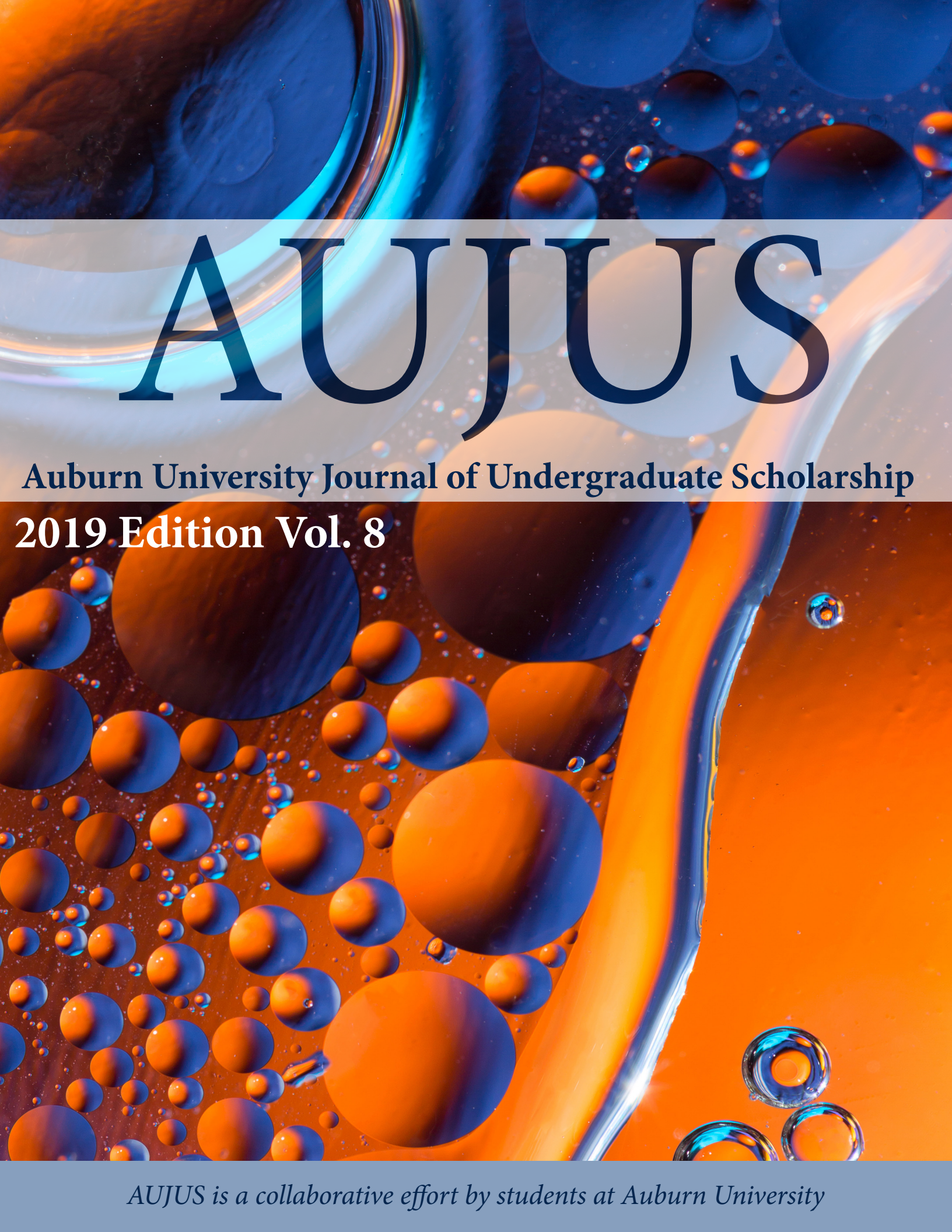

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AUJUS Editorial Team

Production Editor: **Hazl Torres** is a junior pursuing a Bachelor of Chemical Engineering. She is an Office Assistant for the Office of Undergraduate Research. Hazl's other on-campus involvement includes serving as a facilitator for the Alabama Power Academic Excellence Program and being a member of the American Institute of Chemical Engineers. Hazl is also an undergraduate researcher with the Adamczyk Research Group at Auburn University. In her spare time, Hazl enjoys hiking, working out, attending sporting events, and karaoke. Upon graduation, she plans to pursue a career as a chemical engineer.

Associate Editor: **Abby Brittain** completed her Bachelors of Science with the distinction of Honors Scholar in December of 2019. Abigail is continue her education at Auburn University and pursuing her Master of Science. Her research is the field of exercise science and she concentrates on movement assessments in overhead throwing athletes. Abigail is also a member of Team USA Team Handball and competes internationally. She intends to continue both her academic and athletic careers after leaving Auburn Univeristy.

Associate Editor: **Madison Steele** is a junior pursuing a Bachelor of Arts degree in Law and Justice with a minor in Political Science. She works as a Communications Assistant for the Office of Undergraduate Research. Madison's other on-campus involvement includes serving as the Director of Programs for the College of Liberal Arts School's Council, being a member of the Honors College, and being a member of Pi Lambda Sigma (Auburn's Pre-Law Honor Society). In her spare time, Madison enjoys reading, attending concerts, watching Auburn sporting events, and traveling. After graduating, Madison plans to attend law school and focus her career in practicing Civil Rights Law.

Editor-in-Chief: **Dr. Lorraine W. Wolf** is the Auburn University Director of Undergraduate Research and the Lawrence C. Wit Professor in the College of Sciences and Mathematics. She has served as editor-in-chief of AUJUS since 2013. She has been a faculty member in the Department of Geosciences since 1993, where she teaches courses and mentors undergraduate and graduate students in the field of geophysics. Wolf 's main research interests are in earthquake and geologic hazards.

MENTOR OF THE YEAR: Dr. Kathryn Floyd

Dr. Kathryn Floyd first began her journey in research as an undergraduate student, majoring in art history and anthropology, at Vanderbilt University. She credits her interest in educational research to the professors that she encountered there, who talked vividly about their experiences and challenges during their time as researchers. Floyd has focused her research in the history of art exhibitions, primarily those found in twentieth-century Germany. A lot of Floyd's writings have been about a contemporary exhibition called "documenta" that was created in Kassel, Germany in 1955 and occurs every five years.

In her 12 years at Auburn, Floyd has had the opportunity to mentor a number of students through research projects and applications to graduate programs, internships, and jobs including two Undergraduate Research Fellows, both of whom are pursuing graduate degrees in art history at accredited universities. While Dr. Floyd enjoys seeing students develop their skills as researchers and writers, her favorite part of being a mentor is seeing students take established research procedures and adapt them to the quickly changing digital world. She recalls one instance where a student used a popular messaging app to reach out with an internationally renowned artist. Through this innovative idea, the student was able to talk with the artist directly and interview him about his work, even after failing to reach him through more traditional channels. The most rewarding part about mentoring, to Dr. Floyd, is watching students become confident, independent researchers.

Floyd noted "detective work" as her favorite aspect of the research experience." Being able to look at original documents and primary sources," Floyd reflects, "and make connections between them and the scholarship that other researchers have already produced is exciting. Sometimes [one] finds materials that suggest a different way of looking at something or that add a completely new perspective to [ones] understanding of history." When working in archives and in libraries, there is an exciting part about never knowing what one will find or how those materials will route, or reroute, their research. Floyd wants to encourage anyone interested in pursuing research to talk to a research mentor in their field and to take the research process seriously. She also encourages them to pursue the research experience because "skills developed during a student's undergraduate… career… are extremely useful for many different professions- not just academic research."

Dr. Kathryn Floyd, in addition to the Mentor of the Year award, has also been awarded the 2019 Auburn University College of Liberal Arts Advising award, the 2017 Auburn University College of Liberal Arts Teaching Excellence Award, and the 2011 Auburn University Student Government Association Outstanding Faculty Member award for the College of Liberal Arts. She is, also, currently authoring a book about photographs of art installations and exhibitions.

Understanding the Growth and Attachment of Algae on Nanocomposites

Marisa Rodríguez, Derryn W. Herring, Zahra Karimi, David M. Blersch, and Virginia A. Davis

Abstract

The goal of this research is to understand the attachment and growth of algae on nanocomposites that are made from polylactic acid (PLA) and cellulosic materials. In nature, multiple types of algae can grow together on rocks and other surfaces, but the details governing algae attachment and growth are not fully understood. Commercially, cultivated algae can be used for fuel, nutrient supplements, and for pollutant removal. However, different types of algae are preferred for different applications. By better understanding the growth and attachment of algae on different materials, specific substrata could be designed for increasing the yield of specific types of algae. Therefore, a better understanding of the algae attachment and growth would improve the economic viability of algae cultivation for specific applications.

Introduction

Due to the increased media attention on climate change and the negative impacts some fossil fuel processes may have on the environment, many petrochemical companies are investigating alternatives to fossil fuels. For example, the United States is shifting its focus toward the use of biofuels, and plans to replace 20% of fossil fuels with biofuels by 2030.[1] One reason biofuels are of interest for replacing fossil fuels are their lower CO_2 emissions. Algae can have high oil content, making them a promising alternative biofuel that can potentially yield more fuel per unit area than other biofuels.[2] An additional advantage of algae-derived biofuels is that algae can grow rapidly in extreme environments and require less total crop area than is currently used by other biofuel crops.[3]

The high oil content of algae makes it a beneficial nutrient source as well. Fish oil is a popular dietary supplement known for having beneficial omega-3 fatty acids, but current overfishing problems have companies looking for an alternative.[4] Some algae are known to contain those same omega-3 fatty acids in addition to omega-6, vitamin C, vitamin K, and absorbed marine minerals such as iodine, potassium, calcium, and magnesium.[5] Also, the higher concentration of omega-3's in algae means the recommended dosage is less than that of fish oils.

However, the potential benefits of algae for biofuels and nutraceuticals are not being fully realized because of high production costs. The current cost of algae as a biofuel is higher than other biofuel crops. This is largely because of the high cost of harvesting and separating suspended algae from the water in which they grow; this can account for up to 21% of capital costs.[5] Alternative methods of cultivation, where algae is cultivated attached to material surfaces, promises to lower harvesting and separation costs because of operational advantages.[6] In addition, chemical design of material surfaces may allow advantageous selection for desirable algal species, potentially increasing overall yield of bioproducts.[7]

This research explored the attachment of a model planktonic algae, *Scenedesmus dimorphus*, to bio-derived nanocomposites made from polylactic acid (PLA), sulfonated cellulose nanocrystals (SA-CNC), and lignin-coated cellulose nanocrystals (L-CNC). *Scenedesmus dimorphus* was chosen because even though it prefers to be free floating, it can attach to surfaces and is commonly used in investigations of algae attachment on substrates and in studies of biofilm formation.[1,8] PLA was chosen because it is bioderived and biodegradable, and the two types of CNC were chosen because they are also bioderived and have markedly different surface chemistries. The algae were cultivated in flasks in a laboratory, and a method for measuring algae concentration based on UV-vis spectroscopy was developed. Next, the effects of the surface composition on contact angle and algae attachment were measured. The results of this research

provided insights into the attachment of *Scenedesmus dimorphus* on the nanocomposites and established protocols that can be applied to more complex types and mixtures of algae.

Materials and Methods

Algal Culture: The *Scenedesmus dimorphus* culture was contained in a closed 1000 mL Erlenmeyer flask and kept in constant motion on a stir plate at ambient conditions. The algae culture was fed Bold Modified Basal Freshwater Nutrient Solution 50 x (Sigma-Aldrich) once every other week. The attachment testing requires a measured concentration of algae, so the concentration of the overall culture must be known throughout the experiment. The concentration of algae was initially determined using a hemocytometer. A micropipette was used to take 10 µL of the algae culture and inject it into one side of the hemocytometer. A Nikon Eclipse 80i microscope was used to observe the hemocytometer, and the individual algae cells were counted and recorded. This was repeated on the other side of the hemocytometer; then the counted algae cells were used to calculate the concentration of the algae culture. The hemocytometer method requires cells to be individually counted via microscopy, and becomes more inaccurate the larger the algae concentration. Therefore, a UV-vis spectroscopy calibration curve was developed for calculating the algae concentration. A culture with 5.6 x 10^6 cells/mL based on the average of five hemocytometer counts was diluted to 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, and 1:5 algae to water ratios. The diluted samples were put into 10-mm pathway cuvettes and the absorbance of each sample was obtained at a wavelength of 600 nm on a ThermoFisher NanoDrop spectrophotometer.

Substrate Preparation: Nanocomposite substrates were produced using a previously established procedure.[9] A 3 wt% cellulose nanocrystal (CNC) dispersion was created by dispersing either SA-CNC (University of Maine, Orono, Maine) or lignin-coated CNC (Amercian Process Inc., Atlanta, Georgia) into deionized water. The dispersion was then sprayed over liquid nitrogen and freeze-dried to create powdered CNC. This powder was then placed in a ThermoFisher Haake MiniLab compounder along with PLA pellets and allowed to mix at 190 °C and 60 rpm for 20 min to make 8 wt% composites of CNC and PLA. The composite was then extruded into 2-mm filaments. The filaments were then heat pressed into films using a home use electric heat press (Brentwood Appliances) with nonstick surface at medium to high heat. For the plain PLA samples, the PLA pellets were directly heat pressed to create the samples.

Contact Angle Measurements: After the samples were created, their surface energy properties were investigated using a standard goniometer (ramé-hart Instrument Co., Mountain Lakes, New Jersey) and three probe fluids (water, ethylene glycol, and hexadecane). The surface of the material was first cleaned with ethanol and then rinsed using deionized water. Then a drop of the probe fluid was placed on the surface and the contact angle was measured every half second for 15 seconds for a total of 30 measurements per composite sample.

Attachment studies: An attachment test was developed based on modifications to the procedure used in previous literature.[3] A 1000-mL beaker was filled with a 750-mL algae solution with a concentration approximately 100,000 cells/mL. The composites and plain PLA were cut into 1.5 cm x 1.5 cm samples. These samples were each placed in the bottom of the 1000-mL algae-solution beaker, using one sample per type. The beaker was then placed in a dark environment for 24 hr to allow the cells to settle on the surface. The samples were then removed from the beaker and washed on an orbital mixer to remove any unattached or loosely attached cells. The test was repeated for four times. Each composite was analyzed by taking 10 images across the sample using reflected microscopy and a 20X/0.45 LU Plan Fluor objective using a Nikon 80i microscope with Nikon Elements software. The number of algae cells in each 85,300 µm² image were counted; a total of 40 images per substrate were acquired for analysis (with the exception of PLA/L-CNC for which 29 images were analyzed).

Results and Discussion

Figure 1 shows that the absorbance of the algae was linearly related to concentration in accordance with the Beer-Lambert Law, $A = \varepsilon bc$, where A is absorbance, ε is the extinction coefficient, b is the path length, and c is concentration. Based on the slope of Figure 1, $\varepsilon = 3 \times 10^{-7}$ mL (number of cells)$^{-1}$ cm^{-1} for *Scenedesmus dimorphus*. The development of this spectroscopy-

based method for concentration enabled faster determination of the algae concentration than counting with a hemocytometer.

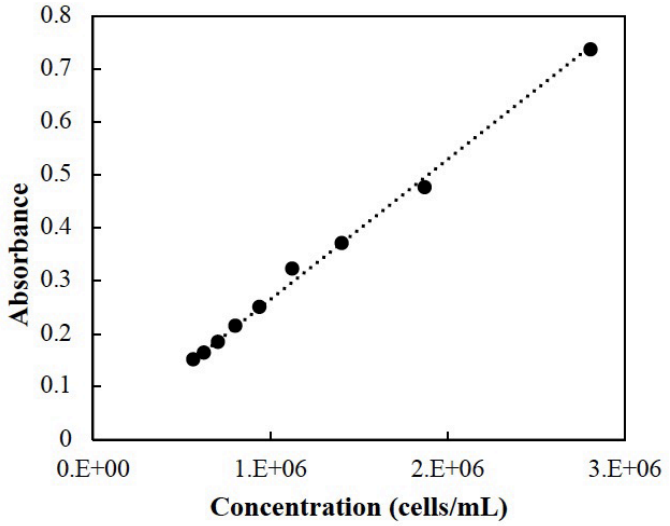

Figure 1. Plot of absorbance versus algae concentration. Equation: $y = 3.0 \times 10^{-7}$; $R^2 = 1.0$

Contact angle measurements were used to obtain information needed for use in the extended Derjaguin-Landau-Verwey-Overbeek (xDLVO) thermodynamic model for predicting the interactions of the substrate and algae cells. This model is commonly used in colloid science to understand how particles interact with each other or surfaces as they approach one another. It has also been used to understand the interaction of *Scenedesmus dimorphus* with some polymeric surfaces[10] but not the nanocomposites used in this research. As described in the literature,[10] the model considers the total Gibbs free energy of interaction as the sum of three components:

$$G^{Tot} = G^{vdW} + G^{AB} + G^{EL}$$

where G^{vdW} is the interaction energy due to van der Waals forces, G^{AB} is the energy due to acid-base interactions, and G^{EL} is the energy due to electrostatic interactions.

Measurements of the contact angle show different values for different materials, across all probe liquids (Table 1). Compared to plain PLA, the contact angles changed significantly with the addition of different types of CNC, where PLA/SA-CNC had a lower contact angle and PLA/L-CNC had a higher contact angle (Table 1). These changes suggest that the incorporation of SA-CNC and L-CNC changes the surface chemistry of the PLA. For example, the measurements using water showed that adding the SA-CNC into PLA made the substrates slightly more hydrophilic, whereas adding the lignin-coated CNC (L-CNC) made the samples more hydrophobic. It is expected that these changes could affect surface thermodynamics that affect algae deposition and attachment.

