurons caused increased muscle contraction. Interestingly, overexpression of wild type UBC-9 or catalytically inactive UBC-9 [UBC-9 (C93S)] in GABA neurons also caused increased muscle contraction. This could be due to SUMO-limiting effects on UBC-9; this possibility is being investigated. Increased pre-synaptic accumulation of GFP-tagged synaptic vesicle proteins in GABA neurons of UBC-9 overexpression worms relative to wildtype worms suggests the increased muscle contraction might result from decreased GABA release. Given the similarities between *C. elegans* and mammalian nervous systems, our results may provide insight related to human neurobiology and may contribute to research on diseases with imbalances in neuronal signaling.

Lysosomes are not the Universal Garbage Disposal: Lysosome-Independent Protein Quality Control (Poster)

Brian J. Snow and Eric M. Rubenstein; Ball State University

Within the endoplasmic reticulum (ER), a protein complex known as the translocon facilitates the transportation of proteins from the cytosol into the ER membrane or lumen. The translocon acts as a pore that is monitored by a system that detects if proteins fail to properly traverse the pore. This system, called ER-Associated Degradation of Translocon-associated proteins (ERAD-T), targets proteins that stall within the translocon for degradation. One protein known to clog the human translocon is apolipoprotein B (apoB), the major protein component of low-density lipoprotein (LDL). LDL (also called "bad cholesterol") can cause many health problems, including heart disease. It is therefore important to understand this pathway and how it is regulated. Normally, apoB partially traverses the translocon. The luminal portion of apoB then associates with lipids, triggering the rest of the apoB protein to be pulled through the translocon and form an LDL particle. If lipid binding is prevented, apoB is degraded by ERAD-T in a manner that depends on the enzyme Hrd1. One method to analyze the pathway cells use to target translocon-associated proteins is the use of an artificial protein known as *Deg1*-Sec62* in the model organism *Saccharomyces cerevisiae*. *Deg1*-Sec62* imitates apoB in that it is degraded by Hrd1-dependent ERAD-T after it aberrantly engages the translocon. However, in strains containing a *HRD1* knockout, *Deg1*-Sec62* is incompletely stabilized, suggesting an alternative degradation pathway. The purpose of this work is to test the hypothesis that lysosomes contribute to the degradation of translocon-associated proteins when ERAD-T is unavailable. *Deg1*-Sec62* stability was analyzed in wild-type yeast, yeast with impaired ERAD-T, and yeast with impaired lysosomal degradation. Impaired lysosomal degradation did not stabilize *Deg1*-Sec62*, suggesting other unidentified mechanisms assist degradation of aberrant translocon-associated proteins.

Multiple cardiogenic roles of the transmembrane receptor Semaphorin 1a (Poster)

Harjas Singh, Kennedy A. Camden, Leah E. Carter, John M. Dalloul, Linda D. Garza, Takoda M. Sons, Andrew J. Kump, Manoj Panta, Srivathsan V. Raghavan, and Shaad M. Ahmad, Indiana State University

Major regulatory components of heart development, including transcription factors and elements of signaling pathways, have been shown to be conserved between vertebrates and *Drosophila melanogaster*. Additionally, mutations in many of these conserved regulators of heart development have also been implicated in various types of human congenital heart disease. Therefore, new insight into the development of the heart in vertebrates, such as humans, may be gained by identifying these cardiogenic regulatory genes in *Drosophila* and understanding the functional processes in which they are involved. With this in mind, we have begun a more detailed analysis of *Semaphorin 1a* (*Sema 1a*), a gene encoding a transmembrane receptor that was identified on the basis of exhibiting cardiac defects when its gene function was knocked down in an RNA interference (RNAi) screen. In both human and *Drosophila* embryos, the heart originates from two bilaterally symmetrical rows of mesodermal cells that migrate most distally from the point of invagination during gastrulation, become committed to a cardiac fate, and ultimately fuse to form a heart tube at the midline. Our

initial genetic analysis with null mutations indicate that *Drosophila* embryos lacking the *Sema 1a* gene exhibit defects in this heart tube closure process, suggesting that *Sema 1a* may be playing a role in the migration of cardiac cells during embryonic development. Furthermore, we detected small increases and decreases in the number of specific cardiac cell types of the heart tube in embryos lacking *Sema 1a* function, suggesting that *Sema 1a* may also be needed for proper cardiac progenitor cell division. Our ongoing experiments are attempting to elucidate the molecular role of *Sema 1a* in both of these cardiogenic processes.

Cell Biology Section continued.....

Suppression of Breast Cancer Cell Proliferation by Fisetin and Luteolin (Poster) **Tawan Beaumont, Mariah Castañon, and Kimberly Baker, University of Indianapolis**

Epidemiological studies suggest that a diet rich in plant flavonoids may prevent cancer. Fisetin and luteolin, dietary flavonoids found in a variety of fruits and vegetables, have been shown to exhibit anticancer activity *in vitro*. In this study, we evaluated the antiproliferative effects of fisetin and luteolin, individually and in combination, using MCF-7 breast cancer cells. We found that both fisetin and luteolin inhibited MCF-7 cell proliferation in a dose dependent manner. Furthermore, we found that co-administration of fisetin and luteolin led to a greater inhibition of cell proliferation. These results suggest that combinatorial treatments using fisetin and luteolin may be an effective chemotherapeutic strategy against breast cancer.

Targeting Both Aberrant Metabolism and Cell Proliferation in Cancer Therapy (Poster) **Bettine Gibbs and Chioniso Patience Masamha**

Mantle Cell Lymphoma (MCL) is an aggressive malignancy that accounts for 6-10% of non-Hodgkin lymphomas. MCL is considered clinically incurable upon relapse, rendering an unfavorable prognosis. Hence, it is imperative to identify novel therapeutic strategies that effectively target deregulated pathways that contribute to MCL. The hallmark of MCL is the constitutive overexpression of the G1-phase cell cycle regulatory oncogene cyclin D1, which is not expressed in B-lymphocytes. This results in uncontrolled cell proliferation. The cyclin D1 driven proliferative phenotype is associated with poor survival in MCL patients. Uncontrolled cell proliferation results in metabolic reprogramming to support the energy and biosynthetic needs of the rapidly dividing cells. These metabolic changes include altered expression of genes involved in metabolism and result in increased glucose and glutamine uptake in tumor cells. We hypothesize that targeting both hyper-proliferation and altered cell metabolism would be more efficacious in inducing cancer cell death. Our Western blot analysis shows that MCL cells overexpress both cyclin D1 and glutaminase, the enzyme that is critical for glutamine metabolism. Treatment targeting cell proliferation alone, or metabolism alone, decreased the cell-viability of MCL cells; however, greater cytotoxic activity was observed with dual treatment. Our preliminary data suggests concurrent targeting of both metabolism and cell proliferation in MCL cells has a synergistic effect that enhances the induction of cancer cell death.

TCGA-Based Approaches to the Development of Predictive Biomarkers in False-Negative HNSCC Metastasis (Poster)

Aidan A. Washer, Rachelle S. Smith, Paige A. Swan, and Colleen L. Doci, Marian University

In head and neck squamous cell carcinoma (HNSCC), regional or distant metastatic disease is highly correlated with poor prognosis, limited therapeutic efficacy, and high mortality. Early diagnosis and cancer screenings have proven effective in many cancer types, but current diagnostic approaches for HNSCC rely on highly invasive surgery or unreliable scanning methods, leading to a nearly 25% false-negative rate of diagnosis. Therefore, it is imperative to identify novel diagnostic approaches that improve reliability and decrease reliance on risky, costly, and often debilitating elective surgery. The lack of representative models has hindered such an approach in the past. Mouse models rarely spontaneously metastasize in the oral compartment and individual cell lines fail to capture the spectrum of cancer phenotypes that exist in the clinic. However, the emergence of The Cancer Genome Atlas (TCGA) database has made it possible to use bioinformatics as a means of identifying potential markers in the primary tumor that can serve as a reliable diagnostic indicator of metastatic disease. We have focused specifically on the issue of false-negative diagnosis and analyzed the genomic, pathologic, and clinical

information available for 246 individuals that were initially diagnosed as free from locoregional disease then upon further histologic examination were either shown to harbor only primary disease (N0-, N=187) or harbored occult metastases (N0+, N=59). This correlated to a false-diagnosis rate of 24%, consistent with prior literature. We then examined a variety of other criteria including age, survival, social behavior, perineural and angiolymphatic invasion to better predict differences between these two groups. Finally, we analyzed the genes differentially expressed between these two cohorts and utilized publicly available databases to assess commonalities in these genes based on transcriptional regulation, gene ontology, and signaling pathways. Overall, we believe this information can be applied to the design of rational hypotheses to test in vitro models of HNSCC cancer metastasis and anticipate that these results may identify novel biomarkers that could contribute to the improvement of diagnostic modalities in the clinic.

Cell Biology Section continued.....

The *Drosophila* zinc finger transcription factor Castor mediates two distinct cardiogenic processes in heart development (Poster)

Andrew J. Kump, Manoj Panta, Srivathsan V. Raghavan, Leah E. Carter, Kennedy A. Camden, Harjas Singh, John M. Dalloul, Linda D. Garza, Takoda M. Sons, and Shaad M. Ahmad, Indiana State University

There is a remarkable conservation among regulators, particularly transcription factors, that mediate heart development in vertebrates and *Drosophila melanogaster*. Furthermore, many of these conserved regulators of heart development are also found to be mutated in various types of human congenital heart disease. Therefore, the identification of regulatory genes involved in cardiac development, and detailed investigations of their functions in *Drosophila*, are likely to provide considerable insight into the related mechanisms controlling cardiogenesis in vertebrates, including human. Here we present preliminary data showing that *castor*, a *Drosophila* gene encoding a zinc finger transcription factor, plays a critical role in cardiogenesis. The *Drosophila* embryonic heart is essentially a tube of muscle morphologically similar to the heart of a 21-day old human fetus that pumps circulatory fluid. We show that *Drosophila* embryos lacking the *castor* gene function exhibit two distinct cardiac phenotypes: (i) small localized increases or reductions in the number of a distinct cardiac cell type along the tube, indicating that *castor* is necessary for proper cardiac progenitor cell division, and (ii) the absence of entire segmental regions of the heart tube along its length, suggesting that *castor* is also needed for an earlier step in cardiogenesis, either proper mesoderm migration or accurate specification of the cardiac progenitors. Given that the vertebrate ortholog of *castor* has also been implicated in heart development, our further analysis of the cardiogenic functions and roles of this gene will be particularly relevant from a biomedical perspective.

The Impact of Obesity-Related Cellular Stressors on Gonadotropin-Releasing Hormone Neuron

Function: Implications for Infertility (Poster)

Lucas T. Knutson, Nigel Dao, Cody W. Grzybowski, Joseph A. Lenkey, David R. Elkins, Noah J. Levi, Heidi E. Walsh, Wabash College

Gonadotropin-releasing hormone (GnRH) is required for fertility in both males and females. This hypothalamic peptide hormone controls both steroid hormone synthesis and gametogenesis by promoting the release of pituitary gonadotropins (luteinizing hormone and follicle stimulating hormone) that act on the gonads. Thus, conditions that reduce GnRH release will impact the entire reproductive axis and thus fertility. Because obesity is a risk factor for infertility, we sought to determine how obesity-related cellular stressors affect GnRH neuron function. At the cellular level, obesity causes endoplasmic reticulum (ER) stress and inflammation due to excess circulating free fatty acids. To characterize how *Gnrh1* gene expression is affected by obesity-related stressors, we used immortalized mouse GnRH neurons (the GT1-7 cell line) as a model system to study expression of the *Gnrh1* gene and activation of stress-induced signaling pathways. Both ER stress and treatment with the saturated free fatty acid palmitic acid (PA) promote JNK and NF- κ B phosphorylation, indicating that both obesity-related stressors activate pro-inflammatory signaling pathways in GnRH neurons. PA treatment also activated ER stress signaling, as measured by induction of the protein CHOP. Furthermore, PA promoted secretion of the pro-inflammatory cytokine interleukin-6. *Gnrh1* gene expression was significantly repressed by ER stress in GT1-7 cells, while expression of a putative *Gnrh1* repressor, *c-fos*, was upregulated by ER stress in a protein kinase C-dependent manner. Preliminary results suggest that PA also represses *Gnrh1* expression. Thus, our work suggests that decreases in GnRH production caused by ER stress and inflammation may contribute obesity-associated infertility.

The investigation of SYD-2 as a potential substrate of the Anaphase Promoting Complex in promoting GABA release at the *C. elegans* neuromuscular junction

Lauryn R. Campagnoli, Daniel K. Lester, and Jennifer R. Kowalski

Neurons communicate at synaptic junctions via chemical neurotransmitters that elicit either excitatory or inhibitory responses in postsynaptic cells. Balance of excitatory to inhibitory signaling (E:I) is governed by the abundance of synaptic proteins. Ubiquitin ligase enzymes tag target proteins with ubiquitin polypeptides, which alters protein location, activity, and abundance. Defects in E:I balance and the ubiquitin system occur in neurological disorders. To study ubiquitin enzyme regulation of E:I balance, we use the neuromuscular junction (NMJ) in *Caenorhabditis elegans*. At the *C. elegans* NMJ, acetylcholine (ACh) neurotransmitter release causes muscle excitation (contraction) whereas GABA neurotransmitter release inhibits muscle excitation (relaxation). These phenotypes can be measured using aldicarb, which causes synaptic ACh buildup, leading to muscle hypercontraction and paralysis. Using aldicarb assays, we showed that the Anaphase Promoting Complex (APC)—a conserved ubiquitin ligase—acts in *C. elegans* GABA-releasing motor neurons to promote GABA release. We hypothesize the APC negatively regulates SYD-2 Liprin α , a conserved protein that acts at GABA neuron presynapses, to promote GABA release. SYD-2 has two APC recognition sequences, and SYD-2 protein levels increase at NMJs in APC loss of function (APC LoF) mutants. SYD-2 LoF and APC LoF;SYD-2 LoF double mutants are both aldicarb resistant, consistent with SYD-2 acting downstream of the APC at the NMJ. SYD-2 overexpression causes aldicarb hypersensitivity, as does APC LoF, also consistent with our model. Current experiments are examining synaptic vesicle accumulation in SYD-2 overexpression animals. Future experiments will test effects of mutating APC recognition motifs within SYD-2 on SYD-2 synaptic protein levels.

Use of Aperio whole slide digital imaging for quantitation of PD-1, PD-L1, and CD8 in Triple Negative Breast Cancer

Katie Beverley^{1,2}, Brianna Bangert³, and George Sandusky¹

¹Indiana University School of Medicine, ²University of Wisconsin School of Medicine and Public Health, Madison, WI, ³Park Tudor School

Cancer is characterized as dysregulated cell proliferation. Breast cancer is one of the most common cancer types in women. 30% of breast cancers are positive for HER-2 neu and respond to standard of care treatment. However, Triple Negative Breast Cancer (TNBC) tumor cells lack the three most common receptor mutations known to drive TNBC cell proliferation—estrogen, progesterone, and HER-2 neu thus standard of care treatments are rendered ineffective. Therefore, immunotherapy has been recently identified as a potential treatment method for TNBC.

A binding event between T-cell programmed death receptor 1 (PD-1) and the ligand, expressed by cancer cells, inhibits the immune system's ability to fight off the cancer cells. Tumor immunotherapy drugs prevent these binding events from occurring. We sought to elucidate whether the tumor immunotherapy markers PD-1 and PD-L1 were expressed in triple negative breast cancer. We hypothesized that both PD-1 and PD-L1 would be present in TNBC cells, and that PD-L1 would be overexpressed. We examined 10 TNBC cases stained for PD-1 and PD-L1 markers which localize to the cell membrane using Aperio whole slide imaging with a positive pixel algorithm.

The tumor microenvironment is critical in understanding cancer pathology and identifying the most effective targets. CD8+ T cells (cytotoxic T lymphocytes) are integral to the tumor microenvironment along with other factors including vasculature and a storm of cytokines and for this reason are viable targets for breast cancer immunotherapy. We hypothesize that there is overexpression of CD8(+) T cells in the microenvironment of TNBC cells. To further elucidate this, 10 TNBC cases were stained for CD8 and imaged using Aperio whole-slide imaging followed by analysis with the positive pixel algorithm.

We observed that PD-1 was minimally expressed and PD-L1 was overexpressed on the cell membrane, as was expected. Our data indicate that CD8 is also overexpressed in the microenvironment and both of these markers could be targets for future immunotherapy drugs for the treatment of TNBC. This type of therapy would allow patients more longevity and lead to fewer side-effects.

Use of Geranylgeraniol to Rescue Osteoblasts, Periodontal Ligament Fibroblasts, and Oral Keratinocytes from Zoledronate-Induced Cell Death (Poster)

Ryan Smith, **Sammy Oberholtzer**, Calli Williams, Terry Beal, Jacob Kellner, Travis Landis, Brooke Johnson, Ben Kawsy, Christian Cuevas, and Dan Jones

Bisphosphonate-induced osteonecrosis of the jaw (BRONJ) is a rare but serious side effect of bisphosphonate treatment. Zoledronate (ZOL) is a potent bisphosphonate used to treat postmenopausal osteoporosis, Paget's

disease, and cancer that has metastasized to bone. Individuals affected by BRONJ are unable to heal from dental procedures, such as extractions and implants, leading to painful oral lesions. Geranylgeraniol (GGOH), an intermediate of the mevalonate pathway, has been shown to block ZOL-induced cell death in primary human osteogenic cell culture, representing a potential treatment for BRONJ. The goal of the work presented here was to investigate the death effects of ZOL as well as GGOH in combination with ZOL in murine MC3T3E1 osteoblasts, human periodontal ligament fibroblasts (HPDLFs), and immortalized human oral keratinocytes (OKF6/TERT2). 72 hours of 50 μ M ZOL treatment in osteoblasts induced 7% apoptosis with simultaneous exposure to 10 μ M GGOH decreasing that level to 3%. In the same cells, ZOL produced 29% necrosis while GGOH treatment significantly reduced necrosis to 14% ($p < 0.01$). In HPDLFs the same ZOL treatment induced 4.5% apoptosis and GGOH treatment reduced apoptosis to 0.96%. ZOL induced 33% necrosis and GGOH treatment reduced necrosis to 13% ($p < 0.01$). Preliminary OKF6/TERT2 data revealed that ZOL significantly increased apoptosis by 40% while GGOH rescue lowered apoptosis below untreated levels. ZOL induced necrosis by 20% but was not rescued. Collectively the data demonstrates the ability of GGOH to rescue osteoblasts, periodontal ligament fibroblasts, and oral keratinocytes from ZOL-induced cell death.

Visualizing miRNA and mRNA Interactions for Next-Gen Sequencing Tumor and Normal Datasets using *MMiRNA-Viewer2*

Yongsheng Bai, Indiana State University

Since the discovery of miRNA and its critical role in gene regulation, various methods were developed to identify the miRNA and mRNA association in different types of cancers. With the goal of identifying cancer or disease candidates of miRNA and mRNA interaction pairs, we developed a novel and user-friendly miRNA-mRNA interaction and visualization tool *MMiRNA-Viewer2* by integrating various cancer-specific databases.

Specifically, we integrated multiple functional annotation and disease association databases to retrieve all the necessary information. Users are able to retrieve the mRNA and miRNA annotation information, signaling cascade pathways and cancer association between miRNAs and mRNAs. Functional enrichments analysis and gene regulatory networks can be performed and generated for miRNA and mRNA pairs with *MMiRNA-Viewer2*. We think *MMiRNA-Viewer2* serves as a multitasking platform which can make a detailed functional annotation between the miRNA-mRNA interaction pairs, which allows users to save time and efforts by providing quick and comprehensive access of various biological information from multiple resources.

Chemistry Section

An Exploration of the Surface Contribution to Second Harmonic Generation in ZnO Nanofluids (Poster)

James D. Adair, Christopher B. Nelson, Tykhon Zubkov, and Mahamud Subir, Ball State University

Zinc oxide (ZnO) in the form of nanoparticles (NPs) is an important nanomaterial due to its catalytic and optoelectronic properties. An interesting aspect of ZnO is that its crystal structure is anisotropic, which leads to strong 2nd order nonlinear response, such as second harmonic generation (SHG). At the nanoscale, NPs exhibit high surface area to volume ratio. Therefore, for the ZnO NPs not only the bulk but its surface can contribute to the overall SHG. The objective of this study has been to characterize SHG from ZnO NPs dispersed in a liquid medium. We have shown that commercially available ZnO NPs of nearly spherical shape (average particle size of 33 ± 13 nm) yield a strong SHG signal. We have further distinguished the surface vs. bulk contribution to the SHG by modifying the NP surface through adsorption of organic dyes. Two different dyes, coumarin 343 (C343, resonant with SHG frequency) and coumarin-3-carboxylic acid (C3, non-resonant with SHG frequency) were studied. Based on SHG polarization anisotropy data, it is revealed that dye modification of NP surface leads to a unique contribution to the overall SHG. To further analyze the influence of the particle size on the SHG, we have synthesized and studied ZnO nanoparticles with diameter less than 10 nm. These smaller size NPs yield weaker SHG and different polarization signature compared to the larger NPs. In this presentation, the size and surface dependent SHG from ZnO NPs will be discussed in the context of molecular structure and dye adsorption onto the ZnO NPs.

Characterization of the Catalytic Oxidation of Alcohols via Various Nickel (II) Diphosphine Complexes (Poster)

Wesley A. Deutscher and Charles J. Weiss, Wabash College

Alcohol oxidation is an important reaction for synthesis, pharmaceuticals, and renewable energy. A nickel complex with formula $\text{Ni}(\text{dcpe})(\text{CH}_3\text{CN})_2(\text{BF}_4)_2$ (dcpe = 1,2-bis(dicyclohexylphosphino)ethane) was synthesized that is capable of oxidizing primary and secondary alcohols into their respective ketones, aldehydes, or esters. Reactions were monitored by ^1H NMR spectroscopy, and it was found that this catalyst is

effective in oxidizing a range of aliphatic and aromatic alcohols. It is believed that the complex proceeds by the transfer-hydrogenation of the acetonitrile solvent, generating imines and/or amines as a byproduct. In addition, a second class of Ni (II) catalysts with formula $Ni(P_2RN_2R')_n(CH_3CN)_m(BF_4)_2$ ($P_2RN_2R' = 1,5-R-3,7-R'-1,5$ -diazia-3,7-diphosphacyclooctane) were synthesized using a series of diphosphine ligands with pendant amines. Kinetic studies of the oxidation of isopropyl alcohol were conducted using UV-vis spectroscopy. It was found that complexes with two diphosphine ligands have an induction period while those with a single P_2N_2 ligand do not.

Chemistry Section continued.....

Chiral Luminescent Materials: Using Lanthanides and Ionic Liquids

Todd Hopkins, Butler University

Circularly polarized light is utilized in applications that are important to industries that generate \$100B's/yr., including film and pharmaceutical. This presentation will describe efforts to make materials that generate circularly polarized light using Ionic Liquids (ILs) and luminescent lanthanides. Ionic liquids are low melting point salts with properties that can be synthetically controlled through the choice of cation and anion. The ILs used in this study have amino acid anions that are chiral and strongly coordinating to lanthanide ions. Complexes with visible luminescent lanthanide ions (Eu^{3+} = red light, Tb^{3+} = green light) are added to the amino acid-based ILs, and the polarization of light is measured with circularly polarized luminescence (CPL) spectroscopic techniques. Results from CPL studies show that these materials emit circularly polarized light, and the preference (left- vs. right-) of polarization can be controlled by choice of IL. This presentation will describe efforts to characterize the structure/CPL spectra relationship in the ILs.

Chlorophyll-Biotin Photosensitizers for Cancer Therapy and Imaging (Poster)

Dewana M. Hammonds and Meden F. Isaac-Lam, Purdue University Northwest

Photodynamic Therapy (PDT) is being explored as a clinically approved, minimally invasive, and target specific treatment for malignant tumors. PDT includes the administration of a photosensitizer (PS) followed by exposure to visible light. PSs are light absorbing compounds that initiate a photochemical reaction by transferring its energy to endogenous oxygen generating highly-reactive cytotoxic singlet oxygen. Specific localization of PS can be achieved by targeting receptors that are over-expressed on certain tumor cells and not on normal cells. Rapidly growing cells such as tumor cells require more vitamins, so they produce more receptors for taking in these nutrients. PS can be chemically bound to biotin, allowing for increased cell uptake. Chlorins are chlorophyll derivatives that show enhanced tumor localization than normal tissues. Synthesized photosensitizers used in this experiment involve a chlorin conjugated to a biotin including its zinc and indium complexes. PDT causes direct cytotoxicity on tumor cells, damage to the tumor vasculature, and local inflammatory response. In this in vitro biological study, the chlorin-biotin conjugate did increase the phototoxicity of PS in colon cancer cells compared to the unconjugated chlorin which serves as control. CT-26 colon cancer cell line was shown by others to exhibit over-expression of biotin receptors. However, the indium complex showed the best photodynamic effect compared to the zinc complex and the unmetallated chlorin-biotin compound. PDT is beneficial to cancer treatment because it involves very low risks of normal tissue toxicity, resistance mechanisms, and organ function damage. This method of treatment can be curative in early stage tumors and can be combined with other antitumor treatments due to its potential for tumor selectivity.

Comparison of the Effectiveness of Hydrogen Peroxide and Sodium Percarbonate in the Catalyzed Degradation of Dyes and Pharmaceuticals (Poster)

Ruth E. Nalliah, Audrey R. Ackley, Nick R. Beery, and Lukas J. Kaylor, Huntington University

Catalyzed oxidation of dyes and pharmaceuticals by either hydrogen peroxide or sodium percarbonate (SPC) holds promise as a potential way for consumers to convert organic pollutants, such as excreted pharmaceuticals, into carbon dioxide and water at the point source. A preliminary comparative survey of the degradation of several dyes and over-the-counter pharmaceuticals was performed in undergraduate teaching laboratories using a proprietary heterogeneous catalyst from Hydrogen Link, a research company in Canada,

along with either hydrogen peroxide or SPC, respectively. The degradation of FD&C dyes Blue 1, Blue 2, Red 3, Red 40, and Yellow 5 was monitored, as well as the degradation of acetaminophen, caffeine, ibuprofen, quinine, and salicylic acid. Concentration conditions were optimized to minimize spectral interference of hydrogen peroxide with spectrophotometric monitoring of the pharmaceuticals. For Blue 1, Blue 2, and Red 40, complete loss of color was observed after 24 hours with SPC, but not with hydrogen peroxide. In contrast, for caffeine and quinine, less degradation was observed with SPC than hydrogen peroxide after 24 hours, while the reverse was shown for acetaminophen. For caffeine, the catalyzed reaction with hydrogen peroxide appears to follow first-order degradation kinetics, with a half-life of 33 min under the conditions studied. This preliminary study indicates that the pH increase from the SPC may have a variable but pronounced effect on the rate and completion of the degradation reactions, depending on the compound being degraded and possibly its concentration. It appears that the use of either hydrogen peroxide or SPC with this catalyst could provide two complementary methods of degradation, which might be used in sequence to degrade a wider variety of compounds.

Detection of Industrial and Pharmaceutical Organics in Aqueous Solution – A Gravimetric Approach Using Quartz Crystal Microbalance (Poster)

Tyler Williams and Mahamud Subir

There is a growing concern for human health and environmental safety due to the presence of man-made pollutants, such as industrial organics and pharmaceuticals and personal care products (PPCPs), within urban water systems. Conventional treatment methods often remove only a small fraction of these compounds because of the diverse mixture of molecular structures and competition with natural organic matter (NOM). Little is known about the role surface chemistry plays in the fate and transport of industrial dyes and PPCPs in the environment. Adsorption is a promising method for removing dyes and PPCPs from aqueous solution due to the simplicity, cost effectiveness and ability to target specific molecules. However, there is a need to better understand how these compounds interact with natural surfaces in order to design and develop better adsorption methods. The goal of this study has been to determine if the quartz crystal microbalance (QCM) can be used to observe the adsorption of environmentally relevant small pollutants to NOM with carboxylate functional groups in situ. QCM is an instrument that measures frequency change as a function of mass uptake, where mass is linearly proportional to the frequency change. We have developed a protocol using malachite green (MG), an industrial organic dye known to bind to carboxylate functional groups, and show that QCM is applicable to study adsorption of organic molecules to the surface of self-assembly monolayer (SAM) mimicking NOM surface. Initial experiments revealed temperature fluctuations, stirring speed, mechanical vibrations and electrical noise, play a crucial role in the observed frequency change. Moreover, electrolyte concentrations can and do contribute to the frequency change. We will present adsorption data of MG and other PPCPs in aqueous solution onto carboxylate terminated thiol groups anchored to gold-coated quartz plates and discuss the potential of this technique to detect and quantify the adsorbed contaminant.

Effects of Copper Doping on PCE of NiWO₄ (Poster)

Amirhossein Hosseini, **Andrew Riley**, Zhihai Li

With much of the developed world looking for alternative renewable energy sources, we are in a time of exponential growth in our understandings of energy and power. One very popular form of alternative energy is solar power. NiWO₄ has recently been shown to have the capacity to effectively convert sunlight to electricity. Using group 11 elements such as copper as a dopant, this effect can be magnified. A precipitation method aided by CTAB in an aqueous solution held between 7 and 7.5 pH, Cu(x)Ni(1-x)WO₄ was able to be synthesized, washed, and then annealed. A paste was made by combining the sample with PEG and acetic acid, and a counter electrode was prepared using platinum ink. Iodine dye was injected and the electrode was sealed. Our results showed an initial PCE for NiWO₄ of 0.15%, which increased dramatically to 0.86% with a 9% doping level of copper. More work is being done to fine tune the synthesis and preparation of electrodes to increase our PCE yield.

Efforts Toward Developing a New Cyclopentannulation Reaction Employing Fischer Carbenes as Linchpin Reagents (Poster)

Andy Romisch, Maggie Somody, and Michael C. Slade, University of Evansville

The cyclopentane core structure is ubiquitous in biologically relevant molecules, which makes it an important target for synthesis. Although widespread in nature, a perceived “synthetic intractability” continues to surround

functionally and stereochemically dense cyclopentanes. There is thus a gap between the molecules that nature makes and those that chemists have the tools to make. This project seeks to fill the gap using a new [4+1] approach to the five-membered ring, complementary to established [3+2] methods. It seeks to establish Fischer carbenes as effective linchpin reagents in a novel interceptive decarboxylative allylation reaction of γ -methylidene- δ -valerolactones. Initial attempts at reaction development have revealed that a dimerization pathway of the Fischer carbenes envisioned as linchpins is competitive with lactone decarboxylation and activation. Approaches to circumvent this issue and future directions will be discussed.

Chemistry Section continued.....

Evaluation of genetically encoded photochemical crosslinkers within self- assembling nanomolecular bacterial secretion machinery (Poster)

Kedric Milholland, Alicija Antonczak and Eric Tippmann

Proteins that self-assemble into higher order structures provide a useful platform to demonstrate the utility of genetically encoded noncanonical amino acids. The target protein here, Hcp1, was first described as part of bacterial Type VI secretion system from *Pseudomonas aeruginosa*. The protein first self-assembles into a hexamer and then the hexamers further stack into a nanotubular structure. Hcp1 monomers were targeted for incorporation with two widely used photoactivatable amino acids: para-benzoyl phenylalanine or para-azidophenylalanine. The ability of these amino acids to form covalent adducts within the Hcp1 self-assembled system was investigated. Multiple residues at the monomer-monomer interface were targeted for mutagenesis. The efficiency of each amino acid to covalently trap self-assembled monomer and form hexamers was determined. The results demonstrate the choice and role of genetically encoded tools applied to complicated biological processes such as self-assembly.

Infrared Spectroscopic Studies of C60 and C70 Nanoparticle Interactions with 2-Pyrrolidone

Joseph L. Kirsch Jr., Daniel Schemenauer, and Austin Engle, Butler University

Fourier transform infrared spectroscopy was used to investigate the interactions between C60 and C70 nanoparticles and 2-pyrrolidone in a toluene solvent environment. Infrared spectra were collected for 2-pyrrolidone in toluene, 2-pyrrolidone in toluene saturated with C60, and 2-pyrrolidone in toluene saturated with C70. Spectra were collected over a concentration range of 200 to 2 μ L's of 2-pyrrolidone per mL of toluene or toluene saturated with the nanoparticles. At the higher concentrations of this range, small broadenings of the carbonyl stretching absorptions on the lower wavenumber side were observed when 2-pyrrolidone was treated with C60 and C70. Significant shifts (~ 15 cm^{-1}) were observed for 2-pyrrolidone treated with C60 at 5 and 2 μ L's of 2-pyrrolidone per mL of toluene saturated with C60. These observed shifts suggest a 2-pyrrolidone — C60 nanoparticle interaction. A small broadening of the carbonyl absorption on the low wavenumber side was observed when 2-pyrrolidone was treated with C70 at 5 and 2 μ L's of 2-pyrrolidone per mL of toluene — C70 solution. Spectral subtraction was used to reveal the carbonyl absorption for the 2-pyrrolidone - C60 complex formed at the higher concentration range. The spectrum of 2-pyrrolidone in toluene (100 μ L's of 2-pyrrolidone per mL of toluene) was subtracted from the spectrum of 2-pyrrolidone in toluene saturated with C60 (100 μ L's of 2-pyrrolidone per mL of toluene saturated with C60), and the subtraction process yielded a spectrum containing a spectral absorption near 1690 cm^{-1} . The 1690 cm^{-1} absorption resulting from the subtraction process is in good agreement with the carbonyl absorption observed, 1687 cm^{-1} , for the 2-pyrrolidone treated with C60 at the low concentration (5.0 μ L of 2-pyrrolidone per mL of toluene saturated with C60).

New Proposed Route to Thiapentalenes (Poster)

Grant M. Carlson, Jessica M. Lokotar, Nathan Tice, and Chad Snyder

Metal η^5 -cyclopenta[c]thienyl complexes are of significant interest in both catalysis and materials chemistry (i.e. polymerization). These relatives of the low-band-gap polymer polybenzo[2,3-c]thiophene show great promise due to their unique electronic properties as environmentally stable, conductive polymers and as energy-efficient, light-emitting diodes (LEDs). Recently, cyclopenta[c]thiophenes have received further attention as studies have shown that these molecules have potential as photodynamic anticancer agents particularly effective against leukemia cells. The current method of synthesizing these compounds is lengthy, costly, and has a very low

yield. Our group has developed an alternative synthetic route fusing making use of traditional organic chemistry reactions (SN2, PCC oxidation, LAH reduction, Dieckmann condensation, etc), proven to consistently work in the laboratory. This new research route is of great value not only as “green” chemistry but also highlights many of the named reactions organic chemistry students learn in the classroom.

Chemistry Section continued.....

Novel and Efficient Allylation of Nucleophilic Oxygen Substrates (Poster)

Jacob C. Davis and Philip A. Albinak, Ball State University

2-Benzyloxy-1-methylpyridinium triflate has been shown to allow for the transfer of benzyl groups to nucleophilic functional groups such as carboxylic acids or alcohols. More recently a new derivative has been designed which features an allyl group as opposed to a benzyl group. The application of allyl functional group transfer has been studied utilizing carboxylic acids as substrates, but has yet to extend to phenols or alcohols. The focus of this project is to extend the utility of an allyl-transferring mechanism to other nucleophilic oxygen substrates that demonstrate a wide variety of electron donating or withdrawing characteristics. The product of these reactions is an allyl ether, a functional group that is widely involved in protecting group strategies for nucleophiles due to its relative ease of removal under mild conditions. This reaction pathway allows for the use of weak bases and shorter reaction times to generate the final product; an improvement over previous methods that utilize strong acids and bases with substantially longer reaction times. The allylation site will be monitored in the presence of competing nucleophilic sites to further define the mechanism. The extension of this reaction could allow for an improvement in efficiency of larger synthesis that utilize the allyl transfer mechanism, as well as a further understanding of the allylation mechanistic pathway.

Orientation Analysis of p-nitrophenolate at Air-Aqueous Interface

Ryan Young, Dan Headley, Maggie Schmits, Milina Quiroz, and Mahamud Subir, Ball State University

Many chemical processes occur at an interface – the molecularly thin region between two bulk phases. Examples of air-water interface include surfaces of environmental water, rain droplets, and aerosol particles in the atmosphere. Often the interfacial chemistry is different from the chemistry in bulk solution. Thus, it is of fundamental importance to understand molecular behavior at an interface. In this work we have used surface second harmonic generation (SHG) spectroscopy to study orientation of p-nitrophenolate (PNP⁻) as a function of its surface coverage at the air-aqueous interface. PNP⁻ is a small organic anion and an aquatic contaminant. Recent studies have shown PNP⁻ to exhibit affinity for the air-water interface. Previously, our group has shown the orientation of PNP⁻ changes with respect to the surface normal as its surface number density increases. This is in contrast to no orientation change for the neutral p-nitrophenol at the air-water interface. Herein, we have further probed this intriguing variation in PNP⁻ orientation at the air-aqueous interface containing zwitterionic lipids (dipalmitoylphosphatidylcholine, DPPC) at the surface and bulk solution of concentrated electrolytes. It is revealed that the lipid monolayer restricts PNP⁻ orientation at the air-aqueous interface as its surface density increases. This orientation angle at the DPPC-PNP⁻ interface was found to be ~32° versus the surface normal, whereas at the air/aqueous interface the angle ranges from ~43° to ~55°. Electrolytic environments of 1M and 2.5M NaCl solutions of PNP⁻ were also probed. The PNP⁻ orientation angle at the NaCl solution surface also increases with PNP⁻ surface population. These studies highlight the importance of electrostatic interaction in molecular ordering at the air-aqueous interface. The experimental results along with their implication in aerosol chemistry and petroleum science will be discussed.

Photodegradation Studies of an Organic Contaminant at Colloidal-Aqueous Interface (Poster)

Mansour Alsarrani, Tyler A. Williams, and Mahamud Subir, Ball State University

Photodegradation is an important natural process that helps breakdown harmful compounds from the environment. In the aquatic system, some organic molecules reside at the surface of naturally occurring colloids,

such as particulate natural organic matter (NOM). Thus, it is useful to understand photodegradation rates at these surfaces and compare it to those in the bulk solution. Malachite green (MG) is widely used as an industrial dye and a well-known organic pollutant that can interact with the surface of NOM. The photodegradation of MG has been investigated when it is bound at the surface of carboxylate functionalized polystyrene colloids (PSCs) suspended in aqueous solution. In this study, we use ultraviolet radiation to start the photochemical reaction and monitor the degradation process over time using second harmonic generation (SHG) spectroscopy. SHG is a surface selective tool that allows the probing of MG attached to the PSC particle surface. A comparative examination of bulk degradation of MG, in the absence of PSC particles, has been measured using UV-Vis spectroscopy. We have compared the SHG and UV-Vis results to determine the photodegradation rate of MG at the PSC particle surface. It was determined that the photodegradation rate of MG is faster at the surface. Moreover, preliminary evidence suggests that the photodegradation products remain attached to the surface. Kinetic data along with spectral identification of the reaction products will be presented. The implication of this work with respect to transport and fate of organic pollutants in the aquatic environment will be discussed.

Photodynamic Evaluation of Chlorophyll-Rexinoid Derivatives in Triple Negative Breast Cancer Cell Line
Angelia C. Jeka, Alexandra D. Mee and Meden F. Isaac-Lam, Purdue University Northwest

Breast cancer is among the most common types of cancer occurring in women, representing close to 30% of newly diagnosed cancers. Photodynamic therapy (PDT) is a binary therapeutic method that offers a minimally invasive option with potential to target even the most difficult subtypes of breast cancer. This procedure involves the selective uptake of a photosensitizer into targeted cells, which are then irradiated with red light, leading to a photochemical reaction producing singlet oxygen and other reactive oxygen species causing direct tumor cell death. PDT is highly selective reducing the potential for damage of normal cells near the tumor. Retinoids are a class of drug that bind retinoic acid receptors (RARs) affecting transcription factors which regulate gene expression, therefore control development, and differentiation. Synthetic retinoids, known as rexinoids, bind specifically to the retinoid X receptor. Rexinoids, including bexarotene, through extensive trials, have demonstrated the desired preventive effect, with reduced side effects when compared to retinoids. Triple-negative breast cancer (TNBC) includes 15-20% of all breast cancers and are particularly difficult to target due to a lack of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor (HER2) expressions. Standard chemotherapy is the only treatment option currently available for women with TNBC. In this study, bexarotene linked to a chlorophyll derivative was synthesized including its zinc and indium complexes, with potential use in the targeted treatment of TNBC. Biological in vitro evaluation using TNBC cell lines upon light irradiation reveal the viability of bexarotene for use in combined therapy with PDT offering a new minimally invasive alternative to TNBC patients with a currently limited selection of treatment options.

Repurposing Sulfur into Functional Polymers
Courtney L. Jenkins, Clayton Westerman, Ball State University

Sulfur is the third most abundant element in petroleum. During the refinement process, sulfur is converted to its elemental form, S₈, for storage. Low demand makes elemental sulfur inexpensive and abundantly available. A recently developed method, "inverse vulcanization," utilizes this waste as a feedstock to develop new materials for IR imaging, Li-S batteries, and with self-healing properties. By employing high temperatures (>160 °C), sulfur bonds are broken forming radicals that can initiate polysulfide formation. Few monomers have been incorporated due to the high temperature requirements. Here we have combined elemental sulfur and divinylbenzene at 185 °C to form poly(S-c-DVB). Less energy is required to break sulfur bonds in this polymeric arrangement allowing poly(S-c-DVB) to act as a pre-polymer. Applying more mild temperatures, ~100 °C, leads to further radical formation. In the presence of additional monomers, these radicals can lead to the formation of terpolymers. Polymer structure was confirmed by NMR. Gel permeation chromatography was used to determine the molecular weight. The sulfur to monomer ratio can be altered to affect the polymer composition including the number of consecutive sulfur bonds. With an emphasis on obtaining disulfide bonds, these polymers are primed for conversion to modifiable polythiol matrices. Within a polymer matrix these highly reactive functional groups can form a cross-linked network, bind to metal ions, or be appended with alternate functional groups via click chemistry.

Synthesis of Malononitrile, Cyanoacetates, and Cyanoacetamides (Poster)
Ebtsam K. Alenezhy and Robert E. Sammelson

Malononitrile and its derivatives are important building-blocks for the synthesis of numerous organic heterocycles that have uses in the agriculture and pharmaceutical industries. This project involves the development of new synthetic organic chemistry for the reductive alkylation of aldehydes with malononitrile in high yields. Malononitrile and the synthesized substituted malononitriles will be reacted with the 2-benzyloxy-1-methylpyridinium triflate (BnOPT) salt to convert them to synthetic cyanoacetamides. Also, the substituted cyanoacetates, which are synthesized from similar reductive alkylation chemistry, will then be transformed into the desired cyanoacetamides by reaction with 1° or 2° amines. The last component of the research will react ethyl cyanoacetate with amines to make cyanoacetamides. These amides will be used to develop the chemistry for the corresponding reductive alkylation with aldehydes. The chemistry will require switching the reaction order for the steps and determine if this reverse sequence will provide a more useful synthesis of the desired cyanoacetamides.

Chemistry Section continued.....

Synthesizing an Adjustable Polymer Framework from Garlic Oils (Poster)

Kristen J. Kamp, Courtney L. Jenkins

Garlic contains the compound diallyl disulfide (DADS) in its oils, which has the potential to be polymerized. The goal of this research is to create a molecular framework from this material. This framework will be natural, easily reproducible, and customizable for further use. This is done by incorporating DADS into a copolymer and then breaking the disulfide bonds to form free thiol groups that can be modified for customizable uses. The DADS is polymerized with methyl methacrylate (MMA) and a reaction initiator, azobisisobutyronitrile (AIBN)—two materials that are cheap, use direct synthetic methods, and are common in the polymer field. The copolymer is then purified and characterized ¹H NMR to confirm the chemical structure and gel permeation chromatography (GPC) to characterize polymer chain length. Once formed, the polymer will be modified by click chemistry—a class of reactions that are high yielding and simple to perform. In order to quantify the amount of modifiable thiol groups, the disulfide bonds will be broken and reacted with maleimide. This reaction is detectable with ultraviolet-visible spectroscopy and will show how much of the product was altered. Preliminary NMR, UV-Vis and GPC data will be presented. Developing one modifiable system for the framework offers a streamlined approach to polymer synthesis. In addition, the polymer incorporates natural resources; the world is moving away from petroleum and looking for renewable materials to replace it.

Synthetic Chlorin-Rexinoid Photosensitizers for Photodynamic Therapy of Triple Negative Breast Cancer (Poster)

Alexandra D. Mee, Angelia C. Jeka, and Meden F. Isaac-Lam, Purdue University Northwest

The key to successful breast cancer treatment lies within strategic combinations of therapy tailored to the individual diagnosis. Unfortunately, the most aggressive subtype, triple negative breast cancer (TNBC), account for 15-20% of all breast cancer diagnoses, and is particularly difficult to treat due to the lack of estrogen, progesterone, and human epidermal growth factor expressions. Several agents are currently being tested for the treatment of TNBC which include derivatives of vitamin A called retinoids. The use of retinoids to inhibit tumor growth have been evaluated in animal models of several cancers, all showing chemopreventive results. Rexinoids (synthetic retinoids) including bexarotene have demonstrated the desired preventive effect, with reduced side effects when compared to retinoids based on numerous research studies. It is possible that the use of rexinoid bexarotene linked to a photosensitizer could provide combined therapy increasing the efficacy of photodynamic therapy (PDT). PDT involves the selective uptake of a photosensitizer (PS) into targeted cells, which are then irradiated with visible light, leading to a localized photochemical chain reaction that induces tumor cell death. The effects of PDT are highly localized to the site of light administration, offering minimal damage to healthy tissues. The success of PDT relies on the targeted delivery of PS. In this study, a chlorin was used as a photosensitizer and linked to a small molecule to target specific receptors in breast cancer cells. Chlorin (chlorophyll derivative) was chemically conjugated to a retinoid bexarotene by a peptide coupling reaction, and the corresponding zinc and indium metal complexes were also prepared in good yields. Molecular structures of the synthesized conjugates were elucidated by spectroscopic methods (¹D/²D NMR, Mass and UV-vis). Biological *in vitro* studies to determine the photodynamic efficacy of the synthesized photosensitizers in breast cancer cell lines will be performed in future projects.

The Differential Impact of Chitin and Chitosan on Heavy Metal Pollution in Water Samples

Jessica Caldwell and James Mendez

Chitin is an abundant biopolymer that can be found in the exoskeletons of a variety of organisms. The most prominently used commercial sources of chitin are shrimp exoskeletons and fungi, but there are many sources including lobster exoskeletons and cicada sloughs. In a previous study, our research group managed to determine that the mechanical and thermal properties of chitin and its derivative chitosan obtained from these sources are very similar. As such, our main chitin/chitosan source was cicada sloughs. For this project, the focus was on utilizing differing ratios of chitin and chitosan to attempt to lower the amount of heavy metals, such as nickel and lead, in water samples. Water samples were collected from Columbus, North Vernon, and Bloomington. The samples were reduced with heat from approximately 3,500 ml to 100 ml to concentrate the metals present. After water samples were reduced, a series of titrations were set up to determine the amount of heavy metals present in each. For the detection of lead, the sample was titrated with 0.0001 M of sodium sulfide. For the detection of nickel, the sample was titrated with 0.0001 M sodium hydroxide. For the detection of chromium, the sample was titrated with 0.0001 M sodium thiosulfate. For the detection of calcium, the sample was titrated with 0.0001 M sodium metasilicate. In order to minimize the amount of heavy metal present in the water, 0.3 g of chitin/chitosan was used to create a filter. 5 ml of a water sample was added to this filter and allowed to sit for thirty minutes before a vacuum was applied. This procedure was repeated utilizing different ratios of chitin and chitosan until the optimum ratio was determined.

Towards the synthesis of aryl tetrazolethiones: Successes and Challenges

Jerry Sheu and Sundeep Rayat*, Ball State University

Our research group is interested in investigating the potential of aryl tetrazolethiones as supramolecular synthons and novel anion binding agents. We have carried out the synthesis of these scaffolds by reacting isothiocyanates with sodium azide under reflux conditions. Some of the required isothiocyanates were commercially obtained, while others were synthesized in our laboratory due to their high cost. Herein, we will discuss various methods for the synthesis of isothiocyanates. At first, we reacted substituted anilines with *N,N*-dimethylcarbamoyl chloride to obtain the desired compounds. However, this method produced low yields. Isothiocyanates were successfully synthesized in good to excellent yields by reacting anilines with carbon disulfide *via* the formation of thiocarbamates followed desulfurylation with di-*tert*-butyl dicarbonate in the presence of a catalytic amount of DMAP. With aryl isothiocyanates in hand, we successfully synthesized phenyl, 3,5-dimethoxyphenyl-, 4-chlorophenyl-, 4-methoxyphenyl-, and 4-iodophenyl tetrazolethiones as well as the 1,1'-(4-methyl-1,3-phenylene)bistetrazolethione. We will also discuss our challenges toward the synthesis of 4-pyridinyl tetrazolethione and 4-(iodoethynyl)phenyl tetrazolethione.

Using 3D Printing and Magnets to Model Chemical Reactions

Karen Smiar, James Mendez

Many colleges, including IUPUC, have experimented with using 3D printing in an educational manner. We wanted to accomplish this task by providing interactive models that help develop a better understanding of molecules and bonds. Previous research accomplished in our lab demonstrated the bonds between two atoms with model, but now we want to show the actual formation of these bonds. We designed plastic pieces with 3D printing to represent different atoms. Each atom has space for a magnet that allows the pieces to attract or repel each other. By applying a small force, shaken in bottle, the initial bonds break and product molecules are formed. If shaken too much, the product bonds would be break again representing decomposition. This model was demonstrated with hydrogen and oxygen forming water but the approach is broad enough to work for other reactions.

Water Chemistry Research at the Ft. Wayne Children's Zoo (Poster)

William Lorenz and Chad Snyder, Grace College

The Ft. Wayne Children's Zoo (FWCZ) contains over 1,000 animals living in four self-contained biomes occupying a footprint of over 40 acres. Of these biomes, the *African Journey*, contains a water system of six artificial, connected ponds that is home to 11 species of bird and mammal inhabitants. The Snyder research group and the FWCZ have partnered to ensure the zoo's pond system health by performing regularly chemical and biological testing of its ponds. Analysis have included pH, surface water temperature, dissolved oxygen, and algae species distribution and concentration (measured in cells/mL). Additionally, the following ions associated with algae growth were measured. Those included calcium, chloride, phosphate, nitrate, and nitrite. The research reported herein displays the pond water analyses collected over a five-month period in 2016.

Working Toward Development of a Compartmental Model of Human Vitamin B-6 Metabolism Which Accommodates Changes in Intake as Well as Oral and Intravenous Dosing. (Poster)

Stephen P. Coburn and Douglas W. Townsend, Indiana University – Purdue University Fort Wayne

Our goal has been to develop a compartmental model of vitamin B-6 metabolism which can respond appropriately to a variety of conditions using data from the literature. Modeling vitamin B-6 metabolism is challenging because vitamin B-6 is interconverted between 7 common forms: pyridoxine, pyridoxine 5'-phosphate, pyridoxal, pyridoxal 5'-phosphate, pyridoxamine, pyridoxamine 5'-phosphate and 4-pyridoxic acid. In addition, in humans 5-pyridoxic acid becomes a significant metabolite with high vitamin B-6 intakes. Development of the model utilized the Simulation, Analysis and Modeling (SAAM) software originally developed at NIH and now available as WinSAAM(<http://www.winsaam.org/>) and SAAM II (<https://tegvirginia.com/solutions/saam-ii/>). The model is designed to achieve a steady state before changes are introduced. We found that several characteristics were required in the model. Because plasma values fluctuate with meal intake, the model needed to reflect meal intake. Because experimental data reveals flushing effects, at least some pool sizes must have limits to generate flushing. Because reducing intake has limited effect on the vitamin B-6 content of muscle, which accounts for about 70% of the body pool, there must be a mechanism to conserve vitamin B-6.

Models provide a method of simulating various situations. In the case of vitamin B-6 this model can be used to estimate turnover rates, the effect of changes in intake on body pools, and the role of erythrocytes in vitamin B-6 metabolism. The model can also be used to determine optimal sampling times for proposed experiments. It can easily be updated as new data become available. We are still working to improve the fit of the model to various data sets. We also hope to add more data on circulation and additional tissues.

X-Ray Crystallographic and Spectroscopic Analysis of Some 3,4-Bis(halomethyl)-2,5-dimethylthiophenes (Poster)

Austin Steppey, Micah Gerakinis, Nathan C. Tice, and Chad A. Snyder

In this presentation we discuss approaches to prepare solid samples for X-ray single crystal analysis as well as spectroscopic analyses that include ¹H and ¹³C NMR spectroscopy and FT-IR Spectroscopy. The solid samples presented serve as important intermediates toward the synthesis of 1,3-cyclopenta[c]thiophenes. Selected bond lengths and bond angles will be presented along with notable pi stacking interaction. These compounds have the potential to serve as conductive materials and as such a thorough study of their structural and electronic components for each of the intermediates is vital. The work presented herein highlights their synthesis, isolation, and purification as well as relevant X-ray data and spectroscopic analysis.

Ecology Section

Comparison of the Prey Base for Eastern Hellbenders between Three Streams

Veronica Yager and Rod Williams, Purdue University

The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a giant, aquatic salamander endemic to the Eastern United States. The species has suffered severe population declines over the past several decades. The Eastern Hellbender is listed as state-endangered in Indiana and is restricted to a single river system. The elimination of Eastern Hellbenders from a river system may cause a trophic cascade by altering the abundance of species such as crayfish and macroinvertebrates. My research compares crayfish and macroinvertebrate abundance in three different rivers with varying Hellbender populations. The Blue River, IN contains a declining Hellbender population, Indian Creek, IN has no known remaining Hellbenders, and Toccoa River, GA contains a healthy Hellbender population. I collected macroinvertebrates using a surber sampler and captured crayfish inside a quadrant at four sites selected within the three rivers between July-October 2016. Our results indicate notable differences in crayfish abundance between the three rivers. The results from this project will help to further describe the important role that Eastern Hellbenders occupy in stream systems.

A Preliminary Survey of the Diversity of Spiders in Morgan – Monroe Forest (Poster)

Leslie Bishop, Earlham College, Marc Milne, University of Indianapolis, Brian Foster, Indiana State University

Spiders are among the first level of consumers in the terrestrial food web with their major prey being herbivorous insects. They are also diverse in life styles, and they are found in every habitat imaginable. Even though spiders

play an important role in terrestrial ecosystems, the study of spiders in the forest habitats of Indiana is in its infancy. We surveyed the spider fauna of Morgan-Monroe State Forest as part of the Indiana Forest Alliance Ecoblitz. To date, we have sampled the forest twice per year, early summer and late summer, from 2013-2016. Each survey date included sampling in creek bottoms, mesic slopes, and dry ridges, during both day and night. Our methods were visual search and litter sampling. We have identified 125 species, including one undescribed species and six species never before recorded in Indiana. Species are considered new state records if not described in the published literature. Each year, we add more species to the list, which indicates our survey is incomplete. These results show that, even within one taxa like spiders, little is known about species diversity in most natural habitats. If we consider all arthropods, our knowledge of biodiversity in mature forests is minimal.

Ecology Section continued.....

A tale of two African *Hibiscus* - differences in floral attraction, pollination, and autonomous delayed selfing between the sympatric *Hibiscus aponeurus* and *H. flavifolius*

Andrew Schnabel, Indiana University South Bend, Juan Carlos Ruiz Guajardo, University of California at Davis, Britnie McCallum, University of North Carolina, Charlotte, Adriana Otero Arnaiz, Office of Agricultural Affairs, USDA, Mexico City, Katherine C. R. Baldock, University of Bristol, Graham N. Stone, University of Edinburgh

The evolution of delayed autonomous self-pollination in plants with mixed-mating systems is most likely when densities are low and environmental conditions are variable. At Mpala Research Centre (MRC) in Kenya, we examined the relationships among floral attraction, insect visitation, and delayed-selfing in the less common, red-flowered *Hibiscus aponeurus*, and the abundant, white-flowered *H. flavifolius*. These species often co-flower in the semiarid, tropical savannahs of MRC, and despite producing similar amounts of pollen and nectar rewards, they are visited by markedly different pollinator guilds. Visitor composition and visitation rates varied significantly across four years of observations, with bee taxa the most common visitors to *H. flavifolius*, and a mixture of bee and butterfly species recorded on *H. aponeurus*. Flowers of *H. flavifolius* received 1.8-10.6 times more visits than did *H. aponeurus* flowers, resulting in significantly higher rates of pollen deposition and removal than in *H. aponeurus*. Both species were capable of using stylar curvature to effect delayed selfing, but *H. aponeurus* used this potential mechanism of reproductive assurance significantly more often than did *H. flavifolius*. A smaller corolla size and downwards orientation of the stylar column suggest that facilitated selfing might be an important mechanism of reproductive assurance in *H. aponeurus*. Field crosses demonstrated very little pollen limitation in either species, a result attributable to high visitation rates in *H. flavifolius* and relatively frequent use of autonomous selfing in *H. aponeurus*. At this site, *Hibiscus aponeurus* populations are either evolving towards a fully selfing reproductive strategy or are showing a phenotypically plastic response to inadequate visitation rates by the appropriate pollinators. Discrimination between these possibilities requires further observations of visitation rates in *H. aponeurus* populations located in other parts of its geographical range, and chemical analyses of nectar composition and floral volatiles that may be deterring pollinators from visiting the species.

An examination of spider diversity in wildflower plots from Northern and Central Florida (Poster)

Joshua Campbell, University of Florida, Marc Milne and **BaoThu Huynh**, University of Indianapolis

Wildflower strips have recently been planted near agricultural fields to increase biodiversity (including pollinators) and to improve habitat health. To investigate the effectiveness of these wildflower strips in increasing biodiversity, we examined the diversity of spiders in agricultural habitats with and without wildflower strips in Northern and Central Florida. In 2015 and 2016 and at eight sites in Northern and Central Florida, we set up a wildflower plot and fallow control plot. We collected spiders by using different trapping method such as bowl traps, pitfalls traps, random sweep net samples during the growing season in Florida (April to November). The data – from just year one – indicate a significant difference in the abundance of spiders between plots with and without wildflower strips. Moreover, there were significant differences between plot types depending on the taxonomic family that was examined. In addition to these data, we discovered new spider distribution records and undescribed species. These data show the importance of wildflower strips to maintaining biodiversity near agricultural plots and the overall lack of knowledge about spider diversity in Florida.

Assessing predator detection and avoidance behavior of rusty crayfish (*Orconectes rusticus*) in the presence of hellbenders (*Cryptobranchus alleganiensis*) and largemouth bass (*Micropterus salmoides*)
(Poster)

Paige Weldy, Erin Kenison, and Rod Williams, Purdue University

Predator prey dynamics are common in nature. Predators and prey may use visual, tactile, chemical, or a combination of cues to communicate. For prey living in a system with multiple predators, they must be able to detect, evaluate, and appropriately respond to perceived risk. Rusty crayfish (*Orconectes rusticus*) are native to a riverine system where they are found in sympatry with largemouth bass (*Micropterus salmoides*) and Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*). We are interested 1) whether rusty crayfish alter their behavior in response to cues from these two predators, 2) how behavioral responses may vary by predator, 3) if prey responses are a function of crayfish size, and 4) how predator diet and conspecific alarm cues influence behavioral responses. We utilized a 2x5 factorial design, with two sizes of crayfish, and five treatment groups (i.e., control, hellbender, bass, hellbender fed crayfish, and bass fed crayfish). We collected predator kairomones with worm and a crayfish diets. We individually exposed crayfish to predator and treatment cues and recorded all freezing behavior, appendage movement, locomotion, and refuge use at one-minute intervals for 20 minutes. We found that crayfish size did not influence behavior, but that crayfish exposed to predator kairomones remained frozen for longer and reduced their movement around the holding tank. These differences were comparable between hellbender and largemouth bass cues. Moreover, crayfish increased their frozen behavior and reduced movement when exposed to conspecific alarm cues in combination with predator kairomones. These behavioral responses may be advantageous in reducing predator detection and subsequent attacks. This study provides definitive evidence for altered crayfish behavior in the presence of predator cues and the importance of conspecific alarm cues in eliciting even stronger defensive responses. This project will benefit future work investigating trophic interactions by providing insight on how crayfish interact with predators, and how they may behave if a predator is removed from a system.

Autumn Bat Activity in Selection Harvests and Intact Forest Stands Located Near Hibernacula
Scott Haulton, Indiana Department of Natural Resources, Division of Forestry.

Harrison-Crawford State Forest (HCSF) is home to several major bat hibernacula, including the well-known Wyandotte Cave complex. HCSF is also a 24,000 acre actively managed forest which provides roosting and foraging habitat for bats of many species during the non-hibernation seasons. The autumn pre-hibernation period is particularly important at HCSF since high concentrations of bats use the caves and surrounding forest to prepare for hibernation or as a temporary stop-over during migration. To determine if habitat use near hibernacula was affected by timber harvesting, bat activity in forest stands that had recently received selection harvests was compared with activity levels observed in "intact" mature forest stands (i.e. >15 years since single-tree selection harvest). Anabat SD2 acoustic detectors were used to monitor bat activity at randomly located sites within 21 paired harvested and intact stands on HCSF during September-November 2012. All paired sampling sites were within 2 miles of a cave entrance serving a known bat hibernaculum. Bat calls were identified to species based on call characteristics using the automated software package BCID (version 2.7). Of the most commonly detected 8 species, 5 had greater activity levels ($P < 0.05$) in recently harvested stands. No species was found to have greater activity levels in intact stands. Among 3 federally listed species known to hibernate in area caves only the gray bat (*Myotis grisescens*) had greater activity levels in harvested sites, while both the northern long-eared bat (*M. septentrionalis*) and Indiana bat (*M. sodalis*) had similar levels of activity between the two stand types. Results indicate that recent selection harvests may be an important resource to foraging bats during fall migration and the pre-hibernation period.

Breeding Ecology of Waterbirds in a Restored Floodplain of the Illinois River (Poster)

Antonio Gioe, Benjamin O'Neal, Franklin College, Heath Hagy, Illinois Natural History Survey, Tyler Beckerman, The National Great Rivers Research and Education Center

The Illinois River Valley provides critical nesting habitat for numerous waterbird species. The Nature Conservancy's Emiquon Preserve, which was restored in 2007, contains >2,700 ha of actively managed emergent marsh. The objectives of our study were to determine nest success, nest density, and nesting characteristics for various waterbird species and compare nests found in hemi-marsh with those in dense emergent cover. We conducted weekly nest searches at randomly generated points in hemi-marsh and dense emergent vegetation. The number of eggs and incubation stage were determined for each nest and environmental covariates associated with each nest were recorded (e.g., dominant vegetation present, turbidity,

water depth, etc.). Nests were monitored weekly until nest fate was determined. From 2013-2016, we observed 193 nests representing 9 waterbird species. Over the course of the study, we estimated nest density to be 1.03 nests/ha for hemi-marsh and 0.98 for dense emergent vegetation. Estimated nest survival was 48% in hemi-marsh and 49% in dense emergent. In 2016, we observed an increase in nest density and a decrease in nest survival relative to the long-term averages for the study. This change was likely associated with record high water levels, which caused a loss of emergent cover and resulted in a concentration of water birds in the remaining habitat. During the summer of 2016, a water control structure was installed with substantial capacity to alter the hydrology of the site. In the next two years, we will investigate how waterbird nesting will respond to changes in hydrology and vegetation following this major change in site management.

Ecology Section continued.....

Can't Grow the Forest for the Trees: Implication of Decades of Deer Browse on a Recalcitrant Layer and Forest Regeneration in a Southwestern Indiana Forest (Poster)

Cris G. Hochwender, Andrew Nunn, Michelle Sonnenberger & Matt Roberts, University of Evansville

Wesselman Woods Nature Preserve (WWNP) has never been subjected to timber harvest. However, deer can completely penetrate WWNP and browse tree seedlings and saplings throughout the forest. In this study, 30 plots (20 x 30 m) were surveyed (1.8 ha total). All trees of every size were identified and categorized into one of four strata based on height—herb layer, shrub layer, midstory, and overstory. Using the Shannon-Weiner Diversity Index, diversity was compared across strata. In the midstory 95% of stems over 150 cm in height and with a dbh < 5 cm were pawpaws (3841 of 4038 stems). Sugar maples comprised 101 of the remaining midstory trees, and only three other species had more than 10 trees in this stratum. Oak trees had been almost completely lost from the midstory. Given its poor representation of canopy species, the midstory layer had significantly lower diversity compared to other strata. Many tree species (including sweetgum, tulip poplar, blackgum, hackberry, and 12 species of oak) have not transitioned into the midstory stratum, suggesting that regeneration of these species into the overstory is limited. In addition, pawpaw appears to have formed a recalcitrant layer and is anticipated to limit forest regeneration even more. While the patterns observed in this survey suggest that forest regeneration may be constrained by deer browsing at WWNP, an experimental study would be needed to confirm that deer (versus other factors, such as fire suppression or shading conditions of the forest) are responsible for limited regeneration. Placed within a forest management perspective, we discuss one possible experiment to examine concerns related to deer browsing and overabundance of pawpaw trees.

Co-occurring invasive plant species: how multiple invasions affect plant community dynamics

Emma Oschrin and H.L. Reynolds, Indiana University

Co-occurring invasive plants are common and likely to become even more so due to climate change-driven range shifts. Yet few studies address simultaneous invasions by multiple plant species. It is therefore critical to extend investigations of invasion dynamics to include co-occurring invasives. Invasive-invasive interactions can be competitive or facilitative as well as symmetric or asymmetric. Furthermore, the impacts of co-occurring invasives can be additive, where impacts of multiple invaders are simply the sum of individual species effects, or non-additive, where impacts of multiple invaders are greater or lesser than individual species effects. If the impacts of co-occurring invasives are non-additive, the species could potentially reduce or enhance each other's effects non-linearly. Such interactions may have potentially profound implications for managing invasives and promoting native communities. We conducted a mesocosm study under controlled greenhouse conditions to investigate interactions among co-occurring invasive species and the effects of such interactions on communities of native prairie species. The sign (competition or facilitation) and magnitude of invasive-invasive interactions were dissected by growing invasive species alone, in pairs, and in three-way combination. Using separate mesocosms, we measured native prairie community responses to each single invader, all possible pairs of invaders, or all three invaders together. We hypothesized that invasive species from dissimilar functional groups would non-additively facilitate each other. We found evidence for both invasive-invasive facilitation and competition when invasive species were grown separately from native communities. Yet the impact of invaders on the native community was consistent among invasion treatments and less than would be expected under additive conditions. These results suggest that interactions with native species modify invasive-invasive

interactions. We discuss a functional group approach as a useful next step in the analysis of co-occurring invasion dynamics.