Values from the contact-angle measurements were used in the xDLVO model to predict the interaction energy between *Scenedesmus dimorphus* and the nanocomposite substrates (Figure 2). A negative G_{Total} value indicates attraction, suggesting the attachment of algal cells to the material. Although the model did not display any primary minima which would indicate strong attraction, the energy profiles showed shallow

Table 1. Contact angle measurements of samples with three probe liquids (errors are standard deviation n=30).

	Water	Ethylene Glycol	Hexadecane
PLA	78.5° +/- 0.1°	56.0° +/- 0.1°	18.6° +/- 0.1°
PLA/SA-CNC	77.3° +/- 0.1°	52.7° +/- 0.1°	17.1° +/- 0.2°
PLA/L-CNC	82.5° +/- 0.1°	58.8° +/- 2.3°	22.0° +/- 1.8°

secondary minima with negative G_{Total} values. These minima are highlighted in Figure 2b; in accordance with colloid thermodynamic theory, they are indicative of attraction between the algae and surface which could result in loosely attached algae on the surface.

The results of the model were compared to the experimental attachment tests. Figure 3a shows the average number of cells per area for each composite resulting from attachment tests. These results show differences in number of attached algal cells among the materials, with average values of 313 cells for PLA, 265 cells for PLA/SA-CNC, and 246 for PLA/L-CNC per 85,300 μm² of surface area. These results were obtained by repeating the attachment experiment four times and analyzing the total number of 40 microscope images per material (with the exception of PLA/L-CNC, for which 29 images were analyzed). The error bars displayed on plot 3a represent the standard deviation value. Additional statistical tests show that differences in the number of cells attached were significantly lower for composites with CNC compared to plain PLA (Figure 3b). This is in agreement with the model results, which also suggested that PLA was more favorable for attachment than PLA/L-CNC. However, the model predicted only negligible difference between the PLA and PLA/SA-CNC. This discrepancy may be due to the significant error in the measurements. One source of error was that the color and opacity of the composites made it difficult to visualize the algae and precluded the use of standard image analysis software. Therefore, the images had to be manually counted; this introduced human error. Another source of error was uneven settling of algae onto the surfaces during the attachment tests. These sources of error are expected to be mitigated by refinement of methods in future experiments.

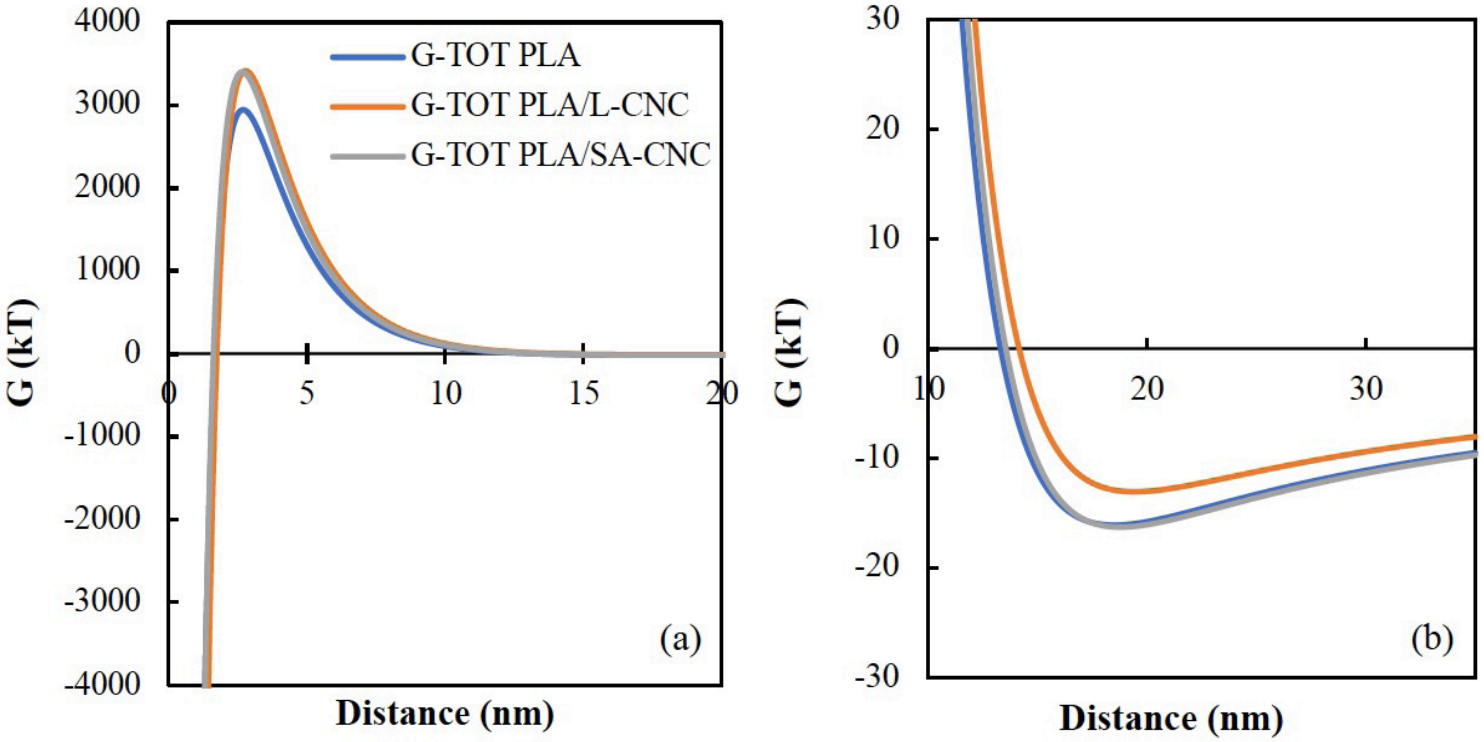

Figure 2. (a)Total interaction energy of samples. (b) Secondary energy minimum from Figure 2a.

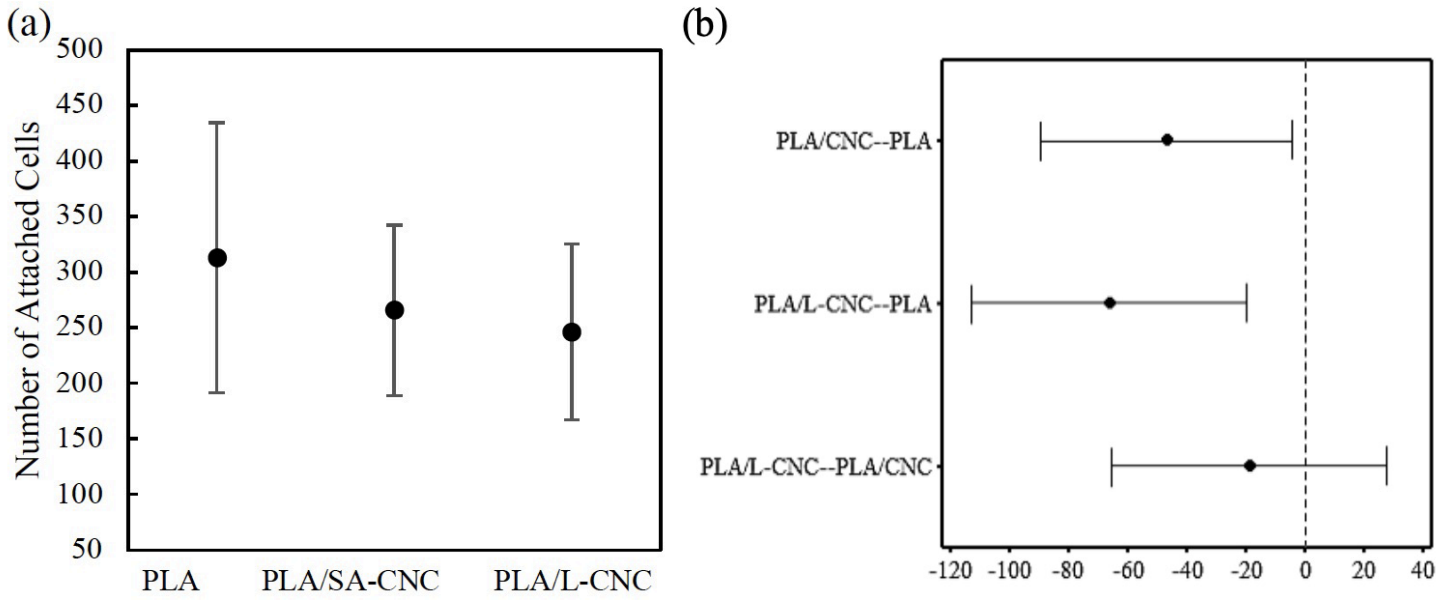

Figure 3. (a) Number of algae cells per image for composites; error bars represent standard deviation.(b) Fisher's statistical significance test for sample pairs; if an interval does not contain zero, the corresponding means are significantly different.

Conclusions

This research developed a rapid spectroscopy-based method for determining the concentration of *Scendesmus dimorphus*, a commonly studied planktonic algae in growing cultures. It also showed that the surface chemistry of a bioderived polymer, PLA, could be modified by the incorporation of hydrophilic SA-CNC or hydrophobic L-CNC. Both thermodynamic modeling and experimental results showed that differences in surface chemistry through CNC addition resulted in differences in the attachment of the model planktonic algae. The protocols established in this l research can be further refined to study substrates with a greater range of surface chemistries, such as additiona PLA nanocomposites or other materials. They can also be extended to the investigation of other algae including industrially relevant attached filamentous algae.

Acknowledgments

The authors acknowledge Joshua Passantino, a former undergraduate researcher in Dr. Virginia Davis' research group for initial experiments and protocol development, and Rachel Howell, an undergraduate research fellow in Dr. David Blersch's research group for assistance with cell counting. Marisa Rodriguez received support through the Auburn University Undergraduate Research Fellowship Program. Additional financial support was provided by the National Science Foundation CMMI -NSF Grant #1563160.

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Porosity and Permeability Modeling of the Paluxy Formation

Jacob Haber Bensinger and Lauren Beckingham

The purpose of this study is to evaluate the pore network structure of a Paluxy sandstone using 3D X-ray Computed Tomography (X-ray CT) images and simulate permeability using pore network modeling. The Paluxy formation is a sandstone formation below Alabama, Mississippi, and much of the adjacent Gulf Coast. It is under consideration as a potential geologic carbon dioxide sequestration site, where it is estimated to have a capacity to store 380 Gt of CO_2. This research is being done as part of a larger project focused on enhancing understanding of the properties and feasibility of using the formations at a natural gas powerplant in Kemper, Mississippi, for storage of carbon dioxide. The Paluxy formation is of particular interest for its commercial potential due to segmentation by the many natural low-permeability baffles and barriers that will serve as impermeable caprocks and prevent upward migration of CO_2. In addition to this, the formation has an upward-fining behavior, so detailed evaluations of samples from different elevations and locations are necessary[1].This work utilizes methods that allow not only for analyzation of the natural, unreacted subsurface formation, but for modelling of the properties of the formation after geochemical reactions take place.

This model utilized a set of 400 black and white segmented 3D X-ray CT images of a core drilled out of the Paluxy formation to create a single representative 3D binary image of the pores and grains. The region of interest for this study was from 5086 ft. The sample 3D image, XCT 5086, was analyzed by the PNExtract program, which generates pore and pore-throat sizes using a medial-surface algorithm. The connectivity was represented by assigning a hierarchy of maximal spheres. This program preserves macroscopic behavior of porous rocks through its ability to analyzes sub-voxel features, including being able to define half-throats and corners[2]. MATLAB® was then used with XCT 5086's distributions to build computational pore networks that can represent porosity and permeability.

Pore network models (PNM) are computationally efficient and able to model field-scale and pore-scale behaviors, such as porosity and permeability[3].

In MATLAB®, the pores were randomly sampled from the distributions, connected by throats, and separated by grains. Figure 1 is a visual representation of a 10 x 10 x 10 PNM. Image porosity of XCT 5086 was determined by the PNExtract code to be 26%. This result is consistent with core analysis of the Paluxy formation, which averages at 23% and is locally > 30%. Permeability has a geometric mean of 220 mD but can exceed 3800 mD locally. The PNMs for XCT 5086 used an average grain size of 73 μm and was found to have an average porosity and permeability of 25% and 1500 mD, respectively. This is a high permeability, but it is within a reasonable graphical margin for Cretaceous sandstone. The next step is to model how porosity and permeability of XCT 5086 changes in potential geochemical reaction scenarios, such as uniform, random, and channelized precipitation and dissolution evolutions.

Statement of Research Advisor

Jacob has assessed the pore network structure and simulated the hydrologic properties of a sandstone formation being considered as a potential reservoir for CO_2 sequestration. His work largely contributes to the understanding of the formation properties and will aid in understanding the dynamic evolution of the system after CO_2 injection.
– *Lauren E. Beckingham, Civil Engineering*

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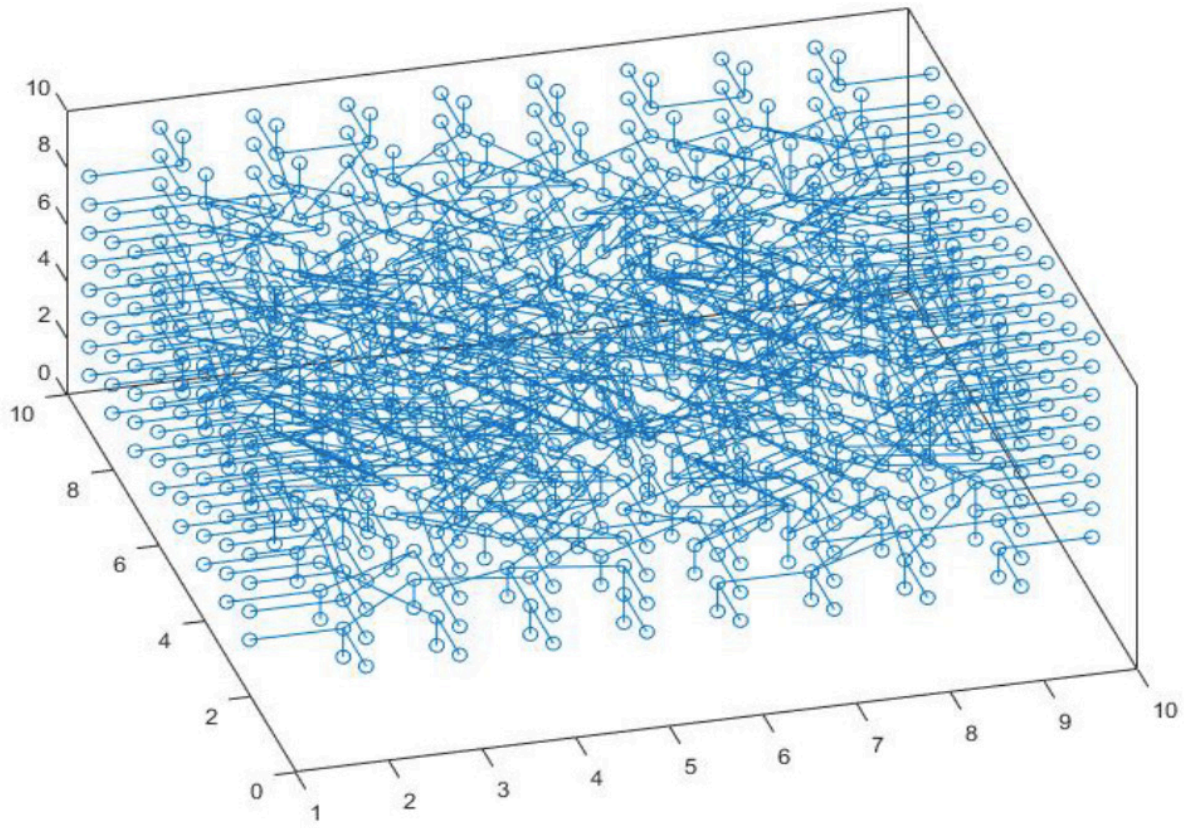

Figure 1: Pore network model defined by a system of spherical pores on a regular, cubic lattice and connected by pore throats.

Neuronal CXCL10/CXCR3 Axis Mediates the Induction of Cerebral Hyperexcitability by Peripheral Viral Challenge

Alison Beverly, Tiffany Petrisko, Jenna Bloemer, Priyanka Pinky, Sharay Setti, Sriraja Srinivas, Gregory Konat, Vishnu Suppiramaniam, and Miranda N. Reed

While peripheral viral infections have been shown to increase the risk for seizures, the cellular/molecular mechanisms of infection-induced seizure hypersusceptibility are poorly understood. We have developed a preclinical murine model to study mechanisms by which peripheral infection exerts its effects on the brain by using an intraperitoneal injection of the epitomic viral mimetic, polyinosinic-polycytidylic acid (PIC). We have demonstrated that PIC challenge induces hyperexcitability of neuronal networks as seen from a profound increase in the basal synaptic transmission (a measure of neuronal communication) and long-term potentiation (a long-lasting increase in signal transmission between two neurons) in hippocampal slices, as well as from hypersusceptibility to kainic acid-induced status epilepticus. Because neuronal hyperexcitability is an invariable feature of seizures, it might provide a mechanistic link for the exacerbating effects of peripheral inflammation on disease progression.