Comparing swimming performance of Bluntnose Minnows *Pimephales notatus* in lentic and lotic systems (Poster)

Crystal C. Nichols and Jason C. Doll, Ball State University and Stephen J. Jacquemin, Wright State University – Lake Campus, Celina, OH

Anthropogenic perturbations, such as culverts, can affect fish species distribution and potentially prevent fish movement. These structures often reduce stream width, thereby increasing water velocity to a rate at which fish cannot overcome. Increased velocities may inhibit species distributions because movement is largely dependent on the physical ability of the fish to cross these barriers. Further, individuals within a species also possess varying swimming abilities based on their sex, size, and the habitat they occur in. Thus, our objective is to assess swimming performance of the Bluntnose Minnow *Pimephales notatus*. Swimming performance was measured using a flow chamber in which the fish was placed in, following the Ucrit procedure. After an hour acclimation period fish were subjected to velocities starting at five and then increasing by five until the fish is unable to keep swimming. We compared Ucrit across habitat where the fish were collected (lentic vs. lotic), sex, and body size on the swimming performance of Bluntnose Minnows. We described swimming performance of Bluntnose Minnows across habitat, sex, and body size. Our results can be used to understand potential hydraulic barriers for this species.

Comparison of leaf litter decomposition rates for native and invasive plant species (Poster)

James Papalia, Yordanos Gebru, and Kristen Short

Native plants can alter forest ecosystems in a number of ways, one of which is by altering nutrient cycling. Several studies have shown that some invasive plants produce higher quality leaf litter that decomposes more quickly than that of native plants, and have proposed that this may affect decomposer communities and nutrient cycling. Our goal in this study was to determine whether invasive autumn olive (*Eleagnus umbellata*) leaves decompose more quickly than native tree leaves. We tested the hypotheses that autumn olive leaves would decompose more quickly than those of silver maple and red oak, and we chose these two tree species because of their abundance at Koinonia Environmental Retreat Center (owned by Manchester University) where the study was conducted. Leaf litter was collected in December 2015, and two grams of leaves were placed into litter bags made of either fine mesh or coarse mesh. Bags were collected for examination in April 2016, and again in December 2016 when leaf litter was weighed again. Results suggested that both autumn olive and silver maple leaves decomposed to a significantly greater extent than red oak leaves. Autumn olive leaves decomposed to a slightly greater extent than silver maple leaves, but this difference was not statistically significant. There was no effect of mesh type on leaf litter decomposition over this time period. Overall, our study suggests that autumn olive leaves are similar to those of other invasive plants in that they decompose more rapidly, and potentially alter nutrient cycling; further studies are needed to investigate the role of autumn olive leaf litter on nutrient cycling.

Effect of *Galerucella* beetles on controlling *Lythrum salicaria* in a newly established wetland

Megan Doucette and Deborah Marr, Indiana University South Bend

Two species of leaf beetles, *Galerucella californiensis* and *Galerucella pusilla* were released in northern Indiana wetlands in 1997 to help control the spread of *Lythrum salicaria* (purple loosestrife), and these beetles have since naturally spread to other wetlands. Retention ponds on the Indiana University South Bend (IUSB) campus were converted into wetlands in 2011, and purple loosestrife became established in 2012. First, we compared *Galerucella* herbivore damage and plant reproductive success in the newly established IUSB population with Bonneyville Mill County Park (BMCP), one of the original sites where *Galerucella* beetles were released almost 20 years prior. *Galerucella* beetle abundance was lower on average at the BMCP site compared to IUSB, but beetle abundance at IUSB was similar to historical data recorded at BMCP between 1997-2007. Inflorescence production was similar between IUSB and 1997 BMCP data, and we have not observed the drop in inflorescence production observed at BMCP by 6-10 years after beetle introduction. Second, we tested how much *Galerucella* beetles move between wetlands on the IUSB campus and whether beetle density per plant affects beetle movement. We did not observe movement of beetles between wetlands separated by more than 400 m. However, within 24-48 hour time periods we observed movement of beetles up to 10 meters. We found significant differences in how much beetles move between plants with low vs. high

density beetles populations. There was less beetle movement on plants with high beetle density (10 or more beetles) compared to plants with low beetle density (less than 10). Further research is needed to look into biocontrol methods that could be paired with the *Galerucella* beetles to more effectively eradicate or even control the purple loosestrife population.

Effects of Emerald Ash Borer on Ash Tree Health and Economic Loss Due to Ash Tree Removal on the Indiana University Southeast Campus in New Albany, Indiana

Jade R. Bilyeu, Indiana University Southeast

The Emerald Ash Borer (EAB), *Agrilus planipennis*, an invasive beetle species native to Asia and accidentally introduced in North America was first detected in 2002. Since initial detection of EAB, ash tree (*Fraxinus* spp.) mortality ranges between 95-99% in regions of the Midwestern U.S. For the current project, ash trees were analyzed from cultivated and non-cultivated areas on campus and data were collected in 2015. The following hypotheses were tested. 1.) There are significant differences in the canopy density of trees located in cultivated compared to non-cultivated areas of campus. 2.) There are significant differences in growth between white (*Fraxinus americana*) and green (*Fraxinus pennsylvanica*) ash trees. 3.) There are individual and groups of ash trees that have a higher survivorship potential measured using canopy density, growth rates, and geographical location. 4.) Maintaining ash trees with treatment is more economically efficient than removing ash trees. The ash tree canopy density in cultivated areas was significantly different compared to that of non-cultivated areas. There were significant differences detected between white and green ash species. Both healthy outliers and groups of healthy trees were found using spatial analyses. The economic value lost from removal of parking lot shade ash trees from 2012-2015 was over \$713,000. The high value of economic loss suggests that treating ash trees is more economically efficient than removing ash trees unless the trees become a safety hazard. It is recommended that healthy outlier ash trees and healthy groups of ash trees be treated, and seeds be collected from these uniquely healthy ash trees. Ash trees in urban (cultivated) areas should continue to be treated to assess survivorship and potential tolerance to EAB, to off-set replacement and removal costs, and allow larger canopy trees to remain, due to their higher economic value, as long as possible in affected areas.

Effects of invasive plant removal on earthworm and microbial biomass (Poster)

Rachael Samm and Ben Miller

Invasive plants are widespread in many Indiana forests, and can profoundly alter native plant communities, decomposer communities, and other aspects of forest ecology. Our goal in this study was to investigate the effects of the experimental removal of multiflora rose (*Rosa multiflora*) and autumn olive (*Eleagnus umbellata*) on earthworm and microbial biomass. Previous studies have shown that both earthworm biomass and microbial biomass are decreased when invasive plants such as honeysuckle and buckthorn are removed, but it is not clear whether this is a pattern that extends to many other invasive plants. We tested the hypothesis that earthworm and microbial biomass would be decreased when either multiflora rose or autumn olive were removed from experimental plots at Koinonia Environmental Retreat Center, a property of Manchester University in Pierceton, Indiana. Experimental plots were set up in early successional forest in May 2016, and earthworm and soil microbe samples were collected in October 2016. Earthworms were collected and weighed after 48 hours in the lab. Microbial active biomass was estimated by measuring the activity of β -1,4-glucosidase in soil cores. Both earthworm biomass and microbial active biomass were lower in *R. multiflora* removal plot than in control plots, though only the microbial biomass result was statistically significant. Earthworm biomass was higher in *E. umbellata* removal plots than in controls, and microbial biomass was not affected; neither result was statistically significant. These early results suggest that multiflora rose may have a facilitative effect on earthworm and microbial communities. Further studies are needed to illuminate the mechanism for this possible effect and to explore whether multiflora rose removal affects other aspects of earthworm and microbial communities.

Eighteen new spider distribution records in Indiana

Brodrick Deno & Marshall Sparenberg

The recorded spider diversity in Indiana is not well known and stands far behind the level of diversity known from the surrounding Midwestern states. In an attempt to increase our knowledge of spider diversity within the state, we sampled Yellowwood State Forest, Morgan-Monroe State Forest, Hoosier National Forest, Goose Pond Fish and Wildlife Area, Dunes National Lakeshore, Glacier's End Nature Preserve, and various caves and other wooded areas throughout the state via the use of pitfall trapping, sweep netting, and hand collecting. Most

of this collecting was done between May-October of 2016. During this time period, we discovered eighteen new distribution records for the state, expanding the ranges for a multitude of species. These discoveries highlight the lack of knowledge about spider diversity in Indiana habitats. These data also reinforce the need to conserve our forests and other natural areas in order to better help preserve the diversity of organisms that has yet to be completely documented.

Evaluating the influence of plant species diversity on biogeochemical cycling in Great Lakes coastal wetlands (Poster)

Adam J. Ares and Kevin H. Wyatt, Ball State University

Freshwater wetlands throughout the Midwest are becoming increasingly colonized by invasive plants, leading to a decrease in local biodiversity. It is not yet clear the impact that invasive plant species will have on biogeochemical cycling in the Great Lakes coastal wetlands. The goal of this study was to examine how changes in plant community composition may influence biogeochemical cycling by altering the composition of dissolved organic carbon (DOC) available to heterotrophic bacteria. To do this, we inoculated DOC (exudates) collected from two native (*Carex crinita* and *Juncus effuses*) and two nonnative (*Phragmites australis* and *Typha x glauca*) plants (in a full factorial design) with a natural bacteria community, and measured decomposition in the laboratory for 16 days. Exudates from all plants degraded rapidly, but exudates containing nonnative DOC typically degraded more rapidly than exudates from native plants. Decomposition was most elevated in treatments with a combination of plant exudates (native and nonnative), suggesting a synergistic effect of multiple carbon sources on heterotrophic metabolism. Our results show that changes in plant community composition, brought about by invasive species, may have implications for biogeochemical cycling within Great Lakes coastal wetlands by altering the composition of carbon sources available for heterotrophic metabolism.

First assessment of soft-release translocation of wild-caught snakes (Poster)

Jillian Josimovich and Bruce Kingsbury; Indiana University – Purdue University Fort Wayne

Wildlife translocation involves moving animals in the wild from one location to another for conservation, mitigation, and/or research purposes. Translocations are often unsuccessful in that relocated animals may suffer higher mortality rates or exhibit different behaviors than controls, such as greater and more frequent movements. Soft-release is a variant of this technique that entails keeping animals in an outdoor enclosure at the release site temporarily in the hopes that they will acclimate to the new environment more readily than those that are immediately released. Here, we report on the status of an ongoing study investigating the utility of soft-releasing massasaugas (*Sistrurus catenatus*), a small, federally threatened rattlesnake. Venomous snakes are often relocated or killed due to potentially negative interactions with humans, so understanding what methods might be used to successfully relocate them is crucial. To our knowledge, past experiments that have attempted to soft-release snakes have only used captive-bred animals, and we are the first to explore whether this technique is effective for relocating wild-caught snakes. Since 2013, we have radio-tracked over 50 translocated and control massasaugas: 12 soft-released (i.e. held in enclosures for approximately two weeks prior to release), 10 hard-released (i.e. released immediately upon relocation), and 29 controls (i.e. released at site of capture). Massasaugas were located approximately three times per week between May and August, and once every three weeks between September and November. We recorded a snake's behavior and location at each sighting, and we are comparing measures of behavior and survival to assess the "success" of the translocation effort. This work is continuing until at least the spring of 2019 and constitutes some of the earliest research of the pros and cons of using soft-release to relocate wild-caught snakes.

Fishing for answers: Bluegill (*Lepomis macrochirus*) habitat preference not related to shoreline type in a small Midwestern impoundment (Poster)

Luke E. Bates, Adrienne C. Funderburg, and F. Collin Hobbs, Huntington University

Several studies have shown that fish change their habitat usage based on shoreline features, with preference for natural shorelines versus those heavily impacted by human use or modification varying between species and ecological context. Lake Sno-Tip, a small man-made lake located on the campus of Huntington University, IN, has two shoreline types – man-made cinderblock walls and natural wooded edges. In order to compare fish habitat usage between these shoreline types, we baited two large cage-style fish traps and set them 5-10m off

shore at ~3m depth, one adjacent to each shoreline type. Traps were retrieved 24hrs later, and each fish was identified to species, counted, its length measured, and released back into the lake. We repeated this sampling procedure five times, catching a total of 56 fish along the natural shoreline and 81 fish along the cinderblock shoreline. All 137 individuals were bluegill (*Lepomis macrochirus*), ranging in size from 12.0 cm to 20.1cm. No statistically significant difference was found in either fish abundance or size between the two shoreline types, though there was a trend towards higher abundance along the cinderblock shoreline. While further replication would strengthen the conclusions our study, the lack of significant differences in fish abundance that we observed in may ultimately be due to the small size and overall disturbed nature of Lake Sno-Tip, causing the natural and cinderblock shorelines to be comparable in habitat quality.

Ecology Section continued.....

Genetic and Geographic Variation in *Elymus Canadensis* (Poster)

T.J. Sullivan and Holly Roberts

Elymus canadensis (Canada wildrye) is native to the central plains of North America. Like many cool-season grasses, it hosts symbiotic, maternally inherited *Epichloë* fungal endophytes. Interactions between grasses and *Epichloë* endophytes can be mutualistic with the endophyte providing defenses against herbivores among other possible benefits, although the ecological effects of this specific symbiosis with *E. canadensis* is not well understood. The Geographical Mosaic Theory of Coevolution predicts that there will be variation in the strength of coevolution in a population based on the specific genotypes of the interacting species. In species that are coevolving, then, we would predict significant geographic correlations between the genotypes of both species. In this study, we describe genetic variation in *E. canadensis* between nine populations ranging from Texas to Minnesota using both microsatellite markers and chloroplast DNA sequence. *E. canadensis* exhibits significant spatial structuring between most populations. Understanding wildrye genetic and geographic variation may offer insight into the nature of the host-endophyte symbiosis.

Gray squirrels (*Sciurus carolinensis*) prefer foraging in urbanized habitat over adjacent woods on a Midwestern university campus (Poster)

Natalie G. Risser, Ellie E. Rife, F. Collin Hobbs, Huntington University

The rapid urbanization occurring in developed and developing countries has greatly influenced the distribution and types of food resources available to wild animals. Eastern gray squirrels (*Sciurus carolinensis*) are a common woodland mammal that are considered to be successful urban adapters, as they seem to quickly adjust their behavioral patterns to take advantage of new opportunities found in urbanized areas. We observed the relative abundance of gray squirrels and their preference for anthropogenic versus natural food sources in urbanized and adjacent wooded areas on the campus of Huntington University during October and November of 2016. To estimate the relative abundance we observed and counted gray squirrels for 2.5 minutes at each of 16 observation areas in wooded and urban habitats on five separate days. To determine preference for anthropogenic versus natural food sources we measured the giving up density (GUD) for natural (sunflower seeds) and anthropogenic (dry cat food) food sources mixed into buckets of sand. Two GUD buckets were placed in each habitat type for 24 hour intervals on five different dates. We found that gray squirrels have significantly higher relative abundances in urban areas (1.03 sightings per observation in urban versus 0.13 in woodland, $p=0.007$). When both food types were considered together, significantly more food was removed from GUD buckets in urban habitat (25.3% urban vs. 7.5% wooded, $p=0.048$). However no significant difference was found in type of food preference, though a trend towards preference of natural food was observed in both habitats (21.7% sunflower vs. 11.2% cat food, $p=0.25$). The higher relative abundances and foraging comfort of squirrels on our campus lend further support to their status as "urban adapters", as it appears squirrels prefer human modified habitats over adjacent natural areas.

Growth-climate relationships of tuliptree (*Liriodendron tulipifera*) in Eastern North America

David C. LeBlanc, Ball State University and Justin Maxwell, Indiana University

This study evaluated relationships between variation in climate and radial growth of tuliptree (*Liriodendron tulipifera*). Annual tree-ring width chronologies for tuliptree at 36 sites distributed across much of the species range were correlated with monthly and seasonal temperature, precipitation, and Palmer Drought Severity Index (PDSI). Positive correlations with precipitation and PDSI during the early growing season (May through July) were strongest and most spatially replicated. Negative correlations with temperature during these same months were weaker and less spatially replicated. These correlations indicate that site water balance during the early growing season has the strongest relationship with tuliptree growth. In general, evidence for relationships between temperature variables and radial growth was weaker. These results suggest future increases in temperature associated with climate change will have limited influence on growth of tuliptree unless this causes increased frequency or severity of early growing season droughts.

Ecology Section continued.....

Increased root-knot nematode suppression in endosymbiotic vs non-symbiotic morning glory roots

(Poster)

Lekeah Durden, Wang Dong, Keith Clay, Indiana University

Some species of morning glories (*Convolvulaceae*) form an endosymbiotic relationship with seed-transmitted fungal symbionts in the genus *Periglandula*. These fungi are known for production of high levels of ergot alkaloids within the plant, which may act as major resistance factors in plant-fungal defensive mutualisms. A previous study focused on allocation of these compounds throughout various plant tissues found that in *Ipomoea tricolor* the alkaloids are distributed to the root system. The agricultural pest, root-knot nematode (*Meloidogyne incognita*), prefers to colonize the youngest root tissues of their host plants, causing systemic damage to the host and often resulting in large crop losses. The aim of this study was to determine whether the presence of the fungal endosymbiont causes a change in root-nematode colonization and host plant fitness. We hypothesized that *I. tricolor* plants infected by the fungal endosymbiont (E+) will have lower numbers of nematodes colonizing their root system than non-symbiotic plants (E-). To test this hypothesis, seeds of morning glories either containing the endosymbiont or not were germinated in the greenhouse. Next, one-week old seedlings were exposed to soil infested with high densities of *M. incognita* nematodes (N+) or no nematodes (N-). Plants were grown for 3 weeks when root colonization was quantified and plant dry weight biomass determined. We found that the presence of the fungal endosymbiont decreased nematode infection by 50%, but there were no significant differences in plant biomass. These results increase understanding of the role of the fungal endosymbiont within the plant host to belowground biotic stressors, such as nematode herbivory. Future research using this system can decrease nematicide applications on important crops by creating a new approaches to deter herbivory, that are safer for the environment.

Influence of Forest Fragment Compositional and Structural Heterogeneity on Ground-Dwelling Arthropod Communities

Andrea L Myers, Jordan M Marshall. Indiana University-Purdue University Fort Wayne

Due to agricultural, urban, and suburban development, forests in the Midwest are often limited to small patches of habitat that are typically isolated. As such, forest conservation efforts are usually conducted by private land owners or organizations, which can be as disjunct as the forests themselves. As part of an established forest ecosystem, ground dwelling arthropod communities can be used as indicators of forest health. In this study, ground dwelling arthropods were collected from ten protected properties across northeast Indiana. Ground dwelling arthropods were periodically sampled via pitfall trapping in May and August 2016. Physical characteristics of the forests were measured to categorize forest structure and composition. Forest types were determined by tree importance values around trapping locations. Arthropod richness and diversity were compared across forest environmental and spatial characteristics. Eight of the ten forests were Sugar Maple types, which is the most common type in northeast Indiana. While there were variations in overstory composition, these forests are likely at the same successional stages (i.e. similar canopy closure, litter and fine woody debris deposition). Results suggest that ground dwelling arthropod communities are relatively similar across the properties. Findings from this study can be used to craft focused and deliberate conservation strategies for regional or state-level organizations. Because the majority of the forests were Sugar Maple types,

broad regional conservation strategies may be helpful for land owners. However, we also found Red Maple and Northern Red Oak forest types, so there is a need for tailored strategies for some properties.

Interspecific Comparison of Hantavirus Prevalence in *Peromyscus* from a Fragmented Agro-ecosystem in Indiana

Jacob L. Berl, **Elizabeth A. Flaherty**, and Robert K. Swihart, Purdue University, and Amy J. Kuenzi, Montana Tech, Butte, Montana

Hantaviruses are rodent borne pathogens that are the causative agents for hantavirus pulmonary syndrome (HPS) and hemorrhagic fever with renal syndrome (HFRS) in humans. Numerous hantavirus genotypes have been identified (e.g., Sin Nombre virus [SNV]), and their prevalence in rodent host populations varies spatially and temporally. Comparatively little is known about hantavirus prevalence within rodent populations from the Midwestern United States, where two species of native mice (prairie deer mice [*Peromyscus maniculatus bairdii*] and white-footed mouse [*P. leucopus noveboracensis*]) are dominant members of rodent communities. We sampled both species in central Indiana and tested individuals for presence of hantavirus antibodies to determine whether seroprevalence (% of individuals with antibodies reactive to SNV antigen) differed between species, or among different habitat types within fragmented agro-ecosystems. Prevalence of hantavirus antibodies varied significantly between species, with seroprevalence in prairie deer mice (21.0%) being nearly 4 times higher than white-footed mice (5.5%). Seroprevalence was almost 8 times higher within the interior of row-crop fields (37.7%) occupied solely by prairie deer mouse populations, relative to field edges (5.2%) where species occurred syntopically or adjacent forest habitat (6.1%) occupied solely by white-footed mice. Understanding species- or habitat-specific differences in hantavirus prevalence in rodents is important for informing human disease risk and management programs. In the fragmented Midwestern agro-ecosystem of this study, prairie deer mice appear to be the dominant hantavirus reservoir with particularly high seroprevalence in populations within the interior of row-crop fields.

Recent Fossils Of Non-Marine Mollusks (Gastropoda) From, Orhaniye-Guvence Basin and The Tarakli Basin, central Anatolia, Turkey

Belkasim Khameiss, Richard Fluegeman, , Eric Lange, and Randall Bernot, Ball State University, and K. Christopher Beard, University of Kansas

Nonmarine mollusk gastropods of the Orhaniye-Guvence and Tarakli Basins of central Anatolia were collected in 2012. Eighty-two shells were collected from a total of 27 localities, which contained four species: *Chondrula lycaonica*, *Metafructicola kizildagensis* sp.n, *Vitrea gostelii* sp.n, and *Zebrina detrita* belonging to three families. The most abundant family was, *Enidae* because their habitat type is adapted to a range of altitudes. The nonmarine mollusks of Turkey are not highly endemic- most of these species are abundant throughout the Mediterranean Sea. The aim of this research was to perform a similar survey of these (invertebrate) animals living around, and identify them in these basins.

Roadkill hotspots in the Dunes National Lakeshore (Poster)

Giesela Pierce, **Vanessa S. Quinn**, Purdue Northwest

Dunes National Lakeshore is a unique ecosystem with high vertebrate diversity. In addition, its location in urban surroundings create a unique problem for the conservation of the wildlife in the Lakeshore. We began this research project with the objective of establishing roadkill hotspots within the Lakeshore. Roadkill hotspots are areas where there is a high density of terrestrial vertebrates being killed in vehicular collisions. We recorded the GIS coordinates of vertebrate roadkill on State Highway 12 in Northwest Indiana in the summer of 2015 and 2016. We photographed the roadkill for future identification. We identified roadkill hotspots and produced an ARC-GIS maps to identify the habitat types most closely related to the location of roadkill hotspots.

The Effects of Secondary Microplastics on the relationship between a trematode parasite, *Schistosoma mansoni*, and its intermediate and definitive hosts (Poster)

Elizabeth M. Reinhart, Purdue University

Plastic materials have generated many societal benefits, however the 'plastic age' has come with downsides. One issue of ecological concern is the accumulation of plastics in the water cycle, and their breakdown into smaller plastics. When plastics breakdown, added chemicals leach into the surrounding waters. One common

chemical, Bisphenol A (BPA), can imitate estrogen. It binds to estrogen activation sites and disrupting the endocrine system. BPA has been shown to induce precocious puberty and other reproductive disorders, however it is not known how it affects the host-parasite relationship. To elucidate the effects, I exposed *Biomphalaria glabrata* snails infected with a trematode parasite, *Schistosoma mansoni*, to multiple treatments; the current average level of BPA found in nature today (.05 µg/L) 100 times the current level (5.0 µg/L of BPA) and lastly a control group (well water). How the chemicals affect the parasites transmission ability is of particular concern, because the next stage is capable of infecting humans. To measure transmission, the number of parasites shed from the snail in response to UV light was measured. Parasite abundance was similar for the group exposed to a higher level of BPA, and the control group, however the group exposed to current levels of BPA had a significantly higher parasite output. This suggests that there is a zone of BPA and therefore microplastic abundance that induces a higher level of transmission for *S. mansoni*.

Ecology Section continued.....

The Taylor University Arboretum Hardwood Reforestation Effort: The Ninth Year Results of Seedling Species Selection, Planting Method, and Deer Herbivory (Poster)

Robert T. Reber and Michaela L. Hecox, Taylor University

In 2008 a 5-hectare oldfield was selected in the Taylor University Arboretum for a hardwood reforestation effort. Planting of hardwood species occurred in the spring of 2008 and 2009. In 2008, 1,000 seedlings were planted. *Quercus alba*, *Q. bicolor*, *Q. imbricaria*, *Q. macrocarpa*, *Q. muehlenbergii*, *Q. rubra*, *Gymnocladus dioicus*, and *Liriodendron tulipifera* were the species selected. 800 of these seedlings were planted in a randomized complete block design that included all species and two planting treatments: a traditional method, using a shovel; or a dibble bar. *Q. rubra* and *Q. macrocarpa* seedlings were planted in a border around the planting, and 60 exclosures were placed around randomly selected border seedlings. In the spring of 2009, an additional 200 *Q. macrocarpa* and 200 *Q. bicolor* seedlings were planted in an adjacent block. Exclosures also were placed around randomly selected seedlings in this block. For the past nine years, research has been conducted to quantify the success of the two planting methods, the survival and growth of hardwood species, and the value of exclosures in reducing deer browse. Measurements of seedling height, seedling diameter, and extent of deer herbivory have been taken every year (beginning of growing season, end of growing season, and mid-autumn). In the original block the first growing season survivorship was significantly greater for *Q. macrocarpa* and *Q. bicolor*. These species continue to attain the highest survivorship through the 2016 growing season, *Q. macrocarpa* (96%) and *Q. bicolor* (89%). *Q. rubra* has the lowest survivorship through the 2016 growing season. In the first growing season, the planting method did not exhibit a significant effect on survivorship of the seedlings. After the ninth growing season, survivorship has been significantly higher for seedlings planted using a shovel (59% shovel, 48% dibble bar). *Q. macrocarpa* and *Q. bicolor* exhibited the greatest height growth during the first growing season, and they also have grown well the last few growing seasons. During the 2016 growing season, *Q. macrocarpa*, *Q. alba*, and *Q. muehlenbergii* produced the greatest height growths, respectively. Deer herbivory has been highly variable from mid-autumn through late spring. In some years it significantly affected height growth the following growing season. The exclosures significantly decreased deer browse in the original border seedlings in the winter of 2015. The exclosures had less effect on deer herbivory in the 2009 block.

Tracking Movements of Cerulean Warblers (*Setophaga cerulea*) in Southern Indiana

Clayton D. Delancey, Garrett MacDonald, Claire Nemes, and Kamal Islam, Ball State University

The Cerulean Warbler (*Setophaga cerulea*), a Neotropical migratory songbird, is listed as a 'Species of Special Concern' by the U.S. Fish and Wildlife Service and state-endangered in Indiana. We have been monitoring Cerulean Warbler breeding populations in Yellowwood and Morgan-Monroe state forests since 2007 as part of a 100-year project called the Hardwood Ecosystem Experiment. This long-term study aims at determining the effects of different forest management techniques on plant and animal communities. Based on previous research, many mature forest-dependent Neotropical migrant fledglings move from mature forest habitat into areas of thick vegetation such as clear-cuts. It is believed that Cerulean Warblers, in particular, fledglings, do not follow this pattern. We are interested in determining where fledgling Cerulean Warblers disperse to after leaving their nests, but before migrating to their wintering grounds. Here we present new information on fledgling

movements through radio-telemetry studies and discuss upcoming research on Cerulean Warblers in Indiana. Information gathered from our research can potentially be used to suggest forest management prescriptions that provide Cerulean Warblers with all of their required habitat needs throughout the breeding season.

Urban Pollinator Diversity and Pollinator Movement Between Wetlands on the IU South Bend Campus
Sara Tabner, Deborah Marr, and Andrew Schnabel, Indiana University South Bend

In recent years, a decline in pollinators, including honeybees and native insects, has been a major concern. We studied pollinator diversity and behavior in urban wetlands to better understand how urban landscaping can improve habitat for pollinators. In the summer of 2016, we estimated pollinator diversity and frequency of floral visitors to nine plant species. We also tracked pollinator movement between plants and between wetlands separated by approximately 230 meters of mowed lawn. Pollen tracking was done by adding fluorescent dye to anthers of focal plants. We identified over 50 taxa from a 24-hour collection from the two wetland areas. *Filipendula rubra* and *Apocynum cannabinum* attracted the highest frequency of floral visitors over a one-month period. Beetles, flies and bees accounted for the majority of floral visitors. The range of pollen distance travelled within wetlands for *Rudbeckia hirta* was 0.04 to 23.4 meters and 0.7 to 26.1 meters for *Asclepias incarnata*. We found no evidence that pollinators travelled between the wetland areas with the exception of one 250 meter pollen transfer in *Asclepias incarnata*.

Using geolocators to examine seasonal movements and migratory connectivity of the Cerulean Warbler (*Setophaga cerulea*), a declining migratory songbird that breeds in Indiana (Poster)
Garrett J. MacDonald, Clayton D. Delancey, and Kamal Islam, Ball State University

Populations of many Nearctic-Neotropical migratory birds are declining. Historically, research has focused on threats on the breeding grounds, but the need for full life cycle monitoring has recently gained attention. Individual light-level data loggers, or geolocators, have become an increasingly reliable and affordable tool for examining the seasonal movements of individuals across broad spatial and temporal scales. The Cerulean Warbler, *Setophaga cerulea*, dependent on mature Eastern deciduous forest during the breeding season, has declined about 3% per year since 1966. It is considered "State Endangered" in Indiana. During Spring 2016, we used a canopy net in combination with audio playback of conspecific song and calls, and a decoy, to capture nine male Cerulean Warblers at our study sites in southern Indiana. These individuals were outfitted with geolocators. We aim to retrieve geolocators in Spring 2017 using the same methods previously used to capture males. Analysis of geocator data will provide information on the movements of individuals throughout the year, allowing us to identify important staging and wintering areas and to begin to elucidate the amount of migratory connectivity shown by the Indiana population. Additionally, these efforts will aid in the conservation of the species both in Indiana and throughout its range by informing conservation actions on staging areas where birds 'refuel' as they make their annual migrations, and on the species' wintering grounds.

Using Isotopes to Trace Nitrogen Assimilation in parasitized *Physa acuta* snails (Poster)
Nicole Chodkowski and Randall J. Bernot

Parasites are infectious agents found throughout all organisms, communities, and ecosystems. Despite parasitism being the most common consumer strategy, the effects of parasites on host nutrient recycling is largely unexplored. Stable isotopes have been used to identify dietary signatures of consumers, trophic positions, and species interactions within an ecosystem. Trematode parasites grow and develop within a mollusk intermediate host and directly feed on host tissues. Given that parasitized hosts maintain a higher required metabolism, nutrient assimilation may differ between parasitized and non-parasitized individuals. We fed ¹⁵N-enriched algae to parasitized and non-parasitized freshwater *Physa acuta* snails collected from the White River in east-central Indiana to trace the assimilation rate of nitrogen through host and parasite tissues over 10 days. Snails ranged in size from 8.0 to 12.5 mm and were dissected to separate snail foot and gonadal tissue from trematode sporocyst, rediae, and cercariae. The samples were dried at 60°C and analyzed at the Marine Biological Laboratory. We hypothesized that parasitized snails will assimilate nitrogen at a faster rate relative to the uninfected treatment. Despite a lot of variation, parasitized individuals have greater ¹⁵N signatures than non-parasitized individuals for all tissue types. These differences in assimilation rates may have important consequences that could help explain the mechanisms of changes in ecosystem nutrient dynamics.

Using predator vision to link trophic levels

Ashley L. Kissick, Jeffrey D. Holland, John B. Dunning, Jr., Patrice Baumhardt, and Esteban Fernández-Juricic, Purdue University

Avoiding predator detection through different mechanisms (e.g., background matching, disruptive coloration) allows many invertebrates to trick the predator's eye and reduce mortality. Here we examine how predator-prey interactions impact community structure with a new approach (i.e., perceptual modeling) that considers the visual conspicuousness of prey from the visual perspective of an avian predator. We used a multitrophic community consisting of wood-boring beetles, their generalist beetle predators, and insectivore birds with a violet- (VS) and ultraviolet- (UVS) sensitive visual system. We then tested whether variations in visual conspicuousness of beetles with backgrounds modulate the degree to which bird abundance impacts beetle abundance. We found significant relationships between the abundance of highly conspicuous prey and avian predators with a violet-sensitive (VS) visual system. These relationships were strongest when we compared predator and prey groups that utilize deadwood as part of their ecological function. Overall we suggest that this novel approach should also include species ecological function to examine predator-prey interactions at the community level.