To determine the molecular link between PIC challenge and neuronal hyperexcitability, we previously examined numerous cytokines, chemokines, and inflammatory mediators after PIC challenge. We demonstrated that in the hippocampus (an ictal site of seizures), only the chemokine CXCL10 was robustly increased, whereas other major inflammatory mediators were either only slightly elevated or unchanged. Because CXCL10 is a potent modulator of neuronal activity, it seems plausible that it might be a putative molecule, which acting through its cognate receptor, CXCR3, drives the development of hyperexcitability.

The present study was undertaken to determine the role of CXCL10 in mediating the development of hyperexcitability in response to PIC challenge. Briefly, young female C57BL/6 mice received an intracerebroventricular infusion of the CXCR3 antagonist, AMG 487 (3 mg/kg) and two hours later were given an intraperitoneal injection of the viral mimetic, PIC (12 mg/kg). Mice underwent examination 24 hours after PIC challenge. Blocking cerebral CXCR3 through intracerebroventricular injection of a specific inhibitor, AMG487, abrogated PIC challenge-induced increase in basal synaptic transmission and long-term potentiation, as well as the reduction of paired-pulse facilitation (a measure of glutamate release). The PIC-mediated abolishment of long-term depression (a long-lasting reduction in the efficacy of neuronal synapses) was also restored after application of AMG487. Moreover, CXCR3 inhibition attenuated seizure hypersensitivity induced by PIC challenge. The efficacy of AMG487 strongly strengthens the notion that CXCL10/CXCR3 axis mediates the induction of cerebral hyperexcitability by PIC challenge.

Statement of Research Advisor

Allison's work strongly indicates that the CXCL10/CXCR3 axis governs the induction of neuronal hyperexcitability and suggests that the CXCL10/CXCR3 axis may play a critical role in the comorbid effect of peripheral viral infections on the progression of major neuropathological diseases, including seizure hypersusceptibility. Future work will identify the how CXCL10/CXCR3 axis signaling mediates the development of hyperexcitability.

– Miranda Reed, Drug Discovery and Development

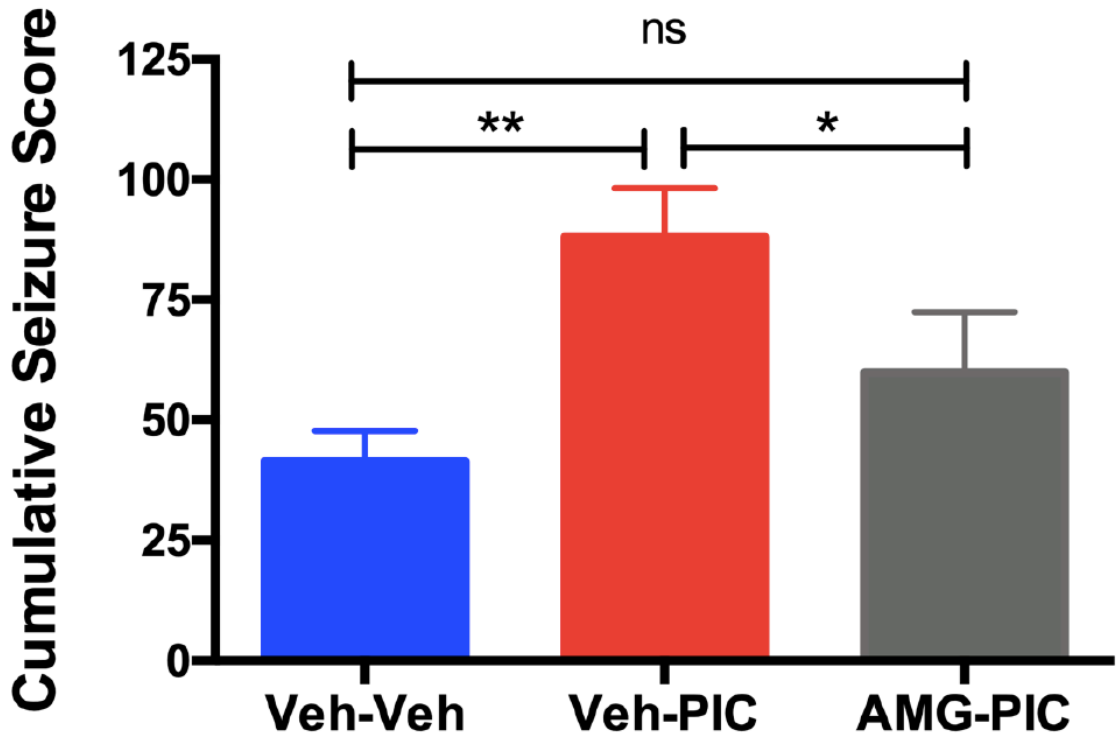

Figure 1: PCXCR3 inhibition attenuates seizure hypersensitivity induced by PIC challenge. Mice received a bolus of intracerebroventricular injection of AMG 487 (3 mg/kg) or vehicle (VEH). Two hours later, the acute antiviral response was induced by intraperitoneal injection of 12 mg/kg of PIC in saline. Mice injected with 100 μL of saline served as vehicle controls. Twenty-four hours after PIC injection, status epilepticus (SE) was induced by subcutaneous injection of 12 mg/kg of kainic acid. Mice injected with saline in lieu of PIC served as controls (VEH). Seizures were expressed as cumulative seizure score using a 6-step scale where 0 is no seizure and 6 is a severe, tonic-clonic seizure. Symbols represent means ± SEM from 3 to 6 mice per group. *$p \leq 0.05$, **$p \leq 0.01$; ns = not significantly different.

Developing MATLAB® Code for Analysis of Heterogeneous Engineered Cardiac Tissue During Contraction

Michaela Bush, Morgan Ellis, Ferdous Finklea, and Elizabeth Lipke

This project attempts to create a comprehensive interface for analyzing videos of calcium propagation through contracting engineered cardiac tissue. Engineered cardiac tissues are stained with a calcium indicator dye and imaged using a high-speed camera to capture the changes in fluorescence associated with calcium propagation. In order to analyze changes in fluorescence, the contracting cardiac tissues must be treated with a compound called blebbistatin to prevent mechanical motion. This compound is cytotoxic and can change cellular behavior. Producing an interface that can account for mechanical motion may give more accurate readings of calcium propagation through the tissue, and the tissue will better mimic hearts *in vivo.* As a result, it is essential to be able to isolate and remove motion artifacts from the videos so propagation can be analyzed.

After reviewing the literature, several methods were tested based on the current experimental setup. The first method tested involved using a block-matching algorithm[1]. In this method, an analysis was conducted to determine when a pixel contained cardiac tissue for every frame. These data were fed into the block-matching algorithm to find motion vectors that were then removed from the initial video data. In practice, the algorithm could not remove all motion effectively enough, and the resulting video data were unusable.

The second method used two videos taken of the same sample. One video captured the fluorescence changes of the contracting tissue, and the second video of the sample used a filter to record only the mechanical contractions. The motion of the second video can theoretically be used to subtract the motion in the first. However, due to the experimental setup, the videos could not be taken at the same time. The filters used also caused the difference in fluorescence between the tissue and the surroundings to be different in the two videos. None of the pairs of videos tested using this method were viable for analysis.

My attempts to account for motion revealed that the current experimental set up would need to be modified to include tissue markers for distinguishing mechanical contraction from changes in fluorescence. Using tissue markers will provide explicit reference points for subtracting motion and allow the researcher to continue imaging without cytotoxic compounds.

Statement of Research Advisor

Michaela's knowledge of computer programming is well beyond that of most undergraduate students. Using her experience from both high school Java courses and her computer science minor, Michaela has been able to have an immediate impact in our lab. The goal of her research project is to develop a MATLAB® code to analyze electrophysiology of engineered cardiac tissues using optical mapping data. She has spent the semester researching and applying current methods of motion subtraction while giving feedback on better ways for collecting optical mapping data.
– *Elizabeth Lipke, Chemical Engineering*

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Circadian Disruption of Core Clock Genes *Bmal1, Reverb-a, Per2, and Cry1* in Adipose Tissue Due to Western Diet-Induced Obesity

Beatriz Carmona, Michael Greene, and Lauren Woodie

The Western diet (WD) is characterized by consumption of a high proportion of fat and sugar and is linked to chronic metabolic diseases including cardiovascular disease, diabetes, and obesity. Approximately 30% of the genes in the mammalian organs follow light-induced circadian rhythms controlled by the suprachiasmatic nucleus (SCN) in the hypothalamus. Disruption of circadian rhythmicity can lead to obesity and chronic metabolic diseases; however, the converse has also been observed: WD-induced obesity can disrupt peripheral circadian rhythms.

Four core clock genes that control how the SCN regulates rhythmicity in the central clock and peripheral tissues (those beyond the hypothalamus) are *Bmal1, Reverb-a, Per2, and Cry1*. By examining core clock and clock-controlled gene expression in various peripheral tissues, we can validate that the WD regulates peripheral clocks in key metabolic tissues related to obesity. Thus, we examined core clock gene expression in epididymal white adipose tissue (eWAT), a type of visceral adipose tissue in mice with a role in whole-body energy and glucose homeostasis.

Male C57NL/6N mice were fed *ad libitum* a Chow diet (n=42) or Western high fat and sugar diet (WD+S) (n=42). After 16 weeks of feeding, the animals were sacrificed every 6 hours (n=7 per time point). Tissue RNA was analyzed with Real Time Quantitative Polymerase Chain Reaction (RT-qPCR) to examine core clock gene expression, with GADPH used as the housekeeping gene. The rhythmicity and expression levels were then compared between the Chow diet control group and the WD+S group and graphed according to the acrophase, which is the timepoint with the highest level of expression over the 24-hour period. To monitor changes in rhythmicity, acrophases were compared between the groups; a difference between these was denoted as a "phase advancement" in hour units. As shown in Figure 1, *Bmal1* and *Per2* had similar differences; both were phase-advanced by 4 hours and dampened, or less expressed, in the WD+S Group compared to the Chow. *Reverb-a* and *Cry1* also shared differences; both were phase-advanced by 12 hours (a directly opposing shift). The overall acrophase timeframe of these genes altogether doubled in range, from a 9-hour window (12-21 hours) to 18 hours (0-18 hours).

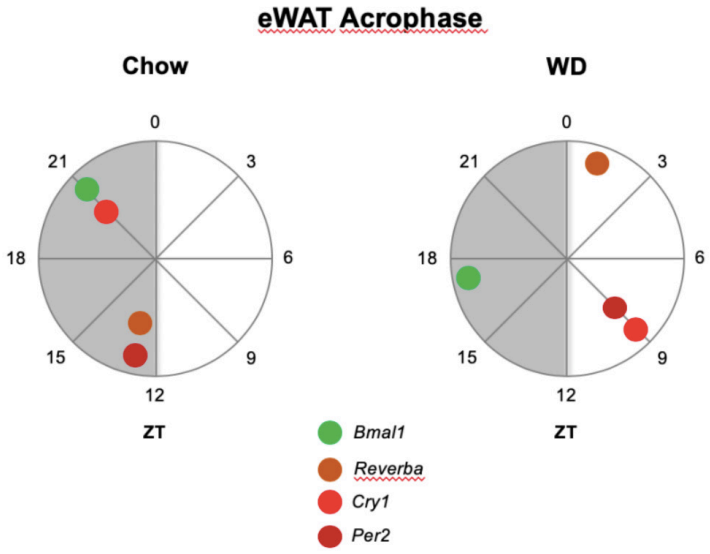

Figure 1: 24-hour clock graphs showing acrophase times of peak expression for each gene A. Acrophase of *Bmal1, Reverb-a, Cry1, and Per2* in Chow group B. Acrophase of *Bmal1, Reverb-a, Cry1, and Per2* in Western diet group.

In addition to eWAT, the core clock genes in the liver have previously been shown to be disrupted in Western diet-induced obesity. In the liver, we observed that *Bmal1, Per2, Reverb-a, and Cry1* were phase-advanced by 12 hours, 4 hours, 0 hours, and 1 hour, respectively. We hypothesize that the hippocampus in the brain, acting as a peripheral tissue, is disrupted in Western diet-induced obesity. Similar opposing shifts were observed in the hippocampus as was observed in the eWAT: *Bmal1* and *Reverb-a* were phase-advanced by

17 hours and 8 hours, respectively, and *Per2*, and *Cry1* were both phase-advanced by 12 hours.

The key impact of this research is that it shows WD+S disrupted the rhythmicity of the core clock in the adipose tissue, which is consistent with our hypothesis that peripheral rhythmicity is disrupted by Western diet consumption.

Statement of Research Advisor

Beatriz has performed key experiments demonstrating adipose tissue clock gene disruption in a mouse model of diet-induced obesity. Her contribution was critical to establishing that circadian disruption occurs in peripheral organs which was necessary for validating our model of obesity-linked circadian disruption.
– Michael Greene, Nutrition, Dietetics & Hospitality Management

Diffusion Tensor Imaging of 3T MRI of Huntington's Disease in Sheep

Erin Batcho, Sai Sheshan Roy Gotoor, Raj Perumal, Marianne Keller, Tim Kuchel, Sundeep Chandra, David Howland, Gopikrishna Deshpande, Heather Gray-Edwards, and Thomas S. Denney Jr.

Huntington's disease (HD) is a dominantly inherited progressive neurogenerative disorder that is often fatal and affects 4-5 people per 100,000 in the United States[1]. It is caused by an expansion of the nucleic acid sequence cytosine-adenine-guanine (CAG) located in exon 1 of chromosome 4 within the huntingtin (HTT) gene. The expansion of this sequence leads to an elongated huntingtin protein, causing it to break up and aggregate in neurons – leading to neuronal degeneration and loss of cell function[2]. This degeneration generates a loss of both connectivity and volume of white matter within the central nervous system. There is currently no clinical treatment or cure for HD and there is a critical need for biomarkers to track the progression of the disease and analyze the efficacy of novel treatments[3]. In 2008, a humanized HD sheep transgenic model was developed for study of HD and to provide a model that recapitulates adult onset HD. This model contains a clinically relevant HD genetic mutation, $(CAG)_{74}$--CAA-CAG, and expresses the expanded human-mutated huntingtin gene (mHTT) throughout the brain.

High-field (3T) magnetic resonance imaging (MRI) was utilized to determine if the HD sheep exhibit traditional neurodegenerative changes typically seen in humans with HD - a loss of connectivity and volume in white matter tracts of the brain. In previous studies, HD patients have exhibited lower values of fractional anisotropy (FA), higher values of apparent diffusion coefficient (ADC), and higher values of radial diffusivity (RD). Using diffusion tensor imaging (DTI), data were analyzed from normal and transgenic sheep using both a region of interest (ROI) analysis and a tract-based analysis to detect white matter alterations. It was predicted that the discrepancies between normal and transgenic sheep would recapitulate the differences typically seen within healthy and diseased humans.

The diffusion images were acquired on a 3T SIEMENS Skyra scanner (Siemens Healthcare, Germany) using a 2D EPI diffusion sequence. For each region segmented, the means of ADC, FA, RD, and axial diffusivity (AD) values were computed. A t-test was used to determine ten total statistically significant differences: one from the ROI-based analysis and nine from the tract-based analysis. For the ROI-based analysis, the difference was found in the ADC mean in the occipital cortex. For the tract-based analysis, the differences were found in the FA mean and RD mean in both the right and left internal capsules, the FA mean and RD mean in the left caudal internal capsule, the FA mean in the parietal cortex, and the ADC mean and RD mean in the occipital cortex. The values for these statistics are shown in Table 1.

While further studies are needed to validate these trends, these results suggest that the HD sheep model exhibits MR based changes that are consistent with human patients and these modalities may be objective measures for testing of novel therapeutics.

Statement of Research Advisor

Diffusion tensor imaging (DTI) is an imaging technique that measures how water diffuses in white matter tracts in the brain and can be used to study how the brain remodels in response to disease. Using DTI, Erin was the first to show that sheep with Huntington's Disease (HD) have differences in white matter that are consistent with HD in humans. She also established analysis protocols that will be used by other students.
– Thomas Denney, Jr., Electrical and Computer Engineering

Table 1. HD Sheep Significant Differences in Tract-Based and ROI-Based Analyses

Analysis	Statistic	Region	Transgenic	Normal	p
ROI	ADC	Occipital Cortex	0.86 ± 0.02	0.80 ± 0.01	0.039
Tract	ADC	Occipital Cortex	0.91 ± 0.01	0.85 ± 0.02	0.036
Tract	FA	Right IC	0.44 ± 0.01	0.49 ± 0.01	0.005
Tract	FA	Left IC	0.45 ± 0.00	0.48 ± 0.01	0.014
Tract	FA	Left Caudal IC	0.43 ± 0.03	0.47 ± 0.01	0.007
Tract	FA	Parietal Cortex	0.43 ± 0.01	0.47 ± 0.01	0.019
Tract	RD	Right IC	0.62 ± 0.01	0.56 ± 0.02	0.033
Tract	RD	Left IC	0.61 ± 0.01	0.56 ± 0.01	0.030
Tract	RD	Left Caudal IC	0.66 ± 0.01	0.61 ± 0.02	0.050
Tract	RD	Occipital Cortex	0.69 ± 0.02	0.63 ± 0.02	0.039

Values are mean ± standard error.

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Body Mass Index and Functional Movement Patterns in Collegiate Students

Abigail R. Brittain and Gretchen D. Oliver

The benefits of sport participation and general physical activity, as well as the harms of high body mass index (BMI), are common knowledge. For instance, high BMI has been associated with greater sport-related injury rates in adolescences[1]. High BMI has also been correlated with lower fundamental movement skill proficiency in adolescent females[2]. Such correlations between high BMI and increased injury, as well as high BMI and decreased movement performance, may be seen in young adult populations as well as adolescences. The purpose of the current study was to identify correlations among BMI, injury, participation in physical activity, and functional movement (defined by the functional movement screen (FMS)) within a university population consisting of young adults.

Twenty college students (21.4 ± 1.5 years, 175.9 ± 8.1 cm, 75.2 ± 4.4 kg) were recruited to participate in the study. The Auburn University institutional review board approved all testing procedures (AU IRB# 18-362 EP 1811). All participants met or exceeded the Center for Disease Control's recommendations for weekly physical activity. Participants completed the FMS under the instruction and grading of an FMS Level 1 certified researcher. Following the FMS, participants completed an online questionnaire using a secure platform. Participants responded "yes" or "no" to having a history of serious injury (defined as requiring a month or more of recovery) and listed modals of their regular physical activity. Height and mass were collected on a uniform scale and stadiometer. All scalar data were transformed to Z-scores prior to running a Pearson product correlation.

Results revealed significant correlations, $\alpha < 0.05$, among BMI, injury, number of physical activities, and FMS scores. A significant positive correlation was found between BMI and the number of physical activities in which participants were involved ($r = 0.509$, $p = 0.022$) and a significant negative correlation was found between BMI and FMS total score ($r = -0.517$, $p = 0.020$). A negative correlation was also found between the number of physical activities and a history of serious injury ($r = -0.471$, $p = 0.036$).

Our findings indicate that those with a higher BMI participated in more physical activities, although they had lower FMS scores. This relationship between high BMI and low movement proficiency agrees with the previous research[1]. Also, in agreement with former findings, participation in fewer physical activities was correlated with an increased history of serious injury[2]. This relationship may be the result of greater exposure to various movement patterns seen in individuals participating in more physical activities. Such exposure could lead to altered bone and tissue density and may lead to increased proprioception, which may make individuals more robust to possible injuries[3,4]. Future studies should explore the degree of adaptations made through participation in numerous physical activities.

Statement of Research Advisor

Abigail's work establishes the groundwork needed for her to further investigate functional movement, pain, and injury in youth athletes specializing in a single sport.

– *Gretchen D. Oliver, Kinesiology*

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Relationship Between Mental Health and Physical Health in Rural, Low-Income, High School Students

Sarah Beth Dolinger, Robyn Feiss, and Melissa Pangelinan

Research shows there are many physical, cognitive, and social factors that influence health and well-being of an individual. These factors are especially prevalent during the period of adolescence. Further, the onset of many common psychiatric disorders occurs during adolescence[1]. It is important that these symptoms are identified early and that individuals seek treatment soon after symptoms are identified, as this has been linked to better outcomes[2]. Research shows that school-based programs can increase these help-seeking behaviors[3].

The purpose of my research was to study the efficacy of a high school wellness fair program on tenth-grade student mental and physical health. The goals of these wellness fairs were to educate students about mental and physical health, increase help-seeking behavior among students, and decrease the stigma associated with mental health burdens.

The wellness fairs were hosted at four local high schools, including one private and three Title I schools. Title I schools are defined as having at least 40% of the students from low-income families receiving free or reduced meals[4]. A total of 146 students participated (62 males, 84 females, 10 gender not provided). This study was approved by the IRB (protocol #18-109 MR 1803); parental consent was obtained prior to each fair.

The fair consisted of physical fitness testing, mental health discussions, and a nutrition session. Students completed the FITNESSGRAM® physical fitness testing that consisted of anthropometrics (height, weight, body mass index [BMI]), body composition (% fat mass, % lean mass), resting heart rate, blood pressure, flexibility (shoulder stretch, sit-and-reach), muscular strength and endurance (push-ups, curl-ups), and aerobic capacity (Progressive Aerobic Cardiovascular Endurance Run [PACER]). Body composition was measured using a TANITA total body composition analyzer (SC-331S Total Body Composition Analyzer, TANITA) and blood pressure and heart rate were measured via an Omron® automatic blood pressure monitor (5 Series Upper Arm Blood Pressure Monitor BP742N, Omron Healthcare).

The mental health discussions covered topics including managing stress, having balance between academics and extracurricular activities (school organizations, sports, home life, work), and developing healthy relationships among peers. These were facilitated by school counsellors, Auburn University faculty, Auburn University graduate students, as well as former and current professional athletes. The nutrition session varied among the different schools in how it was conducted and what information was included due to time constraints. One school participated in a brief discussion of the components of a healthy meal and completed activities to create healthy meals in line with the discussion. The other schools were provided with healthy snacks (e.g., fruits, vegetables, and whole grain snacks) and information about each food option whens students selected snacks.

As part of the wellness fairs, participants completed self-report questionnaires both pre-fair and during the mental health discussions. Questionnaires included PROMIS® Pediatric Anxiety[5], PROMIS Pediatric Depression[5], PROMIS Psychological Stress[6,7], and PROMIS Physical Activity[8]. For the purpose of the present analysis, a limited set of dependent variables were assessed (i.e., questionnaires and BMI). A total of 37.5% of students reported moderate or severe anxiety symptoms, 31.25% reported moderate or severe depressive symptoms, 39% reported moderate or severe psychological stress symptoms, and 72% reported normal levels of physical activity. According to BMI, 16.4% of participants were overweight and 23.3% of participants were obese. In adolescents, BMI ranges are determined based on sex and age of the individual. Spearman correlations were used to examine bivariate relationships between BMI and the PROMIS measures.

A significant correlation was found between BMI and anxiety symptoms ($r = 0.32$, $p < 0.01$). A significant correlation was found between BMI and depressive symptoms ($r = 0.22$, $p = 0.03$). A significant correlation was found between BMI and psychological stress symptoms ($r = 0.22$, $p = 0.04$). No correlation was found between BMI and physical activity levels ($r = 0.02$, $p = 0.88$).

The present results indicate there is a significant relationship between body composition and mental health. In our sample, as BMI increased, self-reported mental health burdens increased. While other studies have not found this relationship to be significant, they have supported a trend towards this relationship, particularly for male adolescents[9]. In addition, these results are similar to findings which suggest that obesity negatively impacts body image, which in turn, impacts self-esteem, anxiety, and depression[9]. The present results add to this literature in that similar relationships were observed in a rural and at-risk population.

There was no relationship between BMI and self-report physical activity. This could be due to the inaccuracies in self-reported physical activity via questionnaires (i.e., overestimating physical activity levels). In comparison to self-reported measures, studies using quantitative measures of physical activity (i.e., accelerometry data) have observed significant associations between BMI and physical activity levels in adolescent samples[10]. Future studies should include quantitatively assessed physical activity via accelerometers, but these devices may be cost-prohibitive in large samples.

In the future, to further understand the relationship between physical and mental health, the physical fitness data collected during the wellness fairs will need to be analyzed. Additionally, by extending and replicating the wellness fairs in the future, we will increase our sample size, thereby improving our ability to analyze our data.

Statement of Research Advisor

Sarah Beth Dolinger is an undergraduate research fellow. Over the course of her first fellowship year, Sarah Beth was a major contributor to the management of the Wellness Fair program, including disseminating information to the schools, distributing and collecting consent forms, as well as managing data collection and data archiving. She contributed to the data analysis for the present study and the interpretation of the study results. Sarah Beth has presented these results at the student research symposium at Auburn University, as well as at two national conferences (National Conference on Undergraduate Research and the annual meeting of the North American Society for Psychology of Sport and Physical Activity). Over the course of her second fellowship year, Sarah Beth will contribute to the replication and extension of this research project. She is currently evaluating data collected from the Wellness Fairs with respect to student athlete mental and physical health outcomes for publication.

– *Melissa Pangelinan, Kinesiology*

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Creating Virtual Reality Immersive Games for Construction Safety Training

Dongnyeok Han, Salman Azhar, and Swarnali Dastider

Jobsite safety has always been a top priority in the construction industry. Although remarkable improvements have taken place in construction technologies and processes in recent years, the safety records in the construction industry continue to be one of the poorest. Unsafe jobsite conditions, inappropriate work planning, insufficient communication between workers and supervisors, and lack of safety training are some of the key contributing factors.

In recent years, several construction companies have tried to improve safety training in an attempt to reduce fatalities and injury rates. However, many companies found it challenging to provide effective safety training to construction workers due to language barriers, limited training time, and use of non-visual safety training methods. This research study was carried out to introduce a new construction safety training paradigm using the Virtual Reality (VR) technology, which is more realistic and independent of language barriers. The most significant advantage of VR technology is gaining real-life experience without exposing students to the danger of the jobsite.

In this research, three VR-based construction safety games were prepared to introduce students to possible hazards as well as safety measures while working in a confined space, at heights on a scaffold, or walking on a construction site cluttered with materials and debris (Figure 1). To begin with, a conceptual framework for design efficiency, execution, time-period, and method of delivery was laid out to improve pedagogical and participation experience about construction safety. Unity 3D® was selected as the developing platform and three VR-based safety games were created for Oculus Go™ VR headset.

The three VR games were introduced to a group of students who were taking the construction safety class at the McWhorter School of Building Science at Auburn University. The students played the three games and compared them with the traditional lectures they received earlier in the semester. Their feedback was collected via a questionnaire survey. The VR training showed significant improvements in correctly identifying hazards and most suitable mitigation plan at the jobsite. The survey results clearly indicate that the VR based safety games/training can play a positive role in improving students' understanding, knowledge, and interest in the construction safety education (Table 1). The following are excerpts from feedback collected from the students through a post-exercise survey:

"Very promising way to assess potential dangers without being put in the actual situation."

"It reminds me of a (video) game. It's very interactive and effective. I think it's an interactive idea for safety training and the immersive environment is definitely useful."

"I believe it will be a great training tool for new construction workers."

Statement of Research Advisor

The thrust of our research was to investigate how emerging visualization technologies such as VR can help construction students and industry workers to improve their learning and knowledge retention in the subject matter. We found that the VR technology was very effective in training students and workers for construction sites without exposing them to any dangers. The research results are valuable for both academic and industry professionals and will help them to revamp construction safety training methods currently in use.

– *Salman Azhar, McWhorter School of Building Science*

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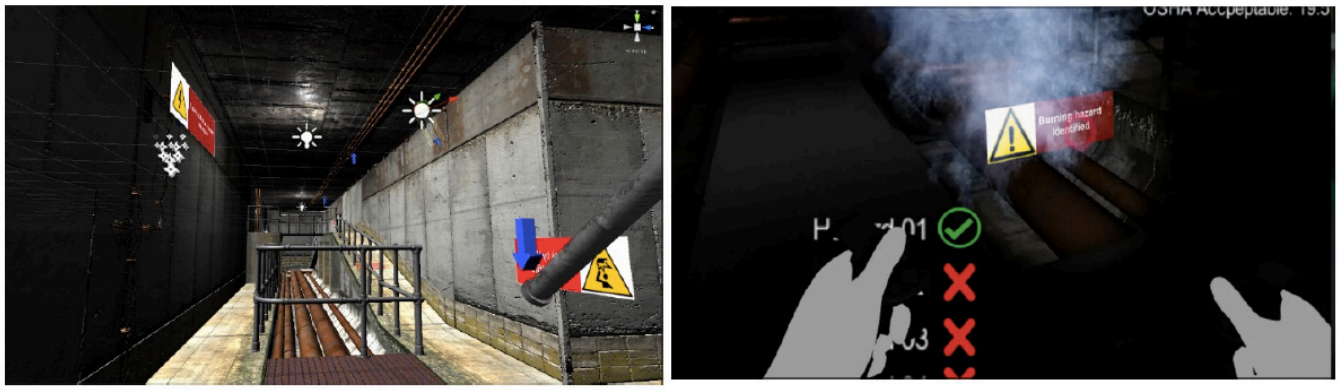

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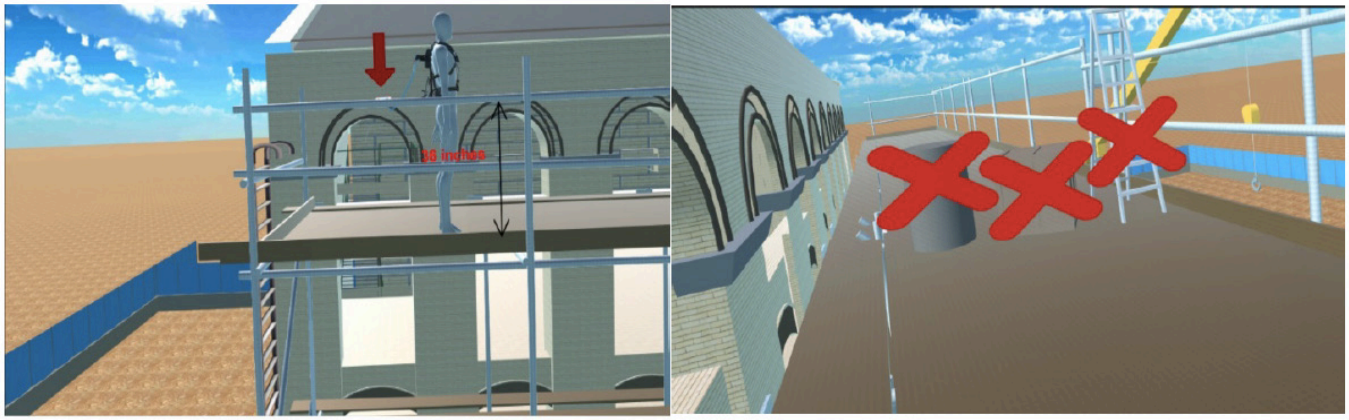

c)

Figure 1: VR-based safety training for (a) working in a confined space; (b) working at heights/on a scalfold; and (c) working on jobsites cluttered with materials and debris.

Table 1: VR Based Safety Training System Effectiveness Scores Based on Students' Responses (Sample Size: 25)

	Questions	Average Score (1: Lowest, 5: Highest)
1	Ease of use (Did you feel comfortable when interacting with the virtual screen of the VR system?)	4.48
2	How real is the virtual environment in the game?	4.12
3	Do you believe the VR system could improve the hazard recognition process for a real construction site?	4.32
4	Do you think the VR games enhanced your safety knowledge or understanding?	4.64
5	Do the VR games improve your long-term memory about hazard recognition	4.09
7	Does the information provide in the VR safety training games more understandable than traditional safety training?	4.36
8	Was the safety content of the VR safety training games helpful in safety cognition?	4.68
9	Do you think the VR safety training games can enhance construction safety education effectively?	4.76
10	Do you think the VR safety training games are more beneficial than the class-based educator's lectures?	4.36

Estimating Illegal Take of White-tailed Deer in East Alabama by Using Bait

James W. Shortnacy, William D. Gulsby, and Stephen S. Ditchkoff

White-tailed deer (*Odocoileus virginianus*) are the most widely hunted game animal in the United States. Deer hunter expenditures on land access, hunting equipment, and supplies provide a major economic benefit to rural communities and conservation funding, particularly in Alabama and other southeastern states. Although deer populations are flourishing throughout most of their range, it is important that deer harvest occurs in a sustainable, ethical manner. Accordingly, many states, including Alabama, prohibit the use of artificially concentrated bait (e.g., corn) to aid in hunter harvest of deer. Specifically, any bait must be ≥ 100 yards away and out of sight of the hunter. Nevertheless, poachers often disregard this law, and we lack sound estimates of the number of deer illegally killed by using bait each year in Alabama. For this study, we collected data to assess baiting prevalence in Alabama and compared it to a state where baiting is legal.

To address this objective, we sampled deer from two deer processing facilities in east Alabama, where hunting over bait is illegal, and three facilities in west Georgia, where hunting by using bait is legal (as a control), during October 2018 to February 2019. At each processing facility, we aged and sexed deer and inspected their oral cavity for the presence of corn (the most popular bait used for deer). We assumed deer with corn present in their oral cavity were harvested with the aid of bait.

We sampled a total of 221 deer in Alabama, 20 (9.0%) of which had corn present in their mouths, and 31 deer in Georgia, 3 (9.7%) of which had corn present in their mouths. The proportion of deer harvested by using bait in each state did not differ statistically ($P = 0.91$; χ^2). The percentage of males (8.3%) versus females (9.7%) harvested by using bait in Alabama did not differ statistically ($P = 0.72$; χ^2).

Our results indicate that harvesting deer by using bait is a relatively common practice, regardless of legality. However, it should be noted that sample size was limited for our control group (i.e., Georgia). Considering this, the impact of a disease outbreak may be amplified through the commonality of baiting because baiting increases animal contact (Thompson et al., 2006). It is also worth noting that because many hunters who harvest deer by using bait do so before the animal has the opportunity to ingest bait from the pile, our estimates of the proportion of deer harvested over bait may be low. These data provide a benchmark to the amount of illegal baiting occurring throughout eastern Alabama and can be used to infer that illegal deer baiting may have important implications for current and future management decisions as well as future white-tailed deer populations.

Statement of Research Advisor

Walker was required to consider and design an appropriate sampling scheme to address his research objective, then coordinate with private businesses to obtain samples. Though his study was relatively small, it revealed useful information on a relevant topic in white-tailed deer management, and established baseline information useful in discussions among state wildlife agency personnel and stakeholders.
– *William D. Gulsby, Forestry and Wildlife Sciences*

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Forced Vital Capacity in Swimmers

Lauren Dunn, Ryleigh Edwards, Sara Ann Pope, and Mary J. Sandage

Swimmers have superior pulmonary capacity (Lazovic-Popovic et al., 2016; Sable, Vaidya, & Sable, 2012; Vaithiyanadane, 2012). The purpose of this study was to compare differences in the lung capacity of competitive swimmers to (a) predicted pulmonary volumes and (b) age-matched controls. Given that swimmers differ from land-based athletes due to horizontal body position and breathing against water pressure, it is important to identify whether differences in pulmonary function exist in the healthy swimmer as compared to age-matched controls. In this study, non-swimmers and swimmers, ages 19 to 40 with no history of asthma or active allergies at the time of data collection were recruited to participate. Participants' forced vital capacity (FVC), or maximum expiration, was measured using a medical-grade spirometer, which is the device typically used for assessing respiratory function and diagnosing pulmonary disease. It was hypothesized that FVC would be greater in swimmers than in non-swimmers and greater than the spirometer-predicted volumes due their unique pulmonary abilities.