Ecology Section continued.....

Utilizing Environmental DNA (eDNA) for the Detection of the Eastern Massasauga (*Sistrurus catenatus*) (Poster)

Jessica L. Merkling and Mark A. Jordan, Indiana University-Purdue University Fort Wayne, Jennifer Moore, Grand Valley State University, Sara Syswerda and A.J. Smith, Pierce Cedar Creek Institute

The detection of organisms from DNA found in environmental samples (eDNA) is a relatively new and increasingly popular technique that is providing opportunities to monitor species that are rare and secretive in behavior. The Eastern Massasauga (*Sistrurus catenatus*) is a species that could benefit from the development and utilization of eDNA methods. Recently elevated to threatened status under the Endangered Species Act, this small, cryptic rattlesnake occupies shallow wetlands in the Great Lakes region (including Indiana). We describe a protocol for detecting the presence of massasaugas using water collected from crayfish burrows that are used by snakes as overwintering sites. Using quantitative PCR (qPCR), we will demonstrate the specificity of primers and probes for *S. catenatus* with respect to other co-occurring snake species at a study site in Michigan. Preliminary assessment of the protocol will be presented on burrow water and soil samples collected in known localities that are both used and not used by *S. catenatus* for overwintering. The development of a verified eDNA assay for the Eastern Massasauga will provide a useful monitoring tool for a species that is traditionally difficult to survey and it will serve as a case study for the possible future application of the eDNA method in other species that utilize crayfish burrows in nature.

Utilizing GIS to Locate Endangered Gravel Hill Prairies of the Wabash River Valley

Ryan Schroeder and Darrell Schulze, Purdue University, Derek Luchik, The Nature Conservancy, and Bob Easter, Gus Nyberg, and Brad Weigel, NICHES Land Trust

The Gravel Hill Prairies (GHP's) of the Wabash River Valley are an endangered ecosystem in the state of Indiana and provide optimal growing conditions for a number of state endangered plants. Currently only four remnants are known to exist near Lafayette, IN, found by a previous study conducted in 1980 by Post, Bacone, and Aldrich (Proceedings of the Indiana Academy of Science, 1984, vol. 94: 457-464). These unique ecosystems have been found to occur almost exclusively on soils classified as Rodman Gravelly Loams and Strawn-Rodman complexes which occur predominantly along the outwash terraces of the Wabash River and its tributaries. Seven Indiana-state endangered plant species, more typical of western shortgrass prairies, are documented on these soils. This research effort aimed to develop GIS maps to scout for and discover areas of unknown GHP remnants. The end goal of the project is to assist conservation groups in the development of a strategy to preserve previously undiscovered remnants. This project relied on spatial analyses with Geographic Information Systems (GIS) and numerous databases including the USDA's gridded Soil Survey Geographic (gSSURGO), which had not been used previously to develop maps and scouting plans. Analysis have identified a total of 972 areas of interest, of which 53 have been visited and had preliminary plant lists put together. These field scouting trips have found five fragments of gravel hill prairie remnants along the Wea Creek and the Wildcat Creek in Tippecanoe Co., one remnant fragment along Bee Run in Warren Co., and numerous high quality open-oak woodlands. These fragments are at varying stages of plant community degradation, however, one of the fragments along the Wea Creek contains the state endangered aromatic aster (*Aster oblongifolius*).

Entomology Section

A newly discovered horned spider in the genus *Epiceraticelus* (Araneae: Linyphiidae)

Michael L. Draney, University of Wisconsin-Green Bay, Marc A. Milne and **Gabrielle Madriz**, University of Indianapolis, Michael Ulyshen, Southern Research Station, U.S. Forest Service, Athens, GA

Most newly described arthropods are found in tropical countries because these locations are immensely understudied while new North American arthropod species are less common due to the history of concentrated research in temperate habitats. Usually the discovery of a new species of arthropod in North America is because it is remarkably small or it is found in a rare, hard-to-reach area (e.g. caves). This new species is no exception to this phenomenon, as it measures approximately 1.2 mm in length. The spider genus *Epiceraticelus* (Araneae: Linyphiidae) was originally described by Crosby and Bishop in 1931 with only one species: *Epiceraticelus fluvialis*. With the discovery of this second species, we have expanded *Epiceraticelus* to include two species. The new species, *Epiceraticelus mandyhowei*, was discovered in the mid-2000's and is named in honor of the late arachnologist, Mandy Howe. It is only known from Virginia and South Carolina, is winter-mature, and has been collected from October – April. The male possesses a modified carapace with a long horn between its eyes, which is likely used during mating. Additionally, pores have been found on the horn which likely function to produce a substance on which the female may feed during mating.

Analysis of Feeding behavior of Soybean thrips infected with *Soybean Vein Necrosis Virus* (Poster) **Geral'n McGee, Sarah Moh, Jinlong Han, Vamsi Nalam and Punya Nachappa**

Thrips are economically important plant pests. Not only does the thrips feeding on the crops cause damage but they also serve as vectors for plant diseases such as Tospoviruses. These viruses cause disease that damage crops and lead to severe yield loss all around the world. Studies with tospovirus, *Tomato Spotted Wilt Virus* have shown that virus infection changes the feeding behavior of thrips, causing them to probe three times as much as uninfected thrips. The tospovirus, *Soybean Vein Necrosis Virus* is an emerging disease of soybean, which is vectored by soybean thrips (*Neohydatothrips variabilis*). The objective of this study was to determine if infection with SVNV causes changes in the feeding behavior of soybean thrips vector. The feeding behaviors of thrips, either infected or uninfected with SVNV, were monitored using the electrical penetration graph (EPG) technique. Electrical penetration graphs (EPGs) record electrical signals or waveforms when mouthparts of piercing-sucking insects, such as thrips, probe into a plant. Different feeding activities produce different waveforms which can be analyzed to determine insect feeding patterns. Our results show that feeding behavior of infected thrips was significantly different compared to uninfected thrips. The most common probe type for the infected thrips was non ingestion probes. Non ingestion probes are not beneficial for the thrips because they are not ingesting the cell content but, non ingestion probes are beneficial for the virus because they allow the virus to be transferred. These results emphasize the coordinated evolution between tospoviruses and their thrips vector.

Bees and Peas: How Apiology Shaped Gregor Mendel's Thinking **Gene Kritsky, Mount St. Joseph University, Cincinnati, OH**

It has long been suggested that Gregor Mendel's methodology to study plant hybridization may have been inspired by the contributions of Johann Dzierzon, a Polish-born Catholic priest and an avid apiologist. Among Dzierzon's many contributions was his discovery in 1835 that male honey bees developed through parthenogenesis. Dzierzon tested his discovery by crossing different strains of honey bees and determining the ratios of the drone bees produced from hybrid queens. He published his theory in 1845 and elaborated on it again in 1854, the year Mendel started his genetics research at the Augustinian Monastery of St. Thomas in Old Brno. Dzierzon's findings were highly controversial and not firmly established during his lifetime. Mendel was exposed to beekeeping as a child, and he kept bees at the monastery, but did not focus on apiology until 1868, after he was elected abbot. He built a bee house, designed a new bee hive, crossed different honey bee strains, examined honey bee foraging, and dealt with foulbrood disease. Unlike his research on plant hybridization, Mendel's apicultural research was respected and his views on the subject were held in high regard by his contemporaries.

Biogeochemical Interactions Between an Invasive Scarab (*Popillia japonica* Newman) and its Subterranean Environment

Garrett Y. Price, Brittany F. Peterson, Michael E. Scharf, Matthew D. Ginzler, and Douglas S. Richmond, Purdue University

The goal of this project is to gain a better understanding of the interactions between invasive Japanese beetle (JB) larvae and their subterranean environment. In support of this goal, we quantified the impact of JB larvae on soil microbial activity (CO₂ flux) and functional diversity (phospholipid fatty acid analysis) in soil microcosms and characterized the microbiota of 1st and 3rd instar larval guts via 16S rDNA sequencing. Findings suggest that JB larvae cause significant and lasting changes to soil microbial diversity and activity; weakening soil fungal and protozoan populations and shifting microbial populations toward bacteria. These changes resulted in increased microbial activity, potentially liberating larger amounts of CO₂ from the soil. Analysis of 16S microbial rDNA revealed taxonomic and functional differences in the microbiota of 1st and 3rd instar larval guts with ammonia oxidizing, nitrogen fixing and organic residue-degrading gut endosymbionts dominating the community and a notable shift toward taxa that are commonly associated with the soil as larval development proceeded. Results suggest that JB infestations may reduce soil organic matter and increase soil CO₂ emissions and imply that interactions between JB larvae and the soil are, in part, microbially-mediated, which could have implications for pest management.

Characterization of the nature of Rag5 mediated resistance to soybean aphids

Kumud Joshi, Indiana University Purdue University Fort Wayne

Soybean (*Glycine max*) is a legume native to Asia but is very popular around the world, especially in the US. In 2016, 4201 million bushels of soybean was planted in the US. The cultivation of Soybean has been severely hampered by the introduction of Soybean aphids (*Aphis glycines*) in the year 2000. The damage caused by soybean aphids accounts to \$3.6-\$4.9 billion annually. Popular management strategies for soybean aphids include scouting and insecticide application on plants that have surpassed the economic threshold of greater than 250 aphids per plant. This method has been effective for soybean aphid management but has several disadvantages such as harmful environmental effects, intensive labor and costs. Host plant resistance is a cheap and economic method of soybean aphid management. The discovery of soybean Rag or Resistance against *Aphis glycines* genes has opened gateways for the discovery of better aphid management techniques. The mechanism of Rag-mediated genes is not fully understood. The plant introduction PI 567301B containing the Rag5 gene exhibits strong non-preference (antixenosis) resistance towards soybean aphids. Preliminary studies on soybean Rag5 shows that soybean aphid performance on Rag5 whole plants is very poor as compared to detached leaves suggesting that the antixenotic response of this plant introduction (PI) is systemic. The objective of this study is to determine the source and nature of Rag5 resistance. By utilizing a variety of techniques including the use of electrical penetration graphs to analyze aphid feeding behavior, grafting assays to determine source of resistance coupled with the analysis of xylem and petiole exudate, we hope to further characterize the nature of resistance contributed by the Rag5 in PI567301B.

Cumulative oxygen consumption during development of two postharvest pests (*Callosobruchus maculatus* Fabricius and *Plodia interpunctella* Huebner) (Poster)

Hannah E. Quellhorst, Scott B. Williams, Larry L. Murdock, and Dieudonne Baributsa, Purdue University

Insect pests cause substantial losses during postharvest storage. Major insect pests of stored grains include the Indian meal moth (*Plodia interpunctella* Huebner), the maize weevil (*Sitophilus zeamais* Motschulsky), the cowpea bruchid (*Callosobruchus maculatus* Fabricius), and the larger grain borer (*Prostephanus truncatus* Horn). Hermetic storage is among the most successful solutions being used by smallholder farmers in Africa and Asia, to protect harvested grain from insect pests. With the increased use of hermetic technologies comes a need to understand the biology of specific storage pests and their responses to hypoxia. We evaluated the effectiveness of a novel non-invasive method (the OxySense 5250i) in measuring the lifetime oxygen consumption of the cowpea bruchid (CPB) and the Indian meal moth (IMM). Results show that oxygen consumed by the CPB during its larval development period is not significantly different from those previously recorded using the Mocon Pac Check 325. An IMM insect (over its larval developmental period) consumes approximately three times the amount of oxygen compared to the CPB. However, daily oxygen consumption of both CPB and IMM are not different. These findings add to our understanding of insect physiology under hermetic conditions, and allows for an assessment of the effectiveness of hermetic technology and its ability to provide protection against major postharvest insect pests.

Diurnal Oviposition of Blow Flies and DNA Identification of Early Arrivers

Janelle Bouman, Kelly Braun, Moriah Carmel, Valparaiso University, Raenah Bailey, Kristi Bugajski, and Beth Scaglione-Sewell

Blow flies (Diptera: Calliphoridae) are usually the first insects to oviposit (lay eggs) on carrion. The timing of blow fly oviposition is critical for determining a postmortem interval (PMI) estimation, which is the time that has passed between death and corpse discovery. The objective of this investigation is to gain more information about the timing of blow fly oviposition so that a more accurate PMI can be calculated. Past research in our lab has shown that blowfly oviposition occurs an average of 4.75 hours after sunrise. This year's research expanded on previous studies by placing three piglets in a remote, wooded area an hour after sunrise. The piglets were checked once an hour until oviposition occurred, and it was recorded whether flies and eggs were present each hour. Egg masses were collected. DNA analysis and BLAST were used to identify the individual blow fly species. The timing of oviposition, in hours after sunrise, was analyzed with respect to temperature, humidity, and light intensity. The research was repeated six times in the fall of 2016. Flies were first seen an average of 2.3 hours after sunrise, and oviposition was observed an average of 4.16 hours after sunrise. The average lux reading at the time of oviposition was 26,755 lux, but ranged between 5,790-52,300 lux. This research has importance in both the scientific and forensic communities, as a more accurate PMI can assist with the validity of a forensic investigation.

Factors Affecting the Population Dynamics of Thrips Vectors of Soybean Vein Necrosis Virus in Indiana
(Poster)

Jonathan Danielson, Tim Loeffler, Stacy Keough, Jordan Marshall and Punya Nachappa

Soybean Vein Necrosis Associated Virus (SVNV) is an emerging *Tospovirus* that was first discovered in soybean fields in Tennessee in 2008. The disease was confirmed in Indiana in 2012. *Tospoviruses* including SVNV are exclusively transmitted from plant-to-plant via insect species called thrips. There is no published information on the factors influencing population dynamics of thrips-infesting soybean and spread of SVNV. The present study was undertaken to better understand weather effects on population dynamics of the primary vector, *Neohydatothrips variabilis* and secondary vectors, *Frankliniella tritici* and *Frankliniella fusca* in 2013 and 2014. Specifically, we evaluated the influence of temperature and precipitation on seasonal abundance of the abovementioned thrips species caught using suction traps from the North Central Regional Soybean Aphid Suction Trap Network located in soybean fields throughout the state of Indiana. Our results show that population of *Neohydatothrips variabilis* peaked between 1-16 August each year. *F. tritici* populations peaked between 1-16 June and a second peak was observed in 1-16 August. *Frankliniella fusca* populations remained low but constant throughout the growing season. Approximately, 25% of the variation in population dynamics of *F. tritici* and 4% of the variation in population dynamics of *F. fusca* can be explained by temperature. Temperature did not affect *N. variabilis* population. Precipitation did not impact either of the three thrips species. We hypothesize that variation in the population dynamics may be due to differences in host plant preferences of the three species. *Neohydatothrips variabilis* is monophagous, feeding mainly on soybean whereas, *F. tritici* and *F. fusca* are polyphagous colonizing many broad-leaved host plants, grasses, and maize during the growing season. Hence, soybean crop distribution, abundance, maturation and senescence likely has larger impact on *N. variabilis* population compared to weather variables. Beyond weather, factors including, agricultural practices, initial thrips population size, thrips behavior, presence of predators and parasites, can affect the number of thrips captured in suction traps. Hence, future studies should include additional parameters beyond temperature and precipitation.

Five recently found undescribed spiders and their mates from Indiana forests

Marc Milne, Brodrick Deno, and Marshall Sparenberg. University of Indianapolis

The extent of spider diversity in Indiana is not well known and lags far behind the level of diversity known from surrounding Midwestern states. In an attempt to remedy this disparity, over the past three years we sampled Yellowwood State Forest, Morgan-Monroe State Forest, Glacier's End Nature Preserve, and various Indiana caves through the use of pitfall trapping, sweep netting, and hand collecting. In addition to discovering many new spider distribution records for the state, we also found five undescribed species or undescribed matching sexes of known species. These discoveries highlight the lack of knowledge about spider diversity in Indiana's forests and the need to conserve these forests and areas like them to preserve diversity about which we may not even be aware.

From Ecoblitz to Identification Blitz: Report on the Beetle (Insecta: Coleoptera) Diversity in Morgan-Monroe and Yellowwood State Forests

Glené Mynhardt, Hanover College

The Indiana Forest Alliance (IFA) is a non-profit organization dedicated to the protection and well-being of Indiana's native forests. One of the IFA's major initiatives includes a comprehensive, multi-year inventory, known formally as an "Ecoblitz," which has been conducted in a 900-acre area in the Morgan-Monroe and Yellowwood State Forests. Taxonomic experts from both academic and non-profit institutions have been aiding the IFA in establishing inventories of the flora and fauna that exist in Indiana's forests, with the goal of establishing baseline diversity estimates as well as documenting the health of these forests. Of particular interest has been the relative species richness of insects, which can serve as important ecological indicators of forest health. Since 2014, insects have been collected by hand and passive collecting methods (Lindgren funnels [2014-2015], Malaise traps [2016]). Due to the large volume of specimens collected, only a report on Coleoptera (beetles) is presented. As of 2015, close to 200 beetle species have been identified, with an additional 60+ species identified for 2016. Species identification is ongoing. Data from three years of the Ecoblitz suggests that collecting efforts should continue and that these efforts be increased over time, space, and in frequency. In addition, involvement in large-scale survey projects like the IFA Ecoblitz offers prime opportunities for undergraduate student engagement and training in the important skills of insect identification, alpha-taxonomy, and basic morphology.

Geographical range and morphological diversity of the brown small minnow mayfly, *Baetis brunneicolor* (Insecta: Ephemeroptera: Baetidae) (Poster)

Sabrina Schipper and Luke M. Jacobus, Indiana University Purdue University Columbus, **Jessica Fang**, Environmental Monitoring and Data Management, Restoration & Infrastructure, Toronto and Region Conservation Authority, Toronto, Ontario, Canada

Baetis brunneicolor, also known as the brown small minnow mayfly, is a species found throughout central and eastern North America, but it is only sparsely represented in the western and far northern parts of the continent. Historically, it has not been found north or west of the prairie provinces of Canada. New data from the Yukon Territory extend its range considerably to the northwest. *Baetis brunneicolor* is part of the *B. vernus* group, which also includes *B. hudsonicus* and *B. bundyae* in North America. Although the *B. vernus* group globally may contain several morphologically cryptic but molecularly distinct taxa, *B. brunneicolor* currently appears to be a monophyletic taxon that is distinct using both lines of evidence. Morphological variability, primarily restricted to color differences, appears to be consistent across its range. The species is identifiable as both a nymph and as a male adult. This makes it one of only a few *Baetis* species that can be studied with some confidence in North America.

Quantification and localization of Soybean vein necrosis virus in its thrips vector, *Neohydathothrips variabilis* (Poster)

Jinlong Han, Vamsi Nalam, and Punya Nachappa, Indiana University-Purdue University, I-Chen Yu, Indiana University School of Medicine- Fort Wayne

Soybean vein necrosis virus (SVNV) is an emerging viral disease affecting soybean. Currently, SVNV is the most prevalent virus in soybean production in the United States. SVNV has been identified as a new species in the genus *Tospovirus*, which are exclusively transmitted by thrips in propagative manner. Soybean thrips, *Neohydathothrips variabilis*, is the only confirmed vector of SVNV. In this study, SVNV acquisition by first instar *N. variabilis* larvae fed on SVNV-infected plants after 6, 12, and 24h Acquisition Access Period (AAP) was investigated, and the dynamics of virus replication within infected thrips was analyzed. Immunofluorescence microscopy was used to show that *N. variabilis* acquired SVNV after 6, 12, and 24h AAP and replication occurring in the various tissues of the alimentary canal and salivary glands as indicated by immunodetection of the nucleocapsid protein (NP). Preliminary results suggests that the midgut epithelial cells are the initial sites of entry for SVNV and that the virus subsequently infects the surrounding muscle cells and eventually the salivary glands. SVNV was able to infect alimentary canal after 6h of AAP but, interestingly, unable to infect salivary glands, which is a pre-requisite for successful transmission of the virus. Hence, successful transmission not only depends on virus acquisition but also replication in the thrips vector. In the long-term, results of this study will increase fundamental and applied knowledge of the biology of thrips vector that affect the epidemiology of SVNV.

Research resources from the Purdue Entomological Research Collection

Luke M. Jacobus, Indiana University-Purdue University Columbus, G. Nearns and J. Zaspel, Purdue University

The Purdue Entomological Research Collection (PERC) is the largest and most important collection of insect specimens in the state of Indiana. Since its establishment in 1896, the PERC acquired numerous historical collections of great significance to the systematic entomology community. The W.S. Blatchley collection, primarily comprised of Hemiptera, Orthoptera, and Coleoptera, is one of the most important historical components of the PERC. The E. B. Montgomery Odonata and George F. Edmunds Ephemeroptera collections are also internationally recognized as noteworthy holdings; the PERC houses the world's largest and most comprehensive collection of Ephemeroptera (mayflies). The PERC also serves as a repository for IDNR, USDA, and CAPS survey specimens and other voucher material from Purdue's forensics program and other organismal research projects. The PERC currently contains approximately 750,000 pinned specimens, 920,000 specimens in vials, 28,000 slides, and over 11,000 dragonflies and damselflies in archival envelopes. The PERC houses over 2,500 primary and/or secondary type specimens of insects. A recently funded National Science Foundation grant will update the existing storage systems in the collection to ensure they are preserved for future research and education. In addition, specimen images and associated data will be stored in an online database freely available to the public. This project will raise awareness of the importance of insects and natural history collections through various public engagement activities, aquatic ecosystem workshops, and rural community youth programs.

The Reproductive Success of Soybean Aphids (*Aphis glycines*) on Drought-Stressed Soybean Plants Affected by The Endosymbiont *Arsenophonus*. (Poster)

Asif Mortuza, Tyler Shuman, Punya Nachappa, Indiana University-Purdue University Fort Wayne

Arsenophonus are mutualistic bacterial endosymbionts that are found in insects from a diversity of insect taxa including aphids. The function of these bacteria in their aphid hosts remains unclear. The soybean aphids are a serious pest of soybeans in North Central United States. The insect is native to Asia and exotic to North America. They reproduce both sexually and asexually and give live birth. The goal of this study was to investigate whether presence of *Arsenophonus* provides a reproductive advantage to soybean aphids during drought stress. Soybean plants subjected to drought stress and well-watered conditions (Control) were populated with soybean aphids with *Arsenophonus* (ARS +) and aphids without *Arsenophonus* (ARS -). The results show that both ARS (+) and ARS (-) aphids did the best under well-watered conditions. ARS (+) and ARS (-) aphid population numbers were reduced on drought-stressed plants with ARS (-) having the lowest reproductive success. This suggests that the presence of *Arsenophonus* provides reproductive advantage to aphids during drought stress, but the exact mechanism is unknown and will need further investigation. Our study will increase our understanding of the endosymbiotic relationship between the *Arsenophonus* bacteria and the aphids which can be crucial for controlling aphids on soybeans.

Engineering Section

Combined Sewer Overflow Abatement, Lafayette and West Lafayette, Indiana, A continuing Story **Victoria Leffel** and Terry West

Applied Geology students under Dr. West's direction have studied the progress made to reduce combined sewer overflow (CSO) in Lafayette, West Lafayette and Purdue University. Kathryn Bosserman (2014) studied the combined sewer overflow problem of the Wabash River in Tippecanoe County. She outlined the areas in both Lafayette and West Lafayette where combined sewers were located. New road construction in southeastern Lafayette and the elimination of combined sewers elsewhere will direct storm water to a retention pond (Durkee's Run). After settling occurs, water will be discharged directly to the Wabash River, bypassing the sewage treatment plant. Earlier a large diameter storage tunnel under Second Street was completed that will hold up to 1.32 million gallons to be processed in due course through the treatment plant. She also addressed Best Management practices (BMPs) to reduce or delay runoff. Ashley Murray (2015) made a detailed study of the progress of CSO abatement in Lafayette. She noted the BMPs accomplished in the area: permeable pavements in north Lafayette, a green roof garden on the Civil Engineering Building at Purdue, biowales and a detailed analysis of the Durkee's Run storm water park. The proposed \$179 million capital improvement on CSO projects was outlined. In 2016 Victoria Leffel reviewed the construction of a 96 inch diameter storm sewer, extending 4300 feet along River Road in West Lafayette, ending at the sewage treatment plant. It will eliminate

two overflow points where storm water is discharged directly into the Wabash River in peak runoff periods. The pipe holds 1.2 to 1.4 million gallons. At present there are about 40 overflow events per year in West Lafayette. She also discussed the Wet Water Treatment facility that stores storm water to be processed later for treatment. It has the capacity of 58,000 gallons. The next phase will be the construction of a 60 and 54 inch diameter pipe to eliminate the third and final overflow point in West Lafayette. All three students have coauthored papers at the Indiana Academy of Science following completion of their studies.

Design of 3-D Printed Hydraulic/Pneumatic Excavator Arm

Farid Breidi and **Keith Pate**, University of Southern Indiana

The purpose of this project is to build a miniature excavator using a unique 3-D printed design, controlled using either hydraulics or pneumatics. Currently available miniature excavator arms include designs that require the use of machines and equipment, which could be expensive and time consuming. This design will include a portable, table top, arm that can be 3-D printed in a short amount of time and assembled/disassembled using a minimum number of tools. The arm will utilize either pneumatics or hydraulics to showcase fluid power systems and engineering. Future implementation would include introducing middle and high school students to science and engineering majors during outreach activities, highlighting the University of Southern Indiana engineering student's activities, and using the excavator in fluid power and data acquisition classes to support the learning experience of engineering students at the University of Southern Indiana. The excavator arm will be manually controlled using joysticks; these joysticks will actuate valves that will transfer either tap water or air to actuators, controlling the motion of the arm. The arm's control will mimic a full-sized excavator and will reinforce the users understanding of fluid power and how it is used in industry. The parts of the arm that will not be 3-D printed include the pump/compressor, fluid reservoir, valves, and cylinders with quick connect fittings for hands-on demonstrations of several hydraulic or pneumatic circuits. The power source for the excavator arm will require a standard 120VAC/15A electrical outlet found in most facilities. This allows the kit to be portable and able to be used in recruiting events, state and county fairs, student callouts, social gatherings and conferences. An interesting and interactive hands-on machine will help draw attention to STEM fields and make people more aware of the University of Southern Indiana engineering programs.

Indium as a Surfactant for Molecular Beam Epitaxial Growth of GaN Nanowires

Kevin Goodman, University of Southern Indiana, Kasra Pourang, University of Notre Dame, Vladimir Protasenko, Cornell University

Research has shown that indium can be utilized to obtain smooth AlGaIn films by molecular beam epitaxy (mbe) [1]. One theory on this phenomena is that the indium will impart more kinetic energy to the adatoms on the substrate surface, allowing a longer diffusion length along the surface [2]. To achieve nanowire growth rather than a two dimensional film, growth conditions are commonly altered to a nitrogen rich environment combined with an increase in substrate temperatures. The question this research poses is "can the addition of indium be utilized to achieve mbe growth of high quality GaN nanowires at substrate temperatures lower than those normally used for GaN nanowire growth?" Growths carried out thus far to investigate the effect of indium during GaN nanowire formation have yielded interesting results. The growth temperature has been lowered from 790 C, where GaN nanowires are usually grown, to 650 C with the addition of indium flux impinging on the substrate, and nanowire formation still occurs. However, keeping all conditions of the 650 C growth the same except for now removing the indium flux, growths at 650 C will not yield nanowires, but rather a porous, grass-like film. This leads to the conclusion that it is possible to replace the kinetic energy the adatoms receive from the substrate heater with energy obtained from energetic indium molecules.

[1] C. Kruse, S. Einfeldt, T. Bottcher, D. Hommel Applied Physics Letters 79 (21) 3425 (2001).

[2] E. Monroy et al. Journal of Applied Physics 93 (3) 1550 (2003)

Reuse Designs for the Tippecanoe County Superfund Sanitary Landfill Site

Terry West and Robert Jacko, Purdue University

The Tippecanoe County Sanitary Landfill, which closed in 1988, was classified as a Superfund site by the US EPA. The landfill was officially closed under environmental standards as managed by Keramida, Inc. of Indianapolis. Sixteen years after the final closure, because of excellent recovery, the Superfund designation may soon be removed, which will qualify it for secondary use. In the Spring semester, 2015, the challenge to

reuse the site was assigned to the graduating Civil Engineers at Purdue University, West Lafayette campus, as the CE 49800 Senior Design Course project. As a result, 15 engineering teams with five to six students each, undertook the challenge. Each team, operating as an independent consulting entrepreneurial entity, in collaboration with Keramida Inc., developed 15 different reuse designs. The presentation will provide details of these 15 ideas, plans and designs.

Strategies for understanding and adapting to heterogeneity in large-scale bioprocesses

Irene M.B. Reizman, Alexander Armstrong, Jonathan K. Lee, and Jessica R. Ames, Rose-Hulman Institute of Technology

A variety of valuable chemical and biological products are produced through fermentation. However, challenges are often encountered during scale-up, due to long mixing times and difficulty in maintaining homogenous conditions in large-scale bioreactors. This can result in formation of oxygen and substrate gradients in the bioreactor, leading to lower biomass yields or formation of unwanted byproducts. In this presentation, I will discuss our recent work to develop a simple scale-down system for simulating oxygen gradients encountered in industrial bioreactors, along with our work to characterize promoters in *E. coli* suitable for controlling gene expression in response to oxygen limitations. For scale-down studies, a standard 3L benchtop bioreactor was modified to function as a two compartment stirred tank reactor-plug flow reactor (STR-PFR) system for exposing cells to short, controlled periods of oxygen limitation. In initial experiments with the STR-PFR system, we have observed reductions in *E. coli* specific growth rate and biomass yield from glucose similar to those reported in the literature upon scale-up from a bench scale bioreactor to a 20 m³ bioreactor. In addition to understanding the response of bacterial cells to oxygen limitation, we hope to use this system to study how control of metabolic enzyme expression could be used to generate more consistent performance across scales. To alter cellular response to periods of oxygen limitation, appropriate promoters for control of gene expression as a function of dissolved oxygen levels are needed. Screening of three promoter systems in *E. coli* has provided some initial leads, and future work will involve testing alternative promoters, as well as exploring in more detail the promoter response across various conditions. This work was supported in part by a Senior Research Grant from the Indiana Academy of Science.

Environmental Science Section

An approach to optimize water quality and streamflow monitoring within Indiana

Jeffrey W. Frey, U.S Geological Survey, IN-KY Water Science Center and Jeremy Webber, Indiana University Purdue University Indianapolis

It is known that federal, state, local agencies and other watershed groups monitor water quality within their respective state. Each group has its own mandate and reason for monitoring including status assessment, trends, enforcement, or general research. Typically, this work is done within the confines of each agency or group and provides invaluable information but the lack of coordination can lead to duplication of efforts. Even within the Indiana water monitoring community, there is not a good understanding of existing active monitoring networks. The Indiana Water Monitoring Council (InWMC) was formed to "Maximize resources through improved communication, coordination, data sharing, and collaboration." The InWMC completed a study to (1) highlight the existing river and stream water quality networks that provide data in Indiana and (2) identify potential sites to augment the existing networks while also examining for potential redundancy of efforts that may be occurring. Additionally, the InWMC and the Indiana Silver Jackets have begun an assessment to optimize the location of streamgages in Indiana to incorporate both water hazards and water quality. This assessment of stream gages will incorporate federal flood prediction needs from the National Weather Service along with input from local Emergency Managers.

Continuous Water Quality Monitoring in the School Branch Watershed

Dawn R. McCausland, and Aubrey R. Bunch, U.S. Geological Survey Indiana-Kentucky Water Science Center

Federal, state, local and academic entities, in collaboration with farmers, are investigating the effects of long-term conservation practices on water quality in School Branch, a small watershed located in Hendricks County, Indiana. The School Branch watershed is dominated by corn and soybean agriculture land use interspersed with small areas of residential land use. The USGS is monitoring in-stream water quality in School Branch which drains into Eagle Creek Reservoir, a primary drinking water source for Indianapolis. A Super Gage site has been established in School Branch downstream from farmland dominated by conservation cropping management.

The Super Gage continuously measures nitrate plus nitrite, orthophosphate, water temperature, specific conductance, pH, dissolved oxygen, turbidity and gage height. Two streamflow gages are in place upstream and downstream from the Super Gage. Discrete water-quality samples taken during variable seasonal and hydrologic conditions will be used with the continuous data to create surrogate models for constituents of greater interest that cannot be measured continuously.

Effects of Annual Ryegrass and Gated Tile Drainage on Subsurface Nutrient Movement (Poster)
Kris Green & Jessi Ghezzi, Ball State University

Fallow season soil degradation in agricultural settings is a concern for Midwest agricultural producers because nutrient leaching and soil erosion contributes to lost fertilizer input revenue and eutrophication of surface waters. The objectives of the study are to examine the relationship of an overwintering annual ryegrass cover crop in conjunction with gated tile drainage to better understand soil nutrient movement in a stagnant soil water environment. It is hypothesized that the use of a gated tile in combination with cover crops would decrease N and P leaching as compared to conventional tile drainage. The study is being conducted in northeastern Delaware County, Indiana through the fallow season months of fall 2015 through spring 2017. Site design includes a conventional tile drainage setup and a gated tile drainage setup on similar soil types to investigate how soil N and P are affected by the annual ryegrass cover crop. Soil and plant analysis will follow procedures outlined under the Wisconsin Procedures for Soil Testing and Plant Analysis. Drainage effluent analysis includes a Bertholet Reaction method for NH_4^+ , a Vernier Nitrate Ion-Selective Electrode for NO_3^- , and Ascorbic Acid method for P analysis. Statistical analysis will be via ANOVA. It is anticipated, from this study, that soil nutrient losses will be reduced through proper timing and management of cover crop applications and soil-water control methods.