Preliminary findings indicate that many swimmers demonstrate a higher FVC than is predicted for their height, weight, sex, age, and ethnicity. The findings from this study will provide evidence for how general pulmonary function in swimmers is measured and interpreted by health-care professionals. Because swimmers train in water, these athletes experience resistance in the area of the ribs and lungs, which may result in their higher lung capacity. Should a competitive swimmer produce a FVC volume within the predicted range, the swimmer may actually be under-functioning for what is required for the sport. Underestimation of swimmer's FVC values on the spirometer may result in under-diagnosis of pulmonary disorders in this population. Health professionals such as pulmonologists and speech-language pathologists must be aware of these differences when diagnosing respiratory disorders and behavioral breathing disorders, such as paradoxical vocal fold motion (Sandage & Zelazny, 2004), in competitive swimmers.

Statement of Research Advisor

Empirical research that characterizes differences between swimmer pulmonary function versus non-swimmer pulmonary function fills an important gap in our current understanding of swimmer pulmonary function in health and disease states. The findings from this investigation merge the disciplines of exercise science and speech language pathology in a novel manner to provide a more thorough understanding of the pulmonary function characteristics of aquatic athletes.

– *Mary Sandage, Department of Communication Disorders*

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Quantifying the Presence of *Toxoplasma gondii* in Songbirds

Alisia Diamond, Geoffrey Hill, Sarah Zohdy, and Christopher A. Lepczyk

Toxoplasma gondii is a parasite found throughout the world, infecting every warm blooded species on Earth, including humans. Felid species (i.e., domestic cats, bobcats, etc.) are the definitive hosts, where reproduction takes place, and are responsible for shedding eggs through their feces. Once ingested, the parasite can have a variety of consequences to the host. Because Toxo is so widespread throughout the world, many different strains have evolved. These different strains of *T. gondii* may lead to different health outcomes in the host species. It is important to understand the potential risks of spreading different Toxo strains as well as the possible effects the strains may have on the hosts. We chose to look at songbirds as potential vectors of Toxo because they have the ability to travel such far distances. We began our research with two objectives: to determine if song birds can be carriers of the parasite *T. gondii* and to determine if migratory birds over one year of age are more likely to be infected with foreign strains of *T. gondii* than resident birds. We specified an age group for the migratory birds because birds under one year will not have migrated yet. To begin studying the prevalence of Toxo as well as the potential spread of different Toxo strains I wanted to determine whether there was Toxo in the birds here in Alabama. Over the past year I opportunistically collected deceased birds from around Auburn. There were no birds harmed for the purpose of this research.

We attempted to identify all birds to species, sex, and age, and whether they were migratory or resident. The sexing of the birds was determined by plumage and aging was done via plumage or skull ossification. Because *T. gondii* forms cysts inside tissue throughout the body, we used a variety of tissue samples to increase the likelihood of detecting the parasite. We collected tissue samples from the brain, heart, and kidneys. Once samples were collected, we extracted DNA and analyzed for *T. gondii* genetic markers via Polymerase chain reaction (PCR). In total, we evaluated 30 samples of various species. Among our samples we had a single positive detection of *T. gondii* from a Purple Finch (*Haemorhous purpureus*). The finch was a female that had been collected about 10 Km from Auburn University in February 2015. Purple Finches are a migratory species that spend the summer in Canada and the northeastern United States and the winters as far south as Florida. Using a binomial equation, we were able to determine that the rate of infection is 3.3% in songbirds in the Auburn area with a confidence interval of 0.08% to 17.22%. Our analysis presents an important detection of *T. gondii* in songbirds of North America.

Statement of Research Advisor

Alisia's work provides a wonderful first step in using incidental bird mortalities to evaluate for a parasite that is of increasing concern to both human and wildlife health.

– Christopher A. Lepczyk, School of Forestry and Wildlife Sciences

Impact Dynamics of a 3D-Printed Piano Hammer

Rebecca Mulholland and Edmon Perkins

The modern piano has remained largely unchanged since the 1800s. The currently used piano and its tonal system has subtle and obvious implications to Western music, such as its equal-tempered tonality. The modern piano is a popular instrument, but it has several limitations, including the need for frequent maintenance, a painstaking fabrication process, and an expensive price. It also has a subtle shortcoming: the modern piano was designed as an equal-tempered instrument. Equal temperament is a tuning system, which is constructed by setting the frequency interval between every pair of adjacent notes to have the same ratio. From an ethnomusical perspective, many pieces played on the modern piano are distorted in this equal temperament (e.g., J.S. Bach's *Well-Tempered Clavier*). To remedy these shortcomings, a three-dimensional (3D) printed grand piano action was manufactured by using a carbon fiber-reinforced thermoplastic. By simplifying the fabrication process and lowering the frequency of maintenance, the cost of the piano can be significantly reduced.

For this project, a standard grand piano action was disassembled manually by the author. Measurements of the small piano action pieces were recorded and drawings were created to turn the wooden design into a virtual design. The wooden action was also thoroughly examined by observing the motions between key components and the specific hardware used to fasten each component together. A computer-aided design (CAD) model was then reverse-engineered from these measurements. CAD models of all twelve parts of the piano action were modeled using SolidWorks™. Since the wooden piano action has many thin components, many of the parts are not suited for 3D-printing. For this reason, many of the parts were significantly restructured so that they could be 3D-printed. The assembled 3D-printed grand piano action is shown in Figure 1.

At this stage of the project, the strength of the carbon fiber-reinforced thermoplastic grand piano action components is found to be sufficient. The geometry of the parts has been successfully modified for effective 3D-printing. A 3D-printed piano action prototype has been designed and fabricated, which is much cheaper and easier to produce than a typical wooden action. With more modifications to the parts, a 3D-printed piano action is a viable option for a cheaper and more adjustable piano.

In the next stages of this project, the action will be improved by adding felt to the 3D-printed piano action. In the wooden piano action, felt plays a key role in adjusting the friction at the joints of the linkage and the density of the hammerhead. In addition, subsequent restructuring of the CAD files will likely be necessary to increase the stability of the linkage. In the final iteration, the hammer position will also be made adjustable in order to change the timbre of the string.

Statement of Research Advisor

During the course of this research, Rebecca completed the initial steps in a broader project, whose goal is to redesign the modern piano. These first steps were completed by reverse engineering a grand piano action in order for it to be effectively 3D-printed. This research has the potential to create a more versatile keyboard instrument.

– Edmon Perkins, Mechanical Engineering

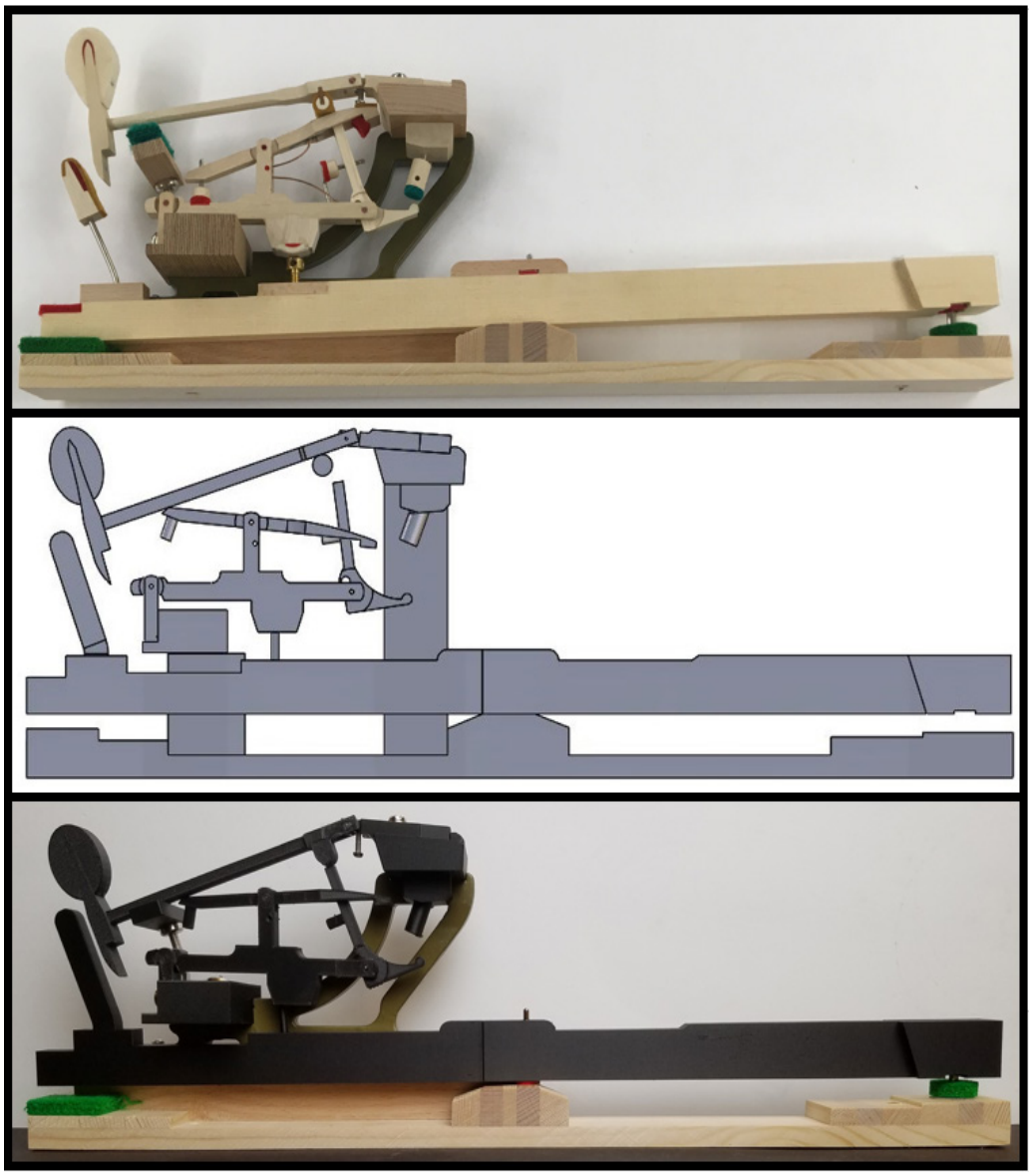

Figure 1.
Top: The original grand piano action is composed of wood with metal and felt components at connection points. Middle: SolidWorks™ CAD of piano action, modified in several ways to allow for 3D-printing. Bottom: The 3D-printed piano action prototype was produced from a Markforged™ printer; the material is nylon with chopped carbon fiber.

Evaluating *Toxoplasma gondii* in Wild Pigs (*Sus scrofa*)

Hannah Short, Christopher A. Lepczyk, and Sarah Zohdy

Wild pigs (*Sus scrofa*; Figure 1) are one of the most destructive invasive species in the southeast United States, including Alabama, where they were introduced in 1988 and subsequently spread across the entire state. One issue with wild pigs is that they contribute to the spread of diseases, such as toxoplasmosis. Toxoplasmosis is a disease caused by *Toxoplasmosis gondii*, a zoonotic parasite that reproduces in felids (i.e., cats). This disease is the second most common food-borne illness in the United States and is linked to behavioral changes in the human brain that contribute to incidence of schizophrenia, epilepsy, Alzheimer's disease, and cancer (Uttah, 2013; Ngô et al., 2017). Given the human health risks associated with *T. gondii*, our objective was to evaluate its presence in wild pigs in Alabama.

To address our objective, we analyzed 50 tongue and tissue samples from wild pigs from Dale, Macon, and Coffee counties in Alabama that were collected by U. S. Fish and Wildlife Services. From these samples, DNA extractions and molecular detection using conventional polymerase chain reaction (PCR) and Sanger sequencing were used to determine whether the pigs were infected with *T. gondii*. We identified three wild pigs that tested positive for *T. gondii*—two from Dale County and one from Coffee County. The positive samples will be used to determine the specific strain of *T. gondii* in future analyses.

Our results confirm that *T. gondii* is present in wild pigs in Alabama and as such may pose a risk to human health. For instance, possible infections from improperly cooked wild pig meat or from the consumption of contaminated food or water could lead to infection. More sampling should be conducted across the state of Alabama to determine the prevalence of toxoplasmosis in human communities.

Statement of Research Advisor

Hannah showed great creativity and enthusiasm in this project, which helps to advance our knowledge of a disease that is of concern to both people and wildlife.
– *Christopher A. Lepczyk, School of Forestry and Wildlife Sciences*

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Figure 1. Image of wild pigs (*Sus scrofa*).

Preclinical Strategies Evaluating the Treatment of Triple Negative Breast Cancer

Elena Skarupa, Shanese "Lani" Jasper, and Robert "Rusty" Arnold

Breast cancer is the second leading cause of cancer death among women. Early detection and diagnosis, along with targeted therapies, have drastically improved survival rates. However, this improvement does not extend to patients with metastatic, triple-negative breast cancer (TNBC). TNBC is an aggressive cancer that is estrogen receptor-negative, progesterone receptor-negative, and HER-2 negative. Therefore, TNBC cannot be targeted via hormone therapies or novel molecularly targeted therapies, such as anti-HER2 therapies. Toxic chemotherapeutics, such as Doxorubicin, remain the primary treatment option. However, Doxorubicin exhibits cumulative dose-limiting cardiotoxicity that limits its clinical utility. In order to create and optimize therapeutic options for the treatment of TNBC, a better understanding of these tumors and their drug interactions is necessary. Often, two-dimensional (2D) *in vitro* models are employed to assess these interactions, but these models do not effectively recapitulate the complexity and multicellular nature of in vivo tumors. Three-dimensional (3D) *in vitro* models present more physiologically relevant models for assessing the interplay between drug and tumor by attempting to mimic the complexity of the tumor microenvironment.

For this study, the potency of Doxorubicin was determined using conventional cell toxicity assays. Both the SRB assay, which measures the total protein content of cells, and MTT assay, which evaluates the mitochondrial enzymatic activity of cells, were performed. The assays were conducted using classic 96-well flat-bottom plates with drug exposures ranging from 100 to 0.0001 uM Doxorubicin in the human TNBC cell-line, MDA-MB-231-LUC-GFP. They were evaluated following 24, 48, and 72 hours of drug exposure. The potency (IC50 value) was calculated after 72 hours of drug exposure. Cytotoxicity correlated positively with Doxorubicin concentration and length of Doxorubicin exposure.

The development and evaluation of the 3D model have been conducted in parallel with the 2D experiments, which serve as a reference platform for evaluating the 3D model using tumor spheroids. Future studies will focus on determining potency of Doxorubicin after the 72-hour time point using a single cell (tumor only) and multicellular (tumor and cells associated with tumor stroma, such as macrophages or fibroblasts) 3D models, as well. Future studies will also focus on evaluating differences in gene and protein expression between the 2D and 3D cell models with particular interest in HIF-1, P21, and other markers associated with tumor growth and metastases. HIF-1 is the Hypoxia-Inducible Factor, which is a transcription factor that responds to decreased available oxygen in the cellular environment. P21 is a cyclin-dependent kinase inhibitor, which inhibits the normal cell cycle. The goal of this work is to determine if 3D models can be used to give a more accurate depiction of the *in vivo* tumor response in an effort to develop better treatment options for TNBC.

Statement of Research Advisor

Over the last year Elena has worked to establish and test a 2D- and 3D-tumor spheroid platform that can be used to examine the effect of individual cell types on tumor growth and responsiveness to chemotherapy. This platform will permit examination of different drug treatment schedules and performance of various nanomedicines with the goal of improving the treatment of aggressive metastatic cancers.

– Robert "Rusty" Arnold, Harrison School of Pharmacy

Vocal Dose for Rhythm-Based Indoor Cycling Instructors: With and Without Amplification

Lauren Allison, Mary J. Sandage, and Aurora Weaver

This study quantified the vocal dose, the distance vocal folds travel during phonation, and perceived vocal effort of 8 rhythm-based indoor cycling (RBIC) instructors. Such research is relevant because fitness instructors are at high risk for occupational-based voice disorders and vocal trauma.[1,2] To date, vocal usage data exist among other aerobic instructors but there is limited research focusing on the voice requirements required for cycling instructors.[3] The purpose of this study was to collect vocal dose of cycling instructors with and without amplification to explore voice usage and related effects. It was hypothesized that vocal dose would be lower in amplified voice versus unamplified voice and perceived phonatory effort (PPE) would be lower in the amplified condition.

In this study, the RBIC instructor's vocal function was quantified by identifying the vocal fold distance dose required while coaching a realistic cycling class under two different conditions: with and without voice amplification. Each instructor completed one trial of both conditions within a two-week window. Related data are shown in Table 1. Data collection occurred in the same local indoor cycling studio, with an average of 17 cycling volunteers participating in each class, exercising to the same cycling routine to maintain ecological validity.

Vocal dose was collected through an accelerometer that was attached just above the sternal notch and quantified using the Ambulatory Phonation Monitor (APM; Model 3200, PENTAX Medical, New Jersey) data logger. Results indicated that vocal dose was similar in both conditions. PPE was lower following the trial with the microphone.