Fish Community Assessment of Marion County Streams
Aubrey R. Bunch, U.S. Geological Survey Indiana-Kentucky Water Science Center

The U.S. Geological Survey sampled fish communities at seven sites along the White River and in six tributaries in Marion County, IN in and around the Indianapolis, IN metropolitan area from 1999-2012. This study was done in conjunction with Citizens (formally the Department of Public Works) to assess the biological community response to changes in waste-water management and the reduction of combine sewage overflows (CSOs). Ten of the thirteen sites are within areas served by CSOs and three are not impacted by CSOs. Differences in fish communities among sites were assessed using biological indices. Changes in the fish communities over time was assessed using multi-variate analysis (non-metric multi-dimensional scaling (MDS)) to group samples by similarities in fish communities. Similarity profile (SIMPROF) was used to identify significantly different groups; and similarity percentages (SIMPER) was used to indicate species that drove differences between groups.

Investigating the Potential Presence of Heavy Metals and Other Elements of Concern in the Bluegrass Fish and Wildlife Area (Poster)
Kendrich Hatfield and **Brian C. Bohrer**

The Bluegrass Fish and Wildlife Area in Warrick County in southwestern Indiana is a natural resource area formed on reclaimed coal mine land. The area is home to approximately two dozen pits totaling over 600 acres of surface water. Due to the twenty-year duration of coal mining operations in the recent history of the area, it seems plausible that minor constituents of any remaining coal residues, including heavy metals and other elements, may be able to leach into these bodies of surface water. The presented work summarizes the results of atomic emission spectroscopy measurements of water samples from the Bluegrass Fish and Wildlife Area as well as samples taken from surface water and wells in the surrounding area. Comparison of these datasets will discern whether or not coal dust and other residues has led to increased abundances of elements such as cadmium, lead, molybdenum, chromium, copper, zinc, nickel, strontium, barium, cobalt, and manganese in the waters of the wildlife area. The findings of this work will be important in defining and building confidence in the environmental quality of the wildlife area and may identify possibilities for remediation to improve environmental quality.

Seasonal Variation in Nitrogen Processing across an Agricultural Stream Network (Poster)
Kara Prior and Adam S. Ward, Indiana University, Caroline A. Davis, IHR- Hydroscience and Engineering, Amy J. Burgin and Terry D. Loecke, University of Kansas, Diego A. Riveros-Iregui, University of North Carolina-Chapel Hill, Steven A. Thomas, University of Nebraska-Lincoln, and Marty A. St. Clair, Coe College

The interaction of agricultural fertilizer use and extremes in drought and flood conditions in 2012-2013 set up conditions for a natural experiment on watershed-scale nutrient dynamics. The region-wide drought in 2012 left surface soils disconnected from stream networks and restricted nutrient use by crops, resulting in an unusually large nitrogen pool in soil columns through the winter. When wet conditions returned to the Midwest in 2013, the unused fertilizer was mobilized, resulting in a six-week period of extremely high in-stream nutrient concentrations. This study analyses three synoptic samples from the Iowa-Cedar River Basin in 2013 to quantify patterns in nitrogen dynamics. We estimate nutrient spiraling metrics by treating the fertilizer pulse as a constant rate nutrient addition across the watershed—a scale on which these processes are increasingly modeled numerically, but on which standard nutrient addition experiments are simply not feasible. Results of our analysis show seasonal differences in the processing of nitrogen, as well as homogenization of processing across spatial scales (from headwater streams to large rivers) in an agricultural watershed.

Environmental Science Section continued.....

Temperature Regulates Microcystin Release from Toxin-Producing Cyanobacteria (Poster)

Jeremy T. Walls, Kevin H. Wyatt, Jason C. Doll, Eric M. Rubenstein, and Allison R. Rober, Ball State University

To evaluate temperature regulation of microcystin release by toxin-producing cyanobacteria, we performed year-round sampling of a eutrophic lake dominated by *Planktothrix agardhii*. Seasonal variation in temperature was replicated in a laboratory incubation experiment designed to evaluate cause-effect relationships between temperature and toxin release. Lake temperature ranged from 3-27°C and cyanobacteria biomass increased with warming up to 18°C, but declined rapidly with further increases in temperature. Extracellular microcystin concentration was coupled with changes in water temperature and was most elevated between 20-25°C, concurrent with the decline in cyanobacteria biomass. A similar trend was observed in laboratory incubations where productivity-specific microcystin release was greater (up to 4-fold) between 20-25°C compared to release rates between 3-19°C. However, productivity-specific microcystin release declined rapidly at 30°C. The results of this study may aid in the ability to forecast elevated toxin levels in eutrophic lakes where blooms can persist year-round with varying degrees of toxin production, thereby minimizing toxin-associated health risks.

Treatment of Oil and Gas Production Wastewater using Green Plants (Poster)

Lucas Clay and J. Pichtel, Ball State University

During hydraulic fracturing ('fracking'), large volumes of high-pressure, chemically-treated water are pumped into subsurface strata to free trapped hydrocarbons. Chemically-enriched water, along with brine and groundwater, collectively termed oil and gas production water (OGPW), are eventually recovered from the well. OGPW may contain hundreds of individual chemicals, some known to be detrimental to public health and the environment. A greenhouse study is investigating the effects of OGPW on soil properties and plant growth. Soils were saturated with OGPW and amended with either composted biosolids, composted food waste, calcium sulfate, or NPK fertilizer. Soils were cultivated to wheat (*Triticum aestivum*) and red clover (*Trifolium repens*). Wheat grew well in the presence of OGPW in all treatments; however, clover growth on several treatments was poor, presumably due to salt sensitivity. Neither species took up appreciable concentrations of Na or K from soil; likewise, heavy metal (Cr, Cu, Pb) uptake was not significantly different from the control. In a second study, a constructed wetland is being used to determine uptake of metals by cattail (*Typha latifolia*) and sedge (*Scirpus fluviatilis*). Preliminary data shows that *Typha* is effective for removal of Cu and Pb. In addition, sediment salinity is markedly reduced in the presence of *Typha*. Both studies should provide useful information regarding phytoremediation methods for removing contaminants from produced water and the recovery of soils contaminated by hydraulic fracturing fluids.

Earth Science Section

Cyclic Alternation of Upper Ordovician Limestone and Mudstone Strata from the Madison, Indiana Area (Poster)

Mason J. Frauhiger, Joanna Stebing, & Jack Carlson, and Benjamin F. Dattilo, Department formerly known as Geosciences

Stratigraphic layers can be separated into various “units,” or “bundles” of rock layers, defined by various characterizing features reflecting the environmental and ecological conditions during and after deposition. A multidisciplinary approach using fossil ecology, rock type, geochemistry, and correlative methods provides a further understanding of the origin of alternating limestone and mudstone strata (~450myo) in the Madison, Indiana area. Limestone samples were taken from three outcrops in and around Madison, Indiana. Faunal, geochemical, and other quantitative and qualitative data were gathered from the samples. Cluster analysis of fossil abundance data displayed various faunal associations and the spatial and temporal arrangement of the taxa and samples. Clusters of taxa and samples will be plotted against the stratigraphy to see if they match how the layers were originally correlated. Thin section analysis was used to quantify phosphate levels and fragmentation in limestone samples; this was based on several classification schemes. Variations in the nature of phosphate formation were recorded, reflecting paleo-environmental conditions and helping correlate layers within the area. Samples will be analyzed with XRF. Carbonate analysis of samples provides geochemical data on the precise composition of strata, further constraining correlations between localities and showing how the strata may have formed. Geomorphic features identified in the Madison, Indiana area, such as waterfalls, refine correlations across the area. Faunal assemblages common to the area were present, reflecting changing environmental conditions. Based on prior research, we expect to find more phosphate in rocks having higher degrees of fragmentation and reworking, suggesting a longer formation period for the phosphate. An integrated correlative approach increases stratigraphic resolution via further paleo-ecological and paleo-environmental parameters. The data collected provides further indication of how cyclic limestone and mudstone strata formed in the Madison, Indiana area, thus allowing for a more complete understanding of the fluctuating environmental conditions.

Solar radiation observed at the South Pole: Variations in atmospheric transmission over a quarter-century

John E. Frederick, University of Chicago

A spectral radiometer located at the South Pole has recorded ground-level solar radiation at wavelengths from 290 nm in the ultraviolet to 600 nm in the visible. Data acquired over 25 observing seasons, covering September to March from 1991-1992 to 2015-2016, provide information on the absorbing and scattering properties of the atmosphere over decadal timescales. The Austral Spring ozone loss distorts the seasonal cycle in ground-level ultraviolet radiation (290-315 nm). Based on solar elevation, maximum irradiances should occur at summer solstice near December 21. However, in some years reduced ozone amounts produce maximum 290-315 nm irradiances in late November or early December with peak values 50% or more above expectations for an unperturbed ozone layer. At wavelengths longer than 315 nm, where effects of ozone are negligible, the total solar energy received over an entire observing season displayed no organized trend prior to the late 1990s. However, between 1996 and 2000 the irradiances began a decline which has persisted. Seasonally-integrated irradiances in 2015-2016 were approximately 2.3-2.4% below those for 1997-1998. The observations imply a small systematic increase in atmospheric attenuation at the South Pole, presumably due to scattering by cirrus clouds, during the current century. The dataset allows a search for links between atmospheric opacity and solar activity. There is no correlation between seasonally-integrated irradiances and the 11-year solar cycle as measured by the 10.7 cm radio flux. On a much shorter timescale, a statistically significant relationship exists between the geomagnetic activity index A_p on one day and ground-level irradiance on the next day. This suggests a small change in the scattering properties of the polar atmosphere following magnetic disturbances, although a physical mechanism for this connection remains unknown.

The Biodiversity of Payton County Park (Poster)

Tessa Aby & Mason Frauhiger, Solomon Isiorho, Department formerly known as Geosciences

Payton County Park (PCP), located in Allen County, Indiana, reveals past topographical alterations, both anthropogenic and geologic in origin, evidenced by remnant structural debris and the glacially-induced wetlands within. In order to compare the degree of biodiversity present between various locations in the park, nine 30 x 30 meter plots were delineated throughout the forty-acre park; these plots were distributed by means of assessing the percentage area of woodlands, wetlands, and open field environments within the park and subsequently allocating a proportional amount of plots to the respective areas. Samples of water and soil from the plots, as well as measures of species richness from within and around the plots, were periodically collected and recorded beginning September 2016 and continuing until November of the same year. Sieve and water quality analyses revealed several north-trending tendencies, encompassed by a gradual increase in elevation, decrease in amount of silt and clay-sized particles, decrease in water saturation, as well as an increasing pH within the PCP pond. Considering all plots, maximum biodiversity occurred where wetland characteristics were present.

Arthropod presence was found to be concentrated in plots of closer proximity to the PCP pond and previously-developed pastures; this was likely due to an abundance of blooming goldenrod. The degree of biodiversity in the plots surrounding Payton County Park's pond may be due to a more extensive undisturbed existence, as opposed to areas of more recently developed/maintained portions of the park, which display characteristics typical of secondary succession. Comparing the degrees of biodiversity between plots provides an initial step towards characterizing the biodiversity of Payton County Park and identifying the internal and external factors which may influence it.

Earth Science Section continued.....

A Miocene Cyprinodontiforme from the Nevada Test Site (Poster)

Michael R. Stoller, Benjamin F. Dattilo, Robert B. Gillespie, Indiana-Purdue University Fort Wayne

Fossilized remains of an unknown species of Cyprinodontiformes (top minnows/pupfish) were recovered on the grounds of the Nevada Test Site. Specimens were found in Miocene volcanoclastic lake sediments and range in size from 6-10 cm. The original bone material of most specimens was missing, leaving behind high-fidelity natural molds in hard silicified silt. Silicone rubber molding compound was used to reproduce the original appearance of the bones. Images of these rubber casts were made with a high-resolution, flat-bed scanner and with a scanning electron microscope. From these images it is possible to compare the skeletal structure of these fish to modern and fossil representatives of related groups. Individual anatomical structures were recognized and identified by utilizing the disarticulated skull of a grass carp, found on the banks of the Ohio River. Notable structures are key to the reconstruction of lost species. Although the exact species has not yet been determined, it appears that only one species is represented, suggesting a stressed environment in these volcanic lakes. The high-resolution casts also show growth lines on the scales and ostracods in the gut, giving a clue to the trophic structure of the paleoecological community. Utilizing the information found in this study, we hope to accurately identify this previously unknown species and gain a general understanding of western N. America during the late Cenozoic era. Furthermore, we hope to accurately identify the ecological and geological environment in which these fishes lived, as well as any environmental stresses to which the fish may have been exposed. Information of this nature could prove useful for conservation efforts pertaining to similar, threatened ecosystems and organisms to date.

Changes in groundwater temperature associated with the ground-source geothermal system at Ball State University (Muncie, Indiana). (Poster)

Matthew Terrell, Carolyn Dowling, Alan Samuelson, Klaus Neumann, Harvie Pollard, and Eric Lange. Ball State University.

This study investigated the changes in temperature of the monitoring wells associated with the Ball State Geothermal Energy System. Numerous wells in geothermal system have been monitored since the system's creation in 2011. For this study, we used temperature profiles from eight wells – four from Phase I (north end of campus) and four from Phase II (south end of campus). Hypothetically, ground-source geothermal system will store heat in the ground to be used when needed during the winter. The normal groundwater temperature for east central Indiana is usually around 12-13°C, with only a half a degree change every 30 meters in depth. Each year, the ground temperatures are expected to return to their original values.

At this time, there is no thermal equilibrium. From the temperature profiles of Phase I, a significant increase can be observed from November 2011 through July 2016. In 2011, the wells in Phase 1 had temperatures around 12°C. In the center of Phase 1 field, the average increase was 7°C/yr until September 2014, when it decreased slightly and has stayed roughly 31°C. However, the wells on the edges of the Phase 1 field have maintained steady albeit smaller annual increases in temperature, with the average rise in temperature being 1.2°C/yr, with the exception of the northernmost well with an overall increase of 0.5°C/yr. A steady increase in temperature can also be seen in the more recently installed Phase II field. Even though data collection in this field began in 2015, the wells show a significant increase in temperature, ranging from 2°C/yr to 6°C/yr. The shape of the temperature profiles is influenced by groundwater flow and stratigraphy. For instance, the locations of the

confining layers (~70 m and 110 m bgs) have become more obvious because of the distinct temperature patterns downhole.

Determining the impact of increasing heat on the groundwater chemistry and bedrock in Phase 2 of the Ball State University ground-source geothermal system (Poster)

Harvie Pollard, Carolyn Dowling, Eric Lange, Klaus Neumann, and Alan Samuelson, Ball State University

Ball State University (BSU) has completed a district-scale ground-source geothermal system in the northern (Phase I) and southern (Phase II) sections of campus. This type of system utilizes the shallow lithology as a thermal reservoir, and the ground temperatures are expected to return to background in an annual cycle. However, the temperatures have been increasing since its installation. To determine if there has been a change in the aqueous chemistry of the groundwater and any impact that it may have on the surrounding bedrock, water samples were collected from several perimeter wells and two center wells from primarily shallow depths (100-200 ft bgs) over the period of 2 years in Phase II. The samples were tested for major cations and anions, and those results were further analyzed using AquaChem and the United States Geological Survey's online alkalinity calculator. The initial data in Phase II indicate that currently there is not enough heat generated from the ground-source geothermal system to affect the groundwater chemistry or the bedrock through the precipitation of calcite. More sample collection and analysis will be conducted in the future.

Geochemical Assessment of Lead Bioavailability in Urban Soils (Poster)

Joshua Pigg, Jennifer Latimer, Matthew Barley, Indiana State University

Even though major sources of lead (Pb) to the environment have been reduced or eliminated, legacy pollution from industrial emissions, leaded fuels, and deteriorating Pb paint have resulted in a significant Pb burden in cities. As a result, children living in urban areas continue to have a higher risk of Pb poisoning than those living in rural areas. Urban areas characterized by high traffic volumes, older housing stock, and a history of industry frequently have elevated soil Pb levels above background values (50 ppm). While many studies have documented spatial patterns of Pb pollution, the bioavailability of Pb in these urban soils is not well known. Our goal was to evaluate soil Pb bioavailability using two different geochemical approaches: a simulated gastric solution to estimate bioavailability following consumption of Pb contaminated soil and a sequential extraction technique to evaluate Pb associations with different soil fractions. All samples used in this study had been previously evaluated for total Pb concentrations. Samples with Pb >200 ppm (n = 210) were subjected to the simulated gastric solution extraction, and samples with Pb >1200 ppm (n=20) were subjected to a Tessier style sequential extraction that isolates Pb that is adsorbed, exchangeable, and associated with Mn-oxides, Fe-oxides, and carbonates. Residual Pb concentrations were determined by difference (sum of all steps subtracted from total Pb). Preliminary results suggest as much as 55-70% of the total soil Pb is bioavailable based on the simulated gastric solution extraction despite the fact that much of the Pb is found geochemically in the residual fraction. Ongoing work includes evaluating Pb bioavailability in the intestines using additional extraction techniques and further analyses of sequential extractions.

Geomorphological study and Rosgen II classification of Walnut Creek, Grant County Indiana (Poster)

Matthew Thornburg, and Michael Guebert, Taylor University

Undergraduate students in a geomorphology course at Taylor University attempted the Rosgen Level II classification of twelve sites along Walnut Creek in Grant County, Indiana. Walnut Creek flows westward from headwaters on the till plain across the Mississinewa Moraine to the confluence with the Mississinewa River. At each site students measured several parameters including bankfull width, depth, cross-section area, width/depth ratio, maximum depth, width of flood-prone area, entrenchment ratio, channel materials D50, water surface slope, and channel sinuosity. From east to west (downstream), bankfull width and width/depth ratio gradually increased, but bankfull depth, maximum depth, cross-section, flood prone area, and entrenchment ratio were variable across the sites. The channel material (D50) was relatively large in the site furthest downstream, but there appeared to be no trend across the sites. The water surface slope was nearly constant across the span of Walnut Creek and sinuosity increased slightly from upstream sites to downstream sites. Extensive human alterations, especially in the headward reaches largely account for these variations in stream geomorphology. Following the Key to the Rosgen Classification of Natural Rivers, students attempted to identify the stream type which tended to be type A4/5 nearer to the headwaters shifting gradually to type F4 farther downstream. This class project contributes to an investigation of Walnut Creek by Flatland Resources, L.L.C., Muncie, Indiana,

who will use this data to determine further action for stream bank restoration along Walnut Creek to abate stream bank erosion.

Heat flow map of the Sunda Arc with detailed basin analysis of the Bali Basin, Indonesia. (Poster)

M. Ingram, M. Terrell, **K. Nicholson**, and R. Fluegeman, Ball State University

The Department of Geological Sciences at Ball State University is in possession of the largest privately owned well log and seismic database covering the southeast Asia-Pacific region. Surface and bottom hole temperatures from wells with depths exceeding 100m, geothermal gradient and lithological information taken from this database will be used in combination with methods developed at the Southern Methodist University Geothermal Laboratory and the Golden Software Surfer program in order to construct a heat flow map of the Sunda Arc, beginning with the South Sumatra and Bali Basins.

The Sunda Volcanic Arc formed as the result of the Indo-Australian plate's subduction beneath Eurasia. This convergent margin is also responsible for regional back arc basins, many of which contain petroleum traps. These hydrocarbon sources allow Indonesia to produce 847,000 billion barrels per day, ranking the country 21st in oil production. Heat flow maps are useful to the exploration of petroleum systems, as well as the exploration of valuable minerals and geothermal energy sources.

Basins located on the western Sunda Arc show high rates of petroleum production and contain many oil and gas fields. This production trend is consistent up to the Bali Basin, where it abruptly ends. This makes the Bali Basin an area requiring further study. Well log and seismic data from the Bali Basin will be entered into IHS Kingdom and Petra computer programs in order to construct a model of the basin. This model, combined with heat flow mapping, will help to better explain truncation of the oil and gas trend at this location and to assess the hydrocarbon potential of the basin.

Hillside Seeps: A Supplemental Method of Groundwater Monitoring in the Upper Wabash River Basin

(Poster)

Dan Deifenbaugh and K.S. Isiorho, Indiana-Purdue University Fort Wayne

Hillside Seeps: A Supplemental Method of Groundwater Monitoring in the Upper Wabash River Basin Dan Deifenbaugh & K. S. Isiorho Geosciences, Indiana-Purdue University Fort Wayne (IPFW) Abstract The seeps along the Upper Wabash River Basin were used as a surrogate for groundwater monitoring. Discharge was measured at each seep and compared to the precipitation data from the area. Water samples from selected seeps, and various streams in the Upper Wabash River Basin were analyzed for temperature, pH, dissolved solids, electrical conductivity, dissolved oxygen, nitrate, nitrite, phosphate, and sulfate to determine the contamination levels in both the surface water and groundwater (seeps). Discharge from at least three seeps is constant but fluctuates depending on the local precipitation. Seepage discharge tends to increase within three or four days of precipitation events. Nitrates ranged from 0.01 — 53.1 mg/L for groundwater and 0.0 — 93.2 mg/L for streams. Nitrites ranged from 0.000 — 9.660 for groundwater and 0.000 — 0.211 mg/L for streams. Phosphates ranged from 0.00 — 49.00 mg/L for groundwater and 0.04 — 32.0 mg/L for streams. Sulfates ranged from 0 — 512 mg/L for ground water and 0 — 112 mg/L for streams. Water analysis reveals nutrient levels frequently exceeded normal parameters in the samples tested. Contamination level appears higher in the groundwater than in the streams.

Linking the Indiana Geologic Names Information System to complementary databases (Poster)

N. R Hasenmueller, W. A. Hasenmueller, R. A. Meyer, and M. S. Daniels, Indiana Geological Survey and G. J. Motz, Indiana University

In 2008, the Indiana Geological Survey (IGS) launched a geologic names website incorporating information for the bedrock units within the state (<https://igs.indiana.edu/IGNIS/>). The website and supporting Indiana Geologic Names Information System (IGNIS) were initially developed by the IGS Geologic Names Committee with assistance from IGS Information Services and Photography and Imaging staff. The primary purpose of the website and IGNIS is to make current information about geologic names that are recognized by the IGS available to a broad spectrum of users from academia, industry, government, and the general public. The IGNIS currently links to and draws information from the following internal sources: (1) the IGS Stratigraphic Names Database; (2) the IGNIS Reference Database of pertinent literature in which bedrock stratigraphic units have been described; (3) the IGS Image Database, which contains photographs and illustrations of Indiana type localities and reference sections, structure and isopach maps, and other figures showing key characteristics of

units; and (4) the IGS Publications Database. Current major external sources of information include: (1) the American Association of Petroleum Geologists Correlation of Stratigraphic Units in North America (COSUNA) chart for the Midwestern basin and arches region; and (2) the online U.S. Geological Survey Geologic Names Lexicon ("Geolex"). In the near future, the IGNIS website will be linking to the Indiana University Paleontology Collection to access: (1) additional fossil images and descriptions; and (2) related information on their stratigraphic distribution, which will help improve and relate the existing knowledge available about the bedrock geology of Indiana.

Earth Science Section continued.....

Manus-Only Sauropod Dinosaur Trackways from the Glen Rose Formation (Lower Cretaceous, Kendall County, Texas)

J.O. Farlow, C. Harter and B. Dattilo, Indiana-Purdue University Fort Wayne, R.T. Bakker and D. Temple Houston Museum of Natural Science, E.E. Deschner, R. Solis, and W. Ward, [deceased], Heritage Museum of the Texas Hill Country, P.L. Falkingham, Liverpool John Moores University

In 1940 R.T. Bird of the American Museum of Natural History examined a sauropod dinosaur trackway in the Glen Rose Formation of southern Texas that consisted mainly of forefoot (manus) impressions. Bird inferred that the huge quadruped had been "punting", pulling itself along the bottom with its forelimbs, its hindquarters suspended in the water column. More recently, manus-only and manus-dominated sauropod trackways have been interpreted instead as transmitted tracks (undertracks) created by differential loading of the forefoot and hindfoot (pes), such that only manus prints were recorded in sediment layers below the one on which the walking animals trod. Discovery of a new Glen Rose tracksite (Coffee Hollow A-Male) in south-central Texas, with three parallel manus-only sauropod trackways, allowed re-examination of the alternative hypotheses. We mapped and measured the trackways, collected cross-sections through prints for sedimentological analysis, and scoured the dinosaur footprint literature for measurements and observations of other sauropodomorph trackways for comparison. The tracklayer is a fine-grained foraminiferal packstone-wackestone. Overlying this layer is a finely-laminated limestone with abundant planar birdseye structures, suggesting an origin as an algal mat. Flame structures from the underlying tracklayer perforate this algal layer. This suggests that the dinosaur footprints as preserved are not deep undertracks, but rather were transmitted only from the algal layer into the immediately underlying tracklayer. If so, the shallowness of the Coffee Hollow prints suggests that the recording tracklayer was fairly firm at the time the dinosaurs crossed it. Two of the Coffee Hollow trackways have bigger manus prints (mean widths c. 65-71 cm) than the third (mean width 52-53 cm). Compared with numerous other sauropod trackways (both manus-only, and manus with pes) from the Glen Rose Formation and other stratigraphic units around the world, the two larger trackways show nothing unusual in manus trackway parameters (step lengths, trackway widths, pace angulations), suggesting that these two dinosaurs may not have been doing anything unusual at the time they crossed the tracksite. The smallest of the three sauropods, in contrast, had a remarkably long pace length and trackway width for the size of its manus prints. An explanation for manus-only trackways that accounts for all of these observations is difficult. Theoretical models of sauropod center of mass position and autopodial loading suggest that a brachiosaurid would likely produce manus-only trackways, whether as "true tracks" or as undertracks, but would also make deeper manus than pes prints. At other Glen Rose Formation tracksites that include sauropod trackways with both manus and pes prints, however, the latter are always as deep as, or deeper than, the former. The more forwardly located center of mass of a brachiosaurid might make it easier for such a sauropod to punt. If any of the Coffee Hollow dinosaurs was doing this, it is most likely to have been the smallest of the three.

Microfacies Analysis Of Early Mississippian Rockford Limestone In The Illinois Basin, Indiana, Illinois (Poster)

Jacob Wojcik, Belkhasim Khameiss, Richard Fluegeman, Jeffry Grigsby, & Shawn Malone, Ball State University

Thirty-five samples are collected from the Indiana Geological Survey, Illinois State Geological Survey, and the Jerseyville Hollow outcrop in Illinois. Initial facies analysis shows these samples range from mudstone to grainstone based on Dunham's classification. Common fossils found in these samples include; Micorinvrtbrtae fauna (ostracodes sp., and foraminifera sp), and Macorfauna (pelecypoda, echinoderm, and bryozoa fragments). The aim of this study is to determine the changing lateral facies across the Illinois Basin and to predict the paleo-environment during the Early Mississippian.

The deepest part of the basin is located in the southern portion of Illinois. At the eastern and western margins of the basin is shallower areas based on composition of thin sections and fauna.

Earth Science Section continued.....

Monitoring Changes in IPFW Well Field along St. Joe River in Fort Wayne, Indiana

David Cole, Indiana-University Purdue-University Fort Wayne (Poster)

The seasonal variation in the quality and quantity of groundwater is an important component of a healthy ecosystem. A well field with 14 observation wells located within a wetland along the St. Joe River on the Indiana-University Purdue-University Fort Wayne (IPFW) campus was monitored for seven weeks to observe any trend in water quality and groundwater flow direction. Bore hole data was also analyzed to develop a lithology model of the area. The total dissolved solids, pH, and temperature of the well waters and surface water from the creek were measured. The pH varied with an increasing trend between 7 and 8.5. TDS ranged from 95 to 1024 ppm. The temperature had a decreasing trend from 20 °C to 14 °C due to change of season. The groundwater level ranged between 35.75 inches above surface level to 5.9 inches below surface level. The results indicate that the pH value is inversely proportional to the groundwater temperature. The well field is a recharge zone during the fall and a discharge zone in the winter. The soils in the borehole data log model could not be confidently identified with the tools available due to the location of the well field. This study shows the varied processes taking place and more data are needed to help in the management and maintenance of a healthy wetland ecosystem.

Nutritional, Economic and Environmental Benefits of Wholesome Insects (Poster)

Mary Pusti & K. S Isiorho, Indiana University-Purdue University Fort Wayne

It is widely accepted that by 2050 the world will host 9 billion people. Today's society already faces nearly 1 billion chronically hungry people worldwide, factoring climate change effects and the alterations in geology there will be devastating effects on food sources. We need to find new ways of growing food and develop back to our original diets as Homo-sapiens. An insectivorous diet is able to provide essential proteins and nutrients to the human body. Insects also offer a significant opportunity to merge traditional knowledge and modern science in both developed and developing countries. Placing insects back into the human diet will allow alternative nutrition, a positive environment, along with economic trade and development. This paper contains two surveys conducted in the hope to raise awareness to the many valuable roles that insects play in sustaining nature and in human life. The first survey with 100 responses focuses shedding light on the misperception people have when considering eating insects. In the end, 39 of the 100 responses chose insect diets over beef. The second survey of 200 responses gives the responders nutritional, environmental, and geographical facts about insects leading to questions in response to the facts given. Based on the second survey conducted, 88% of 200 responders were found to be more willing to try insects and believe in the changes that could happen globally if the diet was embraced. From the two surveys it appears people are more willing to try insects after they have been presented with nutritional and environmental incentive. All it takes is education and exposure to change people's perceptions.

Paying Tributary: A Study of Water Quality in Huntington's Little River

Harlie Summers and K. Solomon Isiorho, Indiana University - Purdue University Fort Wayne

The Little River watershed is an important tributary to the Wabash River, and has been especially useful historically as part of the Wabash and Erie Canal. This river has been part of many geologic studies, however, most of those were strictly stratigraphic. In recent years the Indiana Department of Environmental Management, the United States Geological Survey, and the Lake and River Enhancement Program (through Commonwealth Biomonitoring and Empower Results) have compiled and published some of the only water quality data on the Little River in 2010 and 2009 respectively. In order to have more complete information, this project seeks to compare the Little River in 2016 to the Little River in 2009 and 2010 and view what, if anything, has changed. Water quality data was taken using a meter and probe and by testing water samples in the lab with a colorimeter. Tests focused on pH, conductivity, water temperature, nitrate and phosphate content. Test results showed that pH ranged between 6.95-8.32, conductivity ranged from 447 μ S-1638 μ S, water temperature ranged between 7.2°C-31.5°C, nitrates ranged from 0mg/L-41.4mg/L, and phosphates ranged between 0mg/L-1.56mg/L. By comparing this study to the 2009 and 2010 studies, it was found that pH, water temperature, conductivity, average nitrate content, and average phosphate content were consistent with prior information, however, conductivity and nutrient levels tend to be above Indiana's safety limits.

Earth Science Section continued.....

Sedimentology and geochemistry of the St. Joseph River, Allen County, Indiana (Poster)

Harlie Summers, Dan Deifenbaugh, Aranzazu Pinan-Llamas, Indiana University - Purdue University Fort Wayne

We present preliminary data from sediment sampling and river-bottom surveys that were conducted along the St. Joseph River near the town of Spencerville, Indiana. The main goal of this project is to document bed sediment distribution across the channel, as well as longitudinally, and to assess water chemical changes through time. Sieve analysis was run to analyze grain size distribution; water quality testing included measurements of: pH, conductivity, total dissolved solids, water temperature, and dissolved oxygen, using meters and probes on site. Water samples were analyzed in lab for: nitrates, phosphates, iron, sulfates, and free chlorine using a colorimeter. Coarsest sediments were found near the center of the channel, while sediments fined toward the inner part of meanders. Longitudinally, the highest abundance of coarse grains was found toward the northernmost sample site (upstream). Based on our data, the grain size distribution depends on the geometry of the channel. Geochemical analyses of the water during a time span of five months (October-February) show a general decrease in the concentration of NO₃, PO₄, and SO₄, and a slight increase in the concentration of Fe²⁺ and Cl₂. In the same months, while pH and dissolved oxygen readings were stable, total dissolved solids showed a general decrease. Future work will involve longitudinal expansion of sediment sampling, geochemical analyses of water during the next few months and the characterization of the composition of the river bed sediments.

Soil Explorer – Explore Soil Landscapes in Indiana and Beyond (Poster)

Darrell G. Schulze, Purdue University, and the Isee Network

The Integrating Spatial Educational Experiences (Isee) project utilizes the most detailed US soils information from the USDA SSURGO soil survey dataset, the best available digital elevation models from the National Elevation Data Set, and other data sources to prepare informative maps of a variety of soil properties. Maps for seven U.S. states (Indiana, Illinois, Kentucky, Ohio, Texas, West Virginia, and Wisconsin) are currently online. Maps for Dominant Soil Parent Materials, Natural Soil Drainage Classes, and Soil Orders are available for all the states, and some states have additional maps. These maps can be studied using the new SoilExplorer.net website that replaces a previous website that had maps for only Indiana. The same maps are also available via a free app for the Apple iPad (<https://appsto.re/us/nbdy7.i>). Details of soil landscapes can be explored by zooming to a scale of 1:18,000, while popups provide additional information about each map unit. The project is a collaborative effort of the Isee Network (<http://isee.network>).