Table 1. Participant Ambulatory Phonation Monitor (APM) data

	Participant	Distance Dose (m)	F0 Ave	PPE (%)	SPL (dB)
		Mic (No Mic)	Mic (No Mic)	Mic (No Mic)	Mic (No Mic)
Female Only	F1	2,514 (2,492)	281 (254)	59 (73)	100 (97)
	F2	2,540 (3,040)	263 (319)	25 (84)	107 (107)
	F3	3,480 (2,190)	252 (263)	51 (81)	108 (91)
	F4	2,390 (1,440)	262 (292)	62 (89)	96 (102)
	F5	2,120 (1,990)	290 (312)	61 (81)	87 (87)
	F6	(2,620)	(324)	(82)	(95)
	F7	(2,490)	(287)	(74)	(94)
	M1	2,170 (1,830)	212 (228)	18 (57)	94 (94)
	Range	1,360(1,600)	69(91)	37(16)	21(20)
	Median	3,480(1,440)	252(292)	25(81)	100(95)
	Average	2,608(2,323)	269.6(293)	51.6(80.5)	99.6(96.1)

Note: F0= Fundamental Frequency; PPE=Perceived phonatory effort; SPL= Sound Pressure Level; F=female, M=male

The findings from study support the hypothesis that PPE would be lower with amplification; however, the hypothesis for vocal dose was not supported. This introduces discussion concerning effort load of voice, required effort, and the impact on the RBIC instructors. The broad impact of this research is to more effectively educate and care for the vocal health of all RBIC instructors. Further studies targeted at RBIC could be completed. In addition, exploring other fitness instructors could allow a general understanding of fitness and voice.

Statement of Research Advisor

This project builds logically on my body of research that merges voice science and exercise science into a more holistic study of voice physiology in the occupational voice user. Improving our current voice habilitation and rehabilitation programs for those professionals using their voices in challenging environments requires quantification of the extent and quality of voice, which is accomplished for cycling fitness instructors in this investigation.

– *Mary Savage, Communication Disorders*

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Variation in Jaguar Occupancy in Response to Differential Land Use Practices by Human Communities

Cullen Anderson, Todd Steury, Robert Nipko, and Marcella J. Kelly

Effective conservation necessitates an examination of human influences on wildlife, particularly large carnivores.[1] Jaguars (*Panthera onca*) face anthropogenic pressures (e.g., poaching, deforestation, habitat fragmentation) throughout their geographic range.[2] However, certain human communities may pose greater threats to jaguars and their habitats than other communities due to differential land use.[1] In Belize, many communities (e.g., Creole, Mayan, Mestizo) often practice localized slash-and-burn or small-scale farming operations.[3] Mennonites, originally of European descent, produce much of the food and materials on which the country relies, but also convert tracts of forest to agricultural land at a larger, more intense scale than other rural communities.[3] We hypothesized these current development practices could exclude jaguars from occupying habitat around Mennonite communities compared to other communities.

Using jaguar camera trap data from 2017 and environmental data from two study sites (Hillbank and Yalbac) in Belize, we conducted a single-season occupancy analysis to compare jaguar habitat use near Mennonite and non-Mennonite rural agricultural communities to determine whether jaguars respond differently to these communities. Occupancy analysis estimates presence across an area, while accounting for imperfect detection. We placed 55 cameras on trails and roads to increase jaguar detectability.[4] Using the unmarked package in R,[5] we modeled occupancy and detection and ranked models by AICc, a metric of model competitiveness. We reviewed the literature to pre-select occupancy variables important for modeling jaguar habitat use in the region: distance to rivers, distance to roads, canopy height, and sex.[6,7] We modeled detection with trail-width and sex as covariates.[7,8] We then incorporated our occupancy variables of interest: distance to Mennonite community, distance to non-Mennonite community, and distance to closest human community of either type.

We found jaguar trap success was 4.56 per 100 trap nights, with 119 male captures and 43 female captures, for 162 total jaguar captures. These figures do not refer to numbers of jaguars; rather, they are indicative of jaguar detectability and distribution within the study area. Our top model incorporated distance to Mennonites. Surprisingly, our results suggested occupancy may increase with proximity to Mennonites. Additionally, a marginally competitive model suggested occupancy may decrease with proximity to non-Mennonites. However, the evidence for these relationships is not conclusive due to wide confidence intervals and the presence of several competing models. We do not have the data to definitively explain these results; however, we suggest our findings may result from greater human density and activity at the edges of non-Mennonite communities, pushing jaguars away from non-Mennonites. Alternatively, habitat alteration at the edges of Mennonite communities may benefit jaguar prey species, which may attract jaguars to those areas. However, we cannot examine the validity of these hypotheses with the current data set.

In the coming months, we will collect more habitat data from the same study area and conduct more analyses to attempt to clarify these findings. Our results provide interesting insight into the spatial effects human communities may have on jaguars. Understanding how jaguar habitat use responds to human development will inform more effective conservation of jaguars, other wildlife, and Belize's tropical forests.

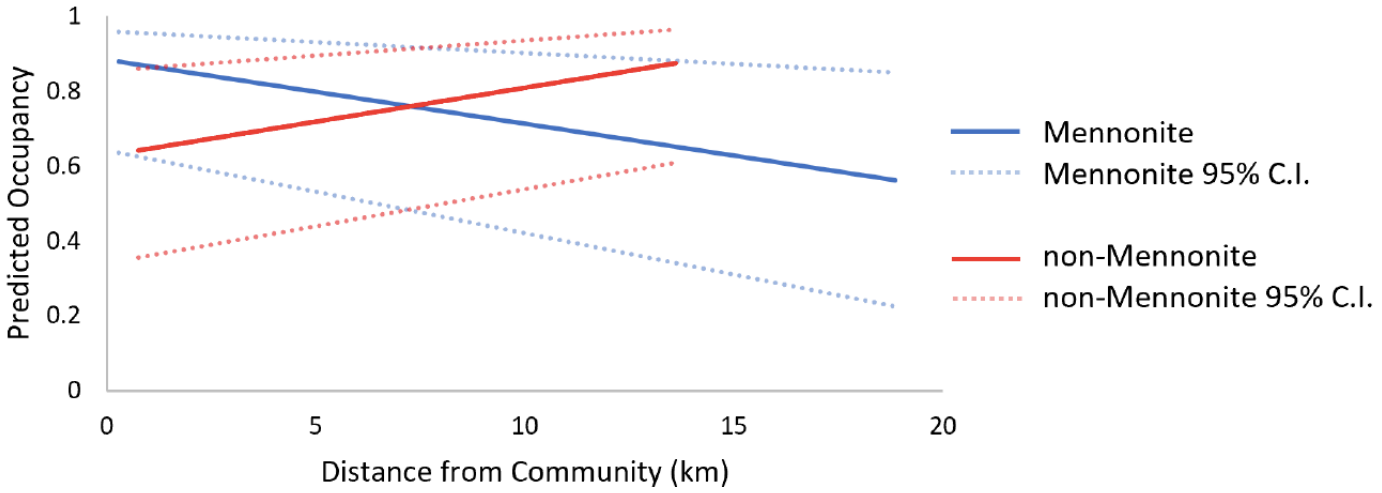

Figure 1. Jaguar occupancy trends with distance to human communities. Approximate estimated trends between male jaguar occupancy and distance from camera stations to human communities from two competing models (two other models were also competing). Trends for female jaguars (not shown) were similar. The solid blue line indicates estimated relationship between occupancy and distance to Mennonite settlements, while the solid red line indicates the estimated relationship between occupancy and distance to non-Mennonite settlements. Dotted lines indicate the 95% confidence intervals. Photo data were collected from 2 private reserves (Hill Bank and Yalbac) in Belize from May to October 2017.

Statement of Research Advisor

Given the ever-increasing direct and indirect effects of humans on wild places, research into how human-driven habitat change influences wild animals is critical to wildlife conservation. This research is a much-needed direct examination of the effects of humans on a top predator; a species that can have wide-ranging impacts on entire ecosystems and is of considerable conservation concern.

– *Todd Steury, Forestry and Wildlife Sciences*

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Hip, Knee, and Ankle Contributions are Affected During Sloped Walking in Individuals with Anterior Cruciate Ligament (ACL) Reconstruction

Katie Corona, Hillary Holmes, Randy Fawcett, and Jaimie Roper

Previous research has found that individuals with anterior cruciate ligament (ACL) reconstruction have a higher propensity to develop degenerative joint disorders such as osteoarthritis.[1] While possible contributing factors to this condition have been studied, very little research has been done on the exact joint moment contributions of the lower extremity during locomotion, specifically during sloped walking.

For this study we recruited 17 individuals who had undergone one ACL tear and reconstructive surgery. All participants were at least four months post operation and cleared to return to their activity by a clinician. Further, no participants had other lower extremity surgery or participated in physical therapy for any lower extremity injury within the last year. All participants walked under three different conditions on our instrumented split-belt treadmill. The split-belt treadmill, designed with two separate belts for each foot with force plates set in both belts, allowed us to gather independent kinetic data for each limb during walking. The participants walked on a level surface, 10 degree incline, and 10 degree decline at 1.3 m/s, 1.0 m/s, and 1.0 m/s, respectively, for two minutes without stopping. We used a 17-camera Vicon (Vicon, Oxford, OH) motion-capture system to record kinematic data. The instrumented treadmill gathered force data, and joint moments were calculated by Vicon Nexus' Plug in Gait Model.

We analyzed the relative joint moment contribution of each limb--that is, the peak moment contributed to the action of propulsion by the ankle, knee, and hip during stance phase. These values are represented as percentages in Figure 1 for comparison of the contribution of each joint. Upon analysis we found no significant differences between limbs for any condition. There was a significant effect of condition at the hip and ankle across all three conditions, and at the knee between flat and decline, and flat and incline conditions.

These results indicate that joint moment contributions changed during walking on incline and decline surfaces. The contribution of knee joint moment increased by around 37% during the decline condition compared to flat and incline conditions. Because we did not find any asymmetries between limbs, these findings reveal that it is possible for healthy young adults to maintain a symmetric gait post-ACLR. Additionally, these results support the possibility that an asymmetric gait may not be a significant contributing factor to the development of osteoarthritis, as previously thought.

Future research should investigate motions in the frontal plane, such as knee abduction (also known as "knock-kneed" pattern), as well as utilize a matched healthy control group for a more complete analysis of potential implications for the development of osteoarthritis.

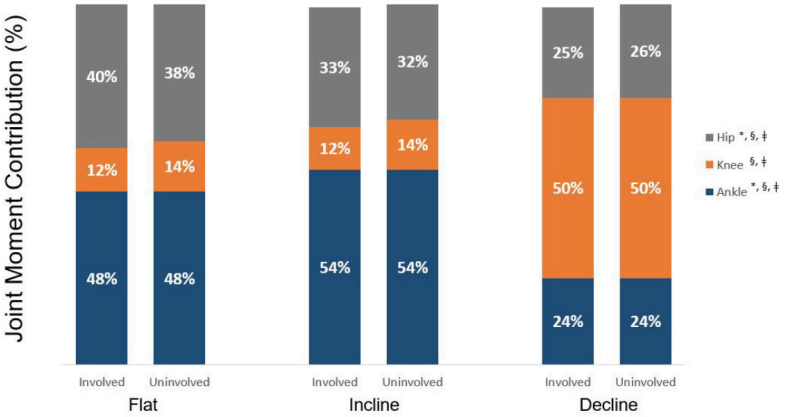

Figure 1. Differences among flat and incline (*), flat and decline (§), and incline and decline (ǂ) (p<0/05). The "Involved" column represents the ACLR limb, while the "Uninvolved" represents the contralateral, healthy limb. Joint Moment Contribution= [(Peak Joint Moment)/ (Σ Hip, Knee, Ankle Joint Moments)] x 100.

Statement of Research Advisor

Katie has executed a study that will inform clinicians and researchers that strive to reduce the onset of re-injury and osteoarthritis development in people with ACL reconstruction. Her work will have a large impact on advising people with ACL reconstruction throughout walking and running during uphill and downhill locomotion.

– *Jaimie Roper, Kinesiology*

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Modeling of Photosynthetic Aeration for Energy Efficient Wastewater Treatment

Bryan L. Holmes, Brendan Higgins

Wastewater treatment is an energy-demanding process with one of the main contributors to energy usage being the intensive aeration that aerobic bacteria require in order to break down organic waste materials[1]. Because of this, aeration alone accounts for roughly 50% of the energy used in the process[2]. Little research has been done to assess and model the effects of utilizing photosynthetic aeration sourced from algal microorganisms. Because algae are known to produce large amounts of dissolved oxygen in water, it is possible that their implementation would reduce the need for mechanical aeration[2,3].

The objective of this project was to quantify the effects that algae have on wastewater oxygenation and the removal of organic material by bacteria. We hypothesized that algae will supply dissolved oxygen to bacteria, thereby speeding degradation of organics. To test this hypothesis, a simplified model of a wastewater treatment process was developed that consisted of a synthetic wastewater media, a model bacterial organism, a model algae, and a single carbon source (2 g/L succinate) consumable by bacteria only. *Auxenochlorella protothecoides* (*A. protothecoides*) and *Escherichia coli* (*E. coli*) were used for the model algae and bacteria, respectively.

Studies were conducted in a 1-L bioreactor equipped with probes to monitor pH, dissolved oxygen, dissolved carbon dioxide, and temperature throughout the trials. This system was then tested both with and without the presence of algae, while monitoring the probe readings over time by use of a Mettler Toledo® M800 transmitter and data loggers. The collected experimental data were then analyzed with MATLAB® using a model that quantified net microbial gas production and consumption. High-pressure liquid chromatography (HPLC) was used to measure changes in succinate, acetate, and fumarate in the medium over time. The latter two acids are generated through cellular metabolism of succinate. After completing analyses on the synthetic system, trials were conducted on primary wastewater acquired from a municipal wastewater treatment facility in Columbus, Georgia. The main difference with these experiments was that our media now contained a plethora of organic compounds and bacterial organisms, making the system much more complex.

The results from our synthetic wastewater experiments indicate that *A. protothecoides* stimulated faster consumption of succinate by *E. coli*. This was likely driven in part by oxygen provision from algal photosynthesis. Dissolved oxygen levels initially depleted in all cultures but recovered 33% faster when algae were present in comparison to bacteria alone (Figure 1). This result indicates that the bacteria were able to consume the organic carbon at a faster rate when cultured with algae. Analysis of organic acids also indicates that more acetic acid, an anaerobic bi-product, was produced in cultures with bacteria than those supplemented with algae. This result, too, confirms that photosynthetic oxygen facilitates the breakdown of organics by bacteria.

As for the experiments regarding real wastewater, observations seem to indicate that the system behaved similarly to that with synthetic wastewater, but the declines in oxygen were much smaller, indicating a lower total organic load. Further analysis by chemical oxygen demand (COD) assay will reveal the extent and rate of organic degradation in the wastewater in the presence and absence of algae. Measurement of nutrient transformation and removal are also needed to determine if algae have a future in the wastewater treatment process. That said, the results from these experiments are conclusive that bacteria were able to perform more efficiently when algae are present, confirming the potential of further research to create a more energy efficient approach to wastewater treatment.

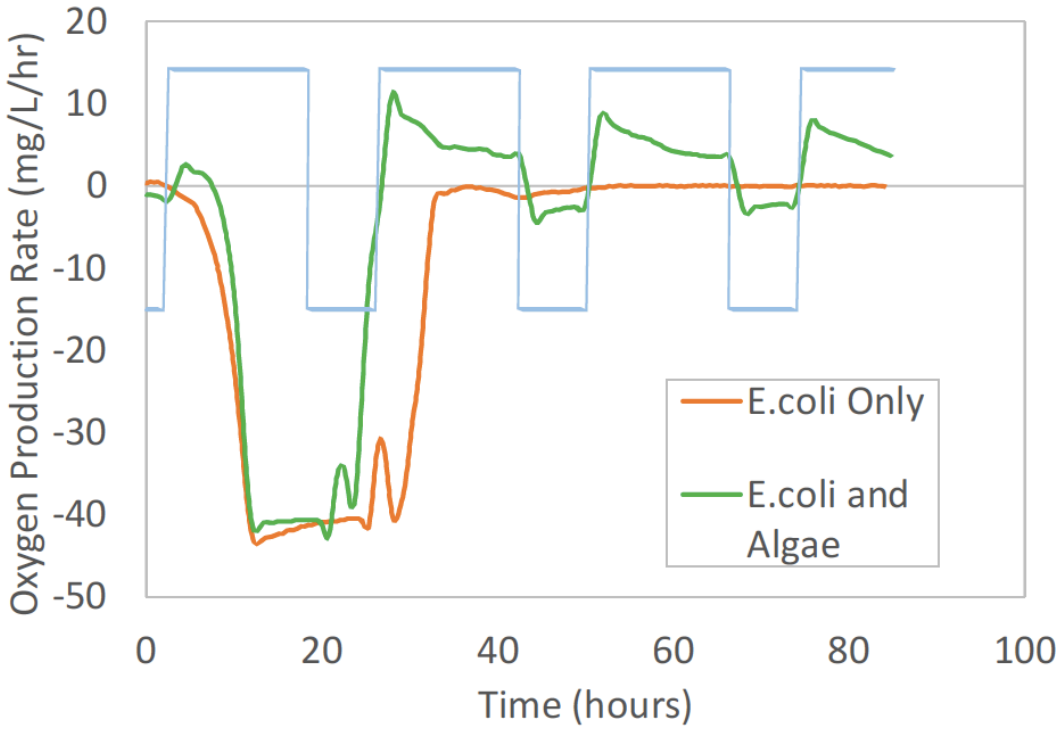

Figure 1. Net dissolved oxygen production (positive) or consumption (negative) by cells in a batch culture. The light was operated on a 14:10 on/off cycle, as designated by the blue line.