Tunnel Channel Networks in the Interlobate Region of Northeastern Indiana: Implications for the Timing, Lobation, and Thermal Regime of the Laurentide Ice Sheet

Anthony H. Fleming, LPG, Nancy R. Hasenmueller, Indiana Geological Survey

The interlobate area in far northeastern Indiana contains extensive, locally anastomosing networks of deeply entrenched meltwater channels whose origins and relationships to surrounding landforms are ambiguous. Recently collected cores and other subsurface data near a large channel network in Kosciusko and Whitley Counties offer insights into the origin and history of these intriguing features.

The earliest fill preserved in these channels consists of subglacially deposited sand containing boulders, basal till clasts, and other rainout debris derived from overlying ice, indicating deposition in tunnel channels rather than ice-walled conduits. Some channels are incised through more than 250 feet of pre-Wisconsin deposits and into bedrock. The great depth of incision suggests that the margin of the ice sheet may have been frozen to the bed, allowing ponding of sufficient subglacial meltwater to generate the large sheet floods invoked by some tunnel channel models. Furthermore, individual trunk channels can be traced for at least 25 miles, transcending known lobe boundaries. The lack of an apparent geometric relationship to individual ice lobes strongly suggests tunnel channel incision occurred beneath a single, unified Laurentide Ice Sheet early in the late Wisconsin glaciation. Differentiation of the ice sheet into discrete lobes occurred after tunnel channel incision and accompanied the transition to a thawed ice margin and deposition of the series of basal till-cored end moraines and outwash fans that outline the classic morphologically defined lobe boundaries in this region. Some of these younger landforms are draped over the older tunnel channel morphology, while others terminate at channel margins. This relationship indicates some segments of the tunnel channel system remained filled with ice through most or all of the late Wisconsin, thus excluding later glaciers and their sediments. At the same time, other segments were not filled with ice and were able to accommodate younger glacial deposits.

Using Substrate to Predict Fish Assemblages in Cedar Creek (Poster)

Gretchen Luchauer, Solomon Isiorho and, Robert Gillespie

Cedar Creek is a highly channelized stream system north of Fort Wayne. It was formed by glacial melt and a series of river captures, but lower Cedar Creek stream system is distinct from many of the other Northeastern Indiana stream systems. This section has a series of mainly forested small valleys carved out of the Wabash Moraine until its confluence with St. Joseph River. Substrate particle size distribution was used to predict fish assemblages at each site. Hydrologic, habitat, and fish data were collected from three sites in lower Cedar Creek. These sites were known as Metea, Hursh, and Tonkel based on nearby a county park or roads respectively. Hydrologic data included temperature, dissolved oxygen (DO), conductivity, pH, nitrates, phosphates, and flow rate. Several fish species known to live in Cedar Creek were separated into groups based on their substrate particle size preference because of their prey's preferences. Temperature, DO, conductivity, pH, nitrates, phosphates, and discharge showed minimal differences among sites. Substrate mineral composition was fairly uniform, with samples comprising mostly quartz, k-feldspar, calcite, organic debris, and asphalt. However, substrate particle size distribution varied among sites. Tonkel had the highest percentage of large gravel and cobble (>2 mm). Hursh had high percentages of both large gravel, small gravel and sand (~2.0mm – 0.063 mm). Metea had the lowest percentage of gravel and had higher proportions of fine sand (0.250mm - 0.125 mm) and silt (< 0.063 mm) instead. Fish with preferences for large gravel, were most common in Hursh. Fish with preferences for small gravel and sand were most common in Hursh and Metea, while fish with preference for mud only appeared in Metea. Although fish that prefer large grain size were fewer than expected at Tonkel, substrate grain size distribution appears to predict fish assemblages in Cedar Creek.

Winter Snow and Dunes from the 2014 Midwest Winter

K. Solomon Isiorho, Indiana University-Purdue University Ft Wayne

Geologic processes take place all the time, some are rather at geologic supersonic speed as such in the case of floods, wind erosion, lightning, etc. while others are never really observed directly such as creep. Scientists make appropriate models such as the natural dune lab being conducted in China to understand how sand dunes form (EARTH, p13, June 2014). Snow may be used to study how sand dunes form by observing snow landforms during winter. The 2014 long and brutal winter provided such an opportunity to examine the formation of dunes.

Within a two-week period, there was a 36-inch snow accumulation. A snowdrift of the accumulated snow resulted in the formation of certain 'dune' patterns. Several dune types from crescent, transverse to linear dunes were observed during this time period. The dune types changed from day to day and in few cases within a day if the wind speed and directions changed.

The light dry snow type, wind speed and direction along with obstacles were responsible for the observed dune types. Crescent shaped dunes were more prevalent in areas with less snow generally found between two structures. In the open field, linear dunes formed easily, but more so where the wind was forced to blow in a given direction for an extended period of time. The wind direction was constrained by the structures; the winds blowing between them along the alley. Although snow 'crystals' are not the same as sand grains, but they can

still be used to simulate sand grain especially for the fine 'dry' fluffy snow type. Nature provides us with opportunity to study the processes if we only pay attention to things happening around us.

Microbiology & Molecular Biology Section

***cmv-2* Incompatibility groups in *Salmonella* multi-drug resistance plasmids (Poster)** **Stephen M. Kuehn** and Samina Akbar, Marian University

Salmonellosis is a significant cause of morbidity and mortality worldwide. Some *Salmonella* species carry multiple antimicrobial resistance genes both in the chromosome and on mobile genetic elements such as plasmids. *Salmonella* species carrying multi-drug resistance plasmids can potentially disperse the *cmv-2* gene conferring β -lactam resistance to new *Salmonella* species as well as to other enteric bacteria commonly found in the human and animal gut. These plasmids are often categorized into incompatibility groups based on their mechanism of replication and maintenance within the bacterial cell. This study seeks to identify the numbers and incompatibility types of plasmids carried by six different *Salmonella* clinical isolates. DNA sequence analyses of raw whole genome as well as secondary DNA fragments (contig) were conducted using the Center for Genomic Epidemiology (Lyngby, Denmark) open source internet databases plasmid Multilocus Sequence Typing (pMLST) and Resistance Finder (ResFinder). Nucleic acid sequences were separated into files, labeled by contig, and uploaded to the consensus databases for analysis against known *Salmonella* resistance genes and plasmid types. Results were returned in table format and identified specific resistance genes, antibiotic susceptibility, and incompatibility groups present with greater than 90% homology between known and analyzed DNA sequences. Results of this analysis show that these bacterial strains carry plasmids which confer multi-drug resistance and that multiple incompatibility groups are present across some but not all analyzed *Salmonella* strains. The analysis will further reveal how many of the plasmids present in these isolates can be conjugatively transferable to other bacteria, increasing the possibility of their spread across species. This study will advance our understanding about how bacteria develop multiple antibiotic resistances and could ultimately help design more effective drugs with greater potential to combat and prevent bacterial diseases.

Antibiotic Resistance Gene Transfer among Bacteria in Wastewater Sludge (Poster) **Jacob Price**, John McKillip, and J. Pichtel, Ball State University

Solids (sludges) from wastewater treatment are known to harbor alarmingly high concentrations of antibiotic compounds. Wastewater biosolids are commonly disposed in landfills and by spreading onto cropland. Therefore, an urgent need exists to quantify the risks of wastewater sludge land disposal. A study is in progress which will determine the abundance of antibiotic resistance genes in bacteria from selected wastewater treatment plants in central Indiana. This data will be correlated to antibiotic concentrations in wastewater sludge in order to formulate a dose-response relationship. The study focuses on four classes of antibiotic compounds (penicillins, macrolides, cephalosporins, and fluoroquinolones). Real-time PCR is being used to determine the abundance of genes among bacterial samples cultured from the sludge; liquid chromatography-mass spectrometry will determine the concentration of antibiotic compounds present. It is anticipated that concentration of a certain class of antibiotics in a sludge sample will have a direct relationship to the number of genes in the bacteria that confer resistance to that class of antibiotics. This study may act to guide future research and regulation for antibiotic concentrations in biosolids. This is becoming increasingly important in order to avoid transference of antibiotic resistance genes to pathogens in the environment.

Investigation of Novel Interactions of PDX 1.2 and PDX2, components of the Pyridoxal 5'-Phosphate Synthase Complex in *Arabidopsis thaliana*

Belinda Petri, John Klem and Elizabeth E. Rueschhoff, Indiana University Southeast

Pyridoxal 5'-Phosphate, the active form of vitamin B6, is an important coenzyme in over one hundred biochemical reactions. Pyridoxal 5'-Phosphate Synthase (PLP Synthase) is the multi-subunit protein complex responsible for the biosynthesis of vitamin B6 in *Arabidopsis thaliana*. Preliminary evidence from our lab suggests that components of this protein complex may be involved in other cellular functions. Our lab is investigating possible protein interactions between two of the proteins of the complex, PDX 1.2 and PDX 2, and other proteins that may be involved in the plant's defenses against stress. We used the Matchmaker Gold Yeast 2-Hybrid system to test for interactions between the PLP synthase subunits and a library of *A. thaliana* proteins. We have isolated a few putative proteins that exhibit interactions with PDX1.2 and PDX2. We are in the process of confirming those findings. One putative interaction that has been identified is between PDX 1.2 and the protein 3-deoxy-8-phosphooctulonate synthase (KDOP synthase). KDOP synthase is responsible, in part, for the biosynthesis of KDO, a component of the plant cell wall. Previously, PDX1.2 has been shown to play a role in both abiotic and biotic stress responses, including defense responses against pathogens. This putative interaction could provide a mechanism for the role of the PDX1.2 protein and its contribution to plant defense responses against pathogenic stress.

Role of Redundant snoRNAs in Pseudouridylation in *Candida albicans* (Poster)

Megan Frederick, Ball State University

In order to better understand fungal virulence, we must better understand what differentiates humans from fungi at the molecular level. *Candida albicans* is the most prevalent human fungal pathogen, responsible for oral thrush and more serious infections in immunocompromised individuals. Pseudouridylation is the most prevalent type of RNA modification and is found in all organisms, but this process has not been examined in any fungal pathogen. It is known that snoRNA molecules act as guides that recruit pseudouridine synthase to specific target sequences within rRNA molecules. Once pseudouridine synthase is brought to the rRNA, it converts specific uridine residues to pseudouridine, which confers different properties than the original residue. The goal of our research is to investigate why multiple *Candida* snoRNAs are predicted to target the same rRNA residue. We predict that different complements of snoRNAs are necessary under different physiological conditions. We are using CRISPR mediated mutagenesis to alter the guide sequences of snoRNAs that target the same uridine residue. By mutating various guide sequences, we will be able to selectively inactivate specific snoRNAs to identify which guides are required for specific modifications. The role of potentially redundant snoRNAs is not known in *C. albicans*. Our work will start to examine the role this unique aspect of RNA modification plays in *C. albicans* biology and virulence.

A G-quadruplex DNA-affinity Approach for Purification of Enzymatically Active G4-Resolvase1 (Poster)

Eric D. Routh and James P. Vaughn, Wake Forest School of Medicine, Steven D. Creacy, YX Genomics, **Peter E. Beerbower** and Philip J. Smaldino, Ball State University, and Steven A. Akman, Department of Hematology and Oncology, Roper St. Francis Hospital

Higher-order nucleic acid structures called G-quadruplexes (G4 structures) can form in regions of both DNA and RNA that contain high numbers of localized guanine. There are ~750,000 possible G4-forming sequences in the human genome, and they are most often found in gene promoter regions, untranslated regions (UTRs), and within telomeres. Due to the potential for these structures to affect cellular processes, such as replication and transcription, enzymes to manage these structures have evolved in humans. One such enzyme is G4 Resolvase1 (G4R1), which was first biochemically characterized in our laboratory (Akman, Vaughn, Smaldino et al.) to bind extremely tightly, as well as unwind both G4-DNA and G4-RNA. G4R1 has been found to be involved in a diverse array of cellular processes including telomere metabolism, embryonic development, gene transcription, hematopoiesis, and immune surveillance. The ability to efficiently express and purify catalytically active G4R1 is of importance for laboratories interested in gaining further insight into the biochemical properties of G4R1. Here, we describe a detailed method for the purification of enzymatically active recombinant G4R1 (rG4R1) from a bacterial expression system. This procedure combines a traditional affinity-based purification of a histidine-tagged rG4R1 expressed in bacteria with a unique second purification step which utilizes the ability of rG4R1 to specifically bind and catalytically unwind G4-DNA. This second step allows us to selectively purify only catalytically active enzyme. Although this protocol is specific to G4R1, it can easily be adapted to other nucleic acid binding enzymes for which the product of the enzymatic reaction is no longer a substrate for binding.

Bacterial diversity and community composition from Nina Mason Pulliam Ecolab at The Marian University (Poster)

Azeem Ahmad, Rachel Bowen, Katherine Timmermann, Marian University

The Nina Mason Pulliam Ecolab is a 55-acre ecological research laboratory located on the campus of Marian University in Indianapolis. Once a very diverse prairie land with many animal and plant species, Ecolab has suffered many environmental stresses and only a few healthy remnants of the original prairie remain. The prairie restoration efforts at the Ecolab have been ongoing for many decades but major hindrance is lack of comprehensive data including unknown microbial composition dynamics of soils, that may prove fundamental in site preparation for crop growth. We plan to investigate compositional relationships of bacterial communities in the soil and water sediments at the Nina Mason Pulliam Ecolab sites. Through 16S ribosomal RNA gene cloning and sequencing, we will characterize total bacterial types and their relative abundance. Site-specific seasonal changes in bacterial communities will also be monitored using denaturing gradient gel electrophoresis (DGGE) to identify major ecological groups. Initially, we have identified 15 research sites to be investigated based on the soil types, vegetation, runoff and many existing stress factors. As a component of prairie ecosystem, bacteria play a unique role in regulating plant growth, mineralizing nutritive elements, increasing and maintaining soil fertility, converting energy forms and cycling materials. Our research on the function and diversity of bacteria at Ecolab will promote sustainable utilization of microorganism in prairie restoration efforts on a larger scale.

Characterization of a putative R2 retrotransposon in the vertebrates *Lampetra aepyptera* and *Lethenteron appendix*. (Poster)

Rex Meade Strange and **Kimberly J. Delaney**, University of Southern Indiana, L. Peyton Russelburg, University of Utah

R2 retrotransposons are a unique class of transposon that move via an RNA intermediate through eukaryotic genomes. These elements have an exquisitely specific genomic location: they are only capable of inserting themselves in the middle of the 28S rDNA gene, thus providing thousands of potential genomic insertion points. The R2 gene is transcribed as part of the larger rDNA cassette and cleaves itself from the pre-rRNA transcript via a ribozyme encoded in its 5'UTR. R2 retroelements have been extensively characterized in arthropods, but there has been almost no description of R2 elements in vertebrate genomes. We have putatively identified an R2 retrotransposon in the genomes of the lamprey species *Lampetra aepyptera* and *Lethenteron appendix*. We are in the process of characterizing these putative transposons via sequencing, locus quantification via qPCR, and a measure of transposition activity via 5' end profiling. Characterization of this gene will lead to biochemical analysis of the structure and function of the R2 protein in vertebrates as well as a look at the phylogenetic conservation of the HSV-like ribozyme that regulates its cleavage from the rRNA transcript and thus plays an important role in transposition.

Diversification and Copy Number Variation of Alkane Monooxygenase Genes in Bacterial Genomes
Hisako Masuda and Peter Tupa, Indiana University Kokomo

Linear alkanes are a major component of petroleum. Despite their toxicity to humans and animals, they are widely released into the environment through improper disposal practices or by accidental oil spills. Some bacteria can metabolize linear alkanes through a series of oxidation reactions. The most well-known enzyme to catalyze the initial rate limiting step of metabolism is the Alkane hydroxylase system. It consists of three components: a hydroxylase subunit (AlkB), an electron transfer protein(s) (rubredoxin), and a reductase. Our analysis of *alkB* sequences on published bacterial genomes revealed that many strains carry multiple copies of *alkB*. While both horizontal gene transfer (HGT) and duplication contributed for the acquisition of multiple copies, intraphyla HGT seems to be the main mechanism of acquisition. Acquired copies seem to be stably maintained over evolutionary time.

Diversity of the cloacal mycobiome of the juvenile green sea turtle (*Chelonia mydas*) (Poster)

Megan Gerber, James T. Price, Frank V. Paladino, and Tanya Soule, Indiana University- Purdue University Fort Wayne, Margaret M. Lamont, United States Geological Survey, Gainesville, Florida, Blair E. Witherington, Disney's Animals, Science, and Environment, Lake Buena Vista, Florida

The animal microbiome has rapidly become of great interest in current research as the microbiome is considered to be of vital importance to the survival of its host. Most studies have focused on bacterial

communities due to their large populations and their smaller genomes. However, there are other understudied groups, such as fungi, which may also be important to improving our knowledge of their roles with their hosts. In this study we aim to identify the fungal communities within the cloaca of green sea turtles (*Chelonia mydas*) with the secondary goal of discovering whether these communities vary by ontogenetic shift in habitat. Pelagic-stage turtles were sampled from the Port of Venice, Louisiana while neritic juveniles were sampled from along the beachfront of Santa Rosa Island, Florida. Cloacal swabs were collected and stored at -20°C until DNA extraction. The fungal ITS regions were amplified using PCR and cloned into the pGEM-T vector for selection and sequencing. Samples from six individual turtles resulted in 30 clones which show that the majority of the fungal communities in the cloaca are dominated by the phylum Ascomycota, which includes the genera *Candida*, *Cladosporium*, *Podospora*, and *Epicoccum*. Other fungi identified include strains of *Exophiala* and *Malassezia*. The overall fungal community of the cloaca is primarily dominated by cellulolytic fungi, especially in the neritic sea turtles. A majority of the fungi identified are common on the dermis of animals, while others, such as *Epicoccum nigrum*, may even have some antimicrobial properties to aid the host in avoiding pathogens. At present, and to the best of our knowledge, there have been no other studies made in an attempt to characterize and identify the mycobiome of healthy, juvenile green sea turtles.

Microbiology & Molecular Biology Section continued.....

Do differences between *Candida albicans* Pseudouridine Synthase 4 alleles lead to distinct functions?
(Poster)

Caleb Embree, and Douglas Bernstein, Ball State University

Candida albicans is the most prevalent human fungal pathogen. The *Candida albicans* genome is diploid and many A and B alleles have different sequences. Recent technological advances have enabled us to begin to investigate if *C. albicans* A and B allele products have distinct functions. Pseudouridine synthases modify uridine to pseudouridine in tRNAs, rRNA, mRNA, snRNA, and snoRNAs. The functions of the *C. albicans* pseudouridine synthases have not been investigated. *C. albicans* pseudouridine synthase 4 (CaPus4) has 13 differences between A and B alleles. This leads to 6 amino acid differences between Pus4A and B, which we hypothesize endows specific functions for each enzyme. In *Saccharomyces cerevisiae*, Pus4 modifies uridine to pseudouridine in cytoplasmic and mitochondrial tRNAs at residue 55. We aim to identify distinct functions of Pus4 A and B in *C. albicans*. We inserted a maltose promoter to selectively turn off or overexpress the PUS4A or B allele. In addition we inserted affinity tags downstream of the A or B alleles. Using these constructs we are testing if Pus4A and B interact with distinct protein binding partners and modify distinct substrates. Furthermore, we are testing how altering the expression of Pus4 A or B affects growth, drug resistance, and virulence of *C. albicans*.

Effect of seasonal changes on antimicrobial defenses in the avian stratum corneum II: Antimicrobial activity in lipids (Poster)

C.R. DeLoney-Marino, T.N. Loppnow, , R.A. Silliman, A.M. Champagne, University of Southern Indiana

The stratum corneum (SC) is the most superficial layer of skin, and is composed of several layers of flattened dead cells called corneocytes embedded in a lipid matrix. These lipids are known to play a critical role in regulating cutaneous water loss. However, these lipids may also play a role in regulating bacterial composition of the SC, as many lipids exhibit antimicrobial activity. In this study, we assessed the antimicrobial activity of lipid extracts of known composition taken from the SC of House Sparrows (*Passer domesticus*). We used bacterial assays to measure minimum inhibitory concentration (MIC) of these lipids against several species of bacteria common on the avian epidermis including *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus licheniformis*. We found that the antimicrobial activity of lipids depends on fatty acid composition and the target bacteria. Our results clarify the role of lipids in regulating bacterial community composition on bird skin.

ETS1 promotes ovarian cancer metastatic colonization through its transcriptional target PTK2 (FAK)

(Poster)

Joshua Scantland, James Haley, Josh Plotnik, Shruthi Sriramkumar, Christopher Thomas, Peter Hollenhorst, Anirban K. Mitra, Indiana University School of Medicine

Ovarian Cancer (OvCa) is known to be one the deadliest gynecological malignancies and is the fifth leading cause of cancer related deaths among women in the United States. Most patients are diagnosed with

disseminated disease and mortality most often results from metastasis rather than the primary tumor. Metastasis commonly occurs by contiguous spread of OvCa to the mesothelial layers of the greater omentum and peritoneum followed by subsequent formation of metastatic lesions. Once attached, OvCa cells and mesothelial cells communicate by reciprocal juxtacrine and paracrine signaling allowing adaptation of the OvCa cells to their new microenvironment. These adaptive changes are mediated by modulating activity of several transcription factors (TFs) including ETS1. We have previously identified ETS1 as an important mediator of the OvCa metastatic process by promoting motility and invasion. Previous in vitro studies with knockdown of ETS1 expression with siRNA demonstrated significantly reduced invasion, colony formation, migration, and proliferation whereas ETS1 overexpression resulted in an opposite response. CHIPseq and RNA-sequencing has confirmed that one of the key direct downstream transcriptional targets of ETS1 is the gene PTK2, which codes for focal adhesion kinase (FAK). OvCa cells have previously been reported to have increased FAK expression as compared to non-neoplastic ovarian epithelial cells. In this study we report that knockdown of FAK with siRNA showed functional similarity to ETS1 knockdown with significant decrease in OvCa cell motility and invasion. Overexpression of FAK rescued the functional effect of ETS1 knockdown which provided further support that PTK2 is a downstream target of ETS1. The result of this study indicates that microenvironment influences expression of FAK through upregulation of ETS1. The increased expression of FAK enhances motility and invasion of OvCa cells during the metastatic process making it a potential target for treatment of metastatic ovarian cancer.

Expression of genes associated with sunscreen biosynthesis in the wild type and a regulatory mutant of the cyanobacterium *Nostoc punctiforme* (Poster)

Janine Bennett, Sejuti Naurin, Gabriela Romo, Tanya Soule, Indiana University-Purdue University Fort Wayne

Cyanobacteria are photosynthesizing organisms that live in environments open to solar ultraviolet radiation. In order to survive in these environments some cyanobacteria produce sunscreen pigments which convey the ability to tolerate harmful solar energy. The indole-alkaloid scytonemin is a sunscreen that is widely produced among the cyanobacteria. Using a mutant strain of the cyanobacterium *Nostoc punctiforme* which lacks the response regulator Npun_F1278 associated with scytonemin biosynthesis, a series of studies were done to validate the relationship between this response regulator and scytonemin biosynthesis, as well as to characterize and compare the mutant to the wild-type strain. The inability of d1278 to produce scytonemin led to the determination that the response regulator Npun_F1278 in *N. punctiforme* is essential for scytonemin biosynthesis. A phenotypic characterization of d1278 suggests that the deletion of Npun_F1278 does not affect the cell morphology or cellular differentiation capability of d1278 compared to the wild type. The mutant, however, had a slower specific growth rate and the amount of phycocyanin for d1278 differed from the wild type. While most of the evaluated effects of this gene appear to be specific for scytonemin, this regulator may also influence the overall health of the cell and specifically, phycobiliprotein synthesis. Furthermore, expression of scytonemin biosynthetic genes in the wild type were measured using qPCR following exposure to UVA, UVB, high light, and oxidative stress to determine the relationship between scytonemin biosynthesis and a variety of environmental conditions. The results showed that genes within the scytonemin operon were up-regulated among all of the tested stress conditions. While up-regulation under UVA was expected for scytonemin, it was surprising to see UVB, high light, and oxidative stress increase expression of scytonemin biosynthetic genes. Future studies will evaluate additional stresses and the ability of *N. punctiforme* to produce scytonemin under several of these conditions.

How CRISPR can be applied as a genome editing tool in fungal pathogens and other organisms.

Douglas Bernstein, Ball State University

Clustered regularly interspaced short palindromic repeat (CRISPR) mediated genome editing has taken the molecular biology world by storm. The evolution of these technologies has dramatically enhanced the scientific community's ability to manipulate the genomes of model organisms and has facilitated significant progress in fields for which classical genetic techniques were impractical. In this hot topic we will discuss some of the foundational discoveries made by scientists in the United States and around the world that have paved the way for these advances. Furthermore, we will discuss the required components of a CRISPR genome editing system and the role these components play in genome editing. We will also discuss how these components can be leveraged by both experienced and novel molecular biologists in the lab. In addition, we will provide specific examples from our research that illustrate how CRISPR technologies can be used by undergraduate and master's students to investigate important fungal pathogens such as *C. albicans*.

Identification of antimicrobial resistance genes and plasmid types in isolates of *Salmonella enterica*

(Poster)

Emily Martin and Samina Akbar, Marian University

Multiple drug resistance is becoming increasingly problematic in the U.S., where antibiotics are overused both in agriculture and healthcare. One way bacteria can develop this resistance is through acquisition of antibiotic resistance genes either on their chromosomes or on plasmids. A plasmid is an autonomous extrachromosomal DNA structure that replicates independently of the bacterial chromosome. Plasmids can be horizontally spread across different bacterial species through conjugation, allowing multiple bacteria the ability to select for a resistant, advantageous phenotype. Typing and characterization of plasmids is important in order to better understand and trace the spread of this resistance. *Salmonella enterica*, one of the major causes of intestinal illnesses worldwide, has acquired resistance to a number of different antibiotics. The objective of this study was to confirm the presence and location of antimicrobial resistance genes and identify plasmid types in clinical isolates of *Salmonella*. This was achieved by analyzing their sequenced genomes using web-based programs put together by Center for Genomic Epidemiology. Three programs, PlasmidFinder, ResFinder, and Plasmid Multi locus Sequence Typing (pMLST) were used to provide better characterization and typing of *S. enterica* plasmids and the resistance genes carried on these plasmids. With the help of these three programs the number of plasmids in three different *Salmonella enterica* clinical isolates were confirmed, the plasmids were divided into different incompatibility groups, and the resistance genes carried by these plasmids were identified. This analysis will be beneficial in comparing the newly identified plasmids to other plasmids belonging to certain incompatibility groups that have been well studied and found to be associated with specific resistance genes. This will greatly aid in assigning these plasmids to lineages that can in turn help with future comparative analysis studies in order to better understand and combat the spread of resistance in *Salmonella*.

Identification of Functions of Pseudouridine Synthase 7 in *Candida albicans* (Poster)

E. S. Pickerill, and D. A. Bernstein, Ball State University

Candida albicans is the most prevalent human fungal pathogen. To identify new antifungal targets we must first better understand what differentiates fungi from humans at the molecular level. This study investigates the effects of CRISPR mediated deletion of Pseudouridine synthase 7 on *C. albicans*. The PUS7 knockout has defects in filamentation and grows more slowly than wild type *C. albicans*. In the future we will identify *C. albicans* Pus7 substrates and test if PUS7 deletion has an effect on virulence.

Identifying the effects of lung cancer-derived exosomes on normal human cells (Poster)

Hana Kubo, Sean E. Humphrey, and Andrea L. Kasinski, Purdue University

MicroRNAs (miRNAs) are small, noncoding RNAs that regulate protein levels typically by interacting with the 3' untranslated region (3'-UTR) of target messenger RNA (mRNAs), and are often aberrantly expressed in cancer. Exosomes, microvesicles secreted by cells to transport cargo, are known to package precursor miRNAs (pre-miRNAs) and miRNA processing proteins in cancer. This results in active miRNA biogenesis inside the exosome. The processed miRNA can then influence gene expression in neighboring cells, allowing for cancer-mediated cell-cell signaling. Our study aims to identify the effects of lung cancer-derived exosomes on normal cells. To do so, we treat normal cell lines with exosomes derived from four lung cancer cell lines, and follow with a colony formation assay to measure the change in anchorage independent growth in the cells. Better understanding cancer-mediated cell-cell signaling can lead to potential therapeutics for inhibiting tumor growth in affected patients.

Identifying the Structural and Mechanistic Changes at the *PLCepsilon*RA2-RAP1A Interface (Poster)

William Mbongo, Angeline M. Lyon and Yvon Rugema: Purdue University

The *phospholipase Cε* (*PLCε*) subfamily is of great interest due to their critical roles in regulating cardiovascular function. *PLC* enzymes are a class of proteins that hydrolyze the lipid *phosphatidylinositol-4,5-bisphosphate* (*PIP2*) to produce *inositol-1,4,5-triphosphate* (*IP3*) and diacylglycerol (*DAG*), key second messengers. *IP3* and *DAG* increase intracellular calcium concentrations and activate *protein kinase C*. Under basal conditions, *PLCε* has low activity and is localized to the cytoplasm. The stimulation of *G protein coupled receptors* (*GPCRs*) and receptor tyrosine kinases (*RTKs*) activates small G proteins, including *Rap1A*, *RhoA*, and *Ras*, which bind directly to *PLCε* and increase its lipase activity while translocating the *G protein-PLCε* complex to the membrane. It has been proposed that activated G proteins release autoinhibition

within *PLCε* and/or increase the affinity of the active site for the membrane. *PLCε* possesses two *Ras association (RA)* domains at its C-terminus and the *RA2* domain is thought to be the primary *G protein* binding site. This project aims to characterize the interactions between *PLCε* and the small *G protein Rap1A*, as this pathway is essential for cardiac contractility and its dysregulation contributes to *hypertrophy* and heart failure. Methods being employed include the isolation of a *Rap1A-PLCε RA2* complex, followed by determination of the X-ray crystal structure. Site-directed mutagenesis and biochemical assays will be to confirm the contributions of residues at the molecular interface. These studies will provide the first insights into the mechanism by which *Rap1A* interacts with and activates *PLCε*.

Impact of Soybean aphid feeding on a host defense regulatory gene *PHYTOALEXIN DEFICIENT 4 (PAD4)* and its splice variants in Soybean (*Glycine max*)

Patrick R Selig and Vamsi Nalam, Indiana University-Purdue University Fort Wayne

Alternative splicing (AS) plays an important role in post-transcriptional regulation in higher eukaryotes. Technological advances in high-throughput sequencing over the past decade have revealed the extent of AS in several plant species. In soybeans, up to 63% of genes undergo AS in response to a variety of stimuli and biotic stressors. With respect to plants defense, AS events have been observed in resistance (*R*) genes. However, there have been no reports of AS occurring in defense regulatory genes. *PHYTOALEXIN DEFICIENT4 (PAD4)* is an important regulator of host defenses and in soybean elevated *PAD4* expression is associated with increased resistance to soybean aphids and to nematodes. Interestingly, our research shows that an alternatively spliced variant of *PAD4* also occurs in soybean plants. Our data shows that the proportion of the *PAD4* splice variant, *PAD4-AS1*, changes in response to soybean aphid infestation. Therefore, the overall objective of the work is to determine the role of *PAD4-AS1* in plant defense. The specific objectives of this study are to: (1) to determine the role of soybean *PAD4* and *PAD4-AS1* in Arabidopsis response to aphid infestation and (2) determine the sub-cellular localization of *PAD4* and *PAD4-AS1*. Towards this end, *PAD4* and *PAD4-AS1* have been cloned into plant expression vectors. Transgenic Arabidopsis plants that over-express soybean *PAD4* and *PAD4-AS1* will be evaluated for response to aphid infestation and to determine the sub-cellular localization of the proteins using confocal microscopy. Findings from this research will provide a better understanding to this important signaling system and better understanding of *PAD4*'s role in host defense.

Investigating the effects of *PUS5* gene deletion on mitochondrial protein expression in *Candida albicans* (Poster)

Allyson R. Morris and Doug Bernstein, Ball State University

While RNA is made of only 4 bases, these bases can be modified in over 100 distinct ways. These modifications play critical roles modulating protein expression. Pseudouridine is the most common modified nucleoside. Pseudouridylation is performed by the pseudouridine synthase (*PUS*) family of enzymes, however, the role of pseudouridine synthases and pseudouridylation is not well understood. We are using budding yeast as a model to investigate the roles of pseudouridylation in biology. We focused on the pseudouridine synthase *PUS5* and its effect on mitochondrial protein expression in *Candida albicans* and *Saccharomyces cerevisiae*. Pseudouridylation is found at 46 sites in *C. albicans* cytoplasmic rRNA and the cytoplasmic ribosome translates thousands of proteins. As such, it is challenging to study the effects of individual sites of pseudouridylation have on translation. However, mitochondria contain their own ribosome and this ribosome is responsible for translating only eight genes encoded by the mitochondrial genome. Unlike the cytoplasmic rRNA, the mitochondrial rRNA contains only one pseudouridine and this modification is catalyzed by *PUS5*. *PUS5* is the only synthase to catalyze pseudouridylation of the mitochondrial RNA. Although this site is highly conserved the role of this single pseudouridylation site in translation has not been investigated. We find that *pus5Δ/pus5Δ* strains are sensitive to drugs that inhibit oxidative phosphorylation such as oligomycin. The effects of oligomycin treatment are heat dependent. We will use quantitative reverse transcription polymerase chain reaction (qRT-PCR) and mass spectrometry to determine the role of pseudouridylation on mitochondrial gene transcription and translation.

Investigating the feasibility of the DNA Endonuclease Argonaute from *N. gregoryi* as a genome editing tool in *E. coli* (Poster)

Arran Liu, Kok Zhi Lee, Archana Kikla, Alex Murfee, Kevin Solomon, Purdue University

CRISPR/Cas9 has revolutionized genetic engineering by making gene editing simpler, more efficient, and widely accessible. Recent studies propose that the Argonaute from *Natronobacterium gregoryi* (NgAgo) is a DNA-

guided endonuclease that may function similarly to the Cas9 endonuclease with the assistance of a 5' phosphorylated single-stranded guide DNA (ssDNA), rather than single-stranded guide RNA (sgRNA). In this poster, I will discuss our studies in *E. coli*. While we are able to express and purify the protein, we, like other groups, have yet to observe in vitro DNA cleavage. We provide evidence, however, that NgAgo and introduced ssDNA interact in an uncharacterized manner at programmed gene locations to repress gene expression. These findings underscore a need for more detailed study of NgAgo and suggests a novel use for NgAgo as a tool for robust and precise gene manipulation.

Investigation of the Short Root Phenotype of *sos4* and *pdx1.3* Mutants by Grafting in *Arabidopsis thaliana*

John Klem and Elizabeth Rueschhoff, Indiana University Southeast

The purpose of this research is to investigate relationships between vitamin B6 pathways and the root development in *Arabidopsis thaliana*. Previous research has shown that mutants of vitamin B6 biosynthesis, *pdx1.3* and *sos4*, exhibit decreased root growth when grown on high sucrose media. Because both mutants also exhibit abnormalities in chloroplast ultrastructure and the chloroplasts of both mutants are also deficient in vitamin B6, we hypothesized that the short root phenotype may be related to this deficiency of chloroplastic B6 in the shoot. To test this hypothesis, we used grafting to determine whether the short root phenotype was a signaled response from the shoot or localized to the roots. WT shoots were grafted onto *pdx1.3* and *sos4* root stock to determine if signaling from the shoot chloroplasts is involved in the short root phenotype. The phenotype of the grafted seedlings was not rescued in either *pdx1.3* or *sos4*. Our findings support an origin for the phenotype that originates in the root cells and is not due to shoot-to-root signaling.

Investigation of the Substrate Specificity of *L-Idonate Dehydrogenase* By Site-directed Mutagenesis (Poster)

Steve Steiner, Allison Terpening, Chelsea McCurdy, Hanover College

The enzyme *L-idonate dehydrogenase* (L-IdDH) is an important enzyme in the pathway for the biosynthesis of tartaric acid from ascorbic acid in grapes. L-IdDH is an NAD⁺/NADH-dependent enzyme which catalyzes the conversion of L-idonate to 5-keto-D-gluconate. Analysis of the amino acid sequence indicates that L-IdDH from *Vitis vinifera* is a member of the family of medium chain dehydrogenases/reductases and is 79% identical with the putative *sorbitol dehydrogenase* (SDH) from *Arabidopsis thaliana*. Despite the high degree of homology, L-IdDH and SDH are each specific for its substrate with neither enzyme showing activity toward the substrate of the other. One of the notable differences in the amino acid sequences of L-IdDH compared to SDH is at position 42 where His is located in L-IdDH in contrast to Tyr in SDH. It is possible that the cationic His in L-IdDH is essential to the binding of the anionic substrates L-idonate and 5-keto-D-gluconate. For this study, site-directed mutagenesis was performed to convert 42H to 42R and to 42Y in L-IdDH. Assay results indicate that mutation of His (positive charge) to Arg (positive charge) causes a significant reduction in activity toward 5-keto-D-gluconate and a mutation to Tyr (uncharged) at this position completely eliminates activity. These findings illustrate the critical role of 42H in the activity and substrate specificity of L-IdDH. *This work is supported by a grant from the Indiana Academy of Science.*

Isolating and characterizing novel bacteriophages from Southern Indiana water samples (Poster)

Angela R. Sabo, Pamela L. Connerly, Indiana University Southeast

Bacteriophages (phages) are viruses that infect and kill bacteria. Recently, phage biology and genomics have become an area of immense interest due to emerging information about host-virus interactions and the striking potential of phage therapy as an alternative to antibiotics. In order to help characterize phages and understand how they interact with their hosts, we are isolating, purifying, and characterizing novel phages that infect *Caulobacter crescentus* and closely related species of bacteria. Water samples collected from many diverse areas were filtered through 0.2 µm filters to isolate phages. The host bacterial culture was plated using a soft agar overlay and the sample filtrate was spotted onto the bacterial lawn to test for phage activity. When phage activity was present in the sample, indicated by clearings on the spot plate, a five-step re-infection process was used to purify the phage. The titer of the sample was found to enable further analysis using TEM imaging, DNA extractions, and restriction enzyme digests. Currently, TEM images of sixteen out of twenty phages have been obtained, showing all to have flexible non-contractile tails characteristic of the siphoviridae family. Multiple novel *Caulobacter* phages have large prolate heads ranging in size from 177nm to 291nm with tail lengths ranging from 259nm to 339nm. DNA extractions and restriction enzyme digests are ongoing. DNA

was isolated using a modified Promega Wizard DNA Clean Up System and a variety of enzymes were used to help characterize the phages and differentiate them from one another. Once data from these processes have been collected on all of the novel phages, the samples will be sent for full genome sequencing. With the full genomes, we will look for similarities and differences between them and compare them with other phage genomes for insight into phage function.

Isolation and Characterization of Antibiotic Producing Bacteria from Nina Mason Pulliam Ecolab at the Marian University (Poster)

Azeem Ahmad, Madison E. McKinney, Aaron H. Smith, Kodilee M. Underwood, and Kimberly S. Cherry Vogt, Marian University

For the last 70 years, antibiotics have been the drugs of choice for treating bacterial diseases. Therefore, it is not surprising that the extensive and indiscriminate use of these antibiotics have led to the emergence of resistant bacteria at a rapid rate. According to the Center for Disease Control (CDC) approximately 2 million people are infected with multiple drug resistant (MDR) bacteria each year. In order to combat common bacterial infections once controlled by antibiotics, we need a fresh batch of novel antimicrobial agents. Historically, the majority of the antibiotics came directly from fungi and bacteria in natural soil samples. Recent studies have speculated that previously uncharacterized soil-dwelling bacteria could potentially provide a source of novel antimicrobial agents. Evaluation of the antimicrobial capacity in soil microbes through purposeful sampling combined with comprehensive screenings could provide support for re-exploring natural sources for novel antimicrobial drugs. We propose to sample diverse soil types at the Marian University's Nina Mason Pulliam EcoLab in Indianapolis for isolating bacteria showing antibacterial activity by using agar well diffusion method. Preliminary sampling has led to the collection of *Pseudomonas spp.* showing antibacterial activity in culture filtrates from a variety of soil types. We are in the process of characterizing these isolates using polyphasic approaches. In addition, we propose to use culture-independent methods to identify specific soil niches supporting antibiotic producing bacterial communities. This preliminary exploration supports the premise that, in the search for novel sources of antibiotics, some environments may provide more diverse and productive sampling targets than others.

Isolation and Expression of *Escherichia coli* Gene Homolog of Salmonella Effector Protein in Infection of Mammalian Host Cells (Poster)

Theresa Emeli, Menghan Li, and Daoguo Zhou, Purdue University

Infection of host cells by bacteria depends on several factors in the infection mechanism of the attacking organism. To understand and treat bacterial infections, one must first understand the mechanism and genes responsible for the initial process of infection of a host cell by an invading bacterium. The purpose of this experiment is to determine if a certain genetic homolog from *E. coli* has the same function in the infection process as Salmonella when infecting host cells. In this experiment, a gene homolog involved in the infection process was isolated from *E. coli* bacteria and replicated via PCR. It was then inserted into a plasmid ring containing an antibiotic marker via ligation reaction. After this reaction, the modified plasmid was then chemically transformed into *E. coli* bacteria. To check for accuracy, the bacteria was lysed and the inserted band and plasmid ring were visualized on 1% agarose gel. The visualization of both the digested plasmid and gene of interest on the gel showed that the target gene was successfully integrated into the plasmid. After isolating and purifying the plasmid clones containing the gene of interest, the plasmids were then transformed into *E. coli* to multiply, recovered, and then transformed into Salmonella bacteria through electroporation. The infection process was then performed, stained, and visualized. The results showed that the gene homolog did not function in during the infection of the host cells by Salmonella.

Microbial Diversity of Biological Soil Crusts of the Indiana Dunes State Park (Poster)

Taylor Smith-Graber, Gabriella Romo, Jordan Marshall, Ben Dattilo, and Tanya Soule, Indiana University-Purdue University Fort Wayne and Scott T. Bates, Purdue University Northwest

Biological soil crusts (BSCs) are microbial assemblages that play important roles in the areas they inhabit, and can influence aspects such as local nitrogen flux, water infiltration, and plant seedling germination. In the dune environment, BSCs can form close connections with sand particles to stabilize dunes at their surface. Despite their potential critical role within Indiana Dunes State Park, little is known about their diversity, ecology, or interactions with plant communities there. The objective of this study, therefore, was to examine abiotic and biotic factors that influence dune BSCs, using Illumina high-throughput sequencing (HTS) to characterize these microbial communities. We hypothesized that specific environmental variables would influence BSC microbial

diversity. In this study we sampled every 50m along a 550m transect, moving from the Lake Michigan shore inward to the hind-dune environment. At each sampling point soil pH, PAR, UV, and chlorophyll and scytonemin pigments were measured, and plant community cover, richness, and diversity were assessed. Moisture, conductivity, and nutrient content (total N and P) were also measured at every 100m. Overall, our HTS effort recovered 1,336,336 bacterial sequences across our 30 samples, and five major phyla were found to dominate our BSC samples, these being *Proteobacteria* (~21% of all sequences), *Acidobacteria* (~16%), *Actinobacteria* (~16%), *Bacteroidetes* (~17%), and *Cyanobacteria* (~10%). While there were no significant trends observed for BSC diversity moving along the transect, we did note a significant negative correlation for biological pigments and soil pH, suggesting a shift in the microbial community structure in response to these environmental parameters. The tendency for pH to structure terrestrial microbial communities has been noted in many studies; therefore, we are currently carrying out additional analyses to examine BSC community composition changes across our transect in relation to measured abiotic variables, with pH being of special interest.

Microbiology & Molecular Biology Section continued.....

Restoration of Mature *let-7* MicroRNA Through Small Molecule Discovery (Poster)

James Welch, Sunghyun Myoung, Bradley P. Loren, , David Thompson and Andrea Kasinski, Purdue University, and Sergey Savinov, University of Massachusetts

MicroRNA's (miRNA) are a class of small non-coding RNA molecules that pair non-perfectly with messenger RNA to repress protein translation. Most miRNAs are transcribed and then processed twice, firstly by the RNase enzyme Drosha and then by Dicer. Although all miRNAs share this mode of biogenesis, an additional layer of processing has been identified for one miRNA family, the *let-7* family. *Let-7* processing by Drosha and Dicer is blocked when unprocessed *let-7* is bound by the RNA binding protein LIN28. Because fully processed *let-7* family members act to repress the oncogenic genes RAS, MYC and LIN28, which are involved in differentiation, proliferation, and development, reduced mature *let-7* levels contribute to uncontrolled growth in cancer. We hypothesize that small molecules can be discovered that interfere with LIN28-*let-7* binding which would then allow mature tumor-suppressive *let-7* to be produced. A library of FDA approved compounds will be tested for their ability to disrupt the *let-7*-LIN28 interactions in a high-throughput screen using a fluorescent polarization assay. Positive hits will be examined in cell culture and eventually chemically refined in an attempt to increase affinity. The crystallography structure of LIN28 bound to *let-7* has been solved and shows a large binding pocket that recognizes a GGAG motif found in all *let-7* family members. Modeling in silico has resulted in several small molecules that mimic the GGAG structure. These rationally designed small molecules will also be tested using the fluorescent polarization assay.

Role of the SidE family effectors in *Legionella longbeachae* virulence (Poster)

Yannan Tian, Purdue University

Legionella pneumophila is a gram-negative, rod-shaped bacterium that is capable of causing Legionnaires' disease. It invades and replicates within alveolar macrophages and epithelial cells upon transmission to the human host. (Zink et al., 2002) Formation of a replicative niche is controlled by a type IV secretion system, designated Dot/Icm, which blocks the endosomal-lysosomal degradation pathway within the host cells. (Zink et al., 2002) *L. pneumophila* is a well-studied model compared to *Legionella longbeachae*. Both species belong to the family Legionellaceae and are genetically homologous. Nearly all cases of Legionnaires' disease are caused by *L. pneumophila* and *L. longbeachae*. The analysis and comparison of *L. longbeachae* genome with *L. pneumophila* has demonstrated common features but also many differences. *L. longbeachae* demonstrates difference in virulence factors from those of *L. pneumophila*, though the Dot/Icm type IV secretion system is still essential for the virulence of *L. longbeachae*. Recent study about *L. pneumophila* effectors revealed that the SidE family effectors (including SidE, SdeA, SdeB, and SdeC) are able to ubiquitinate Rab33b independently of E1 and E2 enzymes in the presence of nicotinamide adenine dinucleotide (NAD). (Qiu et al., 2016) Based on the homology of two effectors, llo_3092 and llo_3095 in *L. longbeachae* to the SidE family in *L. pneumophila*, we predict that they share similar function as the SidE family members in *L. pneumophila*. Thus the specific aims of my proposal are:

1. To determine the function of proteins expressed by *L. longbeachae* genes llo_3092 and llo_3095, testing whether they perform the similar function as SidE family in *L. pneumophila*, that is, to ubiquitinate the substrates

without the help of E1 and E2 enzymes.

2. Create deletion mutants in *L. longbeachae*, to test the defective replication demonstrated by *L. longbeachae* Δ 3092, *L. longbeachae* Δ 3095 as well as the double mutant *L. longbeachae* Δ 3092 Δ 3095.

The roles of non-structural proteins in *Flavivirus* assembly (Poster)

Morgan Schafer, Mike Dibiasio-White, and Richard Kuhn

The recent connection between Zika Virus and microcephaly birth defects and neurological disorders has given way to an emerging public health threat. *Flaviviruses* are a genus of viruses that have a single-stranded RNA genome and include Dengue Fever Virus (DENV), West Nile Virus (WNV), and Zika Virus (ZIKV), which are mosquito-borne. There are currently no vaccines or antiviral therapies available for these viruses.

The *Flavivirus* genome codes for a single polypeptide that is cleaved into three structural and seven nonstructural proteins. Non-structural Protein 1 (NS1) has been shown to be essential for virus replication and have immune evasion properties. It exists in multiple oligomeric forms and is secreted as a hexamer which allows it to interact with the innate immune system. NS1 levels in the serum of patients infected with Dengue have been shown to correlate with disease severity. In an effort to understand interactions with the host that lead to disease pathogenesis, we used a mutagenic approach to identify areas of NS1 that contribute to its function in disease pathology. Preliminary experiments have shown NS1 has a role in viral particle formation, making it an attractive target for antiviral drug development. By better understanding the function of NS1 and how specific residues result in different host interactions, we hope to find a target for a vaccine or antiviral treatment that could minimize the number of people infected and reduce the deaths associated with these diseases in the United States and around the world.

Physics & Astronomy Section

Can we predict electronic structure without electronic orbitals? -- Lessons from visualization

Antonio C. Cancio, Ball State University and Jeremy J. Redd, Utah Valley University

Electron density functional theory is the go-to method for predicting structural and electronic properties of molecules and materials, combining speed and the accurate description of electronic structure at the atomic level. One limitation to this approach is the need to describe the electronic kinetic energy in terms of individual electron orbitals, in order to reproduce important features of the electron density such as atomic shells and chemical bonds. Orbitals becomes impractical for systems like large biomolecules, nanodevices, and matter under extreme conditions, where the number required to describe the system becomes prohibitive and effective orbital-free methods thus become important. We visualize the positive-definite kinetic energy density (KED) in closed-shell atoms, as determined by electronic orbitals, in terms of invariant quantities based only upon the total electron density. We notice a striking fit of the KED within the core of any atom to a gradient expansion model using both the gradient and the Laplacian of the electron density, but one different from that derived from first principles for a slowly-varying electron gas. Correlated with this feature, we notice unexpected structure to the KED near the nucleus that cannot be explained simply by current models. These unexpected features provide potential insights for developing better orbital-free meta-GGA models for the kinetic energy, and provide improved description of molecular binding energies.

Electronic Properties of Hexagonal Boron Nitride and Graphene Nanoribbons

Albert DiBenedetto, Mahfuza Khatun, Antonio Cancio

An actively studied area of condensed matter physics is the use of extremely tiny, two-dimensional nanostructures like hexagonal boron nitride nanoribbons (BNNRs) and graphene nanoribbons (GNRs) for energy storage and device fabrication. We are investigating the electronic properties of both materials. Results of band structure, density of states (DOS), and conductance of both materials will be presented. In addition, we will show how altering atomic edge sites affect the band gap in BNNRs and GNRs. The BNNRs are insulating and on the other hand, GNRs are either metallic or semiconducting. Theories include a Tight-Binding (TB) model with Huckel theory, equilibrium Green's function method, Landauer formalism, and Density Functional Theory (DFT). The TB model is used to calculate the band structures and DOS, and the Green's function theory and Landauer formula are used to obtain the transmission function and the conductance. ABINIT computational software implements DFT to calculate the band structure of boron nitride and VMD software is used to visualize the electron density. The results of DFT will be compared with the TB model.

Electrostatic charging of membranes by adenosine triphosphate

Ryan Z. Lybarger, Bruce D. Ray, and Horia I. Petrache, Indiana University Purdue University Indianapolis

Biological processes rely on energy transfers from chemical sources to molecular machines such as ion pumps, mechanical motors, and molecular switches. This energy transfer often takes place in the vicinity of lipid membranes, however, the interaction of ATP with simple lipid bilayers is yet unknown. We investigate this interaction by NMR spectroscopy, small-angle x-ray scattering, and dynamic light scattering. We find that ATP molecules bind to phospholipid bilayers as measured by a restriction of phosphate motion as well as by a net charging effect of membranes. Interestingly, the membrane charging due to ATP gives rise to an unbinding transition of phosphatidylcholine multilayers which is not seen in the presence of monovalent ionic salts. We quantify this particular electrostatic effect of ATP in terms of surface charge distributions and interbilayer interactions. This study can help descriptions of energy transfers in biomembrane processes.

Physics and Astronomy Section continued.....

Experimental Studies of ^{65}Cu (α , p) ^{68}Zn reaction for Nuclear Astrophysics

Samuel Teye and Mohammed Islam, Ball State University and Richard J. Deboer and Wanpeng Tan, University of Notre Dame

Nuclear reaction rates are needed for understanding the origin and abundance of elements. The r- and s-processes are currently well understood and can be used to explain the abundance of most elements. The abundance of some elements such as proton rich elements cannot be explained using the r- and s- process. A third process, known as p- process may provide an understanding for the proton rich elements. To understand the synthesis of some heavier elements ($A > 60$), produced mainly during a supernova explosion, the p- process is studied. ^{65}Cu (α , p) ^{68}Zn is a reaction to be studied for p-process nucleosynthesis. The experiment was conducted at the Nuclear Facility at the University of Notre Dame using the FN Tandem Van de Graaff Accelerator. The energy of the incident alpha particle ranges between 8 to 11.5 MeV. An array of solid state charged particle detectors placed at angles between 25 to 160 degrees was used so that data can be accumulated at various angles for a given incident energy, all at once. Data at a different energy is obtained by simply changing the energy of the incident particle. Experimental set up and the analysis of data will be reported.

Flow Patterns in Simulated Contact Binaries

Patrick M Motl, Indiana University Kokomo

We present long-term dynamical evolutions of symmetric contact binaries through approximately 90 orbits. Our simulations are conducted with a fully three-dimensional Eulerian code for self-gravitating fluids. While the initial data for the simulations describe two symmetric stars that just reach up to the inner Lagrange point, a steady flow and exchange of material between the two stars is quickly established. We examine this flow and the role that similar features may play in real contact binary systems.

Investigating the Electronic and Structural Properties of Stanene

Brendan Ferris and Antonio Cancio, *Ball State University*

We investigate the structural and electronic properties of two-dimensional tin, or stanene, under compressive and tensile biaxial strain using density functional theory (DFT). Stanene possesses a buckled honeycomb-like structure and is a potential candidate for a quantum spin hall (QSH) insulator in which a quantum hall effect is generated in the absence of a magnetic field due to strong spin-orbit coupling (SOC). This effect, in combination with a strain-tunable band gap, makes stanene an interesting material for spintronic applications. Stanene is stable in both a high-buckled configuration, which is metallic, and a low-buckled configuration which gives rise to a QSH effect, and a transition between the two can be induced through strain. For a monolayer of tin, the high-buckled phase is more stable; we investigate whether multiple layers of tin or a combination of tin and germanium can ensure the low-buckled phase remains the most stable configuration as well as explore the transition from monolayer to bulk tin. We use ABINIT, a plane-wave pseudopotential DFT code that accurately reproduces all-electron calculations of ground-state energies and densities, and which is used to determine the

ground state atomic structure and electronic band structure. We also introduce a Tran-Blaha correction to the standard DFT band structure calculation to improve the estimate of the band gap of the low-buckled phase of stanene.

Modeling of W Ursae Majoris Stars

Alan A. Gavel II and Robert C. Berrington, Ball State University

I present the modeling of two eclipsing binary stars. An eclipsing binary star is defined as a pair of stars that are gravitationally bound and the orbital plane is oriented such that one star eclipses the other as it moves between its companion and Earth. The modeling is completed using the PHysics Of Eclipsing BinariEs (PHOEBE) software. The purpose is to establish orbital periods and other orbital parameters of the two eclipsing binary stars.

The Northern Sky Variability Survey (NSVS) has compiled an extensive temporal record of photometric data. The objective was to detect and investigate any kind of photometric variability. The survey used a robotic system of telephoto lenses equipped with CCD cameras to systematically monitor very large areas of the sky. With an optical range from approximately 8th to 14th magnitude the NSVS observed in Johnson I-band and Johnson V-band. With hundreds of measurements per object, light curves for millions of objects have been created. From this data, several thousands of variable stars have been discovered, classified and cataloged. Because there are so many, relatively few have been extensively studied. Also, the large area nature of the survey means the temporal coverage of the stellar systems is course and sporadic. A lengthy baseline of a year or more is used to generate the light curves, but the lack of the temporal resolution to resolve an individual orbit prevents an extensive photometric study of these systems. I targeted short period eclipsing binary stars because I can achieve greater resolution and thus establish the orbital periods with greater accuracy.

I observed the targeted stars in three band-pass filters, Johnson B, Johnson V and Cousins R. The observations were conducted using the 0.4 meter and 0.6 meter telescopes at the Cooper Science Observatory located on the Ball State University campus. Photometric data was analyzed using the Image Reduction and Analysis Facility (IRAF). The generated light curves show that both of the targeted stars are W Ursae Majoris stars; they are contact binaries. The study provides a preliminary understanding of these stellar systems and will be useful to future investigations.

PUFA: A Raft-Domain Driving Force

Jacob J. Kinnun, Xiaoling Leng, Andrew Meador, and Stephen R. Wassall, Indiana University–Purdue University Indianapolis, Dylan Johnson, Edward Ross Pennington, and Saame Raza Shaikh, East Carolina University, Greenville, NC

It is known that DHA, an omega 3 polyunsaturated fatty acid (PUFA) commonly found in fish oil, can alleviate neurological, inflammatory, and autoimmune disorders. It is currently unknown how DHA benefits the body on a molecular level. Since DHA is implicated in a wide range of disorders and is a fatty acid (which can embed into cellular membranes) its molecular mode of action is believed to be membrane-mediated. As human skin can have spots and marks, so can cell membranes. In cell membranes these “spots” are called rafts, which are relatively ordered and contain molecules that allow cells to communicate with each other (called signaling proteins). By using nuclear magnetic resonance, molecular dynamics simulations, and confocal imaging we were able to observe how the structure of raft domains changed in the presence of DHA. We see that DHA increased overall raft domain size which could potentially allow for the clustering and activation of signaling proteins. Ultimately controlling the size of lipid rafts may be, we propose, an underlying role for PUFAs in the plasma membrane.

Stellar Variability Near the Galactic Mid-Plane

Dylan Griffith, Guillermo Gonzalez, Ball State University

Using a thick chip CCD camera, a 20 inch telescope with a 30 arc second field of view, and a differential photometric data pipeline, we can observe near the galactic mid-plane and detect several stars simultaneously. Interstellar dust and stellar crowding make it difficult to resolve individual stars with enough accuracy to learn much about their characteristics. By observing in the near-infrared, we can compare magnitudes of stars over the course of a year to look for variability while ignoring the effects of interstellar dust in our galaxy. This process is expected to discover several new variable stars as well as exoplanets via transit.

The Ball State University Short-Period Variable Star Program

Robert C. Berrington, Thomas Jordan, Alan Gavel, Michael Holcomb, Jon Van de Water, Erin Tuhey, Ball State University

The Ball State University (BSU) variable star program is a program designed to target variable stars with shorter periods ($p < \sim$ day) that have been recently discovered by large area sky surveys like the northern variability sky survey (nsvs), and the all sky automated survey (asas). these surveys have discovered numerous variable stars with visual magnitudes in the $8 < v < 15$ range. the bsu short-period variable star program will supplement these large surveys by providing the systematic temporal coverage and accurate photometric coverage needed to provide a comprehensive study of these systems. to date most targets are eclipsing variable stars of the *W Ursae Majoris* and β *Lyrae* types but also include *RR Lyrae* pulsating variables. All photometric measurements are obtained by the Cooper Science Rooftop Observatory, which includes a 0.4-meter and a 0.5-meter telescope, and the SARA-KP 1-meter, the SARA-CT 0.6-meter, and the SARA-RM 1-meter telescopes. The modular nature of the study makes this program ideal for students to get involved at all stages of the program. I will summarize the work that has been done to date with both students and faculty.

Physics and Astronomy Section continued.....

Transmission and Circular Currents in a Hex-Quantum Dot Ring

Eric Hedin and Yong Joe, Ball State University

Using a tight-binding model of the Schrodinger equation, we analyze the electron transmission properties of a nano-scale ring structure with six embedded quantum dots (QDs). This system can also provide a model for a molecular benzene ring structure, which we consider in the meta configuration. Calculations show quantum-mechanical circular transmission resonances exist for this asymmetric coupling configuration even in the absence of an external magnetic flux. From these transmission functions, we employ a standard Landauer formulation to calculate the circular current and the system I/V characteristics for variations in the molecule-leads coupling and in the externally applied magnetic flux. The circular current shows particular sensitivity to small variations in flux, when computed over selected bias voltage ranges corresponding to degenerate resonances in the transmission.

Plant Systematics & Biodiversity Section

Comparison of the Structure and Composition of the Woodlands at Skinner Woods and Cooper Woods, Delaware County, Indiana

Ahmed Hubini, Donald Ruch, Megan Crecelius, John Taylor and Kemuel Badger, Ball State University

Approximately 18.8 hectares combined, Skinner Woods and Cooper Woods are located in northwest Muncie, IN (Delaware County). To analyze the structure and composition of the forest in Skinner Woods, twenty-four 15 m radius plots were laid out in four rows [running south to north] with six plots per row [plots running from east to west]. Data collected at each plot included species name, distance from the plot center in m, the azimuth from the plot center, diameter at breast height (dbh: 1.37 m above ground), and the health of the tree. All standing stems, whether dead or alive, were recorded. Tree health was scored on a 0-2 scale, 0 = dead and 2 = no observed health problems. Stems were placed in three size classes, i.e., $dbh \geq 20$ cm, $dbh \geq 10$ cm but < 20 cm, and $dbh \geq 5$ cm but < 10 cm. tree data from cooper woods was provided by dr. kem badger. when only living stems were considered, including *Fraxinus* spp., according to the by Society of American Foresters, both properties would be classified as a "Midland hardwood forest dominated by oak and hickory." The relative importance value for all species of *Carya* and *Quercus* combined was nearly identical for both sites, i.e., 72 for Skinner Woods (SW) versus 69 for Cooper Woods (CW). However, *Carya* spp. had a higher importance at SW (29) then at CW (17). The same three species of *Carya* were found at both sites. Five species of *Quercus* were found at both sites, while *Q. palustris* and *Q. velutina* were only recorded at CW. While present at both sites, the importance of *Q. shumardii* was 4x higher at SW. Between the non-*Carya* and non-*Quercus* species (henceforth referred to as others), CW had a lower diversity (six species) then SW (ten species). Among the other species, four occurred at both sites, i.e., *Acer saccharum*, *Aesculus glabra*, *Ulmus americana*, and *U. rubra*. *Gleditsia triacanthos* and *Acer rubrum* were found only in CW, while *Celtis occidentalis*, living *Fraxinus* spp., *Juglans nigra*, and a *Malus* sp. were found only in SW.

An Inversion Hot Spot in Lobeliaceae Plastid Genomes

Eric B. Knox, Indiana University

The evolution of plastid genomes (= plastomes) in land plants is typically very conservative, with extensive structural rearrangements present in only a few groups, including the Lobeliaceae. Early Southern blot analysis identified two related species (*Lobelia fervens* and *L. erinus*) that minimally required deletion of the plastid gene *accD* and five inversions to account for their plastome arrangement relative to the ancestral organization. Sixty alternative 5-step inversion scenarios could account for the observed pattern of nested inversions, but only one scenario was consistent with the criterion of 'common cause' attributable to a putative rearrangement hot spot at the *accD* deletion-site. Plastome sequencing demonstrated that this previously hypothesized inversion order is the historically accurate sequence of events, but what caused these inversions? Stem-loop and disruption-rescue models were evaluated for each inversion. One inversion has an obvious stem-loop basis, but the other four inversions were primarily caused by serial insertion of foreign (= extra-plastid) DNA bearing large open-reading frames that disrupted plastome organization at the *accD* deletion-site, and complete plastomes were rescued by seemingly arbitrary ligation or fortuitous recombination at the other inversion endpoint. Transposed copies of DNA segments from elsewhere in the plastome are frequently inserted at the inversion junctions, and four junctions are consistent with the stem-loop ligation model.

Fore!!! Vascular Plant County Records and Species of Conservation Concern at Elbel Park, St. Joseph County, Indiana

Scott A. Namestnik, Orbis Environmental Consulting

The Natural Heritage Program within the Indiana Department of Natural Resources – Division of Nature Preserves tracks populations of vascular plant species throughout Indiana that are on the state endangered, threatened and rare (ETR) species list. A fourth category, watch list, is in place for species once listed as ETR but that are now known to be extant in enough locations to no longer warrant ETR status. A vascular plant and natural features inventory was conducted at Elbel Park in St. Joseph County, Indiana in May, July and September 2016. Elbel Park is owned by the City of South Bend and is maintained primarily as a golf course. Mud Lake, a kettle lake, is at the northern end of the park and is surrounded by undeveloped uplands. The 158-acre area included in the study was comprised of nascent floating islands, a large submergent marsh with an emergent and shrubby wetland fringe, and dry-mesic upland forest with varying levels of degradation. Eleven (11) ETR and nine watch list vascular plant species were documented during the inventory. Prior to the study, only two of the ETR and watch list species had been documented from the vicinity of the site. Four of the ETR and watch list species documented during the inventory were previously unknown from St. Joseph County. Mud Lake, including the surrounding upland areas at Elbel Park, is an important botanical and ecological resource that warrants permanent protection.

Gap Analysis: A method to Identify Conservation Priorities in Indiana

Walter Fertig, Indiana University herbarium

Nature reserves are among our most valuable tools for conserving the full array of biological diversity. Gap Analysis is a method to assess how well networks of nature preserves capture native biological diversity and to identify species or vegetation types that are missing or under-represented (gaps). Traditionally, researchers have applied gap methods to vertebrate species or coarse vegetation types using distribution models superimposed over maps of highly protected areas. I applied modified gap procedures to gauge the protection status of the entire flora of Wyoming and the Colorado Plateau region of Utah using herbarium records and species checklists. In Wyoming, I found that 84% of the state's native plant species had at least one highly protected population. Of the 365 taxa falling through the cracks, two-thirds were rare species. Unprotected species tended to be from desert, grassland, and low elevation wetland habitats that were themselves poorly represented in protected areas of Wyoming. In the Colorado Plateau, 32% of the flora was absent from highly protected areas, despite this region having the highest density of national parks in the contiguous U.S. At least 70% of the unprotected species in the Colorado Plateau occurred in just 12 unprotected "hotspots" of biological diversity. Gap methods hold promise for identifying plant species and vegetation types that are poorly represented in protected areas of Indiana. Symbiota, an interactive online repository of herbarium specimen data, could be a platform for observation records from state parks and nature preserves, making it easier to apply gap methods to the Indiana flora.