Statement of Research Advisor

Bryan's research has laid the groundwork for more in-depth study of gas exchange between algae and bacteria in wastewater treatment processes. This research holds the potential to significantly reduce aeration requirements in wastewater treatment plants while also producing algal biomass which has potential value for biofuel and protein production.

– *Brendan Higgins, Biosystems Engineering*

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Effect of Dietary Protein Source and Litter Condition on Mitotically Active Cell and Macrophage Cell Density in the Duodenum of Broiler Chickens at Day 21

Andrew J. Keel, Allan J. Calderon, Oscar J. Tejeda, Jessica D. Starkey, Charles W. Starkey

The small intestine (SI) serves as the site of nutrient absorption and defends against ingested pathogens. Proper nutrient absorption is dependent upon the constant proliferation of mitotically active cells to create new enterocytes, the absorptive cells of the SI, to replace those damaged by the harsh environment within the lumen. Proliferative cells in the small intestine are primarily located at the base of intestinal crypts. As these cells divide, the new cells migrate toward the tip of the villi before being shed. Immune cells, especially macrophages, are necessary to prevent pathogens from causing illness. Macrophages have multiple functions, such as warning other immune cells of pathogen invasion, destroying potential pathogens, and aiding in antibody production. It has been shown that certain dietary protein sources and litter conditions can affect the immune response and microbial population of the SI. Based on this information, it is of interest to determine if different dietary protein sources and litter conditions influence mitotically active cell and macrophage density in the SI of young broilers.

To explore this idea, an experiment with a 3 x 2 factorial treatment arrangement in a randomized complete block design was conducted. The three dietary protein sources used were porcine meat and bone meal, a 50:50 mix of poultry byproduct and poultry feather meal, and soybean meal. The two litter conditions used were new litter, which was fresh pine shavings, and used litter, which was pine shavings used to rear three previous broiler flocks. On day 0, 1,500 female Yield Plus x Ross 708 broiler chicks were placed in an environmentally controlled, raised floor pen facility. Broilers received *ad libitum* access to feed and water. On day 21, six broilers per treatment (total n = 36) were randomly selected for duodenal sample collection. Broilers were injected intraperitoneally with 5'-bromo-2'-deoxyuridine (BrdU) to label mitotically active cells. Duodenal samples were collected approximately 5 cm distal to the duodenal loop. Samples were flushed with phosphate buffered saline, flash frozen in liquid nitrogen, and stored at -80^{0}C for later analysis. Sample analysis began by collecting 5-µm-thick cryosections from each sample on a glass slide. Cryosections were stained using indirect immunofluorescence staining to label BrdU+ cells and macrophages. Taxonomic data were collected by enumerating BrdU+ cells and macrophages to determine the number of mitotically active cells and macrophages per square millimeter of tissue using digital fluorescence microscopy.

Data collected for the study were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, Cary, NC). Dietary protein source and litter condition were the fixed effects, and treatment means were separated using the PDIFF option of SAS. Means were declared significantly different when $P \leq 0.05$. Neither dietary protein source ($P \geq 0.5946$) or litter condition ($P \geq 0.3039$) significantly altered mitotically active cell or macrophage cell density. In conclusion, no treatment effects were observed in the duodenum on day 21. Although no significant differences were detected at this location and time point, further research will be conducted to study potential treatment effects in other locations of the SI at earlier time points.

Statement of Research Advisor

Jake Keel's work is an initiation of a multi-systems approach to evaluate and improve upon overall health and performance of animals raised for food. The major contribution of this research is to advance our knowledge of producing healthy, nutritious, and safe food for the consumer.

– *Charles Starkey, Poultry Science*

The Effects of Phosphoprotein Enriched in Astrocytes-15 (PEA15) on Cerebral Endothelial and Neuronal Cell Density in Domestic Cats

Kacie Florus and Emily Graff

A loss-of-function mutation in phosphoprotein enriched in astrocytes-15 (PEA15) results in severe neurodevelopmental abnormalities in cats. Previous work done in collaboration with HudsonAlpha Institute of Biotechnology explored how PEA15 may be involved in normal neurodevelopment by examining brains in normal cats (unaffected) and those with the mutation (affected). A new RNAseq analysis technique, called cell-type deconvolution, was developed to determine changes in cellular subtype. The cell-type deconvolution study showed that transcripts specific to neuronal cells did not change, while copies of genes specific to endothelial cells were increased in affected compared to unaffected cats, which suggests that affected cats have either increased endothelial cell number or increased endothelial activity with little to no change in neuron cell number.

While these findings are promising, this new technique needs to be independently validated through methods such as immunohistochemistry (IHC). Additionally, a separate analysis, known as differential gene expression, indicated that affected cats have increased expression of genes associated with collagen production. Collagen is an important component of blood vessels and is closely associated with endothelial cells. Changes in cerebral endothelial cell number and function, as well as changes in collagen levels, are associated with various neurodevelopmental abnormalities. These findings may suggest a potential mechanism that contributes to the phenotype observed in cats with a loss of normal PEA15 function. Based on these previous findings, our objective was to (1) validate the cell-type deconvolution data through IHC staining and morphometric analysis, and (2) determine if there are changes in collagen thickness of cerebral vessels.

To address our first goal, sections of the cerebrum from affected (N=5) and unaffected (N=5) cats were independently stained for endothelial cell and neuronal cell detection, digitized, and analyzed using morphometric image analysis software to quantify the density of endothelial and neuronal cells in the samples. We generated multiple algorithms to analyze the same section of cerebrum in order to selectively identify the stain against the background. For endothelial cell detection, we successfully created two algorithms that accurately identified the stain. However, there was no significant difference ($p > 0.9999$ and $p = 0.3095$) between the two groups. This finding contrasted with what our cell-type deconvolution data suggested. Similarly, an algorithm was refined for neuronal cell detection. Consistent with the cell-type deconvolution data, we found that there was no significant difference ($p = 0.47$) between the two groups.

To address our second goal, sections of cerebrum from affected (N=4) and unaffected (N=5) cats were independently stained with Masson's trichrome to distinguish cells from surrounding collagen. Preliminary results suggest that there is an increase in collagen surrounding vessels of affected compared to unaffected cats. In future studies, we plan to measure the ratio of the thickness of the collagen compared to the thickness of the vessel wall for all samples and statistically evaluate difference in ratios between affected and unaffected cats.

In summary, cell-type deconvolution data were able to evaluate neuronal density with findings that were consistent with IHC; however, cell-type deconvolution data did not correlate with IHC staining for endothelial cells. This discrepancy in findings for endothelial cells may reflect a distinct change in endothelial cell activity rather than cell number. For example, endothelial cells can drive changes in collagen within vessels walls, which supports the finding of a subjective increase in collagen in the vessels of affected cats. However further study is needed to determine the significance of these findings.

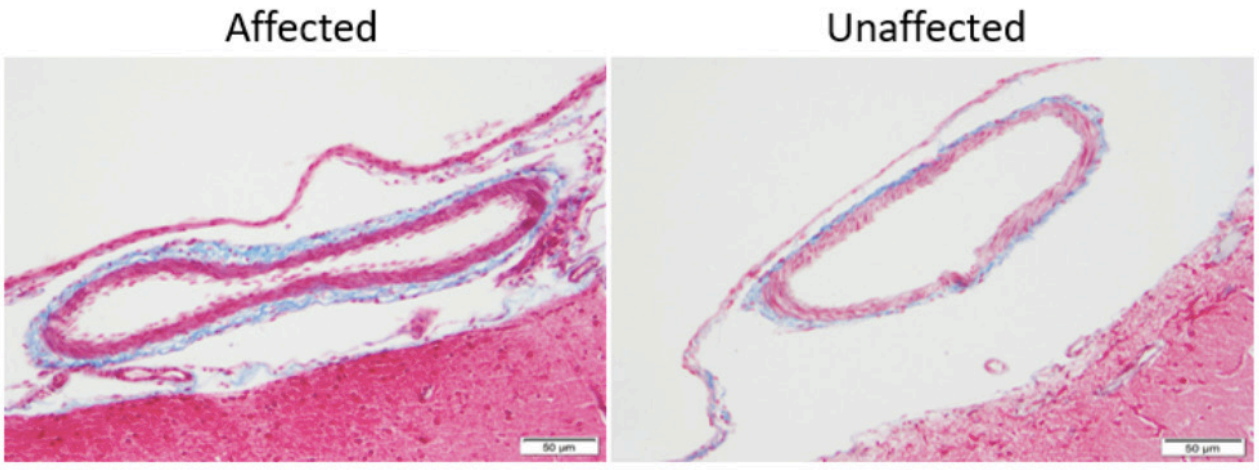

Figure 1. Masson's trichrome stain samples. Sections of cerebrum from affected (N=4) and unaffected (N=5) cats were independently stained with Masson's trichrome. These images represent blood vessels from unaffected cats (right) and affected cats (left). The blue around the blood vessel is collagen.

Statement of Research Advisor

Kacie Florus worked to develop and evaluate IHC stained slides of feline cerebral tissue in order to evaluate mechanisms that contribute to the serve phenotype observed in cats with a loss-of-function mutation in PEA15. Her work is an important contribution that has helped develop and validate new research tools in our lab.

– *Emily Graff, Pathobiology*

Testing Whether Sex-Specific Senescence in the Lizard, *Anolis sagrei*, Translates to the Cellular Level

Milica Courtenay, Amanda Clark, and Tonia Schwartz

Within numerous species, males and females age at different rates resulting in varying susceptibility to age-related diseases. This sexual dimorphism is understood at the organismal level and the trend is that females live longer than males[1]. Our group has found this to be true in the lizard, *Anolis sagrei*, posing this species to be a potential model for studying the basis of sex-specific aging. Seeking to investigate this phenomena on the cellular level, cell culture provides a model to study senescence (the deterioration and loss of function leading to cell death) in a controlled environment. Biomarkers of aging are commonly used to quantify the rate of senescence. Telomeres, found at the ends of chromosomes, are the most commonly used biomarker of aging[2,3]. Upon each genome replication during cell division, telomere length shortens; thus, the length generally shortens with age. Telomere maintenance varies with sex and age in mammals[4]. This study aims to further develop brown anoles as a model for sex-specific aging by (1) establishing cell lines from young lizards, (2) establishing cell lines from old male and female individuals, and (3) testing if telomere length is a good predictor of senescence and sex-specific senescence in these lizards.

The fibroblast cell culture was set up using tail tissue from three-year-old adult males (n = 3), three-year-old adult females (n = 3), and one-month-old hatchling males (n = 3; n_{tot}= 9). At each passage, cells were collected and divided between seeding the next plate, being frozen for long-term storage, and being frozen for analysis. Through this study, we have demonstrated it is possible to establish cultures from this species at the beginning and end of their lifespan (3-4 years in the wild). Culturing methods are continually being optimized for these non-model organisms, so our practices are working to fill a gap in knowledge. The cells frozen for long-term storage are the start of a biobank for future research in our lab. From each sample group, snap-frozen cells from passage two were used in telomere analysis.

Telomere length of the nine cell lines were quantified through DNA isolation, DNA quantification, then quantitative polymerase chain reaction (qPCR)[5,6,7]. We found that on average, telomere length in cells lines started from young lizards were longer than in cell lines started from adults (average starting quantity of 19193 vs 13265), and cell lines from adult female lizards had longer telomeres than adult males at the same (old) age (average starting quantity of 14404 vs 13265). Although not statistically significant at p = 0.05, which is perhaps because of our small sample size in each group, these results are consistent with the hypothesis that telomere dynamics in lizards are similar to mammals in that the telomeres decrease with age and old females have longer telomeres than males of an equivalent age. Viewed cautiously, these findings provide preliminary support for telomeres as a viable biomarker for senescence and sex-specific senescence in this lizard species. Future work will increase the number of cell lines for each age group for further telomere analysis along with other common biomarkers of aging.

In conclusion we have been able to establish fibroblast cell lines from young and old anole lizards that can be used for future experiments, and we have preliminary support for telomeres being a biomarker of senescence in these reptiles. These data and resources further support further study into using the brown anole as a promising model to study sex-specific aging.

Statement of Research Advisor

The field of aging biology has limited vertebrate models to study sex-specific aging. Milica and her PhD student mentor, Amanda Clark, have established the first known set of age-specific cell lines in a reptile species that can be used for future experiments in the context of aging. Furthermore, we have very limited understanding of telomere dynamics in reptiles as a whole, and this is the first study to quantify telomeres in anole lizards. These accomplishments are major

steps forward in using reptiles as models of aging.
– *Tonia Schwartz, Biological Sciences*

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Impacts of Wild Pigs on Acorns as a Food Resource for Native Wildlife

Arielle Fay, Steve Zenas, Mark Smith, and Stephen Ditchkoff

Wild pigs (*Sus scrofa*) are a non-native invasive species that cause $1.5 billion/year in damage to agriculture in the United States, destroy native plant communities, and compete with native wildlife for seasonally available resources (e.g., acorns), especially economically important game species such as white-tailed deer (*Odocoileus virginianus*) and wild turkey (*Meleagris gallopavo*). Our objective was to estimate the consumption of acorns by wild pigs relative to that of other native wildlife species.

For this study, we established 40 monitoring stations beneath acorn producing trees at a 3,406 ha study site of privately owned land in eastern Alabama. At each monitoring station, we placed 5 acorns on a 1m x 1m sand pad and positioned a game camera to record acorn fate (species-specific consumption) during 2-week intervals once a month from November to February, 2018-19. Additionally, we constructed acorn traps from 18.9 L plastic buckets to estimate the total number of acorns that were potentially available for consumption at each monitoring station.

From approximately 3.5 million camera images taken during 55,619 total camera hours, we determined the number of acorns consumed by each species over the 5 months of sampling. From the 1,200 acorns under surveillance, we found that 367 were consumed by 13 species while 183 were lost due to flooding, 322 left on the sand pad at the time of camera failure, and 328 were not consumed. Wild pigs (23.2%) consumed the most acorns followed by white-tailed deer (21.5%) and squirrels (18.8%). Wild turkeys consumed <1% of acorns, which may have been a function of a very low population of turkeys on the study area.

Our observations suggest that wild pigs likely consume a significant amount of acorns and may possibly reduce availability of this pulse resource for other native wildlife species. Additionally, wild pigs may potentially influence oak regeneration as they consume oak seeds (e.g., acorns) before they have a chance to develop. With further research on this project, we will determine if wild pig consumption of acorns depletes this food resource to such an extent that native wildlife are impacted. As part of a larger study, wild pigs will be removed from our study site during the summer and fall of 2019, and we will repeat this study again to estimate changes in acorn consumption by native wildlife after wild pig removal.

Statement of Research Advisor

Arielle's research is the first step in plugging some big holes in our understanding of how invasive wild pigs impact native wildlife species. Her research has been exceptionally well-received by the professional community and is a true, unique contribution to science.

– *Mark Smith, School of Forestry and Wildlife Sciences*

Improving Polymer-Modified Asphalt by Changing the Characteristics of SBS Using Click Chemistry

Tina Huang, Breanna Dobyns, and Bryan Beckingham

The United States has more than 2.7 million miles of paved roads and highways, and 94 percent of those are surfaced with asphalt[1]. Asphalt is made of aggregates (e.g., crushed rock and sand) and mixed with a binder called bitumen, a derivative of crude oil. However, high-volume traffic has resulted in a shorter lifespan for asphalt roads and the permanent strain on asphalt, including rutting and cracking, can make driving rough and dangerous[2]. Increased cost of maintenance is also directly correlated with high traffic volume and strained asphalt. The need for polymer-modified asphalt is now at the forefront with its increased durability and improved viscoelastic properties. Polymer-modified asphalt has stronger heat and cracking resistance than traditional asphalt. Even so, drawbacks such as separation of polymer-asphalt mixture and poor solubility prevent polymer-modified asphalt from replacing traditional asphalt.

This project is aimed at investigating a polystyrene-*block*-polybutadiene-*block*-polystyrene-triblock copolymer (SBS), a thermoplastic elastomer, to improve polymer-modified asphalt so that it can be a possible replacement for traditional asphalt. Thermoplastic elastomers have both rubber and plastic properties that can improve the elasticity of asphalt when added. When SBS and asphalt are mixed together, there is sufficient swelling and uniform dispersion. Both of these are necessary for application. However, the difference in polarity results in unsatisfactory storage stability and incompatibility. SBS is a nonpolar copolymer, while asphalt has polar components mixed in such as sulfur, nitrogen and oxygen[3]. Therefore, polymer-modified asphalt, the SBS and asphalt mixture, must be produced on demand, which increases costs dramatically.

Modification of SBS has the potential to overcome these negative characteristics[3]. In order to modify SBS, functional groups were added to the 1,2 double bonds. The goal was to improve the stability and miscibility of polymer-asphalt mixtures. Click chemistry is a stereospecific, free radical reaction that will selectively add functional groups to the 1,2 double bonds. In my project, 2,2-azobisiobutyronitrile (AIBN) was used as a free radical initiator. It was added in excess due to inhibitors present in sodium 3-mercaptor-1-propanesulfonate (NaMP), the modifying agent, and tetrahydrofuran (THF), the main solvent. Inhibitors were used to prevent compounds from reacting with themselves during storage. The reaction occurred in a nitrogen-filled reaction flask at temperatures above 65 °C to prevent AIBN from reacting with oxygen and to determine the temperature with the most effective conversion ratio. The flask was placed in a silicon oil bath to create a constant temperature environment. The two-neck reaction flask was connected to a condenser in order to prevent THF vapor buildup and increase the surface area for both vapor-liquid contact and heat exchange, as shown in Figure 1. The reacted product was dried in the vacuum oven and characterized with nuclear magnetic resonance (NMR).