Invasive threat of *Pyrus calleryana* (Rosaceae) pear trees in Floyd County Indiana, USA

Sierra Ash, Indiana University Southeast

Pyrus calleryana is a species of pear tree often seen as an ornamental tree. It was brought to the United States in hopes of showing disease resistance to fire blight and was later used for horticultural purposes. It has recently been documented as an escaped species in 25 states and Washington, D. C. On the Indiana University Southeast campus, dozens of this tree have been identified in a thicket area. This is the first report of escaped specimens in Floyd County, IN. The purpose of this study was to examine the characteristics of the escaped *P. calleryana* and better understand their invasive properties. I focused on field ecology data by taking measurements of height, DBH, canopy density, and age. This randomly sampled population (N=50) ranged in age from 1 year to 7 years with the average height = 2.179m, DHB = 36.52mm and canopy density = 49.21%. The relationship between height and DBH was different for the smaller trees (much higher growth in height) than for the larger, fruiting trees. These data were then compared to data collected of purposely-planted *P. calleryana* trees located on campus. While the average DBH and height of the two data sets differ due to age, the height/DBH relationship of the fruiting and mature trees indicate the same growth pattern. Fruit size was also compared by taking 14 fruit samples from each group of trees, showing the size difference was highly significant (p-value < 0.00001). Previous work suggests that *P. calleryana* prefers areas of high light. The target research area had an overall average canopy density of 49.21% and may indicate the ability to thrive in higher shade. Currently, molecular analysis is being done to understand the genetic changes this escapee has undergone and how it contributes to the spread on the IUS campus.

Leaves and fruits of *Pteroceltis* (Cannabaceae) from the Miocene Shanwang Formation of Shandong Province, eastern China

Zhaowen Wu^{1, 2}, William Oki Wong^{1, 3, 4}, and David L. Dilcher⁴,

¹State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, P.R. China,

²University of Chinese Academy of Sciences, Beijing 100049,

³State Key Laboratory of Paleobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing 210008, P.R. China,

⁴Indiana University

Background: *Pteroceltis* (Cannabaceae) is a monotypic genus with a deciduous tree species *P. tartarinowii* (wingedceltis) endemic to China and has been well known for its bark as a key raw material to make high-quality Xuan paper since the Song dynasty. However, relatively little is known about its early evolution and biogeographic origin. Results: On the basis of distinctive leaves and winged fruits, two new fossil-species of *Pteroceltis* were described from the Miocene Shanwang Formation of Shandong Province, eastern China. The new leaf fossil species is characterized by its serrate simple-leaves with a pair of basal secondary veins. The new fruit fossil species possesses orbicular, thin-stipitate, and bilaterally winged fruits. The leaves might be detached from a same species that produced the fruits, representing the same *Pteroceltis* population in the Miocene Shanwang flora. In addition, winged fruits of *Pteroceltis* have been discovered from the late Palaeogene of the United States, Germany, and North Korea. Conclusions: The fossil record indicates that *Pteroceltis* was distributed in the mid-latitudes of North America, Europe and East Asia during the Paleogene. *Pteroceltis* might have become extinct in North America and Europe since the Neogene. East Asia is a wingceltis' refugium, and the Xuan paper utilizing *Pteroceltis* barks was invented by ancient Chinese. Keywords: Biogeography, Cannabaceae, Fossils, Miocene, *Pteroceltis*, Wingedceltis, Xuan paper.

New material of Ginkgoales from the Middle Jurassic of Daohugou, Inner Mongolia, and its implication on paleo-CO₂ reconstruction

Xiao TAN^{1, 2*}, David L Dilcher^{1, 2, 3}, ChunLin Sun^{1, 2}, Qi Wang⁴

¹ Research Center of Palaeontology and Stratigraphy, Jilin University, Changchun 130026, China;

² Key Laboratory for Evolution of Past Life and Environment in Northeast Asia, Ministry of Education, Changchun 130026, China;

³Department of Geology, Indiana University, Bloomington, Indiana 47405, USA;

⁴ State Key Laboratory of Systematic and Evolutionary Botany Institute of Botany, Chinese Academy of Sciences(CAS), Beijing 100093, China.

Recent research on the vertebrate, invertebrate, plants and palynoflora fossils from the Middle Jurassic Daohugou region of northern China indicates that there has been a terrestrial ecosystem with high biodiversity level. As a significant part of this system, the fossil plants have been reported with fewer taxa than insects and animals. Only several species of Bryophyte, Cycads Conifers, Bennettitaleans and possibly angiosperm have been reported. More new taxa are gradually found to supplement.

Our fieldwork on Jurassic outcrops from Daohugou, Inner Mongolia has discovered numerous well-preserved Ginkgoales leaves with cuticle. Those leaves are very common in the Daohugou flora, and the leaves with similar characters were described by Zhou (2007) as associated Ginkgoites leaves of *Yimaia capituliformis* and were mentioned by Wang (2012) as a mimicry plant with hangingfly. However, the epidermal anatomy and the detailed taxonomic information has never been reported before. In present research, the epidermal anatomy of those leaves is provided for the first time. Three taxa are described based on the leaf morphology and epidermal anatomy, and the stomatal parameters are analyzed for the reconstruction of paleo-atmospheric CO₂ concentrations. The stomatal ratio (SR) data collected from nine specimens of the new material yielded a semi-quantitative pCO₂ estimate of 780–1150 ppmv. Combined with the results from the early Middle Jurassic Ginkgoales material (Wu, 2013), it seems to show an increase trend of atmospheric CO₂ concentration between the Aalenian-Bajocian and Bathonia-Callonian, which is agree with the trend revealed by Berner (2006) in GEOCARBSULF model.

Plant Systematics and Biodiversity Section continued.....

Observations of *Thismia rodwayi* (Thismiaceae) in situ: Clues for Locating *Thismia americana*?

Michael A. Homoya, Indiana DNR Division of Nature Preserves, Mark Wapstra, Environmental Consulting Options Tasmania, Wesley Homoya, Tropical Birding, and Barbara Homoya, Veterans Health Administration HSR&D, R. L. Roudebush VA Medical Center.

Thismia Griffith is a genus of mycoheterotrophic herbs distributed primarily in tropical forests. *Thismia americana* Pfeiffer is exceptional in its range and habitat and is the only North American representative of the genus. It was discovered new to science by Norma Pfeiffer in 1912 at a Cook County, Illinois wet prairie about two miles from the Indiana state line. A mere 5 years after its discovery it was lost to the world and has never been observed since, either at the type locality or elsewhere. Within the genus, *Thismia americana* together with *T. rodwayi* F. Muell. and *T. hillii* (Cheeseman) N. Pfeiff. of Australia and New Zealand and *T. huangii* P.Y. Jiang & T.H. Hsieh of Taiwan constitute section *Rodwaya*. Similarities in morphology obviously exist between these species and *T. americana* but there are distinct differences, including perianth color and habitat. The authors recently visited a site in Tasmania to view *T. rodwayi* in situ. Field observations of *T. rodwayi* may prove instructive in the re-discovery of *T. americana*.

The curation and digitization of the Huntington University Herbarium (Poster)

Nicholas A. Bond, Kelly L. Piepenbrink, and F. Collin Hobbs, Huntington University

The Huntington University Herbarium houses approximately 10,000 vascular plant specimens dating back to the late 1800s. Founded in 1903 by Dr. Fred A. Loew, most specimens in the herbarium were collected in northeastern Indiana during the 1930s-1960s. The herbarium remained largely inactive following the 1960s, and had not been curated since this time. Our goals for the first year of this project were to create an initial digital inventory of the collection in its current state and begin the process of curating and imaging each specimen. Pre-curation specimen data (family, genus, species, authority, collector, and date) were recorded in a Microsoft Access database for the entire collection. Specimens were then individually curated to verify their species identification and update taxonomy to currently accepted names. Following curation, high resolution digital images were captured for each specimen and images and basic label data were uploaded to the Consortium of Midwest Herbaria, a Symbiota portal, where these data are now publically accessible. A total of 9,606 specimens were recorded in the pre-curation inventory, representing the work of 75 different collectors, with collecting dates ranging from 1882 to 1991. Of these, 3,588 specimens have been, to date, curated, imaged, and uploaded to the Consortium portal. The original identification of 11.4% of curated specimens was found to have been in error, and a further 18.3% of specimens had taxonomic name changes at the genus or species level. As a whole, our system for cataloging and digitizing worked efficiently and smoothly. Our two person team could image and upload over 50 curated specimens per hour. Our results show that, using the right techniques, herbaria can be efficiently digitized to make botanical information more accessible to the broader scientific community.

Psychology

Are there Gender Differences in Response to Social Exclusion? A Pilot Study (Poster)

Paula B. Eddleman, Adam M. Schwartz, Rodney W. Roosevelt, Indiana University Southeast

Humans have an innate need to belong. When the need to belong is not met, humans can experience social exclusion. Social exclusion reduces positive affect, increases negative affect, has complex effects on aggression, and induces the release of cortisol, a hormone secreted during stressful situations. Cyberball, a widely accepted experimental manipulation used to induce feelings of social exclusion, decreases positive affect but does not convincingly elevate cortisol concentrations or increase negative affect. In our hands, Cyberball concurrent with a sympathetic stressor produced decreased initial aggressive responses, increased negative affect, and elevated cortisol concentrations in a female population. In a male population, we observed increased initial aggressive responses and decreased negative affect. Current investigations are focused on analyzing cortisol concentrations from the male population to determine remaining differences among each of the groups.

Science Education Section

Active Learning in the Organic Chemistry Classroom

Ryan Jeske, Ball State University

Active learning, defined as “anything course-related that all students in a class session are called upon to do other than simply watching, listening and taking notes,” has been shown to be an effective teaching method at the undergraduate level, including in general and organic chemistry classes. I have been using active learning in First Semester Organic Chemistry since the Fall 2015 semester. In this presentation I will discuss the results of my experience, and I will compare the active learning format to a traditional chalkboard lecture in the same course. In addition, I will share anonymous student responses to surveys about the active learning course format that show a high degree of student support for this format.

Does the mode matter? Attitudes and behavior choices for environmental issues under different modes of information delivery (Poster)

Brittany A. Maule, and Melody J. Bernot, Ball State University

In 2006, the United States ranked in the bottom 50% of science literacy globally. Understanding scientific issues is critical to personal, political, and global decisions. Thus, the need for effective science communication is paramount. With up to 60% of Americans consuming science information from online sources, it is necessary to understand what modes of communication are most effective at transmitting information and initiating positive actionable change. One issue where all individuals can make actionable changes is in the emerging issue of pharmaceuticals and personal care products in the environment. Thus, this issue was used as an educational topic to assess what communication modes are most effective at transmitting information and affecting attitudes and behavior choices. This study was done with 291 students at three schools in Delaware County. Students were given a pre-survey consisting of demographic, attitude, and scenario questions followed by exposure to one of three communication modes: website, video, or pamphlet. Following information exposure, students completed a similar post-survey. Student responses indicated the website and pamphlet were more effective at transmitting knowledge in the short-term as well as potentially changing student behavior choices. Additionally, the pamphlet seemed most effective at influencing student attitudes about the environment. While the video did transmit knowledge, and affect student behavior choices, these effects were often less pronounced than the website and pamphlet. These results support that differences in information processing can lead to decreased learning with overstimulating media. Future research replicating this work with other environmental issues and other demographics may yield more insight into whether these findings can be applied broadly.

EPIC: Establishing Practices Integrating Commuter Students

Kristi Bugajski, Mike Watters, Mindy Capaldi, Karl Schmitt, Jon Schoer, Bonnie Dahlke

Commuter students face challenges which distinguish them from residential students. Commuters are more likely to work off campus (of necessity). Commuters are less likely to avail themselves of academic opportunities which are not required for their degrees. Commuters are also less likely to be socially engaged with the wider campus community. These factors have all been shown to have a detrimental impact on student success, causing overall lower graduation rates among commuters.

EPIC (Establishing Practices Integrating Commuters) was funded by the National Science Foundation (Award number: 1564855), for \$999,991 to Valparaiso University to integrate research focused commuter students into the campus community. This talk will discuss the progress of the program in its first year.

Improving STEM Student Retention via Early Research Engagement: A Pilot (Poster)
Michael Watters and Patrice Bouyer, Valparaiso University

We describe here the results of a pilot study, the goal of which was to expose freshman to an ongoing research project during the academic year to promote student growth and improve retention in the STEM disciplines. Freshmen worked with a faculty mentor and were also chaperoned by a more senior student researcher in order that they learn lab techniques and the capacity to work independently. Participants were fully engaged in a research project (performing experiments, analyzing and discussing results), not a classic classroom projects, but discovery based projects. By bringing students into the research lab at this early stage, our aim was to improve retention by allowing science students to actually act as scientists, providing an enhanced experience over the usual freshman survey course content. Of the 6 students who joined the program as freshmen, 5 are still in their major, 4 are still actively engaged in research with a faculty member and have co-authored 11 different papers and conference presentations as of their junior year.

Integrated STEM Education: A TRAILS Approach
Jeffrey D. Holland, and Todd R. Kelley, Purdue University

The TRAILS project is preparing high school teachers to increase knowledge and interest in integrated STEM with lessons at the intersection of biology and engineering design. Students use principles of biomimicry to apply solutions found in nature to real world design problems. Candidate solutions are prototyped with three-dimensional printers and tested. Project success is measured by the increase in teacher self-efficacy and in the students' development of 21st century skills: creativity, critical thinking, collaboration, communication, and conceptual thinking. Transfer problems evaluate students' ability to use solutions in a wider context of problem-solving.

Student Independent Study Projects using *Artemia Salinas* (Poster)
Abigail Borkowski and **Richard C. Roberts**, Grace College

One of the best ways for students at any level to learn about the scientific method is through independent investigation. Such projects can be very rewarding, as students have the opportunity to see the whole scope of scientific investigation, from the identification of a question to address through experimental design and analysis of results. The challenge with such a project at the collegiate level is the shorter duration of time available for such projects to be completed – in the case of Grace College, terms that span only 8 weeks. These projects are a key part of the laboratory experience for advanced classes in both the Plant Biology and Animal Biology courses at Grace. The Plant Biology course has successfully made use of the fast growing model organism *Arabidopsis thaliana* for independent study projects. The desire was to find an equivalent organism for Animal Biology. Desirable traits include ease of culturing, rapid growth (ideally completing the entire life cycle in ~6 weeks), low overall cost and interesting variables for analysis. In this work, we describe the establishment of *Artemia salina* (commonly known as brine shrimp) as a workable test system. We demonstrate simple, straightforward culture conditions that allow the *Artemia* to grow and flourish, with analysis of food source, feeding frequency, and culture vessel cleaning. We present a workable system that will allow for a variety of factors to be examined by students developing independent study projects as part of the Animal Biology course; this system could be adapted for use in a variety of settings, from elementary education to collegiate level.

The Great Lakes Research and Education Center: making national parks a premier place for science
Desiree Robertson, Wendy Smith, Great Lakes Research and Education Center, National Park Service

The Great Lakes Research and Education Center (GLREC) increases the effectiveness and communication of scientific research in eleven national parks in Indiana, Illinois, Michigan, Wisconsin, and Minnesota. Based at Indiana Dunes National Lakeshore, the GLREC is part of a network of Research Learning Centers (RLCs) established by the National Park Service (NPS) to facilitate the use of parks for scientific inquiry, promote science literacy and resource stewardship, and integrate science into park resource management, educational programs, and the visitor experience. The GLREC accomplishes this mission by collaborating with many

partners including universities, K–12 schools, non-profit organizations, community groups, government agencies, and a range of NPS programs. By engaging partners in park research, scholarship, and educational activities, the GLREC is able to leverage in-kind and financial support, broaden the understanding of national park resource issues, and improve stewardship of some of our nation's most treasured landscapes.

Science Education Section continued.....

Uncertainty: a Key Influence in the Decision to Admit Patients with Transient Ischemic Attack (Poster)

Barbara J. Homoya, 1-2; Teresa M. Damush, 1-4; Jason J. Sico, 2, 5-6; Edward J. Miech, 1-3; Gregory W. Arling, 7; Jared B. Ferguson, 1-2; Michael S. Phipps, 9; Eric M. Cheng, 10-11; Dawn M. Bravata, 1-4
1 Center for Health Information and Communication (CHIC); 2 Veterans Health Administration, Health Services Research and Development Service, VA Stroke QUERI Center, Richard L. Roudebush VA Medical Center; 3 Regenstrief Institute, Inc.; 4 Indiana University School of Medicine; 5 VA Connecticut Healthcare System; 6 Yale University School of Medicine; 7 Purdue University; 8 Precision Monitoring to Transform Care (PRIS-M) QUERI; 9 Department of Neurology, University of Maryland School of Medicine; 10 Department of Neurology, VA Greater Los Angeles Healthcare System; 11 Department of Neurology, David Geffen School of Medicine, University of California at Los Angeles

Hospitalization of TIA patients has been shown to improve the timeliness and quality of TIA care yet admission rates for patients presenting with TIA vary considerably across centers. We sought to identify patient, provider, and system level factors linked to the decision to admit a patient with TIA. Trained interviewers conducted face-to-face semi-structured interviews with staff involved in TIA care at diverse Veterans Administration (VA) sites. All transcripts were de-identified and imported into a single NVivo10 project file for data coding and analysis. 70 audiotaped interviews of multidisciplinary clinical staff took place at 14 Veterans Administration Medical Centers (VAMCs). We identified emergent themes and patterns in providers' decision making. Providers' decisions whether to admit TIA patients were related to uncertainty regarding lack of guidance at sites without a facility TIA-specific policy, inconsistent use of the ABCD2 tool and guidelines, limited resources, and concerns about the ability of the system to complete a timely workup. Other factors included symptom resolution, time-elapsd since event, patient perspectives (feeling well, not wanting to wait for tests and results), and recent TIA evaluation/risk factor management. Although there was the perception that most TIA patients are admitted, the majority of sites admitted less than two-thirds of TIA patients. Use of the ABCD2 tool was inconsistent and varied between sites; higher admitting sites reported a greater reliance on the tool and its guidelines. In conclusion, issues related to uncertainty were reported as key factors in clinical decisions concerning admittance of patients presenting with TIA. Quality improvement interventions for TIA care may need to take into consideration how factors like uncertainty relate to the TIA admission decision-making process. Funding: Veterans Health Administration HSR&D QUERI SDP #12-178

Zoology Section

Age-Related Changes in Nuclear Reduced Glutathione Levels in the Rat Kidney

Marianna Zmlauski-Tucker, Ashley Emmons and Bingwei Ye, Ball State University

Aging is associated with change in the cell. There is limited information on the effect of age on nuclear reduced glutathione (GSH) levels. Maintenance of GSH, the major antioxidant inside cells, is important for protection against damage from free radicals produced during oxidative metabolism. The purpose of this study was to

investigate changes in GSH levels in the nucleus of kidney cortex and medulla from Young (i.e., 3 months of age; n= 6) and Old (i.e., 22 months of age; n=6) female Lewis rats. The kidneys were harvested from anesthetized rats and the cortex and medulla were separated and homogenized. The nuclear fractions were isolated using differential centrifugation. The GSH levels were measured by a spectrophotometric assay and compared using a Student's t test. There was a significant decrease in nuclear GSH levels with age in the rat kidney cortex but not in the rat kidney medulla. These findings suggest that molecules in the nucleus from the rat kidney cortex may be susceptible to injury from free radicals with age.

Clutch-level variation in predator avoidance behavior in wood frog (*Lithobates sylvaticus*) tadpoles

Mackenzie M. Spicer, Shelby L. Hart, Brian G. Gall

In nature, there is abundant variation in morphology, physiology, and behavior. Genetics and environmental conditions both contribute to this variability. We tested the predator avoidance behavior of Wood Frog (*Rana sylvaticus*) tadpoles from six clutches to determine if variability existed between independent clutches reared under the same environmental conditions. Tadpoles were exposed to alarm cues from damaged conspecifics and kairomones from a predator (after a learning event) and the corresponding reduction in activity was recorded. Although clutches differed slightly in their responses, we found no significant variation in behavior between clutches, suggesting all clutches exhibit similar intensities of predator avoidance behavior. The potential reasons for the presence of some variation, as well as the lack of overall differences, are discussed.

Delayed nest emergence and dispersion to water in Snapping Turtle (*Chelydra serpentina*) hatchlings: consequences to plasma osmolality and urea content. (Poster)

Jessica R. Filer and Michael S. Finkler, Indiana University Kokomo

Snapping turtle (*Chelydra serpentina*) hatchlings typically emerge from their nests soon after hatching and overwinter in aquatic habitats. Overwintering in nests is extremely rare, even in areas where freezing risks are low. In this study we examined how remaining for three weeks in a terrestrial environment similar to the interior of the nests influences body size and blood plasma hematology compared to animals held in water for three weeks. Hatchlings (7 days post-hatching) were assigned to three different groups. Plasma was harvested from animals in one group ("Initial") at 7 days post-hatching for determination of plasma osmolality. Hatchlings in the other two groups were weighed, had their carapaces measured, and were placed into either moist sand ("Land") or dechlorinated water ("Water") where they were held at 23 °C for 21 days. At Day 28 hatching the hatchlings in the Land and Water treatments were weighed, had carapace length measured, and were sacrificed. Wet carcass mass and wet yolk sac masses were determined, and plasma was harvested for determination of plasma osmolality. Carapace lengths did not change in either Land or Water groups between Day 7 and Day 28 post-hatching. Hatchlings held in sand maintained a stable body mass during the three week period, whereas those held in water gained mass. This gain in mass is attributable to water uptake in the body, as wet carcass mass of Water group hatchlings was higher than in land group hatchlings but neither wet yolk sac mass nor carapace length differed between the two groups. Mean plasma osmolality was higher in Land hatchlings (408 mOsm) and lower in Water hatchlings (266 mOsm) compared to Initial plasma samples (325 mOsm). Mean plasma urea concentrations were higher in Land hatchlings (44.4 mM) than in initial samples (18.7 mM), but Water hatchlings had similar urea concentrations (15.2 mM) to those of initial samples. The increase in urea concentration accounts for ~31% of the difference in plasma osmotic concentration between Land hatchlings and Initial samples, suggesting that increases in other plasma solutes are also important factors contributing to the elevation in plasma osmolality. As wet carcass mass did not change over 21 days in Land hatchlings, elevated concentrations of total plasma solutes may represent a response by the hatching to maintain fluid volume and blood pressure. Nevertheless, sustained continuous elevation of urea and other potential toxic substances in the blood may, in part, account for why Snapping Turtle hatchlings rarely overwinter in the nest.

Is the Marsh Rice Rat (*Oryzomys palustris*) a Potential Animal Model to Study the Effects of Alcohol Administration on Reproduction?

Kent Edmonds, Indiana University Southeast

Chronic alcohol consumption can have significant effects on numerous physiological functions. The studies presented herein addressed whether alcohol can affect reproduction in rice rats. In various experimental paradigms, animals were administered alcohol prenatally, during lactation, postnatally until puberty, as adults, and when aged. The masses of various reproductive as well as nonreproductive organs were examined. In addition, pineal melatonin content was examined after both chronic and acute alcohol administration. Using a

compensatory testicular hypertrophy (CTH) model in juvenile males, both lactational and post-lactational alcohol consumption inhibited CTH at eight weeks of age, but prenatal alcohol had no effect. The blood alcohol concentration was significantly elevated in alcohol-treated animals. In a separate study, lactational alcohol administration inhibited growth and testes development by ~50% at sacrifice at 21 days of age. Postnatal alcohol administration in juvenile females failed to affect reproductive development, growth, and digestive system development. In adult males, 12 weeks on a short photoperiod (12L:12D) caused testicular, seminal vesicle, and Harderian gland regression, but alcohol coupled with a short photoperiod failed to enhance the inhibitory effects of the short photoperiod. Alcohol administration to aged males and females for 10 weeks did not affect body mass, reproduction, or any other non-reproductive organ examined. Finally, both the chronic administration of alcohol (15%) for 3 weeks and an acute injection of alcohol (25%) did not significantly affect pineal melatonin content when assessed during the dark. Taken together, these data suggest that reproduction can be affected by alcohol, but that certain developmental stages (younger vs older animals) may be better suited to study the effects of alcohol on reproduction in rice rats. The age of the animals at the time of alcohol administration may need to be considered in any experimental design involving the use of alcohol.

Zoology Section continued.....

Phylogeographic evidence for post-Pleistocene dispersal of the least brook lamprey (*Lampetra aepyptera*) in the Ohio River system

Rex Meade Strange and Alanna Noland, University of Southern Indiana

The least brook lamprey (*Lampetra aepyptera*) is a common inhabitant of small streams throughout the southeast United States and reaches its northern-most extent near the boundary of the glacial till plains of southern Indiana and Ohio. Although the natural history of *L. aepyptera* is well documented in other parts of its distribution, little is known about the populations occurring in Indiana or their historical relationships with other populations. We examined sequence variation of two mitochondrial genes from specimens of *L. aepyptera* collected from across its distribution to better understand the phylogeographic position of the Indiana populations. Specimens collected from tributaries of southern Indiana and Illinois formed a well-supported monophyletic group with specimens collected from tributaries of the upper Ohio River. In turn, the Ohio River clade is part of a poorly resolved assemblage that includes populations from tributaries of the Green River (central Kentucky) and direct tributaries of the Mississippi River (western Kentucky and Tennessee). The Ohio River clade shows evidence of reduced genetic heterogeneity relative to more southerly populations, consistent with an assemblage of populations that has recently expanded. Our results suggest that the distribution of *L. aepyptera* in the Ohio River valley follows the integration of the Ohio River during the late Pleistocene.

Predator-Prey Interactions Between Burrowing Crayfish and Early Life-History Stages in Amphibians (Poster)

Theresa E. Wrynn and Brian G. Gall, Hanover College

Crayfish can be major predators of vertebrates, and often include amphibian eggs in their diet in addition to plant material and decaying organic matter. We conducted a series of studies examining the role of burrowing crayfish (*Cambarus polychromatus* and *Cambarus diogenes*) on the survivorship of eggs and larvae from several native amphibian species. We found these crayfish readily consume amphibian eggs in captivity and are also capable of consuming recently hatched larvae. Given the habitat overlap between these organisms, these results suggest that burrowing crayfish may be major predators on these vulnerable life-history stages in amphibians.

Stress: A Major Problem in Aquaculture

Ahmed Mustafa, **Alycia Herndon**, Ashley Baum, and Kenneth Saillant

The world's population is rising, and the subsequent demand for high protein sources of food is rising with it. A push for healthier sources of protein is also in increased demand. Protein from current sources, such as chicken, cattle, and other livestock, is not adequate to meet the demand due to a lack of grazing area, climate limitations, long time spans to harvest, and other considerations. Wild fish populations are also not large enough to meet this demand, and many populations are showing signs of overharvesting. Aquaculture, therefore, is rising in prevalence and importance in today's society with the increase in fish consumption and the limitations imposed by normal fishing methods. Aquaculture offers a solution to these issues by providing a high protein

food that requires less space to farm, has a faster harvest time, lowers the cost of production, and is less detrimental to the environment. However, current aquaculture techniques involve the use of antibiotics and other chemicals in order to reduce disease and mortality within the crops. Disease and mortality present a significant problem in farmed fish, which are usually kept in crowded conditions that lead to stress. This increase in stress over time leads to a reduction in immune response and, therefore, a decrease in the ability to fight against diseases. In order to provide solutions to the problems of aquaculture and to provide quality protein to the consumers without the use of potentially harmful substances, many researchers are looking into the use of nutraceuticals in order to decrease stress responses, increase immune responses, increase growth, and increase the nutritional value of farmed fish. In this presentation, we will discuss the usage of different nutraceuticals and their effects on the modulation of stress and immune responses in aquatic animals.

Zoology Section continued.....

The evolution of novelty in insects: the developmental origin of metamorphosis and sociality from a gene family perspective.

Chris R. Smith, Earlham College

Introduction: Novel phenotypes arise from changes in regulation of pre-existing genes, the evolution of novel genes, and/or a combination of both mechanisms. A distinct worker caste represents a novel body plan that arose simultaneously with eusociality. Most studies that have compared worker and queen gene expression have identified many differentially expressed genes, but have only included one or a limited number of developmental stages. Across species comparisons have, by in large, failed to find shared genes that are biased in expression between castes - instead the findings show shared processes.

Methods: Using the red harvester ant, *Pogonomyrmex barbatus*, system we did RNAseq across developmental stages for both worker and queen castes. We also collected and compared other RNAseq datasets from data repositories across insects.

Results/Discussion: The vast majority of genes in the harvester ant are not consistent in caste bias over development, supporting a limited role of caste-specific genes in caste determination. On the other hand, we found a set of genes in a conserved cluster that are worker biased in all stages measured, and further, that have conserved worker-biased expression across multiple origins of sociality. Comparative transcriptomic data suggest that these genes may have been co-opted for pupal development during the evolution of the Holometabola, and further co-opted during the evolution of the worker caste (multiple independent times). These data support a limited role for caste-specific genes, and suggest that a group of genes have repeatedly been recruited into novel phenotypes that are ecologically and evolutionarily important, holometabolous development and sociality.

The Virginia Opossum (*Didelphis virginiana*) as a Possible Predator of Terrestrial and Aquatic Salamander Species

Shelby L. Hart, Mackenzie S. Spicer and Brian G. Gall, Hanover College

The Virginia Opossum is an omnivorous marsupial native to North America. While they are known to consume a variety of small vertebrates and invertebrates, it is unknown whether they are potential predators on amphibians. We set out to test the predator-prey interactions between these species by measuring the palatability, predator avoidance behavior, and flight initiation behavior of Eastern Newts (*Notothalamus viridescens*), Dusky Salamanders (*Desmognathus fuscus*), and Zigzag Salamanders (*Plathodon dorsalis*) in response to opossums (*Didelphis virginiana*). We found that all salamander species are palatable to opossums. Zigzag salamanders exhibit predator avoidance behavior in response to fecal extracts from opossums, but Dusky salamanders do not. Dusky salamanders exposed to a simulated predation event do however flee more rapidly when exposed to these same fecal extracts. These results suggest that opossums may be willing predators of multiple amphibian species, but that multiple predator avoidance and antipredator behaviors have evolved in salamanders to prevent predation.

Using hair to investigate genetic diversity in the bobcat (*Lynx rufus*) population of Clay Hill Memorial Forest (Poster)

Rebekah J. Dickmander, and Pamela R. Pretorius, Hanover College, Allison Niereiter and Gordon Weddle, Campbellsville University, Campbellsville, KY

Bobcats (*Lynx rufus*) have the largest range of all felines in North America. These highly territorial animals rely on urine spraying for intraspecific communication. Due to the solitary nature of these animals, data on genetic diversity within populations is limited. The goal of this project was to examine genetic diversity of the bobcat population located in Clay Hill Memorial Forest in Campbellsville, Kentucky. Hair samples were collected for genetic analysis by applying purchased bobcat urine to 25 rub pad stations, which were monitored by trail cameras to record mammalian visits. Visual inspection of camera footage revealed a range of small predatory mammals, including opossum, raccoon, and long tail weasel among others, visited the rub pads. Collected hair samples were compared to other terrestrial mammals using a dichotomous key to guide identification of differences in medulla structure, shaft diameter, and cuticle pattern. As felines are self-grooming, hair samples could also be recovered from scat identified as bobcat. A modified DNA extraction method was established using exemplar bobcat hairs taken from animals in the Hanover College museum collection. Efforts to assess genetic diversity in identified hairs is ongoing. Known microsatellite repeats in isolated total DNA extracted from bobcat hairs will be amplified by polymerase chain reactions (PCR) and analyzed for genetic relatedness.

Variation in temperature during early development affects embryonic nonpolar lipid contents in Snapping Turtles (*Chelydra serpentina*). (Poster)

Antonio Salas and Michael S. Finkler, Indiana University Kokomo

Temperature variation can profoundly affect rates of development and growth during incubation in oviparous vertebrates, especially during early stages of embryonic development. Less is known about how temperature can affect overall body composition of the embryo during different stages of development. In this experiment we examined the influence of varying temperature on body mass and total nonpolar lipid content of Snapping Turtle (*Chelydra serpentina*) during the first four weeks of embryonic development. Eggs were incubated under four different thermal regimes: three with constant temperatures of 23, 25, and 27 °C, respectively, and a fourth where temperature oscillated from 22 to 28 °C over a seven day period (mean temperature 25 °C). Eggs were opened after 28 days of incubation, the embryo was removed, observed for developmental stage, dried to a constant mass, and analyzed for total nonpolar lipid content. Among the three constant temperature groups, both dry mass and developmental stage at Day 28 increased with increasing temperature, whereas nonpolar lipid content decreased with increasing temperature. Day 28 embryos from the oscillating temperature treatment had greater dry masses and were more developed than those from eggs incubated at a constant 25 °C, but had similar nonpolar lipid contents. Our findings suggest that developmental temperature not only affects embryonic growth and morphological development during early incubation, but also affects overall embryonic composition. Such differences may, in turn, influence body composition at hatching and the ability of the hatchling to survive nest emergence, dispersion, and the first winter post-hatching.