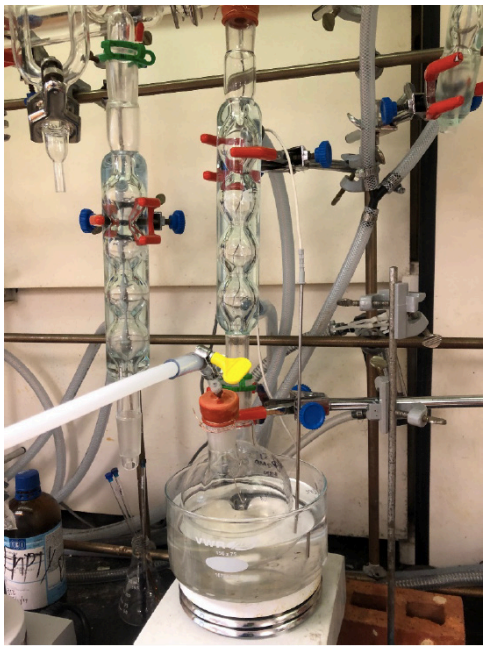

Figure 1: Reaction setup for thiolene click chemistry with SBS using a two-neck flask with a condenser and oil bath.

In the future, trials will be run with cleaned THF (free of water and oxygen) and a higher percentage of excess AIBN to significantly improve the conversion of double bond to function group. Additionally, other modifying agents will be tested to provide a diverse set of modified SBS block copolymers. Once the desired products are obtained, they will be characterized with NMR, differential scanning calorimetry, and gel permeation chromatography. The mechanical properties will be tested and mixed with asphalt binder at The National Center of Asphalt Technology to examine their potential for field application in Alabama roadways.

Statement of Research Advisor

Tina has made good progress as she attempts to produce modified SBS triblock copolymers for use in asphalt pavements. She has developed synthetic methods for performing her target chemistry and we are looking forward to testing the fruit of her efforts with asphalt binders to investigate their potential. – *Bryan B. Beckingham, Chemical Engineering*

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Investigation of the Performance and Energy Consumption of Superconducting Adiabatic Computing Circuits

Keith M. Krause, Michael C. Hamilton, and Uday S. Goetti

Superconducting circuits using adiabatic quantum-flux-parametron (AQFP) logic have been shown to have ultra-low power consumption. The energy dissipation of adiabatic processes are orders of magnitude lower than non-adiabatic processes, which can lead to a high number of logic gates with little power consumption. We have studied the bit-energy dissipation of various superconducting computing circuits that use AQFP logic gates, including NOT gates and Full Adders. In digital binary computation, a NOT gate will give an output "1" when it has an input "0," and it will give an output "0" when it has an input "1." A Full Adder performs an addition operation on three one-bit binary numbers. It produces a sum of the three inputs and a carry value. AQFP logic gates use Josephson Junctions (JJs). These devices consist of a thin layer of non-superconducting material between two layers of superconducting material. When a critical level of current is reached, electron pairs can tunnel across the non-superconducting layer. This is known as the Josephson effect. We have analyzed numerous AQFP logic circuits using the JJ simulator, JSIM.

A NOT gate and Full Adder were separately tested in JSIM (Figures 1 and 2). First, these circuits were verified by testing all possible input combinations and examining the results. Once we confirmed that these circuits were operating correctly, we ran multiple bit-energy dissipation analyses tests. We simulated one bit of input, which correlated to one pulse for each excitation line in the circuit. The energy dissipated in the excitation lines was then calculated. For each consecutive test, the rise/fall time of the trapezoidal excitation current was increased by 10 ps. This process was repeated from 100 ps to 10,000 ps.

In AQFP logic, a NOT gate has two AQFP gates, where the output of the first is negatively coupled with the input of the second. The simulated energy dissipation for the excitation lines in this circuit leveled out with an increasing rise/fall time, well above $k_B T \ln 2$. This refers to the Landauer limit, or the thermal limit for one bit of information. These simulations assume an operating temperature of 4.2 °K, where the Landauer limit would be about 0.04 zJ. In AQFP logic, a Full Adder uses a MAJ gate, where the output corresponds to the majority values of the three inputs. Notably, this circuit had two excitation lines wherein the simulated energy dissipation decreased linearly with increasing rise/fall time, suggesting that they could fall below the Landauer limit with a greater rise/fall time.

In conclusion, we have successfully simulated a functional NOT gate and Full Adder using AQFP gates, which are two essential components of digital logic. The energy dissipation results of the Full Adder indicate that longer rise/fall times can lead to power consumption below the Landauer limit. This shows how AQFP logic could lead to a much greater energy efficiency than both the CMOS technology, which is currently used, and various other superconducting technologies, such as RSFQ and ASL.

Statement of Research Advisor

As we move towards new forms of computing, technologies based on superconductive devices are becoming more intriguing. The inherent, near-zero, power dissipation in superconductors presents opportunities for extremely low energy computing. Adiabatic digital logic circuits, such as the AQFP circuits studied here and other related technologies, are very promising for computing with ultra-high energy efficiency while also maintaining high operation speeds. The work presented here provides the foundation on which our future simulation and experimental efforts will be based.

– Michael Hamilton, Electrical and Computer Engineering

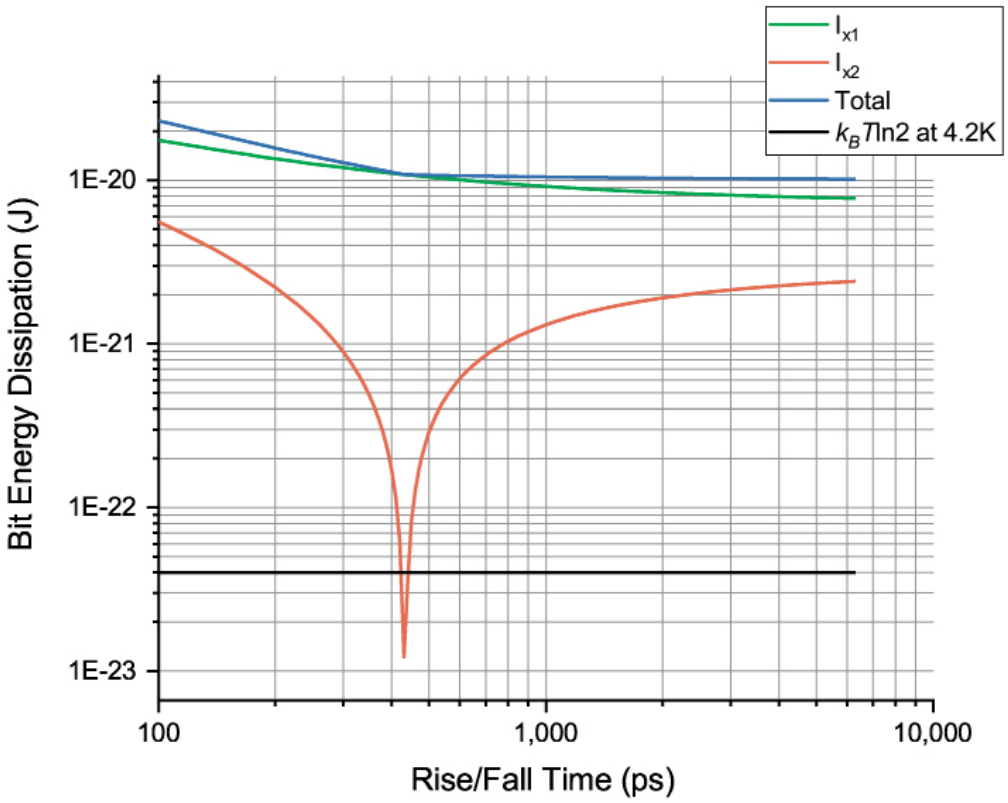

Figure 1: Simulation results from bit energy dissipation analysis of a NOT gate. I_{x1} and I_{x2} represent each excitation line in the circuit, *Total* represents the combined energy dissipated in all excitation lines, and $k_BT$ln2 at 4.2 °K represents the Landauer limit.

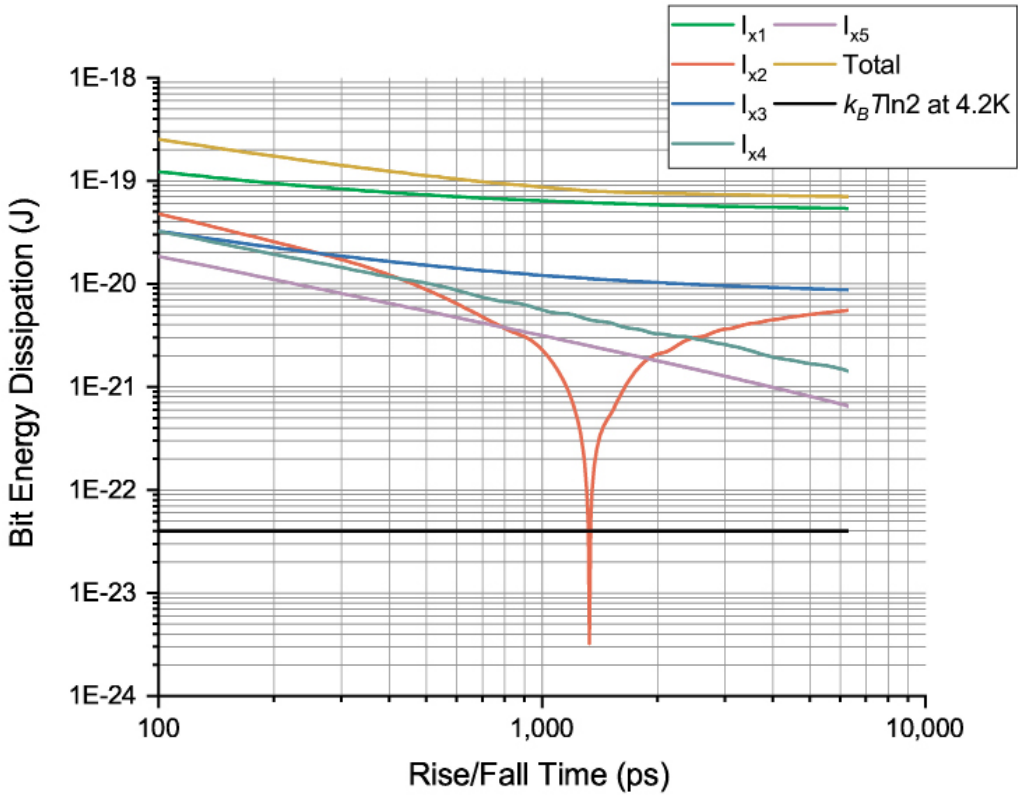

Figure 2: Simulation results from bit energy dissipation analysis of a Full Adder. I*x1*-I*x5* represent each excitation line in the circuit, *Total* represents the combined energy dissipated in all excitation lines, and $k_BT$ln2 at 4.2 °K represents the Landauer limit.

L2 Spanish Learners' Auditory Processing of Gender and its Role in Relative Clause Attachment Resolution

Taylor Mackowski and Daniel Vergara

Second Language Acquisition studies have focused mainly on the instruction, learning and acquisition of grammatical gender (Clahsen & Felser, 1998). However, little to no research has focused on the processing—perception and interpretation—of grammatical gender in learners of Spanish as a second language. This study fills this gap by examining the processing of gender by second language (L2) learners of Spanish to resolve potential ambiguities between noun-adjective agreement in relative clauses (RCs).

Previous research on ambiguous RCs reveals that there are crucial differences in the attachment preferences between native speakers of English and Spanish. Take, for instance, the ambiguous Spanish RC in (1) and its respective English translation in (2):

(1) El profesor enseña en [gimasios]masc. de [colegios]masc. que están [limpios]masc.
(2) 'The professor teaches in gyms of schools that are clean.'

In (1) and (2) the adjective at the end of the RC can potentially agree with both, the higher (i.e., high attachment) and the lower noun (i.e., low attachment). Interestingly, when presented with a sentence like (1), Spanish speakers consistently prefer the high attachment option, whereas when presented with (2), English speakers prefer the low attachment one.

Spanish is a language with a binary gender system (e.g., masculine, feminine), where gender can be morphologically marked in the inflection of nouns and adjectives (e.g., *-o* for masculine, *-a* for feminine) or in the determiner (e.g., *el/ la*). This is illustrated in the unambiguous Spanish RC in (3):

(3) El profesor enseña en [gimnasios]masc. de [escuelas]fem. que son [limpios]masc.
'The professor teaches in gyms of schools that are clean.'

When (3) is presented aurally to learners of Spanish, they must be able to perceive and understand (i.e., process) that the masculine adjective *limpios* modifies the masculine noun *gimnasios* in order to correctly interpret the RC. Failure to do so will result in the application of the English attachment preference by default, erroneously linking the meaning of the adjective *limpios* to the lowest noun, which is feminine. Based on this assumption, we propose the following research question: Do L2 learners of Spanish aurally process gender to resolve the attachment in unambiguous relative clauses?

In order to answer this question, we recruited 31 beginner and 19 intermediate L2 learners of Spanish from an American public university. Participants completed a Spanish proficiency test and were divided according to proficiency level (beginner, intermediate, and advanced). The target stimuli consisted of 32 RCs, in which the adjective inside of the RC matched in gender (masculine, feminine) with either the lower (n= 16) or the higher noun (n=16). The target stimuli were presented aurally and were followed by a question to determine participants' accuracy in adjective-noun gender agreement. The study was administered entirely via Qualtrics.

Analysis of preliminary data revealed that L2 learners of Spanish have difficulties processing gender in RCs regardless of their level of Spanish. In general, participants performed at chance or below chance regarding their accuracy in gender assignment in the RCs presented in the target stimuli. However, we did observe an effect by proficiency, where advanced participants were more accurate in their assignment of gender in cases of high attachment as well as in the presence of a determiner. These results point to emergent L2 strategies for aural processing of gender that have important pedagogical implications.

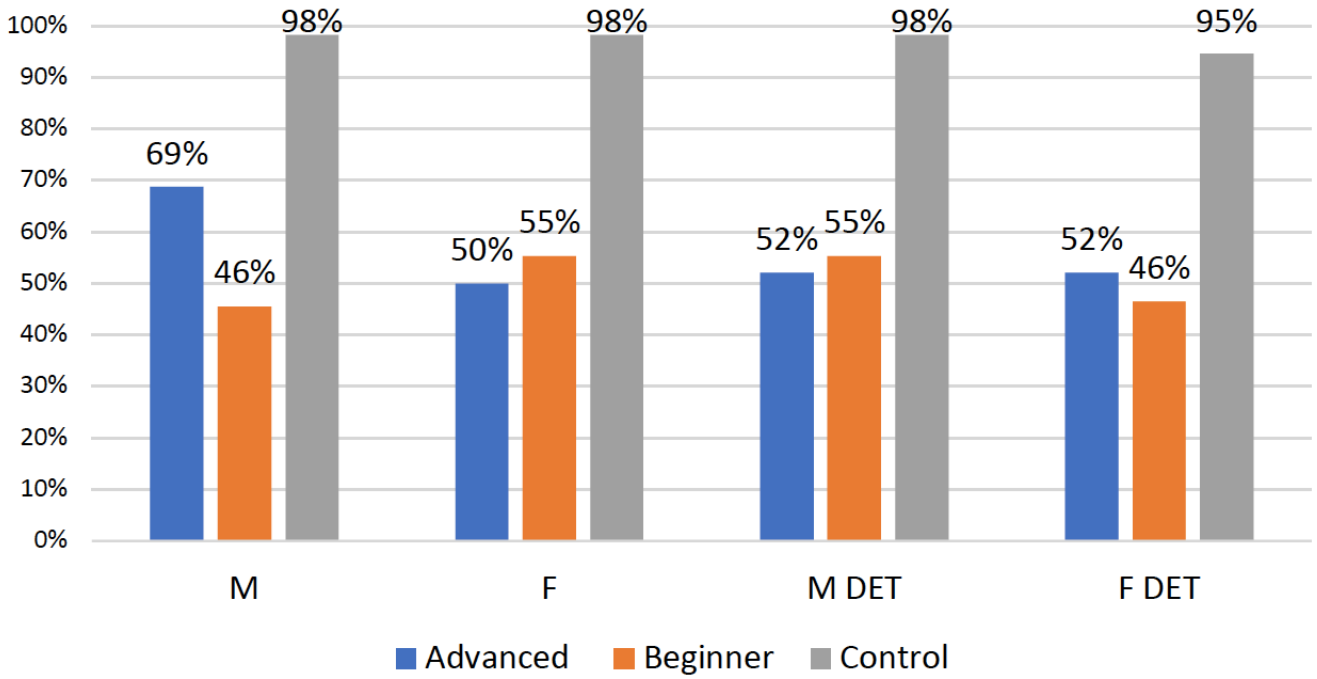

Figure 1: Relative clause attachment accuracy by gender according to proficiency.

Statement of Research Advisor

Foreign language practitioners often focus on providing meaningful language input to students; however, little is known about whether the students process that input. To this end, Taylor's research highlights the importance of teaching gender processing strategies to L2 learners of Spanish in order to facilitate input comprehension. This research has been approved by the Institutional Review Board (IRB) at Auburn University (protocol number:19-045 EX 1902)

– *Daniel Vergara, Foreign Languages and Literatures*

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